Origin and evolution of the unique Australo-Papuan mangrove-restricted avifauna: novel insights form molecular phylogenetic and comparative phylogeographic analyses

By

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Abstract

Coastal mangrove forests of Australo-Papua harbor the world's richest avifauna restricted to mangroves, however their biogeographic origins and evolutionary processes shaping their current distributions are not well understood. Building upon previous work based on field surveys and morphological characters, I am here focusing on elucidating the phylogenetic placement of mangrove-bound species from three different bird families as well as the comparative phylogeographic analysis of eight co-distributed mangrove restricted birds.

In the first molecular phylogenetic analysis of fantails (Aves: Rhipiduride) I document six distinct clades, harboring members spread across large geographic extents. *Rhipidura hypoxantha* is not a true fantail, but rather a member of the Stenostiridae clade that is morphologically and behaviorally convergent with fantails. The Australian mangrove fantails *R. phasiana* and *R. dryas* both evolved recently from Pacific island radiations.

A molecular phylogeny of all extant species of the honeyeater genus *Lichenostomus* (Aves: Meliphagidae) also addresses the relationship of the only mangrove-restricted honeyeaters on Australia's east coast, *L. versicolor* and *L. fasciogularis*. These species were not sisters but rather *L. versicolor* was sister to the pair comprising *L. fasciogularis* and the continental widespread Singing Honeyeater *L. virescens*. The genus *Lichenostomus* is not monophyletic, and instead comprises seven distinct lineages interdispersed within the larger meliphagid assemblage. Based on this

taxonomic and nomenclatural revision, recognition of a novel genus of honeyeater is warranted.

A multilocus molecular phylogeny of gerygones (Aves; Acanthizidae) establishes that the three mangrove endemic species do not form a monophyletic clade, instead indicating three distinct, temporally non-overlapping, radiations into magroves. Moreover, *G. cinerea* from New Guinea is in fact a member of the genus *Acanthiza*, with which it consistently grouped based on 13 distinct molecular loci analyzed.

Comparative phylogeographic analyses of 8 co-distributed mangrove forest endemic birds concludes biogeographic barriers such as the Canning Gap, Bonaparte Gap, and the Carpentarian Gaps all had important, but varying degrees of impact on the species we analyzed. Species with more recent radiations into mangroves include *Rhipidura phasiana, Myiagra ruficollis,* and *Myzomela erythrocephala*, while *Peneoenanthe pulverulenta, Pachycephala melanura*, *P. lanioides, Zosterops luteus*, and *Colluricincla megarhyncha* all had more marked phylogeographic signatures.

My wonderful and kind wife Mona was always on my side and in my heart, providing a comforting and loving feeling during difficult times. During Mona's postdoc in Madison, we have met some old and new friends, who I would like to acknowledge for their wonderful warm company during those cold Wisconsin months.

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Phylogenetic relationships of fantails (Aves: Rhipiduridae)

Abstract

We explore the phylogenetic relationships of fantails (Aves: Rhipiduridae) using molecular characters derived from two nuclear introns and two mitochondrial genes. Our results indicate that *Rhipidura hypoxantha* is not a true fantail, but rather a member of the Stenostiridae clade that is morphologically and behaviorally convergent with fantails. Within the true Rhipiduridae, we identified 6 distinct clades, however phylogenetic relationships among these groups were unresolved. The only well-supported sister relationship was between members of the grey and the rufous fantail complexes. Clades recovered through our model-based phylogenetic analyses generally correspond to previously proposed fantail complexes based on morphological characters. The phylogenetic position of *R. atra* and *R. diluta* remain unclear, as sister relationships varied between analyses for the prior whereas the latter was placed as sister to the New Guinea thicket fantails, *R. leucothorax* and *R. threnothorax*, yet significant node support was not recovered for either taxa. Biogeographically, fantails appear to have radiated rapidly and the six clades are not geographically restricted, but instead span Southeast Asia, New Guinea, Australia, and Pacific Islands.

1. Introduction

Fantails are a well-defined family of small-bodied insectivorous passerine birds distributed across the Oriental, Australasian, and southwest Pacific island regions, with a center of diversity located on New Guinea. Currently, ~44 species are recognized in a single genus, *Rhipidura* (Boles 2006, Dickinson 2003). Diagnostic to the family, all fantails exhibit elongated rectrices, which are held spread apart to form the characteristic fan-shaped tail that in some species may be held cocked, and or swung side to side. Besides obvious inter and intra-specific signal functions, the tail is used, together with partly spread wings, as a "parachute" during foraging, as the bird falls off its perch, tumbling towards the ground for passing insects (Boles 2006).

From a taxonomic point of view, fantails have been suggested to share affinities with Old World flycatchers (Muscicapidae), but this relationship appears to be convergent given their general feeding habits. Recent molecular work has indicated that fantails are part of the large Australo-Papuan songbird radiation, widely separate from the Muscicapidae, and instead part of the Corvoidea, most closely related to monarch flycatchers (Monarchidae), drongos (Dicruridae), and in particular to the Pygmy Drongo, *Chaetorhynchus papuensis* (Barker *et al.* 2002, 2004, Cracraft *et al.* 2004, Irestedt *et al.* 2008). The cohesiveness of the Rhipiduridae has not seen any major challenges, although one species, the Yellow-bellied Fantail (*Rhipidura hypoxantha*) stands out as having smaller body size compared to other rhipidurids, and is the only fantail with a carotenoid based, bright yellow plumage. As such, this distinctive fantail has at times been assigned to a separate genus, *Chelidorhynx* (Boles 1979, 2006).

Within the family, various subgroups and subgenera have been delineated, mainly based on plumage characters and geographic distribution. Probably the best-studied group is the grey fantail complex (Ford 1981, Schodde & Mason 1999, Boles 2006, Christidis & Boles 2008): occurring throughout the Australo-Papuan region, satellite islands, and New Zealand, this complex includes the species *R. albiscapa, phasiana, fuliginosa, albolimbata,* and *hyperythra* (Table 1), all characterized by a somewhat drab plumage and a grey to black dorsum. Ecologically, this group includes the New Guinea lowland species *R. hyperythra*, which is replaced by *R. albolimbata* in the highlands, while on mainland Australia, the more widespread *R. albiscapa* gives way to the mangrove-restricted species, *R. phasiana* (Boles 2006, Schodde & Mason 1999). The central Pacific Islands hold another assemblage hypothesized to be closely linked to the latter complex. This island-endemic group of six species is centered on the Streaked Fantail, *R. verreauxi*, and includes the species R. *personata, nebulosa, drownei, tenebrosa*, and *rennelliana* (Table 1). Previous taxonomic arrangements have acknowledged a close relationship between these two groups by placing these 11 species in the subgenus *Rhipidura* (Watson *et al.* 1986, Boles 2006).

Another large assemblage is the rufous fantail complex, including *R. rufifrons* and 11 other species (Table 1), mostly with rusty-rufous dorsal coloration. Members of this complex are distributed throughout Australia, New Guinea, South and Central Pacific islands, and Indonesia; these species are sometimes placed in the subgenus *Howeavis* (Boles 2006). The northern fantail complex is a smaller group of four species (*rufiventris, diluta, fuscorufa*, and *cockerelli*), extending from the Moluccas through New Guinea, and south to northern Australia (Table 1).

Apart from these major groups, several smaller species clusters have been distinguished within the family, albeit without explicit evolutionary affinities to any of the previously mentioned larger complexes. Examples include the two Philippine endemic species with blue coloration (*R. cyaniceps* and *R. superciliaris*), the New Guinea Thicket Fantails (*R. threnothorax, R. leucothorax,* and *R. maculipectus*), and the distinctive Willie Wagtail (*R. leucophrys*), the largest-bodied member of the family, which is placed in the subgenus *Leucocirca*. Compared to the aforementioned larger rhipidurid groups, the relationships of these remaining fantails, mostly from Southeast Asia, Indonesia, and the Philippines, have received relatively little attention.

In sum, no comprehensive revision of this family has been undertaken, and an overview of phylogenetic relationships of its members is lacking. Herein, we use molecular characters from multiple loci to establish a phylogenetic framework for the Rhipiduridae. We address questions pertinent to (1) the monophyly of the family, (2) the cohesiveness of presently recognized species groups and subgenera, and (3) biogeographic patterns of constituent subgroups.

2.   Materials and Methods

2.1   Taxon sampling and molecular markers

For the present study, the family Rhipiduridae was represented by a total of 35 individuals of 29 species (Table 1). The remaining 13 unsampled species, with the exception of the distinctive *R. phoenicura*, have been considered members of superspecies (Boles 2006), which are represented in this study. For 5 species, we included two samples per species, to confirm species identification and to obtain rough

estimates of intraspecific genetic variation among geographically distinct populations. This study is founded on vouchered genetic samples of fantail species collected throughout the family's range. The choice of outgroup taxa was based on results from recent molecular studies of passerine phylogeny, in which a lineage comprising *Chaetorhynchus papuensis* of the New Guinea highland and *Lamprolia victoriae* of Fiji Islands has been suggested as the closest extant relative of fantails (Barker *et al.* 2002, 2004, Cracraft *et al.* 2004, Irestedt *et al.* 2008). We also included representatives of the Old World flycatchers (Muscicapidae) and the drongos (Dicruridae) as additional outgroup taxa (Table 1).

Total genomic DNA was extracted from frozen or alcohol-preserved tissue samples using standard Qiagen tissue extraction protocols (Qiagen, Valencia, CA). Our choice of molecular markers relied on previously used and established mitochondrial protein-coding genes and two nuclear introns. Sequences of the mitochondrial genes nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2; 1041 bp) and subunit 3 (ND3; 351 bp), the fifth intron of the transforming growth factor ß2 (TGFb2; 590 bp aligned), as well as the fifth intron of the nuclear gene Beta-Fibrinogen (Fib5; 613 bp aligned) were amplified using the primers L5215 – H6313 (Sorenson *et al.* 1999), L10755 – H11151 (Chesser 1999), TGF5 – TGF6 (Primmer *et al.* 2002), and Fib5 – Fib6 (Marini & Hackett 2002), respectively.

All loci were amplified in 25 μl reactions under standard PCR thermocycling protocols using PureTaq™ RTG PCR beads (GE Healthcare Corp.). Amplified double-stranded PCR products were cleaned with ExoSAP-IT™ (GE Healthcare Corp.), and visualized on high-melt agarose gels stained with ethidium bromide. Purified PCR

products were cycle-sequenced with ABI Prism BigDye™ v3.1 terminator chemistry, using the same PCR primers Cycle-sequenced products were further purified using Sephadex™ spin columns (GE Healthcare Corp.), and finally sequenced on an ABI 3130 automated sequencer. Sequences of both strands of each gene were examined and aligned in Sequencher 4.1 (GeneCodes Corp.), and a final data matrix of contiguous sequences assembled using ClustalX 1.83 (Thompson *et al.* 1997). Alignments of the two nuclear introns were further examined by eye and corrected at indel sites as necessary.

2.2. *Phylogenetic reconstruction and analyses*

Sequence evaluation and phylogenetic reconstructions based on the concatenated dataset were performed via maximum likelihood (ML), as implemented in the software PAUP*. ModelTest 3.7 (Posada & Crandall, 1998) was used to determine the most appropriate model of sequence evolution via a hierarchical likelihood ratio test (hLRT) based on the Akaike Information Criterion (AIC). Nodal support was assessed via nonparametric bootstrapping with 100 replicates.

We also conducted Bayesian phylogenetic analyses (BA) using Markov Chain Monte Carlo (MCMC) tree searches using the program MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The concatenated dataset was partitioned by gene and codon positions for the nuclear intron and mitochondrial genes, respectively. ModelTest 3.7 (Posada & Crandall, 1998) was again used to establish the best substitution model according to the AIC (Table 2). Two independent runs of 10^7 generations were conducted using the respective models of sequence evolution, with default chain heating conditions, and sampling every 100 generations. Evaluation of stationarity was

conducted by plotting posterior probabilities from the two runs in the program Tracer (Rambaut & Drummond 2007). Topologies sampled from the first 25% of generations were discarded as an initial "burn-in," so a total of 75,000 trees were summarized to produce a single 50% majority-rule consensus tree.

3.   Results

3.1.   Sequence data characteristics

Sequence alignments for all taxa and genes were straightforward. The mitochondrial data showed no insertions, deletions, or anomalous stop-codons, and base composition was typical for both genes (Table 2), suggesting true mitochondrial origin, as opposed to corresponding to nuclear pseudogenes (Sorenson & Quinn, 1998). The ND3 gene sequence of *R. albiscapa* from Vanuatu included the 'silent base' described in several bird groups, a cytosine insertion at position 174, which does not disrupt the reading frame because it is not translated (Mindell *et al.* 1998). Deletions and insertions were inferred in the nuclear sequences, although these were not coded separately in our analyses.

The complete molecular dataset thus comprised 38 samples of 33 species, of which 30 were ingroup species, and 2595 aligned bases (Table 2). Average pairwise distance (uncorrected *P*) based on the two mitochondrial markers between ingroup and outgroup samples across the entire dataset was 20%; within fantails, pairwise distance ranged from 0.6% between the 2 samples of *R. nigrocinnamomea*, up to 25% between *R. hypoxantha* and *R. tenebrosa*.

3. 2.   The affinities of Rhipidura hypoxantha

The distinctiveness of *R. hypoxantha* compared to other fantails was indicated not only by a large genetic distance, but was also evident in preliminary phylogenetic analyses, where it was consistently recovered outside the fantail family. This peculiarity prompted us to investigate the true relationships of this taxon further, by performing a NCBI GenBank BLAST (Basic Local Alignment Search Tool) search of the ND2 and Fib5 sequences against homologous sequences of other passerines. Search results yielded highest similarities to sequences from several members of the Sylvioidea, in particular to the Stenostiridae (Beresford *et al.* 2005, Johansson *et al.* 2008).

Subsequently, we constructed an additional dataset of ND2 and Fib5 sequences from species sharing highest similarity scores in the BLAST search, as well as a subset of our ingroup taxa; most sequences were drawn from the most recent molecular study of the Passerida (Johansson *et al.* 2008; see Appendix). An additional BA was performed on this new dataset. The results of this analysis indicate conclusively that *R. hypoxantha* is distantly related to true fantails, and instead is part of the recently proposed Stenostiridae family, closely related to the genera *Stenostira, Culicicapa*, and *Elminia* (Figure 1; Beresford *et al.* 2005, Johansson *et al.* 2008, Nguembock *et al.* 2008).

3. 3.   Phylogenetic analyses

ML analyses produced a single topology (likelihood score of -lnL = 20287.43120), which was largely congruent with the consensus tree inferred via BA (Figure 2). Apart from the novel placement of *R. hypoxantha* with the Stenostiridae, true fantail monophyly was recovered with highest statistical support under both search algorithms.

At least 6 distinct fantail clades were recovered, although support for relationships among these clades was weak, effectively producing a large basal polytomy. The only exception to this general lack of support was strong consensus for a sister relationship between the grey and the rufous fantail complexes (clades E and F; Figure 2). Relationships of fantail species within each clade were generally well supported, with only a few exceptions. Most notable among these exceptions was *R. atra*, which was weakly inferred as sister to clade B in the ML analysis, while the BA separated this species from the other clades, placing it with very low support at the base of the fantails. Another taxon that received low phylogenetic support was *R. diluta*, although in this case both methods agreed in placing it sister to *R. leucothorax* and *R. threnothorax* (Figure 2).

4. Discussion

4.1. Phylogenetics and taxonomy

These results constitute the first detailed phylogenetic analysis of fantail relationships using molecular characters. We conclude that the Rhipiduridae, as currently defined, does not constitute a natural group, owing to the misallocation of the Yellow-bellied Fantail (*Rhipidura hypoxantha*), which is in fact a member of heterogeneous African-Eurasian clade, Stenostiridae. The taxonomic affinity of *R. hypoxantha* to other rhipidurids has not been formally disputed, although generic separation into *Chelidorhynx* has been suggested based on its phenotypic distinctiveness relative to the rest of the fantails. *Chelidorhynx* shares plumage colours with the stenostirid genus *Culicicapa*, and a similar long tail – and behavior – is found in

the stenostirid genus *Elminia*. Placement of *R. hypoxantha* within this family received significant support (Figure 1), and thus the Stenostiridae must be expanded to include this new member, under the appropriate scientific name of *Chelidorhynx hypoxantha* (Watson *et al.* 1986, Boles 2006).

Phylogenetic relationships of true fantails are marked by 6 discrete, well-supported groups, while the affinities of two species (*R. atra* and *R. diluta*) were only poorly resolved. The 6 distinct groups are all united by weakly supported nodes, precluding meaningful inferences regarding evolutionary history among these distinct lineages (Figure 2). Two clades, corresponding to the grey and the rufous fantail complexes, were inferred to be sister groups with high support under both of our phylogenetic analyses (clades E and F, Figure 2).

The grey fantail species complex (clade F, Figure 2) includes all 5 "typical" species (Table 1; Boles 2008), but is here redefined to include several members of the Streaked Fantail (*R. verreauxi*) complex, represented in our study by the species *verreauxi, tenebrosa*, and *renelliana*. As reciprocal monophyly was not recovered between the grey and steaked group species, we recognize only one distinct group: within this inclusive clade, the two New Guinea species *hyperythra* and *albolimbata*, a lowland and highland species, respectively, are sister to a separate subgroup of mostly island endemic species (clade F, Figure 2). Within this subgroup, *R. albiscapa* was paraphyletic; samples from Vanuatu and Australia were moderately divergergent from one another (4.7% uncorrected *P* sequence difference; Figure 2) and not sister taxa. The taxonomic complexity of grey fantails has been long recognized (Ford 1981, Schodde & Mason 1999, Boles 2006, Christidis & Boles 2008); our results clearly

indicate the need for a thorough molecular phylogenetic analysis of this broadly distributed group. Based on our results, Pacific Island populations of *R. albiscapa* will likely have to be elevated to full species rank. Overall, our results support the cohesiveness of the grey and streaked fantail groups, as previously suggested based on plumage similarities and by the recommended consolidation of these two groups within the subgenus *Rhipidura* (Watson *et al.* 1986, Schodde & Mason 1999, Boles 2006). In addition to the species that are part of this clade, the traditional taxonomy of streaked fantails also includes the here unsampled island species *drownei, personata* and *nebulosa*.

The rufous fantail species group was consistently recovered as sister to the grey fantails (clade E, Figure 2). Mayr and Moynihan (1946) provided a thorough revision of relationships of the rufous fantail complex based on morphological characters, proposing a series of dispersal events from an ancestral source from which five well defined subgroups emerged. Of these, *R. rufifrons* attained the highest degree of subspeciation. Even though our taxonomic sampling of the rufous fantail group is by no means inclusive, it nevertheless provides a first evaluation of the sequence of speciation events. As such, our results indicate that the New Guinea lowland species *R. rufidorsa* and the highland species *R. brachyrhyncha* branch basally from a well supported subclade containing *dahli, teysmanni, dryas*, and *rufifrons* (Figure 2). This pattern confirms Mayr and Moynihan's phylogenetic hypothesis for this group, which was based on the restriction of ancestral forms to New Guinea, with subsequent colonization of surrounding islands. Previous treatments of rufous fantails did not specifically place *R. brachyrhyncha* within this complex, but a potential link to this

widespread species group has been suggested (Mayr & Moynihan 1946, Boles 2006). According to the traditional classification, this group would also include the species *semirubra* (near *dryas*), *matthiae* and *malaitae* (with *rufidorsa*), and *superflua*, *dedemi*, *opisterythra* and *lepida* (near *teysmanni*). Probably the most interesting finding within this group is that members of the *R. rufifrons* subspecies complex, which currently includes 19 subspecies (Dickinson 2003), have achieved this diversity only recently (assuming that all currently recognized subspecies actually are part of the monophyletic *R. rufifrons*).

Clade D (Figure 2) represents another well-defined, but novel, group. The largest, and morphologically distinct, *R. leucophrys* is sister to a clade of Southeast Asian species, *javanica*, *aureola*, and *albicollis* (plus *albigularis* according to the morphological classification). An affiliation between these species and other major fantail groups has not been proposed, and are here shown to constitute a distinct clade. Evolutionary associations of the widespread Australo-Papuan *R. leucophrys* have also been unclear, although our phylogenetic analysis clearly supports the relationship of this species to a widespread Asian clade, disproving previous distinctions of this taxon as a separate subgenus *Leucocirca* based on morphology and behavior (Harrison 1976, Boles 2006). A sister relationship between clades C and D (Figure 2) was recovered by both phylogenetic analyses, however in both cases this association was weakly supported by ML bootstrap and BA posterior probabilities (<50% and 0.75, respectively). Clade C contains the two thicket fantails of New Guinea, *R. threnothorax* and *R. leucothorax* as sister to *R. diluta*, although support for the inclusion of the latter species in this clade was again quite deficient (Figure 2).

The northern fantail species complex is redefined in our phylogenetic analysis by a novel arrangement of members of clade B (Figure 2). As opposed to the current delineation of this group (see Introduction and Table 1; Boles 2006), our results indicate that *R. diluta* does not belong to this group, and is instead substituted for by the Spotted Fantail, *R. perlata* (Figure 2). Finally, clade A hosts three Philippine endemics, two of which (*R. cyaniceps* and *R. superciliaris*) are the only fantails with blue color in their plumage. The Black Fantail, *R. atra*, was consistently inferred as isolated from all other major fantail groups (Figure 2). This species has been hypothesized to share some affinities with the rufous fantails (clade E, Figure 2; Boles 2006), but we found no support for this relationship (Figure 2).

4. 2.   Biogeographic patterns

Fantails are part of the species-rich Australasian radiation of oscine passerines (Barker *et al.* 2002, 2004), and the present phylogenetic framework provides some important insights into some of the key underlying biogeographic processes through which this family attained its current species distribution. Our sampling for this study included ~70% of extant species diversity, with most omitted species occurring on isolated Pacific islands. Even though we cannot infer a complete biogeographic picture for the family, the overall geographic distribution of rhipidurids (Figure 3) reveals some common themes among the clades, as well as some unique patterns.

New Guinea's close link to the diversification of fantails is abundantly clear, as evidenced by their sister relationship with *Chaetorhynchus papuensis* and the high species diversity present on mainland New Guinea (Mayr & Diamond 2001, Boles 2006). Rhipidurids comprise an important component of the insectivorous guild

throughout New Guinea's lowland and montane bird communities, as high levels of sympatry are maintained through niche partitioning both within and among habitat types. That these communities include members of all fantail clades recovered within our phylogenetic framework, with the exception of the entirely Philippine endemic clade A, clearly demonstrates the integral role New Guinea has played in the evolution of the family.

Phylogenetic relationships and distributions suggest the possibility of colonization events in both directions between islands and continents. Probably the most eloquent example of the historical biogeographic importance of New Guinea is inferred in clades E and F (Figure 3), in which the branching patterns place New Guinea fantails basal to a subset of island species, which in turn are sister to rhipidurids that have recently recolonized mainland Australia. This distinct biogeographic pattern has been recently documented to have occurred also in monarch flycatchers (Monarchidae), where an extensive Pacific Island radiation recolonized mainland Australia, where further speciation events occurred (Filardi & Moyle 2005). By contrast, the prevailing hypothesis of Pacific Island avifaunal diversification assumes a sequential colonization of islands from mainland source populations through a one-directional flow of immigrants (Mayr 1939, 1942, Mayr & Diamond 2001). Integrating results from the present study with groups of taxa sharing similar geographic extents would present a good opportunity to test the timing and geographic fit of speciation events onto a modern view of plate tectonic events throughout SE Asia and the Pacific Islands (Hall 1996, 1997, 1998).

Conclusions

Drawing from a multi-locus molecular dataset, the present phylogenetic foundation of the Rhipiduridae advances understanding of the taxonomic and biogeographic underpinnings of the present species diversity in the family. Striking morphological and behavioral convergence in "*Rhipidura*" (=*Chelidorhynx*) *hypoxantha* has until now obscured the recognition of a new member of the enigmatic Stenostiridae. Among true fantails, the phylogenetic relationships recovered by our dataset clearly indicate rapid radiation of distinct clades, especially through the Pacific Islands, consistently mediated by the influences of New Guinea as a source area and the opportunities for dispersal that arose as a consequence of the large-scale tectonic changes affecting the island arcs along the western margin of the Pacific Ocean from the late Neogene. To broaden our understanding of fantail relationships and biogeography, several avenues of research merit further investigation. Complete taxon sampling of all Pacific island species should be undertaken through assembling modern voucher collections from these poorly known regions in order to fully elucidate the evolutionary history of the family. Relationships within the grey fantail complex as well as several widely distributed lineages including *R. dryas*, *R. ruffifrons*, *R. rufiventris*, and New Guinea's wide ranging lowland and montane taxa should be examined in detail to confirm monophyly and better understand the regional histories of these taxa.

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Table 1:   Taxonomic sampling (alphabetically, and in order of appearance of species groups in the Introduction), voucher sources, and GenBank accession numbers included in this study.

Taxon	Fantail group[1]	Voucher[2]	Sample #	Locality	TGFb2	Fib5	ND2	ND3
Ingroup								
Rhipidura albiscapa	Grey Fantails	KUNHM	6095	Australia, WA, 5 km E Donnybrook	GQ145464	GQ145355	GQ145388	GQ145426
Rhipidura albiscapa	Grey Fantails	LSUMNS	B45814	Vanuatu	GQ145476	GQ145365	GQ145400	GQ145438
Rhipidura albolimbata	Grey Fantails	KUNHM	4595	New Guinea, Morobe Province, Dendawang Camp	GQ145465	GQ145356	GQ145389	GQ145427
Rhipidura fuliginosa	Grey Fantails	LSUMNS	B23324	New Zealand	GQ145475	-	GQ145399	GQ145437
Rhipidura hyperythra	Grey Fantails	KUNHM	4851	New Guinea, Chimbu Province, 2 km WNW Haia	GQ145478	GQ145367	GQ145402	GQ145440
Rhipidura phasiana	Grey Fantails	KUNHM	6194	Australia, WA, 8km SE Carnarvon	GQ145485	GQ145374	GQ145410	GQ145448
Rhipidura rennelliana	Streaked Fantails	AMNH	DOT6602	Solomon Islands, Rennell Island	GQ145486	GQ145375	GQ145411	GQ145449
Rhipidura tenebrosa	Streaked Fantails	ZMUC	612-00	Solomon Islands, Makira Island	GQ145493	-	GQ145418	GQ145456
Rhipidura verreauxi	Streaked Fantails	LSUMNS	B45758	Vanuatu	GQ145491	GQ145380	GQ145416	GQ145454

Species	Group	Museum	Catalog No.	Locality				
Rhipidura atra	Rufous Fantails	KUNHM	4598	New Guinea, Morobe Province, Dendawang Camp	GQ145466	GQ145357	GQ145390	GQ145428
Rhipidura brachyrhyncha	Rufous Fantails	KUNHM	4743	New Guinea, Morobe Province, Dendawang Camp	GQ145468	GQ145359	GQ145392	GQ145430
Rhipidura dahli	Rufous Fantails	KUNHM	5305	New Guinea, East New Brittain, Wanui Camp	GQ145471	GQ145361	GQ145395	GQ145433
Rhipidura dahli	Rufous Fantails	USNM	B4026	New Guinea, New Ireland	GQ145472	GQ145362	GQ145396	GQ145434
Rhipidura dryas	Rufous Fantails	KUNHM	5282	New Guinea	GQ145474	GQ145364	GQ145398	GQ145436
Rhipidura nigrocinnammomea	Rufous Fantails	FMNH	357537	Philippines, Mindanao Island	GQ145482	GQ145371	GQ145407	GQ145445
Rhipidura nigrocinnammomea	Rufous Fantails	FMNH	392297	Philippines, Mindanao Island	GQ145483	GQ145372	GQ145408	GQ145446
Rhipidura rufidorsa	Rufous Fantails	KUNHM	5083	New Guinea, Gulf Province, 7 km N Wabo	GQ145487	GQ145376	GQ145412	GQ145450
Rhipidura rufifrons	Rufous Fantails	KUNHM	12828	Solomon Islands, Makira Island	GQ145488	GQ145377	GQ145413	GQ145451
Rhipidura teysmanni	Rufous Fantails	AMNH	DOT12566	Sulawesi, Goa, Kacamatan, Chicoro	GQ145494	GQ145381	GQ145419	GQ145457
Rhipidura cockerelli	Northern Fantails	AMNH	DOT170	Solomon Islands, Ranongga Island	GQ145469	-	GQ145393	GQ145431
Rhipidura diluta	Northern Fantails	WAM	WA22163	Indonesia, Batu Dulong	GQ145473	GQ145363	GQ145397	GQ145435

Species	Common name	Institution	Specimen	Locality				
Rhipidura fuscorufa	Northern Fantails	WAM	WA25097	Indonesia, Tanimbar Island	GQ145477	GQ145366	GQ145401	GQ145439
Rhipidura rufiventris	Northern Fantails	KUNHM	6867	New Guinea, Oro Province, Uiaku Village	GQ145489	GQ145378	GQ145414	GQ145452
Rhipidura rufiventris	Northern Fantails	USNM	B4008	New Guinea, New Ireland	GQ145490	GQ145379	GQ145415	GQ145453
Rhipidura leucothorax	Thicket Fantails	KUNHM	7305	New Guinea, Madang Province, Tikiam Camp	GQ145481	GQ145370	GQ145406	GQ145444
Rhipidura threnothorax	Thicket Fantails	KUNHM	4857	New Guinea, Chimbu Province, 2 km WNW Haia	GQ145495	GQ145382	GQ145420	GQ145458
Rhipidura albicollis	-	KUNHM	10230	China, Guangxi, Diding Headwater Reserve	GQ145462	GQ145353	GQ145386	GQ145424
Rhipidura albicollis	-	LSUMNS	B36474	Malaysia, Sabah	GQ145463	GQ145354	GQ145387	GQ145425
Rhipidura aureola	-	USNM	B2297	Myanmar	GQ145467	GQ145358	GQ145391	GQ145429
Rhipidura cyaniceps	-	KUNHM	15299	Philippines, Panay Island	GQ145470	GQ145360	GQ145394	GQ145432
Rhipidura hypoxantha	-	USNM	B5724	Myanmar	-	GQ145368	GQ145403	GQ145441
Rhipidura javanica	-	LSUMNS	B46972	Malaysia, Sabah	GQ145479	-	GQ145404	GQ145442
Rhipidura leucophrys	-	KUNHM	6148	Australia, WA, 10 km NE Wubin Station	GQ145480	GQ145369	GQ145405	GQ145443

Rhipidura perlata	-	LSUMNS	B57451	Malaysia, Sabah	GQ145484	GQ145373	GQ145409	GQ145447
Rhipidura superciliaris	-	KUNHM	14144	Philippines, Samar Island	GQ145492	-	GQ145417	GQ145455
Outgroup								
Chaetorhynchus papuensis	-	KUNHM	6974	New Guinea, Oro Province, Uiaku Village	GQ145459	GQ145350	GQ145383	GQ145421
Dicrurus hottentottus	-	KUNHM	14164	Philippines, Samar Island	GQ145460	GQ145351	GQ145384	GQ145422
Monarcha castaneiventris	-	KUNHM	15914	Solomon Islands, Makira Island	GQ145461	GQ145352	GQ145385	GQ145423

[1] Fantail group assignments follow Boles 2008
[2] Institutional abbreviations for voucher sources are as follows: American Museum of Natural History (AMNH), Field Museum of Natural History (FMNH), The University of Kansas Natural History Museum (KUNHM), Louisiana State University Museum of Natural Science (LSUMNS), National Museum of Natural History, Smithsonian Institution (USNM), Western Australian Museum (WAM), Zoological Museum, University of Copenhagen (ZMUC).

Figure 1: Phylogenetic placement of "*Rhipidura*" (=*Chelidorhynx*) *hypoxantha* within Stenostiridae as inferred from Bayesian analysis. Samples contributed by the present study are indicated in bold. An asterisk indicates nodal support of $\geq$ 90 posterior probability.

Figure 2: Maximum likelihood (ML, left) and Bayesian analysis (BA, right) views of phylogenetic patterns implied by analyses of the complete molecular dataset. Support values are indicated by percent bootstrap (ML, left) and posterior probability values (BA, right) above or at each node. Values <50 recovered by each method are not indicated at nodes. Clade letters are referenced throughout the main text.

Figure 3: Schematic consensus tree derived from the ML and BA topologies illustrating geographic distributions of rhipidurids. Clade letters follow Figure 2, and are referenced throughout the main text. Nodal support is schematically indicated via circles: - black circles correspond to nodes supported by ML bootstrap values >75% and BA posterior probability >0.75, white circles denote ML bootstrap values <75% and BA posterior probabilities >0.75, and nodes without circles correspond to ML bootstrap values <75% and BA posterior probabilities <0.75.

Figure 1

Meliphaga reticulata

Monarcha castaneiventris

Dicrurus hottentottus

Chaetorhynchus papuensis

Rhipidura albicollis

Rhipidura cyaniceps

Rhipidura albiscapa   RHIPIDURIDAE

Picathartes gymnocephalus

Certhia familiaris

Parus major

Hyliota flavigaster

"Rhipidura" (= Chelidorhynx) hypoxantha

Stenostira scita

Culicicapa ceylonensis

Elminia albonotata   STENOSTIRIDAE

Bombycilla garrulus

Passer montanus

Regulus calendula

Promerops cafer

Pycnonotus barbatus

Sylvia atricapilla

Hylia prasina

Alauda arvensis

Cinclus cinclus

Figure 2

Figure 3

Rhipidura atra	- New Guinea	
Rhipidura nigrocinnamomea	- Philippines	**A**
Rhipidura cyaniceps	- Philippines	
Rhipidura superciliaris	- Philippines	
Rhipidura perlata	- S Thailand, Malay Peninsula, Sumatra, Borneo	**B**
Rhipidura fuscorufa	- Tanimbar Archipelago	
Rhipidura cockerelli	- Bougainville, Solomons	
Rhipidura rufiventris	- Lesser Sundas, New Guinea + islands, N Australia	
Rhipidura diluta	- Lesser Sundas	
Rhipidura leucothorax	- New Guinea	**C**
Rhipidura threnothorax	- New Guinea	
Rhipidura leucophrys	- Australia, New Guinea + islands, Solomons	**D**
Rhipidura javanica	- SE Asia, Sumatra, Java, Borneo, Philippines	
Rhipidura albicollis	- India, China, SE Asia, Sumatra, Borneo	
Rhipidura aureola	- India, Myanmar, S China, SW Thailand, SC Vietnam	
Rhipidura rufidorsa	- New Guinea	**E**
Rhipidura brachyrhyncha	- New Guinea	
Rhipidura dahli	- Bismark Archipelago	
Rhipidura teysmanni	- Sulawesi	
Rhipidura dryas	- Lesser Sundas, Moluccas, Tanimbar, N Australia	
Rhipidura rufifrons	- N Moluccas, S New Guinea, Solomons, E coast Australia	
Rhipidura albolimbata	- New Guinea highlands	**F**
Rhipidura hyperythra	- New Guinea lowlands	
Rhipidura verreauxi	- New Caledonia, Vanuatu, Fiji	
Rhipidura tenebrosa	- Makira Island (Solomons)	
Rhipidura rennelliana	- Rennell Island (Solomons)	
Rhipidura albiscapa	- New Caledonia, SE Solomons, Vanuatu	
Rhipidura phasiana	- N coast Australia	
Rhipidura albiscapa	- Australia, Tasmania	
Rhipidura fuliginosa	- New Zealand	

Chapter 2**

Systematic dismantlement of *Lichenostomus* improves the basis for understanding relationships within the honeyeaters (Meliphagidae) and historical development of Australo-Papuan bird communities

** Nyári, Á. S. and Joseph L. Systematic fragmentation of Lichenostomus improves the basis for understanding relationships within the honeyeaters (Meliphagidae) and historical development of Australo-Papuan bird communities. EMU. in press.

Abstract

Several recent re-evaluations of relationships among major lineages of honeyeaters (Passeriformes: Meliphagidae) have used dense taxon and nucleotide sampling. The present study, which focuses on the systematically contentious genus *Lichenostomus*, adds to this growing body of phylogenetic analyses of meliphagids. It uses data from the two molecular markers that were common to two major recent studies, the mitochondrial protein-coding gene ND2 and the nuclear intron Fib5. Based on complete species-level sampling of *Lichenostomus*, we confirm the recent finding that *Lichenostomus* is not monophyletic. We find that it comprises seven distinct lineages interdispersed within the larger meliphagid assemblage. The two uniform, unadorned species White-gaped Honeyeater *L. unicolor* and Yellow Honeyeater *L. flavus* were recovered as sister species close to some other taxa currently placed in *Lichenostomus*. The only two species of this group that are essentially mangrove specialists, Varied Honeyeater *L. versicolor* and Mangrove Honeyeater *L. fasciogularis* from north-eastern Australia, were not sisters but *L. versicolor* was sister to the pair comprising *L. fasciogularis* and widespread Singing Honeyeater *L. virescens*. The two New Guinean endemic species Obscure Honeyeater *L. obscurus* and Black-throated Honeyeater *L. subfrenatus* are a sister pair to Yellow-faced Honeyeater *L. chrysops* from eastern Australia. We suggest a revised generic nomenclature for the species recently placed in *Lichenostomus* and erect one new genus-group name, *Bolemoreus*, to include two species that have been previously grouped in *Caligavis* either as a genus or subgenus within *Lichenostomus*.

1.   Introduction

Honeyeaters (Passeriformes: Meliphagidae) are among Australia's most characteristic passerine birds. Most diverse in continental Australia and New Guinea, they are also prominent in major avian diversifications of Australasian island archipelagos where they have reached high levels of endemism (Mayr 1939; Diamond 1977; Schodde and Mason 1999; Mayr and Diamond 2001). The meliphagid radiation has been accompanied by significant morphological and phenotypic diversity, making analysis of relationships within the family challenging and difficult to resolve (Christidis and Schodde 1993; Driskell and Christidis 2004; Gardner *et al*. 2010).

Relationships among meliphagid genera were thoroughly explored by Driskell and Christidis (2004) who used DNA sequence data derived from mitochondrial and nuclear introns, and sampled 32 of the 42 recognized genera. They concluded that the Australian and New Guinean "core" honeyeaters comprise four main clades each with generic level radiations, spinebills *Acanthorhynchus* spp being sister to those four clades. Low support for basal nodes precluded robust estimates of relationships among the four clades, but their results were nevertheless taken as sufficient basis for taxonomic and nomenclatural changes (Higgins *et al*. 2008). More recently, a multilocus study of the superfamily Meliphagoidea to which the Meliphagidae belongs (Gardner *et al*. 2010) examined relationships primarily but not solely among its member families. Emerging from these recent works, however, has been the clear need to reassess the systematic placement of the species currently comprising the genus *Lichenostomus*, the monophyly of which is in doubt.

Lichenostomus as currently construed (Schodde and Mason 1999; Christidis and Boles 2008) is the second most speciose genus of honeyeaters after *Myzomela*. It has 18 species restricted to continental Australia, and three in New Guinea two of which are endemic there. Australian species mostly inhabit arid and semi-arid woodlands and mallee. Two species (*L. versicolor*, *L. fasciogularis*) are essentially mangrove specialists in northeast Australia (Ford 1982, Schodde *et al*. 1979) whereas the two New Guinean endemic species (*L. subfrenatus*, *L. obscurus*), inhabit lowland and montane rainforests. Circumscription of *Lichenostomus* with respect to other meliphagid genera has been challenging (Salomonsen 1967; Schodde 1975; McGill 1976; Keast 1981; 1985). Recent work (Gardner *et al*. 2010) shows two key points: (1) that the species currently placed in *Lichenostomus* are not each other's closest relatives, i.e., it is not a monophyletic group, and (2) that to clarify relationships among the 20 species, all of them need to be analysed along with a broad sampling of other honeyeater genera. The present study aims to do this and in so doing clarify the intrageneric delineations suggested on morphological grounds by Schodde (1975) and Schodde and Mason (1999). These are listed in Table 1 with English names for all species.

Driskell and Christidis (2004) and Gardner *et al*. (2010) included only one or eight species of *Lichenostomus*, respectively, in their analyses, the latter study showing clearly that *Lichenostomus* is not monophyletic, that *L. leucotis* is close to *Entomyzon* and *Melithreptus* (see also Toon *et al*. 2010), and that *L. melanops*, *L. flavus*, and *L. unicolor* are more deeply divergent from the "core" *Lichenostomus* clade (*L. virescens*, *L. flavescens*, *L. penicillatus*, and *L. ornatus*).

Comprehensive taxon sampling can improve overall confidence of phylogenetic analyses, augmenting confidence in branching patterns as well as elucidating the evolutionary history of complex adaptive radiations (Pollock *et al*. 2002; Zwickl and Hillis 2002). To explore relationships of this diverse and challenging group of honeyeaters further, we have included sequence data from all extant species of *Lichenostomus*. These data are then integrated within the higher-level phylogenetic framework of the Meliphagidae, previously delineated by Driskell and Christidis (2004) and Gardner *et al*. (2010). Our aims are to clarify relationships of species currently assigned to *Lichenostomus* relative to *Meliphaga* (Christidis and Schodde 1993), the latter having also been examined by Norman *et al*. (2007) and other genera (Gardner *et al*. 2010), elucidate the phylogenetic placement of the two New Guinean endemic species (*L. subfrenatus* and *L. obscurus*), and address the validity and relationships of previously proposed subgroups within *Lichenostomus* (Schodde 1975; Christidis and Schodde 1993). Testing the monophyly of the species is outside our scope, which is focused on genus-level groupings of taxa. We employ the two molecular markers that were common to previous phylogenetic analyses of honeyeaters (Driskell and Christidis 2004; Norman *et al.* 2007; Gardner *et al.* 2010). We intend that our analysis is a compromise between complete taxon sampling and linkage to existing datasets.

2.   Materials and Methods

2.2.   *Taxon sampling and molecular markers*

We sampled all of the 20 *Lichenostomus* species from vouchered specimens collected by us and others (Table 1). We used the two molecular markers common to

Driskell and Christidis (2004), Norman *et al*. (2007), and Gardner *et al*. (2010) – i.e., the mtDNA protein-coding gene NADH dehydrogase subunit 2 (ND2) and the fifth intron of the nuclear gene Beta-Fibrinogen (Fib5). Total genomic DNA was extracted from frozen or alcohol-preserved tissue samples using standard Qiagen DNeasy™ tissue extraction protocols (Qiagen, Valencia, CA). Target regions were amplified using the primers L5215–H6313 (Sorenson *et al*., 1999) and Fib5 and Fib6 (Marini and Hackett 2002), respectively. All PCR amplifications were performed in 25µl reactions using PureTaq™ RTG PCR beads (GE Healthcare Bio-Sciences Corp.). Amplified double-stranded PCR products were cleaned with ExoSAP-IT™ (GE Healthcare Bio-Sciences Corp.), and visualized on high-melt agarose gels stained with ethidium bromide. Purified PCR products were cycle-sequenced with ABI Prism BigDyeT™ v3.1 terminator chemistry, using the same primers as for each PCR reaction. Cycle-sequenced products were further purified using Sephadex™ spin columns (GE Healthcare Bio-Sciences Corp.), and finally sequenced on an ABI 3130 automated sequencer. Sequences of both strands of each gene were examined and aligned in Sequencher 4.8 (GeneCodes Corp.). Heterozygous base calls in the Fib5 intron were coded as ambiguous according to the International Union of Pure and Applied Chemistry (IUPAC) standards.

Phylogenetic methods

Published sequences of the ND2 (1041bp) and Fib5 (543bp aligned) markers (Driskell and Christidis 2004; Norman *et al.* 2007) were downloaded from GenBank (see Appendix). We added sequence data of the same two markers from the 20 *Lichenostomus* species resulting in a final data matrix of 116 contiguous sequences assembled using ClustalX 2.0.7 (Thompson *et al*., 1997). Alignments were

subsequently scrutinized by eye in Mesquite (Madison and Madison 2009). We analyzed the concatenated dataset through model-based phylogenetic algorithms under both Maximum Likelihood (ML) and Bayesian analyses (BA). ModelTest 3.7 (Posada and Crandall 1998) was used to determine the most appropriate model of sequence evolution via the Akaike Information Criterion (AIC). ML heuristic tree searches were conducted through the program GARLI 1.0 (Zwickl 2008), under a single data partition and the GTR+I+G model of sequence evolution, with parameter values estimated from the data. Nodal support was assessed via 100 non-parametric bootstrap replicates. BA was carried out within the Markov Chain Monte Carlo (MCMC) tree search algorithm framework as implemented in the program MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The concatenated data set was partitioned by gene and codon positions for the nuclear intron and mitochondrial gene, respectively. We ran two independent runs of 10^7 generations, using the previously inferred model of sequence evolution. Search parameters included adjustment of chain heating conditions (temp = 0.1) for improved chain swap acceptance rates, and sampling every 100 generations. Evaluation of stationarity and chain convergence was conducted by plotting posterior probabilities from the two runs in the program Tracer (Rambaut and Drummond 2007). The resulting pool of topologies sampled from the first 25% of generations was discarded as an initial 'burn-in', such that a total of 75,000 trees were finally summarized to produce a single 50% majority-rule consensus tree, rooted with the Striated Grasswren *Amytornis striatus*. We also conducted separate BA on each of the two markers in order to ascertain possible alternative topologies supported by each locus. Given the reported polyphyly of *Lichenostomus* (Gardner *et al.* 2010), we proceeded to evaluate alternative

topologies by enforcing constraints on ML GARLI searches. Site likelihood outputs from the best constrained trees were used in subsequent test against our ML tree via the Approximately Unbiased (AU) test, as implemented in the program CONSEL (Shimodaira and Hasegawa 2001).

3.   Results

After pooling our dataset with the two other studies, we obtained a matrix containing 116 taxa and 1584 base pairs. The ND2 sequences (1041bp) had no insertions, deletions, or anomalous stop-codons. Base composition was typical of avian mtDNA (29%A, 34%C, 12%G, 25%T) consistent with true mitochondrial origin as opposed to nuclear pseudogenes (Sorenson & Quinn 1998). The Fib5 intron (543bp aligned) showed a relatively high presence of indels, making proper alignment crucial for phylogenetic estimation. Given recent issues with phylogenetic importance assigned to indel regions within the Meliphagidae (Driskell and Christidis 2004, Gardner *et al*. 2010), we decided to excise all indels from the intron dataset, and perform analyses on the sequence data alone.

ML and BA analyses both produced congruent topologies, characterized by clear definition of clades but a lack of resolution at the base of the trees (Figure1). Monophyly of the Meliphagidae was strongly supported under both algorithms, but little could be inferred in terms of basal branching patterns within the family. Several individual clades, on the other hand, received moderate-to-strong support. *Lichenostomus flavicollis* and *L. leucotis* are consistently sister species, and are together members of a clade containing the honeyeater genera *Entomyzon*, *Melithreptus*, and *Foulehaio* (node 1,

Figure 1). *Lichenostomus frenatus* and *L. hindwoodi* are similarly sister species and in turn are sister to a clade comprising Australian *Anthochaera* (which now includes *Xanthomyza*; Christidis and Boles 2008) and *Acanthagenys* (node 2, Figure 1).

Most of the remaining *Lichenostomus* species diversity clusters within a larger clade, where two main subclades are evident (nodes 3 and 4, Figure 1). The first at node 3 received low support for the internal branching patterns but nevertheless comprised *L. chrysops* as sister to the New Guinean species pair *L. obscurus* + *L. subfrenatus*, and *L. cratitius* as sister to *L. melanops*. *L. flavus* and *L. unicolor* were also recovered as sister species with strong statistical support. Members of *Melidectes*, *Manorina* and *Purnella* also descend from this node. A second, strongly supported group at node 4 comprises only *Lichenostomus* honeyeaters. It has our Australian sample of *L. versicolor* as sister to the pair of *L. virescens* and *L. fasciogularis*. The same group also contains a strongly supported subclade comprising the remaining six species of *Lichenostomus*. Whereas *Lichenostomus* is indeed highly paraphyletic with respect to other meliphagids, the genus *Meliphaga* is monophyletic and receives strong statistical support. Analyses conducted on the individual loci did present differences in the placement of several species groups, mostly recovered by the Fib5 intron data. These differences however, received low support values, were predominantly between major honeyeater clades, and did not affect the placement of *Lichenostomus* sister species as outlined.

Results from the Approximately Unbiased (AU) tests of four alternative constraint topologies against the recovered ML tree were all statistically significant, except for one, indicating that proposed alternative topologies were all worse hypotheses of

relationships of *Lichenostomus* honeyeaters. Rejected constraint topologies included (1) a monophyletic *Lichenostomus*, (2) reciprocal monophyly of *Lichenostomus* sister species in clade 1 and 2 (Figure 1) and remainder of *Lichenostomus*, and (3) reciprocal monophyly of *Lichenostomus* species in clade 3 and all other *Lichenostomus* species groups. Further, the AU test could not reject the alternative scenario of a monophyletic group of five species (*L. frenatus, L. hindwoodi, L. subfrenatus, L. obscurus* and *L. chrysops*), indicating that this alternative topology is within the 95% confidence interval of our most likely topology, which renders this grouping paraphyletic (Figure 1; see Discussion).

4.   Discussion

Our study is the first molecular systematics analysis of *Lichenostomus* honeyeaters based on complete taxon sampling within the genus as recently construed (e.g., Schodde and Mason 1999; Christidis and Boles 2008). Results from our mitochondrial and nuclear DNA dataset mirror closely the findings of Gardner *et al*. (2010). This similarity is expected, as both studies are based on the solid taxonomic framework sampled by Driskell and Christidis (2004), and also on two molecular markers in common between these studies (ND2 and Fib5). As such, the paraphyly of *Lichenostomus* extends to seven different subgroups (Figure 1, 2). Support for these arrangements, in the form of Bayesian posterior probabilities (BPP) and maximum likelihood bootstrap support (MLBS) values, was generally good, and several species pairs and even a larger "core" assemblage of *Lichenostomus* species were strongly supported (Figure 1). With the need to dismantle *Lichenostomus sensu lato* into

different genera now well-established, we develop below a new generic classification (Table 1; Figure 2) that is a consensus of previous delineations of subgeneric groups (Salomonsen 1967; Schodde 1975; Christidis and Schodde 1993; Schodde and Mason 1999), and well-supported clades recovered from molecular data (present study; Gardner *et al*. 2010). Nomenclatural details used below in making genus-level decisions are derived from Salomonsen (1967).

Strong support was recovered for the sister relationship of *L. leucotis* and *L. flavicollis*, two species previously placed in the "*Nesoptilotis*" subgroup of *Lichenostomus* honeyeaters (Schodde 1975; Christidis and Schodde 1993; Schodde and Mason 1999; Higgins *et al.* 2008). Our dataset places these two sister species as closely related to the Pacific Island endemic Wattled Honeyeater, *Foulehaio carunculatus*, and the clade formed by all three with *Entomyzon* and *Melithreptus* (Figure 1). This result indicates closer relationships, systematically and biogeographically, among these mainland Australian and Pacific Island taxa, than has been previously appreciated (see also Filardi and Moyle 2006; Moyle *et al*. 2009; Nyári *et al*. 2009). We advocate use of *Nesoptilotis* Mathews, 1913 (type species *N. flavicollis*) for these two species. Their geographical replacement of each other coupled with their sister species relationship suggests a history of vicariance splitting an ancestral member of the Bassian avifauna (Schodde and Calaby 1972) between Australian mainland and Tasmanian landmasses, respectively.

Among the five species comprising the "*Caligavis*" subgroup (Iredale 1956; Schodde 1975; Schodde and Mason 1999; Higgins *et al*. 2008), *L. frenatus* and *L. hindwoodi* of eastern Australia are well-supported by our analyses as sister taxa, *L.*

chrysops is similarly well-supported as the sister to *L. obscurus* and *L. subfrenatus*, but all five do not form a monophyletic group (see also Christidis and Schodde 1993). Our best tree similarly indicated non-monophyly of all five. In our analyses *L. frenatus* and *L. hindwoodi* form a pair that is sister to a clade containing the wattlebirds, *Anthochaera* and Spiny-cheeked Honeyeater, *Acanthagenys rufogularis*.

Doubt has surrounded the diagnosis and circumscription of *Caligavis* since Iredale (1956) introduced it for the two New Guinean species, *L. obscurus* and *L. subfrenatus*. He said its purpose was "to act as a lighthouse to warn of the dangers" associated with those two species. This presumably alluded to difficulties associated with their identification and uncertainty about their relationships. Later analyses (cited above) included in *Caligavis* three further species *L. obscurus*, *L. frenatus* and *L. hindwoodi*, the last of which was named by Longmore and Boles (1983). These studies showed there is more phenotypic heterogeneity among the five species than there are traits that can clearly and readily diagnose them as a group (Longmore and Boles 1983; Schodde and Mason 1999; see Figure 3). Given that heterogeneity as well as molecular indications of their non-monophyly, we restrict *Caligavis* Iredale, 1956 (type species *C. obscura*) to the species to be then known as *C. chrysops*, *C. subfrenata* and *C. obscura* (note female endings to epithets with *Caligavis*). No genus-group name is available only for the *L. frenatus*-*L. hindwoodi* pair so whether recognized as a genus or subgenus a new genus-group name is needed. We introduce the following genus-group name:

Family Meliphagidae

Genus *Bolemoreus* Nyári and Joseph, *nomen novum*

Type species: *B. frenatus* (Ramsay, 1875)

Included species: *B. frenatus*, *B. hindwoodi* (Longmore and Boles, 1983)

Diagnosis: The need for recognition of *Bolemoreus* has arisen from molecular data reported herein. Phenotypic traits diagnosing *hindwoodi* and *frenatus* apart from the species with which they have been most closely associated and for which we now advocate a restricted circumscription of *Caligavis* (*obscura*, *subfrenata* and *chrysops*) are difficult to discern, apart, perhaps, from reduced or absent subocular yellow plumage and distinctive vocalizations. The phenotypic diversity, which we hypothesize shows complex patterns of derived traits and retention and loss of ancestral traits especially in the pattern of marking about the heads of these five species, has been reviewed by Longmore and Boles (1983) and Schodde and Mason (1999).

Distribution: The two species are confined to the tropical and subtropical rainforests either side of the Burdekin Gap (Keast 1961; Galbraith 1969) in central eastern and north-eastern Australia.

Etymology: *Bolemoreu*s is a Latinized name of masculine gender that commemorates the work of Walter E. Boles and N. Wayne Longmore. Together and individually, they have contributed enormously to the development of ornithology in Australia, especially systematic ornithology. Of specific relevance here, however, is their role in the discovery and description of the Eungella Honeyeater *B. hindwoodi*, which to date was the most recently discovered and described Australian bird species (Longmore and Boles 1983).

Our Clade 3 includes two strongly supported pairs of sister taxa, *L. flavus* and *L. unicolor*, and *L. cratitius* and *L. melanops*. The former pair comprises the "*Stomiopera*" subgroup (Schodde 1975; Schodde and Mason 1999; Christidis and Schodde 1993;

Higgins *et al*. 2008). They are the most uniformly coloured of *Lichenostomus* honeyeaters, yellow or grey, respectively, and occur in tropical eucalypt woodlands of monsoonal Australia in the Torresian and Irian biogeographical provinces (Schodde and Calaby 1972; Schodde 2006; Bowman *et al.* 2010). We recognize *Stomiopera* Reichenbach, 1852 (type species *S. unicolor*) for this pair. The *L. cratitius-L. melanops* pair, which inhabit Australia's southern mallee and south-eastern temperate eucalypt woodlands and forest-heaths, together were part of the "*Lichenostomus*" subgroup (*sensu* Schodde 1975; Christidis and Schodde 1993; Schodde and Mason 1999). We restrict *Lichenostomus* Cabanis, 1851 to *L. cratitius* (type species) and *L. melanops*. Because the pair *L. cratitius-L. melanops* is not the sister to the pair *L. flavus-L. unicolor*, they should not be combined generically (Figure 2).

The remaining species currently in *Lichenostomus* form a well-supported group in which the "*Gavicalis*" and "*Ptilotula*" subgroups are sister groups (Schodde and Mason 1999). The "*Gavicalis*" subgroup unites *L. versicolor* as sister to *L. virescens* and *L. fasciogularis*. Most notably, the two most mangrove-restricted species of the group under study from the Australian east coast, *L. fasciogularis* and *L. versicolor* (Ford 1982; Higgins *et al*. 2001, 2008) are not sister species in our results. Instead, *L. versicolor* is sister to the pair comprising the widespread *L. virescens* and *L. fasciogularis*. This suggests that the widespread species *L. virescens* might have been derived from eastern Australian mangroves. Lastly, the "*Ptilotula*" subgroup contains *L. ornatus, L. penicillatus*, *L. plumulus*, *L. keartlandi*, *L. fuscus* and *L. flavescens*. All of these taxa occupy a variety of open forests and semi-arid habitats throughout mainland Australia. *Lichenostomus* can no longer apply to these nine species (as argued above).

Ptilotula Mathews, 1912 (type species *Pt. flavescens*) and *Gavicalis* Schodde and Mason, 1999 (type species *G. virescens*) are available, however. *Paraptilotis* Mathews, 1912 (type species *Pa. fusca*) was described on the same page of the same work as *Ptilotula* and so could apply to that subgroup. We advocate use of *Ptilotula* for the species recently known as *L. ornatus, L. penicillatus*, *L. plumulus*, *L. keartlandi*, *L. fuscus* and *L. flavescens* because it has been in recent use (Schodde and Mason 1999). Nonetheless, all nine species are monophyletic so the older generic name, *Ptilotula*, could validly apply to them all.

In contrast to the need to dismantle *Lichenostomus*, data from almost the entire *Meliphaga* radiation clearly suggests its monophyly. Although our study lacks the phylogenetic power to infer well-supported nodes in *Meliphaga* at various levels throughout the topology, it nevertheless makes a continuing case for increased individual, population and taxon sampling in phylogenetic studies of complex radiations. Judging from the branch lengths of our resulting tree, we consider it clear that many honeyeater species have evolved relatively rapidly. This leads to short internodes and long terminal branches, prone to accumulating larger amounts of evolutionary changes in time. We would thus stress the need of studies that test the monophyly of taxonomic species of all meliphagids. At the same time it is of utmost importance to maximize the contribution of phylogenetic signal from every taxon included in the analysis by sampling many loci. In future studies this approach should lead to a more refined understanding image of relationships within this iconic group of largely Australo-Papuan birds.

Acknowledgements

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Table 1: Taxon sampling, voucher information, and GenBank accession numbers of *Lichenostomus* species included in this study. Institutional and regional abbreviations: ANWC – Australian National Wildlife Collection, Canberra; ANSP Academy of Natural Sciences, Philadelphia; KU, University of Kansas, Biodiversity Institute, Lawrence; SA – South Australia; WA – Western Australia; NSW – New South Wales; QLD – Queensland. Taxonomy reflects current and newly proposed generic delineations. GenBank Accession numbers for the remaining ingroup and outgroup taxa included in the study are given in the Appendix.

Taxon	Locality	*Lichenostomus* subgroups (*sensu* Schodde and Mason 1999)	proposed genus	Museum Accession	ND2	Fib5
L. chrysops Yellow-faced Honeyeater	Australia, NSW, Mudgee	*Caligavis*	*Caligavis*	KU 10738	HQ267663	HQ267683
L. frenatus Bridled Honeyeater	Australia, QLD, Atherton Tablelands, Atherton	*Caligavis*	*Bolemoreus*	ANWC 41565	HQ267669	HQ267689
L. hindwoodi Eungella Honeyeater	Australia, QLD, Chelman's Rd., W of Mackay	*Caligavis*	*Bolemoreus*	ANWC 41405	HQ267671	HQ267691
L. obscurus Obscure Honeyeater	Papua New Guinea, Gulf Province, Wabo Village	*Caligavis*	*Caligavis*	KU 7379	HQ267675	HQ267695
L. subfrenatus Black-throated Honeyeater	Papua New Guinea, Morobe Province, Abalgamut Camp	*Caligavis*	*Caligavis*	KU 4792	HQ267679	HQ267699
L. fasciogularis Mangrove Honeyeater	Australia, QLD, Haughton River, 23km NW of Ayr	*Gavicalis*	*Gavicalis*	ANSP 25220	HQ267665	HQ267685
L. versicolor Varied Honeyeater	Australia, QLD, Daintree River Esturay	*Gavicalis*	*Gavicalis*	ANSP 25350	HQ267681	HQ267701
L. virescens Singing Honeyeater	Australia, WA, 15km S of Kalbarri	*Gavicalis*	*Gavicalis*	KU 6160	HQ267682	HQ267702
L. cratitius Purple-gaped Honeyeater	Australia, WA, ca. 7km W of Hopetoun	*Lichenostomus*	*Lichenostomus*	ANWC 31830	HQ267664	HQ267684

L. keartlandi Grey-headed Honeyeater	Australia, QLD, S of Lark Quarry	*Lichenostomus*	*Ptilotula*	ANSP 24418	HQ267672	HQ267692
L. melanops Yellow-tufted Honeyeater	Australia, NSW, Tarcutta, Mate's Gully Road	*Lichenostomus*	*Lichenostomus*	ANSP 22940	HQ267674	HQ267694
L. flavicollis Yellow-throated Honeyeater	Australia, TAS, Maggs Mtn., SW of Mole Creek	*Nesoptilotis*	*Nesoptilotis*	ANWC 45751	HQ267667	HQ267687
L. leucotis White-eared Honeyeater	Australia, WA, 20km W Harrismith	*Nesoptilotis*	*Nesoptilotis*	KU 8763	HQ267673	HQ267693
L. flavescens Yellow-tinted Honeyeater	Australia, WA, Ellendale Station, Mt. Wynne Creek	*Ptilotula*	*Ptilotula*	ANSP 25785	HQ267666	HQ267686
L. fuscus Fuscous Honeyeater	Australia, QLD, Kaban, ca. 8km NNW Ravenshoe	*Ptilotula*	*Ptilotula*	ANSP 25554	HQ267670	HQ267690
L. ornatus Yellow-plumed Honeyeater	Australia, WA, 15km NW of Cranbrook	*Ptilotula*	*Ptilotula*	KU 8758	HQ267676	HQ267696
L. penicillatus White-plumed Honeyeater	Australia, WA, 40km W of Gascoyne Junction	*Ptilotula*	*Ptilotula*	KU 6179	HQ267677	HQ267697
L. plumulus Grey-fronted Honeyeater	Australia, SA, Corunna	*Ptilotula*	*Ptilotula*	ANSP 22636	HQ267678	HQ267698
L. flavus Yellow Honeyeater	Australia, QLD, Kalarka, Cattle Creek Crossing	*Stomiopera*	*Stomiopera*	ANSP 25088	HQ267668	HQ267688
L. unicolor White-gaped Honeyeater	Australia, WA, King Edward River Crossing, Munura	*Stomiopera*	*Stomiopera*	ANSP 26919	HQ267680	HQ267700

Figure 1:　　Phylogenetic relationships of *Lichenostomus* honeyeaters derived from Bayesian analysis of the mitochondrial protein-coding gene ND2 and the nuclear intron Fib5. Outgroup taxa included members of the Pardalotidae and Maluridae (following Driskell and Christidis 2004), rooted with the Striated Grasswren *Amytornis striatus*. The 20 species of *Lichenostomus* contributed by the present study are indicated in bold text style. Taxa in regular font style correspond to samples from the Driskell and Christidis (2004) study, while the *Meliphaga* species shown in grey regular font are from Norman *et al*. (2007). As both search algorithms (BA and ML) produced concordant topologies, we summarize nodal support derived through Bayesian posterior probabilities (BPP) and maximum likelihood bootstrap support (MLBS) by differently shaded circles as follows: black circles indicate 100% BPP and over 95% MLBS, grey circles correspond to above 95% BPP and over 75% MLBS, while white circles show nodes recovered only with more than 75% BPP. The four main *Lichenostomus* groups are flagged by numbered boxes at their nodes.

Figure 2:　　Simplified, diagrammatic overview of Figure 1 to summarize the generic level dismantlement of Lichenostomus proposed here. For graphical convenience, branch lengths have no phylogenetic significance, and we have illustrated branches leading to other meliphagid clades as triangles (see Figure 1 for details).

Figure 3:　　Photograph of specimens of five species formerly placed in *Lichenostomus* and here placed in *Caligavis* and in *Bolemoreus gen. nov*. From left to right with abbreviations of generic names proposed: *B. hindwoodi* (ANWC 41405)*, B.*

frenatus (ANWC 39613)*, C. obscura* (ANWC 1425), *C. subfrenata* (ANWC 4543) and C.

chrysops (ANWC 40727).

Figure 1

Certhionyx niger
Myzomela cardinalis
Myzomela erythrocephala
Myzomela sanguinolenta
Myzomela obscura
Myzomela rosenbergii
Phylidonyris melanops
Glycichaera fallax
Ptiloprora guisei
Ptiloprora plumbea
Acanthorhynchus superciliosus
Acanthorhynchus tenuirostris
Certhionyx variegatus
Prosthemadera novaeseelandiae
Pycnopygius cinereus
Pycnopygius stictocephalus
Certhionyx pectoralis
Lichmera alboauricularis
Lichmera indistincta
Phylidonyris nigra
Phylidonyris novaehollandiae
Phylidonyris pyrrhoptera
Trichodere cockerelli
Grantiella picta
Plectorhyncha lanceolata
Xanthotis flaviventer
Philemon argenticeps
Philemon corniculatus
Philemon buceroides
Philemon citreogularis
Philemon meyeri
Entomyzon cyanotis
Melithreptus albogularis
Melithreptus brevirostris
Foulehaio carunculata
Lichenostomus flavicollis
Lichenostomus leucotis
Ashbyia lovensis
Epthianura albifrons
Epthianura aurifrons
Epthianura tricolor
Epthianura crocea
Melilestes megarhynchus
Melipotes fumigatus
Timeliopsis fulvigula
Timeliopsis griseigula
Conopophila albogularis
Conopophila rufogularis
Ramsayornis fasciatus
Ramsayornis modestus
Acanthagenys rufogularis
Anthochaera carunculata
Anthochaera paradoxa
Xanthomyza phrygia
Anthochaera chrysoptera
Anthochaera lunulata
Lichenostomus frenatus
Lichenostomus kindwoodi
Lichenostomus chrysops
Lichenostomus obscurus
Lichenostomus subfrenatus
Lichenostomus cratitius
Lichenostomus melanops
Manorina flavigula
Manorina melanophrys
Melidectes belfordi
Melidectes ochromelas
Melidectes torquatus
Purnella albifrons
Lichenostomus flavus
Lichenostomus unicolor
Lichenostomus fasciogularis
Lichenostomus virescens
Lichenostomus versicolor
Lichenostomus flavescens
Lichenostomus flavescens
Lichenostomus fuscus
Lichenostomus keartlandi
Lichenostomus plumulus
Lichenostomus ornatus
Lichenostomus penicillatus
Meliphaga albilineata
Meliphaga fordiana
Meliphaga reticulata
Meliphaga albonotata
Meliphaga albonotata
Meliphaga analoga analoga
Meliphaga orientalis orientalis
Meliphaga analoga stevensi
Meliphaga gracilis
Meliphaga gracilis gracilis
Meliphaga gracilis imitatrix
Meliphaga cinereifrons cinereifrons
Meliphaga cinereifrons stevensi
Meliphaga mimikae grenti
Meliphaga montana arcora
Meliphaga flavirictus
Meliphaga aruensis
Meliphaga aruensis aruensis
Meliphaga lewinii amphochlora
Meliphaga lewinii lewinii
Meliphaga notata mixtata
Meliphaga notata notata

Outgroup

0.06

1
2
3
4

Figure 2

Nesoptilotis: *N. leucotis, N. flavicollis*

Bolemoreus: *B. hindwoodi, B. frenatus*

Caligavis: *C. obscura,*
C. subfrenata, C. chrysops

Lichenostomus: *L. cratitius,*
L. melanops

Stomiopera: *S. flavus, S. unicolor*

Gavicalis: *G. versicolor, G. virescens,*
G. fasciogularis

Ptilotula: *P. flavescens, P. ornatus,*
P. penicillatus, P. plumulus,
P. fuscus, P. keartlandi

Figure 3

Chapter 3

Multilocus analysis of the *Gerygone* warblers (Aves: Acanthizidae): phylogenetic relationships, taxonomy and their evolution into the mangroves

Abstract

The Australo-Papuan warblers (Passeriformes: Acanthizidae) have been the subject of recent molecular phylogenetic analyses. Taxon sampling for one member genus *Gerygone*, however, has been incomplete. This has limited our ability to draw meaningful conclusions about the evolutionary history and historical biogeography of *Gerygone*. Here we report on a phylogenetic analysis of *Gerygone* based on comprehensive taxon sampling and a multilocus dataset of thirteen loci spread across the avian genome (eleven nuclear and two mitochondrial loci). Since *Gerygone* includes three species restricted to Australia's coastal mangrove forests, we sought to understand the biogeography of their evolution in that ecosystem. Analyses of individual loci, as well as of a concatenated dataset drawn from previous molecular studies indicates that the genus as currently defined is not monophyletic, and that the Grey Gerygone (*G. cinerea*) from New Guinea is a basal member of the genus *Acanthiza*. Evolution into mangrove ecosystems occurred repeatedly, in three non-overlapping time frames. Our results highlight recurrent difficulties of recovering strongly supported species trees from multilocus datasets, particularly in groups that have undergone rapid radiations.

1.   Introduction

Among the members of the Australo-Papuan passerine family Acanthizidae, the genus *Gerygone* is the most geographically widespread. Its 19 currently recognized member species occur in Australia, New Guinea, New Zealand, Pacific Islands, and Indonesia as well as on many offshore islands. One species, *G. sulphurea*, is found north of Wallace's Line from Thailand to the Philippines, and *G. insularis* of Lord Howe Island became extinct following predation by introduced rats in the early 19th century (Ford 1986). All *Gerygone* are small, relatively drab, and forage arboreally. Habitats range from closed canopy moist forests to open arid zone woodlands, and at least three species (*G. magnirostris, G. tenebrosa, G. levigaster*) occur predominantly in coastal mangrove forests (Ford 1982, 1986, Schodde and Mason 1999, Christidis and Boles 2008). Given the diverse biogeographic and ecological patterns exemplified by gerygones – a mainly Australo-Papuan clade with several members on offshore islands, and several mangrove forest specialists – they rank among the groups best-suited for elucidating the origin of Australia's rich mangrove avifauna (Ford 1982, Schodde et al. 1979, Schodde 2006).

Despite Ford's (1986) pioneering attempt to analyze *Gerygone* phylogenetically, the birds' conservative morphology has inhibited development of a comprehensive phylogenetic framework. This in turn has complicated interpretations of biogeographic patterns. A recent phylogenetic study of the largest radiation of Australasian songbirds, the Meliphagoidea (Gardner et al. 2010), included the first molecular analysis of acanthizids including *Gerygone*. The eight species of *Gerygone* from Australia and New Guinea comprised a monophyletic group, which, together with the monotypic Fernwren

Oreoscopus gutturalis, was basal to all other acanthizids. Support for the monophyly of the eight species was high but relationships within the genus were not well resolved and there were only a few well-supported clades.

Several molecular phylogenetic studies have now documented the importance of island radiations in diversification of continental avifaunas (Filardi and Moyle 2006, Moyle et al. 2009, Nyári et al. 2009). They have led to the conclusion that islands are not necessarily evolutionary dead ends, but rather that they are important sources of biological diversity for mainland groups through back-colonization events. By analogy, the role of Australo-Papua's mangrove forests as ecological islands for closed-canopy-dwelling birds during Australia's long history of aridification (Byrne et al. 2008) might also be tested. This could assess whether several avian families evolved and speciated solely within mangrove forests (Ford 1982). While it is currently hypothesized that the rich Australo-Papuan mangrove avifauna has evolved mainly from continental sources (Ford 1982, Schodde 2006, Loynes et al. 2009, Nyári and Joseph *in press*), examples of contributions of novel mangrove-restricted species from island radiations have also been documented (Nyári et al. 2009).

Our use of multilocus datasets here reflects two now well-established observations: that individual gene trees can differ from the true species tree, and that these datasets offer richer windows into the evolutionary history of lineages than studies based on mitochondrial DNA (mtDNA) (Edwards et al. 2005, Jennings and Edwards 2005, Hackett et al. 2008, Loynes et al. 2009, Nyári et al. 2009, Christidis et al. 2010, Li et al. 2010, Toon et al. 2010, Flórez-Rodríguez et al. 2011). Gene tree – species tree discordances can be due to stochastic sorting of ancestral polymorphisms, or varying

degrees of gene flow following lineage-splitting events at different depths within the phylogenetic history of a group of organisms (Degnan and Rosenberg 2006, Liu and Edwards 2009). All of these processes call for increased complexity and thoroughness of model-based phylogenetic estimations from multilocus datasets. These range from individual gene tree analysis, concatenation and partitioning of an entire multilocus dataset, to Bayesian Estimation of Species Tree methods, which estimate the joint posterior distribution of gene trees for each locus and use the resulting joint posterior distribution of gene trees to approximate the Bayesian posterior distribution of the species tree based on coalescent theory (Liu and Pearl 2007, Edwards et al. 2007). The implications of these methodological advances are far reaching. Anomalous gene trees (Degnan and Rosenberg 2006) are known to be quite common, particularly in groups that have seen rapid bursts of speciation (Moyle et al. 2009).

Accordingly, we here use comprehensive taxon sampling and an analysis of sequence data derived from 13 loci spread across the avian nuclear and mitochondrial genomes to test the (1) monophyly of the acanthizid genus *Gerygone*, (2) monophyly of the set of mangrove-restricted species (*G. magnirostris, G. tenebrosa,* and *G. levigaster*), and (3) biogeographic influence of island species and timing of speciation events tied to mangrove forests.

2.   Materials and methods

2.1.   Taxon sampling and laboratory protocols

Our ingroup of 16 *Gerygone* species comprised single samples per taxon and so was not designed to test species limits, which mostly are uncontroversial. Unsampled

taxa included extinct *G. insularis* and extant populations of *G. dorsalis* and *G. albofrontata* from the Lesser Sundas and Chatham Islands, respectively. Outgroup taxa were chosen based on results of previous higher-level phylogenetic studies of passerines and included diverse acanthizids: *Oreoscopus gutturalis* (Fernwren), *Smicrornis brevirostris* (Weebill), and *Acanthiza apicalis* (Inland Thornbill).

Genomic DNA was extracted from frozen or ethanol preserved tissue samples from vouchered specimens collected by us and researchers from other institutions (Table 1) via the standard Qiagen DNeasy™ tissue extraction protocols (Qiagen, Valencia, CA). We amplified and sequenced 13 distinct loci distributed across the avian nuclear and mitochondrial genomes using a published set of primers and protocols (Table 2). A detailed list of GenBank accession numbers for all loci and species is presented in Table 3. All PCR amplifications were performed in 25µl reactions using PureTaq™ RTG PCR beads (GE Healthcare Bio-Sciences Corp.). Amplified double-stranded PCR products were cleaned with ExoSAP-IT™ (GE Healthcare Bio-Sciences Corp.), and visualized on high-melt agarose gels stained with ethidium bromide. Purified PCR products were subsequently cycle-sequenced with ABI Prism BigDyeT™ v3.1 terminator chemistry using the same primers as for each PCR reaction. Cycle-sequenced products were further purified using Sephadex™ spin columns (GE Healthcare Bio-Sciences Corp.), and finally sequenced on an ABI 3130 automated sequencer. Sequences of both strands of each gene were examined and aligned in Sequencher 4.8 (GeneCodes Corp.). We did not attempt to reconcile the allelic phase of heterozygous base calls, but rather coded them as ambiguous according to the International Union of Pure and Applied Chemistry (IUPAC) standards.

2.2. Data matrix construction and phylogenetic analyses

Complementary gene sequence contigs derived from all 13 loci for all taxa were aligned using ClustalX 2.0.7 (Thompson et al., 1997), and scrutinized further by eye in Mesquite 2.74 (Madison and Madison 2010). Separate data matrices of 19 taxa (16 ingroup and 3 outgroup) were assembled for each of the 11 nuclear loci, while the two mitochondrial genes (ND2 and ND3) were combined in a single dataset. Subsequent analyses examined individual loci and a partitioned dataset through model-based phylogenetic algorithms under both Maximum Likelihood (ML) and Bayesian analysis (BA) approaches. ModelTest 3.7 (Posada and Crandall 1998) was used to determine the most appropriate model of sequence evolution via the Akaike Information Criterion (AIC).

ML heuristic tree searches were conducted using the program GARLI 1.0 (Zwickl 2008), under a single data partition and the GTR+I+G model of sequence evolution, with parameter values estimated from the data. Nodal support was assessed via 1000 non-parametric bootstrap replicates. BA was carried out within the Markov Chain Monte Carlo (MCMC) tree search algorithm framework as implemented in the program MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The concatenated data set was partitioned by each locus, and by codon position for the mitochondrial genes. We ran two independent runs of 10^7 generations, using the previously inferred model of sequence evolution specified for each locus. Search parameters included unlinking of all partition-specific rates and models of evolution, adjustment of chain heating conditions (temp = 0.1 – 0.05) for improved chain swap acceptance rates, and sampling every 100 generations. Evaluation of stationarity and chain convergence was conducted

by plotting posterior probabilities from the two runs in the program Tracer (Rambaut and Drummond 2007). The resulting pool of topologies sampled from the first 30% of generations was discarded as an initial 'burn-in', such that 70,000 trees were finally summarized to produce a single 50% majority-rule consensus tree, rooted with the Fernwren *Oreoscopus gutturalis*. Lastly, we proceeded to evaluate the monophyly of the 3 mangrove-restricted gerygones by enforcing their monophyly as a constraint on ML GARLI searches. Site likelihood outputs from the best constrained trees were used in subsequent test against our ML tree via the Approximately Unbiased (AU) test, as implemented in the program CONSEL (Shimodaira and Hasegawa 2001).

Additionally, a species tree was estimated from the joint distribution of individual gene trees via the program BEST 1.6 (Liu et al. 2007, 2008). The dataset was again partitioned by locus, each with an appropriately specified model of evolution. We assigned default settings for the parameter values of the Bayesian search, as recommended by the authors: flat priors, inverse gamma distribution with values of α = 3 and β = 0.003 for priors of population size, and a uniform distribution with bounds of 0.5 and 1.5 for priors of the mutation rates. Two runs with four separate chains (one heated and three cold) were run simultaneously for 5×10^7 generations, sampling every 1000 generations. A consensus topology from the two separate runs was obtained after discarding an initial burn-in of 30% of the sampled topologies.

2.3. Phylogenetic affinities and divergence timing of diversification of G. cinerea

Initial examination of the data revealed that sequences of the Grey Geryone, *G. cinerea*, from the highlands of New Guinea were substantially distinct from other *Gerygone* species. This prompted us to consider further testing of the phylogenetic

placement of *G. cinerea* within the Meliphagoidea in which *Gerygone* itself is embedded. Gardner et al.'s (2010) study of Meliphagoidea shared three markers with our dataset. Accordingly, we assembled a separate data matrix from published and newly derived sequences for nuclear exons of RAG1 and RAG2 and the mtDNA gene ND2 to examine relationships of *G. cinerea* within the Acanthizidae specifically and Melipgaoidea more generally (Appendix 1).

We performed a Bayesian analysis using the program MrBayes 3.1.2 as described above, partitioning our data by gene and by codon for the two nuclear and the mitochondrial genes, respectively. This larger dataset was also used to estimate relative timing events of cladogenesis using the program BEAST 1.4.8 (Drummond and Rambaut, 2007) by producing an ultrametric tree with 95% confidence intervals for node heights. Given the lack of reliable fossil calibration points for acanthizids, we opted not to place dates on the ultrametric tree, but rather focus on relative differences in the sequence of splitting events. A topological constraint in the form of the Bayesian consensus tree was placed onto the MCMC run, such that rates were allowed to vary only along this given scenario. A relaxed clock model (Drummond et al., 2006) with uncorrelated rates drawn from a lognormal distribution was selected, and two MCMC runs of 10^7 generations with parameters logged every 100 generations. The first 40% of generations of each run were discarded as burn-in after inspection of likelihood scores and parameters for stationarity. The final ultrametric tree was generated from the combined tree files of the two MCMC runs.

3.   Results

3.1. Phylogenetic analyses of gene trees and species tree reconstruction

Alignment of sequence data derived from all thirteen loci was straightforward, resulting in a total of 8124 base pairs (bp). Overall sequence length ranged from 279 bp to 1350 bp for nuclear loci, whereas the two mitochondrial genes were 1041 bp and 351 bp in length (Table 2). Among the nuclear loci, Mame AL-23, MUSK, and TGFb2 were the most variable; however, Mame AL-16, CDC132 and Fib5 had the highest percentage of informative sites (Table 2). The two mtDNA protein-coding genes ND2 and ND3 had no insertions, deletions, or anomalous stop-codons. Base composition was typical of avian mtDNA (Table 2), consistent with true mitochondrial origin as opposed to nuclear pseudogenes (Sorenson & Quinn 1998). Information content in the two mitochondrial loci was significantly higher than in the nuclear loci: out of the total number of variable sites, ND2 and ND3 had over 70% and 64% parsimony informative sites, respectively (Table 2).

Resolution of individual gene trees varied at diverse nodes throughout the topology, most loci showing consistent patterns of sister species relationships (Figure 1). G3PDH was the least informative locus and also the shortest sequence, but all other nuclear loci showed at least four strongly supported nodes (Bayesian posterior probabilities and ML bootstrap support; Figure 1). The combined mitochondrial dataset (ND2 and ND3) featured the best-resolved topology, and all but two nodes had the highest possible support. Analysis of the combined datased under a single partition and separated by gene and codon region for the two mtDNA protein-coding genes recovered similar topologies and statistical support as our species tree estimate (Figure 2, see below). Nodal support was strong throughout the concatenated and partitioned

datasets: only some terminal nodes received relatively low statistical support (Figure 2). Compared to the species tree estimate, the concatenated and partitioned datasets differed in placement of *G. magnirostris* relative to other mangrove species. Further differences are also evident along subsequent nodes, although the three different data analysis schemes agreed on the sister relationship of *G. igata* and *G. modesta*.

G. cinerea was consistently recovered by all loci as not closely related to other ingroup species rendering *Gerygone* polyphyletic (Figure 1). Analysis of our 13-locus dataset placed this species with the three outgroup members, specifically with *Acanthiza apicalis*.

All gene trees indicated clearly that the three mangrove restricted species *G. magnirostris*, *G. tenebrosa*, and *G. levigaster*, do not form a monophyletic group. Strong support was evident in all gene trees for two sister species relationships, one between *G. chrysogaster* and *G. mouki*, and the other between *G. igata* and *G. modesta*. The mtDNA dataset further indicated strong support for sister species relationships between *G. chloronota* and *G. palpebrosa* (also supported by Fib5), between *G. inornata* and *G. olivacea* (also supported by MUSK, HMG2, AL16), and between *G. fusca* and *G. levigaster* (also supported by RAG2, TGFb2, HMG2, CDC132).

The species tree inferred from all 13 loci mirrored closely the consensus among the underlying gene trees and the analysis of the concatenated and partitioned dataset. Again, *Gerygone* was not monophyletic and the sister species relationships of *G. chrysogaster/G. mouki*, and *G. igata/G. modesta* were strongly supported (Figure 2). Similarly, the three mangrove specialists were not a monophyletic group, and their constrained monophyly constitutes a significantly worse likelihood under the AU test.

Most other nodes in the species tree received low-to-moderate support, except that uniting *G. chloronota, G. inornata* and *G. olivacea*.

3.2. *Phylogenetic affinities of* G.cinerea *and timing of speciation events*

Based on the broader, three gene dataset, we addressed the phylogenetic placement of *G. cinerea* within acanthizids generally. The dataset comprised 3429 bp from RAG1 (1350 bp), RAG2 (1038 bp) and ND2 (1041 bp) (Appendix 1). Results clearly supported our previous phylogenetic inferences based on the 13-locus dataset, where *G. cinerea* clusters not with other gerygones but with *Acanthiza*, the second largest group of acanthizid warblers. Placement of *G. cinerea* within *Acanthiza* received very strong nodal support (Figure 3): *G. cinerea* is relatively basal within *Acanthiza* where it is sister to *A. lineata* and *A. nana*, both of which are endemic to Australia.

The same extended dataset was used to infer a sequence of splitting events under a relaxed-clock model coupled with an enforced topological constraint from the Bayesian consensus tree. The resulting ultrametric tree illustrates important variation in the 95% confidence intervals for node heights (Figure 3). As such, we can clearly distinguish differences in evolutionary rates between the two prominent acanthizid groups, *Gerygone* and *Acanthiza*, the former clearly having radiated later, and with increased speciation rate, whereas the clade containing *Acanthiza*, *Sericornis*, and other Australo-Papuan acanthizids is relatively older and has had slower rates of diversification. Due to the unavailability of a calibration point in this analysis, we report estimates of mitochondrial sequence divergence to be used as rough guidelines in the estimation of divergence times. Based on uncorrected sequence divergences of the two mitochondrial genes, the genetically most distinct gerygones (excluding *G. cinerea*)

were *G. palpebrosa* and *G. mouki* at 13.5%. Highest divergences values within the clade containing the three mangrove-bound species (Figure 3) were at 8.1% between *G. magnirostris* and *G. igata*. The three mangrove endemic species differed by 7.7% (*G. magnirostris* vs. *G. tenebrosa*), 7.3% (*G. magnirostris* vs. *G. levigaster*), and 4.0% (*G. levigaster* and *G. tenebrosa*).

4. Discussion

4.1. Multilocus phylogenetic analysis and taxonomy of gerygones

Our study represents the first comprehensive phylogenetic analysis of the acanthizid warbler genus *Gerygone*, using a broadly sampled, multilocus dataset. While multilocus phylogenetic analyses have been successfully employed throughout a diverse array of avian groups (McGuire et al. 2007, Pasquet et al. 2007, Wright et al. 2008, Fregin et al. 2009, Loynes et al. 2009, Parra et al. 2009), the present study is among the few that make use of high numbers of unlinked loci spread across the avian nuclear and mitochondrial genomes (Hackett et al. 2008, Lovette et al. 2010, Toon et al. 2010, Flórez-Rodríguez et al. 2011). Moreover, we directed our study towards a group of diverse evolutionary and ecological histories, to understand better the implications of individual gene histories and their influence on species tree estimation. Overall, several common phylogenetic patterns emerged from the individual gene trees and their differences also highlight complexity of the group's evolutionary history. The Bayesian estimate of species tree relationships and the Bayesian analyses of the concatenated and partitioned dataset resulted in very similar topologies. Below, we highlight detail of some of these commonalities and differences among analytical methods.

Ford (1986) reviewed the taxonomy of *Gerygone* based on numerical analysis of morphological characters. He noted inherent difficulties in reconstructing relationships based solely upon morphology, but nevertheless derived important hypotheses regarding sister species relationships of gerygones, some of which were corroborated here with the aid of multilocus data. For example, two relationships suggested by Ford, that of *G. inornata* of the Lesser Sundas being closely related to Australo-Papuan *G. olivacea*, and Australian *G. fusca* being closely related to the mangrove forest endemic *G. levigaster*, were affirmed here in the species tree, three of the gene trees, and the mtDNA tree (Figure 1 and 2). Further, Ford's hypothesis that *G. mouki* is one of the basal members on the *Gerygone* tree, is again in agreement with our species tree, combined gene trees, four different gene trees and the mitochondrial tree (Figure 1 and 2).

The most novel relationship concerning *Gerygone* that we recovered is the exclusion from *Gerygone* of *G. cinerea*, which clearly belongs in *Acanthiza* (Figure 2 and 3). Based on plumage and biogeography, Ford (1986) suggested that *G. cinerea* was closely related to *G. chloronota*. We conclude that *G. cinerea* should be assigned to *Acanthiza* Vigors and Horsfield, 1827, and so be known as *A. cinerea* (Salvadori, 1876).

Other novel relationships within *Gerygone* include the eastern Australian endemic *G. mouki* as sister to *G. chrysogaster* from the lowlands of New Guinea. This relationship was supported almost unequivocally in our different data analyses (Figures 1, 2, and 3). *G. chloronota* grouped with *G. inornata* and *G. olivacea*, although support for this arrangement came only from the species tree (Figure 2). However, individual

gene trees consistently placed two of these three taxa in close phylogenetic proximity (Figure 1). Another unequivocally supported sister species relationship was between the endemics of New Zealand and Norfolk Island, *G. igata* and *G. modesta*, respectively. Ford (1986) had alternatively concluded that *G. modesta* and *G. igata* are not sister taxa and that the former is possibly more closely affiliated to mangrove-restricted *G. levigaster*. Nonetheless, as in our analyses, he had repeatedly found *G. levigaster* to be close to widespread Australian *G. fusca.*

Several *Gerygone* species were characterized by weakly-supported phylogenetic placements in the species tree analysis. An example is *G. palpebrosa*, which was recovered from a deeper node in the topology of our species tree, as well as our concatenated and partitioned 13-locus dataset, where it received high nodal support (Figure 2). Individual gene trees did not show particularly strong support for placement of this taxon, while the ntDNA gene tree identified it as sister to *G. chloronota*. Similarly, our separate gene tree analysis indicated that the northernmost species, *G. sulphurea,* is also characterized by labile phylogenetic placement, migrating from deeper to more shallow nodes throughout the *Gerygone* clade between analyses (Figure 1). The species tree together with the combined and partitioned phylogenetic analyses nevertheless placed *G. sulphurea* with moderate to strong support at the node preceding the clade containing all three mangrove restricted *Gerygone* species (Figure 2). The mtDNA gene tree, on the other hand, placed *G. sulphurea* as sister to one of the mangrove endemics, *G. magnirostris*.

The New Guinean highland endemic, *G. ruficollis*, is another species with uncertain evolutionary history. The species tree places it with low support as sister to

the *G. fusca*/*G. levigaster* pair (Figure 2), but the concatenated and partitioned dataset analysis instead strongly support it as sister to *G. igata/G. modesta* (Figure 2). Interestingly, our mtDNA dataset includes *G. ruficollis* as sister to a clade containing both of these other sister species pairs.

4.2. Biogeographic patterns and the evolution of mangrove-restricted gerygones

Complex evolutionary and biogeographic scenarios in the history of *Gerygone* are clearly apparent from our results. Consensus was achieved in identifying *G. chrysogaster* and *G. mouki* as basal to the rest of *Gerygone*. This is consistent with an Australo-Papuan center of diversity for the group. The geographic distributions of these two taxa correspond to Australo-Papuan tropical lowland (Irian) and subtropical-montane rainforest (Tumbunan) avifaunas (Schodde and Calaby 1972, Schodde and Mason 1999, Schodde 2006).

The clade formed by *G. chloronota* as sister to *G. inornata* and *G. olivacea*, includes species from northwest Australia and New Guinea, the Lesser Sundas, northeast Australia and southeast New Guinea, respectively. The sister relationship between insular *G. inornata* and continental *G. olivacea* illustrates the broader geographic extent of the Australo-Papuan Torresian influence within this clade (Schodde 2006). The only *Gerygone* species extending beyond Wallace's Line, *G. sulphurea*, has radiated well into the Malay Peninsula, Greater Sundas, and the Philippines, where it occupies forests as well as coastal mangroves. The phylogenetic placement of this wide-ranging species amidst different clades of mostly Australo-Papuan gerygones aptly illustrates the capability of rapid dispersal and speciation within

this group of acanthizid warblers, in direct contrast with the other constituent members of the family (Figure 3).

The remaining species of *Gerygone* are from continental Australian, New Guinea, and Pacific Islands (Figure 2). Prominent in this group are the three mangrove endemic species *G. magnirostris, G. tenebrosa*, and *G. levigaster*. Although our data do not recover a single unequivocal pattern of relationships among these species, there is no support for them representing a single radiation into mangrove ecosystems. Rather, they appear to represent three independent, repeated colonizations of mangroves from continental or island sister species. We were unable to infer with certainty which species arrived first in Australia's mangroves, as the species tree placed *G. tenebrosa* as earliest to enter mangroves (Figure 2), while the concatenated and partitioned datasets, as well as the extended taxon sampling dataset supported *G. magnirostris* as the earliest mangrove gerygone (Figure 2 and 3). *G. levigaster*, the most recently arrived mangrove gerygone, currently occupies coastal mangrove forests mostly east of the range of *G. tenebrosa*; these two species overlap only along a short stretch of the Kimberley coast (Ford 1982). Conversely, the broad sympatry of *G. magnirostris* and *G. levigaster* along the northern and north-eastern Australian seaboard coasts (Ford 1982, Schodde and Mason 1999, Schodde 2006) is probably due to some degree of niche partitioning. *G. magnirostris*, for example, also explores resources in nearby swamplands and riparian forests besides its main, mangrove-preferred habitat (Ford 1982, Johnstone 1990, Noske 1996).

As is clearly evident from our data, establishing a definite sequence of speciation events tied to mangroves remains problematic, even with the contribution of multilocus

phylogenetic analysis. This is most likely due to the comparatively recent evolution of this clade of acanthizids (Figure 3), but can be also due to potential hybridizations between taxa such as *G. magnirostris* and *G. tenebrosa* (Johnstone 1975, Ford 1983), further complicating species tree inferences. Concerning the temporal framework of evolution in *Gerygone*, it is clear that the sequence of speciation events within this final clade occurred relatively quickly, potentially predating the Pleistocene based solely on uncorrected sequence divergences and a mitochondrial clock of 2% per m.y. This is supported by the lack of consensus in phylogenetic resolution of all the relevant taxa (Figure 2 and 3). Thus, all three methods we have used had difficulties in discerning a common pattern. Variable placements of the Solomon Islands endemic *G. flavolateralis*, the New Guinean highland endemic *G. ruficollis*, and the widespread interior Australian endemic *G. fusca* all illustrate this. Multilocus phylogenetic analysis has seen a surge of attention in recent years, although difficulties associated with obtaining well-supported phylogenetic topologies from such a large and diverse array of loci can lead to a sense of low return given the considerable effort required for generating such datasets. Differences in topologies and support can derive from difficulties in proper model parameterization of such large datasets, further complicated by rapid rates of speciation over broad geographic scales and ecological niches. We are, however, certain that such repeated efforts in generating well-sampled datasets for non-model organisms will lead to an increased understanding of their intricate evolutionary histories, highlighting the need for further research towards novel approaches in data collection and analysis.

5.   Conclusions

Employing a diverse array of molecular markers to elucidate the evolutionary history of gerygones still has proven difficult in recovering an overall well-supported phylogenetic hypothesis. Mangrove-bound gerygones were shown to have evolved repeatedly and not as a single evolutionary lineage, lending further support for a case-by case exploration of the rich Australo-Papuan mangrove avifauna. Further phylogeographic analysis of relationships among the three gerygones tied to coastal mangroves will provide additional insights into the levels of intraspecific genetic markup, influence of geographic barriers, as well as putative hybridization events. Contrasting these molecular findings with morphological data based on plumage, song and behavior will broaden our understanding of historical biogeography within this group.

Acknowledgements

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Table 1: Taxon sampling, voucher information, and locality information of *Gerygone* species included in the present study.

Taxon	Voucher	Locality
Gerygone chloronota	ANWC 39172	Australia, WA, Mitchell Falls
Gerygone chrysogaster	KUNHM 7504	New Guinea, Western Province, Ekame Camp
Gerygone cinerea	KUNHM 16404	New Guinea, Central Province, Mt. Simpson Bush Camp
Gerygone flavolateralis	AMNH DOT6559	Solomon Islands, Rennell Island, Tahamatangi
Gerygone fusca	ANWC 40265	Australia, NT, Kunoth Bore, NW of Alice Springs
Gerygone igata	MV B10851	New Zealand
Gerygone inornata	WAM 23458	Indonesia, Sabu
Gerygone levigaster	ANWC 39335	Australia, QLD, SE of Gladstone
Gerygone magnirostris	ANWC 39961	Australia, QLD, N of Innisfail
Gerygone modesta	ANWC 40523	Australia, Norfolk Island Territory
Gerygone mouki	ANWC 39196	Australia, NSW, NNE of Kempsey
Gerygone olivacea	ANWC 26490	New Guinea, Central Province, Port Moresby
Gerygone palpebrosa	ANWC 39361	Australia, QLD, Miriam Vale
Gerygone ruficollis	ANWC 26963	New Guinea, Gulf Province, Mountain Camp
Gerygone sulphurea	AMNH DOT12621	Indonesia, Sulawesi, Bangai
Gerygone tenebrosa	ANWC 39184	Australia, WA, Point Torment
Acanthiza apicalis	ANWC 24367	Australia, QLD, S of Winton
Smicrornis brevirostris	ANWC 24332	Australia, NSW, NW of Cootamundra
Oreoscopus gutturalis	ANWC 39536	Australia, QLD, Longlands Gap, S of Atherton

Institutional abbreviations for voucher sources are as follows: American Museum of Natural History (AMNH), Australian National Wildlife Collection (ANWC), The University of Kansas Natural History Museum and Biodiversity Institute (KUNHM), Museum Victoria (MV), Western Australian Museum (WAM).

Table 2:   Summary of the thirteen loci included in the present study.

Locus	Length (aligned bp)	Category, chromosome #[a]	Substitution model	A,C,G,T frequency	Variable sites (% of total)	Informative sites (% of total / % of variable)	Source
Mame AL-06	415	anonymous locus	TrN	0.267, 0.169, 0.270, 0.293	47 (11.32)	15 (3.61 / 31.91)	Lee and Edwards (2008)
Mame AL-16	387	anonymous locus	HKY+G	0.241, 0.230, 0.213, 0.314	66 (17.05)	24 (6.20 / 36.36)	Lee and Edwards (2008)
Mame AL-23	428	anonymous locus	TrN+I	0.324, 0.234, 0.177, 0.264	88 (20.56)	25 (5.84 / 28.40)	Lee and Edwards (2008)
CDC132	597	intron, 2	TVM+G	0.264, 0.171, 0.216, 0.347	93 (15.57)	39 (6.53 / 41.93)	Backström et al. (2008)
HMG2	494	intron, 4	TVM	0.314, 0.172, 0.203, 0.309	76 (15.38)	15 (3.03 / 19.73)	Backström et al. (2008)
Fib5	621	intron, 4	HKY+G	0.299, 0.176, 0.201, 0.323	96 (15.46)	41 (6.60 / 42.70)	Marini and Hackett (2002)
G3PDH	279	intron, 1	HKY	0.260, 0.337, 0.185, 0.216	37 (13.26)	9 (3.22 / 24.32)	Fjeldså et al. (2003)
TGFb2	563	intron, 3	GTR+I	0.229, 0.243, 0.211, 0.315	105 (18.65)	33 (5.86 / 31.42)	Primmer et al. (2002)
MUSK	560	intron, Z	HKY+I	0.298, 0.168, 0.194, 0.337	117 (20.89)	22 (3.92 / 18.80)	F.K. Barker (pers.comm.)
RAG1	1350	exon, 5	TrN+I+G	0.316, 0.219, 0.232, 0.232	108 (8.00)	41 (3.03 / 37.96)	Barker et al. (2002)
RAG2	1038	exon, 5	HKY+I+G	0.289, 0.210, 0.238, 0.262	94 (9.05)	25 (2.40 / 26.04)	Barker et al. (2002)
ND2	1041	mitochondrial	GTR+I+G	0.298, 0.389, 0.104, 0.206	359 (34.48)	255 (24.50 / 71.03)	Sorenson et al. (1999)
ND3	351	mitochondrial	TrN+I+G	0.325, 0.361, 0.097, 0.215	133 (37.89)	86 (24.50 / 64.66)	Chesser (1999)

[a] Locus information and chromosome number was inferred from the genome map of the chicken genome on GenBank

Table 3: GenBank accession numbers for the Gerygone species and the 13 loci included in the present study.

NOTE: to be updated upon acceptance of sequence submission.

Taxon	Mame AL-06	Mame AL-16	Mame AL-23	CDC132	HMG2	Fib5	G3PDH	TGFb2	MUSK	RAG1	RAG2	ND2	ND3
Gerygone chloronota													
Gerygone chrysogaster													
Gerygone cinerea													
Gerygone flavolateralis													
Gerygone fusca													
Gerygone igata													
Gerygone inornata													
Gerygone levigaster													
Gerygone magnirostris													
Gerygone modesta													
Gerygone mouki													
Gerygone olivacea													
Gerygone palpebrosa													
Gerygone ruficollis													
Gerygone sulphurea													
Gerygone tenebrosa													
Acanthiza apicalis													
Smicrornis brevirostris													
Oreoscopus gutturalis													

Figure 1:   Phylogenetic estimates of gene trees obtained via Bayesian analysis of individual loci. Locus acronyms follow Table 2 and references therein. Strong support in form of Bayesian posterior probabilities of >95% are indicated by dark circles at nodes. The mitochondrial protein-coding genes ND2 and ND3 have been combined in a single partition, indicated as "mtDNA". Mangrove specialists are highlighted in green.

Figure 2:   Phylogenetic analysis of the combined 13-locus dataset. All topologies are rooted with the Fernwren *Oreoscopus gutturalis*, not shown for brevity of branch length. Support values in form of Bayesian posterior probabilities are given at each node, with dark circles emphasizing strong support (>95%). LEFT panel illustrates the species tree obtained under the BEST algorithm. CENTER panel depicts phylogenetic hypothesis based on the Bayesian analysis of the entire dataset under a single, concatenated partition. RIGHT panel represents topology derived from a Bayesian analysis of the entire dataset partitioned by locus and codon position for the two mitochondrial protein-coding genes. Mangrove specialists are highlighted in green.

Figure 3:   Phylogenetic hypothesis of relationships within the broader family Acanthizidae, highlighting the placement of *Gerygone cinerea* within the genus *Acanthiza*. Results are based on a three gene extended dataset (RAG1, RAG2, ND2) derived from the study of Gardner et al. (2010). Nodal support in form of Bayesian posterior probabilities are given at each node. Also illustrated are 95% confidence intervals around node heights as derived from the ultrametric tree generated in the

program BEAST. For overview purposes, the genus *Gerygone* is colored red, while

Acanthiza is blue, and the mangrove specialists are again indicated in green.

Figure 1:
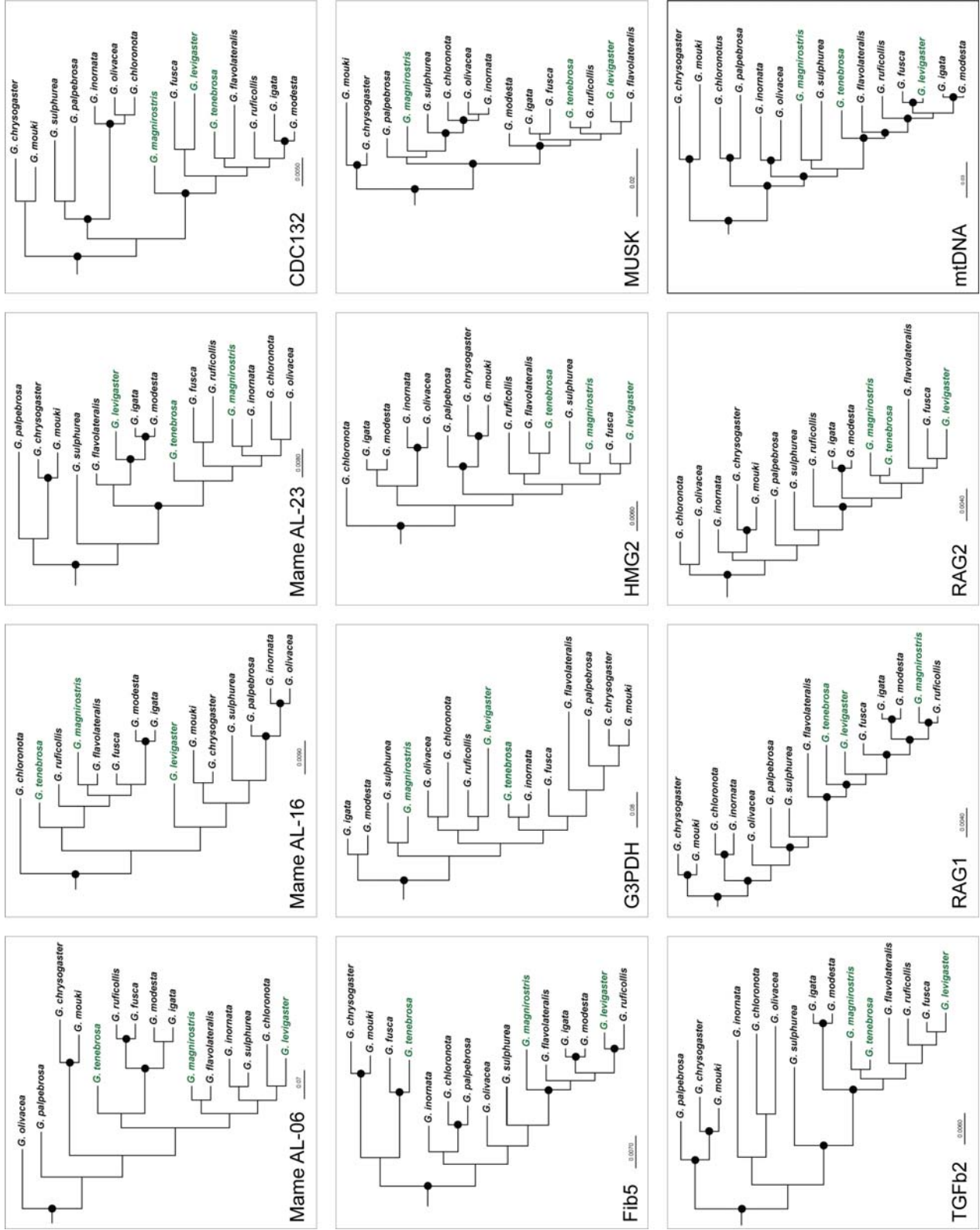
Figure 2:

Figure 3:
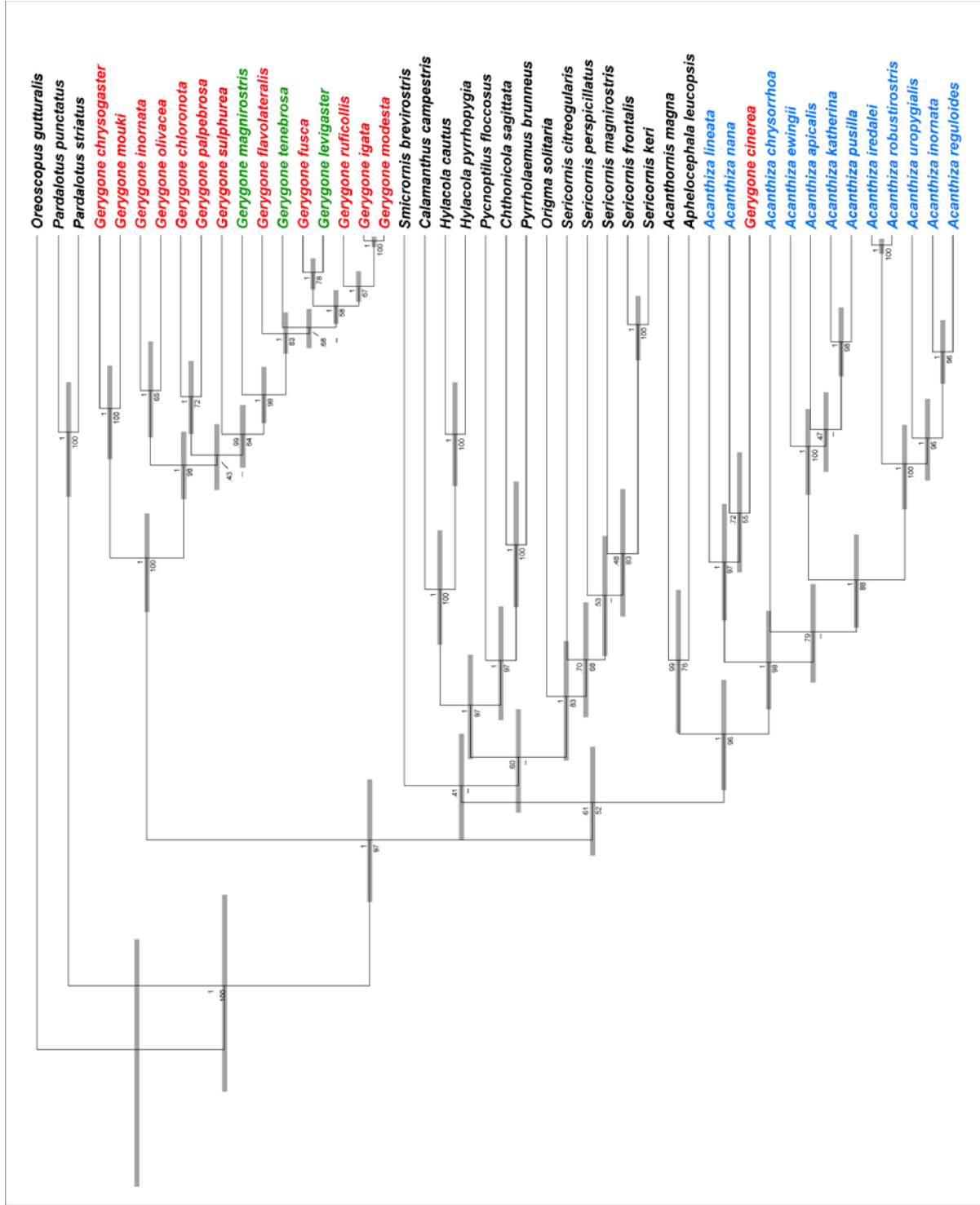
Chapter 4

Comparative phylogeography of Australo-Papuan mangrove-restricted and mangrove-associated avifaunas

Abstract

Australia and New Guinea feature the world's richest mangrove-restricted avifauna; however, the intraspecific genetic variation and the differentiation of the species involved are almost completely unknown. Here, we use sequence data derived from two mitochondrial protein-coding genes sampled to study the evolutionary history of 8 co-distributed mangrove-restricted and mangrove-associated birds from the Australian part of this region. Utilizing a comparative phylogeographic framework, we conclude that the region's mangrove forest birds present coincident phylogeographic breaks across their shared geographic distribution. Barriers such as the Canning Gap, Bonaparte Gap, and the Carpentarian Gaps all had important, but varying degrees of impact on the studied species. Statistical phylogeographic simulations were able to discern among alternative scenarios involving 6 different geographic and temporal population separations. Species exhibiting recent colonization of mangroves include *Rhipidura phasiana, Myiagra ruficollis,* and *Myzomela erythrocephala*, while *Peneoenanthe pulverulenta, Pachycephala melanura*, *P. lanioides, Zosterops luteus*, and *Colluricincla megarhyncha* all had deeper histories, reflected as more marked phylogeographic divergences.

1. Introduction

Climatic fluctuations and their associated effects on distributional changes of species have played important, but disparate, roles in shaping the present ranges of global biota. For birds, molecular phylogeographic studies have documented numerous instances of varying degrees of intraspecific genetic structuring that have been directly tied to repeated Pleistocene climatic fluctuations (Avise and Walker 1998, Holder et al. 1999, Milá et al. 2007, Peters et al. 2005, Zink 1996). During these past climatic changes, processes driving distributional shifts of taxa have depended chiefly on persistence of habitable areas that served as refugia, while other, unfavorable areas served as barriers to gene flow between populations.

1.1. *Biogeographic importance of Australo-Papuan mangrove forests*

On the Australian continent, climatic variation from the late Middle Miocene to the Pleistocene involved pronounced aridification of previously predominant subtropical rainforest cover across much of the landscape (reviews in Schodde 2006, Bowman et al. 2010). Specifically, recent studies of Australian terrestrial avifaunas (i.e. not in mangroves) indicate that birds with once widespread distributions underwent range contractions into isolated refugia around the coast and in the center of the continent. While these species have been subdivided into multiple subspecies by taxonomist, but their genetic signatures point in some cases only to a separation between western and eastern clades or single range expansions (Joseph and Wilke 2006, 2007, Toon et al. 2007, Joseph and Omland 2009). An important role in shaping the present composition and distribution of Australian avifaunas, however, is expected from the persistence of a land bridge to New Guinea (Arafura Platform) during the low-sea-level cool periods of

the Pleistocene. Several avian groups are thought to have escaped the pronounced continental aridification by tracking suitable habitats from the eastern rainforests of Australia (Tumbunan and Irian avifaunas) and the eucalypt woodlands and scrubs of the northern parts of the continent (Torresian avifaunas) via the Arafura Platform into New Guinea, where most of these taxa apparently underwent further radiation and speciation (Schodde and Calaby 1972, Schodde and Mason 1999, Schodde 2006).

A particularly interesting aspect of the climatic fluctuations, continental aridification and faunal exchange between Australia and New Guinea is the origin and evolution of a mangrove-tied component of the avifauna. Especially noteworthy are the mangrove-specialized birds of Australia, where numerous taxa confined are confined entirely to mangroves (12 species); other taxa occupy mangroves only in parts of their range (16 species); and a large part of the Australian avifauna (80-90 species) visits mangroves only to forage opportunistically (Schodde et al. 1979, Ford 1982, 1983, Simpson and Day 1999, Schodde 2006). These high numbers contrast significantly with the patterns of mangove-inhabiting birds from other parts of the world (Luther and Greenberg 2009). As such, Australia harbors the world's greatest concentration of endemic, and habitat-restricted mangrove forest birds.

Mangrove forests extend narrowly in Australia from Shark Bay in the west all the way around the northern rim east to Sydney on the east coast (Ford 1982). In the western part of the range, arid coastal climates accentuate environmental gradients between mangrove forests and adjacent scrubby vegetation, whereas in the more humid northeast, mangroves form more of a continuum with other closed-canopy vegetation types, such as tropical rainforests, monsoonal forests, and gallery forests.

These differences in vegetation composition appear to have shaped the way in which some bird species have adapted to using mangrove forests, with more mangrove-restricted taxa found exclusively in the northwestern Australian mangroves (Ford 1982). In contrast, populations from the eastern part of the range (Cape York Peninsula) may occur also in closed canopy forests abutting mangroves. Ford (1982) and Schodde et al. (1979) provided an excellent summary of the avifauna tied to varying degrees to mangroves, as well as several hypotheses on their origin, speciation and distribution within the habitat. The development of the Arafura Platform during Pleistocene glacial cycles, coupled with shifts in vegetation composition due to climate fluctuation, is proposed as key in shaping the current distribution of mangrove taxa.

1.2.   *Mangrove-inhabiting birds as model system for comparative phylogeography*

Beyond traditional morphology-based taxonomy, molecular data analyzed in a phylogenetic and population genetic framework offer promising new tools for elucidating questions related to evolution and speciation. Previous molecular studies of Australian biogeographic areas have focused mostly on taxa of the Tumbunan and Irian biogeographic region (Schodde and Calaby 1972) and more broadly in the Atherton Plateau Wet Tropics and Eastern Queensland (Cracraft 1991). These studies (James and Moritz 2000, Joseph et al. 1995, 2001, Hugall et al. 2002, Schneider et al. 1998, 1999) have documented consistent phylogeographic structuring, coinciding with past climatic (Plio-Pleistocene) vicariant speciation events. On the other hand, little or no phylogeographic differentiation has been documented in studies of a suite of more widespread Australian birds (Joseph and Wilke 2006, 2007, Toon et al. 2007).

Because of the aforementioned different extent of "mangrove-dependency" manifested by sympatrically distributed birds, these taxa are excellent candidates for testing hypotheses of biogeographic history of areas of endemism around the northern Australian rim (Figure 1). A recent study investigating the Pleistocene effects of sea-level changes on freshwater shrimp populations in Indo-Australian waters (De Bruyn and Mather 2007) have reported distinct haplotypes corresponding to discrete biogeographic areas from Western Australia, Northern Territory, and the Cape York Peninsula, pointing to persistence of natural barriers through the Pleistocene. These barriers correspond to the Canning Gap (around Eighty Mile Beach), Bonaparte Gap, Carpentarian Gaps, and the Burdekin Gap (Schodde 2006, Figure 1).

Here, we focus on the comparative phylogeography of 8 species of mangrove-endemic and mangrove-associated birds (Ford 1982, Table 1). We use sequence data derived from mitochondrial protein-coding genes to investigate underlying patterns of population genetic and phylogeographic structure. Recently developed analytical frameworks for testing alternative hypotheses within a statistical phylogeographic framework provide a model-based testing ground for discriminating among alternative population histories across suites of co-distributed taxa (Knowles and Madison 2002, Richards et al. 2007, Carstens and Richards 2007, Knowles 2009). Given the dynamic nature of the biogeography of the northern Australian mangrove forests (Schodde et al. 1979, Ford 1982, Schodde 2006, Bowman et al. 2010) and the diverse evolutionary histories of its constituent endemic avifauna, our aim is to examine effects of hypothesized historical barriers on population genetic subdivision, and to test alternative

historical scenarios of mangrove bird population processes via coalescent methods in a statistical phylogeographic framework.

2.   Materials and Methods

2.1.   *Laboratory protocols and sequence data acquisition*

Our sampling scheme focuses on 8 bird species endemic or partially endemic to coastal mangrove forests, distributed across the putative refugia of the Pilbara, Kimberley Plateau, Arnhem Land, Cape York Peninsula, and East Queensland (Figure 1, Tables 1 and 2). Genomic DNA was extracted from frozen or ethanol-preserved tissue samples from vouchered specimens collected by us and other institutions (Appendix 1) via the standard Qiagen DNeasy™ tissue extraction protocols (Qiagen, Valencia, CA). We amplified and sequenced the mitochondrial protein-coding genes NADH dehydrogase subunit 2 (ND2, 1041bp), and 3 (ND3, 351bp), using primers L5215 – H6313 (Sorenson et al. 1999) and L10755 – H11151 (Chesser 1999). All PCR amplifications were performed in 25 µl reactions using PureTaq™ RTG PCR beads (GE Healthcare Bio-Sciences Corp.). Amplified double-stranded PCR products were cleaned with ExoSAP-IT™ (GE Healthcare Bio-Sciences Corp.) and visualized on high-melt agarose gels stained with ethidium bromide. Purified PCR products were subsequently cycle-sequenced with ABI Prism BigDyeT™ v3.1 terminator chemistry using the same primers as for each PCR reaction. Cycle-sequenced products were purified further using Sephadex™ spin columns (GE Healthcare Bio-Sciences Corp.) and finally sequenced on an ABI 3130 automated sequencer. Sequences of both strands of each gene were examined and aligned in Sequencher 4.8 (GeneCodes Corp.), and complete

data matrices were assembled via Mesquite 2.74 (Maddison and Maddison 2010) for subsequent phylogeographic and population genetic analyses.

2.2.   Phylogenetic and population genetic analyses

Since both of our loci are mitochondrial protein-coding genes, they can be regarded as a single functional and genetic unit. We combined these two loci for all subsequent analyses. Mitochondrial gene trees were constructed via model-based phylogenetic algorithms under Bayesian (BA) and Maximum likelihood (ML) criteria. For each species' dataset we used ModelTest 3.7 (Posada and Crandall 1998) to determine the most appropriate model of sequence evolution via the Akaike Information Criterion (AIC).

ML heuristic tree searches were conducted using the program GARLI 1.0 (Zwickl 2008), under a single data partition and the appropriate model of sequence evolution (Table 1), with parameter values estimated from the data. Nodal support was assessed via 100 non-parametric bootstrap replicates. BA was carried out within the Markov Chain Monte Carlo (MCMC) tree search algorithm framework as implemented in the program MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Datasets were partitioned by gene and by codon position. Focusing solely on unique haplotypes for increased processing speed, we ran two independent runs of 10^7 generations, using the previously inferred model of sequence evolution. Search parameters included unlinking of all partition-specific rates, adjustment of chain heating conditions for improved chain swap acceptance rates, and sampling every 100 generations. Evaluation of stationarity and chain convergence was conducted by plotting posterior probabilities from the two runs in the program Tracer (Rambaut and Drummond 2007). The resulting pool of

topologies sampled from the first 30% of generations was discarded as an initial 'burn-in', such that 70,000 trees were summarized to produce a single 50% majority-rule consensus tree. Outgroup choices for each species were selected according to the most recent molecular phylogenetic studies of familial relationships (Appendix 1).

Intraspecific haplotype networks were reconstructed using TCS 1.18 (Clement et al. 2000) via parsimony using a 95% connection limit. DNAsp 5 (Librado and Rozas, 2009) was used to calculate number of polymorphic sites (S), number of haplotypes (H), haplotype (h) and nucleotide diversity (π), and net divergence (Da). We also tested whether constituent populations of each species have undergone demographic expansion by calculating Fu's Fs (Fu 1997), Tajima's D (Tajima 1989), and R_2 (Ramos-Onsin and Rozas 2002). All calculations were performed on geographic populations identified through haplotype network and phylogenetic analyses. Analysis of molecular variance (AMOVA) was performed via Arlequin 3.5 (Excoffier and Lischer 2010) for each species to test levels of genetic variation between and within intraspecific phylogroups. Statistical significance was evaluated based on 10,000 nonparametric permutations.

2.3.   Statistical phylogeography

Based on the regional biogeographic history and areas of endemism (Figure 1), we chose to test 3 different phylogeographic scenarios involving various degrees of population differentiation. Phylogeographic population structure could have been attained under at least 3 tractable scenarios: one involving simultaneous fragmentation of a continuously distributed population along a single barrier (Figure 2A), early divergence across a single barrier (Bonaparte Gap) followed by subsequent divergence

of Pilbara-Kimberley and Arnhem-Gulf of Carpentaria populations (Figure 2B), and lastly, according to a sequential colonization of current areas of endemism via isolation by distance from east to west (Figure 2C). As relative time frames of splitting events, we used a latest divergence time of 12,000 years based on studies of the duration of Last Glacial Maximum land bridge connectivity in the Gulf of Carpentaria and Joseph Bonaparte Gulf (Yokoyama et al. 2001). Older splitting events within the mid-Pleistocene (700,000 years before present) and early Pleistocene (1.5 million years before present) were also included in the alternative hypotheses testing scenarios (Figure 2). As such, through a combination of 3 geographic topologies and an additional 3 time-frame variants, 6 alternative scenarios in all were tested for the 8 mangrove birds (Figure 2 – A1, A2, A3, B1, B2, C).

We used Migrate 3.2 to estimate effective population size N_e for each species from $\theta = 2N_e\mu$ using coalescence simulations (Beerli 2006). Mesquite 2.74 (Maddison and Maddison 2010) was used to generate phylogeographic topologies in the form of population trees and to simulate 500 gene trees under the coalescent process onto these topologies for each taxon. Timing of phylogeographic splitting events was defined as number of generations along branch lengths. We then simulated a DNA data matrix of equivalent number of base pairs, number of individuals, and under the same inferred model of sequence evolution as our observed dataset (Table 1). Besides accurately parameterizing each simulated DNA matrix, we also verified that maximum intraspecific sequence divergence of simulated DNA matrices was similar to our observed dataset by adjusting the scaling factor for character model specifications (Madison and Madison 2010). The resulting 500 data matrices of each of the six alternative phylogeographic

hypotheses (Figure 2) were used to derive a new set of phylogenetic trees to serve as a null distribution against which our observed datasets were tested.

Slatkin and Maddison's (1989) *S* statistic, which measures discord between a gene tree and constituent population tree subdivisions, was used to assess significance of each hypothesis. We also measured the amount of discord (i.e. number of deep coalescents, nDC) between the simulated datasets and the population topology, and compared the distribution of this value to our observed dataset (Knowles and Madison 2002, Richards et al. 2007, Carstens and Richards 2007, Knowles 2009), rejecting phylogeographic scenarios if our observed values were outside the 95% confidence interval.

3   Results

3.1. *Genetic diversity and structure*

A total of 315 samples from 8 species of Australo-Papuan mangrove-restricted birds (Appendix 1) were sequenced for the two mitochondrial protein-coding gene fragments, ND2 and ND3. Both genes were combined to form a concatenated dataset of total sequence length varying from 1251 to 1392 base pairs (1041 bp ND2, 351 bp ND3, Table 1). Alignment of sequences was straightforward, with only few samples in which ambiguous base-calls towards the ends of the ND2 gene had to be excised. Base frequencies were concordant with previously reported studies of avian mtDNA. Moreover, all sequences translated into amino acids without the presence of aberrant stop codons or double peaks in chromatograms, thus indicating true mitochondrial origin as opposed to nuclear pseudogenes, or numts (Sorenson and Fleischer 1996,

Sorenson and Quinn 1998). As expected, sequence variation and substitution models within the concatenated dataset were species-dependent, where *Zosterops luteus* and *Colluricincla megarhyncha* had highest numbers of variable sites, while *Peneoenanthe pulverulenta* and *C. megarhyncha* showed the highest percentage of informative sites. On the other hand, *Rhipidura phasiana* and *Myiagra ruficollis* sequences had lowest numbers of informative sites.

A summary of genetic diversity indices is given in Table 2, and Figure 3 illustrates intraspecific haplotype networks based on a 95% parsimony connectivity limit. All haplotype networks showed strong geographic structure, including unique haplotypes from 5 different biogeographic regions. However, two species (*Rhipidura phasiana* and *Zosterops luteus*), contained shared haplotypes involving singletons from the Kimberley region together with samples from the Pilbara and Arnhem regions. Four species had two or more haplotype groups that could not be connected under the 95% limit. Haplotype diversity (*h*) ranged from 0.46 in the Kimberley populations of *Pachycephala lanioides* to the > 0.90 for most other species, and even reaching 1.00 for our New Guinea populations of *Colluricincla megarhyncha* (Table 2). Nucleotide diversity (*π*), on the other hand, was generally lower, from 0.04% in *P. lanioides*, reaching upper values of 0.6%, and even a maximum of 5.7%, again for the New Guinean samples of *C. megarhyncha*. On average, populations of *P. lanioides* had lowest values of *h* and *π*, while *C. megarhyncha* showed the highest values of the two indices. All populations of *P. pulverulenta* showed high levels of *h*, while *π* values for corresponding populations showed proportionally lower values. A similar trend was observed in *Z. luteus*. From a geographic point of view, samples from western regions

(Pilbara, Kimberley, and Arnhem) generally had lower h and π values compared to populations from the Gulf of Carpentaria and East Queensland.

Estimates of population size changes and selection generally indicated non-neutrality. *Peneoenanthe pulverulenta* and *Zosterops luteus* had 3 populations with significant Fu's Fs values, while *Pachycephala melanura, Myzomela erythrocephala* and *Myiagra ruficollis* each had one population with significant Fs values. Values of Tajima's D were mostly negative and non-significant, except for *Z. luteus*, for which populations of Pilbara and East Queensland were significant at the $p < 0.05$ level. In contrast, calculations of Ramos-Onsin and Roza's R_2 were all nonsignificant, however, *Z. luteus* and *C. megarhyncha* each had populations with low R_2 values, suggesting population expansion. Results from the analysis of molecular variance (AMOVA) showed a significant contribution of variation among populations for 5 mangrove birds (*P. pulverulenta, P. lanioides, P. melanura, Z. luteus* and *C. megarhyncha*), while 3 species (*R. phasiana, M. erythrocephala* and *M. ruficollis*) had their intraspecific variation distributed more equitatively among and within populations (Table 3).

3.2. *Phylogenetic analysis*

Intraspecific phylogenetic analyses were conducted on the concatenated mtDNA dataset using the estimated model of sequence evolution for each species (Table 1). Results of gene trees are presented alongside halpotype networks in Figure 3. Previous studies demonstrated paraphyly between East Queensland populations of *Zosterops luteus* and eastern populations of *Z. lateralis* (Degnan 1993, Degnan and Moritz 1993, Moyle et al. 2009). As a result, we included 29 additional samples from populations of *Z. lateralis* alongside the mangrove-endemic *Z. luteus* (Appendix 1). Phylogenetic

resolution varied among the 8 species, mirroring findings derived from the intraspecific haplotype networks. As such, for each species, 2 – 4 distinct geographic phylogroups were identified (Figure 3, Table 2). Nodal support was higher at the clade level and generally lacking statistical support within individual phylogroups. Topologies inferred for *R. phasiana*, *M. erythrocephala* and *M. ruficollis* showed the least support for individual clades, while the remaining species, especially *Z. luteus* and *P. melanura*, had very well-supported nodes throughout.

Net sequence divergence between individual clades (*Da*) differed again among species; least differentiation was documented in the case of the two populations of *R. phasiana* and *M. ruficollis*, and also between the Pilbara and Kimberley populations of *P. lanioides* (Table 2). Alternatively, highest sequence divergence values were observed within *P. pulverulenta*, *Z. luteus,* and *C. megarhyncha* (at the Arnhem – Gulf of Carpentaria barrier; Table 2, Figure 1).

3.3. *Statistical phylogeography*

Analyses of alternative phylogeographical hypotheses were summarized according to 3 geographic and 3 additional temporal scenarios (Figure 2). Results based on the number of deep coalescents (*nDC*) and Slatkin's *S* derived from simulated datasets under each scenario indicate that simultaneous divergence of populations at the end of the Last Glacial Maximum could not be rejected in *Rhipidura phasiana, Pachycephala lanioides, Myzomela erythrocephala, Myiagra ruficollis,* and *Zosterops luteus* (Table 4). Simultaneous divergence was rejected unilaterally as a historical scenario in *Pachycephala melanura*, while *S* values for *Peneoenanthe pulverulenta* could not reject simultaneous divergence at older time frames of the early Pleistocene

(Table 4). Scenarios involving the Bonaparte Gap as an important geographic break at the mid to early Pleistocene could generally not be rejected, as each species had at least one of the test statistics return non-significant values (Table 4). The hypothesis of sequential divergence from east to west also received mixed results, as we could only significantly reject this scenario based on nDC, and not Slatkin's S, for *R. phasiana, P. lanioides, M. erythrocephala,* and *M. ruficollis*, while *C. megarhyncha* was the only species for which solely a scenario of sequential population divergence was found plausible (Figure 2, Table 4).

4.   Discussion

The present study constitutes the first analysis of molecular variation among Australia's rich mangrove endemic birds (Schodde et al. 1979, Ford 1982, 1983, Schodde 2006). Using two protein-coding mitochondrial genes in a comparative phylogegeographic framework, we were able to illustrate commonalities and differences among 8 co-distributed birds tied to mangrove forests (Figure 1). Overall, the 8 species showed similar major population subdivisions within Australia's coastal mangroves (Figure 3). We elaborate on the observed patterns for each species below.

4.1.   Phylogeographic patterns

Rhipidura phasiana – This species diverged recently as part of a rich and recent Pacific radiation of fantails (Rhipiduridae), and is the current sister lineage to a clade comprised of the mainland Australian form of *R. albiscapa* and the New Zealand fantail *R. fuliginosa* (Nyári et al. 2009). Phylogeographic analysis uncovered a shallow topology, corresponding to at least two distinct major lineages separated only by very

few base changes between populations (Figure 3). Remarkable is the shallow but almost completely sorted mitochondrial population tree (Joseph and Omland 2009), with only one haplotype shared between the Pilbara and Kimberley regions. Low genetic diversity indices (π and h) and negative values of Tajima's D and Fu's Fs (but non-significant at the 95% level) point towards a recently expanding population. This result was also evident in our statistical phylogeographic analysis, wherein we could not reject a scenario of recent simultaneous divergence at the end of the Pleistocene (Table 4, Figure 2). It is also noteworthy that this species does not include multiple plumage-based subspecies (Ford 1982, Schodde and Mason 1999, Simpson and Day 1999, Christidis and Boles 2008f). From the phylogeographic patterns observed in this taxon, we believe that, given sufficient time and cessation of gene flow among the 4 populations, quantifiable morphological differences will eventually result.

Peneoenanthe pulverulenta – Part of the Australo-Papuan robin (Petroicidae) radiation, this taxon has been long labeled as of uncertain taxonomic affinities, even in light of modern multilocus phylogenetic analyses (Loynes et al. 2009). It is clearly part of an older lineage, exhibiting deep intraspecific divisions. Our phylogeographic analysis detected 4 distinct subpopulations separated by divisions corresponding to the Gulf of Carpentaria and the Bonaparte Gap (Figure 1 and 3). Levels of genetic diversity were moderate, while values of Tajima's D and Fu's Fs were negative for all 3 populations east of the Kimberley, reaching their highest significance on the East Queensland coast (Table 2). These patterns point toward sequential eastward expansion of populations, an idea corroborated by our simulations, wherein we could not reject hypotheses of sequential population differentiation (Table 4). Based on Slatkin's S, however,

simultaneous divergence of populations in the mid-to-late Pleistocene could also have been possible. Three subspecies on the coastlines of Australia are recognized (Ford 1982, Schodde and Mason 1999, Simpson and Day 1999, Christidis and Boles 2008), all differing in morphology and vocalizations. Given reciprocally monophyletic lineages and marked sequence divergences, populations from Pilbara and Kimberley, Arnhem, and east of the Gulf of Carpentaria could each be recognized as distinct species. The herein-unsampled populations of New Guinea would provide additional insights to the geographic origins of this enigmatic robin.

Pachycephala lanioides – Sister to the continental Rufous Whistler (*P. rufiventris*), this mangrove endemic whistler (Pachycephalidae) is also part of a diverse lineage of Australo-Papuan birds (Jønsson et al. 2010). Haplotype networks for this Australian endemic species showed completely sorted and reciprocally monophyletic geographic lineages, corresponding to 3 populations. All three exhibited negative (but not-significant) values of Tajima's *D* and Fu's *Fs*, indicative of population expansion (Figure 3, Table 2). A recent population subdivision was also supported by our statistical phylogeographic simulations, which could not reject a hypothesis of a late-Pleistocene simultaneous population divergence (Table 4). The Bonaparte Gap also proved to be an important barrier for this species as an alternative explanation of observed phylogeographic patterns. Similar to *P. pulverulenta*, 3 subspecies with slight morphological trait variations are recognized (Ford 1982, Schodde and Mason 1999, Simpson and Day 1999, Christidis and Boles 2008). Although sequence divergence between populations was relatively low, as with *R. phasiana*, current monophyletic lineages could well be interpreted as following distinct evolutionary trajectories.

Pachycephala melanura – Another mangrove-restricted member of the whistler radiation, this species is part of the hyper-variable *P. pectoralis* species complex that spans the entirety of the Australo-Papuan and Oceania regions (Galbraith 1956, Mayr and Diamond 2001, Jønsson et al. 2008). This species exhibits marked phylogeographic subdivision, with at least 4 distinct populations (Figure 3, Table 2). With the exception of East Coast populations, all other subclades had moderate genetic diversity indices and negative values of neutrality tests (Table 2). East Coast populations are regarded as outliers in this analysis, as they are linked phylogenetically instead to *P. m. dahli* populations from the Bismark Archipelago (Nyári, *pers. obs.*). Our topological test scenarios were able to reject unequivocally a simultaneous divergence model in favor of older, sequential population separations, influenced again by the Bonaparte Gap, which was responsible also for the largest sequence divergence within this taxon (Table 4). More work including multilocus datasets covering the entire geographic extent of the *P. pectoralis*/*P. melanura* complex is necessary to elucidate their elaborate historical biogeography.

Myzomela erythrocephala – This species lies at the base of the diverse honeyeater (Meliphagidae) radiation, exhibiting the largest geographic extent of any honeyeater genus (Driskell and Christidis 2004, Gardner et al. 2010). Similar to *R. phasiana*, this honeyeater features shallow intraspecific divergences, suggestive of recent splits (Joseph and Omland 2009). Two major population subdivisions were recognized, focused around the Gulf of Carpentaria (Figure 3). Populations east of the Gulf had moderate to high genetic diversity indices, although populations from Arnhem and the Kimberley had negative but nonsignificant values of Tajima's *D* and Fu's *Fs*, a

signature of recent population expansion (Table 2). Based on our alternative phylogeographic test settings, we were unable to reject a simultaneous recent divergence (Table 4). Insufficient statistical power led to inability to discern among a predominant role of the Bonaparte Gap and a sequential divergence.

Myiagra ruficollis – As a member of the Australasian monarch flycatchers (Monarchidae), this species is part of a larger complex that has radiated into Pacific Islands from mostly continental sources (Filardi and Moyle 2006). On the Australian continent, one subspecies, *M. r. mimikae* extends from the Kimberley east through the Gulf of Carpentaria all the way to the East Coast (Figure 1). Our phylogeographic analysis identified a very shallow network of haplotypes, as in *M. erythrocephala*, grouped in two mail geographic areas (Figure 3). Featuring low sequence divergence, low genetic diversity indices and negative values of Tajima's *D* and Fu's *Fs*, this species has most likely witnessed recent population expansions (Table 2). The historical scenario most favored by our simulations was one of recent simultaneous divergence, although as was the case of *M. erythrocephala*, we believe that statistical power was insufficient to distinguish between temporal effects of the Bonaparte Gap and sequential divergence of populations (Table 4).

Zosterops luteus – One of the most intriguing constituent species of mangrove dependent endemics, *Z. luteus* evolved within an unprecedentedly rapid white-eye radiation (Zosteropidae), which spans the entire Old World Tropics, reaching numerous archipelagos of the Atlantic, Indian, and Pacific Ocean within the last 2 million years (Moyle et al. 2009). The mitochondrial paraphyly between East Queensland populations of *Z. luteus* and eastern populations of *Z. lateralis* demonstrated by previous studies

(Degnan 1993, Degnan and Moritz 1993) led us to include broader sampling of *Z. lateralis* in our phylogeographic analysis. Indeed, we confirmed the previous findings of incomplete mitochondrial lineage sorting between the two species, with one sample of *Z. luteus* having the exact same haplotype as several *Z. lateralis* individuals (Figure 3). This pattern is most likely attributed to recent hybridization events of the isolated *Z. luteus* populations on Australia's eastern shore (Figure 1 and 3). Nevertheless, a preliminary analysis of the entire dataset with the addition of a nuclear intron (TGFb2) was unable to confirm reciprocal monophyly of the two species, as previously reported based on RFLP analysis (Degnan 1993). This result was expected given the rapid radiation of the group, where the nuclear genome would still lack complete sorting (Zink and Barrowclough 2008, Joseph and Omland 2009). Our analysis identified 4 main populations featuring moderate genetic diversity and negative values of Tajima's *D* and Fu's *Fs*, suggestive of recent expansion. Sequence divergence between populations on either side of the Gulf of Carpentaria exceeded 4% (Table 2) – equivalent to almost half of the sequence divergence observed in the entire *Zosterops* radiation (Clade B of Moyle et al. 2009). Statistical phylogeographic simulation results based on values of nDC could not distinguish well between alternative scenarios, however, values of Slatkin's *S* rejected all scenarios except for that of sequential divergence, in which case both estimators were in agreement (Table 4, Figure 2). Considering the complex history of this group of birds (Clegg et al. 2002, Moyle et al. 2009), more detailed analyses featuring the entire radiation and the use of multilocus or genomic datasets are warranted (Edwards et al. 2005, Edwards 2007, Lerner and Fleischer 2010).

Colluricincla megarhyncha – Another member of the extended whistler family (Pachycephalidae), this species has seen extensive radiation in the Australo-Papuan region, with over 31 recognized subspecies (Schodde and Mason 1975, Ford 1978, Schodde and Mason 1999). On the Australian continent, it occupies diverse closed-canopy habitats, though is restricted to mangroves only around the western edge of its distribution in Arnhem and around the Gulf of Carpentaria (Ford 1982, Schodde and Mason 1999, Simpson and Day 1999, Christidis and Boles 2008). It is therefore the only taxon in our analysis that utilizes mangroves only in part of its range. New Guinea populations included in the present study were intended to sergve as a geographic outgroup for the mangrove-restricted subspecies *C. m. parvula* and *C. m. aelptes* (Schodde and Mason 1975, Ford 1978, Schodde and Mason 1999). Phylogeographic analysis recovered deep lineage splits, where New Guinea populations were basal in the topology, sister to the mangrove-restricted populations of *C. m. parvula* and *C. m. aelptes*, while the remaining populations were distributed along the Gulf of Carpentaria and Australia's east coast (Figure 1 and 3). Deep divergences were observed between samples from New Guinea and Arnhem (5.7%), and between Arnhem and Gulf of Carpentaria (6.7%; Table 2), although individual lineages shared haplotypes between regions (Figure 3). Based on the statistical phylogeographic analysis, we were able to reject all hypotheses except the sequential divergence scenario (Table 4). Our analysis reveals the distinctiveness of the Arnhem population *C. m. parvula*, while *C. m. aelptes* samples fall within a larger clade of populations from the Gulf of Carpentaria and Cape York, attributed to *C. m. normani* (Schodde and Mason 1975, Ford 1978, Schodde and Mason 1999).

4.2.   Geographic barriers across coastal mangrove forests

Comparing phylogeographic patterns across our 8 mangrove-restricted and mangrove-associated birds revealed several important parallels of geographic barriers of coastal mangroves and habitats further inland (Ford 1982, Cracraft 1991, Schodde 2006, Bowman et al. 2010). At their western-most extent, around Shark Bay, mangrove forests are depauperate, consisting of only a single tree species. From there, tree species diversity increases eastwards: 5 in Pilbara, 17 in Kimberley, and 22 in the Northern Territory, to a high of 28 species along the eastern coast of the Cape York Peninsula, after which it decreases along the Gulf of Carpentaria to 13 in central Queensland, and to 3-7 species in New South Wales (Semeniuk et al. 1978, Ford 1982, Ricklefs and Latham 1993, Ellison et al. 1999). In contrast, numbers of mangrove-restricted birds are highest in western and northern parts of Australia, decreasing significantly towards the east, reaching lowest numbers of endemic species along the East Coast (Ford 1982, Schodde et al. 1979).

In the westernmost coastal barrier, the Canning Gap around Eighty Mile Beach, mangroves are completely absent, providing an extensive arid barrier to gene flow between the Pilbara and Kimberley regions. *R. phasiana*, *P. pulverulenta, P.lanioides, P. melanura,* and *Z. luteus* all have populations on either side of the barrier, although only *P. pulverulenta, P. lanioides* and *P. melanura* have reciprocally monophyletic lineages on either side of the Canning Gap (Figure 1 and 3). In the case of *R. phasiana*, only one haplotype from the Kimberley groupes with the Pilbara clade, whereas *Z. luteus* showed increased gene flow across the barrier (Figure 3). Therefore, the present

study is among the few that have examined the biogeographic implications of the Canning Gap (Bowman et al.2010).

The Bonaparte Gap separates the regions of the Kimberley Plateau and Arnhem (Figure 1), and is considered an important biogeographic barrier, given the exposure of the Sahul Shelf and the formation of lacustrine environments in the Joseph Bonaparte Depression during low sea levels (Yokoyama et al. 2001), extending further inland with the Ord Arid Intrusion. All 8 species havd genetically distinct populations isolated by the Bonaparte Gap, a few showing moderate levels of genetic divergence across this barrier (*P. pulverulenta, P. melanura*; Table 2). These patterns have been documented in a diverse array of organisms (reviewed in Bowman et al. 2010), most notable for birds being the multilocus study of Jennings and Edwards (2005) describing timing of population divergence across the Bonaparte Gap in *Poephila* grass finches to have occurred 300,000 years ago. Our suite of mangrove-associated taxa suggests important contributions of the Bonaparte Gap in population subdivision, although estimates of divergence timing were found to be earlier, in the middle to early Pleistocene (Table 4).

Northern Australia's biogeographic history was influenced predominantly by the processes surrounding the Gulf of Carpentaria, especially during the Pleistocene sea level fluctuations that have led to the exposure of the Arafura Platform, thus connecting Australia and New Guinea facilitating a rich faunistic exchange (Schodde 2006). During the presence of this land bridge, the newly formed Lake Carpentaria was surrounded by low-laying plains, and an extensive marshy environment, as well as more widespread mangrove forest cover (Chivas et al. 2001, Yohoyama et al. 2001). Examples of population divergences around the Carpentarian Gap have been reviewed by Bowman

et al. (2010). Our mangrove-based system appropriately expands the pool of organisms influenced by this geographic barrier, since all 8 mangrove-bound birds exhibited marked population subdivisions around the Gulf of Carpentaria (Figure 3). With the exception of *P. lanioides, M. erythrocephala* and *C. megarhyncha*, all taxa had unique haplotypes on either side of the barrier. Sequence divergences across the Gulf ranged from 0.2% in *R. phasiana* and *M. ruficollis*, to 4.11% in *Z. luteus* and 6.78% in *C. megarhyncha* (Table 2).

Only few of our mangrove species had populations reaching Australia's east coast, making it difficult to evaluate the influence of the Burdeking Gap on mangrove inhabiting birds. Since the East Coast features less mangrove-restricted species (Schodde et al. 1979, Ford 1982, Schodde 2006), this barrier is likely to have acted as a minor influence on shaping the overall biogeography of these birds. It is noteworthy, however, that *Z. luteus* and *P. melanura* both have well-differentiated isolated populations along the east coast. Populations of *P. pulverulenta* showed only minor demarcation across the Burdeking Gap, being divided instead between the eastern and western sides of the Cape York Peninsula. While not confined to mangroves along the east coast, *C. megarhyncha* nevertheless had distinct populations across this barrier, albeit with signs of past gene flow (Figure 3).

4.3. Geographic origin of Australian mangrove birds

Our phylogeographic analysis suggests an important role of the Arhem Land as basal geographic region for *P. pulverulenta*, *Z. luteus* and *C. megarhyncha*. However, in *P. melanura*, populations from the Kimberley and Pilbara were recovered as basal (Figure 3). All other species had low support for the branching sequences owing to

smaller sampling size and low intraspecific variation. The role of the Arnhem region as a source of mangrove birds can be explained by its implication in the Arafura Platform during low sea levels of the Pleistocene (Yokoyama et al. 2001), where taxa could have spread through mangroves along the western shorelines of the landbridge and finally become separated by recurring sea level rise. This historical pattern is supported also by the fact that with the exception of *P. lanioides* and *Z. luteus*, all other Australian mangrove birds also have isolated populations along the southern coast of New Guinea (Ford 1982). A scenario of direction of evolution between Australia and New Guinea remains to be investigated with additional sampling.

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Table 1: Taxon sampling, sequence attributes, and substitution models for 8 mangrove-restricted and -associated passerines included in the comparative phylogeographic analysis.

Taxon	Common name	Family	n	total bp aligned	Substitution model	A, C, G, T frequency	Variable sites (% of total)	Informative sites (% of total / % of variable)
Rhipidura phasiana	Mangrove Fantail	Rhipiduridae	21	1357	TrN	0.29, 0.31, 0.12, 0.28	18 (1.32)	9 (0.66 / 50.00)
Peneoenanthe pulverulenta	Mangrove Robin	Petroicidae	41	1392	GTR+I	0.31, 0.35, 0.11, 0.23	84 (6.03)	72 (5.17 / 85.72)
Pachycephala lanioides	White-breasted Whistler	Pachycephalidae	37	1275	HKY	0.33, 0.27, 0.10, 0.30	35 (2.74)	22 (1.72 / 62.85)
Pachycephala melanura	Mangrove Golden Whistler	Pachycephalidae	44	1392	TrN+I	0.33, 0.27, 0.11, 0.29	65 (4.67)	48 (3.45 / 73.84)
Myzomela erythrocephala	Red-headed Honeyeater	Meliphagidae	31	1392	TrN+I	0.27, 0.36, 0.13, 0.24	40 (2.87)	26 (1.87 / 65.00)
Myiagra ruficollis	Broad-billed Flycatcher	Monarchidae	21	1331	HKY+I	0.29, 0.31, 0.14, 0.26	14 (1.05)	7 (0.52 / 50.00)
Zosterops luteus	Yellow White-eye	Zosteropidae	54	1392	TrN+I	0.32, 0.34, 0.10, 0.24	151 (10.85)	114 (8.20 / 75.50)
Colluricincla megarhyncha	Little Shrike-thrush	Pachycephalidae	66	1251	TrN+G	0.31, 0.31, 0.11, 0.27	247 (19.74)	220 (17.58 / 89.06)

Table 2: Taxon sampling, number of individuals and genetic diversity within 8 species of mangrove-endemic and -associated birds. Number of samples for each population corresponds to Figure 1 and to results from the haplotype network and phylogeographic analysis (Figure 3).

Taxon	Population and sample region[1]	N	D_a (%)[3]	S	H / h	π (%)	Tajima's D	Fu's Fs	Ramos-Onsin and Rozas' R_2
Rhipidura phasiana	Pilbara & Kimberley	15		9	6 / 0.64	0.13	-1.06 (-1.73, 1.79)	-0.84 (-3.86, 3.82)	0.18 (0.09, 0.22)
	Arnhem & Gulf of Carpentaria	6	0.22	7	4 / 0.86	0.23	0.12 (-1.40, 1.76)	0.31 (-3.45, 4.62)	0.18 (0.12, 0.32)
Peneoenanthe pulverulenta	Pilbara & Kimberley	5		7	4 / 0.90	0.25	0.49 (-1.16, 1.74)	-0.03 (-2.37, 4.36)	0.20 (0.12, 0.40)
	Arnhem	14	1.07	14	10 / 0.94	0.26	-0.63 (-1.77, 1.72)	-3.65 (-4.34, 4.85)*	0.10 (0.09, 0.22)
	Gulf of Carpentaria	10	(3.07)	6	6 / 0.77	0.11	-0.97 (-1.79, 1.75)	-2.40 (-3.29, 3.23)*	0.13 (0.11, 0.25)
	East Queensland	12	1.51	11	10 / 0.97	0.21	-0.71 (-1.75, 1.70)	-5.82 (-4.06, 3.98)**	0.10 (0.09, 0.23)
Pachycephala lanioides	Pilbara	12		2	3 / 0.53	0.04	-0.38 (-1.45, 1.75)	-0.32 (-1.32, 2.53)	0.17 (0.15, 0.27)
	Kimberley	8	0.31	3	3 / 0.46	0.06	-1.44 (-1.44, 1.72)	-0.30 (-1.83, 2.98)	0.23 (0.16, 0.33)
	Arnhem & Gulf of Carpentaria	17	0.63	16	7 / 0.71	0.24	-1.35 (-1.75, 1.72)	-0.30 (-4.69, 4.88)	0.10 (0.08, 0.21)
Pachycephala melanura	Pilbara & Kimberley	8		6	6 / 0.92	0.14	-0.70 (-1.63, 1.81)	-2.67 (-2.93, 4.28)*	0.14 (0.12, 0.27)
	Arnhem	15	1.68	6	5 / 0.56	0.10	-0.68 (-1.76, 1.81)	-0.53 (-3.17, 3.86)	0.12 (0.09, 0.23)
	Gulf of Carpentaria	17	(0.88)	16	8 / 0.78	0.24	-1.10 (-1.72, 1.78)	-0.92 (-4.74, 4.93)	0.09 (0.08, 0.20)
	East Queensland	4	0.62	18	2 / 0.50	0.64	-0.85 (-0.85, 2.09)	5.38 (-1.32, 4.41)	0.43 (0.09, 0.40)
Myzomela erythrocephala	Arnhem & Kimberley	11		14	9 / 0.96	0.29	-0.61 (-1.79, 1.69)	-3.44 (-3.89, 4.39)*	0.10 (0.09, 0.23)
	Gulf of Carpentaria & Cape York	20	0.60	29	11 / 0.85	0.75	1.04 (-1.74, 1.71)	0.77 (-5.10, 5.25)	0.16 (0.07, 0.19)
Myiagra ruficollis	Arnhem & Kimberley	11		8	4 / 0.63	0.14	-1.16 (-1.71, 1.75)	0.72 (-3.6, 4.14)	0.15 (0.10, 0.25)
	Gulf of Carpentaria & Cape York	10	0.21	6	6 / 0.77	0.10	-1.49 (-1.79, 1.75)	-2.92 (-2.29, 3.34)**	0.11 (0.11, 0.26)
Zosterops luteus	Pilbara & Kimberley	21		29	13 / 0.91	0.29	-1.90 (-1.74, 1.73)*	-4.49 (-5.24, 5.53)*	0.07 (0.07, 0.19)
	Arnhem	9	0.61	4	5 / 0.72	0.08	-0.68 (-1.60, 1.91)	-1.99 (-2.36, 3.78)*	0.13 (0.12, 0.27)
	Gulf of Carpentaria & Cape York	22	(4.11)	13	12 / 0.87	0.15	-1.45 (-1.71, 1.78)	-6.81 (-4.72, 4.48)**	0.07 (0.07, 0.20)
	East Queensland[2]	3 (+15[2])	1.66	63	11 / 0.88	0.41	-2.43 (-1.72, 1.71)*	-0.76 (-4.68, 5.29)	0.17 (0.08, 0.19)
Colluricincla megarhyncha	New Guinea	7		15	7 / 1.00	5.70	0.62 (-1.51, 1.55)	1.10 (0.66, 5.41)	0.20 (0.10, 0.26)
	Arnhem	13	5.79	9	7 / 0.84	0.16	-1.22 (-1.74, 1.77)	-2.29 (-3.69, 4.21)*	0.09 (0.09, 0.23)
	Gulf of Carpentaria & Cape York	22	(6.78)	34	13 / 0.87	0.69	-0.36 (-1.70, 1.72)	-0.87 (-5.47, 5.51)	0.11 (0.07, 0.19)
	East Queensland	24	1.24	49	18 / 0.96	0.68	-1.34 (-1.71, 1.74)	-5.20 (-5.64, 5.82)*	0.07 (0.07, 0.18)

1   Populations are ordered from west (Pilbara) to east (East Queensland). See Figure 1 for details.
2   East Queensland populations of *Z. luteus* are paraphyletic with eastern populations of *Z. lateralis*, and have been here analyzed together
3   Estimates of genetic divergence are between geographically adjacent pairs of populations. For species with 4 distinct populations, an additional estimate between two adjacent
    population-pairs is given in brackets.
*   Statistically significant at $p < 0.05$
** Statistically significant at $p < 0.01$

Table 3:   Results of the AMOVA performed among and within populations. Populations are defined according to

Figure 1, Table 2, and results from the haplotype network and phylogeographic analysis (Figure 3).

Taxon	Sum of squares		Variance components		Percent of variation		Fixation index
	among	within	among	within	among	within	
Rhipidura phasiana	14.58	21.70	1.57	1.14	57.86	42.14	0.57
Peneoenanthe pulverulenta	476.60	55.22	15.92	1.49	91.43	8.57	0.91
Pachycephala lanioides	151.35	30.38	6.34	0.89	87.66	12.34	0.87
Pachycephala melanura	246.28	57.83	7.93	1.44	84.58	15.42	0.84
Myzomela erythrocephala	63.27	119.04	4.17	4.10	50.39	49.61	0.50
Myiagra ruficollis	15.70	15.92	1.42	0.84	62.87	37.13	0.62
Zosterops luteus	1179.65	95.46	23.04	1.46	94.09	5.91	0.94
Colluricincla megarhyncha	1406.10	416.13	29.72	6.71	81.58	18.42	0.81

Table 4:   Results of the statistical phylogeographic analysis performed under 6 alternative phylogeographic scenarios. For each species, estimates of θ_{Total} and N_e are given alongside observed values of Slatkin and Madison's S, and the number of deep coalescents (nDC). Tested phylogeographic scenarios (A, B, C) and temporal divergence within these topologies correspond to the schematic diagrams in Figure 2 (A1, A2, A3, B1, B2, C).

Taxon	θ_{Total}	N_e	Observed (S / nDC)	Simultaneous divergence (A)			Bonaparte Gap divergence (B)		Sequential
				12k ybp (1)	700k ybp (2)	1.5mill ybp (3)	700k ybp (1)	1.5mill ybp (2)	divergence (C)
Rhipidura phasiana	0.0065	16250	4 / 5	** / ns	** / **	** / **	ns / ns	ns / **	ns / **
Peneoenanthe pulverulenta	0.0202	50500	4 / 5	** / **	ns / **	ns / **	** / ns	** / ns	ns / ns
Pachycephala lanioides	0.0089	22250	4 / 7	** / ns	** / **	** / **	ns / ns	ns / ns	ns / **
Pachycephala melanura	0.0200	50000	5 / 5	** / **	** / **	** / **	** / ns	** / ns	ns / ns
Myzomela erythrocephala	0.0115	28750	3 / 6	ns / ns	** / **	** / **	ns / ns	ns / ns	ns / **
Myiagra ruficollis	0.0055	13750	5 / 8	ns / **	** / **	** / **	ns / **	ns / ns	ns / **
Zosterops luteus	0.0262	65500	10 / 19	** / ns	** / **	** / **	** / ns	** / ns	ns / ns
Colluricincla megarhyncha	0.0988	247000	5 / 14	** / **	** / **	** / **	** / **	** / **	** / ns

**   Statistically significant at $p < 0.05$
ns   Statistically not significant at $p < 0.05$

Figure 1:   Map of sampling localities for eight species of mangrove endemic and mangrove-associated Australo-Papuan birds distributed along the Australian coastline and New Guinea. Designation of important areas of endemism follow Cracraft (1991), and are color coded to their respective coastal extent. Phylogeographic barriers along the coastline are indicated following Ford (1982) and Schodde (2006).

Figure 2:   Schematic representation of alternative biogeographic topologies applied as individual, species-specific statistical phylogeography testing frameworks for eight species of mangrove-endemic and mangrove-associated Australo-Papuan birds distributed along the Australian coastline and New Guinea. Three main geographic categories aim to test the historical influence of barriers between areas of endemism, and are designated A, B, C. Geographic scenarios A and B each have 3 and 2 respective additional temporal constraints imposed upon them, indicated by numbers.

Figure 3:   Intraspecific parsimony haplotype networks (95% connection limit; upper panel) and model-based phylogeographic trees (lower panel) for eight species of mangrove endemic and mangrove-associated Australo-Papuan birds distributed along the Australian coastline and New Guinea. Population coloring scheme follows that of Figure 1 and is based on the coastal extent of areas of endemism and major geographic barriers. Circle size of haplotype networks is proportional to the number of samples contained within each group. Circles with single haplotypes are not numbered, and black dots represent inferred steps of changes. Phylogeographic trees follow the same coloring scheme, listing also sample catalog numbers. Black circles at nodes

correspond to >95% Bayesian posterior probability. The scale bar indicates a

proportional amount of 0.005 changes/site. Note that the *Zosterops luteus* tree also

contains samples of *Z. lateralis*, with which it is paraphyletic.

Figure 1

Colluricincla megarhyncha
(n = 66) ▣

Zosterops luteus
(n = 54) ⊗

Myiagra ruficollis
(n = 21) ◈

Myzomela erythrocephala
(n = 31) △

Pachycephala melanura
(n = 44) ●

Pachycephala lanioides
(n = 37) ☆

Peneoenanthe pulverulenta
(n = 41) ✳

Rhipidura phasiana
(n = 21) ⊙

PILBARA

Canning
Gap

KIMBERLEY
PLATEAU

Bonaparte
Gap

ARNHEM
LAND

GULF
OF
CARPENTARIA

CAPE
YORK
PENINSULA

Carpentarian
Gaps

Burdekin
Gap

EAST QUEENSLAND

Figure 2

Rhipidura phasiana, Peneoenanthe pulverulenta, Pachycephala lanioides, Pachycephala melanura, Zosterops luteus

Myzomela erythrocephala, Myiagra ruficollis

Colluricincla megarhyncha

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Figure 3

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Appendix

Chapter 1: GenBank accession numbers of taxa used in the phylogenetic framework to establish taxonomic affinities of *Rhipidura* (= *Chelidorhynx*) *hypoxantha*.

Taxon	ND2	Fib5
Meliphaga reticulata	DQ673232	DQ673252
Picathartes gymnocephalus	DQ125989	EU739155
Certhia familiaris	FJ177333	EU680633
Parus major	AY732696	DQ320586
Hyliota flavigaster	DQ125983	EU680653
Stenostira scita	DQ125993	EU680689
Culicicapa ceylonensis	DQ125979	EU680640
Elminia albonotata	EU652714	EU680645
Bombycilla garrulus	FJ177331	EU680629
Passer montanus	AY030144	EU626752
Regulus calendula	AY329435	EU680681
Promerops cafer	DQ125990	EU680676
Pycnonotus barbatus	DQ402232	EF626746
Sylvia atricapilla	DQ125994	EU680691
Hylia prasina	AY136606	EU680652
Alauda arvensis	DQ125975	EF626747
Cinclus cinclus	FJ177334	EU680638

Chapter 2: Table of GenBank accession numbers for the mtDNA protein-coding gene

NADH dehydrogase subunit 2 (ND2) and the fifth intron of the nuclear gene Beta-

Fibrinogen (Fib5) for the entire dataset included in the analyses. Further details on

sample and voucher information can be found in Driskell and Christidis (2004) and

Norman *et al.* (2007).

Taxon	sample information	ND2	Fib5
Driskell and Christidis 2004			
Stipiturus mallee	MEW1	AY488328.1	AY488485.2
Malurus splendens	SW 683	AY488327.1	AY488484.2
Malurus lamberti	VW 104	AY488326.1	AY488483.2
Amytornis striatus	SGW1	AY488325.1	AY488482.2
Sericornis perspicillatus	ANWC E313	AY488324.1	AY488481.2
Sericornis frontalis	MV 228	AY488323.1	AY488480.2
Pardalotus striatus	ANWC B471	AY488322.1	AY488479.2
Pardalotus punctatus	ANWC B479	AY488321.1	AY488478.2
Gerygone chrysogaster	ANWC E670	AY488320.1	AY488477.2
Gerygone chloronotus	ANWC E122	AY488319.1	AY488476.2
Dasyornis broadbenti	MV 2172	AY488318.1	AY488475.2
Acanthiza chrysorrhoa	MV 116	AY488317.1	AY488474.2
Acanthiza apicalis	MV 158	AY488316.1	AY488473.2
Xanthotis flaviventer	ANWC E594	AY488315.1	AY488472.2
Xanthomyza phrygia	ANWC F724	AY488314.1	AY488471.2
Trichodere cockerelli	ANWC 42941	AY488313.1	AY488470.2
Timeliopsis griseigula	ANWC E714	AY488312.1	AY488469.2
Timeliopsis fulvigula	ANWC E233	AY488311.1	AY488468.2
Ramsayornis modestus	ANWC C900	AY488310.1	AY488467.2
Ramsayornis fasciatus	MV 1230	AY488309.1	AY488466.2
Pycnopygius stictocephalus	ANWC C035	AY488308.1	AY488465.2
Pycnopygius cinereus	ANWC C057	AY488307.1	AY488464.2
Ptiloprora guisei	ANWC E173	AY488306.1	AY488462.2
Prosthemadera novaeseelandiae	MNZ 11/1996	AY488305.1	AY488461.2
Plectorhyncha lanceolata	ANWC C379	AY488304.1	AY488460.2
Phylidonyris novaehollandiae	ANWC B685	AY488303.1	AY488458.2
Phylidonyris nigra	MV 198	AY488302.1	AY488457.2
Phylidonyris albifrons	ANWC D361	AY488301.1	AY488455.2
Philemon meyeri	ANWC E683	AY488300.1	AY488454.2
Philemon corniculatus	ANWC C720	AY488299.1	AY488453.2
Philemon citreogularis	ANWC D008	AY488298.1	AY488452.2
Philemon buceroides	ANWC C863	AY488297.1	AY488451.2
Philemon argenticeps	ANWC JCW095	AY488296.1	AY488450.2

Myzomela sanguinolenta	ANWC C402	AY488295.1	AY488449.2
Myzomela rosenbergii	ANWC E240	AY488294.1	AY488448.2
Myzomela obscura	ANWC C531	AY488293.1	AY488447.2
Myzomela cardinalis	2494 SI	AY488292.1	AY488445.2
Melithreptus brevirostris	MV 371	AY488291.1	AY488444.2
Melithreptus albogularis	ANWC JC100	AY488290.1	AY488443.2
Melipotes fumigatus	ANWC E332	AY488289.1	AY488442.2
Meliphaga gracilis	ANWC C753	AY488288.1	AY488441.2
Meliphaga albonotata	ANWC E471	AY488287.1	AY488440.2
Melilestes megarhynchus	ANWC E557	AY488286.1	AY488439.2
Melidectes torquatus	ANWC E389	AY488285.1	AY488438.2
Melidectes ochromelas	ANWC E360	AY488284.1	AY488437.2
Melidectes belfordi	ANWC E168	AY488283.1	AY488436.2
Manorina melanophrys	ANWC 42737	AY488282.1	AY488435.2
Manorina flavigula	ANWC 42856	AY488281.1	AY488434.2
Lichmera indistincta	ANWC C271	AY488280.1	AY488433.2
Lichmera alboauricularis	ANWC E629	AY488279.1	AY488432.2
Lichenostomus flavescens	ANSP 52785	AY488278.1	AY488431.2
Grantiella picta	MV 2673	AY488277.1	AY488430.2
Glycichaera fallax	ANWC E663	AY488276.1	AY488429.2
Foulehaio carunculata	2077 SI	AY488275.1	AY488428.2
Epthianura aurifrons	ANWC D156	AY488274.1	AY488425.2
Epthianura albifrons	ANWC D328	AY488273.1	AY488424.2
Entomyzon cyanotis	ANWC F274	AY488272.1	AY488423.2
Conopophila rufogularis	MV 1300	AY488271.1	AY488422.2
Conopophila albogularis	MV 1216	AY488270.1	AY488421.2
Certhionyx variegatus	SAM W036	AY488269.1	AY488420.2
Certhionyx pectoralis	ANWC C912	AY488268.1	AY488419.2
Certhionyx niger	ANWC C954	AY488267.1	AY488418.2
Ashbyia lovensis	ANWC D173	AY488266.1	AY488417.2
Anthochaera paradoxa	ANWC B736	AY488265.1	AY488416.2
Anthochaera lunulata	MV 175	AY488264.1	AY488415.2
Anthochaera chrysoptera	ANWC B792	AY488263.1	AY488414.2
Anthochaera carunculata	ANWC C257	AY488262.1	AY488413.2
Acanthorhynchus tenuirostris	ANWC B873	AY488261.1	AY488412.2
Acanthorhynchus superciliosus	MV 248	AY488260.1	AY488411.2
Acanthagenys rufogularis	MV 1122	AY488259.1	AY488410.2
Ptiloprora plumbea	ANWC C173	AY488409.1	AY488463.2
Phylidonyris pyrrhoptera	ANWC B651	AY488408.1	AY488459.2
Phylidonyris melanops	ANWC D451	AY488407.1	AY488456.2
Myzomela erythrocephala	MV 1198	AY488406.1	AY488446.2
Epthianura tricolor	ANWC D229	AY488405.1	AY488427.2
Epthianura crocea	ANWC D175	AY488329.1	AY488426.2

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Meliphaga reticulata	RJ996	DQ673232.1	DQ673252.1
Meliphaga orientalis orientalis	ANWC 26771	DQ673231.1	DQ673251.1
Meliphaga notata notata	ANWC 39741	DQ673230.1	DQ673250.1
Meliphaga notata mixtata	ANWC 39527	DQ673229.1	DQ673249.1
Meliphaga montana aicora	ANWC 26714	DQ673228.1	DQ673248.1

Meliphaga mimikae granti	AM O.59188	DQ673227.1	DQ673247.1
Meliphaga lewinii amphochlora	ANWC 39738	DQ673226.1	DQ673246.1
Meliphaga lewinii lewinii	ANWC 39451	DQ673225.1	DQ673245.1
Meliphaga gracilis imitatrix	ANWC 39509	DQ673224.1	DQ673244.1
Meliphaga gracilis gracilis	ANWC 39862	DQ673223.1	DQ673243.1
Meliphaga fordiana	ANWC 39176	DQ673222.1	DQ673242.1
Meliphaga flavirictus	ANWC 26479	DQ673221.1	DQ673241.1
Meliphaga cinereifrons stevensi	ANWC 27018	DQ673220.1	DQ673240.1
Meliphaga cinereifrons cinereifrons	ANWC 27099	DQ673219.1	DQ673239.1
Meliphaga aruensis	ANWC 26588	DQ673218.1	DQ673238.1
Meliphaga aruensis aruensis	AM O.59185	DQ673217.1	DQ673237.1
Meliphaga analoga stevensi	ANWC 27038	DQ673216.1	DQ673236.1
Meliphaga analoga analoga	AM O.59191	DQ673215.1	DQ673235.1
Meliphaga albonotata	ANWC 24488	DQ673214.1	DQ673234.1
Meliphaga albilineata	NTM 1633	DQ673213.1	DQ673233.1

Chapter 4: Catalog numbers, collecting locality and geographic coordinates for the samples of 8 mangrove-bound birds included in the comparative phylogeographic analysis. Additional samples of *Zosterops lateralis* included are given at the end of the table. Catalog numbers correspond to the Australian National Wildlife Collection (ANWC) holdings, while numbers preceded by an asterisk correspond to samples from The University of Kansas Biodiversity Institute and Natural History Museum (KUNHM).

SAMPLE #	CATALOG #	TAXON	STATE	LOCALITY	LATITUDE	LONGITUDE
1	29491	Rhipidura phasiana	QLD	NORMAN RIVER, KARUMBA	-17.47222	140.82750
2	32680	Rhipidura phasiana	QLD	ALBERT RIVER, NEAR MOUTH, NE OF BURKETOWN	-17.60000	139.75000
3	33075	Rhipidura phasiana	WA	ELLY CREEK, C. 2.4KM N OF DELLY CREEK, C.24 KM N OF DE GREY STATION HOMESTEAD	-19.98944	119.31694
4	33134	Rhipidura phasiana	WA	BOODARIE STATION, 29 KM NW OF PORT HEDLAND	-20.36417	118.46111
5	33217	Rhipidura phasiana	WA	YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
6	33218	Rhipidura phasiana	WA	YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
7	33219	Rhipidura phasiana	WA	YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
8	33263	Rhipidura phasiana	WA	OLD ONSLOW AREA	-21.70333	114.93972
9	33264	Rhipidura phasiana	WA	OLD ONSLOW AREA	-21.70333	114.93972
10	33265	Rhipidura phasiana	WA	OLD ONSLOW AREA	-21.70333	114.93972
11	33883	Rhipidura phasiana	WA	OLD ONSLOW AREA	-21.70333	114.93972
12	33972	Rhipidura phasiana	NT	VICTORIA RIVER MOUTH, N OF BULLO RIVER HOMESTEAD	-14.97056	129.59944
13	33973	Rhipidura phasiana	NT	VICTORIA RIVER MOUTH, N OF BULLO RIVER HOMESTEAD	-14.97056	129.59944
14	34469	Rhipidura phasiana	WA	MARY ISLAND NORTH, KING SOUND, NW OF DERBY	-17.29417	123.54306
15	48561	Rhipidura phasiana	NT	TIMRAMBU, 2 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.44278	130.68361

#	Reg.	Species	State	Location	Latitude	Longitude
16	50515	Rhipidura phasiana	WA	MARY ISLAND SOUTH, KING SOUND, NW OF DERBY	-17.31472	123.54778
17	50516	Rhipidura phasiana	WA	MARY ISLAND SOUTH, KING SOUND, NW OF DERBY	-17.31472	123.54778
18	50719	Rhipidura phasiana	WA	ROEBUCK BAY, C.20 KM SE OF BROOME	-18.05694	122.38000
19	50755	Rhipidura phasiana	WA	ROEBUCK BAY, C.20 KM SE OF BROOME	-18.03667	122.37750
20	50906	Rhipidura phasiana	WA	PENTECOST RIVER, HOME VALLEY STATION	-15.60361	127.85500
21	* 6194	Rhipidura phasiana	WA	CARNARVON, 8 KM SE; UENDO CREEK	-25.05000	113.68333
1	28771	Peneoenanthe pulverulenta	QLD	MURRAY CREEK, C.50 KM N OF MACKAY	-20.90778	148.84222
2	28772	Peneoenanthe pulverulenta	QLD	MURRAY CREEK, C.50 KM N OF MACKAY	-20.90778	148.84222
3	28805	Peneoenanthe pulverulenta	QLD	ESTUARY OF VICTORIA CREEK, C.15 KM E OF INGHAM	-18.62389	146.32917
4	29496	Peneoenanthe pulverulenta	QLD	NORMAN RIVER, C.5 KM SW OF KARUMBA	-17.54333	140.80111
5	29497	Peneoenanthe pulverulenta	QLD	NORMAN RIVER, C.5 KM SW OF KARUMBA	-17.54333	140.80111
6	29762	Peneoenanthe pulverulenta	QLD	WENLOCK RIVER, N OF WEIPA, CAPE YORK PENINSULA	-12.20833	141.91667
7	29763	Peneoenanthe pulverulenta	QLD	WENLOCK RIVER, N OF WEIPA, CAPE YORK PENINSULA	-12.20833	141.91667
8	29764	Peneoenanthe pulverulenta	QLD	WENLOCK RIVER, N OF WEIPA, CAPE YORK PENINSULA	-12.20833	141.91667
9	31126	Peneoenanthe pulverulenta	QLD	MURRAY CREEK, C.50 KM N OF MACKAY	-20.90778	148.84222
10	31127	Peneoenanthe pulverulenta	QLD	MURRAY CREEK, C.50 KM N OF MACKAY	-20.90778	148.84222
11	31288	Peneoenanthe pulverulenta	QLD	CATTLE/ELEANOR CREEKS ESTUARY, HALIFAX BAY, SE OF INGHAM	-18.86667	146.26667
12	31289	Peneoenanthe pulverulenta	QLD	CATTLE/ELEANOR CREEKS ESTUARY, HALIFAX BAY, SE OF INGHAM	-18.86667	146.26667
13	31313	Peneoenanthe pulverulenta	QLD	VICTORIA CREEK ESTUARY, C.15 KM E OF INGHAM	-18.62389	146.32917
14	31314	Peneoenanthe pulverulenta	QLD	VICTORIA CREEK ESTUARY, C.15 KM E OF INGHAM	-18.62389	146.32917
15	31369	Peneoenanthe pulverulenta	QLD	DAINTREE RIVER ESTUARY	-16.28333	145.41667
16	31370	Peneoenanthe pulverulenta	QLD	DAINTREE RIVER ESTUARY	-16.28333	145.41667
17	31371	Peneoenanthe pulverulenta	QLD	DAINTREE RIVER ESTUARY	-16.28333	145.41667
18	33095	Peneoenanthe pulverulenta	WA	BOODARIE STATION, 29 KM NW OF PORT HEDLAND	-20.33889	118.45194
19	33132	Peneoenanthe pulverulenta	WA	BOODARIE STATION, 29 KM NW OF PORT HEDLAND	-20.36417	118.46111

#	ID	Species	State	Location	Latitude	Longitude
20	33133	Peneoenanthe pulverulenta	WA	BOODARIE STATION, 29 KM NW OF PORT HEDLAND	-20.36417	118.46111
21	33731	Peneoenanthe pulverulenta	NT	ISLAND OFF GLYDE POINT, GUNN POINT, E OF DARWIN	-12.15306	131.11583
22	33756	Peneoenanthe pulverulenta	NT	MOUTH OF LEADERS CREEK, GUNN POINT, E OF DARWIN	-12.19139	131.10361
23	33757	Peneoenanthe pulverulenta	NT	MOUTH OF LEADERS CREEK, GUNN POINT, E OF DARWIN	-12.19139	131.10361
24	33758	Peneoenanthe pulverulenta	NT	MOUTH OF LEADERS CREEK, GUNN POINT, E OF DARWIN	-12.19139	131.10361
25	33957	Peneoenanthe pulverulenta	NT	VICTORIA RIVER, N OF BULLO RIVER HOMESTEAD	-15.29500	129.85278
26	33958	Peneoenanthe pulverulenta	NT	VICTORIA RIVER, N OF BULLO RIVER HOMESTEAD	-15.30611	129.85639
27	33974	Peneoenanthe pulverulenta	NT	VICTORIA RIVER MOUTH, N OF BULLO RIVER HOMESTEAD	-14.97056	129.59944
28	33975	Peneoenanthe pulverulenta	NT	VICTORIA RIVER MOUTH, N OF BULLO RIVER HOMESTEAD	-14.97056	129.59944
29	48659	Peneoenanthe pulverulenta	NT	GOOSE CREEK (ANDRANANGOO), MELVILLE ISLAND	-11.38278	130.92417
30	48660	Peneoenanthe pulverulenta	NT	GOOSE CREEK (ANDRANANGOO), MELVILLE ISLAND	-11.38278	130.92417
31	48661	Peneoenanthe pulverulenta	NT	GOOSE CREEK (ANDRANANGOO), MELVILLE ISLAND	-11.38278	130.92417
32	48666	Peneoenanthe pulverulenta	NT	GOOSE CREEK (ANDRANANGOO), MELVILLE ISLAND	-11.34583	130.87611
33	50517	Peneoenanthe pulverulenta	WA	MARY ISLAND SOUTH, KING SOUND, NW OF DERBY	-17.31472	123.54778
34	50518	Peneoenanthe pulverulenta	WA	MARY ISLAND SOUTH, KING SOUND, NW OF DERBY	-17.31472	123.54778
35	50902	Peneoenanthe pulverulenta	WA	PENTECOST RIVER, HOME VALLEY STATION	-15.60361	127.85500
36	50903	Peneoenanthe pulverulenta	WA	PENTECOST RIVER, HOME VALLEY STATION	-15.60361	127.85500
37	54584	Peneoenanthe pulverulenta	NT	OLD AQUAFARM AREA, PORT ROPER	-14.7653	135.2044
38	54597	Peneoenanthe pulverulenta	NT	ROPER RIVER	-14.742	135.292
39	54609	Peneoenanthe pulverulenta	NT	ROPER RIVER	-14.793	135.158
40	54624	Peneoenanthe pulverulenta	NT	ROPER RIVER	-14.713	135.284
41	54625	Peneoenanthe pulverulenta	NT	ROPER RIVER	-14.713	135.284
1	29492	Pachycephala lanioides	QLD	NORMAN RIVER, KARUMBA	-17.47222	140.82750
2	29493	Pachycephala lanioides	QLD	NORMAN RIVER, C.5 KM SW OF KARUMBA	-17.54333	140.80111
3	29494	Pachycephala lanioides	QLD	NORMAN RIVER, C.5 KM SW OF KARUMBA	-17.54333	140.80111

4	32677	Pachycephala lanioides	QLD	ALBERT RIVER, NEAR MOUTH, NE OF BURKETOWN	-17.60000	139.75000
5	32678	Pachycephala lanioides	QLD	ALBERT RIVER, NEAR MOUTH, NE OF BURKETOWN	-17.60000	139.75000
6	32693	Pachycephala lanioides	QLD	NORMAN RIVER, C.5 KM SW OF KARUMBA	-17.54333	140.80111
7	32694	Pachycephala lanioides	QLD	NORMAN RIVER, C.5 KM SW OF KARUMBA	-17.54333	140.80111
8	32695	Pachycephala lanioides	QLD	NORMAN RIVER, C.5 KM SW OF KARUMBA	-17.54333	140.80111
9	32696	Pachycephala lanioides	QLD	NORMAN RIVER, C.5 KM SW OF KARUMBA	-17.54333	140.80111
10	33078	Pachycephala lanioides	WA	ELLY CREEK, C.24 KM N OF DE GREY STATION HOMESTEAD	-19.98944	119.31694
11	33079	Pachycephala lanioides	WA	ELLY CREEK, C.24 KM N OF DE GREY STATION HOMESTEAD	-19.98944	119.31694
12	33080	Pachycephala lanioides	WA	ELLY CREEK, C.24 KM N OF DE GREY STATION HOMESTEAD	-19.98944	119.31694
13	33131	Pachycephala lanioides	WA	BOODARIE STATION, 29 KM NW OF PORT HEDLAND	-20.36417	118.46111
14	33208	Pachycephala lanioides	WA	YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
15	33209	Pachycephala lanioides	WA	YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
16	33210	Pachycephala lanioides	WA	YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
17	33828	Pachycephala lanioides	WA	ELLY CREEK, C.24 KM N OF DE GREY STATION HOMESTEAD	-19.98944	119.31694
18	33882	Pachycephala lanioides	WA	BOODARIE STATION, 29 KM NW OF PORT HEDLAND	-20.33889	118.45194
19	33968	Pachycephala lanioides	NT	VICTORIA RIVER MOUTH, N OF BULLO RIVER HOMESTEAD	-14.97056	129.59944
20	33978	Pachycephala lanioides	NT	VICTORIA RIVER MOUTH, N OF BULLO RIVER HOMESTEAD	-14.94222	129.61167
21	33979	Pachycephala lanioides	NT	VICTORIA RIVER MOUTH, N OF BULLO RIVER HOMESTEAD	-14.94222	129.61167
22	34207	Pachycephala lanioides	WA	ELLY CREEK, C.24 KM N OF DE GREY STATION HOMESTEAD	-19.98944	119.31694
23	34473	Pachycephala lanioides	WA	MARY ISLAND NORTH, KING SOUND, NW OF DERBY	-17.29417	123.54306
24	50489	Pachycephala lanioides	WA	KING SOUND, NW OF DERBY	-17.29444	123.60889
25	50490	Pachycephala lanioides	WA	KING SOUND, NW OF DERBY	-17.29444	123.60889
26	50512	Pachycephala lanioides	WA	MARY ISLAND SOUTH, KING SOUND, NW OF DERBY	-17.31472	123.54778
27	50513	Pachycephala lanioides	WA	MARY ISLAND SOUTH, KING SOUND, NW OF DERBY	-17.31472	123.54778
28	50567	Pachycephala lanioides	WA	KING SOUND, C.18 KM N OF DERBY	-17.17889	123.64361

No.	Reg.	Species	State	Locality	Latitude	Longitude
29	50717	Pachycephala lanioides	WA	ROEBUCK BAY, C.20 KM SE OF BROOME	-18.05694	122.38000
30	50754	Pachycephala lanioides	WA	ROEBUCK BAY, C.20 KM SE OF BROOME	-18.03667	122.37750
31	* 6195	Pachycephala lanioides	WA	CARNARVON, 8 KM SE; UENDO CREEK	-25.05000	113.68333
32	* 6196	Pachycephala lanioides	WA	CARNARVON, 8 KM SE; UENDO CREEK	-25.05000	113.68333
33	54484	Pachycephala lanioides	NT	GOONDI LANDING, MCARTHUR RIVER	-15.851	136.619
34	54485	Pachycephala lanioides	NT	MCARTHUR R DOWNSTREAM FR KING ASH BAY, NR GOONDI LANDING	-15.865	136.63
35	54602	Pachycephala lanioides	NT	ROPER RIVER	-14.742	135.292
36	54603	Pachycephala lanioides	NT	ROPER RIVER	-14.742	135.292
37	54626	Pachycephala lanioides	NT	ROPER RIVER	-14.713	135.284
1	29384	Pachycephala melanura		LEICHHARDT RIVER MOUTH, NE OF BURKETOWN	-17.59278	139.75583
2	29385	Pachycephala melanura		LEICHHARDT RIVER MOUTH, NE OF BURKETOWN	-17.59278	139.75583
3	29432	Pachycephala melanura		NICHOLSON RIVER MOUTH, N OF BURKETOWN	-17.50583	139.60583
4	29433	Pachycephala melanura		NICHOLSON RIVER MOUTH, N OF BURKETOWN	-17.50583	139.60583
5	29524	Pachycephala melanura		NORMAN RIVER, SE OF KARUMBA	-17.54333	140.80111
6	29525	Pachycephala melanura		NORMAN RIVER, SE OF KARUMBA	-17.54333	140.80111
7	29627	Pachycephala melanura		STAATEN RIVER, INKERMAN STATION, SW CAPE YORK PENINSULA	-16.35083	141.43917
8	29628	Pachycephala melanura		STAATEN RIVER, INKERMAN STATION, SW CAPE YORK PENINSULA	-16.35083	141.43917
9	29658	Pachycephala melanura		STAATEN RIVER, INKERMAN STATION, SW CAPE YORK PENINSULA	-16.35083	141.43917
10	32686	Pachycephala melanura		NICHOLSON RIVER MOUTH AREA	-17.50583	139.60583
11	32703	Pachycephala melanura		STAATEN RIVER, INKERMAN STATION, CAPE YORK PENINSULA	-16.35083	141.43917
12	33097	Pachycephala melanura		BOODARIE STATION, 29 KM NW OF PORT HEDLAND	-20.33889	118.45194
13	33098	Pachycephala melanura		BOODARIE STATION, 29 KM NW OF PORT HEDLAND	-20.33889	118.45194
14	33207	Pachycephala melanura		YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
15	33262	Pachycephala melanura		OLD ONSLOW AREA	-21.70333	114.93972
16	33725	Pachycephala melanura		ISLAND OFF GLYDE POINT, GUNN POINT, E OF DARWIN	-12.15306	131.11583

17	33726	Pachycephala melanura		ISLAND OFF GLYDE POINT, GUNN POINT, E OF DARWIN	-12.15306	131.11583
18	33754	Pachycephala melanura		MOUTH OF LEADERS CREEK, GUNN POINT, E OF DARWIN	-12.19139	131.10361
19	33755	Pachycephala melanura		MOUTH OF LEADERS CREEK, GUNN POINT, E OF DARWIN	-12.19139	131.10361
20	33956	Pachycephala melanura		VICTORIA RIVER, N OF BULLO RIVER HOMESTEAD	-15.29500	129.85278
21	33959	Pachycephala melanura		VICTORIA RIVER, N OF BULLO RIVER HOMESTEAD	-15.30611	129.85639
22	33960	Pachycephala melanura		VICTORIA RIVER, N OF BULLO RIVER HOMESTEAD	-15.30611	129.85639
23	33961	Pachycephala melanura		VICTORIA RIVER, N OF BULLO RIVER HOMESTEAD	-15.30611	129.85639
24	34428	Pachycephala melanura		ROEBUCK BAY, C.20 KM SE OF BROOME	-18.05694	122.38000
25	34474	Pachycephala melanura		MARY ISLAND NORTH, KING SOUND, NW OF DERBY	-17.29417	123.54306
26	43800	Pachycephala melanura		SHOALWATER BAY ARMY TRAINING RESERVE, N OF ROCKHAMPTON	-22.41389	150.29861
27	48662	Pachycephala melanura		GOOSE CREEK (ANDRANANGOO), MELVILLE ISLAND	-11.38278	130.92417
28	48664	Pachycephala melanura		GOOSE CREEK (ANDRANANGOO), MELVILLE ISLAND	-11.34583	130.87611
29	48665	Pachycephala melanura		GOOSE CREEK (ANDRANANGOO), MELVILLE ISLAND	-11.34583	130.87611
30	48726	Pachycephala melanura		COAST DUE S OF PICKERTARAMOOR, MELVILLE ISLAND	-11.85056	130.85306
31	50511	Pachycephala melanura		MARY ISLAND NORTH, KING SOUND, NW OF DERBY	-17.29417	123.54306
32	50720	Pachycephala melanura		ROEBUCK BAY, C.20 KM SE OF BROOME	-18.05694	122.38000
33	50899	Pachycephala melanura		PENTECOST RIVER, HOME VALLEY STATION	-15.60361	127.85500
34	50900	Pachycephala melanura		PENTECOST RIVER, HOME VALLEY STATION	-15.60361	127.85500
35	50901	Pachycephala melanura		PENTECOST RIVER, HOME VALLEY STATION	-15.60361	127.85500
36	51358	Pachycephala melanura		CAMP ISLAND, OFF ELLIOT RIVER MOUTH, C.60 KM SE OF AYR	-19.85083	147.89833
37	51359	Pachycephala melanura		CAMP ISLAND, OFF ELLIOT RIVER MOUTH, C.60 KM SE OF AYR	-19.85083	147.89833
38	51360	Pachycephala melanura		CAMP ISLAND, OFF ELLIOT RIVER MOUTH, C.60 KM SE OF AYR	-19.85083	147.89833
39	52425	Pachycephala melanura	NT	PORT ROPER, ROPER RIVER	-14.7559	135.3184
40	54440	Pachycephala melanura	NT	MCARTHUR RIVER KING ASH BAY	-15.925	136.515
41	54441	Pachycephala melanura	NT	MCARTHUR RIVER KING ASH BAY	-15.925	136.515

No.	ID	Species	State	Location	Latitude	Longitude
42	54449	Pachycephala melanura	NT	MCARTHUR RIVER KING ASH BAY	-15.922	136.518
43	54450	Pachycephala melanura	NT	MCARTHUR RIVER KING ASH BAY	-15.922	136.518
44	54522	Pachycephala melanura	NT	KING ASH BAY MCARTHUR RIVER	-15.926	136.511
1	29523	Myzomela erythrocephala	QLD	NORMAN RIVER, SE OF KARUMBA	-17.54333	140.80111
2	29639	Myzomela erythrocephala	QLD	STAATEN RIVER, INKERMAN STATION, SW CAPE YORK PENINSULA	-16.39222	141.29667
3	29640	Myzomela erythrocephala	QLD	STAATEN RIVER, INKERMAN STATION, SW CAPE YORK PENINSULA	-16.39222	141.29667
4	29723	Myzomela erythrocephala	QLD	LEITHEN CREEK, WEIPA	-12.69083	141.81750
5	32679	Myzomela erythrocephala	QLD	ALBERT RIVER, NEAR MOUTH, NE OF BURKETOWN	-17.60000	139.75000
6	33728	Myzomela erythrocephala	NT	ISLAND OFF GLYDE POINT, GUNN POINT, E OF DARWIN	-12.15306	131.11583
7	33729	Myzomela erythrocephala	NT	ISLAND OFF GLYDE POINT, GUNN POINT, E OF DARWIN	-12.15306	131.11583
8	33759	Myzomela erythrocephala	NT	MOUTH OF LEADERS CREEK, GUNN POINT, E OF DARWIN	-12.19139	131.10361
9	41659	Myzomela erythrocephala	QLD	KARUMBA	-17.50000	140.83333
10	41660	Myzomela erythrocephala	QLD	KARUMBA	-17.50000	140.83333
11	43030	Myzomela erythrocephala	QLD	EASTERN MCILWRAITH RANGE LOWLANDS, CAPE YORK PENINSULA	-13.70556	143.53611
12	43059	Myzomela erythrocephala	QLD	EASTERN MCILWRAITH RANGE LOWLANDS, CAPE YORK PENINSULA	-13.88611	143.58333
13	48555	Myzomela erythrocephala	NT	TIMRAMBU, 2 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.44278	130.68361
14	48556	Myzomela erythrocephala	NT	TIMRAMBU, 2 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.44278	130.68361
15	48725	Myzomela erythrocephala	NT	COAST DUE S OF PICKERTARAMOOR, MELVILLE ISLAND	-11.85056	130.85306
16	48739	Myzomela erythrocephala	NT	C.17 KM SE OF PICKERTARAMOOR, MELVILLE ISLAND	-11.88278	130.90389
17	48778	Myzomela erythrocephala	NT	C.19 KM SE OF PICKERTARAMOOR, MELVILLE ISLAND	-11.90556	130.92222
18	50569	Myzomela erythrocephala	WA	KING SOUND, C.18 KM N OF DERBY	-17.17889	123.64361
19	50570	Myzomela erythrocephala	WA	KING SOUND, C.18 KM N OF DERBY	-17.17889	123.64361
20	50571	Myzomela erythrocephala	WA	KING SOUND, C.18 KM N OF DERBY	-17.17889	123.64361
21	51431	Myzomela erythrocephala	QLD	KALPOWAR STATION, PRINCESS CHARLOTTE BAY, CAPE YORK PEN	-14.36056	144.21694
22	51463	Myzomela erythrocephala	QLD	KALPOWAR STATION, PRINCESS CHARLOTTE BAY, CAPE YORK PEN	-14.48361	144.14944

#	No.	Species	State	Location	Latitude	Longitude
23	51715	Myzomela erythrocephala	QLD	KOWANYAMA REGION, SOUTH MITCHELL RIVER, CAPE YORK PEN	-15.49111	141.49167
24	51767	Myzomela erythrocephala	QLD	KOWANYAMA REGION, SOUTH MITCHELL RIVER, CAPE YORK PEN	-15.36194	141.55250
25	51768	Myzomela erythrocephala	QLD	KOWANYAMA REGION, SOUTH MITCHELL RIVER, CAPE YORK PEN	-15.36194	141.55250
26	51769	Myzomela erythrocephala	QLD	KOWANYAMA REGION, SOUTH MITCHELL RIVER, CAPE YORK PEN	-15.36194	141.55250
27	54454	Myzomela erythrocephala	NT	3 KM W MUGGS MISTAKE, MCARTHUR R, CA 5 KM E KING ASH BAY	-15.913	136.544
28	54455	Myzomela erythrocephala	NT	3 KM W MUGGS MISTAKE, MCARTHUR R, CA 5 KM E KING ASH BAY	-15.913	136.544
29	54483	Myzomela erythrocephala	NT	GOONDI LANDING, MCARTHUR RIVER	-15.851	136.619
30	54585	Myzomela erythrocephala	NT	PORT ROPER	-14.7561	135.3189
31	54617	Myzomela erythrocephala	NT	PORT ROPER	-14.713	135.284
1	29354	Myiagra ruficollis	QLD	ALBERT RIVER, NEAR MOUTH, NE OF BURKETOWN	-17.60000	139.75000
2	29386	Myiagra ruficollis	QLD	LEICHHARDT RIVER MOUTH, NE OF BURKETOWN	-17.59278	139.75583
3	29431	Myiagra ruficollis	QLD	NICHOLSON RIVER MOUTH, N OF BURKETOWN	-17.50583	139.60583
4	29490	Myiagra ruficollis	QLD	NORMAN RIVER, KARUMBA	-17.47222	140.82750
5	29659	Myiagra ruficollis	QLD	STAATEN RIVER, INKERMAN STATION, SW CAPE YORK PENINSULA	-16.35083	141.43917
6	29733	Myiagra ruficollis	QLD	HEY RIVER, WEIPA	-12.78333	141.91667
7	33561	Myiagra ruficollis	NT	KOOLPINYAH STATION, E OF DARWIN	-12.42000	131.22417
8	33659	Myiagra ruficollis	NT	KOOLPINYAH STATION, E OF DARWIN	-12.41083	131.20000
9	33760	Myiagra ruficollis	NT	MOUTH OF LEADERS CREEK, GUNN POINT, E OF DARWIN	-12.19139	131.10361
10	43031	Myiagra ruficollis	QLD	EASTERN MCILWRAITH RANGE LOWLANDS, CAPE YORK PENINSULA	-13.70556	143.53611
11	43818	Myiagra ruficollis	QLD	SHOALWATER BAY ARMY TRAINING RESERVE, N OF ROCKHAMPTON	-22.39444	150.21611
12	48667	Myiagra ruficollis	NT	GOOSE CREEK (ANDRANANGOO), MELVILLE ISLAND	-11.34583	130.87611
13	48781	Myiagra ruficollis	NT	C.19 KM SE OF PICKERTARAMOOR, MELVILLE ISLAND	-11.90556	130.92222
14	50514	Myiagra ruficollis	WA	MARY ISLAND SOUTH, KING SOUND, NW OF DERBY	-17.31472	123.54778
15	50568	Myiagra ruficollis	WA	KING SOUND, C.18 KM N OF DERBY	-17.17889	123.64361
16	50712	Myiagra ruficollis	WA	ROEBUCK BAY, C.20 KM SE OF BROOME	-18.05694	122.38000

No.	Reg.	Species	State	Location	Latitude	Longitude
17	50713	Myiagra ruficollis	WA	ROEBUCK BAY, C.20 KM SE OF BROOME	-18.05694	122.38000
18	50714	Myiagra ruficollis	WA	ROEBUCK BAY, C.20 KM SE OF BROOME	-18.05694	122.38000
19	50715	Myiagra ruficollis	WA	ROEBUCK BAY, C.20 KM SE OF BROOME	-18.05694	122.38000
20	54442	Myiagra ruficollis	NT	MCARTHUR RIVER KING ASH BAY	-15.925	136.515
21	54459	Myiagra ruficollis	NT	3 KM W MUGGS MISTAKE, MCARTHUR R, CA 5 KM E KING ASH BAY	-15.913	136.544
1	29331	Zosterops luteus		2 KM NE OF BURKETOWN AT ALBERT RIVER	-17.73333	139.59167
2	29332	Zosterops luteus		2 KM NE OF BURKETOWN AT ALBERT RIVER	-17.73333	139.59167
3	29333	Zosterops luteus		2 KM NE OF BURKETOWN AT ALBERT RIVER	-17.73333	139.59167
4	29549	Zosterops luteus		C.4 KM S OF KARUMBA	-17.50417	140.84056
5	29550	Zosterops luteus		C.4 KM S OF KARUMBA	-17.50417	140.84056
6	29604	Zosterops luteus		C.1.3 KM N OF KARUMBA AIRPORT	-17.43750	140.84611
7	29635	Zosterops luteus		STAATEN RIVER, INKERMAN STATION, SW CAPE YORK PENINSULA	-16.39222	141.29667
8	31250	Zosterops luteus		CROMARTY BOAT RAMP, HAUGHTON RIVER, C.23 KM NW OF AYR	-19.33611	147.09750
9	31251	Zosterops luteus		CROMARTY BOAT RAMP, HAUGHTON RIVER, C.23 KM NW OF AYR	-19.33611	147.09750
10	31252	Zosterops luteus		CROMARTY BOAT RAMP, HAUGHTON RIVER, C.23 KM NW OF AYR	-19.33611	147.09750
11	33050	Zosterops luteus		7 KM N OF PARDOO STATION HOMESTEAD	-20.07833	119.57528
12	33051	Zosterops luteus		7 KM N OF PARDOO STATION HOMESTEAD	-20.07833	119.57528
13	33063	Zosterops luteus		7 KM N OF PARDOO STATION HOMESTEAD	-20.07833	119.57528
14	33076	Zosterops luteus		ELLY CREEK, C.24 KM N OF DE GREY STATION HOMESTEAD	-19.98944	119.31694
15	33214	Zosterops luteus		YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
16	33215	Zosterops luteus		YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
17	33216	Zosterops luteus		YAN YARE RIVER MOUTH, C.30 KM W OF KARRATHA	-20.83917	116.45750
18	33260	Zosterops luteus		OLD ONSLOW AREA	-21.70333	114.93972
19	33261	Zosterops luteus		OLD ONSLOW AREA	-21.70333	114.93972
20	33732	Zosterops luteus		ISLAND OFF GLYDE POINT, GUNN POINT, E OF DARWIN	-12.15306	131.11583

#	No.	Species	Location	Latitude	Longitude
21	33733	Zosterops luteus	ISLAND OFF GLYDE POINT, GUNN POINT, E OF DARWIN	-12.15306	131.11583
22	33953	Zosterops luteus	VICTORIA RIVER, N OF BULLO RIVER HOMESTEAD	-15.29500	129.85278
23	33954	Zosterops luteus	VICTORIA RIVER, N OF BULLO RIVER HOMESTEAD	-15.29500	129.85278
24	34063	Zosterops luteus	ELLY CREEK, C.24 KM N OF DE GREY STATION HOMESTEAD	-19.99944	119.31694
25	34159	Zosterops luteus	KING SOUND, NW OF DERBY	-17.29444	123.60889
26	34453	Zosterops luteus	YARDOOGARRA CREEK, THANGOO STATION, C.35 KM S OF BROOME	-18.24806	122.20972
27	34455	Zosterops luteus	YARDOOGARRA CREEK, THANGOO STATION, C.35 KM S OF BROOME	-18.24806	122.20972
28	41649	Zosterops luteus	GLENORE STATION, NORMAN RIVER, SE OF NORMANTON	-17.86667	141.13333
29	41650	Zosterops luteus	GLENORE STATION, NORMAN RIVER, SE OF NORMANTON	-17.86667	141.13333
30	41658	Zosterops luteus	KARUMBA	-17.50000	140.83333
31	48557	Zosterops luteus	TIMRAMBU, 2 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.44278	130.68361
32	48558	Zosterops luteus	TIMRAMBU, 2 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.44278	130.68361
33	50491	Zosterops luteus	KING SOUND, NW OF DERBY	-17.29444	123.60889
34	50638	Zosterops luteus	14.5 KM E OF YEEDA STATION HOMESTEAD, C.15 KM S OF DERBY	-17.66861	123.56528
35	50639	Zosterops luteus	14.5 KM E OF YEEDA STATION HOMESTEAD, C.15 KM S OF DERBY	-17.66861	123.56528
36	50718	Zosterops luteus	ROEBUCK BAY, C.20 KM SE OF BROOME	-18.05694	122.38000
37	50779	Zosterops luteus	YARDOOGARRA CREEK, THANGOO STATION, C.35 KM S OF BROOME	-18.24806	122.20972
38	50780	Zosterops luteus	YARDOOGARRA CREEK, THANGOO STATION, C.35 KM S OF BROOME	-18.24806	122.20972
39	50781	Zosterops luteus	YARDOOGARRA CREEK, THANGOO STATION, C.35 KM S OF BROOME	-18.24806	122.20972
40	50891	Zosterops luteus	PENTECOST RIVER/BINDOOLA CK JUNCTION, HOME VALLEY STATION	-15.70361	127.85167
41	50892	Zosterops luteus	PENTECOST RIVER/BINDOOLA CK JUNCTION, HOME VALLEY STATION	-15.70361	127.85167
42	50908	Zosterops luteus	PENTECOST RIVER, HOME VALLEY STATION	-15.60361	127.85500
43	51362	Zosterops luteus	KALPOWAR STATION, PRINCESS CHARLOTTE BAY, CAPE YORK PEN	-14.48361	144.14944
44	51363	Zosterops luteus	KALPOWAR STATION, PRINCESS CHARLOTTE BAY, CAPE YORK PEN	-14.48361	144.14944
45	54437	Zosterops luteus	MULE CK BOAT RAMP ENVIRONS, SE OF BING BONG	-15.642	136.419

No.	ID	Species	State	Location	Latitude	Longitude
46	54438	Zosterops luteus		MULE CK BOAT RAMP ENVIRONS, SE OF BING BONG	-15.642	136.419
47	54456	Zosterops luteus		3 KM W MUGGS MISTAKE, MCARTHUR R, CA 5 KM E KING ASH BAY	-15.913	136.544
48	54457	Zosterops luteus		3 KM W MUGGS MISTAKE, MCARTHUR R, CA 5 KM E KING ASH BAY	-15.913	136.544
49	54523	Zosterops luteus	NT	KING ASH BAY MCARTHUR RIVER	-15.926	136.511
50	54524	Zosterops luteus	NT	KING ASH BAY MCARTHUR RIVER	-15.926	136.511
51	54589	Zosterops luteus	NT	PORT ROPER LANDING, PORT ROPER	-14.756	135.319
52	54606	Zosterops luteus	NT	ROPER RIVER	-14.713	135.284
53	54627	Zosterops luteus	NT	ROPER RIVER	-14.713	135.284
54	54628	Zosterops luteus	NT	ROPER RIVER	-14.713	135.284
1	28803	Colluricincla megarhyncha	QLD	MOUTH OF CRYSTAL CREEK, HALIFAX BAY, SE OF INGHAM	-18.92694	146.31833
2	28830	Colluricincla megarhyncha	QLD	ANNAN RIVER ESTUARY, COOKTOWN	-15.51667	145.21667
3	28940	Colluricincla megarhyncha	QLD	3 KM N OF MARIAN, W OF MACKAY	-21.12500	148.95000
4	29129	Colluricincla megarhyncha	QLD	KENNEDY RIVER, 34 KM NW OF LAURA, CAPE YORK PENINSULA	-15.42028	144.18417
5	29629	Colluricincla megarhyncha	QLD	STAATEN RIVER, INKERMAN STATION, SW CAPE YORK PENINSULA	-16.35083	141.43917
6	29630	Colluricincla megarhyncha	QLD	STAATEN RIVER, INKERMAN STATION, SW CAPE YORK PENINSULA	-16.35083	141.43917
7	29780	Colluricincla megarhyncha	QLD	WENLOCK RIVER, N OF WEIPA, CAPE YORK PENINSULA	-12.26667	141.90000
8	29781	Colluricincla megarhyncha	QLD	WENLOCK RIVER, N OF WEIPA, CAPE YORK PENINSULA	-12.26667	141.90000
9	29782	Colluricincla megarhyncha	QLD	WENLOCK RIVER, N OF WEIPA, CAPE YORK PENINSULA	-12.26667	141.90000
10	31175	Colluricincla megarhyncha	QLD	MATHERS LANDING, MURRAY CREEK, C.50 KM N OF MACKAY	-20.90778	148.84222
11	31199	Colluricincla megarhyncha	QLD	MOUTH OF ROCKY PONDS CREEK, C.40 KM SE OF AYR	-19.82028	147.66806
12	31201	Colluricincla megarhyncha	QLD	MOUTH OF ROCKY PONDS CREEK, C.40 KM SE OF AYR	-19.82028	147.66806
13	31216	Colluricincla megarhyncha	QLD	LOWER WALLACE CREEK, C.30 KM SE OF AYR, UPSTART BAY	-19.73611	147.55528
14	31238	Colluricincla megarhyncha	QLD	HAUGHTON RIVER, C.23 KM NW OF AYR	-19.43333	147.11667
15	31296	Colluricincla megarhyncha	QLD	MOUTH OF CRYSTAL CREEK, HALIFAX BAY, SE OF INGHAM	-18.92694	146.31833
16	31297	Colluricincla megarhyncha	QLD	MOUTH OF CRYSTAL CREEK, HALIFAX BAY, SE OF INGHAM	-18.92694	146.31833

No.	ID	Species	State	Location	Latitude	Longitude
17	31389	Colluricincla megarhyncha	QLD	CREB TRACK, HILDA CREEK AREA, C.16 KM NNE OF DAINTREE	-16.16389	145.30556
18	31390	Colluricincla megarhyncha	QLD	CREB TRACK, HILDA CREEK AREA, C.16 KM NNE OF DAINTREE	-16.16389	145.30556
19	31423	Colluricincla megarhyncha	QLD	S FOOT OF MT COOK, COOKTOWN	-15.49889	145.27222
20	31424	Colluricincla megarhyncha	QLD	S FOOT OF MT COOK, COOKTOWN	-15.49889	145.27222
21	31487	Colluricincla megarhyncha	QLD	C.2 KM N OF COOKTOWN AIRPORT	-15.43722	145.17750
22	31537	Colluricincla megarhyncha	QLD	RIDGE ABOVE SHIPTONS FLAT, C.50 KM S OF COOKTOWN	-15.79167	145.23333
23	31563	Colluricincla megarhyncha	QLD	RIDGE ABOVE SHIPTONS FLAT, C.50 KM S OF COOKTOWN	-15.79167	145.23333
24	31564	Colluricincla megarhyncha	QLD	RIDGE ABOVE SHIPTONS FLAT, C.50 KM S OF COOKTOWN	-15.79167	145.23333
25	32707	Colluricincla megarhyncha	QLD	WENLOCK RIVER, N OF WEIPA, CAPE YORK PENINSULA	-12.26667	141.90000
26	32721	Colluricincla megarhyncha	QLD	WENLOCK RIVER, N OF WEIPA, CAPE YORK PENINSULA	-12.12083	141.89667
27	33536	Colluricincla megarhyncha	NT	KOOLPINYAH STATION, E OF DARWIN	-12.42000	131.22417
28	33537	Colluricincla megarhyncha	NT	KOOLPINYAH STATION, E OF DARWIN	-12.42000	131.22417
29	33611	Colluricincla megarhyncha	NT	KOOLPINYAH STATION, E OF DARWIN	-12.37556	131.21472
30	33712	Colluricincla megarhyncha	NT	KOOLPINYAH STATION, E OF DARWIN	-12.41667	131.21139
31	33713	Colluricincla megarhyncha	NT	KOOLPINYAH STATION, E OF DARWIN	-12.41667	131.21139
32	41380	Colluricincla megarhyncha	QLD	5 KM NE OF SARINA, S OF MACKAY	-21.40000	149.24167
33	41384	Colluricincla megarhyncha	QLD	5 KM NE OF SARINA, S OF MACKAY	-21.40000	149.24167
34	41423	Colluricincla megarhyncha	QLD	DALRYMPLE SCHOOL HOUSE, CLARKE RANGE, W OF MACKAY	-21.10000	148.52222
35	41430	Colluricincla megarhyncha	QLD	DALRYMPLE SCHOOL HOUSE, CLARKE RANGE, W OF MACKAY	-21.10000	148.52222
36	41517	Colluricincla megarhyncha	QLD	KILLYMOON CREEK, 25 KM S OF TOWNSVILLE	-19.40000	146.98333
37	41533	Colluricincla megarhyncha	QLD	3 KM S OF CAPE CLEVELAND, S OF TOWNSVILLE	-19.28333	147.03333
38	42892	Colluricincla megarhyncha	QLD	EASTERN MCILWRAITH RANGE LOWLANDS, CAPE YORK PENINSULA	-13.88611	143.50833
39	42923	Colluricincla megarhyncha	QLD	EASTERN MCILWRAITH RANGE LOWLANDS, CAPE YORK PENINSULA	-13.55000	143.53889
40	43259	Colluricincla megarhyncha	NSW	ORARA RIVER, COUTT'S CROSSING, N OF COFFS HARBOUR	-29.98611	152.89778
41	43302	Colluricincla megarhyncha	NSW	ORARA RIVER 15 KM N OF GLENREAGH, N OF COFFS HARBOUR	-29.92139	152.93611

#	Species	State	Locality	Latitude	Longitude
42	Colluricincla megarhyncha	NSW	15KM N OF GLENREAGH, N OF COFFS HARBOUR	-29.92139	152.93611
43	Colluricincla megarhyncha	QLD	KROOMBIT TOPS, DAWES RANGE PLATEAU	-24.37222	150.99444
44	Colluricincla megarhyncha	QLD	SHOALWATER BAY ARMY TRAINING RESERVE, N OF ROCKHAMPTON	-22.80972	150.60000
45	Colluricincla megarhyncha	QLD	SHOALWATER BAY ARMY TRAINING RESERVE, N OF ROCKHAMPTON	-22.80556	150.60000
46	Colluricincla megarhyncha	QLD	SHOALWATER BAY ARMY TRAINING RESERVE, N OF ROCKHAMPTON	-22.67361	150.67667
47	Colluricincla megarhyncha	QLD	SHOALWATER BAY ARMY TRAINING RESERVE, N OF ROCKHAMPTON	-22.67750	150.34500
48	Colluricincla megarhyncha	NSW	BIG SCRUB, WHIAN WHIAN STATE FOREST, TWEED VALLEY	-28.63611	153.32500
49	Colluricincla megarhyncha	NT	TARACUMBI FALLS, 22 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.60389	130.71250
50	Colluricincla megarhyncha	NT	36 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.71972	130.67333
51	Colluricincla megarhyncha	NT	36 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.71972	130.67333
52	Colluricincla megarhyncha	NT	36 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.71972	130.67333
53	Colluricincla megarhyncha	NT	TARACUMBI FALLS, 22 KM S OF SNAKE BAY, MELVILLE ISLAND	-11.60389	130.71250
54	Colluricincla megarhyncha	NT	KOOLPINYAH STATION, E OF DARWIN	-12.37556	131.21472
55	Colluricincla megarhyncha	NT	KOOLPINYAH STATION, E OF DARWIN	-12.37556	131.21472
56	Colluricincla megarhyncha	NT	MCARTHUR RIVER KING ASH BAY	-15.922	136.518
57	Colluricincla megarhyncha	NT	1 KM W BATTEN CK	-15.883	136.512
58	Colluricincla megarhyncha	NT	1 KM W BATTEN CK	-15.883	136.512
59	Colluricincla megarhyncha	NT	ROPER RIVER	-14.742	135.292
60	Colluricincla megarhyncha	PNG	HAIA, 2 KM WNW; TAU-TELO CAMP	-6.69583	144.97517
61	Colluricincla megarhyncha	PNG	HAIA, 2 KM WNW; TAU-TELO CAMP	-6.69583	144.97517
62	Colluricincla megarhyncha	PNG	13.5 KM 317 SW FROM VIAKU VILLAGE, COLLINGWOOD BAY, W BANK WAI-IO-O RIVER, BASE MT. SUCKLING	-9.54667	149.07000
63	Colluricincla megarhyncha	PNG	13.5 KM 317 SW FROM VIAKU VILLAGE, COLLINGWOOD BAY, W BANK WAI-IO-O RIVER, BASE MT. SUCKLING	-9.54667	149.07000
64	Colluricincla megarhyncha	PNG	40 KM 10 DEGREES FROM KIKORI AIRSTRIP, SIREBI RIVER, DARK END CAMP	-7.06833	144.31167
65	Colluricincla megarhyncha	PNG	116 KM 314 DEGREES FROM MANDANG AIRSTRIP, TIKIAM CAMP	-4.48250	145.03167
66	Colluricincla megarhyncha	PNG	116 KM 314 DEGREES FROM MANDANG AIRSTRIP, TIKIAM CAMP	-4.48250	145.03167

Note: entries 60, 61, 62, 63, 64, 65, 66 are prefixed with *

#	Species	Location	Latitude	Longitude
1	Zosterops lateralis	ATHERTON, ATHERTON TABLELAND	-17.26667	145.48333
2	Zosterops lateralis	CHELMAN'S ROAD, CLARKE RANGE, W OF MACKAY	-21.03333	148.56667
3	Zosterops lateralis	MIDDLE ISLAND, ARCHIPELAGO OF THE RECHERCHE	-34.10000	123.18333
4	Zosterops lateralis	SHOALWATER BAY ARMY TRAINING RESERVE, N OF ROCKHAMPTON	-22.67750	150.34500
5	Zosterops lateralis	CAPE BORDA, KANGAROO ISLAND	-35.75000	136.58333
6	Zosterops lateralis	5 KM W OF BOOL LAGOON, SW OF NARACOORTE	-37.15000	140.63333
7	Zosterops lateralis	CAROLINE FOREST AREA, 23 KM SE OF MT GAMBIER	-37.95278	140.96167
8	Zosterops lateralis	TABLELAND ROAD, C.20 KM ENE OF TENTERFIELD	-29.01667	152.25000
9	Zosterops lateralis	ARM RIVER FORESTRY CAMP, MAGGS MOUNTAIN, SW OF MOLE CREEK	-41.69139	146.20556
10	Zosterops lateralis	WAIT-A-WHILE ROAD, SW OTWAY RANGE	-38.68861	143.46944
11	Zosterops lateralis	CULLEN BULLEN STATE FOREST, C.25 KM NW OF LITHGOW	-33.28333	150.05000
12	Zosterops lateralis	BOSTON BAY, C.6 KM N OF PORT LINCOLN, EYRE PENINSULA	-34.66722	135.85278
13	Zosterops lateralis	MT CRAWFORD FOREST, NORTH MT LOFTY RANGE	-34.70972	138.82806
14	Zosterops lateralis	PEG IN STUMP ROAD, MEBBIN STATE FOREST, TWEED VALLEY	-28.46306	153.16083
15	Zosterops lateralis	23 KM SE OF ELLISTON, EYRE PENINSULA	-33.74944	135.09000
16	Zosterops lateralis	THEVENARD AREA, CEDUNA	-32.14889	133.65500
17	Zosterops lateralis	MILLEWA STATE FOREST, C.19 KM E OF MATHOURA	-35.78917	145.06083
18	Zosterops lateralis	RIDGE ABOVE SHIPTONS FLAT, C.50 KM S OF COOKTOWN	-15.79167	145.23333
19	Zosterops lateralis	BROCKMAN STATE FOREST, C.12 KM ENE OF PEMBERTON	-34.46222	116.10972
20	Zosterops lateralis	TOOMPUP SOUTH ROAD, 15 KM S OF ONGERUP/GNOWANGERUP ROAD	-34.08917	118.47139
21	Zosterops lateralis	BARREN GROUNDS NATURE RESERVE	-34.68333	150.70000
22	Zosterops lateralis	5 KM NW OF BAIRNSDALE	-37.80000	147.58333
23	Zosterops lateralis	WEST MACKAY	-21.15000	149.16667
24	Zosterops lateralis	BANNITUP LAKE AREA, C.17 KM E OF ESPERANCE	-33.81833	122.06667
25	Zosterops lateralis	C.43 KM NW OF MT BARKER	-34.42361	117.27472

26	50303	Zosterops lateralis		HUME HIGHWAY, 6 KM E OF JUGIONG	-34.81944	148.37222
27	* 6094	Zosterops lateralis	WA			
28	* ATP07-44	Zosterops lateralis		NEW ZEALAND		
29	LSUMNS 45835	Zosterops lateralis		VANUATU		