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Phylogeny and evolution of the Australo-Papuan honeyeaters (Passeriformes, Meliphagidae)

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Abstract

We analyzed nucleotide variation at four loci for 75 species to produce a phylogenetic hypothesis for the Meliphagidae, and to examine the evolution and biogeographic history of the Meliphagidae. Both maximum parsimony and Bayesian methods of phylogenetic analysis were employed. The family was found to be monophyletic, though the genera *Certhionyx*, *Anthochaera*, and *Phylidonyris* were not. Four major clades were recovered and the spinebills (*Acanthorhynchus*) formed the sister clade to the remainder of the family in most analyses. The Australian endemic arid-adapted chats (*Epthianura*, *Ashbyia*) were found to be nested deeply within the family Meliphagidae. No evidence was found to support the hypothesis of separate New Guinean and Australian endemic radiations, nor of a close phylogenetic relationship between taxa from the New Guinea highlands and those from Australian northern rainforests.

Keywords: Phylogenetic analysis; Avian systematics; Biogeography; Meliphagidae

1. Introduction

One of the dominant groups of birds in Australia and New Guinea, both numerically and ecologically, is the passerine family Meliphagidae, the honeyeaters. In certain habitats more than 12 species of honeyeater can co-occur seasonally (Keast, 1985). Although the family has its centers of diversity in Australia and New Guinea, meliphagids are also important endemic elements of the biota of many of the islands in the south Pacific.

Honeyeaters are diverse in size, morphology, and diet. They can be either nectarivorous, insectivorous, frugivorous, or more commonly, a combination of nectar- and insect-eating. Although many species have long, narrow, decurved bills, presumably adapted for nectar-feeding, these species are often insectivorous during certain seasons of the year (Lea and Gray, 1935; Rand and Gilliard, 1968). In Australia, honeyeaters are major pollinators of many endemic plant groups, including *Banksia*, *Dryandra*, *Melaleuca*, *Hakea*, and *Eucalyptus* (Paton and Ford, 1977; Recher, 1981). The Meliphagidae are an ecologically and evolutionarily significant element of the Australo-Papuan fauna and yet phylogenetic relationships within this large family are almost entirely unknown.

Traditionally the Meliphagidae were linked with the Nectariinidae (sunbirds) and other nectarivorous birds (Cracraft, 1981; Wetmore, 1960). DNA–DNA hybridization studies (Sibley and Ahlquist, 1985, 1990) and allozyme evidence (Christidis, 1991; Christidis and Schodde, 1991) demonstrated that the honeyeaters belong to a clade originating in the Australo-Papuan region and composed of the Meliphagidae, the Pardalotidae–Acanthizidae (Australasian warblers and allies), and Maluridae (Australasian fairy-wrens and grasswrens). Mitochondrial and nuclear sequence data (Barker et al., 2002; Cracraft and Feinstein, 2000; Ericson et al., 2002a,b) confirm close affinities between the Meliphagidae, Pardalotidae–Acanthizidae, and Maluridae.

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Relationships within the Meliphagidae are poorly understood. There have been no phylogenetic studies of the entire family, but a few studies have examined relationships among some genera (Christidis and Schodde, 1993; Christidis et al., 1993). Published taxonomies for the Meliphagidae lack classification levels between family and genus: no subfamilies or tribes have been proposed (Christidis and Boles, 1994; Schodde, 1975; Schodde and Mason, 1999; Sibley and Monroe, 1990). The number of monotypic genera and the morphological distinctness of most genera have been cited as impediments to determining interrelationships within the family (Schodde, 1975; Schodde and Mason, 1999).

In the last decade, molecular and biochemical studies have modified the traditional composition of the family Meliphagidae. Sibley and Ahlquist (1990) demonstrated that the South African sugarbirds *Promerops* and New Guinean longbills *Oedistoma* and *Toxorhamphus* were not honeyeaters but more closely related to the Nectariniidae (*Promerops*) and Melanocharitidae (*Oedistoma* and *Toxorhamphus*). Sibley and Ahlquist also showed that the Australian chats, *Epthianura* and *Ashbyia* (formerly the Epthianuridae), were honeyeaters, a result which was supported by allozyme data (Christidis et al., 1993). In addition, DNA–DNA hybridization (Sibley and Ahlquist, 1990) and DNA sequence (Slikas et al., 2000) studies established that south Pacific *Cleptornis* was not a honeyeater but instead was closely related to the white-eyes (Zosteropidae). The genus *Apalopteron* from Bonin Island has likewise been shown to be a white-eye rather than a honeyeater, based on DNA sequence data (Springer et al., 1995). Another genus, *Macgregoria*, traditionally classified as a bird-of-paradise, was shown to be a honeyeater based on combined analyses of DNA sequence and morphological data (Cracraft and Feinstein, 2000).

Modifying the taxonomic review of the Meliphagidae by Sibley and Monroe (1990) with the recent changes described above, the family now comprises 182 species in 42 genera. Two of these genera (*Moho* and *Chaetoptila*) are extinct on Hawaii (Pratt et al., 1987). Australia has over 70 species of honeyeaters (Christidis and Boles, 1994), and New Guinea over 60 species (Beehler et al., 1986). A few genera are distributed across the Lesser Sunda Islands, the Moluccas and Sulawesi. One species of honeyeater, *Lichmera limbata*, crosses Wallace's line, but occurs only as far west as Bali (Coates and Bishop, 1997). In the south Pacific, honeyeaters are distributed northwards from New Guinea to the Mariana Islands, as far south as New Zealand, and east to Hawaii (Pratt et al., 1987).

The main goal of this paper is to introduce a phylogenetic hypothesis for the family Meliphagidae. More specific goals are to: (1) examine systematic relationships of some taxonomically unstable genera (e.g., *Certhionyx*, *Phylidonyris*, and *Meliphaga sensu lato*); (2) determine the phylogenetic relationship of the Australian chats (*Epthianura*, *Ashbyia*), which are remarkable among the Meliphagidae for their adaptation to arid habitats; and (3) examine the biogeographical history of the family, especially the relationships among the New Guinean and Australian honeyeater faunas. In addition, we wanted to explore the utility of three different categories of genetic loci (mitochondrial protein-coding genes, mitochondrial ribosomal DNA, and a nuclear intron) for reconstructing phylogenetic relationships in such a large, divergent, and relatively old family of passerines.

2. Methods

2.1. Taxon sampling, DNA extraction, amplification, and sequencing

The sample of the family Meliphagidae consisted of 63 species, representing 32 of the 41 described meliphagid genera listed in Sibley and Monroe (1990). To test for intraspecific sequence variation, multiple individuals were sampled for 10 species. Nine species, representing five genera, of the Pardalotidae (*sensu* Sibley and Monroe, 1990), the apparent sister group of the Meliphagidae (Christidis and Schodde, 1991; Sibley and Ahlquist, 1985), were also sampled. To establish monophyly of the ingroup (Meliphagidae) and the sister relationship of the Meliphagidae and Pardalotidae, four species of the Maluridae, generally considered the next-most closely allied family, were also included. Specimens, collection localities, and voucher specimen locations and numbers are listed in Appendix A.

DNA was extracted from ethanol-preserved tissue. Blood was used as a DNA source only for the New Zealand Tui, *Prosthemadera novaeseelandiae*. DNA was obtained using standard proteinase-*k* digestion followed by either phenol–chloroform extraction or the protein precipitation method as implemented in the Puregene kit (Gentra Systems). Standard PCR amplification and automated sequencing techniques were used to sequence part or all of one nuclear gene, β-fibrinogen intron 5 (FIB5), and three mitochondrial genes: cytochrome-*b* (CYTB), 12S rDNA (12S), and NADH dehydrogenase subunit 2 (ND2). Both strands of all gene regions were sequenced and portions of most were sequenced multiple times. Each resequencing essay was preceded by reamplification from the original DNA extract to provide additional insurance against lab errors. Primers used for amplification and sequencing are listed in Table 1.

2.2. Data verification and sequence alignment

Although it is impossible to completely guard against the amplification of nuclear copies of mitochondrial

Table 1
Primers used for amplification and sequencing in this study

Gene	Primer	Sequence	Reference
Cytochrome *b*	L14990	CCATCCAACATCTCAGCATGATGAAA	Kocher et al. (1989); modified L14841
	L15191	ATCTGCATCTACCTACACATCGG	Lanyon and Hall (1994)
	H15298	CCCCTCAGAATGATATTTGTCCTCA	Kocher et al. (1989); modified H15149
	L15656	ACCTACTAGGAGACCCAGA	Helm-Bychowski and Cracraft (1993)
	H15916	ATGAAGGGATGTTCTACTGGTTG	Lanyon and Hall (1994)
	H16065	GGAGTCTTCAGTCTCTGGTTTACAAGAC	Helm-Bychowski and Cracraft (1993)
NADH dehydrog. subunit 2	L5206	CTAATAAAGCTTTCGGGCCCATAC	Kirchman et al. (2001); L5208
	L5575	AAACTAGGACTAGTGCCATTCCA	S.J. Hackett, unpublished
	H5802	GAGAATAATGGTTATTCATCC	Kirchman et al. (2001); H5804
	H6313	TTCTACTTAAGGCTTTGAAGGC	Kirchman et al. (2001); H6315
12S rDNA	L1276	CACTGAAGATGCCAAGATGG	This study
	L1746	GCTTCAAACTGGGATTAGATACC	Kocher et al. (1989); modified L1091
	H1811	CCTTAGAGTTTAAGCGTTTGTGC	This study
	H2512	GCAGAGGGTGACGGGCGGTGTGT	Kocher et al. (1989); modified H1478
β-fibrinogen intron 5	FIB5	CGCCATACAGAGTATACTGTGACA	F.K. Barker and S.J. Hackett, unpublished
	FIB6	GCCATCCTGGCGATTCTGAA	F.K. Barker and S.J. Hackett, unpublished

Each is listed in 5′ to 3′ orientation, numbered with reference to the complete mtDNA sequence of the chicken (Desjardins and Morais, 1990), and designated as H or L with respect to their location on the heavy or light strand of the mitochondrial genome (published source in parentheses, along with original primer name if different than numbered designation).

gene regions (*numts*) and it can be impossible to identify them in a data set (reviewed by Quinn, 1997), a number of precautions were taken. Only one of the tissue samples used was blood, which previous avian studies have shown to be particularly prone to *numt* contamination (Quinn, 1997). Larger fragments of target genes were amplified whenever possible, except in the case of 12S, which was amplified in two segments. Each data partition was inspected for taxa with significantly different base compositions using the "pairwise base differences" option PAUP* v. 4.0b10 (Swofford, 2002). The sequence data for both protein coding genes for each species was aligned to published sequences, translated and checked for termination codons, insertions, and deletions. In addition, the alignment of the 12S data to a structural model (below) allowed recognition of loss of stem complementarity or conserved binding motifs, which might be expected in a non-functional nuclear copy (Houde et al., 1997). The absence of termination codons and deletions in the protein coding genes, and lack of suspicious deletions in stems or other aspects of the 12S data allows moderate confidence that amplification and sequencing of nuclear pseudogene copies of these genes was successfully avoided.

Very low levels of intraspecific sequence variation (<1.0% in CYTB and ND2, and 0.0% in 12S and FIB5) were observed. Allelic variation within individuals was, however, apparent in the nuclear FIB5 data set. Differences of 1–12 bp between the two alleles of the intron were observed in 38 of the specimens sequenced (22 of these had 1 bp difference between the two alleles), and the majority of these base differences (66%) were transitions. These differences were observed as double peaks when directly sequencing PCR products. The double peaks were verified as the consequence of different alleles by cloning PCR products using a topo-TA cloning kit (Stratagene) and sequencing multiple (up to 10) clones. In addition to sequence differences, three individuals exhibited alleles varying in length by 5–12 bp (manifested as single insertion–deletion events). PCR products of these individuals were cloned. Allelic differences in nucleotide sequence were represented in the alignment used for phylogenetic analysis by ambiguity codes. However, in every instance the two alleles from an individual were more similar in sequence to each other than to those from any other taxon. For specimens whose alleles varied in length, both alleles were included in an initial phylogenetic analysis. In all instances, the two alleles from a single individual grouped together with high bootstrap support, and in later analyses only the allelic sequence without the unique insertion or deletion was used to represent the taxon.

All output from the automated sequencer was checked for accuracy in base identification. Sequences of a gene from a specimen were compiled using the program Sequencher v. 3.1 (Genecodes 1991–1998) and then imported into PAUP* (Swofford, 2002) for alignment.

Sequences of the β-fibrinogen intron were aligned by eye. Within this data set, and within each of the families in this study, insertions and deletions (indels) ranging in size from 1 to 47 bp in length were present. Aside from those involving only one base, most insertions in the FIB5 data set appeared to involve repeated motifs. The inserted regions were copies (sometimes multiple copies) of flanking sequence and identification and alignment

was relatively straightforward. Two regions containing insertions and any ambiguously aligned flanking sites (amounting 81 bp of the overall alignment) were excluded from phylogenetic analyses. One of these regions (2 bp) was exclusive to two outgroup congeners. The other excluded region is the result of at least four and probably more indel events and exact homology among nucleotides was difficult to assess. As a conservative measure, this entire region was excluded. All remaining gaps in the alignment were treated as missing data for phylogenetic analysis. Twelve of these indel gaps were coded as binary characters.

To create amore general avian 12S model to serve as a template for alignment of our 12S data, published 12S sequences for the chicken (Desjardins and Morais, 1990) and falcon (Mindell et al., 1997) were aligned according to a model of secondary structure published for the falcon (Mindell et al., 1997). Use of the model simplified identification of the stem regions flanking each loop, and isolated all of the length variation to loop regions. All ambiguously aligned regions, which amounted to 11 of 62 variable loops, were excluded from phylogenetic analysis. A total of 114 bp of the total 12S sequence (12.5%) was excluded in this fashion.

2.3. Molecular characterization and phylogenetic analysis

Saturation was assessed graphically by plotting transition and transversion differences between pairs of taxa against their Jukes–Cantor distance (Jukes and Cantor, 1969). Graphs were produced separately for each codon position in the two protein coding genes, for stem and loop regions in 12S, and for FIB5. A data partition was considered to be saturated if one of two criteria was met (Griffiths, 1997): (1) a levelling-off or plateau of the plotted data was apparent, or (2) most ingroup comparisons were as great as outgroup comparisons.

To assess the homogeneity of phylogenetic signal contained within different data partitions, incongruence length difference tests (ILD tests; Farris et al., 1995a,b), as implemented in PAUP* (Swofford, 2002), were conducted. Invariant sites were removed from the data sets before analysis as recommended by Cunningham (1997) and tests used 1000 replicates.

The mitochondrial and fibrinogen data sets were subjected to both separate and combined heuristic searches under the parsimony criterion using PAUP* vers. 4.0b10 (Swofford, 2002); these searches employed 100 random addition sequences (RAS) and tree-bisection-reconnection (TBR) branch swapping. Each random addition sequence in the separate FIB5 analysis was limited to 20 min of branch swapping. The nuclear data set provided little or no resolution at very shallow nodes and excessive time was spent branch-swapping essentially unresolvable nodes. As a means of exploring signal and noise in the mitochondrial data set, analyses were performed with all sites weighted equally, and with saturated data partitions downweighted relative to other partitions through the implementation of step matrices. Bootstrap analysis with the full heuristic option, 10 RAS, and TBR branch swapping was used to evaluate nodal support.

An estimate of the maximum likelihood topology of the combined data set was produced using Bayesian analysis as implemented by MRBAYES (Huelsenbeck, 2000). The program MODELTEST 3.0 (Posada and Crandall, 1998), which performs a series of likelihood ratio tests (Huelsenbeck and Crandall, 1997; Huelsenbeck and Rannala, 1997), was used to explore the best-fit maximum likelihood models for each of the four data partitions. Eight independent analyses were performed, each with 1,000,000 generations with four simultaneous chains. Trees were sampled every 1000 generations. Likelihood scores for each tree were plotted against generation and visually inspected to ascertain the point at which the chain appeared to reach stationarity. Trees from the generations preceding stationarity were discarded in each analysis. The remaining trees from each run were combined and posterior probabilities were calculated using majority rules consensus (Larget and Simon, 1999). Comparing the likelihood scores for each topology from each generation identified the most likely topology from each run.

The combined data set was also subjected to a parsimony search constrained to contain a clade found in the separate FIB5 consensus topology (Fig. 2). The constraint required only that 10 taxa form a monophyletic group. The particulars of this parsimony and bootstrap analysis were the same as above.

Parametric bootstrapping was conducted to test whether the difference in tree length between the unconstrained and constrained topologies was statistically significant (Goldman et al., 2000; Swofford et al., 1996). MESQUITE 1.0 (Maddison and Maddison, 2003) was used to simulate 500 data sets on each of the two equally parsimonious topologies resulting from the constrained search. Data was simulated using a $GTR + I + \Gamma$ model with the parameters estimated by MODELTEST 3.0 (Posada and Crandall, 1998). Each of the 1000 data sets was subjected to a constrained parsimony search (employing the same constraint as above) and an unconstrained search using PAUP* with 20 RAS and TBR branch-swapping. MESQUITE was then employed to calculate the tree length differences for each test data set, construct a distribution of tree length differences, and to estimate the critical value for a significance level $\alpha = 0.05$. The difference in tree length from constrained and unconstrained searches of the actual data set was compared to this critical value and an assessment of the statistical significance of the difference was made.

3. Results

The total alignment of sequences (including gaps and indels) from CYTB (1046 bp), ND2 (1040 bp), 12S (910 bp), and FIB5 (547 bp) was 3843 base pairs (alignments are available from the first author's website; see Acknowledgements). All sequences were deposited in GenBank under Accession Nos. AY353241, AY353242, AY488184–AY488485. The aligned nexus file and associated trees have been submitted to TreeBASE (http://www.treebase.org/treebase) and can also be downloaded from the author's website (see Acknowledgments).

3.1. Molecular characterization

Total length and levels of variation for the four gene regions are reported in Table 2. The ND2 gene had the highest proportion of variable positions (62%), with 91% of these being potentially parsimony-informative. The sequences of both CYTB and ND2 included in analyses were of similar length, and nearly all third positions of both genes were variable (97 and 99%, respectively). However, considerably more first and second positions were variable in the ND2 gene. Of the 910 bp of 12S included for analysis, only 36% were variable, and loop regions were more variable than stem regions. Of the variable positions of the FIB5 intron only 55% were potentially parsimony-informative, which indicates a high proportion of autapomorphic changes in this gene region. Although the two protein coding genes had the highest proportions of potentially parsimony informative characters, nearly 80% of these characters had a consistency index (CI) lower than 0.5 (Table 2), indicating a relatively high level of homoplasy in these data. The most variable partitions of these genes, third positions, had even higher levels of homoplasy (87% of CYTB third positions and 97% of ND2 third positions had $CI < 0.5$). In contrast, the majority of potentially informative FIB5 characters had $CI = 1.00$, denoting a relatively low level of homoplasy. Within the Meliphagidae, uncorrected CYTB sequence divergences ranged from 2.7 to 19% and ND2 sequence divergences ranged from 3.8 to 30%. Divergences at the 12S and FIB5 loci were much lower and similar to each other, ranging from less than 1% to about 10%.

Twelve FIB5 indels were coded as binary characters (Tables 3a and 3b). Only six of these characters were included in parsimony analyses of the individual FIB5 nucleotide data and the combined data set. The remaining six were exclusive to the outgroup taxa. Inclusion of the six binary indel characters had no effect on the topology resulting from parsimony analysis, but when mapped onto a topology (Figs. 2 and 4) provide strong corroborative evidence for particular nodes (see below).

Based on saturation plots (available from the first author's website; see Acknowledgements), transitions at third codon positions of ND2 and CYTB and at the first codon positions of ND2 showed evidence of leveling off at higher divergences. In these three instances, many of the ingroup comparisons were as great as or greater than comparisons between the ingroup and outgroup. These two signs were taken as evidence of saturation at higher levels of divergence as *per* Griffiths, 1997. There is no clear evidence for saturation in any of the remaining data partitions; in no instance do transversion

Table 2
Comparative variability of genes and partitions within genes and consistency indices for characters in each gene and partition

Gene Partition	Number of basepairs	# variable characters (%)	# parsimony inform. characters (%)[a]	% with $CI < 0.5$	% with $CI = 1.00$
FIB5	547	304 (56)	168 (55)	12	66
12S	910	331 (36)	255 (77)	48	36
Stem regions	457	212 (27)	85 (83)	45	40
Loop regions	336	118 (35)	86 (73)	52	32
CYTB	1046	508 (49)	447 (88)	75	15
1st positions	348	127 (36)	95 (75)	54	32
2nd positions	349	43 (12)	24 (56)	37	49
3rd positions	349	338 (97)	328 (97)	87	4
ND2	1040	646 (62)	585 (91)	79	13
1st positions	347	197 (57)	166 (84)	65	22
2nd positions	347	106 (30)	78 (73)	50	35
3rd positions	346	343 (99)	341 (99)	97	$\ll 1$

The total number of basepairs in each gene and partition, the number of variable characters (proportion of characters variable), number of parsimony informative characters (proportion of variable characters), proportion of characters with consistency indices less than 0.5, and the proportion with consistency indices of 1.0 are listed. For the calculation of consistency indices, invariant and autapomorphic characters were excluded, and characters were mapped onto the consensus topologies resulting from parsimony analysis of each gene. FIB5, β-fibrinogen intron 5; 12S, 12S ribosomal DNA; CYTB, cytochrome-*b*; ND2, NADH dehydrogenase subunit 2; CI, consistency index; and $\ll 1$, less than 1%.

[a] proportion of variable characters that are also parsimony informative.

Table 3a
Description of β-fibrinogen intron 5 indels coded as binary characters

Char.	Sequence position	# b.p.	Relative type	Taxa (number of species)
1.	42–44	3	Deletion	*C. niger*, *G. fallax*, *Myzomela* (5 spp.), *P. melanops*, *Ptiloprora* (2 spp.)
2.	110–127	18	Insertion	*G. fallax*, *C. niger*, *Myzomela* (5 spp.), *P. melanops*, *Ptiloprora* (2 spp.), Pardalotidae (7 spp.), Maluridae (4 spp.)
3.	175	1	Deletion	*Philemon* (5 spp.)
4.	197–243	var.	Insertion	*E. cyanotis*, 11 bp; *Foulehaio carunculata*, 36 bp; *Melithreptus albogularis*, 47 bp; *M. brevirostris*, 33 bp; see Table 3b
5.	247–254	8	Deletion	*Myzomela* (5 spp.)
6.	280	1	Deletion	*Certhionyx niger, Myzomela* (5 spp.)
NI	183	1	Deletion	Maluridae (4 spp.)
NI	309	1	Deletion	Maluridae (4 spp.)
NI	336–343	8	Deletion	*Acanthiza* (2 spp.), *Sericornis* (2 spp.)
NI	348–349	2	Deletion	Maluridae (4 spp.)
NI	522–525	4	Deletion	*Malurus* (2 spp.)
NI	648–664	17	Insertion	*Acanthiza* (2 spp.)

For each indel, the position in the sequence alignment, size, relative type, and taxa in which it appears are listed. The sequence position is numbered from the 3′ end of the primer FIB5. The relative type depends on whether the insertion or deletion is most common (e.g., deletion is the relative type if fewer taxa possess it). Character numbers refer to the characters mapped onto Figs. 2 and 4. Char., character number; NI, not included for phylogenetic analysis; # b.p., indel length in number of basepairs; and var., of variable length.

Table 3b
Elaboration of indel character 4 from Table 3a

Taxon	Indel character 4 sequence
Glycichaera fallax	GCTCACACTT----------AAATA
Epthianura aurifrons	GTTCACACTT----------AAGTA
Entomyzon cyanotis	GCTCACACTTAAGTACTACTT----------AAGTA
Foulehaio carunculata	GCTCACACTTAAGTACTACTTAAGTACTACTTAAGTACTAC----------TACTTAAGTA
Melipthreptus albifrons	GCTCACACTTAAGTACTACTTAAGTACTACTTAAGTACTACTTAAGTACTACTACTTAAGTA
Melipthreptus brevirostris	GCTCACACTTAAGTACTACTTAAGTACTACTTAAGTACTACTT----------AAGTA

Epthianura aurifrons and *Glycichaera fallax* are included to illustrate the common (uninserted) condition. The other four taxa possess inserted sequence of varying length.

differences appear to saturate. Based on these plots, only transitions at the third positions of the protein coding genes and at first position in ND2 were considered saturated and weighting schemes were applied only to these partitions.

3.2. Phylogenetic analyses

None of the ILD tests between mitochondrial genes produced significant results. The test between the FIB5 data and the combined mitochondrial genes resulted in a $p = 0.054$. This would be considered borderline significant using a traditional α level of 0.05 (but see Cunningham, 1997), so potential incongruence between the mitochondrial and nuclear partitions was investigated by topological comparisons. There were no conflicting nodes in the 75% bootstrap trees resulting from separate phylogenetic analysis of the nuclear and mitochondrial data sets and this was interpreted as a lack of strongly supported conflict between the data sets.

Unweighted parsimony analysis of the mitochondrial data set resulted in one most parsimonious tree (Fig. 1). The Meliphagidae form a strongly supported monophyletic group and all genera, with the exception of *Anthochaera*, *Certhionyx*, and *Phylidonyris*, are strongly supported as monophyletic. Although many of the deeper nodes in the tree have little or no bootstrap support, a number of novel relationships among genera are well-supported. Downweighting transitions in the three saturated partitions (CYTB and ND2 3rd positions and ND2 1st positions) resulted in no serious change in topology (results not shown): two unsupported nodes collapsed. However, downweighting did increase bootstrap (BS) support for a number of relatively deep nodes within the family. This increase indicates that homoplasy in the saturated partitions is compromising resolution at these nodes and downweighting homoplasious partitions can boost the signal in the remaining partitions. However, as no significant differences in topology obtained with downweighting, the combined analyses were unweighted.

Parsimony analysis of the FIB5 data set resulted in 8290 equally parsimonious trees. The strict consensus of these trees (Fig. 2) has a well-supported monophyletic Meliphagidae and all genera, with the same exceptions as the mitochondrial data, are well-supported and monophyletic. Resolution at the tips of the tree is poorer with the FIB5 data than the mitochondrial data; this

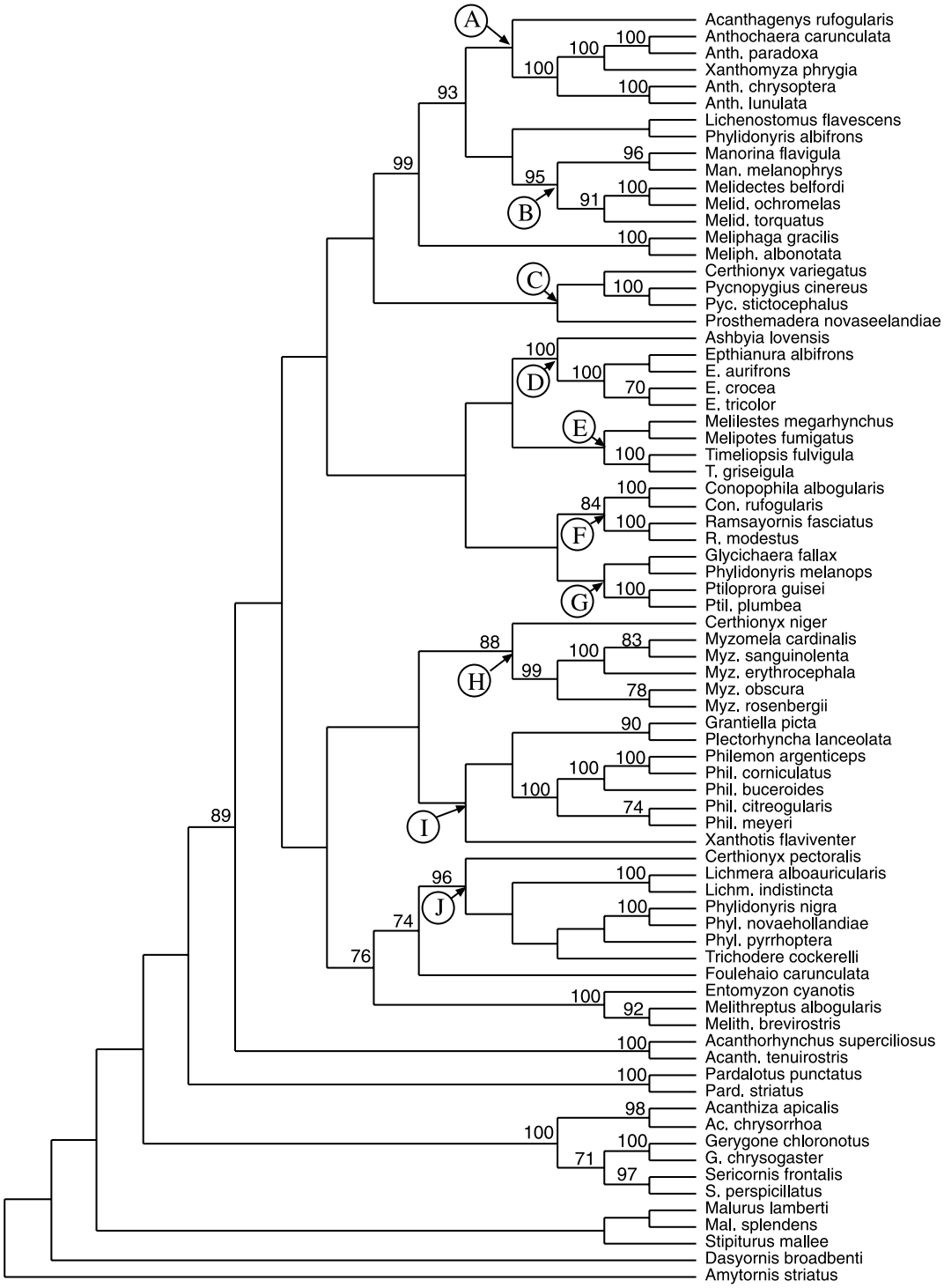

Fig. 1. The most parsimonious tree resulting from unweighted analysis of the three mitochondrial loci (CYTB, ND2 and 12S). Bootstrap support values greater than 70% are shown. Letters are used to label nodes for comparison with the nuclear consensus topology (Fig. 2).

result is not unexpected given the low levels of divergence in the nuclear data set. Although both data sets resolve many of the same groups of genera (labelled in Figs. 1 and 2), deeper nodes in the tree, corresponding to relationships among these well-supported groups of genera, are quite different. However, due to the poor resolution in the middle levels of the tree, the differences in resolution cannot be considered serious conflict.

Analysis of the combined nuclear and mitochondrial data sets under the parsimony criterion produced two equally parsimonious trees (Fig. 3). The two topologies differed in the relative positions of the three major

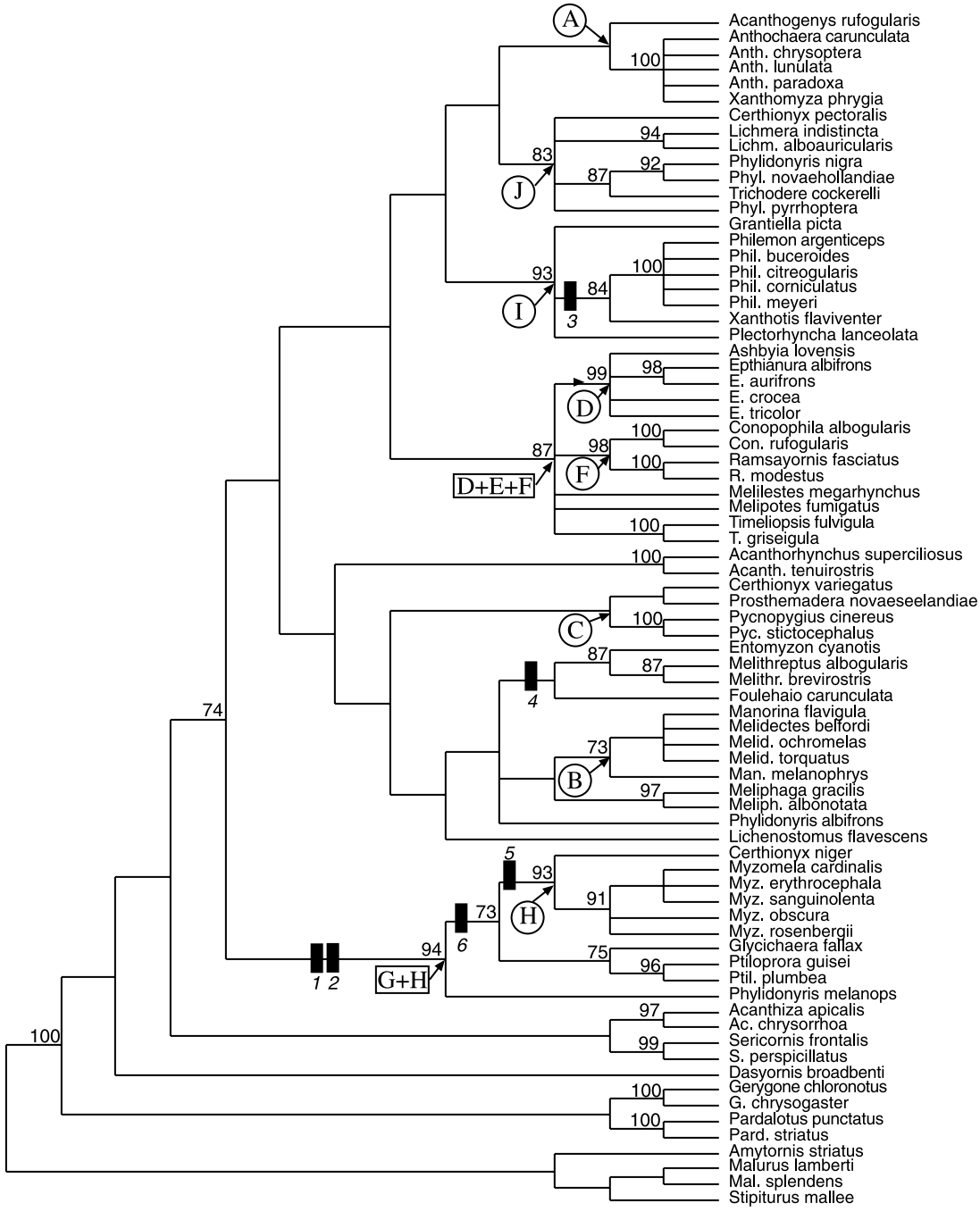

Fig. 2. A strict consensus of the 8290 equally parsimonious trees resulting from unweighted parsimony analysis of the nuclear FIB5 data set. Bootstrap support values greater than 70% are shown. Letters are used to label nodes for comparison with the mitochondrial topology (Fig. 1). Shaded boxes correspond to the most parsimonious mapping of the indel characters listed in Tables 3a and 3b.

pardalotid lineages and not in any ingroup relationships. The topologies resulting from the combined analysis and the separate mitochondrial analysis are nearly identical and there are no well-supported differences. Therefore, the primary result of the addition of the FIB5 data to the mitochondrial data set was a general increase in many bootstrap support values, particularly those at deeper nodes within the Meliphagidae.

For Bayesian analysis a model with six substitution classes, unequal base frequencies and rate heterogeneity was employed. The results of the MODELTEST analysis indicated that each of the partitions required a substantially different Γ shape parameter. Therefore, instead of modeling rate variation with a single parameter for all four partitions, site specific rates were calculated for each data partition separately. Based on

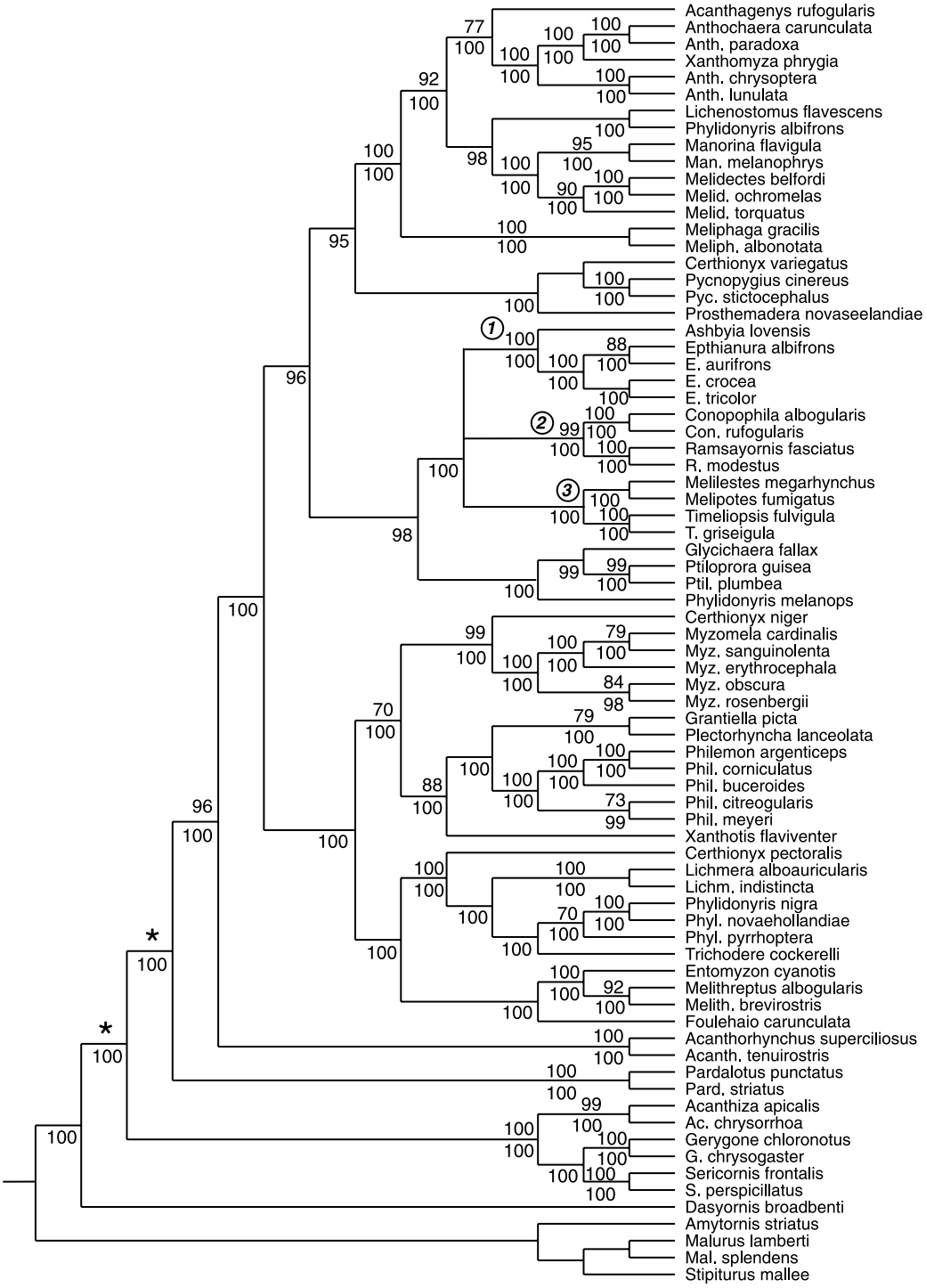

Fig. 3. The topology equivalent to the maximum *a posteriori* estimate resulting from Bayesian analysis and the consensus topology (of two equally parsimonious tres) resulting from parsimony analysis of the combined mitochondrial and nuclear data set. Bayesian posterior probabilities greater than 95% are shown below the nodes and bootstrap support values greater than 70% are shown above the nodes. The two nodes marked by asterisks are unresolved in the parsimony consensus topology and the three numbered nodes are referred to in the text.

visual examination, all eight MCMC runs appeared to reach stationarity at approximately 30,000 generations. As a conservative measure, the trees from the first 50,000 generations were excluded from the calculation of posterior probabilities. The maximum a posteriori (MAP) estimates of the topology, which are point estimates of the maximum likelihood topology (Huelsenbeck and Bollback, 2001), were identical from all eight runs. Posterior probabilities (PP) of all nodes save two were >95% and the majority were 100% (Fig. 3).

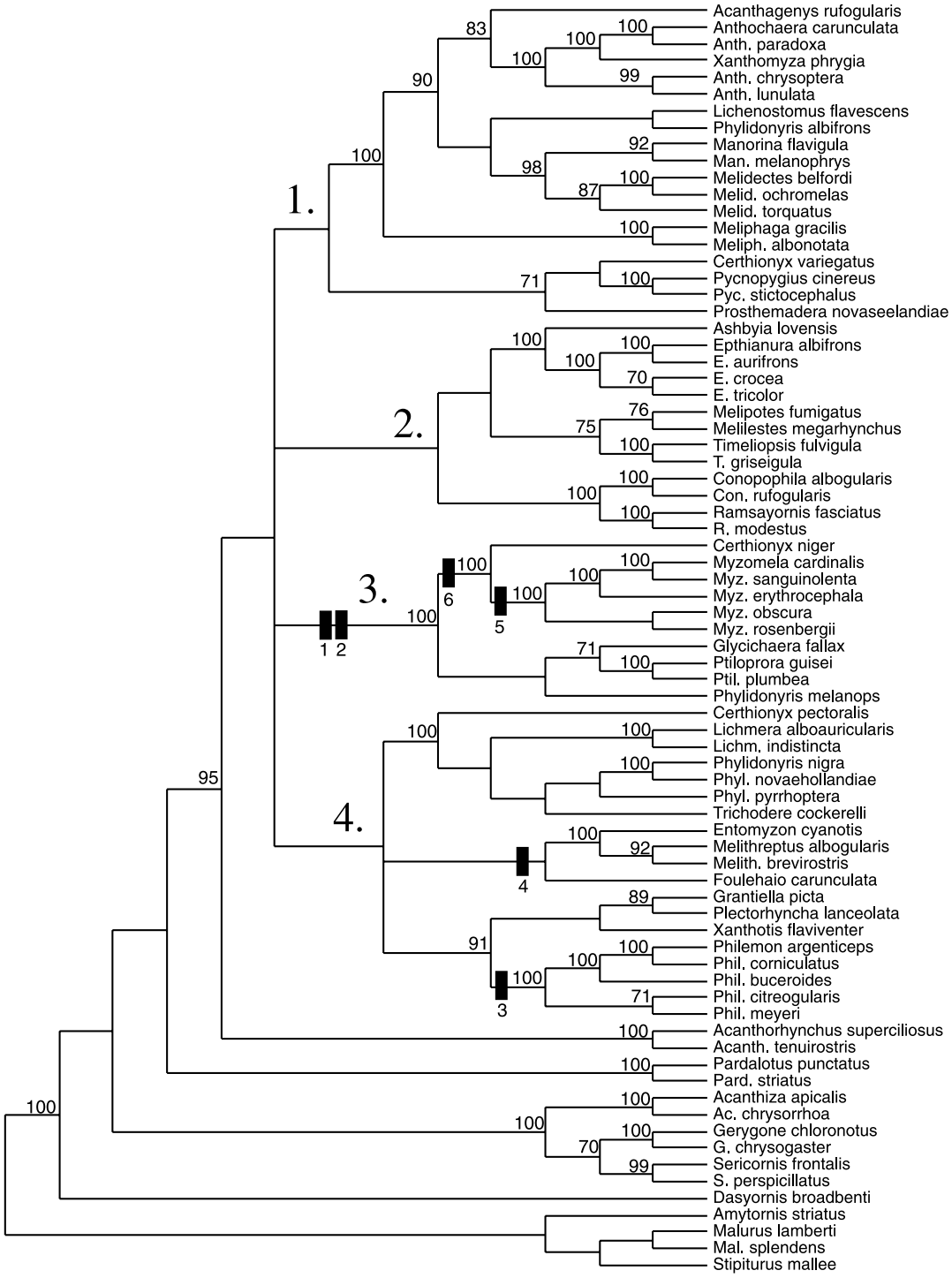

Fig. 4. A strict consensus of the two equally parsimonious trees resulting from a constrained parsimony search. The node numbered "3" was the constrained node. Although 14 steps longer, his topology was not found to be significantly different from the topology in Fig. 3 using parametric bootstrapping. Bootstrap support values greater than 70% are shown. Shaded boxes correspond to the most parsimonious mapping of the indel characters listed in Tables 3a and 3b. Numbered clades are referred to in the text.

The MAP topology resulting from Bayesian analysis was nearly identical to the consensus topology resulting from the unweighted parsimony analysis. Where parsimony analysis was unable to resolve the phylogenetic positions of the three pardalotid clades, in the Bayesian topology these lineages were resolved as successive outgroups to the Meliphagidae, with high PP. The only instance of conflict between the parsimony consensus topology and the MAP topology was in the relative positions of three meliphagid lineages (labelled 1, 2, and

3 in Fig. 3). In the parsimony topology, the trichotomy was resolved as (1,3)2 with BS support of 62%. The MAP topology had another resolution of these clades: (1,2)3 with a PP of 61%. As neither method of analysis furnished a well-supported resolution of these three clades, relationships among them should be considered equivocal and unresolved.

One strongly supported node in the FIB5 consensus tree unites *Myzomela*, *Certhionyx niger*, *Glycichaera*, *Ptiloprora*, and *Phylidonyris melanops* (clade G + H in Fig. 2, and hereafter referred to as the "nuclear" arrangement of the taxa) and is not present in either the separate mitochondrial tree (Fig. 1) or in the trees resulting from analysis of the combined data set (Fig. 3). Instead, in these other topologies *Myzomela* + *C. niger* are associated with a large clade of *Philemon* and its relatives, while *Ptiloprora*, *Glycichaera*, and *Phylidonyris albifrons* form a clade elsewhere in the tree (henceforth referred to as the "mitochondrial" arrangement of these taxa). The nuclear arrangement of these taxa is further bolstered by the presence of two indel characters: a 3-bp deletion shared by *C. niger*, *P. melanops*, *Ptiloprora*, *Glycichaera*, and *Myzomela*, and an 18-bp insertion shared by these taxa and both outgroup families (Tables 3a and 3b and Figs. 2 and 4). The constrained search of the combined data set produced two equally parsimonious trees (Fig. 4) which were 14 steps longer than the trees resulting from the unconstrained search. Parametric bootstrapping showed that the null hypothesis of the constrained topology could not be rejected by the data (the critical value was 29 steps). Therefore the constrained and unconstrained topologies are not significantly different.

4. Discussion

4.1. Comparative information content of the four loci

We believe the proportion of parsimony informative characters is an insufficient measure for comparing the phylogenetic utility of different data partitions, or for determining whether a partition should be down-weighted for analysis (*contra* Allard et al., 1999; Sennblad and Bremer, 2000). Among the four loci we sampled, the more "parsimony informative" characters contained in a partition, the lower the average CI of characters in that partition (Table 2). An extreme example of this is ND2 third positions, of which 99% are parsimony informative and 97% have CI less than 0.5. Thus, in these data, characters classed as parsimony informative are actually very homoplasious.

In our data, FIB5 has a higher proportion of variable characters, but 12S has more phylogenetically informative characters (Table 2). Although FIB5 has more autapomorphic changes, the level of homoplasy in this partition is much lower. In addition, a large amount of variation in the 12S data occurs in the form of indels in the loop regions of the molecule. These indels make many loop regions difficult to align unequivocally, and force the exclusion of these regions from analyses. In this process, a potential source of phylogenetic information is lost (Lutzoni et al., 2000). Despite the fact that much of the molecular evolution of the FIB5 intron also takes the form of indels, these indels are generally not as difficult to align as the indels in 12S. Furthermore, variation in the FIB5 indel regions is quite low, and many of indels themselves can be profitably coded and included in phylogenetic analysis, making the nuclear intron a more fruitful source of phylogenetic information than 12S.

Although of a similar length, ND2 provides considerably more variable characters than CYTB for phylogenetic analysis (Table 2). This is a consequence of the higher variability of the first and second codon positions in ND2. Levels of homoplasy in the two protein coding genes appear similar, and therefore ND2 appears to have greater phylogenetic utility than CYTB.

4.2. Molecular systematics of the Meliphagidae

The family Meliphagidae as constituted here is strongly supported as monophyletic. The genus *Pardalotus* may be more closely related to the honeyeaters than to other genera in the family Pardalotidae, but these relationships are not robustly resolved in our analyses. Certainly, phylogenetic relationships among pardalotid taxa require further study. Within the Meliphagidae, all genera for which we sampled more than one species, with the exceptions of *Anthochaera*, *Phylidonyris*, and *Certhionyx*, are monophyletic.

The Regent Honeyeater, *Xanthomyza phrygia*, is nested within the wattlebird genus *Anthochaera*. The large-bodied wattlebirds (*Anthochaera carunculata* and *Anthochaera paradoxa*) are more closely related to *X. phrygia* than they are to the smaller-bodied wattlebirds (*Anthochaera lunulata* and *Anthochaera chrysoptera*). While this result is unexpected, monotypic *Xanthomyza* is behaviorally similar to the large wattlebirds (A. Keast, pers. comm.), while Schodde and McKean (1976) noted that the eggs of *Xanthomyza* have a similar color and pattern to those of *Anthochaera*. In addition, Veerman (1992) reported some of the vocalisations of *Xanthomyza* resembled those of *Anthochaera*. Although this was interpreted as evidence of vocal mimicry by *Xanthomyza*, it may in fact reflect the shared phylogenetic history of the two genera.

Species composition of *Phylidonyris* and *Certhionyx* has traditionally been in flux and a number of authors have questioned the validity of the genera as currently described (reviewed in Christidis and Boles, 1994). In our phylogeny, both genera are polyphyletic. The three

species comprising *Certhionyx* are not closely related to one another and belong to three different clades. The five species of *Phylidonyris* included in this study form three separate lineages. *Phylidonyris novaehollandiae*, *Phylidonyris nigra*, and *Phylidonyris pyrrhoptera* form a monophyletic group that is disassociated from *P. melanops* and *P. albifrons*, both of which appear in different clades. These five *Phylidonyris* species are restricted to Australia and the genus has no members in northern Australia or New Guinea (Sibley and Monroe, 1990). The remaining two species are restricted a few south Pacific islands: *Phylidonyris notabilis* to Vanuatu and southern Melanesia, and *Phylidonyris undulata* to New Caledonia. The unusual geographic distribution of *Phylidonyris*—present only in Australia and some south Pacific islands, but absent from New Guinea—is unique among passerines (Sibley and Monroe, 1990). As the five Australian species do not form a monophyletic group, it seems unlikely the two south Pacific species will prove to be closely related to their current congeners.

Schodde (1975) split the large genus *Meliphaga* into three genera: *Lichenostomus*, *Xanthotis*, and *Meliphaga*, and this division was supported by allozyme data (Christidis and Schodde, 1993). Our results show that these three genera do not form a monophyletic group. *Xanthotis*, distributed in rainforests in northernmost Australia and in New Guinea, is more closely related to the friarbirds (*Philemon*) and their allies and not at all closely related to either *Lichenostomus* or *Meliphaga*. The latter two genera are two of the most species-rich honeyeater genera and are contained within the same clade, but not as each other's closest relatives. However, many species in these two genera remain to be sampled and phylogenetic relationships in this clade may change with the addition of taxa. Therefore a definitive statement on the relatedness of *Lichenostomus* and *Meliphaga* cannot be made at this time.

In the constrained parsimony consensus topology, (Fig. 4) all meliphagid taxa except the spinebills (*Acanthorhynchus*) are contained in one of four large clades. Each of these four clades is comprised of New Guinean and Australian endemics as well as more widely ranging taxa. The lack of bootstrap support for the three unconstrained clades arises from a paucity of characters supporting this part of the tree, and therefore we do not perceive phylogenetic relationships within the family as fully resolved at this level. However, each clade includes one or more strongly supported subclades.

Clade 1. The first clade (labelled 1 in Fig. 4) is primarily comprised of a large well-supported subclade containing the wattlebirds (*Anthochaera*), Regent Honeyeater (*Xanthomyza phrygia*), miners (*Manorina*), and White-fronted honeyeater (*P. albifrons*) from Australia, the New Guinean montane endemic *Melidectes*, and the genera *Lichenostomus* and *Meliphaga* which occur in both Australia and New Guinea. One unexpected result is the sister relationship between *Manorina* and *Melidectes*. Their close relationship has interesting implications for the evolution of cooperative breeding in the family. The miners (*Manorina*), arguably the only truly obligate cooperative breeders in the family Meliphagidae (Clarke, 1995), are not most closely related to other cooperatively breeding honeyeater genera such as *Melithreptus* and *Lichenostomus*, but instead to *Melidectes*, in which this complex behavioural trait has not been observed.

Also part of clade 1 is a subclade composed of the New Zealand endemic *P. novaeseelandiae*, the New Guinean endemic *Pycnopygius*, and the Australian endemic *Certhionyx variegatus* Biogeographically, this clade is a hodge–podge assemblage and although these taxa appear together in all analyses, relationships among them are not robustly resolved. Furthermore, a number of monotypic south Pacific and Indonesian endemic honeyeater genera, including another New Zealand taxon (*Anthornis*), were not sampled in the current study. With the addition of these enigmatic taxa, which may be potential relatives of members of this subclade, a more coherent biogeographical pattern may emerge.

Clade 2. The second major clade is a poorly resolved trichotomy involving the Australian endemic chats (*Epthianura* and *Ashbyia*), the primarily Australian *Conopophila* and *Ramsayornis*, and the New Guinean endemics *Melilestes*, *Melipotes*, and *Timeliopsis*. Both *Conopophila* and *Ramsayornis* range into southern-most New Guinea, but neither genus has an endemic New Guinean species. Therefore, if the endemic chats (*Ashbyia* + *Epthianura*) and *Conopophila* + *Ramsayornis* are in fact sister groups, this clade would represent a substantial Australian radiation with a few ancient New Guinean lineages.

The Australian chats (*Epthianura* and *Ashbyia*) reliably resolve as members of the second clade. At one time classified in their own family Epthianuridae (e.g., Schodde, 1975), the chats are remarkable for their adaptation to arid habitats (Schodde and Mason, 1999). Our result supports those of Sibley and Ahlquist (1990) and Christidis et al. (1993) and establishes the chats not only as members of the Meliphagidae, but in a position nested well within the family.

Clade 3. This clade is strongly supported by the nuclear data, and further strengthened by two unequivocal indel characters. While it is possible that the indel characters are homoplastic, we consider it highly unlikely that both characters would show the same pattern of homoplasy and we take these two indel characters as firm evidence for this clade. One component of clade 3 is a subclade consisting of the New Guinean endemic *Ptiloprora*, the Australian endemic *P. melanops*, and *Glycichaera fallax*. Monotypic *Glycichaera* has a very limited distribution in Australia, but is much more widespread in New Guinea (Sibley and Monroe, 1990).

Based on the structure of its bill and skull, Schodde and Mason (1999) re-classified *G. fallax* in the New Guinean genus *Timeliopsis*, but our results show that these two genera are not closely related. Both *Glycichaera* and *Timeliopsis* primarily feed by gleaning foliage for insects (Beehler et al., 1986), which is a rare specialty among honeyeaters (Blakers et al., 1984). Both genera are also characterized by a moderately long, straight bill, which is also rare among meliphagids. It is possible that the straight bills of *Timeliopsis* and *Glycichaera*, adapted for foliage gleaning, appear more similar than they truly are, when compared with the longer, more curved bills of the majority of honeyeaters.

In the other subclade, our results show the Australian endemic Black Honeyeater, *C. niger*, is sister to the five representative species of the genus *Myzomela*. Although *C. niger* was originally classified in the genus *Myzomela* (Gould, 1838), it is more likely that *C. niger* is the sister taxon to *Myzomela*, rather than nested within it, for two reasons. First, although the sample of *Myzomela* species included in the present study is small (5 of 30 species), it includes taxa from Australia, New Guinea, and islands in the south Pacific. Yet, *C. niger* is, on average across all four genes, 12.0% divergent from the *Myzomela* species (range of divergences within sampled *Myzomela*: 5.2–10.5%). Also, although *C. niger* shares a unique deletion in FIB5 with *Myzomela*, it also lacks a FIB5 deletion unique to the 5 species of *Myzomela*. Given the indel data, it is difficult to postulate a phylogenetic position for *C. niger* within *Myzomela*, and these data support instead a sister position.

Clade 4. The fourth primary clade, contains three well-supported subclades, one of which is substantiated by a complex indel character. Included in clade 4 are the species-rich and wide-ranging genera *Philemon* and *Lichmera*, the primarily New Guinean *Xanthotis*, the primarily Australian *Melithreptus* and *Entomyzon*, the Australian endemics *Certhionyx pectoralis*, *Grantiella*, *Plectorhyncha*, *Trichodere*, *P. novaehollandiae*, *P. nigra*, and *P. pyrrhoptera*, and the south Polynesian endemic *Foulehaio*. This clade contains the broadest geographic coverage of the three, with representatives from Indonesia through to the south Pacific.

Within one subclade, the friarbirds (*Philemon*) group with *Grantiella*, *Plectorhyncha*, and *Xanthotis*. Within the friarbirds, species group predictably with regard to morphological similarity: the three species with knobbed bills and bare black skin on the head (*Philemon argenticeps*, *Philemon corniculatus*, and *Philemon buceroides*) form one clade, while the two straight-billed, more fully feathered species (*Philemon citreogularis* and *Philemon meyeri*) form a second. An unanticipated pairing is the sister relationship between the Painted Honeyeater (*Grantiella picta*) and the Striped Honeyeater (*Plectorhyncha lanceolata*). Both of these species are morphologically disparate and a close relationship between them has never been suspected. However, both *Grantiella* and *Plectorhyncha* build nests with similar structure (N. W. Longmore, pers. comm.).

Another well-supported subclade contains three species of *Phylidonyris*, the monotypic White-streaked honeyeater (*Trichodere cockerelli*), *Lichmera*, and *C. pectoralis*. *Trichodere* is sister to the *Phylidonyris* clade and these four taxa do share bright yellow patches of color on the wing with some members of their sister genus *Lichmera*. However, presence of a yellow wing patch is a poor defining character for the group, as this character is widespread among other honeyeaters and strikingly similar in some unrelated taxa (e.g., *Grantiella*, *Xanthomyza*, and *P. albifrons*).

In the third subclade, the Blue-faced Honeyeater (*Entomyzon cyanotis*) is sister to *Melithreptus* (two species examined). *Entomyzon* was classified within the genus *Melithreptus* without explanation by Storr (1977, 1984), but most authors (e.g., Schodde, 1975) have considered it to be more closely related to the larger-bodied miners (*Manorina*) and wattlebirds (*Anthochaera*). The plumage of the Blue-faced Honeyeater is remarkably similar to that of *Melithreptus*, but it is possible that the great size difference between the two genera has obscured the significance of this fact. A definitive statement on whether *Entomyzon* should be included within *Melithreptus*, as advocated by Storr, waits on the sampling of the remaining four species of *Melithreptus*. Sister to *Melithreptus* + *Entomyzon* is the south Polynesian endemic *Foulehaio*. This grouping is supported by the presence of a large insertion in the FIB5 intron in these taxa relative to the remaining Meliphagidae (Tables 3a and 3b). The inserted sequence is a different length in all four species, but nucleotide sequence of these four inserted regions are identical and differ from one another by deletions within the insertion. The most parsimonious reconstruction of the acquisition of this insertion would be if it occurred in the common ancestor of *Foulehaio*, *Melithreptus*, and *Entomyzon*.

The spinebills, *Acanthorhynchus*, which are perhaps the most obviously specialized nectarivores in the family and inhabit dry heathland and woodlands (Longmore, 1991), have no close relatives and appear as sister to all remaining Meliphagidae in all but the nuclear consensus topology. Heathlands were widespread in Australia during the Tertiary and became fragmented as rainfall became more seasonal during the Quaternary (Specht, 1979). Although the exact age of the Meliphagidae is unknown, the order Passeriformes has recently been posited to be Cretaceous in origin (Cooper and Penny, 1997; Cracraft, 2001; Hedges et al., 1996; Paton et al., 2002), which could place the origin of the Meliphagidae within the widespread-heathland period of the mid-Tertiary. Our finding that heathland-adapted spinebills were one of the earliest established lineages of the

Meliphagidae habitat corresponds well to a mid-Tertiary origin of the family.

4.3. Comparison with previous studies

The DNA–DNA hybridization study of Sibley and Ahlquist (1985, 1990) included considerably fewer honeyeater taxa than the present study (20 genera, compared to 31). Some of the same species are present in both studies, but a number of the species used in the DNA–DNA hybridization study are not identified. This species identification is particularly important for the genus *Certhionyx*, which occupies three different positions in our results. Although Sibley and Ahlquist's methods and analysis have been criticized from many standpoints (Cracraft, 1987; Harshman, 1994; Houde, 1987; Mindell, 1992), it is the only previously published phylogenetic hypothesis for the entire family Meliphagidae. Our topology shares two aspects with Sibley and Ahlquist's topology: the chats (*Epthianura* and *Ashbyia*) are nested within the Meliphagidae and are related to the genus *Ramsayornis*; and *Entomyzon* is sister to the genus *Melithreptus*. Nearly all other taxa are placed in different positions in the two topologies. Other than the DNA–DNA hybridization study, only two studies grouped some of the honeyeater taxa based on morphological (Schodde, 1975) and biochemical (Sibley, 1970) characteristics. Sibley's study contained very few honeyeater taxa and has only one group in common with the current study: the grouping of *Lichmera* with *Meliornis* (*P. novaehollandiae* and *P. nigra* in the present study). Schodde (1975) grouped many Australian and New Guinean genera into two "lines" based on morphological and plumage characteristics, but there are as many differences as similarities between Schodde's groups and our tree. However, the majority of the genera in Schodde's "line 1" also belong to a monophyletic group in our topology: *Melidectes*, *Anthochaera*, *Acanthagenys*, *Xanthomyza*, *Manorina*, *Meliphaga*, and *Lichenostomus*.

4.4. Biogeography of the meliphagidae

Based on current taxonomy, the honeyeater faunas of New Guinea and Australia are each composed of a number of endemic genera, with additional genera and species shared between the two (Sibley and Monroe, 1990). A reasonable hypothesis for the generation of this pattern of distribution is that the endemic taxa arose at a point in time when Australia and New Guinea were relatively isolated from one another. This would produce a phylogenetic tree with largely monophyletic Australian and New Guinean endemic radiations. However, our topology does not support this hypothesis. Certainly there is very little evidence for a New Guinean radiation. Endemic New Guinean genera are found in four different clades, and are not necessarily each other's closest relatives even within a clade. Indeed, it seems unlikely that the New Guinean genera arose at the same time. The New Guinean endemic *Melidectes* is more closely related to its sister taxon, the Australian endemic *Manorina* (average uncorrected genetic distance across all four genes 7.9%), than are the three New Guinean genera *Melilestes*, *Melipotes*, and *Timeliopsis* to each other (average genetic distance 12.4%). In fact, *Melidectes* is more closely related to *Manorina* than the two species of *Timeliopsis* are to each other (9.2%). This suggests that *Melidectes* is more recently derived than these other New Guinean endemics. Of course, alternative explanations for this phenomenon exist: the rate of molecular evolution in *Melidectes* may be slower than in the other New Guinean endemics, or *Melilestes* and *Melipotes* may be the extant remnants of much larger clades and hence their level of genetic divergence appears to be greater.

There is very little evidence in the topology for the "Tumbunan" hypothesis, which predicts a close relationship between taxa from the New Guinea highlands and those from the Australian rainforests (Schodde and Calaby, 1972). Species in the New Guinean genus *Melidectes* are all found in montane areas above 1200 m elevation. Yet their closest relatives are the Australian miners (*Manorina*), which inhabit woodlands and forests, but not rainforests (Blakers et al., 1984). Other New Guinean highland taxa are found in clade 2 (Fig. 1), but their sister relationships are not resolved. Nonetheless, the remaining taxa in these two clades are not rainforest inhabitants, although *Ramsayornis* and *Conopophila* inhabit swamps and mangroves in northern Australia (Blakers et al., 1984). The present study does not support the highlands-southern rainforests link proposed by Schodde and Calaby (1972), but this link might be upheld by studies at a lower phylogenetic level. An examination of the phylogenetic relationships within the large genera *Meliphaga* and *Lichenostomus*, which have member species in both New Guinea and Australia, might better test this hypothesis.

5. Conclusions

The family Meliphagidae, as constituted here, is monophyletic, although the genera *Anthochaera*, *Certhionyx*, and *Phylidonyris* are not. Four major clades are recovered, and the overwhelming majority of honeyeater taxa belong to one of these four clades. The exception is the genus *Acanthorhynchus* (spinebills) which are sister to the remaining meliphagids and have no close relatives. The arid-adapted chats (*Epthianura*, *Ashbyia*) are nested deeply within the family, although their sister group is not identified. Each of the four major clades contains a mix of New Guinean and Australian

endemics, along with more wide-ranging taxa. There is no evidence for the occurrence of separate Australian and New Guinean endemic radiations. There is also little evidence for the "Tumbunan" hypothesis of a close phylogenetic relationship between New Guinea highland and eastern Australia rainforest taxa.

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Appendix A

List of specimens sequenced in the present study

Family Species	Spec. No.	Voucher	Collection locality
Meliphagidae			
Acanthagenys rufogularis	MV1122	MV	Stockyard HS, NT
Acanthorhynchus superciliosus	MV248	MV	Albany, WA
Acanthorhynchus tenuirostris	B873	ANWC	Tenterfield, NSW
Anthochaera carunculata	C257	ANWC	Stuart's Point, NSW
Anthochaera chrysoptera	B792	ANWC	Upper Blessington, TAS
Anthochaera lunulata	MV175	MV	Esperance, WA
Anthochaera paradoxa	B736	ANWC	Upper Blessington, TAS
Ashbyia lovensis	D173	ANWC	Koonchara Dune, SA
Certhionyx niger	C954	ANWC	Winton, QLD
Certhionyx pectoralis	C912	ANWC	Musgrave, QLD
Certhionyx variegatus	W036	SAM	Mabel Creek, SA
Conopophila albogularis	MV1216	MV	Gunn Point, NT
Conopophila rufogularis	MV1300	MV	Cape Crawford, NT
Entomyzon cyanotis	F274	ANWC	Chillagoe, QLD
Epthianura albifrons	D328	ANWC	Eyre Peninsula, SA
Epthianura aurifrons	D156	ANWC	Innaminka, SA
Epthianura crocea	D175	ANWC	Koonchara Dune, SA
Epthianura tricolor	D229	ANWC	William Creek, SA
Foulehaio carunculata	2077	RF UV	Unknown
Glycichaera fallax	E663	ANWC	Veimari River, PNG
Grantiella picta	MV2673	MV	Killawarra State Forest, VIC
Lichenostomus flavescens	D029	ANWC	Timber Creek, NT
Lichmera alboauricularis	E629	ANWC	Port Moresby, PNG
Lichmera indistincta	C271	ANWC	Stuart's Point, NSW
Manorina flavigula	42856	ANWC	Charters Tower, QLD
Melidectes ochromelas	E360	ANWC	Tetebedi, PNG
Manorina melanophrys	42737	ANWC	Kempsey, NSW
Melidectes belfordi	E168	ANWC	Tetebedi, PNG
Meliphaga albonotata	E471	ANWC	Tetebedi, PNG
Meliphaga gracilis	C753	ANWC	McIlwraith Range, QLD
Melipotes fumigatus	E332	ANWC	Tetebedi, PNG

Appendix A (*continued*)

Family Species	Spec. No.	Voucher	Collection locality
Melipthreptus albogularis	JC100	ANWC	Burra Range, QLD
Melipthreptus brevirostris	MV371	MV	Ceduna, SA
Myzomela cardinalis	2494	RF UV	Unknown
Myzomela eythrocephala	MV1198	MV	Gunn Point, NT
Myzomela obscura	C531	ANWC	Cathu, QLD
Myzomela rosenbergii	E240	ANWC	Tetebedi, PNG
Myzomela sanguinolenta	C402	ANWC	Agnes Waters, QLD
Philemon argenticeps	JCW095	ANWC	Coen region, QLD
Philemon buceroides	C863	ANWC	Silver Plains, QLD
Philemon citreogularis	D008	ANWC	Pine Creek, NT
Philemon corniculatus	C720	ANWC	Musgrave, QLD
Philemon meyeri	E683	ANWC	Veimari River, PNG
Phylidonyris albifrons	D361	ANWC	Sinclair's Gap, SA
Phylidonyris melanops	D451	ANWC	Big Desert, VIC
Phylidonyris nigra	MV198	MV	Raventhorpe, WA
Phylidonyris novaehollandiae	B685	ANWC	Launceston, TAS
Phylidonyris pyrrhoptera	B615	ANWC	Mt. Lofty Ranges, SA
Plectorhyncha lanceolata	C379	ANWC	Agnes Waters, QLD
Prosthemadera novaeseelandiae	11/1996	MNZ	New Zealand
Ptiloprora guisei	E173	ANWC	Tetebedi, PNG
Ptiloprora plumbea	C173	ANWC	Efogi, PNG
Pycnopygius cinereus	C057	ANWC	Efogi, PNG
Pycnopygius stictocephalus	C035	ANWC	Efogi, PNG
Ramsayornis fasciatus	MV1230	MV	Finniss River, NT
Ramsayornis modestus	C900	ANWC	Silver Plains, QLD
Timeliopsis fulvigula	E233	ANWC	Tetebedi, PNG
Timeliopsis griseigula	E714	ANWC	Veimari River, PNG
Trichodere cockerelli	42941	ANWC	Silver Plains, QLD
Xanthomyza phrygia	F724	ANWC	Sutton, NSW
Xanthotis flaviventer	E594	ANWC	Kokoda, PNG
Pardalotidae			
Acanthiza apicalis	MV158	MV	Norseman, WA
Acanthiza chrysorrhoa	MV116	MV	Port Augusta, SA
Dasyornis broadbenti	MV2172	MV	Aireys Inlet, VIC
Gerygone chrysogaster	E670	ANWC	Veimari River, PNG
Gerygone chloronotus	E122	ANWC	Tetebedi, PNG
Pardalotus punctatus	B479	ANWC	Sutton, NSW
Pardalotus striatus	B471	ANWC	Sutton, NSW
Sericornis frontalis	MV228	MV	Albany, WA
Sericornis perspicillatus	E313	ANWC	Tetebedi, PNG
Maluridae			
Amytornis striatus	SGW1	SPJ UV	Hattah-Kulkyne, N.P., VIC
Malurus lamberti	VW104	BCP	Brookfield Cons. Park, SA
Malurus splendens	SW683	BCP	Brookfield Cons. Park, SA
Stipiturus mallee	MEW1	SPJ UV	Hattah-Kulkyne, N.P., VIC

Species are organized by family. Specimen number (Spec. No.) is the collector's field number, in most instances, or the tissue or Accession number. Voucher is the location of the voucher specimen corresponding to the tissue specimen. Collection locality is the nearest named location to the specimen's collection locality. ANWC = Australian National Wildlife Collection, C.S.I.R.O., Canberra, Australia; BCP = Brookfield Conservation Park, SA; MNZ = Museum of New Zealand Te Papa Tongerewa, Wellington, New Zealand; MU = Massey University, Palmerston North, New Zealand; MV = Museum Victoria, Melbourne, VIC; RF = Rob Fleischer, Smithsonian Institution, Washington, DC; SPJ = Steve Pruett-Jones, University of Chicago, Chicago, IL; WAM = Western Australia Museum, Perth, WA; NT = Northern Territory; QLD = Queensland; SA = South Australia; SAM = South Australian Museum, Adelaide, SA; TAS = Tasmania; PNG = Papua New Guinea; VIC = Victoria; and UV = unvouchered.

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