Phylogeny and evolution of the Meliphagoidea, the largest radiation of Australasian songbirds.

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Abstract

The Meliphagoidea comprises the largest radiation of Australasian passerines. Here we present the first detailed molecular phylogenetic analysis of its families and genera, particularly the Acanthizidae, using sequences from nine gene regions including both mitochondrial and nuclear DNA. Our results support some suggested relationships but challenge other groupings, particularly in Meliphagidae and Acanthizidae. Maluridae is sister to all other members of the superfamily. We provide the first strong molecular evidence for bristlebirds, *Dasyornis*, as a separate family, Dasyornithidae, sister to Acanthizidae + Pardalotidae + Meliphagidae. Pardalotidae is sister to Acanthizidae, but whether it is retained as a separate family is arbitrary. The meliphagid genus *Lichenostomus* is polyphyletic. We find no support for the current subfamily structure within Acanthizidae but recognise a clade that includes members of the subfamily Sericornithinae excluding *Oreoscopus* and *Acanthornis*. Subfamily Acanthizinae is paraphyletic. Surprisingly, the Tasmanian island endemic *Acanthornis magnus* of mesic habitats is sister to the *Aphelocephala* of mainland Australian xeric zones.

1. Introduction

Some 5,740 species of birds comprise the largest avian order, the Passeriformes, or perching birds. Its largest suborder, the oscine songbirds, originated in the Australo-Papuan region (Sibley and Ahlquist 1985; Barker et al. 2002, 2004; Ericson et al. 2002). Two main subgroups of oscines are recognized. One is the "Corvida", a paraphyletic grade of mainly Australian and New Guinean (hereafter Australo-Papua) lineages diverging basally in the oscine tree and representing a Gondwanan radiation within it. Dispersal from Australo-Papua or Africa of one of the most recently diverging corvidan lineages gave rise to the Passerida (see Jønsson et al. 2007; Jønsson and Fjeldsa 2006a). The Passerida are a monophyletic group which today contain the bulk of passerine diversity and comprise the majority of northern hemisphere passerines and secondary radiations in the southern hemisphere.

Here we focus on the Meliphagoidea, the largest radiation of Australasian passerines. It is one of five basal lineages of oscine songbirds (Barker et al. 2004; Hackett et al. 2008), others being Menuridae (lyrebirds), Climacteridae (treecreepers) plus Ptilonorhynchidae (bowerbirds), Pomatostomidae (babblers) and Orthonychidae (logrunners). The Meliphagoidea contains some 276 species of which ca. 145 are Australian. Although the centre of diversity of the superfamily is in Australia and New Guinea some species occur in the south Pacific. Currently, four families (Maluridae, Meliphagidae, Acanthizidae, Pardalotidae) are recognized (Schodde and Mason 1999; Christidis and Boles 2008). They display great diversity in ecology, morphology and behaviour, and occupy a broad range of habitats from desert to rainforest.

The Maluridae, comprising grasswrens (*Amytornis*), fairy-wrens (*Malurus, Clytomyias, Sipodotus*) and emu-wrens (*Stipiturus*), are a distinctive and divergent group of very small to small (most 5-10g, largest 40g) insectivores that feed predominantly on the ground or in low, dense vegetation; a few grasswrens have bills adapted for granivory (Rowley and Russell, 1997). They are weak fliers, with characteristically long, often cocked tails and long legs. All are sexually dimorphic. Of the 27 species of malurid, five are restricted to New Guinea (*Malurus grayi* broad-billed fairy-wren, *M. cyanocephalus* emperor fairy-wren, *M. alboscapulatus* white-shouldered fairy-wren, *Clytomyias insignis* orange-crowned wren and *Sipodotus wallacii* Wallace's wren), while the remaining 22 species are Australian.

The Acanthizidae are a diverse assemblage of very small to medium-sized, primarily insectivorous Australo-Papuan passerines. In total there are 63 spp in 14 genera of which 7 are monotypic. The Australian fauna comprises 41 spp in 13 genera, 10 of which are endemic. The systematic position of *Dasyornis*, the bristlebirds (3 species), has long been contentious. At present they are included in this family although they differ in morphology and are widely considered to warrant a separate family (see Schodde and Mason 1999; Christidis and Boles 2008). This proposal needs confirmation and is addressed in the current work.

The Pardalotidae has just four species in one genus, *Pardalotus* and all are Australian. They are small (7-14g), hollow-nesting insectivores that feed in the foliage of *Eucalyptus*. Convergent morphological similarities with the Asian-Pacific flowerpeckers led to their erroneous placement in the Dicaeidae, but DNA studies have since revealed that they are a specialised, divergent group allied to Acanthizidae (review in Schodde and Mason, 1999).

The Meliphagidae (honeyeaters) are by far the dominant family, with approximately 182 species in 42 genera. There are more than 70 Australian species and over 60 in New Guinea. A few species occur in the South Pacific and New Zealand. One species occurs north of Wallace's Line, as far west as Bali (Coates and Bishop, 1997). This family displays great diversity in size and morphology but most have a characteristic, long, narrow, curved bill adapted for nectar feeding. Many species combine nectarivory and insectivory.

To date there has been no detailed phylogenetic study of the entire Meliphagoidea. Cracraft and Feinstein (2000) showed it to be monophyletic, as suggested by earlier authors (Sibley and Ahlquist, 1990; Christidis and Schodde, 1999) and subsequently affirmed (Driskell and Christidis, 2004; Norman et al., in press). Other studies have examined the relationships within particular families or among particular genera (e.g., Meliphagidae: Driskell and Christidis, 2004; Norman et al., 2007; *Sericornis*: Christidis et al., 1988; Christidis and Schodde, 1991; Joseph and Moritz, 1993; thornbills *Acanthiza*: Nicholls, 2001; Nicholls et al., 2000; fairywrens *Malurus* and Maluridae: Christidis and Schodde, 1997). At present we have a poor understanding of the systematic relationships both within and between the constituent families. There is confusion about which shared traits are the result of convergence, which states are ancestral and which are derived. This is particularly evident in the Acanthizidae where the arrangement of genera has been based largely on morphological data and is complex and controversial. Most contentious are the systematic placement of seven monotypic acanthizid genera: pilotbird *Pycnoptilus*, rock warbler *Origma*, fernwren *Oreoscopus*, scrubtit *Acanthornis*, redthroat *Pyrrholaemus*, speckled warbler *Chthonicola* and weebill *Smicrornis*. Accordingly, there is a strong need to determine whether molecular data can clarify relationships,

especially within the Acanthizidae, as well as more broadly among the families of the Meliphagoidea.

Here we construct a phylogenetic hypothesis for the Meliphagoidea and examine systematic relationships among families and taxonomically unstable genera, particularly those in the Acanthizidae. We concentrate largely on the Australian species, with a few representatives from New Guinea. From the Meliphagidae (honeyeaters), we have included only 30 species in 13 genera as our aim was to understand relationships of honeyeaters to the other meliphagoid families rather than to address systematics within Meliphagidae. However, our results do permit some significant conclusions about generic relationships within Meliphagidae. We sample the three other families in greater depth, having 20 species in four genera of Maluridae, 45 species in 15 genera of Acanthizidae *sensu lato*. and three species of Pardalotidae.

2. Materials and Methods

2.1 Taxa and data

Species and gene sequence regions initially were selected according to whether data were held in the GenBank 'nr' database. Every GenBank record from Maluridae, Acanthizidae and Pardalotidae was examined, and genera of Meliphagidae were selected to sample broadly across the phylogeny proposed by Driskell and Christidis (2004). Four species from the families Menuridae, Climacteridae and Ptilonorhynchidae were chosen as outgroups to root a meliphagoid tree.

We did additional sequencing to extend both the number of species sampled and the number of species sequenced for each gene. We did not aim for complete

coverage of all genes previously sequenced for any species. We restricted our sequencing to five genes chosen as potentially informative of relationships across a range of time intervals, and so extended the taxonomic range of the data set. Each species and gene sequenced was represented by at least two specimens and most by several. Tissue for sequencing was sourced from the Australian National Wildlife Collection, Canberra.

2.2 Molecular Methods

DNA extraction, PCR amplification and sequencing

DNA was extracted from preserved tissue with standard Proteinase K digestion and precipitation methods (Bruford *et al.*, 1998). Fragments of the nuclear genes RAG-1 and RAG-2 and the mitochondrial 12S ribosomal RNA gene were amplified with PCR primers designed using avian gene sequences obtained from GenBank. Approximately 1400bp of the first exon of RAG-1 were amplified with the primers RAG-1-F1b (aaaaacagcctctgatgacagt) and RAG-1-R2 (tcccacttctgtgttagtgga); approximately 1100bp of the single exon of RAG-2 were amplified with the primers RAG-2-F1 (gaagagatcctgccccact), and RAG-2-R2 (cacgtgatccagtagcctgt); and approximately 1000bp of the mtDNA 12S gene were amplified with the primers L1276mod (cactgaagatgtcaagatgg) a modification of L1276 in Driskell and Christidis (2004), and trnVR (tcaggtgtaagctgaatgc). Fragments of the mitochondrial ND2 and CO1 genes were amplified using primers from Sorenson et al. (1999). Approximately 1200bp of ND2 were amplified with the primers L5143 and H6313 and approximately 1550bp of CO1 were amplified with the primers L6615 and H8121.

Twenty microlitre PCRs contained 1U of Taq DNA polymerase and reaction buffer at 1X concentration (Qiagen), $MgCl_2$ at 1.5mM to 3.0mM, forward and reverse primers at 0.2μM, the four dNTPs each at 0.2mM and approximately 40ng of genomic DNA template. Cycling conditions were the same for all reactions: an initial three minute denaturing step was followed by 38 amplification cycles comprising 30 seconds of denaturing at 94°C, 30 seconds of annealing, initially at 66°C then reduced by 3°C every third cycle to reach a final annealing temperature of 48°C, and 45 seconds of extension at 72°C.

Sequencing templates were prepared by precipitating PCR products with ammonium acetate and ethanol and resuspending in water. Templates were sequenced with the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems) and electrophoresed and detected with an Applied Biosystems 3100 genetic analyzer. With the exception of CO1 templates (see below), we sequenced both strands of all templates with the primers used for their amplification and with additional internal primers. RAG-1 templates were sequenced with the internal pair of primers RAG-1-F2 (gattctgtcacaactgttggagt) and RAG-1-R1 (ccttgtcaaagacaggaggt), and RAG-2 and 12S templates were sequenced with the internal reverse primers RAG-2-R1 (gtagccaccaacaaggaca) and 12S-R2(caggcatagtggggtatcta) respectively. ND2 templates were sequenced with the internal primers L5758 and H5766 (Sorenson et al. 1999). CO1 templates were not sequenced off the H8121 primer used for their amplification but were sequenced with the other member of the amplification pair, L6615, and the internal primers L7036, L7122 and H7548 (all from Sorenson et al. 1999).

Base calls were checked and edited by visualizing and aligning the multiple chromatograms for each sample for each gene into contigs using Sequencher v3.1

(Genecodes). The redundancy provided by sequencing each template off multiple primers, such that most sequence positions were represented in two or more chromatograms, facilitated editing and provided unambiguous verification of most of the sequence we generated. A few 12S templates could not be sequenced effectively off the outside reverse primer trnVR, presumably due to a long run of consecutive cytosine bases that were apparent in these templates near the trnVR priming position. The partial 12S sequences for these samples therefore comprised approximately 800bp spanned by the forward primer L1276mod and the internal reverse primer 12S-R2.

2.3 Phylogenetic methods

2.3.1 Sequence selection and alignment

From GenBank we obtained data from nine separate gene regions for between two and 56 species. The regions represented mitochondrial genes 12S rDNA, 16S rDNA, CO1, ND2, and Cyt *b*, nuclear protein coding genes RAG-1 and RAG-2, and nuclear introns from Gd3ph and Beta Fibrinogen (Beta5 intron). In some cases more than one accession for a gene region from a species was available in GenBank. We downloaded all available accessions and constructed a multiple alignment using either CLUSTAL (Thompson et al., 1994) or MUSCLE (Edgar 2004) at default settings. This alignment was examined both by pairwise distances and by maximum likelihood trees estimated in GARLI v0.951 (Zwickl, 2006), at default settings, to confirm that accessions annotated as being from the same species clustered together. Suspect sequences were discarded and a single representative sequence was chosen from the

remaining sequences for each gene, for each species, based on the length and quality of the accession.

Sequences newly estimated by us were aligned together with all previously selected GenBank sequences and compared using both pairwise distances and maximum likelihood analyses in GARLI. Again, one sequence was chosen to represent each gene, for each species, based on the length and quality of the edited sequence product.

2.3.2 Sequence Alignment, noise reduction and data matrix

Each of our nine gene regions was aligned separately using an alignment cost-minimising program, either CLUSTAL or MUSCLE, at default settings, to obtain an initial alignment. This cost-minimising alignment was then adjusted by hand as necessary to preserve structural features. In protein-coding regions we retained the triplet pattern of codons. In rDNA and intron sequence we aimed at consistency of alignment across taxa within repetitive regions. The sequences from ribosomal genes and introns were not so dissimilar, across our taxa, as to require alignment methods based on secondary structure prediction. For aligned protein coding genes we used MacClade v3.08 (Maddison, 1992) to test for appropriate translation into protein. The other loci were examined in light of Morrison's (2006) concept of a phylogenetic alignment; regions in which alternative, plausible hypotheses about past evolutionary events would imply a change to the alignment were excluded from analysis. Several such short, ‘unalignable’ regions were found in the 12S rDNA alignment but none in the other loci. Each aligned region was also examined for any parsimony-informative patterns of shared indels, and these were coded as a small set of additional, binary

characters for use in cladistic parsimony analysis. Because some Genbank accessions had different start or end points some segments of some alignments represented data from fewer than four taxa. These uninformative segments were removed and the nine aligned gene regions were concatenated into a single matrix.

We investigated the effect of saturation (multiple hits) by two methods. First, we made a series of scatter plots of pairwise transition-transversion ratios against the uncorrected (p)-distance and against GTR distances. These results suggested that mtDNA third codon positions were saturated. Accordingly, we made phylogenetic estimates in three ways: from the entire gene region, from codon positions 1+2, and from codon position 3. Bootstrap sampling (Felsenstein, 1985) was used to measure branch support. The phylogeny was not affected by the inclusion or exclusion of the saturated characters. Third codon positions mostly supported shallow nodes that were supported by other characters and failed to provide support toward the base of the tree. Only in one case was a group resolved differently, and equivocally, by the full data than by mtDNA third codon positions alone (bootstrap scores of 88% vs 56% for two alternative arrangements of three *Pardalotus* species).

As a second and, we think, a novel method of assessing the effect of noise from saturation we calculated a maximum-likelihood tree using the whole of the data and then used the 'reweight characters' command in PAUP 4.0b10 (Swofford, 2002) to identify characters which are strongly homoplasious on that tree. We built ‘exclusion sets’ for characters showing a rescaled consistency index of either 0, or <0.1, or <0.3, and ran maximum likelihood bootstrap analyses on those exclusion sets. Each exclusion set included some but not all mtDNA third codon positions as well as some characters from other parts of the data. Bootstraps showed that each exclusion set contained hierarchical signal consistent with non-excluded data, chiefly relating to

shallow nodes, and no signal that was significantly in conflict. We conclude that both methods of noise reduction are equivalent and character set exclusion to counter saturation is not necessary.

To examine base usage we evaluated each gene separately and, in mtDNA protein-coding genes, we evaluated first and second coding positions separately from thirds. We ran chi-square tests for homogeneity of base usage using the 'base frequencies' command in PAUP. Most tests indicated stationarity except those for third positions in Cyt *b* and ND2. Therefore we ran paired sets of maximum likelihood bootstraps, one using data from all characters showing homogeneity of base usage, and the other using either Cyt *b* or ND2 third positions. No bootstrap of ≥70% in one run was contradicted at ≥70% in the other run of either pair, except that Cyt *b* third positions supported alternative arrangements of four species of *Malurus*. For our stated aims and scope, it is not necessary to exclude either Cyt *b* or CO1 third codon position characters from the analysis.

Dasyornis broadbenti was represented in the alignment by six genes excluding CO1 and *D. brachypterus* by CO1 alone. All tree-estimation algorithms showed these two taxa adjacent to each other, but for lack of data in common they did not form a group. We therefore assumed monophyly of *Dasyornis* and analysed a single composite terminal taxon "*Dasyornis* spp". Our final matrix thus has 101 not 102 terminal taxa.

2.3.3 Tree estimation

We tested whether our phylogenetic trees were robust to variation in the method of analysis and choice of an evolutionary process model. First, we used

unweighted and 2:1 transversion-weighted parsimony in PAUP 4.0b10 (Swofford, 2002). Binary characters describing shared indels were included in these analyses. A parsimony ratchet search procedure was used as described by Nixon (1999) and batch commands for the PAUP searches were created using PAUPMacRat (Sikes and Lewis, 2001). Bootstrap runs used PAUP's fast-heuristic algorithm with 1000 bootstrap pseudoreplicates.

Second, two maximum likelihood searches were run in GARLI 0.951 (Zwickl, 2006) using a single data partition with the GTR+I+G model of evolutionary process and parameter values estimated from the data. Bootstrap runs used 100 pseudoreplicates. A five-partition maximum likelihood analysis of the final data set was conducted in RAxML (Stamatakis et al., 2005, 2008) on the CIPRES supercomputer (Portal 1) at <www.phylo.org>. The data were partitioned into nuclear coding, nuclear non-coding, mitochondrial ribosome, mitochondrial first plus second coding position, and mitochondrial third codon position characters. Each partition was given its own overall rate and set of base-change relativities. A bootstrap run under the same model used 100 pseudoreplicates.

Third, a single-data-partition Bayesian likelihood analysis was conducted in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) on the CIPRES supercomputer (Portal 1). Settings were 2 runs at 4 chains per run, chain temperature 0.2, for 2m generations sampled every 1000 generations. A four-partition Bayesian likelihood analysis was conducted in MrBayes 3.1 on an Intel Mac. The data were partitioned into nuclear coding, nuclear non-coding, mitochondrial ribosomal, and mitochondrial protein coding characters. Each partition was given its own set of GTR+I+G parameters and rates were unlinked (a 19-parameter model). The analysis

was run until well past the point of apparent convergence to a total of 8 million generations. Trees were sampled every 1000 generations.

A singularly unexpected result concerning relationships of the acanthizid genera *Acanthornis* and *Aphelocephala* prompted re-estimation of species-level relationships in that part of the tree. We used a new method for joint estimation of gene trees and their species tree within a Bayesian framework (Edwards 2009). Edwards (2009) has described this methodological advance as a paradigm shift in phylogenetics, but at present the calculations can only be done on small numbers of taxa. We used MBBEST (Liu and Pearl, 2007; Liu et al., 2008; see also Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) to examine a four-taxon subset of our data (*Acanthornis magnus, Aphelocephala leucopsis, Sericornis citreogularis*, and *Hylacola pyrrhopygia* as outgroup), *Sericornis* being the genus to which *Acanthornis* has often been aligned or synonymised. The data were partitioned into the five genes for which we had complete data for these taxa. MtDNA third codon positions were not treated separately because they must be inherited as a unit together with first and second codon position characters. Haploid and diploid sources were identified to the program and an unlinked, six-rate model with invariant positions and gamma-distributed rates (GTR+I+G) was applied to each partition (a 35-parameter model). The analysis was run for 10 million generations with two runs and four chains per run. We also ran a 10-taxon subset which comprised those four species plus *Pycnoptilus floccosus, Oreoscopus gutturalis, Acanthiza pusilla, Gerygone mouki* and *Smicrornis brevirostris*, with *Pardalotus striatus* as outgroup. The 10-taxon analysis was set up in the same way and run for 100m generations with two runs and two chains per run, but the MCMC process failed to converge.

3. Results

3.1 Taxa and Data

GenBank provided sequence data from nine gene regions for between two and 56 of the 102 selected species. To these sequences ex GenBank we were able to add 36 species for 12S rDNA, 35 for RAG-1, 36 for RAG-2, 45 for CO1 and 56 for ND2. Our data matrix contains 12S rDNA sequence from 57 species, 16S rDNA from 20 species, CO1 from 52 species, ND2 from 93 species, Cyt *b* from 56 species, RAG-1 from 40 species, RAG-2 from 38 species, Gd3ph from 16 species and Beta5 from 31 species. GenBank accession numbers of the sequences used in our analyses are listed in Table 1. [Lab codes will be replaced by GenBank codes prior to publication.]

The final, aligned data matrix is available at [supp. information; insert web address]. This matrix of 101 taxa and 8974 alignment positions is 43% complete, with 57% of cells coded as either alignment gaps or non-sequenced genes. Character sets, taxon sets showing the taxon coverage for each gene region, and the RC≤0.1 exclusion set from the second of the noise reduction experiments, are listed in a PAUP block at the end of the matrix. Notes on data provenance, alignment, and sequence choice are provided as comments within the DATA block. The preliminary alignment results, saturation plots and other noise-reduction results are not reported here.

3.2 Phylogenetic results

All phylogenetic methods and models applied to the final data set yielded similar trees. No method gave any relationships at a bootstrap score ≥70% or a posterior probability ≥80% that was contradicted at that level by any other method except in *Malurus* (see Methods above). Bootstrap scores and clade credibility values never declined and for some nodes improved as additional parameters were added, and we suspect, given the disparate nature of our data and the non-linearity of our pairwise GTR distance vs. transition-transversion ratio plots, that even our most-comprehensive models (RAxML 5-partition model and MrBayes 4-partition model) are under-parameterised. Nonetheless the tree topology is stable across the entire range of our analyses: only some bootstrap and clade credibility scores might be under-estimated.

Transversion-weighted parsimony with heuristic search via the parsimony ratchet gave 201 best-fit trees (Fig. 1). A fast heuristic bootstrap indicated support >70% for every branch shown in Figs. 2-3 as having good support by either maximum likelihood or Bayesian methods. Maximum likelihood using GARLI and a single data partition, with process model GTR+I+G, gave an identical tree topology to that in Fig. 1. Branch lengths were not noticeably different from the lengths in Fig. 1. Bootstrap support was >70% for every branch in Figs. 2-3 that is reported there with a bootstrap score within that range. The GARLI bootstrap score was 100% for every branch having a bootstrap score of 100% in the RAxML results. Partitioned maximum likelihood analysis using RAxML gave the single best-fit tree in Fig. 1. Bootstrap scores in the RAxML bootstrap are reported as the second of the two scores on branches in Figs. 2-3. Partitioned Bayesian maximum-likelihood gave a single best tree topology very similar to that in Fig. 1, except that some branches poorly resolved under conventional ML were resolved equally poorly but differently under Bayesian

ML. Figs. 2-3 are built from the Bayesian consensus tree of 2001 trees, after eliminating the first 6000 trees or 6 million generations as 'burnin', and after collapsing any branch that had neither a clade credibility value (i.e., "posterior probability") ≥95% nor a bootstrap score in the RAxML bootstrap ≥70%.

Figs. 1 and 4 revealed a wholly unanticipated sister relationship between *Acanthornis* and *Aphelocephala*, at bootstrap score 97% and posterior probability 100%. Further testing of this hypothesis using MBBEST gave the species phylogeny in Fig. 4a in which a sister relationship between *Acanthornis* and *Aphelocephala* is supported with clade credibility score 98%. The various 10-taxon analyses in which the MCMC process failed to converge gave a lower score or else failed altogether to resolve the species-level tree. Fig. 4b shows a typical result. Significantly, every analysis that resolved *any* part of the species tree included within it a sister-group relationship between *Acanthornis* and *Aphelocephala*. Neither of those terminals ever associated with any other taxon.

4. Discussion

Based on sequences from nine gene regions of both mitochondrial and nuclear DNA we provide the first robust, well-resolved molecular phylogeny of the oscine songbird superfamily Meliphagoidea. Our results provide support for many suggested relationships within and between constituent families, but also challenge previously contentious groupings, particularly the sequence and arrangement of genera in the Acanthizidae, and the classification of Meliphagidae proposed by Driskell and Christidis (2004).

4.1 Family relationships within the Meliphagoidea

The Meliphagoidea are a monophyletic clade, within our analysis, with Maluridae sister to all the other members of the group as previously suggested (Sibley and Ahlquist, 1990, Cracraft and Feinstein, 2000; Jønsson and Fjeldså, 2006b). We provide the first strong molecular evidence consistent with recognition of bristlebirds as a separate family, Dasyornithidae (Johnstone and Storr, 2004). It is sister to the Acanthizidae + Pardalotidae + Meliphagidae assemblage (Driskell and Christidis 2004; Christidis and Boles, 2008). We retain the Pardalotidae as a separate family, sister to Acanthizidae, in accordance with Schodde and Mason's (1999) conclusion based largely on morphology and behaviour, and in contrast with Driskell and Christidis (2004) who placed Pardalotidae as sister to honeyeaters rather than acanthizids. However, our data suggest that the pardalotes could equally be included in an expanded Acanthizidae so the decision is arbitrary. Meliphagidae (honeyeaters) are more closely related to the Acanthizidae + Pardalotidae clade than to Maluridae, as also previously shown by Sibley and Ahlquist (1985) and Jønsson and Fjeldså (2006b). Bootstrap and Bayesian support scores for all of these family-level relationships are particularly strong (Fig. 2).

4.2 Maluridae

The Maluridae comprises two subfamilies: the Amytornithinae for *Amytornis* grasswrens and the Malurinae comprising *Malurus*, *Stipiturus* and *Clytomias*, as suggested by Christidis and Schodde (1997) on allozyme evidence. *Sipodotus* from New Guinea was not included here but is unremarkably considered part of the

Malurinae. The position of the monotypic *Clytomias* within Maluridae was not resolved by Schodde and Mason (1997), who presented various lines of evidence for its alignment with either *Stipiturus* or *Malurus*. Pending inclusion of *Sipodotus*, we show clearly that *Clytomias* is sister to *Malurus* (bootstrap 100%).

We agree with Christidis and Schodde (1997) that *Malurus* comprises two major clusters, although the component species are at odds with their analysis. Our analysis did not include two New Guinea species of *Malurus*, *M. grayi* (broad-billed) and *M. cyanocephalus* (emperor). One lineage we define comprises the morphologically distinct bicoloured wrens, *M. melanocephalus* (red-backed), and *M. leucopterus* (white-winged) (New Guinean species *M. alboscapulatus (*white-shouldered) not sampled)). The second lineage includes the remaining species. We have not been able to clearly ascertain the internal phylogenetic structure within this lineage except that, given our taxon sampling, the blue fairy-wrens *M. cyaneus* (superb fairy-wren) and *M. splendens* (splendid fairy wren) form a pair that in turn is most probably sister to *M coronatus* (purple-crowned fairy-wren). The chestnut-shouldered group *M. amabilis* (lovely fairy-wren), *M lamberti* (variegated fairy-wren) and *M. pulcherrimus* (blue-breasted fairy-wren) may be monophyletic as suspected. The position of *M. elegans* (red-winged fairy-wren) endemic to south-western Australia is equivocal: maximum-likelihood analysis places it as sister to the *amabilis-pulcherrimus-lamberti* group with bootstrap score 72% but Bayesian likelihood analysis places it as sister to all of our second group (*amabilis-pulcherrimus-lamberti-coronatus-cyaneus-splendens*) with posterior probability 0.60. The apparent non-stationarity in base usage across some mitochondrial third codon positions in this part of the tree (see deleted Methods) may be implicated here and more work is needed. In Fig. 3a we depict this part of the tree as unresolved.

458 Excluding *M. coronatus*, the blue wren and chestnut-shouldered groups share
459 iridescent blue plumage and semi-erectile blue ear tufts, and accordingly have been
460 thought to be closely related (Christidis and Schodde, 1997), as shown here. Of the
461 blue group, *M. cyaneus* and *M. splendens* are more closely related to one another than
462 to *M. coronatus*, reflecting these differences in plumage characters. In addition, a
463 recent study shows *M. coronatus* to be behaviourally distinct from all other *Malurus*
464 (Kingma et al., 2009).
465
466 *4.3 Meliphagidae (honeyeaters)*
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468 Branching patterns in our dataset of 30 species are in partial agreement with
469 those described by Driskell and Christidis (2004) who recovered four major clades
470 plus *Acanthorhynchus*, the spinebills, although the relationships among their four
471 groups were unresolved. In particular, our phylogeny supports *Acanthorhynchus* as
472 sister to all other honeyeaters, and we recover clade #1 of Driskell and Christidis
473 (2004): *Meliphaga* + *Acanthagenys* + *Anthochaera* + *Xanthomyza* + *Manorina* + a
474 majority of the genus *Lichenostomus* (Fig. 3b). Of their clade #2 we have only one
475 representative, *Ramsayornis fasciatu*s (bar-breasted honeyeater), and this is firmly
476 placed as sister to clade #1. Among the remaining taxa, Driskell and Christidis (2004)
477 recovered discordant phylogenetic signal between nuclear beta Fibrinogen intron and
478 the three mtDNA genes (Cyt *b*, ND2 and 12S). They resolved this by relying on the
479 Beta5 signal over the mitochondrial signal and constrained their tree search so as to
480 create their clade #3 (*Certhionyx*, *Myzomela*, *Glychichaera*, *Ptiloprora* and
481 *Phylidonyris (=Glyciphila) melanops*) separate from their clade #4. Of those five
482 genera, our data set includes only *Myzomela (M. obscura)* and *Phylidonyris* (*P.*

novaehollandiae and *P. nigra*) but we find them to be widely separated and nested deep within what would otherwise represent Driskell and Christidis' clade #4.

We investigated this discrepancy between our results and those of Driskell and Christidis (2004) by re-aligning their data for comparison with ours. We found no disagreement between phylogenetic signal from Beta5 and that from Cyt *b*, ND2 and 12S (we used the same Beta5 data from GenBank). Further, we note that Driskell and Christidis' decision to prefer the Beta5 signal over the other three genes was strongly influenced by two large indels in the Beta5 sequences, of 18 and 12 bases, shared among taxa they constrained as their clade #3. In our alignment there are no such shared indels, and neither is there a third indel of 15 bases that Driskell and Christidis report from *Stipiturus mallee*. In fact, at 598 aligned positions our phylogenetic alignment (*sensu* Morrison, 2006) of the Beta5 intron is 119 positions shorter than the Driskell and Christidis alignment, and 16 positions shorter than a default CLUSTAL alignment. We conclude that Driskell and Christidis (2004) used a suboptimal alignment that does not satisfy Morrison's (2006) later criteria of a phylogenetic alignment. Their topological constraint artificially grouped a set of genera, two at least of which we find should not be so grouped. In place of clades #3 and 4 of Driskell and Christidis (2004) we find a clade comprising *Myzomela*, *Philemon*, *Entomyzon*, *Melithreptus*, *Lichmera* and *Phylidonyris* within which all relationships are robustly supported (Fig. 3b).

Lichenostomus leucotis (white-eared honeyeater) also appears within this clade (*Entomyzon* + *Melithreptus)*. Its position here is notable given that its putative congeners appear, albeit in two widely separated places, within the group which Driskell and Christidis (2004) called clade #1. This species was represented in our study by two independent specimens sourced from the Australian National Wildlife

Collection, and was sequenced for ND2 and CO1. Both genes and both specimens support the sister relationship to *Entomyzon* + *Melithreptus*. Our findings that *Lichenostomus* is polyphyletic and *L. leucotis* is not closely related to either of two other clades in this genus are supported by independently derived molecular data (pers. comm. A. Nyari).

Schodde (1975) split the genus *Meliphaga* (*s.l.*) into three: *Lichenostomus*, *Xanthotis* and *Meliphaga (*s.s.*)*. Driskell and Christidis (2004) showed that the three genera do not form a monophyletic group, and foreshadowed major rearrangements. Our analysis, which includes eight *Lichenostomus* species and three *Meliphaga* species but not *Xanthotis*, confirms that *Meliphaga* and *Lichenostomus* are not each other's closest relatives. *Lichenostomus* is polyphyletic, forming two clades plus *L. leucotis*. *L. flavus* (yellow honeyeater) and *L. unicolor* (white-gaped honeyeater) are sister species. This is reflected in the similarity of their eggs, which differ from those of other *Lichenostomus* (Beruldsen 2003), and their song, with pairs in both species performing duets (Higgins at el., 2001). This pair of species in turn is sister to *L. melanops* (yellow-tufted honeyeater). The second clade, well separated from these by two strongly supported branches, comprises *L. virescens* (singing honeyeater), *L. flavescens* (yellow-tinted honeyeater), *L. penicillatus* (white-plumed honeyeater) and *L. ornatus* (yellow-plumed honeyeater*)*. Further work is being done to resolve the systematics of *Lichenostomus* and the other species-rich genera, and their relationships with other honeyeaters (A. Nyari, pers. comm.).

We recover *Entomyzon* as sister to *Melithreptus* as shown by Driskell and Christidis (2004). Previously, a close relationship between *Entomyzon* and the larger-bodied honeyeaters *Manorina* miners and *Anthochaera* wattlebirds had been suggested (Schodde 1975). Storr (1977; 1984) included it within *Melithreptus* which

533 it resembles but for its larger size. Driskell and Christidis (2004) sequenced two
534 *Melithreptus* species (*M. albogularis* and *M. brevirostris*); we added one additional
535 species, *M. lunatus* (white-naped honeyeater) represented in our data set by Cyt *b*
536 from Cracraft and Feinstein (2000) and new ND2 and CO1 sequences. Elsewhere, one
537 of us, LJ, will present a phylogeny of all *Melithreptus* (A. Toon and L. Joseph, in
538 prep).

539 We support Driskell and Christidis (2004) in finding that the Regent
540 honeyeater (*Xanthomyza phrygia*) is nested within the wattlebirds (*Anthochaera*) and
541 is more closely related to the large-bodied species, represented here by *A. carunculata*
542 (red wattlebird), than to the small-bodied species *A. chrysoptera* (little wattlebird),
543 and *A. lunulata* (western wattlebird). Similarly, *Phylidonyris novaehollandiae* (New
544 Holland honeyeater) and *P. nigra* (white-cheeked honeyeater) are sister species and
545 sister to *Lichmera indistincta* (brown honeyeater) as shown by Driskell and Christidis
546 (2004). Our data set does not include *P. melanops* (tawny-crowned honeyeater),
547 which Driskell and Christidis (2004) placed apart from its congeners, and which is
548 often placed in monotypic *Glyciphila*. We predict it will ultimately align with other
549 *Phylidonyris*.

550 We confirm the suspected close relationship between *Meliphaga lewinii*
551 (Lewin's honeyeater), *M. notata* (yellow-spotted honeyeater) and *M. gracilis*
552 (graceful honeyeater). *M lewinii* and *M. notata* are each other's closest relative and
553 sister to *M. gracilis*. This trio is morphologically very similar, all displaying
554 prominent yellowish gape stripes and yellow spots on their ear coverts, and
555 accordingly are easily confused in the field where their ranges overlap in the Wet
556 Tropics of north east Queensland. The three are, however, readily distinguishable by
557 vocalizations (Higgins et al., 2001). *M. gracilis* differs from the other two in its

smaller size and in the colour and patterning of its eggs, which are richly coloured as opposed to being plain with small spots (Higgins et al., 2001). This relationship supports previous suggestions that *M. lewinii* and *M. notata* represent an *in situ* allopatric speciation event (Christidis and Schodde 1993; Norman et al., 2007). In contrast, *M. gracilis* is thought to have dispersed into north-eastern Australia from New Guinea (Christidis and Schodde, 1993; Norman et al., 2007).

4.4 Pardalotidae

The small insectivorous, foliage-dwelling pardalotes are retained here as a separate family, reflecting morphological and molecular differences (Christidis and Schodde, 1991; Cracraft and Feinstein, 2000), as discussed elsewhere (Schodde and Mason, 1999). However, the decision is arbitrary, as the group could equally be retained as a subfamily in an extended Acanthizidae, reflecting their sister relationship.

4.5 Acanthizidae

We present the first molecular phylogeny of relationships within the family. It provides a novel but well-supported arrangement of genera. The previous, complex sequence of genera, which was based largely on morphological data, is controversial. The relative positions of the seven monotypic genera have been particularly problematic. Our data refute the traditional view that Acanthizidae comprises two subfamilies, the Sericornithinae (*Pycnoptilus, Origma, Oreoscopus, Crateroscelis, Sericornis, Acanthornis, Hylacola, Calamanthus, Pyrrholaemus, Chthonicola*) and Acanthizinae (*Smicrornis, Gerygone, Acanthiza, Aphelocephala*). Instead, we recover

583 a well-resolved set of generic relationships in which monotypic *Oreoscopus* is sister
584 to all other genera; *Gerygone* and the remaining genera are sister taxa; *Acanthornis* +
585 *Aphelocephala* are sister to *Acanthiza*, and the sister taxon to that set of three genera
586 includes the remaining group, with *Smicrornis* sister to the rest (Fig. 3c). Overall, our
587 data support a restricted subfamily Sericornithinae comprising a clade that excludes
588 *Oreoscopus* and *Acanthornis*, but subfamily Acanthizinae is paraphyletic. Most of our
589 changes from the previous classification result from new associations of the
590 monotypic genera.
591 We provide strong evidence that *Oreoscopus gutturalis*, the fernwren, is sister
592 to all other acanthizids. This species is endemic to the montane Wet Tropics of north-
593 eastern Australia and is osteologically divergent from all other acanthizids (Schodde
594 and Mason, 1999). Traditionally it was placed nearest to *Sericornis* (scrubwrens) and
595 the New Guinean *Crateroscelis* (mouse-warblers) (Schodde, 1975) although
596 differences in skull characters, egg patterning, call and bill morphology (which have
597 been suggested to be derived and adapted for foraging under litter) have clouded its
598 taxonomic position (Schodde and Mason 1999).
599 Previous arrangements have placed *Gerygone* with *Smicrornis*, *Acanthiza* and
600 *Aphelocephala* (review in Schodde and Mason 1999). However, our phylogeny places
601 *Gerygone* and the remaining acanthizid species as sister taxa, a relationship that is
602 well-supported by high bootstrap values and Bayesian posterior probabilities.
603 *Gerygone* is a morphologically distinct group that has the widest distribution of all
604 Acanthizidae, having radiated into New Zealand, Pacific islands, Indonesia and the
605 Philippines.
606
607 *4.5.1 Acanthornis–Aphecephala-Acanthiza assemblage*

We found strong support for a close relationship between *Acanthiza* (thornbills) and *Aphelocephala* (white-faces), as suggested elsewhere (Schodde and Mason 1999), but our finding that *Acanthornis* and *Aphelocephala* are sisters is remarkable. The scrubtit *Acanthornis magnus* is restricted to the wet forests of the continental island Tasmania and its offshore island King Is. and is insectivorous. In contrast, the three species of *Aphelocephala* are dry woodland, semi-arid and arid zone species of mainland Australia. They have bills and digestive tracts morphologically adapted for seed-eating (Schodde and Mason, 1999). In external phenotype, *Acanthornis* closely resembles some species of *Sericornis* but has little if any similarity to *Aphelocephala* (Fig. 5). Eggs of *Acanthiza*, *Aphelocephala* and *Acanthornis* are, however, similar and differentiated from *Sericornis.* Circumscription of *Sericornis* has variously been expanded to include *Acanthornis,* (e.g., Keast, 1978) or exclude it (see review in Christidis et al., 1988), although with little comment in either case. Schodde and Mason (1999) curiously remarked that *Acanthornis* is an "ancestral" form. They noted that among acanthizids it has at least one particularly divergent character of cranial osteology, well-developed vomerine horns. They placed it close to *Calamanthus* and *Hylacola* in a linear sequence of genera because of the karyological and protein data of Christidis (1990). This is the most explicitly argued previous hint that *Acanthornis* may not be close to *Sericornis*.

We took several steps to test the strong support in our analyses for the unexpected sister relationship between *Acanthornis* and *Aphelocephala*. First, we extracted and sequenced additional samples of *Acanthornis*; sequences generated were the same for all *Acanthornis* samples (n = 3). Next, we addressed recent concerns that under some circumstances the most likely gene tree for any given gene

633 is almost certain to differ from the true species tree (i.e., where there are short
634 branches deep within a tree, see Degnan and Rosenberg, 2006; Kubatko and Degnan,
635 2007). Our reanalysis with MBBEST (Liu and Pearl, 2007; Liu et al., 2008) yielded a
636 posterior probability of 0.98 for the *Aphelocephala + Acanthornis* relationship in a
637 four-taxon analysis. However, MBBEST provided no indication of a relationship
638 other than *Aphelocephala* and *Acanthornis* being sister taxa. The MBBEST results
639 (0.98 and 0.87 posterior probability; Fig. 4a/b) thus are consistent with the
640 conventional species tree estimation methods (Fig. 3c) that show 1.00 posterior
641 probability and 97% phylogenetic bootstrap score for this novel clade. We conclude
642 that *Aphelocephala* has diverged morphologically, possibly in part due to
643 morphological adaptations associated with granivory; *Aphelocephala* are the only
644 primarily granivorous species in the Acanthizidae, the rest are primarily insectivorous.
645 The thornbills, *Acanthiza*, are recovered as monophyletic. Although the deep
646 branches within this clade are not well resolved the arrangement of species largely
647 follows that suggested by Schodde and Mason (1999) based on plumage, behaviour
648 and voice, and subsequently the mtDNA data of Nicholls et al. (2000). Nicholls et al.
649 identified five clades with *A. robustirostris* (slaty-backed thornbill) sister to all other
650 *Acanthiza*. We recover *A. robustirostris* as sister to *A. iredalei* (slender-billed
651 thornbill), which reduces the five clades to four. We provide good support for this
652 group as sister to the *A. uropygialis* (chestnut-rumped thornbill) + *A. reguloides* (buff-
653 rumped thornbill) + *A. inornata* (western thornbill) assemblage, as suggested by
654 Nicholls et al. (2000). The suggested relationship of New Guinean *A. murina* (Papuan
655 thornbill) to *A. nana* (yellow thornbill) and *A. lineata* (striated thornbill) (Nicholls et
656 al., 2000) is also confirmed here, reflecting similarity in plumage patterns and
657 behaviour.

658

659 *4.5.2 Smicrornis, Pycnoptilus-Pyrrholaemus-Chthonicola, Calamanthus-Hylacola,*

660 *and the Origma-Crateroscelis-Sericornis complex*

661

662 In general, the relationships among species in this clade are uncontroversial

663 with most associations well-established. The two exceptions are the monotypic genera

664 *Smicrornis* (weebill) and *Pycnoptilus* (pilotbird) where affinities previously were

665 uncertain. We find that *Smicrornis* is sister to all other genera in this group.

666 *Smicrornis* was previously aligned with *Gerygone*, although a range of characters set

667 it apart (bill, skull, nest and eggs; Schodde and Mason, 1999) as well as its specialised

668 foraging niche. Presumably, the morphological character states by which it was

669 previously associated with *Gerygone* should now be re-interpreted as shared ancestral

670 traits (symplesiomorphies).

671 Monotypic *Pycnoptilus* is clearly placed in our analysis as sister to

672 *Pyrrholaemus* + *Chthonicola*. Traditionally, this species has been viewed as having

673 features both of the bristlebirds (*Dasyornis*) and the acanthizids, and accordingly it

674 has been seen as a "link" supporting the placement of *Dasyornis* within Acanthizidae.

675 Alternatively, *Dasyornis* -like character states have been suggested to result from

676 convergence (del Hoyo et al., 2007). Accordingly, various arrangements have been

677 suggested: Schodde (1975) and Schodde and Mason (1999) placed *Pycnoptilus* closest

678 to *Dasyornis* with both retained in Acanthizidae, although in different subfamilies,

679 whereas Christidis and Boles (2008) recognised *Dasyornis* as a separate family but

680 retained *Pycnoptilus* in the Acanthizidae pending molecular data. Our analysis

681 provides strong evidence for *Pycnoptilus* deep within Acanthizidae and separate from

682 *Dasyornis*, supporting the 'morphological convergence' hypothesis. Interestingly, all

683 three genera in this group have uniformly dark eggs, purplish in *Pycnoptilus* and
684 reddish in *Pyrrholaemus* + *Chthonicola* (Higgins and Peter 2002).
685 The relationships between *Hylacola* + *Calamanthus* and *Chthonicola* +
686 *Pyrrholaemus* and their association with *Sericornis* + *Crateroscelis* are as proposed
687 by Schodde and Mason (1999). Schodde and Mason merged the heathwrens *Hylacola*
688 with the fieldwrens *Calamanthus*. All methods we employed support these genera as
689 sister taxa. Whether *Hylacola* needs to be retained as a separate genus appears to be a
690 matter of choice. The clade *Hylacola* + *Calamanthus* is sister to the *Chthonicola* +
691 *Pyrrholaemus* + *Pycnoptilus* assemblage. We show that the two monotypic genera
692 *Chthonicola* (*C. sagittata* speckled warbler) and *Pyrrholaemus* (*P. brunneus*
693 redthroat) are each other's closest relatives, providing support for Schodde and
694 Mason's (1999) reversion of *Chthonicola* to *Pyrrholaemus*. This relationship is
695 reflected in the strong similarity in the appearance of the eggs (they are the only
696 acanthizids to have plain chocolate-brown eggs) and both species are the primary
697 hosts of the brood parasitic black-eared cuckoo (*Chrysococcyx osculans*). This sister
698 relationship is also supported by Christidis (1990).
699 The monotypic rockwarbler *Origma solitaria* is sister to the New Guinea
700 mouse-warblers *Crateroscelis* and they are sister to *Sericornis*. Previous arrangements
701 have placed *Origma* closer to *Pyrrholaemus* + *Chthonicola* and *Calamanthus* +
702 *Hylacola* as these genera share similarities in osteology, morphology and general
703 biology. In these characters they are closer to one another than to *Sericornis* (Schodde
704 and Mason, 1999). However, the pale eggs of *Origma*, its strikingly unusual pendant
705 nests, specialized niche and restricted distribution, confined to the rocky outcrops of
706 the Hawkesbury sandstone belt of central eastern New South Wales in eastern
707 Australia, suggest differences between *Origma* and the rest.

Monophyly of the scrubwrens *Sericornis* is affirmed in our best-estimate tree (Fig. 1) as argued elsewhere (Christidis et al., 1988). Whether *S. citreogularis* (yellow-throated scrubwren) is sister to all congeners is poorly resolved (Fig. 3c) although our best-fit tree (Fig. 1) indicates that the conflicting results of Christidis et al. (1988) and Joseph and Moritz (1993a) may be resolved in favour of the latter. *S. citreogularis* aside, the two New Guinean species *S. perspicillatus* (buff-faced scrubwren) and *S. papuensis* (Papuan scrubwren) form a clade that is sister to the remaining five species group and all seven form a well-resolved clade. We support retention of three species in the *frontalis* complex. The Tasmanian endemic *S. humilis* (Tasmanian scrubwren) is strongly supported as the sister to *S. keri* (Atherton scrubwren) a restricted range endemic of rainforests at the opposite end of eastern Australia and not to the morphologically similar and geographically closer *S. frontalis* (white-browed scrubwren), as previously suggested (Christidis et al., 1988; Schodde and Mason, 1999, Joseph and Moritz, 1993a) and notwithstanding hybridization between *S. frontalis* and *S. keri* (Joseph and Moritz 1993b). Accordingly, we find no support for combining *S. humilis* and *S. frontalis* at the species level, as suggested from genetic distances by Christidis and Schodde (1991). The sister of the *frontalis* complex is *S. magnirostris* (large-billed scrubwren) and New Guinean *S. nouhusyi* (large scrubwren), as shown by Christidis et al. (1988).

4.6 Relationships between the Australian and New Guinea fauna

We find no support for separate New Guinean and Australian endemic radiations within Acanthizidae. This is consistent with Driskell and Christidis' (2004) finding for the Meliphagidae. We show that *Sericornis* species from New Guinea do

not form a monophyletic group. A parsimonious reconstruction of the biogeography would involve two separate range extensions with subsequent loss of connectedness to the parent population for the ancestor of *Sericornis nouhuysi* and the ancestor of *S. perspicillatus* + *S. papuensis*. However, not all New Guinea species are included in our analysis.

Conclusions

Our first main aim was to resolve family-level relationships in the Meliphagoidea. We generated a robust phylogeny that argues for recognition of five families not four as in the current classification. *Dasyornis* is not sister to the remaining Meliphagoidea, as was suggested by Driskell and Christidis (2004), nor is it closely related to *Pycnoptilus* within Acanthizidae, but it is sister to Meliphagidae + Pardalotidae + Acanthizidae, and *Pardalotus* is sister to Acanthizidae. The family relationships can be represented as (Maluridae (Dasyornithidae (Meliphagidae (Pardalotidae, (Acanthizidae))))).

Our analysis confirms the two subfamilies of Maluridae and places *Clytomias* sister to *Malurus*, but the relationships among *Malurus* species need further work. We have no information about relationships within *Dasyornis* because our sample did not extend to all three species, and we obtained equivocal results for relationships amongst our three-species sample of *Pardalotus*. Differences in gene trees resulting from incomplete lineage sorting, or under-parameterization of the model may have caused this result but broader samples are needed. The position of Pardalotidae as sister to Acanthizidae is, however, well supported.

Within Meliphagidae our major finding is that the classification proposed by Driskell and Christidis (2004) is likely compromised by analytical errors. We offer an alternative arrangement of genera in which their clade #3 is split and distributed within their clade #4. Our sample was representative of this family rather than comprehensive, and further work is required. Also clear is the non-monophyly of *Lichenostomus*, which falls into three separate parts within our limited sample of species.

A second aim of our study was to provide a first molecular phylogeny of the Acanthizidae. Our conclusions here appear robust, with strong bootstrap support and posterior probability scores and we find no substantial disagreement across different models or methods of tree inference. *Oreoscopus* is sister to all other acanthizid genera, and *Gerygone* is sister to all remaining genera. A sister taxa relationship between *Acanthornis* and *Aphelocephala*, which we tested with closer scrutiny and could not reject, must surely be one of the most remarkable cases of external morphology misleading phylogenetic inference in the Australo-Papuan avifauna. The sister relationship emerges strongly despite their very different habitats and feeding modes. Egg patterning shows strong similarities previously ignored or interpreted as shared ancestral traits, but which we suggest are shared derived traits. This has consequences for the interpretation of egg morphological characters across the entire family. More work is required. The sister to *Acanthornis* + *Aphelocephala* is *Acanthiza*, not *Sericornis* as has been previously proposed.

Sericornis is sister to a clade comprising *Origma* + *Crateroscelis*. The biogeographical implications of this are profound because *Origma* is restricted to the Hawkesbury sandstone belt of central coastal New South Wales whereas

Crateroscelis is endemic to New Guinea; *Sericornis* occurs widely in mostly mesic Australia and New Guinea and one species (*S. beccarii*) spans both areas.

The sister to (*Sericornis* (*Origma* + *Crateroscelis*)) comprises the genus-pairs *Calamanthus/Hylacola* and *Pyrrholaemus/Chthonicola*. In both cases the decision to use either a single or two generic names is arbitrary. *Pycnoptilus* being sister to *Pyrrholaemus/Chthonicola* it is thus also firmly within Acanthizidae and not close to Dasyornithidae. This opens the way for further work on the morphological similarities between those two taxa, which presumably arose by convergence. Likewise, the finding that *Smicrornis* is not particularly closely related to *Gerygone* suggests that the various similarities by which they were previously grouped together are either convergent or shared ancestral traits.

Overall, our new phylogeny of the Meliphagoidea provides a strong foundation for subsequent study and reinterpretation of character evolution and biogeography in a large radiation of Australo-Papuan passerines. The phylogenetic coverage of this work clearly needs to be extended to include additional taxa, and our tree needs to be tested with further gene regions. Beyond these obvious extensions, a large number of morphological, behavioural, ecological and biogeographic traits, that may have been misinterpreted due to incorrect phylogenetic assumptions or never examined for want of a robust phylogenetic hypothesis, can now be examined afresh.

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952 FIGURE LEGENDS

953

954 Figure 1. Best-estimate maximum-likelihood tree from RAxML partitioned analysis.

955

956 Figure 2. Supported relationships at family level, showing the placement of genera
957 *Dasyornis* and *Pardalotus*. Scores are Bayesian posterior probability and RAxML
958 bootstrap percent.

959

960 Figure 3. The Bayesian consensus tree showing supported relationships within a)
961 Maluridae; b) Meliphagidae; c) Acanthizidae. Scores are Bayesian posterior
962 probability score and RAxML bootstrap percent. Branches with both a posterior
963 probability <0.95 and a bootstrap score <70% have been collapsed.

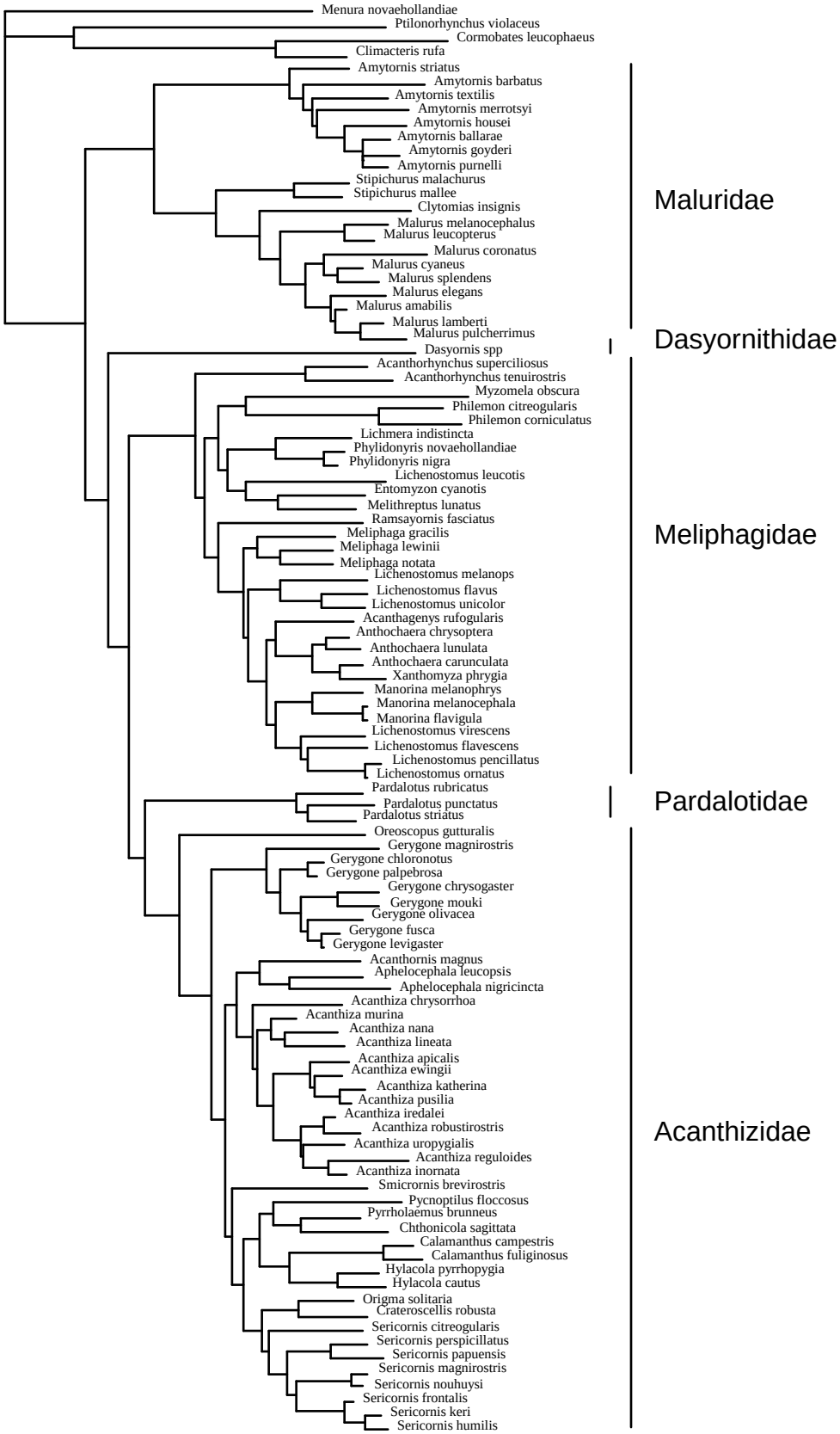
964

965 Figure 4. Species tree estimates and clade credibility values (x100) reported by
966 program MBBEST: a) result from a four-taxon analysis with *Hyacola* as outgroup; b)
967 result from a 10-taxon analysis with *Pardalotus* as outgroup.

968

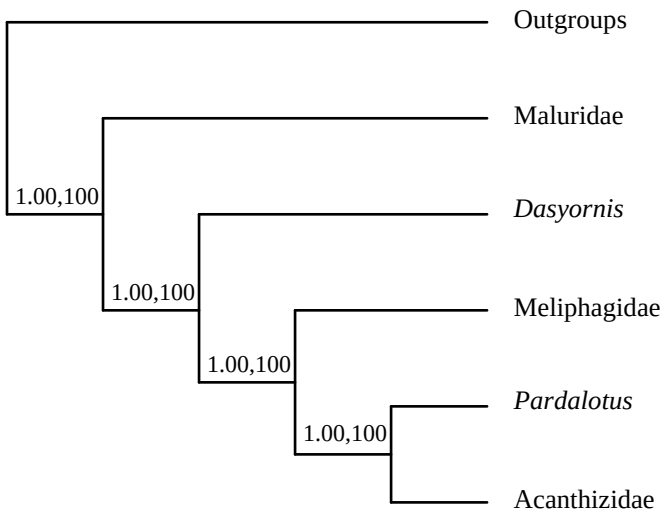
969 Figure 5. Plumage and morphological (eg bill) differences among related scrubwren,
970 scrubtit and whiteface species; a) lateral and b) ventral views. From L to R (ANWC
971 accession numbers): *Sericornis humilis* (45774), *S. frontalis* (20401), *S. frontalis*
972 (17637), *Acanthornis magnus*(45993), *A. magnus* (38941) and *Aphelocephala*
973 *leucopsis* (12246).

974 Fig 1
975



976

977 Fig 2
978



979
980

Fig 3a

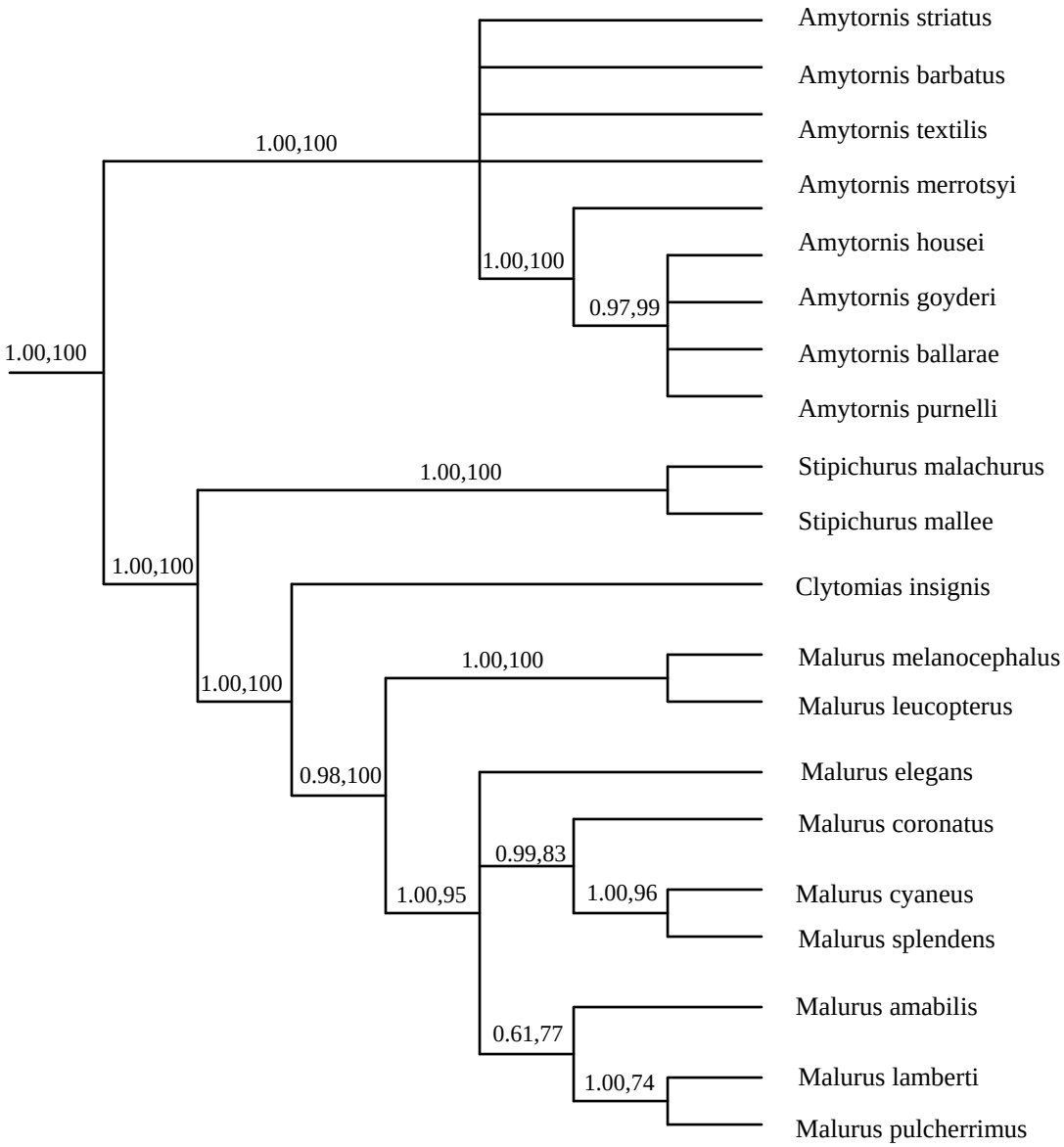
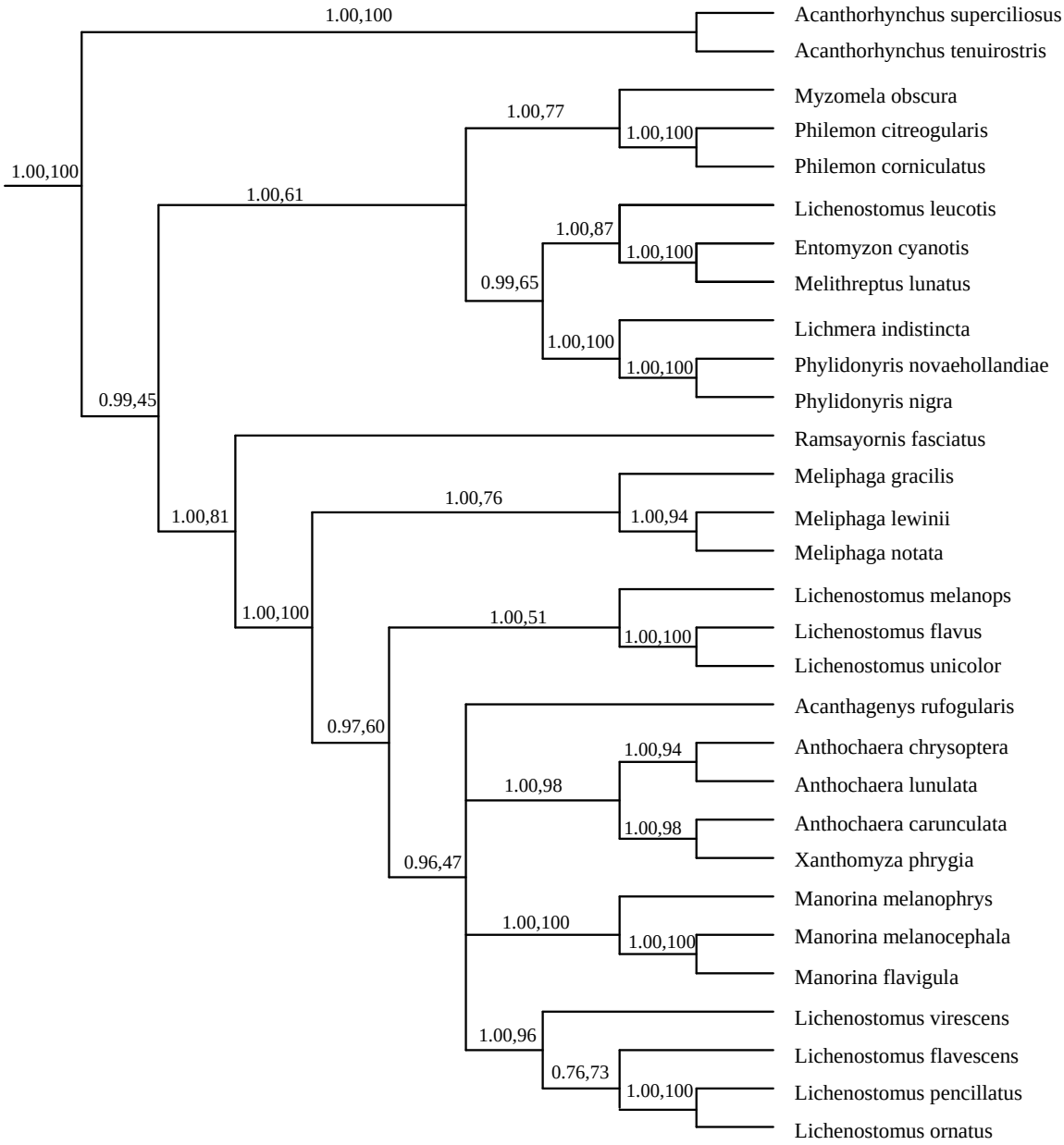

Amytornis striatus
Amytornis barbatus
Amytornis textilis
Amytornis merrotsyi
Amytornis housei
Amytornis goyderi
Amytornis ballarae
Amytornis purnelli
Stipichurus malachurus
Stipichurus mallee
Clytomias insignis
Malurus melanocephalus
Malurus leucopterus
Malurus elegans
Malurus coronatus
Malurus cyaneus
Malurus splendens
Malurus amabilis
Malurus lamberti
Malurus pulcherrimus
1.00,100
1.00,100
0.97,99
1.00,100
1.00,100
1.00,100
1.00,100
1.00,100
0.98,100
0.99,83
1.00,95
1.00,96
0.61,77
1.00,74

Fig 3b


1.00,100
Acanthorhynchus superciliosus
Acanthorhynchus tenuirostris
1.00,77
Myzomela obscura
1.00,100
Philemon citreogularis
1.00,100
Philemon corniculatus
1.00,61
Lichenostomus leucotis
1.00,87
1.00,100
Entomyzon cyanotis
0.99,65
Melithreptus lunatus
1.00,100
Lichmera indistincta
1.00,100
Phylidonyris novaehollandiae
0.99,45
Phylidonyris nigra
Ramsayornis fasciatus
Meliphaga gracilis
1.00,76
1.00,81
1.00,94
Meliphaga lewinii
Meliphaga notata
1.00,100
Lichenostomus melanops
1.00,51
1.00,100
Lichenostomus flavus
Lichenostomus unicolor
Acanthagenys rufogularis
0.97,60
1.00,94
Anthochaera chrysoptera
1.00,98
Anthochaera lunulata
1.00,98
Anthochaera carunculata
0.96,47
Xanthomyza phrygia
Manorina melanophrys
1.00,100
Manorina melanocephala
1.00,100
Manorina flavigula
Lichenostomus virescens
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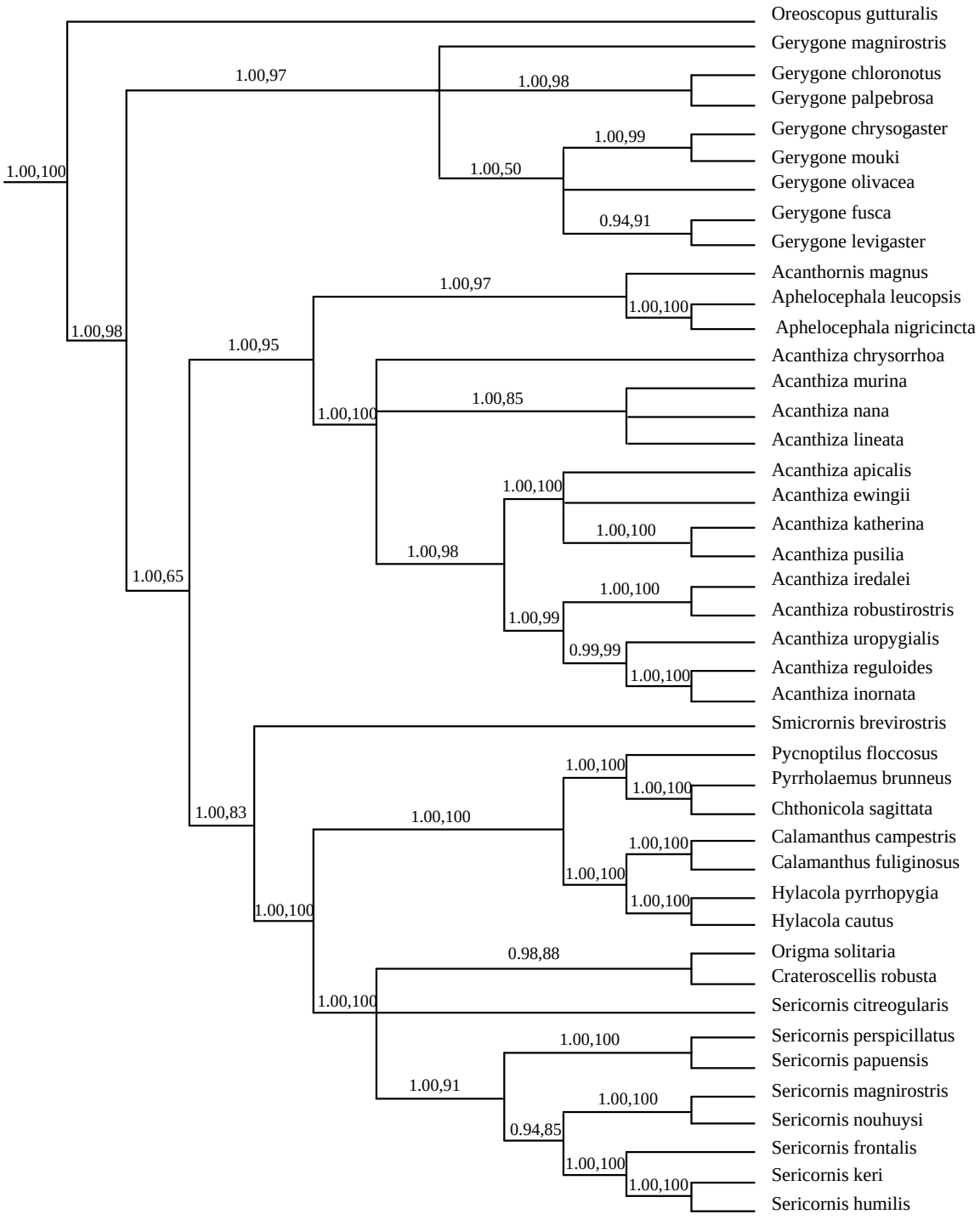
Lichenostomus flavescens
0.76,73
1.00,100
Lichenostomus pencillatus
Lichenostomus ornatus

Fig 3c


Oreoscopus gutturalis
Gerygone magnirostris
Gerygone chloronotus
Gerygone palpebrosa
Gerygone chrysogaster
Gerygone mouki
Gerygone olivacea
Gerygone fusca
Gerygone levigaster
Acanthornis magnus
Aphelocephala leucopsis
Aphelocephala nigricincta
Acanthiza chrysorrhoa
Acanthiza murina
Acanthiza nana
Acanthiza lineata
Acanthiza apicalis
Acanthiza ewingii
Acanthiza katherina
Acanthiza pusilia
Acanthiza iredalei
Acanthiza robustirostris
Acanthiza uropygialis
Acanthiza reguloides
Acanthiza inornata
Smicrornis brevirostris
Pycnoptilus floccosus
Pyrrholaemus brunneus
Chthonicola sagittata
Calamanthus campestris
Calamanthus fuliginosus
Hylacola pyrrhopygia
Hylacola cautus
Origma solitaria
Crateroscellis robusta
Sericornis citreogularis
Sericornis perspicillatus
Sericornis papuensis
Sericornis magnirostris
Sericornis nouhuysi
Sericornis frontalis
Sericornis keri
Sericornis humilis
1.00,100
1.00,97
1.00,98
1.00,99
1.00,50
0.94,91
1.00,97
1.00,100
1.00,98
1.00,95
1.00,100
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1.00,100
1.00,100
1.00,98
1.00,65
1.00,100
1.00,99
0.99,99
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1.00,100
1.00,100
1.00,83
1.00,100
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0.98,88
1.00,100
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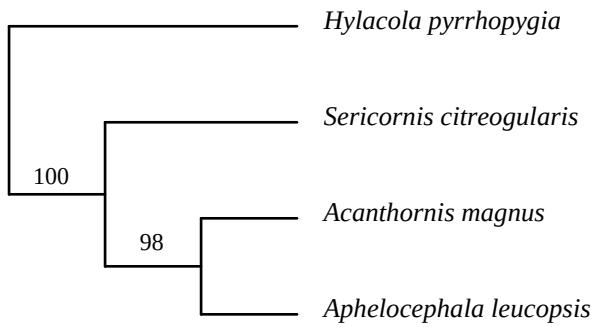
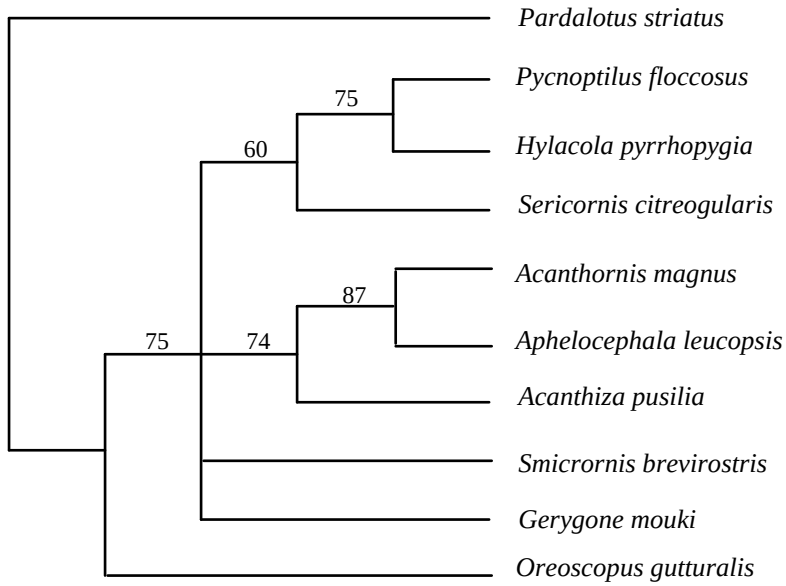
0.94,85
1.00,100
1.00,100

1002 Fig 4
1003

a



b



1004
1005

Fig 5

a)

b)

1015 Table 1. GenBank accession numbers of sequences used. For sequences generated in this study, ANWC (Australian National Wildlife

1016 Collection) accession numbers are given in brackets.

1017

Species	**12S**	**RAG-2**	**RAG-1**	**16S**	**Cyt *b***	**ND2**	**Beta5**	**Gd3ph**	**CO1**
Malurus melanocephalus		AY443162	AY057001			43297_M_m			pas039 (29510)
Malurus leucopterus		M_l_32001				33327			
Malurus cyaneus	M_c_42458	M_c_42458	M_c_42458		AF197845	42458_M_c.cyaneus			AF197846
Malurus splendens	M_s_40517	M_s_40517	M_s_40517		AY488403	AY488327	AY488484		
Malurus lamberti	M_l_32811	M_l_32811	M_l_32811		AY488402	AY488326	AY488483		
Malurus amabilis			AY037847		AY228088	AY064752			
Malurus elegans	M_e_29154	M_e_29154	M_e_29154			31938_M_el			
Stipichurus malachurus	S_m_31755		S_m_31755			31755_S.malchurus			
Stipichurus mallee	AY488258				AY488404	AY488328	AY488485		
Amytornis striatus	A_s_28865		A_s_28865 revcomp		AY488401	AY488325	AY488482		
Clytomias insignis	C_i_26949					26656			
Pardalotus punctatus	P_p_32659	P_p_32659	P_p_32659		AY488397	AY488321	AY488478		
Pardalotus striatus	P_s_29313	P_s_29313	P_s_29313		AF197847	AY488322	AY488479		AF197848
Dasyornis spp	D_b_40391	D_b_40391	D_b_40391		AY488394	AY488318	AY488475		Dbrachpas044 (34386)
Pycnoptilus floccosus	P_f_45256	P_f_45256	P_f_45256			pas064			pas064 (45256)
Origma solitaria	O_s_46238	O_s_46238	O_s_46238						pas063 (46238)
Oreoscopus gutturalis	O_g_31499	O_g_31499	O_g_31499			pas062			pas062 (31499)
Sericornis citreogularis	S_c_33397	S_c_33397	S_c_33397		U22042	pas068			pas068 (33397)
Sericornis frontalis	S_f_RM1159	S_f_RM1159	S_f_RM115	AF129171	AF197849	AY488323	AY488480	AF129247	AF197850
Sericornis keri					U22039	31567or31569			
Sericornis magnirostris					U22038	pas069 (29207)			pas069 (29207)
Sericornis perspicillatus	AY488254				AY488400	AY488324	AY488481		pas003 (31825)
Acanthornis magnus	A_m_45994	A_m_45994	A_m_45994			38926 A.magnus1			pas053 (45994)

Pyrrholaemus brunneus	P_b_33280	P_b_33280	P_b_33280			pas066			pas065 (33833)
Chthonicola sagittata	C_s_JG116	C_s_JG116	C_s_JG116			pas067			pas067
Calamanthus campestris		C_c_49967	C_c_49967			pas055			pas055 (49967)
Hylacola pyrrhopygia	C_p_43301	C_p_43301	C_p_43301			pas057			pas057 (43301)
Hylacola cautus		C_c_49625				pas056 (49625)			pas056 (49625)
Acanthiza katherina				AF129192	AF129231	28844		AF129254	
Acanthiza pusilia	A_p_DG244	A_p_DG244	A_p_DG244	AF129199	AF129237	pas072		AF129255	pas072
Acanthiza apicalis	AY488246	A_a_49264		AF129180	AY488392	AY488316	AY488473	AF129251	pas048 (49264)
Acanthiza ewingii				AF129184	AF129222	pas020		AF129253	pas020 (20555)
Acanthiza reguloides	A_r_DE034	A_r_DE034	A_r_DE034	AF129201	AF129239	42069		AF129258	
Acanthiza inornata	A_i_31702	A_i_31702	A_i_31702	AF129187	AF129225	pas049 (31702)		AF129257	pas049 (31702)
Acanthiza iredalei				AF129189	AF129227	pas024 (48407)		AF129256	
Acanthiza chrysorrhoa	A_c_29158	A_c_29258	A_c_29158	AF129182	AF197851	AY488317	AY488474	AF129252	AF197852
Acanthiza uropygialis	A_u_29266	A_u_29266	A_u_29266	AF129207	AF129245	pas052 newND2data		AF129259	pas052 (29266)
Acanthiza nana	A_n_29232	A_n_29232	A_n_29232	AF129196	AF129234	pas051 (29232)		AF129262	pas051 (29232)
Acanthiza lineata	A_l_46334	A_l_46334	A_l_46334	AF129194	AF129232	pas050 (46334)		AF129261	pas050 (46334)
Acanthiza robustirostris				AF129205	AF129243	pas007 (40245)		AF129260	pas007 (40245)
Acanthiza murina				AF129198	AF129236				
Smicrornis brevirostris	S_b_33814	S_b_33814	S_b_33814	AF129175	AF129213	33019 S brev1		AF129250	pas075 (33814)
Gerygone chrysogaster	AY488250				AY488396	AY488320	AY488477		
Gerygone chloronotus	AY488249				AY488395	AY488319	AY488476		
Gerygone olivacea	G_o_49262	G_o_49262	G_o_49262	AF129179	AF129217				pas061 (49262)
Gerygone fusca		G_f_49648 revcomp		AF129177	AF129215	pas058 (49648)		AF129249	pas058 (49648)
Gerygone mouki	G_m_29205	G_m_29205	G_m_29205	AF129178	AF129216	pas060 (29205)			pas059 (29206)
Aphelocephala leucopsis	A_l_29281	A_l_29281	A_l_29281	AF129173	AF129211	pas054 (29281)		AF129248	pas054 (29281)
Acanthagenys rufogularis	AY488184		A_r_49683		AY488330	DQ097571	AY488410		pas074 (49683)
Anthochaera chrysoptera	AY488188				AY488334	AY488263	AY488414		
Anthochaera carunculata	AY488187				AY488333	AY488262	AY488413		
Lichenostomus virescens						DQ097606			

Lichenostomus pencillatus	L_p_33100		L_p_33100			33100_Lich			
Manorina melanophrys	M_m_32043	M_m_32043	M_m_42735		AY488355	AY488282	AY488435		
Manorina melanocephala	M_m_34179	M_m_34179	M_m_34179		AF197859	AY064753			AF197860
Manorina flavigula	AY488208				AY488354	AY488281	AY488434		
Lichmera indistincta	AY488207				AY488353	AY488280	AY488433		
Phylidonyris novaehollandiae	AY488231				AY488377	AY488303	AY488458		
Phylidonyris nigra	AY488230				AY488376	AY488302	AY488457		
Ramsayornis fasciatus	AY488239				AY488385	AY488309	AY488466		
Myzomela obscura	AY488220				AY488366	AY488293	AY488447		
Meliphaga gracilis	AY488215				AY353241	AY488288	DQ673243		
Meliphaga lewinii						DQ673225	DQ673245		
Philemon citreogularis	AY488225				AY488371	AY488298	AY488452		
Cormobates leucophaeus	C_l_29228	C_l_29228	C_l_29228						
Ptilonorhynchus violaceus	P_v_32548	P_v_32548	AY057026		X74256	AY064759			AF197833
Climacteris rufa			AY037846		U58501	AY064746			
Menura novaehollandiae	AY542313	AY443171	AY057004	AY542313	AY542313	AY542313			AY542313
Philemon corniculatus	AY488226				AY488372	AY488299	AY488453		
Entomyzon cyanotis	AY488197				AY488343	AY488272	AY488423		
Anthochaera lunulata	AY488189				AY488335	AY488264	AY488415		
Acanthorhynchus superciliosus	AY488185				AY488331	pas037	AY488411		
Acanthorhynchus tenuirostris	AY488186				AY488332	AY488261	AY488412		
Melithreptus lunatus					AF197853	pas019 (43479)			pas032 (41975)
Amytornis ballarae						pas002 (41737)			pas002 (41737)
Sericornis papuensis						pas004 (24425)			pas004 (24425)
Sericornis nouhuysi						pas005 (24396)			pas005 (24396)
Lichenostomus flavescens						AY488278			
Aphelocephala nigricincta						pas009 (40088)			pas009 (40088)
Crateroscellis robusta									pas010 (24439)
Calamanthus fuliginosus						pas011 (38940)			pas012 (38186)
Amytornis barbatus						pas013 (40054)			pas013 (40054)

Amytornis textilis									pas014 (40176)
Amytornis goyderi									pas015 (40080)
Amytornis housei						pas016 (24307)			pas016 (24307)
Amytornis purnelli						pas018 (42889)			pas017 (40220)
Lichenostomus ornatus						49652			pas022 (46738)
Amytornis merrotsyi									pas023 (28202)
Lichenostomus leucotis						pas26 (42440)			pas026 (42440)
Gerygone magnirostris						pas036 (32144)			pas036 (32144)
Malurus coronatus						ANUPC9			pas077
Gerygone levigaster						pas035 (29007)			
Gerygone palpebrosa						pas041 (29756)			
Pardalotus rubricatus						pas042 (33106)			
Lichenostomus flavus						51481			
Lichenostomus melanops						46323			
Lichenostomus unicolor						50847			
Malurus pulcherrimus						28233			
Meliphaga notata						31299			
Xanthomyza phrygia						42003			
Sericornis humilis						38908			

1018