Mitochondrial and nuclear DNA phylogenies reveal a complex evolutionary history in the Australasian robins (Passeriformes: Petroicidae)

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ARTICLE INFO

Article history:
Received 8 December 2010
Revised 1 August 2011
Accepted 9 August 2011
Available online 17 August 2011

Keywords:
Petroicidae
Australasian robins
Avian systematics
Avian biogeography
Avian phylogenetics
Australian songbirds
Ecology

ABSTRACT

The Australasian robins (Petroicidae) comprise a relatively homogeneous group of small to medium-sized insectivorous birds. Their center of diversity is Australia and New Guinea (40 species) but seven species have managed to colonize geographically distant islands such as Tanimbar, New Britain, New Zealand, New Caledonia, Norfolk Island, Vanuatu, Solomon Islands, Fiji and Samoa. To resolve the evolutionary relationships within the Petroicidae, we here present the results of a phylogenetic analysis of sequence data from two mitochondrial genes (ND2, CO1) and one nuclear intron (β-Fibrinogen intron 5) for all 14 genera and 40 of the 46 currently recognized species. All phylogenetic analyses identified six primary lineages, treated here as subfamilies, within the Petroicidae: (1) Eopsaltriinae comprising Eopsaltria (excluding E. flaviventris), Tregellasia, Peneothello, Melanodryas, Poecilodryas and Heteromyias; (2) Drymodinae comprising Drymodos; (3) Microcininae comprising Microeca, Monachella and Eopsaltria flaviventris; (4) Petroicinae comprising Petroica and Eugerygone; (5) Pachycephalopseinae comprising Pachycephalos; and (6) Amalocichlininae comprising Amalocicha. The genera Eopsaltria, Microeca, Peneothello and Poecilodryas were found to be paraphyletic. Based on assessments of phylogenetic branching patterns and/or DNA divergence it also was apparent that Eopsaltria australis, Tregellasia leucops, Melanodryas cucullata, Heteromyias albispecularis, Drymodos superciliosus and Microeca flavigaster may each comprise more than one species. The Petroicidae display a complex biogeographical history involving repeated radiations both within, and across Australia and New Guinea. It appears that dispersal into smaller islands such as New Britain, Tanimbar and the South Pacific has only been undertaken by species with a “flycatcher” body form.

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1. Introduction

The Australasian robins (Petroicidae) comprise a relatively homogeneous group of small to medium-sized insectivorous birds. They occur in all wooded habitats from rainforest through temperate woodland to arid scrub, as well as treeless alpine regions. Most are somewhat plump, upright perching birds that employ a ‘sit and pounce’ foraging technique, but some are more flycatcher-like and a few resemble larger thrushes or small warblers. Their center of diversity is Australia and New Guinea where 40 of the 46 species, and all 14 genera occur (based on Boles (2007)). Three species occur in New Zealand, with one each in New Caledonia and Tanimbar, and one extending from Norfolk Island through Vanuatu and the Solomon Islands to Fiji and Samoa.

DNA–DNA hybridization data (Sibley and Ahlquist, 1990) aligned the Petroicidae (=Eopsaltriidae) with the Australasian centered Corvoidea (crows, whistlers, cuckoo-shrikes, fantails and allies), while allozyme data (Christidis and Schodde, 1991) instead, identified closer affinities with the Australasian Meliphagoidea (honeyeaters, fairy-wrens and Australasian warblers). Both placements were consistent with the distributional center of the Petroicidae. However, several DNA sequence studies (Ericson et al., 2002a; Barker et al., 2002, 2004; Beresford et al., 2005; Irestedt and Ohlson, 2008) indicate that the Petroicidae occupies a basal position in the largely northern hemisphere centered Passerida assemblage (warblers, swallows, finches, thrushes and allies). Such a basal phylogenetic position for the Australasian Petroicidae suggests that the family has had a long evolutionary history. Elucidating the phylogenetic and biogeographical history of the

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Petroicidae takes on additional significance, as Australasia is established as the center of origin for the world’s oscine songbirds (Barber et al., 2002; Ericson et al., 2002b; Jönsson et al., 2011).

Based on plumages, eggs, skull characters and behavior, Schodde and Mason (1999) divided the family into three subfamilies: Drymodinae (Drymodius); Petrocinia (Petroica, Euerhypura, Microeca, Monarchella and possibly Pachycephalops); and Eopsaltriinae (Eopsaltria, Tregellasia, Peneoenanthe, Peneothello, Melanolodrys, Heteromyrias, Poeclydrasys). Amalaccia was included in the Acanthisiidae, but DNA sequence data (Norman et al., 2009) firmly place this genus within the Petroicidae. Apart from the configuration of a single skull character, the vomer in the Eopsaltriinae, there were few characters identified by Schodde and Mason (1999) that defined the subfamilies from a phylogenetic perspective, and relationships within each subfamily were also poorly resolved.

A multi-locus DNA phylogenetic study on the Petroicidae by Loynes et al. (2009) was partially congruent with the taxonomy of Schodde and Mason (1999). The DNA data supported recognition of the Eopsaltriinae and Drymodinae, but the Petroicidae was found to be paraphyletic. Phylogenetic relationships of the enigmatic species Peneoenanthe pulvulecta and Eopsaltria flaviventris were also resolved. Although the study of Loynes et al. (2009) had good coverage of Australian taxa, it lacked the New Guinean genera Monachella, Euyrgygona and Anomalochila, and representation from Microeca, Poeclydrasys, Petroica and Heteromyrias was restricted to Australian species. A DNA study by Miller and Lambert (2006) only examined relationships among the New Zealand species of Petroica.

To resolve the evolutionary relationships within the Petroicidae, we here present the results of a phylogenetic analysis of sequence data from two mitochondrial genes (ND2, CO1) and one nuclear intron (β-fibrinogen intron 5) for all 14 genera and 40 of the 46 currently recognized species. We use the phylogeny to better elucidate the biogeographical history of the family and to assess the patterns of ecological diversity.

2. Materials and methods
2.1. Taxon sampling

We sampled all 14 genera and 40 of the 46 of the currently recognized species (Boles, 2007). For several species multiple individuals and subspecies were examined (Table 1). No DNA data were obtained for the following six species: Poeclydrasys brahychura, Peneothello cryptoleuca, Microeca hemixantha, Petroica traversi, Petroica archibaldi and P. bivittata. The absence of these species from the analysis has differing effects on the phylogenetic and biogeographical conclusions. M. hemixantha is generally regarded to be an island representative of M. flavigaster (Boles, 2007), while DNA analysis has revealed a sister relationship between P. traversi and P. macrocephala (Miller and Lambert, 2006). The affinities of P. brachyrhura are less clear, but it is most similar to P. hypoleuca in overall appearance. P. cryptoleuca is believed to be a sister species of P. cyamus (Boles, 2007). More significant are the absence of P. archibaldi and P. bivittata from the analyses as their affinities are very poorly understood (Schodde and Mason, 1999), and their restriction to the montane regions of New Guinea increases their importance in understanding the biogeographical history of the genus Petroica.

The taxon sampling also included representatives from Callaeatidae (Callaeas cinerea), Picathartidae (Picathartes gymnocephalus) and the large Passerida radiation (Hirundinidae: Hirundo rustica, Paridae: Parus major, Passeridae: Passer montanus and Sturnidae: Sturnus vulgaris), as these lineages have been suggested to be closely related to Petroicidae (Barker et al., 2002, 2004; Ericson et al., 2002a; Irestedt and Ohlson, 2008). The trees were rooted with Corvidae (Corvus corone). All sequences obtained in the present study have been lodged on GenBank with the accession numbers JN597010 to JN597237 and JN607437 TO JN607440.

As sequence data for all three genes were not obtained from all specimens included in this study, the taxon sampling for the analysis of the concatenated data was slightly reduced. Specimens from which both nuclear and mitochondrial sequences where available were generally included in the combined analysis. However, when the sequences from multiple specimens of the same taxon were found to be identical (or almost identical), some of these individuals were excluded from the analysis (mainly to reduce the amount of data for the time consuming phylogenetic analyses). In three cases (Petroica multicolor, Petroica australis and E. flaviventris), species were included in the combined analysis based on sequence data from only a single gene.

2.2. DNA extraction, PCR and sequencing

DNA was extracted from frozen and ethanol-preserved tissue using the salt-chloroform extraction procedure of Gemmell and Akiyama (1996). Primers for the amplification of the mitochondrial ND2 (Hackett, 1996; Kirchman et al., 2001) and CO1 (Christidis et al., 2010) genes, and the nuclear gene region β-fibrinogen intron 5 (Driskell and Christidis, 2004) were sourced from the literature. The choice of genes was largely based on previous success in elucidating intrageneric and intergeneric relationships in Australasian songbirds (e.g. Driskell and Christidis, 2004; Norman et al., 2007; Christidis et al., 2010).

PCR amplifications were performed in 25 μL reaction volumes using standard conditions as described in Norman et al. (1998), or in the presence of GoTaq Green Mastermix (Promega Corp., Wisconsin USA) with a final MgCl2 concentration of 3 mM. Annealing temperatures ranged from 48 °C to 52 °C, while extension times ranged from 45 to 80 s depending on the product length. Amplified products were purified using AMPure (Beckman Coulter Inc., Massachusetts, USA), magnetic bead separation or the GFX Gel Band and PCR Purification Kit (Amersham Bioscience Corp., Piscataway, New Jersey, USA). Cycle sequencing reactions and purifications were performed as described in Norman et al. (2007). Sequenced products were separated on a MegaBACE 1000 capillary DNA sequencer as described in Norman et al. (2007). Sequences were aligned and edited using the program Sequencher v4.1.4 (Gene Codes Corp., Michigan, USA) or ProSeq 3 (Filatov, 2002).

2.3. Phylogenetic analysis

We used Bayesian inference and Maximum Likelihood to estimate phylogenetic relationships. All phylogenetic analyses were performed at the CIPRES Science Gateway [http://www.phylo.io/portal2/login/input.action] (Miller et al., 2010). The models for nucleotide substitutions used in the analyses were selected for each gene individually, and by codon position for the two mitochondrial genes by applying the Akaike Information Criterion using the program MrModelfest 2.2 (Nylander, 2004) in conjunction with PAUP*(Swofford, 1998).

In the Bayesian analyses, posterior probabilities of trees and parameters in the substitution models were approximated with MCMC and Metropolis coupling using the program MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003). Analyses were performed for the individual genes separately and for the concatenated data set of all genes. For the concatenated data set, three different partitioning strategies were compared: (1) two partitions – one for β-fibrinogen intron 5 and one for the mitochondrial DNA; (2) four
partitions – one for $b$-fibrinogen intron 5 and one for each codon position in the mitochondrial DNA; and (3) seven partitions – one for $b$-fibrinogen intron 5 and one for each codon position in ND2 and CO1 separately. To select the best partitioning strategy, we compared the log of Bayes Factors as suggested by Kass and Raftery (1995). Tracer v1.5 (Rambaut and Drummond, 2009) was used to calculate, and to compare, the Bayes Factors. For all partitions, two analyses, each with four Metropolis-coupled Markov Chain Monte Carlo simulations (one cold and three heated), were performed. We ran 10 million generations for the individual genes

Table 1
Species and subspecies examined along with their distributions (Distr): NG = New Guinea; Aust = Australia; NZ = New Zealand; SI = Solomon Islands; NC = New Caledonia. Specimens examined are listed under the individual genes sequenced. Those specimens that were not included in the combined analysis are underlined. Tissue samples are listed according to their museum voucher field numbers. Prefixes for the various museums are: MV = Museum Victoria; AM = Australian Museum; UC = University of Copenhagen; NTAMAG = Northern Territory Museum and Art Gallery; SRI = Massey University. All other tissue samples are from the Australian National Wildlife Collection (CSIRO). More detailed locality and voucher information can be provided on request.

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Fig. 1. Bayesian tree (with branch lengths) based on the ND2 sequences. Posterior probabilities are shown at the nodes; values of 1.00 are indicated with an asterisk. Specimen details are listed in Table 1.
Fig. 2. Bayesian tree (with branch lengths) based on the CO1 sequences. Posterior probabilities are shown at the nodes; values of 1.00 are indicated with an asterisk. Specimen details are listed in Table 1.
and 25 million generations for the concatenated data set, with trees sampled every 1000 generations. We used Tracer v1.5 (Rambaut and Drummond, 2009) to estimate when the chains had reached the apparent target and to ensure that the ESS for each
parameter was appropriate. Trees sampled during the burn-in phase were discarded.

For the Maximum Likelihood analysis of the concatenated data we used RaxML v7.04 (Stamatakis, 2008) and seven partitions; one for β-fibrinogen intron 5, and one for each codon position in the two mitochondrial genes. In the RaxML analyses we used default parameters and 100 tree search replicates. Node stability on partitioned data sets was estimated with 100 non-parametric bootstrap replicates (Felsenstein, 1985).

3. Results

3.1. Sequence variation and selection of substitution models

Except for a few cases where species lacked a short segment of a studied gene region, the aligned sequences from the mitochondrial ND2 and CO1 genes were 1041 and 653 bp, respectively. The non-coding β-fibrinogen intron 5 sequences could be easily aligned by eye, due to the low number of insertions/deletions. All gaps were treated as missing data in the analyses. The alignment of the nuclear β-fibrinogen intron 5 consisted of 572 bp (the individual sequences ranged between 548 and 569 bp), while the concatenated data set of all three gene regions consisted of 2266 bp. Uncorrected p-distances among the ingroup taxa (Petroicidae) revealed sequence divergences of 0–26% for ND2, 0–19% for CO1, and 0–15% for β-fibrinogen intron 5.

Most of the indels (insertions or deletions) in the alignment of β-fibrinogen intron 5 were observed in comparisons between the outgroup and ingroup taxa. Within the ingroup taxa, some indels were restricted to single species or consisted of single nucleotides in highly repetitive regions. There were five phylogenetically informative indels among the ingroup taxa: (1) a 20 bp deletion shared between Amalocichla incerta and Amalocichla schaterrana; (2) a 12 bp deletion shared between the genera Drymodos, Heteromyias, Poecilodryas, Melanodryas, Peneothello, Eopsaltria and Tregellasia (β-fibrinogen intron 5 data was lacking for Peneonanthe); (3) a 7 bp deletion shared between the genera Monachella and Microeca (β-fibrinogen intron 5 data was lacking for E. flaviventris); (4) a 5 bp deletion shared between all species in the genus Petroica; and (5) a 1 bp deletion shared between Melanodryas, Peneothello, Eopsaltria, Tregellasia and Poecilodryas placens (β-fibrinogen intron 5 data was lacking for Peneonanthe). The alignment for the β-fibrinogen intron 5 sequences is provided as online Supplementary material (Supplementary Table 1).

The prior selection of substitution models selected the GTR + I + Γ model for the total ND2 and CO1 sequences, the 1st, 2nd and 3rd codon positions in ND2, and the 1st codon position in CO1. The HKY + Γ model was selected for the 2nd codon position in CO1 and for β-fibrinogen intron 5, while the GTR + Γ model was selected for the 3rd codon position in CO1. Assessment of the log of Bayes Factors indicated that the best partition strategy for the concatenated data set of all genes was seven partitions (one for β-fibrinogen intron 5, and one for each codon position in ND2 and CO1). This was the partition strategy employed for the concatenated data set. After discarding the burn-in phase, the final inference was based on total samples of 16,000 and 18,000 (concatenated from two runs each) from the posterior for the individual loci, and 32,000 samples (concatenated from two runs) from the total concatenated data set.

The GTR + I + Γ model was used for all partitions in the Maximum Likelihood analysis of the concatenated data using RaxML v.7.0.4 (Stamatakis et al., 2008).

3.2. Phylogenetics

The trees obtained from the Bayesian analyses of the individual gene partitions (Figs. 1–3) exhibit different degrees of resolution and are not topologically fully congruent. Overall, the ND2 tree contains the most strongly supported nodes (above 0.95 posterior probability) while the CO1 and β-fibrinogen intron 5 tree are less resolved and have fewer strongly supported nodes. Basal nodes are generally most strongly supported in the β-fibrinogen intron 5 tree. Even though the topologies from the individual gene trees are somewhat difficult to compare, the topological conflicts almost exclusively concern weakly supported nodes (<0.95 posterior probability). It is also noticeable that nodes that receive strong support values in the tree obtained from the concatenated data set (Fig. 4), are generally supported by both the mitochondrial genes (most often the ND2 tree) and the nuclear β-fibrinogen intron 5 tree (although several terminal nodes in the latter tree are not recovered).

The tree obtained from the Maximum Likelihood analysis of the concatenated data set (not depicted) is almost fully congruent with the Bayesian concatenated tree (Fig. 4). Only three topological differences occur and they all involve nodes that are weakly supported by posterior probabilities (<0.80) and bootstrap support values (<60%). The topologies of both concatenated trees are also in good congruence with the less taxon dense phylogeny presented by Loynes et al. (2009).

4. Discussion

4.1. Phylogenetic relationships

All phylogenetic analyses identified six primary lineages within the Petroicidae: (1) Eopsaltriines comprising Eopsaltria (excluding E. flaviventris), Tregellasia, Peneothello, Melanodryas, Poecilodryas and Heteromyias; (2) Drymodines comprising Drymodos; (3) Microecines comprising Microeca, Monachella and E. flaviventris; (4) Petroicines comprising Petroica and Eugerygone; (5) Pachycephalopines comprising Pachycephalophasis; and (6) Alamocichlinae comprising Alamocichla. In the concatenated tree (Fig. 4), each of these received posterior probabilities of 1.00 and bootstrap support of 100%. These lineages are consistent with results from the taxonomically limited DNA-based study of Loynes et al. (2009), which did not include Monachella, Eugerygone or Alamocichla or any non-Australian representatives of Microeca, Petroica, Poecilodryas and Heteromyias.

These DNA-based assessments of relationships are similar to the morphological-based treatment of Schodde and Mason (1999) which recognized three subfamilies (Eopsaltriinae, Petrocinia, Drymodinae). However, several major differences also exist between the DNA-based phylogenies and the treatment of Schodde and Mason (1999). Whereas, Schodde and Mason (1999) combined the petroicines and microecines in a single subfamily, the DNA data clearly suggests that they are not each other’s closest relatives. Alamocichla was considered by Schodde and Mason (1999) to be a member of the Acanthizidae, but Norman et al. (2009) and the present study confirm that it is in fact part of the Petroicidae.

Here we treat the six primary lineages as subfamilies: Eopsaltriinae Matheus, 1946; Drymodinae Wolters, 1975–1982; Microecinae Loynes et al., 2009; Petrociniae Matheus, 1920; Pachycephalopines subfam. nov.; and Alamocichlinae subfam. nov. The new subfamily names Pachycephalopines and Alamocichlinae are proposed in accordance with Article 11.7 of the International Commission on Zoological Nomenclature (1999). Pachycephalopines: one genus Pachycephalophasis Salvadorti, 1879, with two species, Pachycephalophasis poliosa (Sharpe, 1882) [type] and Pachycephalophasis hartmannensis (Meyer, 1874). Alamocichlinae: one genus Alamocichla De Vis, 1892, with two species, A. schaterrana De Vis, 1892 [type] and A. incerta (Salvadorti, 1875).
Fig. 4. Bayesian tree (with branch lengths) based on concatenated DNA sequences. Posterior probabilities and bootstrap support values from Maximum Likelihood analysis are indicated at the nodes: filled circles indicate 1.00 posterior probability and 100% bootstrap support; filled diamonds indicate $P_{0.95}$ posterior probability and $P_{75}\%$ bootstrap support; and open circles indicate $P_{0.75}$ posterior probability and $P_{50}\%$ bootstrap support. Specimen details are listed in Table 1. Phylogenetically informative indels are indicated by bars. Numbers refer to the subfamilies of Petroicidae recognized here: 1 = Eopsaltriinae; 2 = Drymodinae; 3 = Microecinae; 4 = Petroicinae; 5 = Pachycephalopsinae; 6 = Amalocichlinae. Species and subspecies in gray refer to those occurring in New Guinea. All other are from Australia unless followed by the following prefixes: NZ = New Zealand; NC = New Caledonia; SI = Solomon Islands.
4.1.1. *Eopsaltria*inae

Within the *Eopsaltria*inae, two subclades were recovered in all analyses. The first comprised *Tregellasia*, *Eopsaltria*, *P. placens*, *Peneothello*, *Melanodryas* and *Peneoenanthae*, while the second comprised *Heteromyias* and *Poecilodryas* (less placens). These two clades were also recovered by *Loynes et al.* (2009). The first subclades further divided into two groups: (1) *Tregellasia, Eopsaltria, P. placens*; and (2) *Peneothello, Melanodryas, Peneoenanthae*.

The DNA trees did not recover *Eopsaltria* (australis, griseogularis, georgiana, and flaviventris) as a monophyletic genus. The concatenated (Fig. 4) and ND2 (Fig. 1) trees of the present study and that of *Loynes et al.* (2009) linked *Eopsaltria* georgiana with *Tregellasia*, while our CO1 (Fig. 2) and Fib 5 (Fig. 3) trees isolated the species as an independent lineage, as did the nuclear gene trees of *Loynes et al.* (2009). In considering the various tree topologies, georgiana is best segregated into monotypic *Quoyornis*. Even more divergent was New Caledonian *E. flaviventris*. Both the present study and *Loynes et al.* (2009) placed it within the *Microecinae*, and its affinities are discussed in the section dealing with that subfamily.

All trees (Figs. 1–4) linked New Guinean *P. placens* with *Eopsaltria* and *Tregellasia*. *Boles* (2007) suggested that with its strong yellow plumage it could be included in *Eopsaltria*. However, it is not sister to *Eopsaltria* so it is best segregated in its own genus, for which the names *Genneadryas* is available. Recognition of the four genera, *Eopsaltria* (australis, griseogularis), *Tregellasia* (leucops, capito), *Quoyornis* (georgiana) and *Genneadryas* (placens) is consistent with the relative depths of the nodes leading to each lineage.

The level of uncorrected ND2 sequence distance (6.5–6.8%) separating *Eopsaltria* australis (eastern Australia) and *E. griseogularis* (western Australia) is consistent with species-level separation, supporting current taxonomic treatments (e.g. *Schodde and Mason*, 1999; *Boles*, 2007; *Christidis and Boles*, 2008). Within *E. australis*, two mtDNA haplotype groups were identified which differed by uncorrected CO1 sequence distances of 5.7–5.8% *Loynes et al.* (2009) recorded an ND2 distance of 5% between two *E. australis* haplotype groups. These divergences are comparable to those separating *E. australis* and *E. griseogularis*, thereby suggesting that each haplotype group warrants species level recognition. However, the two haplotype groups do not correspond to the two currently recognized subspecies *australis* and *chrysoorhros* (*Schodde and Mason*, 1999). Consequently, further phylogeographic work is required to ascertain the species limits within *E. australis*.

Ten subspecies of *Tregellasia* leucops are currently recognized (*Boles*, 2007), of which three were examined in this study: *albigularis* (far northeastern Australia), *nigriceps* (Southern Highlands of New Guinea) and *albificies* (southeastern New Guinea). Two clades, differing by an average ND2 distance of 5.3% were identified: (1) *albigularis*, *nigriceps* and (2) *albificies*. This value is higher than that recorded between the two subspecies of *Tregellasia* capito (ND2 distance of 2.9%) and is comparable to species level divergences in *Eopsaltria*. Although it apparent that additional species level diversity exists within *T. leucops*, greater coverage of subspecies is required to elucidate species composition within the complex.

*Melanodryas cucullata* was not recovered as a monophyletic species with respect to its congener, *Melanodryas vittata*. The three individuals of *M. cucullata* from eastern Australia (subspecies *cucullata* and *picata*) and Tasmanian *M. vittata* formed one clade, while the two individuals of *M. cucullata* from western Australia (*westralensis*) comprised a second clade. Western *M. cucullata* differed by up to 4.3% ND2 distance from eastern *M. cucullata*, whereas the ND2 distance between the latter and *M. vittata* was only 2.6%. Such a pattern of relationships conflicts with the placement of *M. cucullata* and *M. vittata* into different subgenera by *Schodde and Mason* (1999). Given the paraphyly of *M. cucullata*, three species need to be recognized in *Melanodryas*: *M. vittata*, *M. westralensis* and *M. cucullata* (includes *picata*). We recommend that the English names Eastern Hooded Robin and Western Hooded Robin be applied to *M. cucullata* and *Melanodryas westralensis*, respectively.

*Melanodryas* is part of a clade that includes *Peneothello* (New Guinea) and *Peneoenanthae* (coastal northern Australia and New Guinea). The three species of *Peneothello* examined (*cyanus, sigillata* and *bimaculata*) were not recovered as a monophyletic group with respect to *Peneoenanthae*, which is in agreement with the findings of *Loynes et al.* (2009). Consequently, we here treat *Peneoenanthae* as a subspecies of *Peneothello*. *P. cryptoleuca* was not examined but is thought to form a sister pair with *P. cyanus* in the subgenus *Papulestes* (*Walters*, 1975–1982; *Boles*, 2007). According to *Walters* (1975–1982) and *Boles* (2007), *P. sigillata* and *P. bimaculata* are also sister species in the subgenus *Peneothello*. However, only the ND2 tree (Fig. 1) recovered a sister relationship between the two (c.f. *Loynes et al.* , 2009). Further DNA analysis including *P. cryptoleuca* is required to resolve subgeneric limits within *Peneothello*.

The other clade of *Eopsaltria*inae comprises *Poecilodryas* (less placens) and *Heteromyias*. When *Mayr* (1941) rationalized the limits of *Poecilodryas*, he combined six species that had previously been placed in five genera by *Mathews* (1930). *Mayr* (1986) retained this arrangement, other than for the removal of *pulverulenta* to *Peneothello*. *Boles* (2007) raised the possibility that *P. placens* and *Poecilodryas albonotata* might be better placed elsewhere. The DNA trees confirm that *placens* does not belong here and is part of the *Eopsaltria–Tregellasia* assemblage. The other four species in this genus examined (*P. hypoleuca, P. superciliosa, P. cerviniventris* and *P. albonotata*) were recovered as a monophyletic group. Although *P. brachyura* was not examined, it shares similar plumage patterns and foraging behavior with *P. hypoleuca, P. superciliosa* and *P. cerviniventris* (*Boles*, 2007). Conversely, *P. albonotata* lacks the facial and wing patterns of the others, has a more massive but flattened bill and forages primarily by the flycatching rather than pouncing. These morphological and behavioral differences are also reflected in the DNA-based trees, with *P. albonotata* being the most divergent member of *Poecilodryas*. As suggested by *Boles* (2007), it is here separated generically into monotypic *Plesiodryas*.

The relatively high ND2 distance (8.5%) between *Poecilodryas superciliosa* and *Poecilodryas cerviniventris* is consistent with the recent practice of treating them as separate species (*Schodde and Mason*, 1999; *Boles*, 2007; *Christidis and Boles*, 2008).

Although some authors have included *Heteromyias* in *Poecilodryas* (e.g. *Schodde*, 1975; *Schodde and Mason*, 1999; *Dickinson*, 2003), the relatively high level of ND2 distance (15.5–20.6%) is such, that retention of the two is the more appropriate treatment. *Heteromyias cinereifrons* of northeastern Australia is sometimes included in New Guinean *Heteromyias albispecularis* (*Schodde*, 1975; *Schodde and Mason*, 1999; *Dickinson*, 2003). The level of ND2 distance between *H. cinereifrons* and *H. albispecularis* (10.5–11.3%) is more consistent with separate species treatment. The latter species has several forms which break up into three subspecies groups (*Boles*, 2007). Representatives from two of these groups were examined here: *H. a. armitii* (southeastern New Guinea) and *H. a. centrals* (northern and central New Guinea). The 5.7% ND2 sequence distance recorded between *armitii* and *centrals* suggests that *H. albispecularis* may comprise more than one species. Greater coverage of subspecies is required to elucidate species composition within the *H. albispecularis* complex.

4.1.2. Drymodinae

The CO1, β-fibrinogen intron 5 and concatenated trees (Figs. 2–4) recovered three deeply diverged lineages within *Drymodina*: *D. brunneoppia* (southern Australia); *D. superciliiars brevirostris* (New Guinea); and *D. s. superciliiars* (northeastern Australia).
ND2 data were not obtained for last taxon. The New Guinean and Australian forms of Drymodes supercililars are arguably best separated out as species. We retain the English name Northern Scrub-robin for the Australian form D. supercililars, and refer the three New Guinean subspecies (beccarii, nigriceps and brevirostris) to D. beccarii Salvadori, 1876. The English name New Guinean Scrub-robin is applied to the latter.

4.1.3. Microecinae

Morphological assessments (e.g. Boles, 2007) have suggested a close association between Monachella, E. flaviventris and Microeca. All DNA trees (Figs. 1–4) not only confirmed this, but also included a clade with Microeca fascinans and M. flavigator, which in turn was linked to E. flaviventris (only COI data were available for the latter). Microeca flavivorescens and Microeca griseocephs were recovered as sister taxa as were M. fascinans and M. flavigator. Microeca papuana is believed to represent the most divergent member of the genus (e.g. Schodde and Mason, 1999) and the DNA trees (Figs. 1–4) separated it as a monotypic lineage.

Because Monachella and E. flaviventris are embedded in Microeca, both could be included in a broader concept of that genus. However, the observed level of ND2 distances between major lineages within the Microecinae ranged from 17% to 20.3% which is comparable to the distance separating Heteromyias and Poecilodryas. Consequently, the action taken here is to divide Microeca into three genera, retain Monachella for muelleriana, and place flaviventris in a new monotypic genus. The name Microeca now applies to M. fascinans, flavigator (and presumably hemixantha); papuana is segregated into Devicoea; while flavivorescens and griseocephs are placed in Kempiella. As no available generic name exists for flaviventris, we propose a new genus for this species.

Cryptomicroeca, gen. nov. Christidis, Irestedt, Rowe, Boles and Norman, this study

Type species: Eopsaltria flaviventris Sharpe, 1903.
The name is derived from kryptos, (Greek, hidden) and Microeca (the genus of petroicine robins to which this species is related); it alludes to this long undetected relationship.

Within M. fascinans, southeastern fascinans and northwestern pallida have sometimes been treated as a species, separate from southwestern assimilis (e.g. Sharpe, 1879). Representatives of all three forms were included in the present study. Both mitochondrial trees (Figs. 1 and 2) recovered M. f. fascinans and M. f. pallida as a clade separate from M. f. assimilis. Nevertheless, the ND2 distance between the two clades was low (c. 1.7%) supporting recognition of only one species.

There were, however, two deep lineages recovered in Microeca flavigator: (1) M. f. flavissima (Cape York Australia and southeastern New Guinea) and M. f. laetissima (northeastern Australia); and (2) M. f. flavigator (northern Australia). ND2 distances between the two clades were in the order of 4.2–4.3% ND2; comparable to other sister species level distances recorded within Petroicidae. Inclusion of DNA data on M. f. tormenti (northwestern Australia), M. f. laeta (northern New Guinea) and M. f. tarara (southernwestern New Guinea) is critical to determining whether additional species should be recognized within the M. flavigator complex. The low ND2 distance (0.7%) separating M. f. flavissima and M. f. laetissima is more consistent with the treatment of Mayr (1986) who only recognized one subspecies in eastern Australia (cf. Schodde and Mason, 1999).

4.1.4. Petroicinae

A close association between Petroica and monotypic Eugerygone was recovered in all the DNA trees and is consistent with morphologically-based assessments (Schodde and Mason, 1999; Boles, 2007). The levels of uncorrected ND2 sequence distance between Eugerygone and Petroica (14.1–17.0%) are consistent with generic recognition.

Schodde and Mason (1999) maintained four subgenera within Petroica: Petroica (boodang, multicolor, goodenovii, macrocephala and bivittata); Littlera (phoenicea); Erythrodryas (rosea, rodinogaster); and Miro (australis, traversi). The subgenus Petroica was not recovered in any of the DNA trees. A sister relationship between P. (P.) boodang and P. (Littlera) phoenicea was consistently recovered, though with low support. Although the combined tree of Lownes et al. (2009) identified P. (P.) boodang and P. (P.) goodenovii as sister species, this was influenced strongly by the two sharing identical B5 sequences. Their other gene trees were more consistent with the present study in identifying a closer relationship between Petroica boodang and Petroica phoenicea. In our study, P. boodang was not sister to P. multicolor, even though the two have been treated as either a single species (e.g. Mayr, 1986) or as sister species (Schodde and Mason, 1999). Our results, based on a single gene (COI), instead, suggest a closer relationship between P. multicolor and Petroica goodenovii.

Within P. boodang, eastern Australian boodang and Tasmanian leggii were almost identical in DNA sequences while western Australian campbellii was more distant (Figs. 1, 3 and 4). Although Schodde and Mason (1999) tentatively separated P. b. leggii from P. b. boodang, the DNA data are more consistent with the treatment of Mayr (1986) who only recognized one subspecies across eastern Australia and Tasmania.

Both mtDNA gene trees (Figs. 1 and 2) did not align P. macrocephala with other members of the subgenus Petroica, and instead recovered it as the first diverged lineage. Miller and Lambert (2006) recorded a sister relationship between P. macrocephala and P. (Miro) traversi, while P. (Miro) australis was either weakly linked to P. multicolor or to the P. macrocephala–traversi group, depending on the tree building algorithm used. In our study, only β-fibrinogen intron 5 was available for P. australis and this species was recovered as an early diverged lineage in the tree (Fig. 3).

Pink-breasted P. rosea and P. rodinogaster were recovered as sister species in the concatenated (Fig. 4) and ND2 (Fig. 1) trees, but not in the CO1 (Fig. 2) or β-fibrinogen intron 5 (Fig. 3) trees. Similarly, in the Lownes et al. (2009) study the nuclear gene trees did not recover the two as sister species. Although Schodde and Mason (1999) maintained the two in the subgenus Erythrodryas they noted that it may constitute a paraphyletic group.

The DNA data indicates that the subgeneric circumscription within Petroica requires a major re-assessment. Whether additional genera could be recognized is also worthy of further investigation. Better coverage of mitochondrial and nuclear DNA data across all species of Petroica, including New Guinean P. archboldi and P. bivittata, is needed before such taxonomic revision is possible.

4.2. Ecology

Christidis and Norman (2010) commented that within Australian songbirds, sympatric congeners were generally part of separate lineages and were rarely sister taxa. Examples included Acanthisa thornbills (Nicholss et al., 2000), Amytornis grasswrens (Christidis, 1999), Malurus fairy-wrens (Christidis and Schodde, 1997) and Meliphaga honeyeaters (Norman et al., 2007). Such a pattern is consistent with the conclusions of Grant and Grant (1997) and Peterson et al. (1999) that most speciation in birds occurs in geographical, not ecological dimensions. Nevertheless, examples of ecological speciation have been suggested in birds-of-paradise (Irestedt et al., 2009) and honeyeaters (Norman et al., 2007). Within the Microecinae, Kempiella (formerly Microeca) flavivorescens (lowland New Guinean rainforest) and K. griseocephs
(lower montane New Guinean and northeastern Australian rainforest) were recovered as sister species. The two are generally considered to be altitudinally separated ecological counterparts (Diamond, 1972; Schodde and Mason, 1999) and they may represent another example of ecological speciation.

Loyes et al. (2009) noted that the Petroica robins of eastern Australia (phoenicea, boodang, rodinogaster and rosea) share breeding ranges and were strongly distinguished from one another in terms of genetic divergence, suggesting a long evolutionary history. Although the ND2 (Fig. 1) and concatenated (Fig. 4) trees recovered P. rosea and P. rodinogaster as sister species (though statistical support was low), the two differ in foraging behavior. Petroica rosea is a warbler-like foliage gleaner while P. rodinogaster employs a typical robin ‘perch and pounce’ method and the two cannot be considered as ecological replacements. The woodland-adapted P. phoenicea has been considered to be one of the more divergent members of the genus (e.g. Schodde and Mason, 1999), but the mtDNA trees (Figs. 1 and 2) and combined tree (Fig. 4) recovered it as sister to P. boodang, though with low support. Further resolution of the interrelationships between the species of Petroica is needed to better assess the role of ecological differentiation in generating taxonomic diversity.

4.3. Biogeography

An examination of the deeper divergences within the Petroicidae indicates that most are centered within New Guinea. The Amallocichlinae and Pachycephalopsinae are restricted to New Guinea. Within the Microcininae, the basal taxon Devioca (formerly Microeca) papua, Kembaliella flavovirens and Kembaliella griseiceps are largely restricted to New Guinea, with only the last species also occurring in Australia (Cape York Peninsula). The only other microcine species that occur in Australia, M. flavigaster and M. fascinans represent terminal branches. This suggests a New Guinean center of origin for the Microcininae. The Drymodinae are represented in both Australia and New Guinea and it is not possible, on current data, to determine their likely biogeographical origin. Although the Eopsaltriinae are distributed across New Guinea and Australia, basal lineages such as Genneadryas (formerly Poecilodryas) placens and Plesiodyras (formerly Poecilodryas) albonotata are restricted to New Guinea. Within the Petroicininae, Eugygone is restricted to New Guinea, while Petroica is widespread across the Australo-Pacific region. Unfortunately, the New Guinean members of Petroica were not examined.

Christidis and Norman (2010) reviewed the role of several biogeographic barriers in generating species diversity within Australia. One of the most significant barriers identified was the Carpentarian Barrier in northern Australia. Several recent molecular studies have identified major taxonomic breaks coinciding with this barrier that do not correspond to variations in plumage patterns. Examples include fairy-wrens Malurus melanocephalus (Jennings and Edwards, 2005) and honeyeaters Melithreptus albobularis (Toon et al., 2010). In both these cases, plumage-based assessments identify a different subspecies in central-eastern Australia from that occurring in northeastern Australia and across northern Australia. DNA data instead, place the major taxonomic break as occurring between the northern Australian and northeastern Australian populations (Jennings and Edwards, 2005; Toon et al., 2010).

The DNA data obtained on the Petroicidae further demonstrate the significance of the Carpentarian Barrier in generating taxonomic diversity. Within M. flavigaster, the two major lineages identified by the DNA data corresponded to a northern Australian clade and a northeastern Australian clade (including eastern New Guinea). The genetic divergence between the two clades was suggestive of species level separation. The present study also substantiates the species level divergence between northern/northwestern P. cerviniventris and northeastern P. superciliosa. The other species of Petroicidae for which DNA are available for populations on either side of the Carpentarian Barrier is Peneothello (Peneonanthus) pulvinalenta. Loyes et al. (2009) obtained ND2 sequence data from northeastern Australian leucura, while the present study examined alligator from the Northern Territory. In this instance the ND2 distance between the two (2.1%) was consistent with subspecies level differentiation.

Although the New Guinean populations of M. flavigaster were found to be closely related to those of northeastern Australia, there were examples in the Petroicidae where species level divergences were observed between Australian and New Guinean populations. The New Guinea and northern Australian forms of D. superciliea were as diverged from one another as they were from D. brunneopygia. Separation of New Guinean and Australian populations of Heteromyias was confirmed by the DNA data and there was an indication that some New Guinean populations of T. leucops could be specifically separated from Australian populations. The honeyeater Meliphaga gracilis (Norman et al., 2007) and the logrunner Orthonyx tendimcinckii (Norman et al., 2002) are additional examples of species that once were thought to be shared between Australia and New Guinea, but were revealed by DNA data to each comprise separate Australian and New Guinean species. Further study on other presumed shared species between Australia and New Guinea could reveal additional species level divergence.

Toon et al. (2010) identified another case in honeyeaters where plumage-based assessments of species limits did not accord with DNA data, this time in southern Australia. Eastern populations of Melithreptus lunatus formed a clade with Tasmanian M. affinis separate from western populations of M. lunatus. In the present study, an identical pattern of discordance between plumage pattern and DNA lineages was found in Melanodryas. Eastern M. cucullata and Tasmanian M. vittata formed one clade, while western populations of M. cucullata formed a separate clade. A closer evolutionary relationship between eastern Australian and Tasmanian taxa, relative to western ones, is common and established examples include honeyeaters (Acanthorhynchus, Anthochaera), finches (Stagonopleura), black-cockatoos (Calyptrhynchus) and Petroica boodang (present study: Fig. 1). In each of these cases, plumage patterns are consistent with an eastern Australian/Tasmanian clade and a western clade. Conversely, Melithreptus and Melanodryas add to a growing number of examples in which DNA differentiation does not track plumage patterns but is consistent with biogeographical barriers. Such patterns suggest that plumage may be more influenced by environmental factors than previously thought.

The situation in Melanodryas is particularly interesting. The Tasmanian form M. vittata is monomorphic with adults resembling females/imatures of dimorphic mainland forms of M. cucullata. Interestingly, two other species complexes have representatives in Tasmania that resemble mainland immatures. The adult Green Rosella (Platycercus caledonicus) from Tasmania resembles immature mainland Crimson Rosellas (P. elegans). Similarly, the Tasmanian Forty-spotted Pardalote (Pardalotus quadragintus) resembles immature mainland Spotted Pardalotes (P. punctatus). Why juvenile and immature plumages should be retained in these Tasmanian species is worthy of further research. Price (2007) has noted that in several species complexes, the “duller” plumaged species tend to occur on islands.

The present study has revealed that within the Petroicidae, vicariant barriers have played a significant role in generating taxonomic diversity in Australian and New Guinea, with dispersal playing a minor role. Miller and Lambert (2006) suggested that two invasions of Petroica from Australia gave rise to the 3–4 species currently found in New Zealand. Apart from New Zealand Petroica, only four other species have expanded away from Australia and...
New Guinea; *M. hemixantha* on Tanimbar, *Monachella muellieri* on New Britain, *P. multicolor* in the south-west Pacific, and *Cryptomicroeca* (formerly *Eopsaltria*) *flavinventris* on New Caledonia. All four share a “flycatcher body-plan”. Boles (2007) noted that unlike other members of the genus, populations of *P. multicolor*, from the Solomon Islands and south-west Pacific, are very similar to the microeines in foraging behavior and body proportions. One could surmise from this that a “flycatcher body-plan” has facilitated dispersal across water barriers. Dispersal as a generator of species diversity in the Indo-Pacific is now well documented in monachine flycatchers (Filardi and Moyle, 2005), whistlers (Jønsson et al., 2008, 2010a), cuckoo-shrikes (Jønsson et al., 2010b) and white-eyes (Moyle et al., 2009). Christidis and Norman (2010) noted that the ability to adapt to mangrove habitats appeared linked to island dispersal in several Australasian songbird genera. Future studies need to consider the role of ecological adaptations in facilitating dispersal across water barriers.

4.4. Taxonomic revision of Petroicidae

A taxonomy based on the findings from the present study is detailed below. Three areas require comment. Firstly, within *Peneothello*, the composition of the subgenera *Peneothello* and *Papualestes* adopted here requires further confirmation. Secondly, whether *P. brachyura* could be separated into the subgenus advocated by Wolters (1975–1982), was not resolved as that species was not available for DNA analysis. Finally, subgenera clearly not resolved as that species was not available for DNA analysis. Finally, subgenera clearly not resolved as that species was not available for DNA analysis.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.08.014.