DIVERSIFICATION OF THE TROPICAL PACIFIC AVIFAUNA

By

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Abstract

I investigated the origins and diversification of Pacific avifaunas. Chapters 1, 2, and 4 elucidate the evolutionary history of three classically polytypic species complexes of Pacific island birds using multilocus phylogeographic approaches. The focal taxa were: *Ceyx lepidus* (Aves: Alcedinidae), *Pachycephala pectoralis* (Aves: Pachycephalidae), and *Todiramphus chloris* (Aves: Alcedinidae). In chapter 3, I examined the systematic relationships of 14 species of Pacific honeyeaters (Aves: Meliphagidae) relative to continental lineages. Each of these studies revealed novel biogeographical patterns heretofore underappreciated in Pacific birds. All three species complexes underwent rapid diversification with extensive genetic and phenotypic differentiation across widespread island archipelagos spanning thousands of kilometers from southeast Asia to Polynesia. This pattern was evidenced by phylogenies with short basal internodes, long stem lineages, and shallow divergences within each taxon. *Todiramphus* was noteworthy because it has attained extensive reproductive isolation, despite the recency of the radiation, as evidenced by multiple sympatric taxa throughout the Pacific. The work on meliphagid honeyeaters found extensive paraphyly of Pacific lineages with respect to their presumed continental congeners. I found evidence for a Central Polynesian radiation that included taxa from the eastern Solomon Islands, Fiji, Samoa, and Tonga. Throughout this dissertation I draw inferences on the processes of origination, diversification, and extinction in Pacific avifaunas using a comparative framework across multiple lineages at different scales of differentiation.

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# Introduction

I am inspired by evolutionary diversity in nature and how it is partitioned across the globe. From intra-specific populations and their constituent genealogies to higher-level biological classification at or above the level of species, I am fascinated by the evolutionary history in the tree of life. This fascination—combined with a life-long passion for birds—guides my over-arching research goal, which is to study the patterns and processes that generated the exquisite diversity of the world's birds. Specifically, my dissertation research investigated the origins and diversification of birds in the tropical southwest Pacific.

I incorporate specimen-based fieldwork and DNA sequence data to study evolutionary processes behind geographic partitioning of biological diversity on islands. To study these processes, I explore patterns of genetic and phenotypic diversification in widespread Pacific radiations as well as those that are endemic to particular archipelagos. This geographically nested approach enables comparative studies of avian lineages at multiple spatial scales and relative diversification times. In short, I aim to study the tempo and mode of evolution in birds on Pacific islands.

Island archipelagos are ideal theaters for the study of biogeography and diversification. Their isolation, discrete geographic boundaries, and relatively well-known geologic histories have influenced a wealth of evolutionary theory (Darwin 1859; Wallace 1881; Lack 1940; Wilson 1959, 1961; MacArthur and Wilson 1963, 1967; Diamond 1975; Diamond et al. 1976; Boag and Grant 1981; Grant 1991; Wagner and Funk 1995; Lomolino 2000; Heaney 2007; Rosindell and Phillimore 2011; Gillespie et al. 2012). Adaptive radiation is a common theme throughout this literature; it is an especially common research program of biologists working in the remote Hawaiian and Galápagos Archipelagos (Boag and Grant 1981; Grant 1981; Price et al.

1984; Fleischer et al. 1998; Lovette et al. 2002; Lerner et al. 2011), and also the Greater Antilles (Williams 1972; Irschick et al. 1996; Jackman et al. 1997; Jackman et al. 1999). Adaptive radiations such as the Hawaiian honeycreepers and silverswords, Galápagos Finches, and Caribbean *Anolis* have provided a wealth of important information on the evolutionary processes behind adaptation (Givnish and Sytsma 1997; Schluter 2000). While these radiations are remarkable, they are far from the biological norm. In fact, evolutionary radiations have evolved equally rapidly on islands without producing overt adaptive consequences or morphological novelty (Arbogast et al. 2006; Moyle et al. 2009). For example, the geologically complex region of the southwest Pacific is home to numerous geographic radiations of birds—widespread, polytypic species complexes that comprise phenotypically differentiated forms from island to island. It was these geographic radiations, not their adaptive cousins, that inspired insular speciation theory in Melanesia for the better part of the 20$^{th}$ Century (summarized by Mayr and Diamond 2001).

Geographic radiations are a conspicuous component of the avifauna in the tropical southwest Pacific. Examples include the *Monarcha* and *Symposiachrus* radiations of monarch-flycatchers (Coates et al. 2006). In particular, the *Monarcha castaneiventris* species complex—perhaps more so than any other—is a poster child of geographic radiations, and it continues to provide a fruitful study system for evolutionary biologists (Uy et al. 2009a; Uy et al. 2009b). Zosteropid white-eyes are another example of a geographic radiation (van Balen 2008), albeit a much more widespread and speciose group than *Monarcha. Zosterops* is the quintessential explosive avian radiation that has produced dozens of species throughout the Old World tropics (Moyle et al. 2009). The *Z. griseotinctus* species complex was a central component behind ideas such as the paradox of the great speciators (Diamond et al. 1976; Diamond and Mayr 1976), in

which Diamond et al. pitted dispersal ability (gene flow) against diffentiation and asked the question: *why are the species most capable of long-distance dispersal also the most geographically well-differentiated from island-to-island in an archipelago?*

Several of the most diverse geographic radiations in the Pacific transcend biogeographical boundaries of single archipelagos. To date, few widespread Pacific radiations have been studied in a molecular phylognetic context with comprehensive geographic sampling (Moyle et al. 2009; Irestedt et al. 2013; Cibois et al. In press). This dearth of research is due in large part to the inherent difficulties of sampling fresh genetic source material across expansive insular distributions that often comprise numerous archipelagos and political governments. In this dissertation I investigated several of the most widespread, and by extension, diverse geographic radiations in the Pacific. I generated multilocus DNA sequence datasets with robust geographic and population-level sampling for three classically polytypic species complexes: *Ceyx lepidus* and *Todiramphus chloris* (Aves: Alcedinidae) and *Pachycephala pectoralis* (Aves: Pachycephalidae). These three studies comprise three chapters of my dissertation. The fourth chapter examines 14 lineages of Pacific meliphagids (honeyeaters) in a phylogenetic context to investigate their monophyly and taxonomic affinities relative to continental congeners from Australia and New Guinea.

A common theme I discovered in my investigation of the three species complexes was one of explosive and widespread diversification. My data show phylogenetic signatures suggesting that these lineages underwent massive range expansions from their ancestral origin in a rapid burst of colonization followed by cessation of gene flow and subsequent diversification in allopatry. As a result of this rapid diversification, phylogenetic relationships among constituent lineages are often equivocal, and it remains an open question whether more data (i.e., high-

throughput sequencing) will resolve these polytomies or if they truly are hard polytomies. An unfortunate consequence of basal polytomies is they preclude accurate estimates of biogeographic origins of ancestral lineages. Overall, these results are concordant with those found in the few Pacific bird lineages that have been examined recently, including white-eyes, *Alopecoenas* ground-doves, *Erythropitta erythrogaster*, and some *Myiagra* species (Moyle et al. 2009; Jønsson et al. 2011; Irestedt et al. 2013; Moyle et al. 2013; Fabre et al. In press). All of these studies faced similar plights of biogeographic interpretation, at least at critical nodes of the phylogenies. Throughout this dissertation, I refrain from over-interpretation of historical biogeography. Instead, I believe the phylogenetic signal (i.e., rapid diversification rooting to a polytomy) is a real biological phenomenon worthy of discussion, regardless of our ability to accurately pinpoint biogeographic origins of any one clade. Biogeographic origin(s) of Pacific avifaunas are worth considering, despite my addmitedly conservative approach to the topic in this dissertation.

The prevailing biogeographic origin hypothesis is one of a unidirectional stepping-stone model of colonization with insular lineages derived from continental origins (Mayr 1940a, b, 1942; MacArthur and Wilson 1963, 1967). This hypothesis makes intuitive sense because the continents are relatively speciose compared to islands; however, the paradigm was challenged by Filardi and Moyle (2005), who found phylogenetic evidence that archipelagos can generate diversity, as well. Furthermore, they noted "upstream" colonization of monarch flycatchers from islands to continents, against the grain of paradigm. This was an important paper, in part for the biogeographical findings, but perhaps more so because it highlighted how rudimentary knowledge of the phylogeny implicated a reinterpretation of long-held ideas on the unidirectional colonization of archipelagos.

The Filardi and Moyle (2005) paper was by no means the first study (nor the last) to illustrate paraphyly of traditional taxonomic groupings using modern phylogenetics. However, they did draw attention to the geographic region (southwest Pacific) and group of birds (monarch-flycatchers) that were featured so prominently in seminal works on speciation theory and biogeography (Mayr 1942; Diamond 1974, 1975; Diamond et al. 1976). Many who work in the field of avain systematics criticize Ernst Mayr and Jared Diamond for their persistent interpretations of ecological and evolutionary phenomena through the lens of the Biological Species Concept; myself included. Indeed, it is easy to use their interpretations as strawman arguments in modern systematics studies, but I suggest that if one looks past the species debate and focuses on phenotypically and genetically diagnosable lineages as units of study, one will find that many of their ideas were not without merrit. In fact, over the course of my dissertation, I have come to appreciate the nuiances of their ideas and I find their voluminous writing to be among the first literature I turn to throughout the scientific process.

This dissertation comprises four data-rich chapters on the systematics of Pacific birds. It lays fundamental ground work for continued research in the Pacific, especially as the field moves from elucidating phylogenetic patterns to understanding evolutionary processes that govern origination, diversification, assembly, and extinction of biological diversity on islands. Some of the groups of birds I studied had not seen rigorous systematic work in more than 60 years since Mayr last curated the collection from the Whitney South Sea Expedition. It is my hope that I did these groups the justice they deserve. Some of my conclusions and interpretations, especially those of species limits, naturally are the product of the time period in which this work was conducted. Biological species are falling from favor in avian systematics; most are being “split” by way of phylogenetic/lineage-based species. If in 50 or 100 years science finds reason for this

pendulum to swing back, I would not be upset to see my interpretations in this dissertation amended. Regarding the more evolutionary interpretations herein (e.g., those pertaining to biogeography and diversification), this dissertation surely does not answer all the relevant questions, which is OK. Instead, I think of it as a living document that will continue to fine-tune my focus for future research endeavors, both in the field and the lab. If nothing else, I believe the nature of field-based systematics is such that scholarship can and should always be improved with increased sampling and more efficient and robust means of collecting and analyzing data. Ultimately, understanding the diversity of life is a never-ending endeavor—one that builds upon theoretical and empirical achievements of our predecessors—and that makes it all worthwhile and fun.

# Chapter 1*

## Phylogeography of the Variable Dwarf-Kingfisher *Ceyx lepidus* (Aves: Alcedinidae) inferred from mitochondrial and nuclear DNA sequences

* Andersen, M. J., Oliveros, C. H., Filardi, C. E., and R. G. Moyle. 2013. Phylogeography of the Variable Dwarf-Kingfisher *Ceyx lepidus* (Aves: Alcedinidae) inferred from mitochondrial and nuclear DNA sequences. Auk 130:118–131.

Abstract

We reconstructed the phylogeographic relationships of the Variable Dwarf-Kingfisher (*Ceyx lepidus*) using DNA sequence data. Maximum Likelihood and Bayesian analysis methods were used to reconstruct trees from a multilocus dataset of all 15 named subspecies of the *Ceyx lepidus* species complex. The concatenated dataset length was 2,471 bp and included two mitochondrial genes and two non-coding nuclear introns. Support for the monophyly of *Ceyx lepidus* was equivocal; instead, we found support for a clade including all *C. lepidus* subspecies plus two endemic Philippine taxa: *C. argentatus* and *C. cyanopectus*. Relationships among subspecific taxa were not well resolved, and many nodes were collapsed into polytomies suggesting a rapid and widespread colonization. *In situ* diversification likely played a role in generating current diversity within four archipelagos: the Philippines, Malukus, Bismarcks, and Solomons. Some biogeographic patterns recovered for the Solomon Islands taxa match those seen in other bird species, such as the close relationship of taxa on Bougainville, Choiseul, and Isabel; whereas the sister relationship between populations on Guadalcanal and the New Georgia Group is novel. We discuss species limits and make taxonomic recommendations to treat all 15 subspecies of *Ceyx lepidus* as species.

# Introduction

The islands of Southeast Asia and the western Pacific are home to some of the most phenotypically diverse avian species complexes in the world. Birds such as the Island Thrush (*Turdus poliocephalus*), Golden Whistler (*Pachycephala pectoralis*), and Collared Kingfisher (*Todiramphus chloris*) are well known for their widespread geographic distributions and diverse phenotypes, each having more than 50 subspecies (Galbraith 1956; Woodall 2001; Peterson 2007). These hyperdiverse species have served as exemplars by ornithologists and biogeographers to study evolutionary processes that lead to geographic partitioning of biological diversity on islands (Mayr and Diamond 2001).

The Variable Dwarf-Kingfisher (*Ceyx lepidus*) is another widespread, phenotypically diverse species that has long puzzled ornithologists. *Ceyx lepidus* is a highly variable species with 15 recognized subspecies (Fry et al. 1992; Woodall 2001; Clements et al. 2011). Indeed, within its more limited distribution, *C. lepidus* is nearly as diverse as the more widespread species complexes cited above (Clements et al. 2011). Each subspecies is defined by distinctive phenotype based on variation in breast, mantle, and rump coloration, and bill color and shape. Subspecies are distributed allopatrically on islands from the Philippines to the Solomon Islands, including the Maluku Archipelago, New Guinea, and the Bismarck Archipelago (Fig. 1.1). *Ceyx lepidus* is a biogeographic enigma; no other bird species shares its distribution. Indeed, no biogeographic term exists to circumscribe this region (Lomolino et al. 2010). Interestingly, among terrestrial vertebrates, this distribution is mirrored closely by *Platymantis* frogs (Allison 1996; Duellman 1999); however, *Platymantis* extends east to Fiji, whereas the Solomon Islands mark the eastern boundary of *C. lepidus*.

The general plumage pattern of *Ceyx lepidus* is blue or black above and rufous below. The breast and belly generally are rufous with a paler throat; the crown, back, wings, rump, and tail are blue or black, and a rufous loral spot and pale post-auricular stripe are present. Mayr and Diamond (2001) considered close relationships among some subspecies based on these generalized plumage patterns; however, they considered *Ceyx lepidus dispar*, *C. l. meeki*, and *C. l. gentianus* to be phenotypically disparate enough to warrant status as so-called "megasubspecies." *Ceyx l. gentianus*, for example, is the only taxon with a fully white breast and *C. l. dispar* is the only one with sexually dichromatic plumage (Fry et al. 1992). Notable plumage patterns also occur in the polymorphic Philippine endemic subspecies, *C. l. margarethae*, which has sympatric pale- and dark-backed morphs similar to the polymorphism described in *C. erithacus* of mainland Southeast Asia and the Sunda Shelf (Lim et al. 2010). In addition to plumage variation, bill structure and coloration vary dramatically within *Ceyx lepidus*. Bills are either red or black; red bills tend to be dorso-ventrally compressed and black bills tend to be laterally compressed. Two taxa, *C. l. nigromaxilla* and *C. l. sacerdotis*, have intermediate bill colors with black or dusky maxillae and orange mandibles (Fry et al. 1992; Woodall 2001). Indeed, the amount of variation expressed in *C. lepidus* bill morphology matches that seen across the entire clade to which it belongs: the pygmy-kingfishers (subfamily Alcedininae).

Few attempts have been made to elucidate the phylogenetic relationships of *C. lepidus* with other pygmy-kingfishers. Fry (1980) hypothesized a closer relationship with the Philippine endemic *C. argentatus* than with the sympatric *C. melanurus* based on plumage characters. More recently, studies using molecular data recovered *C. lepidus* in a well-supported clade of 3-toed pygmy-kingfishers in the genus *Ceyx* (Moyle 2006; Moyle et al. 2007) that included *C. cyanopectus*, *C. argentatus*, *C. melanurus*, and *C. erithacus*. Furthermore, Moyle et al. (2007)

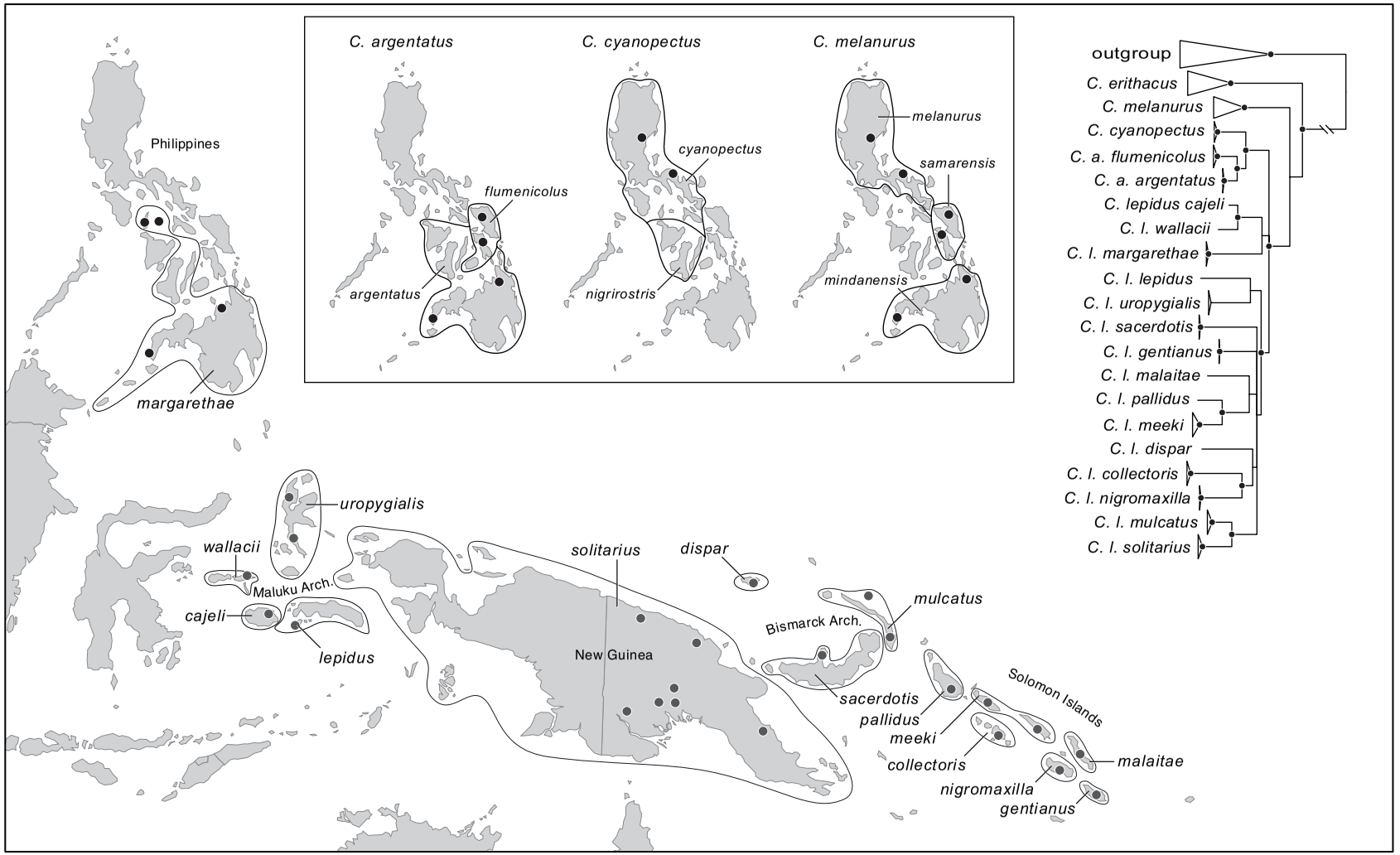



Figure 1.1. Map showing the distribution of the fifteen subspecies of *Ceyx lepidus* in the Philippines, Wallacea, New Guinea, and Melanesia. Inset panel depicts the distribution of closely-related Philippine endemic species *C. argentatus*, *C. cyanopectus*, and *C. melanurus*. Black dots represent sampling localities used in this study; some dots represent more than one individual per locality. The reader is referred to Table 1 for the complete sampling list. The phylogeny is a cartoon of Figure 2 for topological reference. Black circles on nodes denote Bayesian posterior probability = 0.95 and Maximum Likelihood bootstrap support ≥ 70.

found evidence of a paraphyletic *C. lepidus*, but the extent of paraphyly was not known owing to sampling deficiencies. Traditional taxonomy, based largely on plumage characters and operating within the confines of the biological species concept, has for a long time treated *C. lepidus* as one species with 15 diagnosable subspecies (Cottrell et al. 1945; Clements et al. 2011); however, historically, nine of the 15 taxa were described as species (all except *C. l. uropygialis*, *C. l. mulcatus*, *C. l. pallidus*, *C. l. collectoris*, *C. l. malaitae*, and *C. l. nigromaxilla*). In this paper, we reconstruct a molecular phylogeny of the *C. lepidus* species group and its closely-related taxa in order to elucidate the evolutionary history and assess species limits of this group.

## Methods

### *Taxon sampling*

Ingroup sampling included all 15 named taxa of *Ceyx lepidus* (Clements et al. 2011) as well as representative subspecies of *C. erithacus*, *C. melanurus*, *C. cyanopectus*, and *C. argentatus* (2/5, 3/3, 1/2, and 2/2, respectively; Table 1.1). Outgroup sampling included all remaining taxa in the 3-toed pygmy-kingfisher clade as circumscribed by Moyle et al. (2007) and *Alcedo websteri*, which was used to root trees. We sequenced 1–11 individuals per taxon, but, whenever possible, more than one sample per taxon was used to guard against errors of misidentification, mislabeling, or sample contamination.

### *DNA sequencing*

Total genomic DNA was extracted from frozen or alcohol-preserved muscle tissue using a Qiagen tissue extraction protocol (Qiagen, Valencia, CA). All tissue samples have associated

Table 1.1. Samples used to reconstruct the phylogeny of *Ceyx lepidus* including voucher institution and locality.

| Taxon | Voucher[a] | Sample | Locality |
|---|---|---|---|
| *Alcedo hercules* [d] | KUNHM | 10160 | China: Guangxi Province |
| *Ceyx azureus* | KUNHM | 96095 | Papua New Guinea: Gulf Province |
| *Ceyx fallax* [b, d, e] | AMNH | 299259 | Indonesia: Sulawesi |
| *Ceyx pusillus* | UWBM | Bu67896 | Solomon Islands: Western Province, New Georgia Island |
| *Ceyx websteri* | USNM | 608680[f] | Papua New Guinea: Bismarck Archipelago |
| *Ceyx argentatus argentatus* | KUNHM | 18103 | Philippines: Mindanao Island |
| *Ceyx argentatus argentatus* | KUNHM | 19071 | Philippines: Mindanao Island |
| *Ceyx argentatus argentatus* | KUNHM | 19252 | Philippines: Mindanao Island |
| *Ceyx argentatus argentatus* | KUNHM | 19268 | Philippines: Mindanao Island |
| *Ceyx argentatus argentatus* | KUNHM | 19269 | Philippines: Mindanao Island |
| *Ceyx argentatus* | KUNHM | 14284 | Philippines: Leyte Island |
| *Ceyx argentatus* | KUNHM | 14289 | Philippines: Leyte Island |
| *Ceyx argentatus* | KUNHM | 14241 | Philippines: Samar Island |
| *Ceyx cyanopectus* | KUNHM | 17990 | Philippines: Luzon Island |
| *Ceyx cyanopectus* | KUNHM | 18068 | Philippines: Luzon Island |
| *Ceyx cyanopectus* | KUNHM | 20334 | Philippines: Luzon Island |
| *Ceyx erithacus erithacus* | KUNHM | 10417 | China: Guangxi Province |
| *Ceyx erithacus motleyi* | LSUMNS | B38586 | Malaysia: Borneo, Sabah |
| *Ceyx erithacus motleyi* | KUNHM | 12359 | Malaysia: Borneo, Sarawak |
| *Ceyx erithacus motleyi* [c, d] | KUNHM | 12650 | Philippines: Palawan Island |
| *Ceyx erithacus motleyi* [c, d] | KUNHM | 12808 | Philippines: Palawan Island |
| *Ceyx lepidus cajeli* [b, c, d, e] | AMNH | 637134 | Indonesia: Maluku Province, Buru Island |
| *Ceyx lepidus collectoris* [d] | UWBM | Bu66054 | Solomon Islands: Western Province, New Georgia Island |
| *Ceyx lepidus collectoris* [d] | UWBM | Bu68064 | Solomon Islands: Western Province, New Georgia Island |
| *Ceyx lepidus collectoris* | UWBM | Bu68077 | Solomon Islands: Western Province, New Georgia Island |
| *Ceyx lepidus dispar* | KUNHM | 5611 | Papua New Guinea: Manus Province, Manus Island |
| *Ceyx lepidus gentianus* | KUNHM | 12801 | Solomon Islands: Makira-Ulawa Province: Makira Island |
| *Ceyx lepidus gentianus* | KUNHM | 13530 | Solomon Islands: Makira-Ulawa Province: Makira Island |
| *Ceyx lepidus gentianus* | KUNHM | 13540 | Solomon Islands: Makira-Ulawa Province: Makira Island |
| *Ceyx lepidus lepidus* [b, c, d, e] | AMNH | 637099 | Indonesia: Maluku Province, Ambon Island |
| *Ceyx lepidus malaitae* | UWBM | Bu66025 | Solomon Islands: Malaita Province: Malaita Island |
| *Ceyx lepidus margarethae* | KUNHM | 14022 | Philippines: Camiguin Sur Island |
| *Ceyx lepidus margarethae* | KUNHM | 14031 | Philippines: Camiguin Sur Island |
| *Ceyx lepidus margarethae* | KUNHM | 14355 | Philippines: Camiguin Sur Island |
| *Ceyx lepidus margarethae* | KUNHM | 14384 | Philippines: Camiguin Sur Island |
| *Ceyx lepidus margarethae* | KUNHM | 14397 | Philippines: Camiguin Sur Island |
| *Ceyx lepidus margarethae* | KUNHM | 19259 | Philippines: Mindanao Island |
| *Ceyx lepidus margarethae* | FMNH | 344953 | Philippines: Sibuyan Island |
| *Ceyx lepidus margarethae* | FMNH | 358316 | Philippines: Sibuyan Island |
| *Ceyx lepidus margarethae* | FMNH | 358317 | Philippines: Sibuyan Island |
| *Ceyx lepidus margarethae* | KUNHM | 14484 | Philippines: Tablas Island |

| Taxon | Voucher[a] | Sample | Locality |
|---|---|---|---|
| *Ceyx lepidus margarethae* | KUNHM | 14485 | Philippines: Tablas Island |
| *Ceyx lepidus meeki* | UWBM | Bu63203 | Solomon Islands: Choiseul Province, Choiseul Island |
| *Ceyx lepidus meeki* [c, d] | UWBM | Bu60194 | Solomon Islands: Isabel Province, Isabel Island |
| *Ceyx lepidus meeki* | AMNH | DOT6641 | Solomon Islands: Isabel Province, Isabel Island |
| *Ceyx lepidus mulcatus* [e] | LACM | 91032 | Papua New Guinea: New Ireland Province, New Ireland Island |
| *Ceyx lepidus mulcatus* [d, e] | LACM | 91033 | Papua New Guinea: New Ireland Province, New Ireland Island |
| *Ceyx lepidus mulcatus* [b, c, d, e] | AMNH | 335499 | Papua New Guinea: New Ireland Province, Tabar Island |
| *Ceyx lepidus nigromaxilla* | KUNHM | 15880 | Solomon Islands: Guadalcanal Province, Guadalcanal Island |
| *Ceyx lepidus nigromaxilla* | KUNHM | 15892 | Solomon Islands: Guadalcanal Province, Guadalcanal Island |
| *Ceyx lepidus nigromaxilla* | UWBM | Bu60341 | Solomon Islands: Guadalcanal Province, Guadalcanal Island |
| *Ceyx lepidus pallidus* | KUNHM | 5633 | Papua New Guinea: Bougainville Province, Bougainville |
| *Ceyx lepidus sacerdotis* | UWBM | Bu67945 | Papua New Guinea: West New Britain Province, ~12 km SE |
| *Ceyx lepidus sacerdotis* | UWBM | Bu68050 | Papua New Guinea: West New Britain Province, ~12 km SE |
| *Ceyx lepidus solitarius* | KUNHM | 5157 | Papua New Guinea: Chimbu Province |
| *Ceyx lepidus solitarius* | UWBM | Bu68037 | Papua New Guinea: Chimbu Province |
| *Ceyx lepidus solitarius* | KUNHM | 9539 | Papua New Guinea: East Sepik Province |
| *Ceyx lepidus solitarius* | KUNHM | 5192 | Papua New Guinea: Gulf Province, Ivimka Camp |
| *Ceyx lepidus solitarius* | UWBM | Bu67992 | Papua New Guinea: Gulf Province, Ivimka Camp |
| *Ceyx lepidus solitarius* | UWBM | Bu68021 | Papua New Guinea: Gulf Province, Ivimka Camp |
| *Ceyx lepidus solitarius* [d] | KUNHM | 7229 | Papua New Guinea: Madang Province |
| *Ceyx lepidus solitarius* | KUNHM | 7295 | Papua New Guinea: Madang Province |
| *Ceyx lepidus solitarius* | KUNHM | 6977 | Papua New Guinea: Oro Province |
| *Ceyx lepidus solitarius* | KUNHM | 6982 | Papua New Guinea: Oro Province |
| *Ceyx lepidus solitarius* | KUNHM | 7526 | Papua New Guinea: Western Province |
| *Ceyx lepidus uropygialis* [e] | YPM | 74993 | Indonesia: North Maluku Province, Bacan Island |
| *Ceyx lepidus uropygialis* [d, e] | YPM | 74989 | Indonesia: North Maluku Province, Halmahera Island |
| *Ceyx lepidus uropygialis* [b, c, | AMNH | 637110 | Indonesia: North Maluku Province, Halmahera Island |
| *Ceyx lepidus wallacii* [b, c, d, e] | AMNH | 637152 | Indonesia: North Maluku Province, Mangole Island |
| *Ceyx melanurus melanurus* | KUNHM | 18046 | Philippines: Luzon Island |
| *Ceyx melanurus melanurus* | KUNHM | 20203 | Philippines: Luzon Island |
| *Ceyx melanurus* | KUNHM | 18184 | Philippines: Mindanao Island |
| *Ceyx melanurus* | KUNHM | 19006 | Philippines: Mindanao Island |
| *Ceyx melanurus samarensis* | KUNHM | 14304 | Philippines: Leyte Island |
| *Ceyx melanurus samarensis* | KUNHM | 14226 | Philippines: Samar Island |

[a] Institutional abbreviations for voucher sources are as follows: American Museum of Natural History (AMNH), Field Museum of Natural History (FMNH), University of Kansas Natural History Museum (KUNHM), Los Angeles County Museum (LACM), Louisiana State University Museum of Natural Science (LSUMNS), Burke Museum University of Washington (UWBM), National Museum of Natural History Smithsonian Institution (USNM), Yale Peabody Museum (YPM).

[b, c, d] Denotes samples for which data are lacking from ND3, Myo2, and/or GAPDH, respectively.

[e] Denotes samples for which DNA was extracted from museum study skins.

[f] This sample is the same as "B04021" from Moyle et al. (2007).

museum study-skin vouchers. For taxa with no available tissue samples, DNA was extracted from toepads of museum study skins (Table 1.1) in lab space separate from other *Ceyx* tissue extractions to minimize contamination risk (Mundy et al. 1997).

We sequenced the entire second and third subunits of mitochondrial nicotinamide adenine dinucleotide dehydrogenase (hereafter ND2 and ND3, respectively), the second intron of the nuclear Myoglobin gene (hereafter Myo2), and the 11$^{th}$ intron of the nuclear glyceraldehyde-3-phosphate dehydrogenase gene (hereafter GAPDH). Target DNA fragments were amplified using polymerase chain reaction (PCR) with external and internal primers. ND2 and Myo2 primers are described by Moyle (2006) and Moyle et al. (2007). Additionally, we used internal primers 503L (Oliveros and Moyle 2010) and 562H1 (designed for this project; 5'-GATRATAATRGCYATTCAKCC-3') to amplify ND2 and the internal primer KingMyo620R (5'-AGGTTGCAGAGCCTGGAAATATCTC-3') to amplify Myo2 on some samples. The primer combinations L10755 and H11151 (Chesser 1999) and G3P13b and G3P14b (Fjeldså et al. 2003) were used to amplify ND3 and GAPDH, respectively.

PCR amplifications were performed in 25 μl reactions using 5-PRIME HotMaster *Taq* DNA polymerase with a touchdown protocol for mtDNA and GAPDH (annealing temperature: 58, 54, and 50 °C). We used an annealing temperature of 52 °C for Myo2 following Kimball et al. (2009). Amplified PCR products were screened on high-melt, 2% agarose gels stained with ethidium bromide, and purified with 10% Exo-SAP-IT™ (GE Healthcare Bio-Sciences Corp.). We cycle-sequenced purified PCR products in both directions with the same primers used in PCR for 25 cycles using the ABI Big Dye Terminator Cycle-Sequencing Kit version 3.1 (Applied Biosystems Inc., Foster City, CA). Sequencing was performed on an ABI Prism 3730 high-throughput capillary electrophoresis DNA analyzer and aligned sequences by hand using

Sequencher 4.9 (GeneCodes Corp.). Nuclear intron alignments were done by hand and checked against an automated alignment in MUSCLE (Edgar 2004).

*Phylogenetic analysis and topology tests*

Phylogenetic reconstruction was performed both on the concatenated data and on each individual locus. Maximum Likelihood (ML) tree searches were performed using GARLI 1.0 (Zwickl 2006) following the recommended default settings. We conducted 1,000 non-parametric bootstrap replicates (Felsenstein 1985) to assess clade credibility and SumTrees 1.1.1, part of the DendroPy 2.3.0 package (Sukumaran and Holder 2010), was used to create bootstrap consensus trees and calculate bootstrap values. Models of DNA sequence evolution for all phylogenetic analyses were tested using Akaike's Information Criterion (AIC) employed in jModelTest 2.1.1 (Guindon and Gascuel 2003; Darriba et al. 2012).

Bayesian Analysis (BA) was conducted using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003; Altekar et al. 2004; Ronquist et al. 2012) implemented with BEAGLE (Ayres et al. 2012). The data were partitioned by codon position for mtDNA and by gene for the nuclear introns. Two independent MCMC runs of 20 million generations were conducted using default number of chains (n=4) and heating conditions, sampling every 1,000 generations. TRACER 1.5 (Rambaut and Drummond 2007) and Are We There Yet? (AWTY; Wilgenbusch et al. 2004; Nylander et al. 2008) were used to assess convergence of parameter estimates and tree splits, respectively. The average standard deviation of split frequencies (ASDSF) was used to determine topology convergence between runs. The appropriate burn-in generations (25% for all analyses) were discarded based on convergence assessments of the ASDSF passing below 0.01. The remaining trees were summarized in a 50% majority-rule consensus tree.

Finally, the monophyly of *Ceyx lepidus* was evaluated using the approximately unbiased (AU) test (Shimodaira 2002). Using the same settings as the GARLI analyses described above, 200 ML searches were performed; 100 unconstrained and 100 with a topological constraint of *C. lepidus* monophyly. Per-site likelihoods were estimated for each tree under a partitioned model and an AU test was performed on these values using CONSEL v0.1i (Shimodaira and Hasegawa 2001). The *P*-value reported is the largest *P*-value of all trees inferred under the constraint.

## Results

### *Sequence attributes*

The aligned dataset was 2,471 bp and included 75 samples from 27 named taxa. All sequences are deposited in GenBank (Accession Nos. KC112595–KC112848). We obtained DNA sequences for all genes for all samples with the exception of those taken from museum skins, for which we were only able to sequence mitochondrial genes (Table 1.1). Alignment lengths were 1,041 bp (ND2), 352 bp (ND3), 709 bp (Myo2), and 370 bp (GAPDH). The aligned dataset contained 629 variable characters (25.5%) and 481 (19.5%) parsimony-informative characters. Pairwise distances in ND2 (uncorrected *p*; Table 1.2) ranged 8.0–11.6% between outgroup taxa and *C. lepidus* and 2.6–6.8% (mean = 4.7%) among *C. lepidus* taxa.

The ND3 gene sequence contained a single cytosine insertion at position 174 in all samples, an insertion reported in several other bird groups and turtles (Mindell et al. 1998). This insertion does not disrupt the reading frame because it is not translated. Apart from this insertion in ND3, the mitochondrial data showed no other insertions, deletions, or anomalous stop-codons; thus there was no evidence that mtDNA sequences were of nuclear origin (i.e., pseudogenes; Sorenson and Quinn 1998). The relative divergence among codon positions was typical for

Table 1.2. Uncorrected ND2 pair-wise *p*-distances. Mean pair-wise distances are reported for taxa with more than one sample. Column headers are abbreviated with the first three letters of the subspecific epithet.

| | *eri.* | *mot.* | *mel.* | *min.* | *sam.* | *arg.* | *flu.* | *cya.* | *caj.* | *col.* | *dis.* | *gen.* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ceyx erithacus erithacus* | — | | | | | | | | | | | |
| *C. erithacus motleyi* | 0.048 | — | | | | | | | | | | |
| *C. melanurus melanurus* | 0.088 | 0.085 | — | | | | | | | | | |
| *C. m. mindanensis* | 0.085 | 0.076 | 0.026 | — | | | | | | | | |
| *C. m. samarensis* | 0.084 | 0.084 | 0.019 | 0.025 | — | | | | | | | |
| *C. argentatus argentatus* | 0.085 | 0.080 | 0.070 | 0.065 | 0.070 | — | | | | | | |
| *C. argentatus flumenicolus* | 0.092 | 0.083 | 0.074 | 0.070 | 0.077 | 0.025 | — | | | | | |
| *C. cyanopectus cyanopectus* | 0.092 | 0.080 | 0.072 | 0.068 | 0.073 | 0.034 | 0.041 | — | | | | |
| *C. lepidus cajeli* | 0.080 | 0.075 | 0.059 | 0.056 | 0.060 | 0.045 | 0.046 | 0.054 | — | | | |
| *C. l. collectoris* | 0.098 | 0.088 | 0.084 | 0.079 | 0.084 | 0.062 | 0.064 | 0.069 | 0.057 | — | | |
| *C. l. dispar* | 0.089 | 0.083 | 0.073 | 0.068 | 0.077 | 0.057 | 0.058 | 0.059 | 0.045 | 0.065 | — | |
| *C. l. gentianus* | 0.092 | 0.088 | 0.073 | 0.066 | 0.080 | 0.054 | 0.057 | 0.063 | 0.041 | 0.067 | 0.053 | — |
| *C. l. lepidus* | 0.086 | 0.082 | 0.069 | 0.064 | 0.065 | 0.045 | 0.050 | 0.057 | 0.047 | 0.063 | 0.055 | 0.057 |
| *C. l. malaitae* | 0.093 | 0.088 | 0.075 | 0.070 | 0.081 | 0.059 | 0.061 | 0.059 | 0.053 | 0.061 | 0.057 | 0.054 |
| *C. l. margarethae* | 0.086 | 0.080 | 0.073 | 0.066 | 0.073 | 0.055 | 0.064 | 0.063 | 0.046 | 0.061 | 0.062 | 0.062 |
| *C. l. meeki* | 0.085 | 0.085 | 0.074 | 0.067 | 0.077 | 0.056 | 0.060 | 0.061 | 0.047 | 0.068 | 0.056 | 0.063 |
| *C. l. mulcatus* | 0.099 | 0.086 | 0.077 | 0.067 | 0.078 | 0.062 | 0.062 | 0.063 | 0.052 | 0.067 | 0.062 | 0.052 |
| *C. l. nigromaxilla* | 0.094 | 0.089 | 0.078 | 0.071 | 0.081 | 0.061 | 0.064 | 0.065 | 0.048 | 0.053 | 0.059 | 0.059 |
| *C. l. pallidus* | 0.083 | 0.083 | 0.076 | 0.068 | 0.080 | 0.052 | 0.062 | 0.062 | 0.049 | 0.070 | 0.060 | 0.061 |
| *C. l. sacerdotis* | 0.100 | 0.092 | 0.076 | 0.073 | 0.082 | 0.060 | 0.061 | 0.069 | 0.047 | 0.073 | 0.061 | 0.051 |
| *C. l. solitarius* | 0.093 | 0.085 | 0.078 | 0.073 | 0.082 | 0.066 | 0.067 | 0.070 | 0.044 | 0.066 | 0.065 | 0.054 |
| *C. l. uropygialis* | 0.089 | 0.082 | 0.071 | 0.067 | 0.077 | 0.051 | 0.052 | 0.061 | 0.051 | 0.065 | 0.058 | 0.054 |
| *C. l. wallacii* | 0.083 | 0.076 | 0.062 | 0.058 | 0.061 | 0.050 | 0.048 | 0.058 | 0.016 | 0.061 | 0.054 | 0.048 |

| | *lep.* | *mal.* | *mar.* | *mee.* | *mul.* | *nig.* | *pal.* | *sac.* | *sol.* | *uro.* | *wal.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. l. lepidus* | — | | | | | | | | | | |
| *C. l. malaitae* | 0.056 | — | | | | | | | | | |
| *C. l. margarethae* | 0.060 | 0.064 | — | | | | | | | | |
| *C. l. meeki* | 0.045 | 0.053 | 0.062 | — | | | | | | | |
| *C. l. mulcatus* | 0.060 | 0.058 | 0.060 | 0.060 | — | | | | | | |
| *C. l. nigromaxilla* | 0.058 | 0.056 | 0.064 | 0.057 | 0.058 | — | | | | | |
| *C. l. pallidus* | 0.051 | 0.050 | 0.062 | 0.032 | 0.060 | 0.068 | — | | | | |
| *C. l. sacerdotis* | 0.061 | 0.060 | 0.067 | 0.063 | 0.060 | 0.063 | 0.066 | — | | | |
| *C. l. solitarius* | 0.060 | 0.061 | 0.066 | 0.064 | 0.033 | 0.065 | 0.068 | 0.063 | — | | |
| *C. l. uropygialis* | 0.048 | 0.056 | 0.060 | 0.057 | 0.055 | 0.063 | 0.053 | 0.062 | 0.061 | — | |
| *C. l. wallacii* | 0.052 | 0.056 | 0.050 | 0.052 | 0.056 | 0.060 | 0.053 | 0.048 | 0.054 | 0.055 | — |

mtDNA (3 > 1 > 2). Four deletions were noted in Myo2, but all were autapomorphic in the following samples: *Ceyx pusillus* (2 bp), *C. erithacus* (1 bp in each: B38586 and 12359), and *C. l. margarethae* (2 bp; 14384). A synapomorphic 1-bp deletion was shared by all *C. erithacus* and *C. melanurus* samples in GAPDH. Based on the results of model testing, we used the GTR+I+G model of sequence evolution for all three mtDNA codon positions, HKY+I for Myo2, and

HKY+G for GAPDH. All ML analyses with GARLI were done with the GTR+I+G model across the entire dataset.

### *Phylogenetic relationships*

Individual gene trees were highly concordant (Fig. 1.2). The topologies recovered from analyses of mtDNA showed greater resolution than those derived from nuclear introns, which was expected given the higher rates of sequence evolution in animal mtDNA compared to nuclear DNA (Brown et al. 1979). No well-supported clades recovered from the analysis of individual genes conflicted with those from other gene trees or those from the concatenated dataset so we focused our discussion on phylogenetic relationships inferred from the concatenated dataset (Fig. 1.3). The inferred topologies from multiple independent ML and BA runs were highly concordant. The best ML topology had a –ln likelihood score of 9605.1289, as reported in GARLI.

We recovered a well-supported clade (i.e., Bayesian posterior probability > 95% and ML bootstrap > 70%) that included two outgroup taxa (*C. erithacus* and *C. melanurus*) and the ingroup clade (Fig. 1.3, Clade A), which comprised *C. lepidus*, *C. cyanopectus*, and *C. argentatus*. However, relationships among *C. erithacus*, *C. melanurus*, and Clade A were unresolved, a result similar to those obtained by Moyle et al. (2007) and Lim et al. (2010). The synapomorphic indel observed in the GAPDH intron supports a sister relationship between *C. melanurus* and *C. erithacus*, but this hypothesis requires further investigation.

Support for Clade A was unequivocal, but monophyly of *C. lepidus* received no support. Instead, basal relationships within Clade A consisted of a polytomy among four well-supported clades: (1) the Philippine endemics *C. cyanopectus* and *C. argentatus* (Fig. 1.3, Clade B); (2)

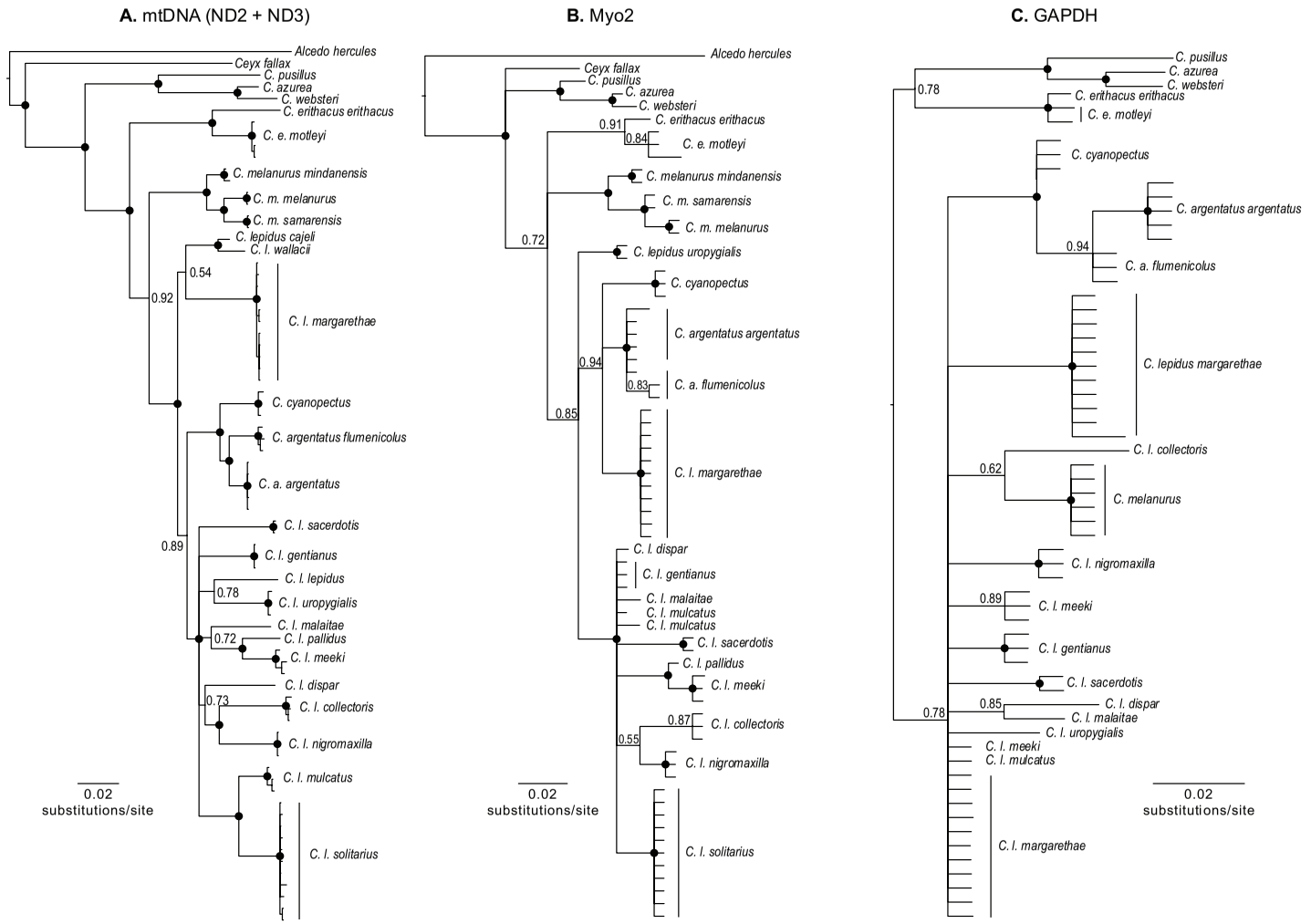



Figure 1.2. Bayesian phylogenies of the *Ceyx lepidus* complex for each of the three independently-segregating loci. (A) mtDNA: ND2+ND3, (B) Myo2, (C) GAPDH. Black circles on nodes denote Bayesian posterior probability = 0.95 and Maximum Likelihood bootstrap support ≥ 70. Numbers by nodes indicate Bayesian PP. Some taxa are missing from trees B and C because data are lacking for Myo2 and GAPDH, respectively.

Wallacean *C. l. cajeli* and *C. l. wallacii* (Fig 1.3, Clade C); (3) Philippine endemic *C. l. margarethae* (Fig. 1.3, Clade D); and (4) a clade including the remaining 12 subspecies of *C. lepidus* (Fig. 1.3, Clade E). Although the polytomy raised the possibility that *C. lepidus* is paraphyletic, this relationship is best considered unresolved. Indeed, an AU test failed to reject *C. lepidus* monophyly ($P = 0.230$). Despite the lack of resolution at the bases of Clades A and E, each of the 15 *C. lepidus* subspecies was monophyletic, and several sister pairs were well supported: *Ceyx l. mulcatus* + *C. l. solitarius*, *C. l. collectoris* + *C. l. nigromaxilla*, and *C. l. pallidus* + *C. l. meeki*.

Within *C. argentatus* phylogeographic structure was concordant with named subspecies (see Discussion section below). Conversely, we found no discernable genetic structure within two widespread *C. lepidus* subspecies: *C. l. margarethae* and *C. l. solitarius*, despite broad sampling within their ranges. This result was somewhat expected for *C. l. solitarius* and likely suggests a high amount of gene flow across the island of New Guinea. However, the lack of genetic differentiation in *C. l. margarethae* across multiple Philippine oceanic islands—and representing two color morphs—is noteworthy.

Finally, removal of the five taxa represented by only one gene sequence in our dataset (*Ceyx l. dispar, C. l. malaitae, C. l. cajeli, C. l. wallacii,* and *C. l. lepidus*) had little effect on results of Bayesian analysis of the concatenated dataset. Bayesian posterior probabilities and the backbone topology were extremely similar between analyses with and without the five taxa (results not shown).

Figure 1.3. Bayesian phylogeny of the *Ceyx lepidus* complex based on a concatenated dataset of two mitochondrial coding genes and two nuclear introns. Black circles on nodes denote Bayesian posterior probability (PP) = 0.95 and Maximum Likelihood (ML) bootstrap support ≥ 70. Numbers by nodes detail unresolved nodes, with numbers above branches indicating Bayesian PP and those below branches ML bootstrap.

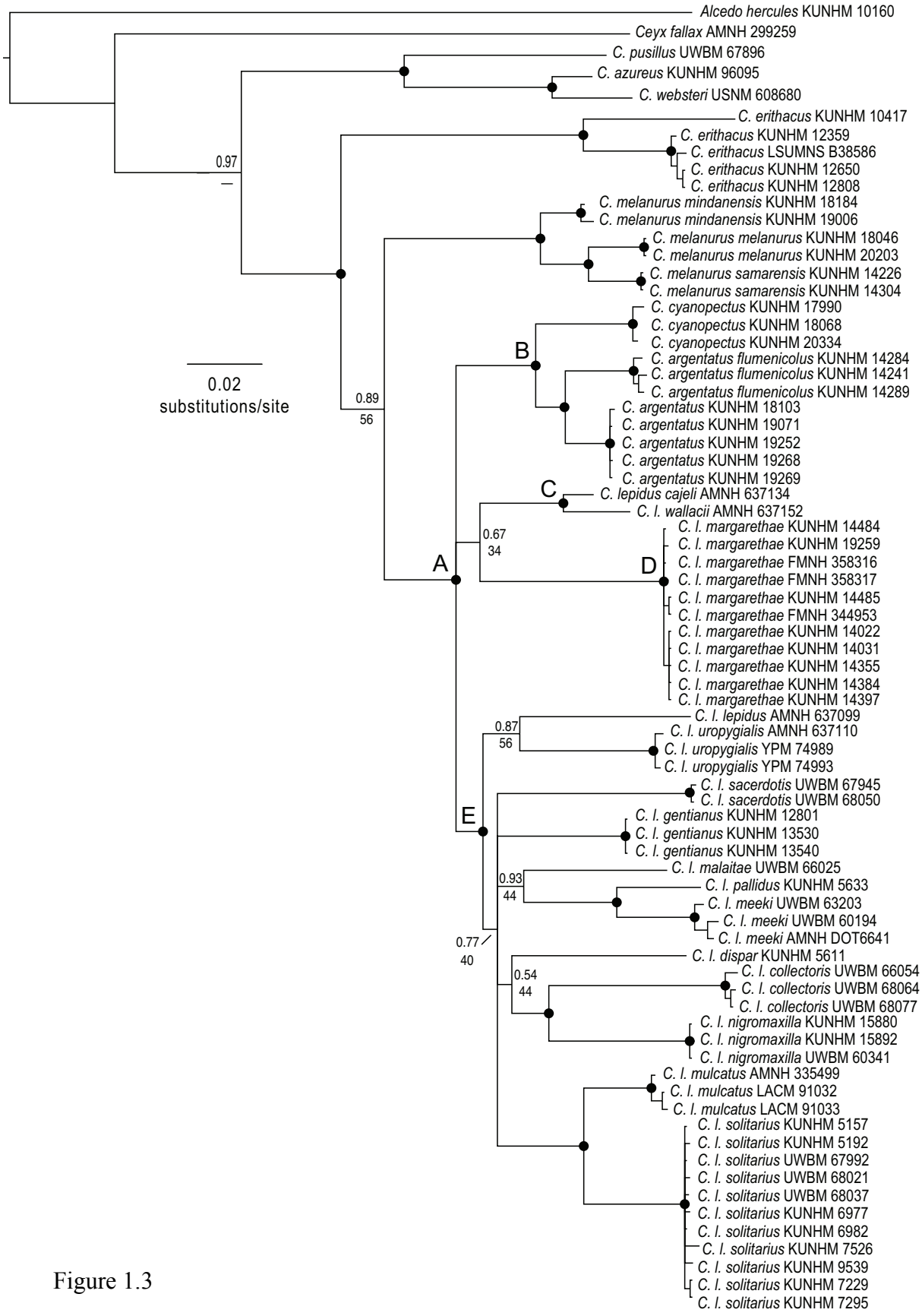

Alcedo hercules KUNHM 10160
Ceyx fallax AMNH 299259
C. pusillus UWBM 67896
C. azureus KUNHM 96095
C. websteri USNM 608680
C. erithacus KUNHM 10417
C. erithacus KUNHM 12359
C. erithacus LSUMNS B38586
C. erithacus KUNHM 12650
C. erithacus KUNHM 12808
C. melanurus mindanensis KUNHM 18184
C. melanurus mindanensis KUNHM 19006
C. melanurus melanurus KUNHM 18046
C. melanurus melanurus KUNHM 20203
C. melanurus samarensis KUNHM 14226
C. melanurus samarensis KUNHM 14304
C. cyanopectus KUNHM 17990
C. cyanopectus KUNHM 18068
C. cyanopectus KUNHM 20334
C. argentatus flumenicolus KUNHM 14284
C. argentatus flumenicolus KUNHM 14241
C. argentatus flumenicolus KUNHM 14289
C. argentatus KUNHM 18103
C. argentatus KUNHM 19071
C. argentatus KUNHM 19252
C. argentatus KUNHM 19268
C. argentatus KUNHM 19269
C. lepidus cajeli AMNH 637134
C. l. wallacii AMNH 637152
C. l. margarethae KUNHM 14484
C. l. margarethae KUNHM 19259
C. l. margarethae FMNH 358316
C. l. margarethae FMNH 358317
C. l. margarethae KUNHM 14485
C. l. margarethae FMNH 344953
C. l. margarethae KUNHM 14022
C. l. margarethae KUNHM 14031
C. l. margarethae KUNHM 14355
C. l. margarethae KUNHM 14384
C. l. margarethae KUNHM 14397
C. l. lepidus AMNH 637099
C. l. uropygialis AMNH 637110
C. l. uropygialis YPM 74989
C. l. uropygialis YPM 74993
C. l. sacerdotis UWBM 67945
C. l. sacerdotis UWBM 68050
C. l. gentianus KUNHM 12801
C. l. gentianus KUNHM 13530
C. l. gentianus KUNHM 13540
C. l. malaitae UWBM 66025
C. l. pallidus KUNHM 5633
C. l. meeki UWBM 63203
C. l. meeki UWBM 60194
C. l. meeki AMNH DOT6641
C. l. dispar KUNHM 5611
C. l. collectoris UWBM 66054
C. l. collectoris UWBM 68064
C. l. collectoris UWBM 68077
C. l. nigromaxilla KUNHM 15880
C. l. nigromaxilla KUNHM 15892
C. l. nigromaxilla UWBM 60341
C. l. mulcatus AMNH 335499
C. l. mulcatus LACM 91032
C. l. mulcatus LACM 91033
C. l. solitarius KUNHM 5157
C. l. solitarius KUNHM 5192
C. l. solitarius UWBM 67992
C. l. solitarius UWBM 68021
C. l. solitarius UWBM 68037
C. l. solitarius KUNHM 6977
C. l. solitarius KUNHM 6982
C. l. solitarius KUNHM 7526
C. l. solitarius KUNHM 9539
C. l. solitarius KUNHM 7229
C. l. solitarius KUNHM 7295
0.97
0.02
substitutions/site
0.89
56
A
B
C
D
E
0.67
34
0.87
56
0.93
44
0.77
40
0.54
44

Figure 1.3

## Discussion

### *Biogeography*

This paper presents the first fully-sampled molecular phylogeny of *Ceyx lepidus*. Although the abundance of unresolved relationships precluded quantitative biogeographic analysis, some biogeographic insights are evident from our results.

Overall, the most striking aspect of the phylogeny is that each taxon in the *C. lepidus* complex is monophyletic and substantially diverged from all other taxa (2.6–6.8% divergent in uncorrected ND2 *p*-distance). We interpret this pattern of shallow internodes at the base, long stem lineages, and shallow divergences within each taxon as support for a scenario in which *C. lepidus* achieved its full geographic distribution rapidly followed by little or no subsequent gene flow among most island populations. This biogeographic pattern of rapid and widespread colonization across Southeast Asia and the Pacific islands is thought to have occurred in other widespread polytypic species complexes such as *Todiramphus chloris*, *Pachycephala pectoralis*, and *Turdus poliocephalus* (Mayr and Diamond 2001). However, densely sampled phylogenetic hypotheses are not available to test this hypothesis in these groups (but see Jones and Kennedy 2008b; Jonsson et al. 2008a).

The highest diversity in this group of dwarf-kingfishers (under present taxonomy) occurs in the Philippine archipelago, where one to three species are present on each major island. It appears that *in situ* diversification of *C. argentatus* and *C. cyanopectus* (Clade B) in the Philippines played a role in generating this diversity. Our results also indicate that multiple colonization events contributed to the diversity of dwarf-kingfishers in the Philippines: the ancestors of *C. erithacus* appear to have invaded the western Philippines from the Sunda Shelf and at least two other colonization events were responsible for the presence of *C. melanurus*, *C. cyanopectus*, *C. argentatus*, and *C. l. margarethae* in the archipelago. These four taxa occur in

sympatry on some islands; however, it is uncertain whether they occur syntopically. For instance, *C. argentatus*, *C. melanurus*, and *C. l. margarathae* all occur on Mindanao, and although natural history data are sparse for these taxa, preliminary observations suggest that *C. argentatus* is a stream-associated species, whereas *C. melanurus* and *C. l. margarethae* are forest species with no affinity to water (P. Hosner pers. comm.). This pattern suggests that at least some level of ecological partitioning helps separate these otherwise broadly sympatric taxa.

Some geographic insight can be gleaned from sister relationships in the *C. lepidus* species group. For instance, dwarf kingfishers of the Malukus are derived from two well-supported but unrelated pairs of sister taxa (*C. l. cajeli* and *C. l. wallacii*; *C. l. lepidus* and *C. l. uropygialis*), indicating the combined role of colonization and local diversification in generating diversity. *In situ* diversification is also evident in the Solomon Islands with the recovery of two pairs of sister taxa within the island group. The first pair, *C. l. pallidus* and *C. l. meeki*, reflects the close affinities of Bougainville, Choiseul, and Isabel, which form part of the Pleistocene island of Greater Bukida (Mayr and Diamond 2001). The close affinity of fauna within Greater Bukida, especially between Choiseul and Isabel, is documented in multiple avian lineages (Smith and Filardi 2007; Uy et al. 2009a) and also has been observed in bats (Pulvers and Colgan 2007). Bougainville tends to have taxa more divergent from the rest of the Greater Bukida islands (Mayr and Diamond 2001), and this pattern is also seen in *Ceyx*. The second sister pair within the Solomon Islands, *C. l. collectoris* and *C. l. nigromaxilla*, reveals a close relationship between the New Georgia Group and Guadalcanal, a biogeographic pattern not recovered in other avian studies (Filardi and Smith 2005; Smith and Filardi 2007; Uy et al. 2009a). The mostly unresolved relationships among the lineages in Clade E obscures the number of colonization events of the Solomon Islands. Lastly, the sister relationship of *C. l. solitarius* and *C. l. mulcatus*

unites New Guinea with the northern Bismarck Archipelago islands of New Ireland, New Hanover, Tabar, and Lihir. This result suggests that at least two independent colonization events were involved in assembling the dwarf-kingfishers of the Bismarcks. This sister pairing is cohesive with respect to plumage because they are nearly identical—both are rufous below with whitish throats, pale rufous loral spots, dark blue backs, and black bills. This pattern of similarly-plumaged sister taxa was not upheld throughout the rest of the tree, which highlights the need for revisionary taxonomy not based solely on plumage patterns in polytypic, insular species complexes (Peterson 2007).

### *Plumage polymorphism*

Examples of plumage polymorphism in birds are numerous and have received much attention (Roulin 2004). *Ceyx l. margarethae*, an endemic of central and southern Philippines, is the only *C. lepidus* subspecies for which polymorphism within single-island populations occurs. Only one other *Ceyx* species is polymorphic within a population: *C. erithacus* (Fry et al. 1992). Lim et al. (2010) found evidence for polymorphism in *C. erithacus* as a result of admixture of historically separate and genetically well-differentiated populations across southeast Asia and the Sunda Shelf. We sampled widely throughout the range of *C. l. margarethae*, including pale- and dark-backed individuals from the Philippine islands of Camiguin Sur, Tablas, Mindanao, and Sibuyan; however, we failed to recover genetic structure in *C. l. margarethae* with respect to geography or plumage polymorphism. Recent studies have found that single point mutations in the melanocortin-1-receptor gene are associated with plumage polymorphisms in bananaquits and monarchs (Theron et al. 2001; Uy et al. 2009b). It is possible that a single point mutation is

driving plumage polymorphism in *C. l. margarethae*, and investigations on the role of this gene in polymorphism in *Ceyx* species is recommended.

The subspecies *Ceyx l. dispar,* from the Admiralty Islands of Papua New Guinea, is sexually dichromatic—the male has the typical blue head, while the female is orange-headed. This pattern is reminiscent of the *Ispidina* pygmy-kingfishers of Africa (Fry et al. 1992). Only one other ingroup taxon, *C. cyanopectus*, is sexually dichromatic; males have a double breast band, while females have only one breast band (Kennedy et al. 2000); thus, sexual dichromatism appears to have evolved twice in the ingroup. Indeed, other instances of differential patterns of sexual dichromatism in polytypic insular bird species are known. For example, *Turdus poliocephalus niveiceps* and *T. p. carbonarius* are sexually dichromatic on Taiwan and New Guinea, respectively, but not elsewhere (Peterson 2007). Interestingly, *Pachycephala pectoralis feminina* on Rennell Island in the Solomon Islands is sexually monochromatic; in this instance the male reverts to female plumage (Galbraith 1956).

### *Taxonomy*

Our discussion of taxonomy is based largely on an evolutionary species concept (Simpson 1961; Wiley 1978) and its extension, the general lineage-based species concept (de Queiroz 1999). We draw upon details of genetic divergence, biogeography, and plumage pattern as the most prescient evidence. Application of lineage-based species concepts to island systems is preferable to the biological species concept (Mayr 1963) because reproductive isolation between allopatric insular taxa cannot be assessed. Instead, we employ a lineage-based species concept to recognize ancestor-descendant populations with unique evolutionary histories.

Two Philippine species warrant discussion on species limits: *C. argentatus* and *C. melanurus*. *Ceyx argentatus* is distributed throughout the central and southern Philippines (Fig. 1.1, inset). Two subspecies of *C. argentatus* are described: *C. a. argentatus* and *C. a. flumenicolus*, though both were originally described as species (Cottrell et al. 1945). We recovered these two subspecies as sister clades diverged by 2.3% ND2 uncorrected *p*-distances. Our results support the suggestion of Collar (2011), which was based on morphological data, to treat *C. argentatus* and *C. flumenicolus* as full species, but we acknowledge that this pair of taxa requires further investigation to determine whether there is gene flow between them. On the other hand, *C. melanurus* consists of three subspecies, which are distributed along the eastern arc of the Philippines (Fig. 1.1, inset): *C. m. melanurus*, *C. m. samarensis*, and *C. m. mindanensis*. We sampled all three subspecies and found strong support for the sister relationship of *C. m. mindanensis* and a clade comprising *C. m. melanurus* and *C. m. samarensis*. Morphologically, these forms differ in the extent of black on the wings and the presence or absence of a blue streak on the side of the head (Fry et al. 1992). A comprehensive study of the genetic structure and morphological variation in this species is ongoing (P. Hosner, unpublished data), thus, we refrain from recommending taxonomic changes in this group. In both *C. argentatus* and *C. melanurus*, our data demonstrate the genetic distinctiveness of species on the eastern Philippine islands of Samar, Leyte, and Bohol, despite their land connection to Mindanao during the last glacial maximum. This result provides another example showing the distinctiveness of avian populations in this group of islands (Sánchez-González and Moyle 2011; Sheldon et al. 2012) and supports a nascent, but growing, body of studies recognizing that the paradigm of late Pleistocene aggregate islands explaining the distribution of diversity in the Philippines proposed by Heaney (1986) is overly simplistic for mammals, reptiles, amphibians, and birds (Evans et al.

2003; Jones and Kennedy 2008a; Esselstyn and Brown 2009; Linkem et al. 2010; Oliveros and Moyle 2010; Siler et al. 2010).

Plumage differences between the 15 *C. lepidus* subspecies are described in (Fry et al. 1992; Woodall 2001) and summarized in Appendix I. We discuss in detail an example of highly divergent plumage and an example in which the plumage differentiation is minimal, and the reader is referred to the appendix for details of plumage differences that are not discussed in the text. *Ceyx l. gentianus* from Makira Island in the Solomon Islands is one of the most morphologically disparate taxa. It is entirely white below, lacking the rufous tones found in most other forms of *C. lepidus* (Fry et al. 1992; Dutson et al. 2011). Other described taxa have more subtle plumage differences, and the two most similar taxa occur in the Solomon Islands. The Bougainville taxon, *C. l. pallidus*, one of the few taxa originally described as a subspecies of *C. lepidus*, is slightly paler than *C. l. meeki* from Choiseul and Isabel Islands. In his description Mayr (1935a) noted that *C. l. pallidus* is "similar to *Ceyx lepidus meeki*, but [its] under parts [are] pale yellowish buff, instead of golden-yellowish ochre." *C. l. pallidus* appears to be only weakly differentiated from *C. l. meeki* in plumage, but our data support a well-differentiated genetic split (3.3% ND2 uncorrected *p*-distance) between these sister taxa. This divergence is substantially higher than the 2.3% divergence between *C. a. argentatus* and *C. a. flumenicolus*, two morphologically divergent sister taxa. It appears that in the case of *C. l. pallidus* and *C. l. meeki*, morphology was conserved while their populations diverged.

Although our phylogeny does not resolve the apparent rapid and widespread geographic diversification of *Ceyx lepidus* in a bifurcating fashion, it does provide a basis for a reevaluation of species limits in this group. We propose recognizing all 15 *C. lepidus* subspecies as species for the following reasons: (1) each subspecies is morphologically distinct; (2) these taxa exhibit a

relatively uniform and high degree of genetic differentiation among lineages (2.6–6.8% in ND2 uncorrected *p*-distance, Table 1.2), which is higher than in two sister taxa (*C. argentatus* and *C. flumenicolus*) that are closely related to *C. lepidus*; and (3) the 15 subspecies have allopatric distributions and therefore are experiencing their own evolutionary fate.

These results support an improved understanding of the high degree of morphologic and cryptic genetic diversity not only in Philippine birds (Lohman et al. 2010) but more broadly in the archipelagos of Southeast Asia and the Pacific. Recognizing subspecies of *C. lepidus* as full species will have important conservation implications, especially because most taxa are endemic to small islands or island groups.

# Chapter 2*

## Molecular systematics of the world's most polytypic bird: the *Pachycephala pectoralis*/*melanura* (Aves: Pachycephalidae) species complex

* Andersen, M. J., Nyári, Á. S., Mason, I., Joseph, L., Dumbacher, J. P., Filardi, C. E., and R. G. Moyle. In press. Molecular systematics of the world's most polytypic bird: the *Pachycephala pectoralis*/*melanura* (Aves: Pachycephalidae) species complex. Zoological Journal of the Linnean Society doi: 10.1111/zoj.12088

Abstract

With more than 70 described subspecies distributed from Java to Fiji, the Golden Whistler species complex (Aves: *Pachycephala pectoralis*/*melanura*) is the world's most geographically variable bird species. We sequenced 10 genes totaling 5743 bp from 202 individuals and 32 nominal subspecies, mostly from the Australasian and Polynesian lineages. We used concatenated maximum likelihood and Bayesian inference, as well as coalescent species tree analysis, to reconstruct a phylogeny. The resulting phylogeny is the most densely sampled and robust estimate of this group's evolutionary history to date and many novel relationships are revealed. The ingroup comprised three well-supported clades. An Australasian clade inclusive of Vanuatu was sister to a clade including the Bismarck Archipelago, the Solomon Islands, and the Polynesian taxa minus Vanuatu, and sister to these two clades was *Pachycephala citreogaster collaris* of the Louisiade Archipelago. Some species-level taxa endemic to the Pacific were found to be embedded in the ingroup (e.g., *P. feminina*, *P. flavifrons*, and *P. jacquinoti*), whereas others were found to be outside of the species complex (e.g., *P. implicata*). Generally, most nodes in the tree had strong support with the exception of several Polynesian lineages whose relationships remain equivocal. Relationships within each clade are discussed in detail, and current taxonomic treatments are critiqued in light of our results.

## Introduction

Islands are ideal laboratories to study evolution and geographic partitioning of biological diversity, because of their isolation, discrete geographic boundaries, and relatively well-known geologic histories. Indeed, islands have long been recognized as special geographic entities populated with evolutionary novelties (Darwin 1859; Wallace 1881). The importance of islands spawned a quarter-century of intensive research on the ecology and evolution of insular species' distributions (MacArthur and Wilson 1963, 1967; MacArthur 1972; Wagner and Funk 1995). The utility of islands as 'natural laboratories' of evolution is exemplified in patterns of differentiation in widespread, phenotypically variable avian lineages (Mayr and Diamond 2001; Grant and Grant 2002; Lovette et al. 2002; Filardi and Moyle 2005; Smith and Filardi 2007; Ricklefs and Bermingham 2008).

A conspicuous element of island bird faunas, especially in the southwest Pacific, is the profusion of widespread 'polytypic' species that contain many nominal subspecies (Mayr and Diamond 2001). These species occur on many islands—often across multiple archipelagos (e.g., Collared Kingfisher Todiramphus chloris (Boddaert, 1783), Variable Dwarf-kingfisher Ceyx lepidus Temminck, 1836, Island Thrush Turdus poliocephalus Latham 1802, and Monarcha Vigors & Horsfield, 1827 flycatchers; Woodall 2001; Collar 2005; Coates et al. 2006). Although the various subspecies or island populations of these species are apparently closely related, many differ markedly in plumage pattern or coloration. Classification of these distinct allopatric populations has challenged taxonomists working under the Biological Species Concept (Mayr 1942, 1963) because reproductive isolation among allopatric populations was impossible to assess. As a result, up to several dozen distinctive populations were recognized as subspecies within single 'species complexes'. Although a frustration for taxonomists, these broadly

distributed but well-differentiated populations have proved excellent study systems for the development of classic concepts in evolutionary biology (Mayr 1942; Diamond 1974, 1975; Diamond et al. 1976) and, more recently, hypothesis testing using modern data sources and analytical methods (Moyle et al. 2009; Uy et al. 2009a; Uy et al. 2009b).

One of the most striking examples of a polytypic species is the Golden Whistler *Pachycephala pectoralis* (Latham, 1802), which comprises ca. 60–70 nominal subspecies spanning the Indo-Pacific (Galbraith 1956; Boles 2007). Most of the subspecies correspond to phenotypically distinct, single-island populations. Often, subspecies on adjacent islands are more disparate in plumage than are subspecies on islands separated by greater distances. Overall, plumage distinctiveness in Golden Whistlers comprises variation in a limited number of traits. Most subspecies are dorsally olive-green to black and ventrally yellow. Subspecies differ in combinations of throat color (white or yellow), presence or absence of a black nuchal collar, yellow loral spots and nape, intensity of ventral yellow, and other minor plumage details on the wings and tail (Boles 2007). The population on Rennell Island of the Solomon Islands, *P. feminina*, is an extreme in plumage variation, its males being female-plumaged (i.e., sexually monochromatic). In addition to plumage differences, bill morphology and overall size also vary between subspecies. For instance, a greater than two-fold difference in mass occurs across all subspecies (e.g., P. p. kandavensis is 25 g and P. p. orioloides is 58 g; Boles 2007).

These patterns of diversity have led to an array of alternative taxonomic treatments (summarized in Table 2.1). Mayr focused on Pacific lineages and treated most of the complex as one polytypic species (Mayr 1932a, b, 1945; Mayr and Diamond 2001) apart from a few exceptions that he recognized as aberrant species-level taxa (*P. feminina* and *P. sanfordi*; Mayr 1931b, c). Galbraith (1956) proposed splitting the entire complex into eight ‘subspecies groups’

spanning Indonesia to Polynesia. Galbraith's groups were largely consistent with discrete geographic entities such as archipelagos. He retained one widespread group, however, suggesting a degree of difficulty in circumscribing species limits that link plumage patterns to geography in the complex.

Later, Galbraith (1967) and Diamond (1976) recognized that closely related taxa in this group in Australia and the Bismarck Archipelago maintain reproductive isolation by habitat choice despite instances of parapatry. Thus, *P. melanura* Gould, 1843, and its associated subspecies have since been recognized as a distinct species having affinities for mangrove habitats in Australia and small islets in the Bismarcks. Dickinson (2003) recognized Galbraith (1956) eight groups as species and subsequent authors have adopted this taxonomic framework (Dickinson 2003; Dutson et al. 2011; Gill and Donsker 2012; Clements et al. 2013). Some authors, however, still adhere to the 'Mayrian' view of 60–70 subspecies of *P. pectoralis* and five of *P. melanura* (Boles 2007). Here for consistency, we adopt the taxonomy of (Dickinson 2003), including prevalent use of subspecies names.

Two previous studies addressed the molecular systematics of this group (Smith and Filardi 2007; Jonsson et al. 2008a). Smith and Filardi (2007) sequenced mitochondrial DNA (mtDNA) for 13 individuals from the Solomon Islands and Australia. Jonsson et al. (2008a) added 16 samples from the Bismarcks, Australia, and the Solomon Islands to the former dataset, and this still only amounted to less than 20% of nominal subspecies of *P. pectoralis*. Both studies provided valuable preliminary windows into the phylogenetic relationships within this species complex but their taxon sampling was inevitably limited. In this paper, we reconstruct the most densely sampled to date, multi-locus phylogeny of the *P. pectoralis*/*melanura* species complex

Table 2.1. Summary of four taxonomic treatments of the *Pachycephala pectoralis*/*melanura* species complex. Species names are in bold followed by the subspecies ascribed to each species. Note that Galbraith (1956) split the complex into "groups," but he did not assign names to them.

| Galbraith (1956) | Dickinson (2003) | Gill and Donsker (2012) | Clements et al. (2013)* |
|---|---|---|---|
| ***P. pectoralis*** **(Lesser Sundan group A):** *fulviventris, javana, fulvotincta, everetti, teysmanni* | ***P. fulvotincta*****:** *teysmanni, everetti, javana, fulvotincta, fulviventris* | ***P. fulvotincta*****:** *teysmanni, everetti, javana, fulvotincta, fulviventris* | ***P. caledonica*** **(New Caledonia group):** *caledonica, littayei* |
| ***P. pectoralis*** **(Moluccan group B):** *mentalis, tidorensis, obiensis* | ***P. macrorhyncha*****:** *calliope, sharpie, dammeriana, par, compar, fuscoflava, macrorhyncha, buruensis, clio, pelengensis* | ***P. macrorhyncha*****:** *calliope, sharpie, dammeriana, par, compar, fuscoflava, macrorhyncha, buruensis, clio, pelengensis* | ***P. caledonica*** **(Vanuatu group):** *cucullata, chlorua, intacta, vanikorensis* |
| ***P. pectoralis*** **(Solomons group C):** *bougainvillei, orioloides, cinnamomea, sanfordi, melanonota, melanoptera, centralis, feminina, christophori* | ***P. mentalis*****:** *tidorensis, mentalis, obiensis* | ***P. mentalis*****:** *tidorensis, mentalis, obiensis* | ***P. vitiensis*****:** *utupuae, ornata, kandavensis, lauana, vitiensis* |
| ***P. pectoralis*** **(Fijian group D):** *graeffii, aurantiiventris, torquata, bella* | ***P. pectoralis*****:** *balim, pectoralis, xanthoprocta, contempta, youngi, glaucura, fuliginosa* | ***P. pectoralis*****:** *balim, pectoralis, xanthoprocta, contempta, youngi, glaucura, fuliginosa* | ***P. graeffii*****:** *koroana, torquata, ambigua, optata, graeffii, aurantiiventris, bella* |
| ***P. pectoralis*** **(Northern Australian group E):** *melanura, violetae, spinicauda, dahli, whitneyi, balim* | ***P. citreogaster*****:** *collaris, rosseliana, citreogaster, sexuvaria, goodsoni, tabarensis, ottomeyeri* | ***P. citreogaster*****:** *collaris, rosseliana, citreogaster, sexuvaria, goodsoni, tabarensis, ottomeyeri* | ***P. flavifrons*** |
| ***P. pectoralis*** **(Southern Australian group F):** *fuliginosa, glaucura, pectoralis, queenslandica, contempta, xanthoprocta* | ***P. orioloides*****:** *bougainvillei, orioloides, centralis, melanoptera, melanonota, pavuvu, sanfordi, cinnamomea, christophori, feminina* | ***P. orioloides*****:** *bougainvillei, orioloides, centralis, melanoptera, melanonota, pavuvu, sanfordi, cinnamomea, christophori, feminina* | ***P. jacquinoti*** |
| ***P. pectoralis*** **(Southern Melanesian group G):** *caledonica, littayei, cucullata, chlorura, vanikorensis* | ***P. caledonica*****:** *vanikorensis, intacta, cucullata, chlorura, littayei, caledonica* | ***P. caledonica*****:** *vanikorensis, intacta, cucullata, chlorura, littayei, caledonica* | ***P. implicata*****:** *implicata, richardsi* |
| ***P. pectoralis*** **(Widespread group H):** *calliope, sharpei, dammeriana, fuscoflava, macrorhyncha, buruensis, clio, pelengensis, collaris, citreogaster, ottomeyeri, tabarensis, goodsoni, ornata, utupuae, kandavensis, vitiensis, lauana, melanops* (=*jacquinoti*) | ***P. vitiensis*****:** *utupuae, ornata, kandavensis, lauana, vitiensis, bella, koroana, torquata, aurantiiventris, ambigua, optata, graeffii* | ***P. vitiensis*****:** *utupuae, ornata, kandavensis, lauana, vitiensis* | ***P. citreogaster*****:** *tabarensis, ottomeyeri, goodsoni, citreogaster, sexuvaria, collaris, misimae, rosseliana* |
| ***P. flavifrons*** | ***P. jacquinoti*** | ***P. graeffii*****:** *bella, koroana, torquata, aurantiiventris, ambigua, optata, graeffii* | ***P. orioloides*****:** *whitneyi, bougainvillei, orioloides, cinnamomea, sanfordi, pavuvu, centralis, melanoptera, christophori* |
| | ***P. melanura*****:** *dahli, spinicaudus, melanura, robusta, whitneyi* | ***P. jacquinoti*** | ***P. feminina*** |
| | ***P. flavifrons*** | ***P. melanura*****:** *dahli, spinicaudus, melanura, robusta, whitneyi* | ***P. fulvotincta*****:** *javana, teysmanni, everetti, fulvotincta, fulviventris* |

| Galbraith (1956) | Dickinson (2003) | Gill and Donsker (2012) | Clements et al. (2013)* |
|---|---|---|---|
| | ***P. implicata*:** *richardsi, implicata* | ***P. flavifrons*** | ***P. macrorhyncha*:** *pelengensis, clio, buruensis, macrorhyncha, calliope, compar, par, dammeriana, sharpie, fuscoflava* |
| | | ***P. implicata*:** *richardsi, implicata* | ***P. mentalis*:** *mentalis, tidorensis, obiensis* |
| | | | ***P. pectoralis*:** *balim, pectoralis, youngi, glaucura, contempt, xanthoprocta, fuliginosa* |
| | | | ***P. melanura*:** *dahli, melanura, robusta, spinicaudus* |

* Earlier versions of the sixth edition of Clements et al. (2013) treated most subspecies within *P. pectoralis*.

and focus on the Australasian and Polynesian lineages in order to elucidate the evolutionary history of this classically polytypic species.

## Methods

### *Taxon sampling*

Sampling comprised 175 ingroup individuals from 32 nominal taxa within *Pachycephala pectoralis*/*melanura* and 27 outgroup samples, of which nine were taken from the literature (Smith and Filardi 2007; Jonsson et al. 2008a; Jonsson et al. 2008b; Jonsson et al. 2010) and 16 were newly sequenced (Table 2.2, Fig. 2.1). Broad outgroup sampling was included to ensure correct phylogenetic placement of taxa for which there was no *a priori* molecular phylogenetic hypothesis (e.g., *P. implicata* and *P. leucogastra*). The clade comprising *Pachycephala inornata*, *P. olivacea*, and *P. nudigula* was used to root trees because Jonsson et al. (2010) found it sister to the rest of the *Pachycephala* lineage. Whenever possible we sequenced multiple individuals per population (i.e., per island) to guard against errors of misidentification, mislabeling, or sample contamination.

Figure 2.1. Sampling sites for ingroup *Pachycephala* used in this study. Color-coded circles, stars, and squares represent sampling points. The symbols and colors simply reflect clades on the tree; like symbols and colors do not reflect phylogenetic relationships between clades. Sampling points are not scaled to the number of individuals (the reader is referred to Table 2.2 for sampling numbers). The Bayesian phylogeny from Fig. 2.2 is reproduced here with node support denoted by black (PP=1.0, 70≤BS≤100) and gray circles (0.95≤PP≤0.99, 50≤BS≤69). Three inset panels offer greater geographic resolution of sampling localities in (A) the Bismarck Archipelago and southeast Papua New Guinea, (B) the Solomon Islands, and (C) Fiji.

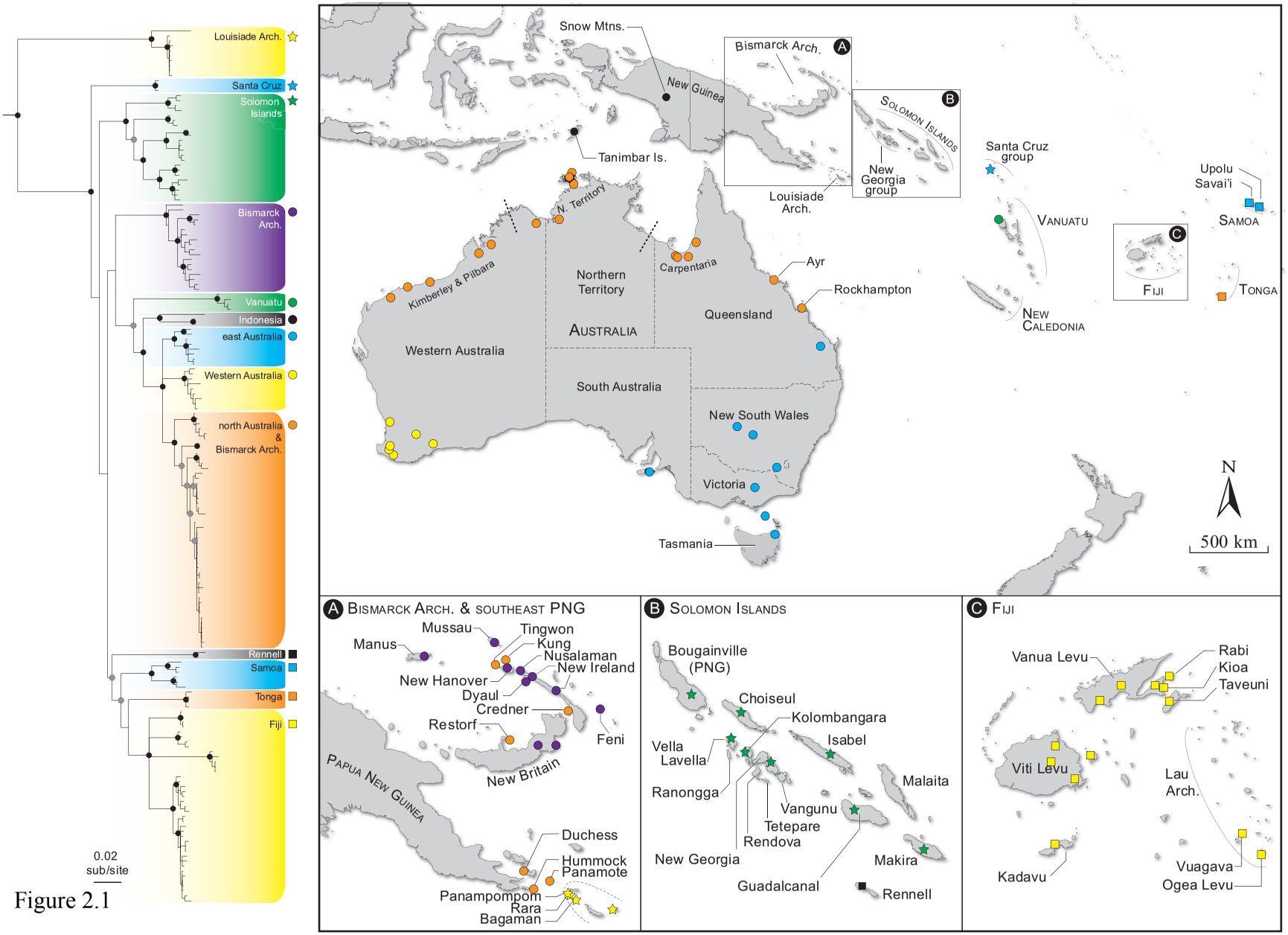

Louisiade Arch.
Santa Cruz
Solomon Islands
Bismarck Arch.
Vanuatu
Indonesia
east Australia
Western Australia
north Australia & Bismarck Arch.
Rennell
Samoa
Tonga
Fiji
0.02 sub/site
Snow Mtns.
New Guinea
Bismarck Arch.
A
B
Solomon Islands
New Georgia group
Louisiade Arch.
Tanimbar Is.
N. Territory
Kimberley & Pilbara
Northern Territory
Carpentaria
Ayr
Rockhampton
Queensland
AUSTRALIA
Western Australia
South Australia
New South Wales
Victoria
Tasmania
Santa Cruz group
VANUATU
NEW CALEDONIA
C
FIJI
Upolu
Savai'i
SAMOA
TONGA
N
500 km
A BISMARCK ARCH. & SOUTHEAST PNG
Mussau
Tingwon
Manus
Kung
Nusalaman
New Ireland
New Hanover
Dyaul
Credner
Restorf
Feni
New Britain
PAPUA NEW GUINEA
Duchess
Hummock
Panamote
Panampompom
Rara
Bagaman
B SOLOMON ISLANDS
Bougainville (PNG)
Choiseul
Kolombangara
Isabel
Vella Lavella
Ranongga
Malaita
Vangunu
Tetepare
Rendova
New Georgia
Makira
Guadalcanal
Rennell
C FIJI
Vanua Levu
Rabi
Kioa
Taveuni
Viti Levu
Lau Arch.
Kadavu
Vuagava
Ogea Levu

Figure 2.1

*DNA sequencing*

Total genomic DNA was extracted from frozen or alcohol-preserved muscle tissue using a noncommercial guanidine thiocyanate method (Esselstyn et al. 2008). All muscle tissue samples have associated museum study-skin vouchers. For taxa with no available tissue samples, DNA was extracted from toepads of museum study skins (Table 2.2) with dedicated equipment in lab space separate from other *Pachycephala* pre-PCR products to minimize contamination risk (Mundy et al. 1997). Thirteen unvouchered blood samples were used from remote islands in Milne Bay Province, Papua New Guinea, but most of these individuals were supplemental to vouchered tissue samples from the same islands (Table 2.2).

We sequenced the entire second and third subunits of mitochondrial nicotinamide adenine dinucleotide dehydrogenase (hereafter ND2 and ND3, respectively). Eight nuclear gene regions were sequenced: the coiled-coil domain containing protein 132 (CCDC132), the fifth intron of the Beta-fibrinogen gene (Fib5), the 11$^{th}$ intron of the nuclear glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH), the high mobility group protein B2 (HMGB2), the third intron of the Z-linked muscle-specific kinase gene (MUSK), the second intron of the nuclear myoglobin gene (Myo2), introns 6–7 and exon 7 of the ornithine decarboxylase gene (ODC), and the fifth intron of the transforming growth factor β2 (TGF). Target DNA fragments were amplified using polymerase chain reaction (PCR) with external and internal primers. External primers are described as follows: L5215 (ND2; Hackett 1996) and H6313 (ND2 Johnson and Sorenson 1998), L10755 and H11151 (ND3 Chesser 1999), CDC132L and CDC132H (Backström et al. 2008), Fib5 and Fib6 (Marini and Hackett 2002), G3P13b and G3P14b (GAPDH; Fjeldså et al. 2003), HMG2L and HMG2H (Backström et al. 2008), MUSK-

I3F and MUSK-I3R (Kimball et al. 2009), Myo2 (Slade et al. 1993) and Myo3F (Heslewood et al. 1998), OD6 and OD8R (Friesen et al. 1999; Primmer et al. 2002) and TGF5 and TGF6 (Primmer et al. 2002). Additionally, we used internal primers to amplify 200–250 bp fragments of toepad samples (Table 2.3). PCR amplifications were performed in 13 μl reactions using Promega Go*Taq* DNA polymerase. A touchdown protocol was used in PCR for ND2, ND3, CCDC132, GAPDH, HMGB2, and ODC with annealing temperatures of 58, 54, and 50 °C. Annealing temperatures were held constant for Fib5 (54 °C), MUSK (50 °C), Myo2 (52 °C), and TGF (58 °C) following recommendations by Kimball et al. (2009). Amplified PCR products were screened on high-melt, 2% agarose gels stained with GelRed, and purified with 10% Exo-SAP-IT™ (GE Healthcare Bio-Sciences Corp.). We cycle-sequenced purified PCR products in both directions with the same primers used in PCR for 25 cycles using the ABI Big Dye Terminator Cycle-Sequencing Kit version 3.1 (Applied Biosystems Inc., Foster City, CA). Sequencing was performed on an ABI Prism 3730 high-throughput capillary electrophoresis DNA analyzer.

### *Model selection and phylogenetic analysis*

Sequence contigs were assembled in Geneious 5.6 and individual nuclear intron alignments were constructed by hand and checked against an automated alignment in MUSCLE (Edgar 2004). Appropriate models of sequence evolution for each of the 10 partitions were identified (Table 2.4) using Akaike's Information Criterion (AIC), as implemented in MrModelTest 2.3 (Nylander 2004).

Phylogenetic reconstruction was performed on the total concatenated data, on separate concatenated mtDNA and nDNA, and separately on each individual locus. Maximum

Table 2.2. List of samples used in the study following taxonomy of Clements et al. (2013). Ancient DNA samples derived from museum specimens (i.e., toepads) and unvouchered blood samples are noted. Institutional abbreviations: AMNH, American Museum of Natural History; ANWC, Australian National Wildlife Collection; CAS, California Academy of Sciences; DMNH, Delaware Museum of Natural History; FMNH, Field Museum of Natural History; KUNHM, University of Kansas Natural History Museum; LSUMNS, Louisiana State University Museum of Natural Science; MV, Museum Victoria; SNZP, Smithsonian National Zoological Park; USNM, United States National Museum; UWBM, University of Washington Burke Museum; WAM, Western Australia Museum; ZMUC, Zoological Museum University of Copenhagen. Papua New Guinea is abbreviated as "PNG." Samples included in the *BEAST species-tree analysis and their respective species assignments are denoted in column, "#": (1) *P. citreogaster*, (2) *P. feminina*, (3) *P. orioloides*, (4) *P. intacta*, (5) *P. ornata*, (6) *P. vitiensis*, (7) *P. fuliginosa*, (8) *P. pectoralis*, (9) *P. melanura*, (10) *P. macrorhyncha*, (11) *P. collaris*.

| Genus | Species | Subspecies | # | Institution | Sample | Locality |
|---|---|---|---|---|---|---|
| Ingroup | | | | | | |
| *Pachycephala* | *caledonica* | *intacta* | 4 | LSUMNS | B45385 | VANUATU: Espiritu Santo |
| *Pachycephala* | *caledonica* | *intacta* | 4 | LSUMNS | B45398 | VANUATU: Espiritu Santo |
| *Pachycephala* | *caledonica* | *intacta* | 4 | LSUMNS | B45759 | VANUATU: Espiritu Santo |
| *Pachycephala* | *caledonica* | *intacta* | 4 | LSUMNS | B45791 | VANUATU: Espiritu Santo |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | KUNHM | 5306 | PNG: Bismarck Arch.; New Britain Is. |
| *Pachycephala* | *citreogaster* | *goodsoni*† | 1 | KUNHM | 5615 | PNG: Admiralty Islands; Manus Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | KUNHM | 27694 | PNG: Bismarck Arch.; New Ireland Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | KUNHM | 27721 | PNG: Bismarck Arch.; New Ireland Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | KUNHM | 27730 | PNG: Bismarck Arch.; New Ireland Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | KUNHM | 27742 | PNG: Bismarck Arch.; New Ireland Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | KUNHM | 27853 | PNG: Bismarck Arch.; Dyaul Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | KUNHM | 27859 | PNG: Bismarck Arch.; Dyaul Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | ANWC | 52360 | PNG: Bismarck Arch.; New Britain Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | ANWC | 52361 | PNG: Bismarck Arch.; New Britain Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | ANWC | 52364 | PNG: Bismarck Arch.; New Britain Is. |
| *Pachycephala* | *citreogaster* | *citreogaster* | 1 | ANWC | 52373 | PNG: Bismarck Arch.; New Britain Is. |
| *Pachycephala* | *citreogaster* | *citreogaster*†[GB] | 1 | ZMUC | 95287 | PNG: Bismarck Arch.; Dyaul Is. |
| *Pachycephala* | *citreogaster* | *citreogaster*†[GB] | 1 | ZMUC | 95288 | PNG: Bismarck Arch.; Feni Is. |
| *Pachycephala* | *citreogaster* | *citreogaster*†[GB] | 1 | ZMUC | 95289 | PNG: Bismarck Arch.; New Ireland Is. |
| *Pachycephala* | *citreogaster* | *citreogaster*†[GB] | 1 | ZMUC | 95290 | PNG: Bismarck Arch.; New Britain Is. |
| *Pachycephala* | *citreogaster* | *citreogaster*†[GB] | 1 | ZMUC | 95291 | PNG: Bismarck Arch.; New Hanover Is. |
| *Pachycephala* | *citreogaster* | *sexuvaria*† [GB] | 1 | ZMUC | 95286 | PNG: Bismarck Arch.; Mussau Is. |
| *Pachycephala* | *citreogaster* | *collaris* | 11 | CAS | 96792 | PNG: Louisiade Arch.; Rara Is. |
| *Pachycephala* | *citreogaster* | *collaris* | 11 | CAS | 96796 | PNG: Louisiade Arch.; Panapompom Is. |
| *Pachycephala* | *citreogaster* | *collaris* | 11 | CAS | 96831 | PNG: Louisiade Arch.; Panapompom Is. |
| *Pachycephala* | *citreogaster* | *collaris* | 11 | CAS | 96832 | PNG: Louisiade Arch.; Panapompom Is. |
| *Pachycephala* | *citreogaster* | *collaris** | 11 | CAS | 96841 | PNG: Louisiade Arch.; Bagaman Is. |
| *Pachycephala* | *citreogaster* | *collaris** | 11 | CAS | 96842 | PNG: Louisiade Arch.; Rara Is. |
| *Pachycephala* | *citreogaster* | *collaris** | 11 | CAS | 96852 | PNG: Bonvouloir Islands; Panamote Is. |
| *Pachycephala* | *citreogaster* | *collaris** | 11 | CAS | 96853 | PNG: Bonvouloir Islands; Panamote Is. |
| *Pachycephala* | *citreogaster* | *collaris** | 11 | CAS | 96854 | PNG: Bonvouloir Islands; Panamote Is. |
| *Pachycephala* | *citreogaster* | *rosseliana* | 11 | SNZP | TKP2004057 | PNG: Louisiade Arch.; Rossel Island |
| *Pachycephala* | *feminina* | | 2 | AMNH | DOT6601 | SOLOMON ISLANDS: Rennell Is. |
| *Pachycephala* | *feminina*† [GB] | | 2 | ZMUC | 95292 | SOLOMON ISLANDS: Rennell Is. |
| *Pachycephala* | *flavifrons*† | | 6 | KUNHM | 104114 | SAMOA: Upolu Is. |
| *Pachycephala* | *flavifrons*† | | 6 | KUNHM | 104115 | SAMOA: Upolu Is. |
| *Pachycephala* | *flavifrons*† | | 6 | KUNHM | 104123 | SAMOA: Upolu Is. |
| *Pachycephala* | *flavifrons*† | | 6 | KUNHM | 104126 | SAMOA: Savai'i Is. |
| *Pachycephala* | *flavifrons*† | | 6 | KUNHM | 104129 | SAMOA: Savai'i Is. |
| *Pachycephala* | *flavifrons*† | | 6 | KUNHM | 107654 | SAMOA: Savai'i Is. |
| *Pachycephala* | *graeffii* | *graeffii* | 6 | KUNHM | 22502 | FIJI: Central Division; Viti Levu Is. |
| *Pachycephala* | *graeffii* | *graeffii* | 6 | KUNHM | 22537 | FIJI: Central Division; Viti Levu Is. |

| Genus | Species | Subspecies | # | Institution | Sample | Locality |
|---|---|---|---|---|---|---|
| *Pachycephala* | *graeffii* | *graeffii* | 6 | KUNHM | 22555 | FIJI: Central Division; Viti Levu Is. |
| *Pachycephala* | *graeffii* | *graeffii* | 6 | KUNHM | 22567 | FIJI: Western Division; Viti Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 24229 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 24245 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 24257 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 24265 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 24277 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 24281 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 24288 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *torquata* | 6 | KUNHM | 24297 | FIJI: Northern Division; Taveuni Is. |
| *Pachycephala* | *graeffii* | *torquata* | 6 | KUNHM | 24299 | FIJI: Northern Division; Taveuni Is. |
| *Pachycephala* | *graeffii* | *torquata* | 6 | KUNHM | 24323 | FIJI: Northern Division; Taveuni Is. |
| *Pachycephala* | *graeffii* | *torquata* | 6 | KUNHM | 24349 | FIJI: Northern Division; Taveuni Is. |
| *Pachycephala* | *graeffii* | *graeffii* | 6 | KUNHM | 24366 | FIJI: Western Division; Viti Levu Is. |
| *Pachycephala* | *graeffii* | *ambigua* | 6 | KUNHM | 26449 | FIJI: Northern Division; Rabi Is. |
| *Pachycephala* | *graeffii* | *ambigua* | 6 | KUNHM | 26458 | FIJI: Northern Division; Rabi Is. |
| *Pachycephala* | *graeffii* | *ambigua* | 6 | KUNHM | 26462 | FIJI: Northern Division; Rabi Is. |
| *Pachycephala* | *graeffii* | *ambigua* | 6 | KUNHM | 26469 | FIJI: Northern Division; Rabi Is. |
| *Pachycephala* | *graeffii* | *ambigua* | 6 | KUNHM | 26479 | FIJI: Northern Division; Kioa Is. |
| *Pachycephala* | *graeffii* | *ambigua* | 6 | KUNHM | 26487 | FIJI: Northern Division; Kioa Is. |
| *Pachycephala* | *graeffii* | *ambigua* | 6 | KUNHM | 26493 | FIJI: Northern Division; Kioa Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 26510 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 26513 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 26520 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *aurantiiventris* | 6 | KUNHM | 26523 | FIJI: Northern Division; Vanua Levu Is. |
| *Pachycephala* | *graeffii* | *optata* | 6 | KUNHM | 30491 | FIJI: Eastern Division, Ovalau Is. |
| *Pachycephala* | *graeffii* | *optata* | 6 | KUNHM | 30505 | FIJI: Eastern Division, Ovalau Is. |
| *Pachycephala* | *graeffii* | *optata* | 6 | KUNHM | 30506 | FIJI: Eastern Division, Ovalau Is. |
| *Pachycephala* | *jacquinoti*† | | 6 | DMNH | 11331 | TONGA: Vava'u Is. |
| *Pachycephala* | *jacquinoti*† | | 6 | DMNH | 11332 | TONGA: Vava'u Is. |
| *Pachycephala* | *jacquinoti*† | | 6 | AMNH | 250556 | TONGA: 'Euakafa Is. |
| *Pachycephala* | *jacquinoti*† | | 6 | AMNH | 250567 | TONGA: 'Euakafa Is. |
| *Pachycephala* | *macrorhyncha* | *fuscoflava* | 10 | WAM | 25185 | INDONESIA: Tanimbar Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | KUNHM | 27666 | PNG: Bismarck Arch.; Restorf Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | KUNHM | 27795 | PNG: Bismarck Arch.; Nusalaman Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | KUNHM | 27797 | PNG: Bismarck Arch.; Nusalaman Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | KUNHM | 27798 | PNG: Bismarck Arch.; Nusalaman Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | KUNHM | 27799 | PNG: Bismarck Arch.; Nusalaman Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | KUNHM | 27800 | PNG: Bismarck Arch.; Nusalaman Is. |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 29385 | AUSTRALIA: Queensland |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 29432 | AUSTRALIA: Queensland |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 29433 | AUSTRALIA: Queensland |
| *Pachycephala* | *melanura* | *melanura* | 9 | ANWC | 33097 | AUSTRALIA: Western Australia |
| *Pachycephala* | *melanura* | *melanura* | 9 | ANWC | 33207 | AUSTRALIA: Western Australia |
| *Pachycephala* | *melanura* | *melanura* | 9 | ANWC | 33262 | AUSTRALIA: Western Australia |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 33754 | AUSTRALIA: Northern Territory |
| *Pachycephala* | *melanura* | *melanura* | 9 | ANWC | 34428 | AUSTRALIA: Western Australia |
| *Pachycephala* | *melanura* | *melanura* | 9 | ANWC | 34474 | AUSTRALIA: Western Australia |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 43800 | AUSTRALIA: Queensland |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 48664 | AUSTRALIA: Northern Territory |
| *Pachycephala* | *melanura* | *melanura* | 9 | ANWC | 50720 | AUSTRALIA: Western Australia |
| *Pachycephala* | *melanura* | *melanura* | 9 | ANWC | 50901 | AUSTRALIA: Western Australia |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 51358 | AUSTRALIA: Queensland |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 51359 | AUSTRALIA: Queensland |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 52425 | AUSTRALIA: Northern Territory |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 54440 | AUSTRALIA: Northern Territory |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 54441 | AUSTRALIA: Northern Territory |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 54449 | AUSTRALIA: Northern Territory |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 54450 | AUSTRALIA: Northern Territory |
| *Pachycephala* | *melanura* | *robusta* | 9 | ANWC | 54522 | AUSTRALIA: Northern Territory |
| *Pachycephala* | *melanura* | *dahli* | 9 | UWBM | Bu67949 | PNG: Bismarck Arch.; Restorf Is. |

| Genus | Species | Subspecies | # | Institution | Sample | Locality |
|---|---|---|---|---|---|---|
| *Pachycephala* | *melanura* | *dahli* | 9 | UWBM | Bu68054 | PNG: Bismarck Arch.; Restorf Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | CAS | 96787 | PNG: Engineer Group; Hummock Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | CAS | 96793 | PNG: Engineer Group; Hummock Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | CAS | 96794 | PNG: Engineer Group; Hummock Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | CAS | 96795 | PNG: Engineer Group; Hummock Is. |
| *Pachycephala* | *melanura* | *dahli** | 9 | CAS | 96838 | PNG: Engineer Group; Hummock Is. |
| *Pachycephala* | *melanura* | *dahli** | 9 | CAS | 96839 | PNG: Engineer Group; Hummock Is. |
| *Pachycephala* | *melanura* | *dahli** | 9 | CAS | 96840 | PNG: Engineer Group; Hummock Is. |
| *Pachycephala* | *melanura* | *dahli** | 9 | CAS | 96844 | PNG: D'Entrecasteaux Arch.; Duchess Is. |
| *Pachycephala* | *melanura* | *dahli** | 9 | CAS | 96845 | PNG: D'Entrecasteaux Arch.; Duchess Is. |
| *Pachycephala* | *melanura* | *dahli** | 9 | CAS | 96846 | PNG: D'Entrecasteaux Arch.; Duchess Is. |
| *Pachycephala* | *melanura* | *dahli** | 9 | CAS | 96850 | PNG: D'Entrecasteaux Arch.; Duchess Is. |
| *Pachycephala* | *melanura* | *dahli** | 9 | CAS | 96851 | PNG: D'Entrecasteaux Arch.; Duchess Is. |
| *Pachycephala* | *melanura* | *robusta* [GB] | 9 | MV | 1248 | AUSTRALIA: Northern Territory |
| *Pachycephala* | *melanura* | *dahli* | 9 | SNZP | TKP2003069 | PNG: D'Entrecasteaux Arch.; Duchess Is. |
| *Pachycephala* | *melanura* | *dahli* | 9 | SNZP | TKP2003070 | PNG: D'Entrecasteaux Arch.; Duchess Is. |
| *Pachycephala* | *melanura* | *dahli*† [GB] | 9 | ZMUC | 95283 | PNG: Bismarck Arch.; Kung Is. |
| *Pachycephala* | *melanura* | *dahli*† [GB] | 9 | ZMUC | 95284 | PNG: Bismarck Arch.; Tingwon Is. |
| *Pachycephala* | *melanura* | *dahli*† [GB] | 9 | ZMUC | 95285 | PNG: Bismarck Arch.; Credner Is. |
| *Pachycephala* | *orioloides* | *bougainvillei* | 3 | KUNHM | 5283 | PNG: Bougainville Is. |
| *Pachycephala* | *orioloides* | *christophori* | 3 | KUNHM | 13527 | SOLOMON ISLANDS: Makira Is. |
| *Pachycephala* | *orioloides* | *christophori* | 3 | KUNHM | 13536 | SOLOMON ISLANDS: Makira Is. |
| *Pachycephala* | *orioloides* | *cinnamomea* | 3 | KUNHM | 15879 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Pachycephala* | *orioloides* | *cinnamomea* | 3 | KUNHM | 15900 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Pachycephala* | *orioloides* | *orioloides* | 3 | UWBM | Bu60214 | SOLOMON ISLANDS: Isabel Is. |
| *Pachycephala* | *orioloides* | *orioloides* | 3 | UWBM | Bu60289 | SOLOMON ISLANDS: Isabel Is. |
| *Pachycephala* | *orioloides* | *orioloides* | 3 | UWBM | Bu60314 | SOLOMON ISLANDS: Isabel Is. |
| *Pachycephala* | *orioloides* | *cinnamomea* | 3 | UWBM | Bu60347 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Pachycephala* | *orioloides* | *centralis* | 3 | UWBM | Bu63131 | SOLOMON ISLANDS: New Georgia Is. |
| *Pachycephala* | *orioloides* | *orioloides* | 3 | UWBM | Bu63227 | SOLOMON ISLANDS: Choiseul Is. |
| *Pachycephala* | *orioloides* | *orioloides* | 3 | UWBM | Bu63262 | SOLOMON ISLANDS: Choiseul Is. |
| *Pachycephala* | *orioloides* | *centralis* | 3 | UWBM | Bu66074 | SOLOMON ISLANDS: New Georgia Is. |
| *Pachycephala* | *orioloides* | *centralis* | 3 | UWBM | Bu66075 | SOLOMON ISLANDS: New Georgia Is. |
| *Pachycephala* | *orioloides* | *melanonota* | 3 | AMNH | DOT153 | SOLOMON ISLANDS: Vella Lavella Is. |
| *Pachycephala* | *orioloides* | *melanonota* | 3 | AMNH | DOT155 | SOLOMON ISLANDS: Vella Lavella Is. |
| *Pachycephala* | *orioloides* | *centralis* | 3 | AMNH | DOT190 | SOLOMON ISLANDS: Kolombangara Is. |
| *Pachycephala* | *orioloides* | *centralis* | 3 | AMNH | DOT257 | SOLOMON ISLANDS: Kolombangara Is. |
| *Pachycephala* | *orioloides* | *bougainvillei* | 3 | AMNH | DOT14982 | PNG: Bougainville Is. |
| *Pachycephala* | *orioloides* | *bougainvillei* | 3 | AMNH | DOT14984 | PNG: Bougainville Is. |
| *Pachycephala* | *orioloides* | *christophori* [GB] | 3 | ZMUC | 139460 | SOLOMON ISLANDS: Makira Is. |
| *Pachycephala* | *orioloides* | *christophori* [GB] | 3 | ZMUC | 139478 | SOLOMON ISLANDS: Makira Is. |
| *Pachycephala* | *pectoralis* | *fuliginosa* | 7 | KUNHM | 6093 | AUSTRALIA: Western Australia |
| *Pachycephala* | *pectoralis* | *fuliginosa* | 7 | KUNHM | 6118 | AUSTRALIA: Western Australia |
| *Pachycephala* | *pectoralis* | *fuliginosa* | 7 | KUNHM | 6132 | AUSTRALIA: Western Australia |
| *Pachycephala* | *pectoralis* | *fuliginosa* | 7 | KUNHM | 6175 | AUSTRALIA: Western Australia |
| *Pachycephala* | *pectoralis* | *youngi* | 8 | ANWC | 29282 | AUSTRALIA: New South Wales |
| *Pachycephala* | *pectoralis* | *youngi* | 8 | ANWC | 31665 | AUSTRALIA: New South Wales |
| *Pachycephala* | *pectoralis* | *fuliginosa* | 7 | ANWC | 31704 | AUSTRALIA: Western Australia |
| *Pachycephala* | *pectoralis* | *fuliginosa* | 7 | ANWC | 31781 | AUSTRALIA: Western Australia |
| *Pachycephala* | *pectoralis* | *youngi* | 8 | ANWC | 42504 | AUSTRALIA: South Australia |
| *Pachycephala* | *pectoralis* | *pectoralis* | 8 | ANWC | 43411 | AUSTRALIA: Queensland |
| *Pachycephala* | *pectoralis* | *glaucura* | 8 | ANWC | 45375 | AUSTRALIA: Tasmania |
| *Pachycephala* | *pectoralis* | *glaucura* | 8 | ANWC | 45665 | AUSTRALIA: Tasmania; Deal Is. |
| *Pachycephala* | *pectoralis* | *fuliginosa* | 7 | ANWC | 50360 | AUSTRALIA: Western Australia |
| *Pachycephala* | *pectoralis* | *balim*† | 10 | AMNH | 341498 | INDONESIA: Papua; Bele River |
| *Pachycephala* | *pectoralis* | *balim*† | 10 | AMNH | 341500 | INDONESIA: Papua; Bele River |
| *Pachycephala* | *pectoralis* | *youngi* | 8 | UWBM | Bu57458 | AUSTRALIA: New South Wales |
| *Pachycephala* | *pectoralis* | *fuliginosa* | 7 | UWBM | Bu60858 | AUSTRALIA: Western Australia |
| *Pachycephala* | *pectoralis* | *fuliginosa* [GB] | 7 | MV | 2658 | AUSTRALIA: Western Australia |
| *Pachycephala* | *pectoralis* | *youngi* [GB] | 8 | MV | 3477 | AUSTRALIA: Victoria |

| Genus | Species | Subspecies | # | Institution | Sample | Locality |
|---|---|---|---|---|---|---|
| *Pachycephala* | *vitiensis* | *ornata* | 5 | KUNHM | 19400 | SOLOMON ISLANDS: Santa Cruz Group; Nendo Is. |
| *Pachycephala* | *vitiensis* | *ornata* | 5 | KUNHM | 19410 | SOLOMON ISLANDS: Santa Cruz Group; Nendo Is. |
| *Pachycephala* | *vitiensis* | *ornata* | 5 | KUNHM | 19418 | SOLOMON ISLANDS: Santa Cruz Group; Nendo Is. |
| *Pachycephala* | *vitiensis* | *kandavensis* | 6 | KUNHM | 24405 | FIJI: Eastern Division; Kadavu Is. |
| *Pachycephala* | *vitiensis* | *kandavensis* | 6 | KUNHM | 24411 | FIJI: Eastern Division; Kadavu Is. |
| *Pachycephala* | *vitiensis* | *kandavensis* | 6 | KUNHM | 24412 | FIJI: Eastern Division; Kadavu Is. |
| *Pachycephala* | *vitiensis* | *kandavensis* | 6 | KUNHM | 25220 | FIJI: Eastern Division; Kadavu Is. |
| *Pachycephala* | *vitiensis* | *lauana* | 6 | KUNHM | 26324 | FIJI: Eastern Division; Lau Arch., Ogea Levu Is. |
| *Pachycephala* | *vitiensis* | *lauana* | 6 | KUNHM | 26326 | FIJI: Eastern Division; Lau Arch., Ogea Levu Is. |
| *Pachycephala* | *vitiensis* | *lauana* | 6 | KUNHM | 26330 | FIJI: Eastern Division; Lau Arch., Ogea Levu Is. |
| *Pachycephala* | *vitiensis* | *lauana* | 6 | KUNHM | 26337 | FIJI: Eastern Division; Lau Arch., Ogea Levu Is. |
| *Pachycephala* | *vitiensis* | *lauana* | 6 | KUNHM | 26412 | FIJI: Eastern Division; Lau Arch., Vuagava Is. |
| Outgroup | | | | | | |
| *Pachycephala* | *caledonica* | *caledonica*† GB | | FMNH | 268487 | NEW CALEDONIA |
| *Pachycephala* | *cinerea* | | | KUNHM | 12751 | PHILIPPINES: Palawan Is. |
| *Pachycephala* | *cinerea* GB | | | ZMUC | 118870 | PHILIPPINES |
| *Pachycephala* | *homeyeri* | | | KUNHM | 15340 | PHILIPPINES: Panay Is. |
| *Pachycephala* | *hyperythra* | | | KUNHM | 7889 | PNG: West Sepik Prov. |
| *Pachycephala* | *hyperythra*† GB | | | FMNH | 280631 | INDONESIA: Papua |
| *Pachycephala* | *implicata* | *implicata*† | | DMNH | 11918 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Pachycephala* | *implicata* | *implicata*† | | DMNH | 11921 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Pachycephala* | *implicata* | *richardsi*† | | AMNH | 222855 | PNG: Bougainville Is. |
| *Pachycephala* | *implicata* | *richardsi*† | | AMNH | 226336 | PNG: Bougainville Is. |
| *Pachycephala* | *inornata* GB | | | ANWC | 38742 | AUSTRALIA: New South Wales |
| *Pachycephala* | *lanioides* | | | KUNHM | 6195 | AUSTRALIA: Western Australia |
| *Pachycephala* | *leucogastra* | *meeki* | | SNZP | TKP2004065 | PNG: Milne Bay Prov.: Louisiade Arch.: Rossel Island |
| *Pachycephala* | *leucogastra* | *meeki* | | SNZP | TKP2004067 | PNG: Milne Bay Prov.: Louisiade Arch.: Rossel Island |
| *Pachycephala* | *lorentzi* GB | | | FMNH | 280615 | INDONESIA: Papua; Snow Mountains |
| *Pachycephala* | *modesta* | | | KUNHM | 4736 | PNG: Morobe Prov. |
| *Pachycephala* | *nudigula* | | | WAM | 22678 | INDONESIA: Flores Is. |
| *Pachycephala* | *olivacea* GB | | | MV | 1826 | AUSTRALIA |
| *Pachycephala* | *philippinensis* | | | KUNHM | 17983 | PHILIPPINES: Luzon Is. |
| *Pachycephala* | *rufiventris* | | | KUNHM | 6174 | AUSTRALIA: Western Australia |
| *Pachycephala* | *rufiventris* | | | UWBM | Bu57510 | AUSTRALIA: Queensland |
| *Pachycephala* | *schlegelii* | | | KUNHM | 5079 | PNG: Chimbu Prov. |
| *Pachycephala* | *schlegelii* GB | | | ANWC | 24574 | PNG: Oro Prov. |
| *Pachycephala* | *simplex* | | | KUNHM | 7250 | PNG: Madang Prov. |
| *Pachycephala* | *simplex* GB | | | MV | 1183 | AUSTRALIA |
| *Pachycephala* | *soror* | | | KUNHM | 7888 | PNG: West Sepik Prov. |
| *Pachycephala* | *soror* GB | | | ANWC | 26736 | PNG: Oro Prov. |

*, denotes samples for which DNA was extracted from blood.

†, denotes samples for which DNA was extracted from toepads.

GB, denotes samples for which sequence data were downloaded from GenBank.

Table 2.3. Newly-designed primers to sequence samples derived from museum specimen toepads.

| Locus | Primer name | 5' to 3' sequence |
|---|---|---|
| CCDC132 | CDC132.PachyH | CTGCCACAAAATTCTTCTC |
| | CDC132.PachyL | GTCTAACTTCAAATACGACG |
| | CDC132.Pachy173L | GCATTTTGATGCCAGTTTC |
| | CDC132.Pachy230H | CTACCTCTCCCAAATACATC |
| | CDC132.Pachy395L | GAGCAGAAAAATACTGTGG |
| | CDC132.Pachy450H | CTGTCAGTTCACAGTCTC |
| | CDC132.Pachy534L | GGCTCTTTKTCTCTCTGTG |
| | CDC132.Pachy605H | CAGAGCACCAATGTTACATTG |
| Fib5 | Fib5.Pachy.ext | GCCATACAGAGTATACTGTGACAT |
| | Fib5.Pachy258 | GCTGATGCAGAATAGGACACTTC |
| | Fib6.Pachy383 | AGAACTTGAAGGACGGCCTG |
| | Fib6.Pachy.ext | ATTCTGAATCAAAGTCCAGCC |
| GAPDH | G3P13.Pachy160L | GATCCAGGTGGATACACAG |
| | G3P14.Pachy218H | GGAGGCAGCTACAATAATTTC |
| HMG2 | HMG2.Pachy155L | GTGTCTTACACCCAAACCG |
| | HMG2.Pachy239H | GAATCCTCACAGGGAACCTG |
| | HMG2.Pachy362L | CAGTCAGACTCCAAAGCAC |
| | HMG2.Pachy387H | GGCAAAAGAACATAYAGTGCAGAC |
| Myo2 | Myo2.Pachy166 | GCTCTCCCTCAAGTTCAAGG |
| | Myo2.Pachy370 | GACTGGACACAAGGGACATAC |
| | Myo2.Pachy537 | GATCAGCGTCAGAGCTAGG |
| | Myo3.Pachy240 | CTGTGGTGTTTGGAATGGGAAATC |
| | Myo3.Pachy427 | CATGCCCTGTGTTTGTATAAC |
| | Myo3.Pachy583 | CTGGAGAGACAGTGAGGTC |
| ND2 | Pachy170L | ACGAGCYATTGAAGCTGCAAC |
| | Pachy183H | GYTGAAGCAGTGGCTTGTAC |
| | Pachy247H | TTAATTGAGTAATRTCTCATTG |
| | Pachy320L | AGCCATTCAATAAAAYTAGG |
| | Pachy381L | GGCTCTYCNCTRATCACAGG |
| | Pachy399H | AATGTRATTGGTGGGAATTTTAT |
| | Pachy507L | AGCYCTAGGRGGATGAATAGG |
| | Pachy555H | ATAATRGTYATTCATCCTAGGTG |
| | Pachy641L | TATATGYTYTAATAACTACAGC |
| | Pachy697H | TGAAGGTRTTTTTGTTCATGC |
| | Pachy719L | CTGCATGAACAAAAAYACCTTCAC |
| | Pachy766L | TATCTTTAGCCGGCCTGCCC |
| | Pachy794H | CATTATTCAAGAAYTAACTAAACA |
| | Pachy885L | GGRCTRTTCTTYTAYCTYCG |
| | Pachy909H | GATTTGTRGTRTGAGGRGGYAG |
| ND3 | ND3.PachyH.ext | CTAATTAAGACAGTTGATTTCG |
| | ND3.PachyL.ext | GGTTTAAACCCAGAGAAGAG |
| | ND3.Pachy142L | GGYTTCGACCCACTAGGATCAG |
| | ND3.Pachy218H | GGCTCATGGTAGTGGTAGT |
| ODC | OD6.Pachy106 | GACCTTGCCATTGTTGGAG |
| | OD6.Pachy288 | GTAGTTTCCATGTTGGAAGTGG |
| | OD6.Pachy459 | GCTAGCTAAGGCACTGACTTC |
| | OD8R.Pachy172 | GCAAAGGCATCTCTATTGTC |
| | OD8R.Pachy306 | CAGAAATGGCTTGAACAAAGG |
| | OD8R.Pachy498 | GGAGTTTTGCCAAGCTGGTC |

likelihood (ML) heuristic tree searches were performed using GARLI 2.0 (Zwickl 2006). To avoid local optima, 250 independent searches were performed, each starting from a random tree. GARLI's default parameters were adjusted to terminate searches when no topological improvements were found after 100,000 generations (genthreshfortopoterm = 100000); otherwise, default settings were used. We selected the topology with the best likelihood as our maximum-likelihood stimate. Statistical support for this topology was obtained by running 1,000 non-parametric bootstrap replicates (Felsenstein 1985) in GARLI to assess clade credibility and SumTrees 3.3.1, part of the DendroPy 3.12.0 package (Sukumaran and Holder 2010), was used to create a 50% majority-rule consensus tree. Nodes with >70% bootstrap support were considered well-supported (Hillis and Bull 1993; Wilcox et al. 2002).

Bayesian analysis (BA) was conducted using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003; Altekar et al. 2004; Ronquist et al. 2012) implemented with BEAGLE (Ayres et al. 2012). The data were partitioned by codon position for mtDNA and by gene for the nuclear introns. Four independent MCMC runs of 50 million generations were conducted using four chains per run (nchains=4) and incremental heating of chains (temp=0.1), sampling every 5,000 generations. A species tree analysis was conducted in *BEAST 1.7.5 (Heled and Drummond 2010) on the full ingroup dataset. First, sequences were phased in DnaSP (Librado and Rozas 2009) with output threshold of 0.7 using algorithms provided by PHASE (Stephens et al. 2001; Stephens and Donnelly 2003). Branch tips were defined by assigning species based on well- supported clades from the concatenated MrBayes analysis (see Table 2.2 for assignments).

All samples from Fiji, Samoa, and Tonga were treated as one species due to deficient data at some loci for Samoan and Tongan samples. We ran 10 independent MCMC runs of 250 million generations sampled every 12,500 generations. The first 40% of trees were discarded as

Table 2.4. Summary statistics for the ten loci used in this study.

| Locus | Aligned length | Category, chromosome # | Substitution model | A, C, G, T frequency | Variable sites | Parsimony informative sites | Source |
|---|---|---|---|---|---|---|---|
| CCDC132 | 586 | intron, 2 | HKY+I+G | 0.286, 0.137, 0.232, 0.345 | 67 | 46 | (Backström et al. 2008) |
| Fib5 | 534 | intron, 4 | HKY+I | 0.300, 0.194, 0.191, 0.315 | 26 | 11 | (Marini and Hackett 2002) |
| GAPDH | 299 | intron, 1 | HKY+G | 0.224, 0.213, 0.326, 0.237 | 40 | 20 | (Fjeldså et al. 2003) |
| HMG2 | 495 | intron, 4 | HKY+I | 0.309, 0.183, 0.217, 0.291 | 43 | 33 | (Backström et al. 2008) |
| MUSK | 489 | intron, Z | GTR | 0.292, 0.186, 0.205, 0.318 | 43 | 22 | (Kimball et al. 2009) |
| Myo2 | 697 | intron, 1 | GTR+I | 0.274, 0.224, 0.243, 0.259 | 38 | 19 | (Slade et al. 1993; Heslewood et al. 1998) |
| ODC | 686 | intron, 3 | HKY+G | 0.273, 0.174, 0.209, 0.345 | 54 | 26 | (Friesen et al. 1999; Primmer et al. 2002) |
| TGFβ2 | 565 | intron, 3 | GTR | 0.238, 0.243, 0.210, 0.309 | 33 | 20 | (Primmer et al. 2002) |
| ND2+ND3 | 1392 | mitochondrial | | | | | (Sorenson et al. 1999) |
| | | codon pos. 1: | GTR+I+G | 0.358, 0.305, 0.138, 0.199 | 160 | 134 | |
| | | codon pos. 2: | GTR+I+G | 0.180, 0.309, 0.090, 0.421 | 67 | 43 | |
| | | codon pos. 3: | GTR+I+G | 0.465, 0.252, 0.044, 0.239 | 373 | 327 | |

burn-in and we combined tree sets from the seven runs to produce a maximum-credibility consensus tree. The posterior distribution of species trees was visualized in DensiTree 2.1.7 (Bouckaert 2010).

For all Bayesian analyses, TRACER 1.5 (Rambaut and Drummond 2007) and Are We There Yet? (AWTY; Wilgenbusch et al. 2004; Nylander et al. 2008) were used to assess convergence of parameter estimates and tree splits, respectively. For MrBayes analyses, the average standard deviation of split frequencies (ASDSF) and the potential scale reduction factor (PSRF) were used to determine topology convergence between runs. For *BEAST analyses, TRACER was used to assess convergence of independent runs as well as parameter estimates and effective sample sizes (ESS) to ensure they reached >200. The appropriate burn-in generations (25% for all analyses) were discarded based on convergence assessments of the

ASDSF passing below 0.01. The remaining trees were summarized in a 50% majority-rule consensus tree.

## Results

### *Sequence attributes*

The aligned dataset was 5,743 bp and included 202 samples (summary statistics presented in Table 2.4). All new sequences are deposited in GenBank. We obtained complete DNA sequences for all genes for all fresh samples. For samples from museum skins, or for those downloaded from GenBank, it was not possible to obtain complete sequences for certain genes. Alignment lengths were 1041 bp (ND2), 351 bp (ND3), 586 bp (CCDC132), 534 bp (Fib 5), 299 bp (GAPDH), 495 bp (HMGB2), 489 bp (MUSK), 697 bp (Myo2), 686 bp (ODC), and 565 bp (TGF). The aligned dataset contained 944 variable sites (16.4 %) and 701 (12.2 %) parsimony-informative sites. Uncorrected pairwise distances in ND2 (*p*-distance) between subspecies ranged from 0.008 (*P. vitiensis graeffii* and *P. v. aurantiiventris*) to 0.052 (*P. v. graeffii* and *P. pectoralis fuliginosa*). The *p*-distance across the basal split between *P. citreogaster collaris* and the rest of the ingroup was 0.087.

The mitochondrial data showed no insertions, deletions, or anomalous stop-codons; thus, there was no evidence that mtDNA sequences were of nuclear origin (i.e., pseudogenes; Sorenson & Quinn, 1998). The relative divergence levels among codon positions was typical for mtDNA (3 > 1 > 2). A 2bp indel in ODC was observed in all *P. vitiensis ornata* and three of four *P. caledonica intacta* samples. Several unique substitutions and heterozygous bases surrounding this indel suggested either gene flow or incomplete lineage sorting between *P. vitiensis ornata* and *P. caledonica intacta* (see Table 2.5 for details of this indel).

*Phylogenetic relationships*

The topologies recovered from analyses of mtDNA (Appendix II.A) showed greater resolution than those derived from nuclear introns (Appendix II.B–J); this was expected given the higher rates of sequence evolution in animal mtDNA compared to nuclear DNA (Brown et al. 1979). The topologies inferred from multiple independent ML and BA runs were highly concordant and the *BEAST species tree resolved some equivocal nodes from the concatenated ML and BA runs (see below). Stationarity was achieved in MrBayes (i.e., the ASDSF remained < 0.01) after 16.58 million generations. The PSRF values for all parameters were 1.0. We report well-supported nodes as defined by Bayesian posterior probability (PP) > 0.95 and ML bootstrap (BS) > 70.

The ingroup was defined by a well-supported clade that included all taxa presumed *a priori* to be part of the species complex based on taxonomy and geography (Fig. 2.2, clade A: PP=0.98, BS=70). Within clade A, samples from the Louisiade Archipelago (*P. citreogaster collaris*) of southeast Papua New Guinea formed a well-supported clade (clade B: PP=1.0, BS=100), which was sister to the rest of the ingroup (clade C: PP=1.0, BS=100). Within clade C, we found support for five clades (clades D–H), whose relationships to each other were equivocal.

Clade D (PP=1.0, BS=100) comprised samples from the Santa Cruz group, Solomon Islands (*P. vitiensis ornata*), whereas clade E (PP=1.0, BS=96) comprised samples from the main Solomon Islands archipelago, exclusive of *P. feminina* from Rennell Island. *Pachycephala citreogaster* from the Bismarck Archipelago formed a well-supported clade (clade F: PP=1.0, BS=100). Modest geographic structure was found within *P. citreogaster*, including a well-supported clade composed of samples from New Britain, New Ireland, New Hanover, and nearby islands all referable to nominate *P. c. citreogaster*. This clade was distinct from single samples

Table 2.5. Summary of an indel in the ODC locus.

| Taxon | Indel Sequence |
|---|---|
| | ODC (position 201–227) |
| Remainder of alignment | TTTGCCAAATA--GCAACTGATAGTTT |
| *Pachycephala caledonica intacta* B45791 | TTTGCCAAATA--GCAACTGATAGTTT |
| *Pachycephala caledonica intacta* B45759 | TTTGCCAAMTAATACAAATGAKAGTTT |
| *Pachycephala caledonica intacta* B45385 | TTTGCCAAMTMATACAAATGAGAGTTT |
| *Pachycephala caledonica intacta* B45398 | TTTGCCAACTCATACAAATGAGAGTTT |
| *Pachycephala vitiensis ornata* KUNHM 19400 | TTTGCCAACTCATACAAATGAGAGTTT |
| *Pachycephala vitiensis ornata* KUNHM 19410 | TTTGCCAACTCATACAAATGAGAGTTT |
| *Pachycephala vitiensis ornata* KUNHM 19418 | TTTGCCAACTCATACAAATGAGAGTTT |

from Manus (*P. c. goodsoni*) and Mussau Islands (*P. c. sexuvaria*), but relationships among these three subspecies were unresolved.

*Pachycephala caledonica intacta* of Vanuatu was the basal lineage of clade G, followed by divergence of a lineage that contained three Indonesian samples representing two sister taxa (*P. macrorhyncha* and *P. pectoralis balim*). This Indonesian lineage was sister to a large clade (clade H: PP=1.0, BS=91) that in turn comprised three clades among which relationships were unresolved. These three clades were clade I (PP=1.0, BS=88), which included three of the four Australian subspecies (nominotypical *P. p. pectoralis*, *P. p. youngi* and *P. p. glaucura*); J (PP=1.0, BS=100), which comprised only *P. p. fuliginosa* of western and southern Australia; and clade K (PP=1.0, BS=98), which comprised all *P. melanura* samples. Interestingly, the species tree analysis found strong support for the sister relationship of clades I and J, which was sister to clade K (Fig. 2.4).

Clade K (*P. melanura*) contained five well-supported subclades. Several samples representing *P. m. melanura* from Kimberley and Pilbara in Western Australia formed a clade sister to the rest of clade L. *P. m. robusta* was not monophyletic because it consisted of three

well-supported clades from (1) Queensland, (2) Northern Territory west of Darwin, and (3) the Gulf of Carpentaria, but the Gulf of Carpentaria clade was sister to *P. m. dahli*, a well-supported clade from Papua New Guinea.

Samples from the Solomon Islands formed a well-supported clade (clade K: PP=1.0, BS=97) including all *P. orioloides* samples; however, the relationship of *P. feminina*, a species-level taxon from Rennell Island, was equivocal. These lineages were strongly supported as sister in the species tree (Fig. 2.4), but this relationship was not supported in the concatenated BA and ML analyses (Fig. 2.2). Instead, the concatenated analyses found *P. feminina* to be the basal lineage of a largely Polynesian clade (clade L). Strong geographic structure was found within the Solomon Islands, including several well-supported clades corresponding to nominal subspecies. Nominotypical *P. o. orioloides* of Choiseul and Isabel Islands was sister to *P. o. bougainvillei* from Bougainville Island. This clade was sister to the samples from the New Georgia group, of which there were two well-supported clades: *P. o. melanonota* of Vella Lavella and *P. o. centralis* of New Georgia and Kolombangara Islands. Finally, *P. o. christophori* of Makira Island and *P. o. cinnamomea* of Guadalcanal Island branched sequentially from the base of the clade.

Clade L comprised a group of Polynesian taxa including samples from Samoa, Tonga, and Fiji. Samples from each archipelago received high support as clades (PP≥0.98), despite the topology being equivocal with respect to *P. jacquinoti* of Tonga and *P. feminina* of Rennell Island. We found a well-supported Fijian clade (clade M: PP=0.98, BS=77) with evidence of geographic structure within the archipelago. Four Fijian clades were well-supported, of which three correspond to single subspecies distributed in discrete geographic areas (i.e., *P. vitiensis lauana*, Lau Archipelago; *P. v. kandavensis*, Kadavu Island; and *P. graeffii torquata*, Taveuni Island). The fourth clade comprised three nominal subspecies distributed on four islands.

Figure 2.2. Molecular phylogeny of the ingroup *Pachycephala pectoralis*/*melanura* species complex. The tree is the Bayesian maximum consensus tree from the concatenated, partitioned analysis. Node support is denoted as Bayesian posterior probabilities and maximum likelihood bootstrap support (PP/BS). Sequences of taxa labeled with "(GB)" were downloaded from GenBank. Clades A–M are discussed in the text. Photos illustrate representative phenotypes in the complex and corresponding clades are numbered accordingly: 1. *P. citreogaster collaris collaris* Rara Island, Louisiade Archipelago, Papua New Guinea; 2. *P. vitiensis ornata* Ndende Island, Santa Cruz Group, Solomon Islands; 3. *P. orioloides christophori* Makira, Solomon Islands (KUNHM 98857); 4. *P. orioloides cinnamomea* Guadalcanal, Solomon Islands; 5. *P. citreogaster citreogaster* New Ireland, Bismarck Archipelago, Papua New Guinea; 6. *P. pectoralis glaucura* Tasmania, Australia; 7. *P. pectoralis youngi* Canberra, Australia; 8. *P. melanura dahli* Milne Bay Province, Papua New Guinea; 9. *P. flavifrons* Upolu, Samoa (KUNHM 104114); 10. *P. graeffii torquata* Taveuni, Fiji; 11. *P. vitiensis kandavensis* Kadavu, Fiji; 12. *P. vitiensis lauana* Ogea Levu, Lau Archipelago, Fiji; 13. *P. graeffii optata* Ovalau, Fiji; 14. *P. graeffii aurantiiventris* Vanua Levu, Fiji; 15. *P. graeffii ambigua* Rabi, Fiji.

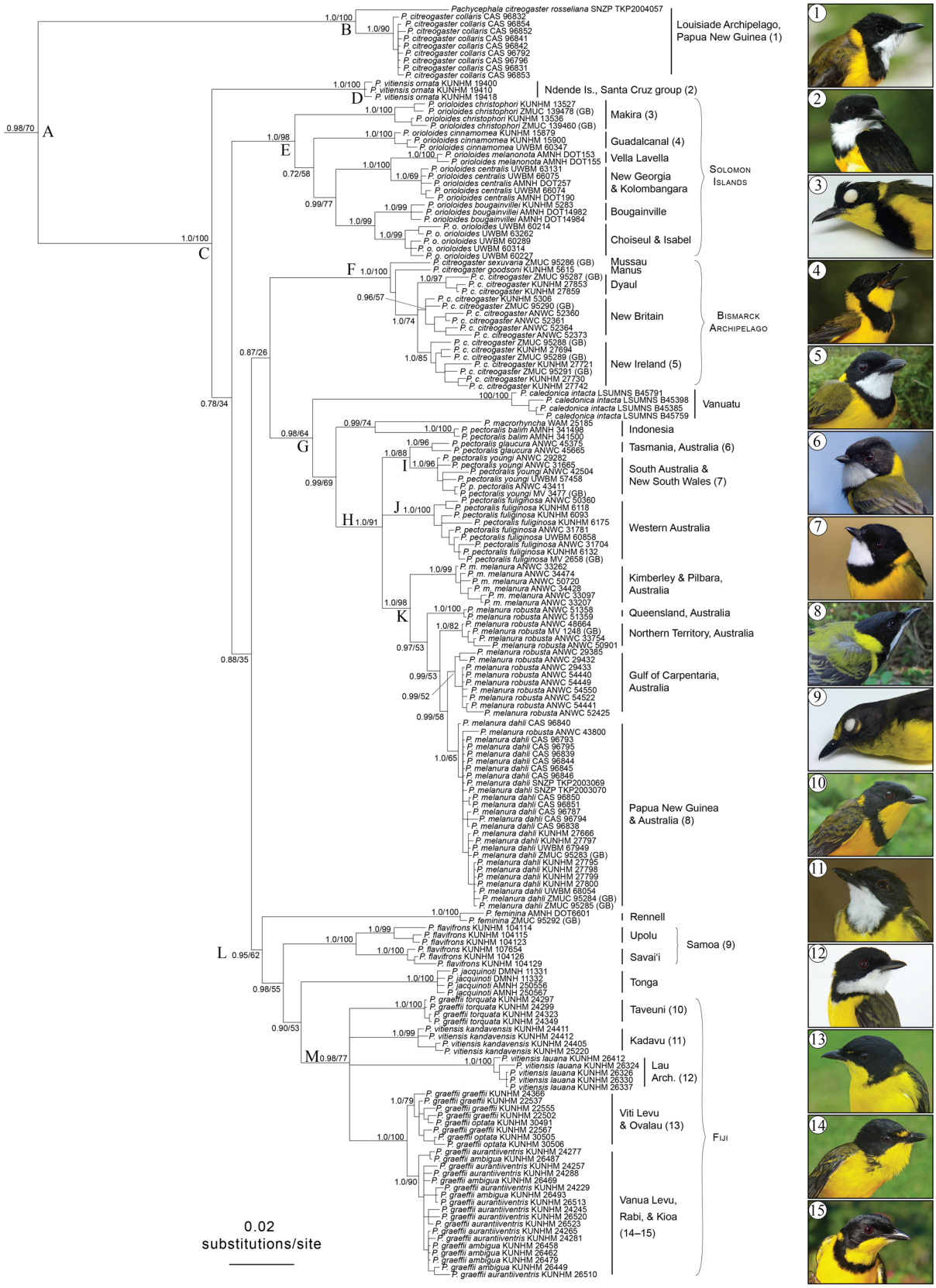

Pachycephala citreogaster rosseliana SNZP TKP2004057
P. citreogaster collaris CAS 96832
P. citreogaster collaris CAS 96854
P. citreogaster collaris CAS 96852
P. citreogaster collaris CAS 96841
P. citreogaster collaris CAS 96842
P. citreogaster collaris CAS 96792
P. citreogaster collaris CAS 96796
P. citreogaster collaris CAS 96831
P. citreogaster collaris CAS 96853
Louisiade Archipelago, Papua New Guinea (1)
P. vitiensis ornata KUNHM 19400
P. vitiensis ornata KUNHM 19410
P. vitiensis ornata KUNHM 19418
Ndende Is., Santa Cruz group (2)
P. orioloides christophori KUNHM 13527
P. orioloides christophori ZMUC 139478 (GB)
P. orioloides christophori KUNHM 13536
P. orioloides christophori ZMUC 139460 (GB)
Makira (3)
P. orioloides cinnamomea KUNHM 15879
P. orioloides cinnamomea KUNHM 15900
P. orioloides cinnamomea UWBM 60347
Guadalcanal (4)
P. orioloides melanonota AMNH DOT153
P. orioloides melanonota AMNH DOT155
Vella Lavella
P. orioloides centralis UWBM 63131
P. orioloides centralis UWBM 66075
P. orioloides centralis AMNH DOT257
P. orioloides centralis UWBM 66074
P. orioloides centralis AMNH DOT190
New Georgia & Kolombangara
P. orioloides bougainvillei KUNHM 5283
P. orioloides bougainvillei AMNH DOT14982
P. orioloides bougainvillei AMNH DOT14984
Bougainville
P. o. orioloides UWBM 60214
P. o. orioloides UWBM 63262
P. o. orioloides UWBM 60289
P. o. orioloides UWBM 60314
P. o. orioloides UWBM 60227
Choiseul & Isabel
Solomon Islands
P. citreogaster sexuvaria ZMUC 95286 (GB)
Mussau
P. citreogaster goodsoni KUNHM 5615
Manus
P. c. citreogaster ZMUC 95287 (GB)
P. c. citreogaster KUNHM 27853
P. c. citreogaster KUNHM 27859
Dyaul
P. c. citreogaster KUNHM 5306
P. c. citreogaster ZMUC 95290 (GB)
P. c. citreogaster ANWC 52360
P. c. citreogaster ANWC 52361
P. c. citreogaster ANWC 52364
New Britain
P. c. citreogaster ANWC 52373
P. c. citreogaster ZMUC 95288 (GB)
P. c. citreogaster KUNHM 27694
P. c. citreogaster ZMUC 95289 (GB)
P. c. citreogaster KUNHM 27721
P. c. citreogaster ZMUC 95291 (GB)
P. c. citreogaster KUNHM 27730
P. c. citreogaster KUNHM 27742
New Ireland (5)
Bismarck Archipelago
P. caledonica intacta LSUMNS B45791
P. caledonica intacta LSUMNS B45398
P. caledonica intacta LSUMNS B45385
P. caledonica intacta LSUMNS B45759
Vanuatu
P. macrorhyncha WAM 25185
Indonesia
P. pectoralis balim AMNH 341498
P. pectoralis balim AMNH 341500
P. pectoralis glaucura ANWC 45375
P. pectoralis glaucura ANWC 45665
Tasmania, Australia (6)
P. pectoralis youngi ANWC 29282
P. pectoralis youngi ANWC 31665
P. pectoralis youngi ANWC 42504
P. pectoralis youngi UWBM 57458
P. p. pectoralis ANWC 43411
P. pectoralis youngi MV 3477 (GB)
South Australia & New South Wales (7)
P. pectoralis fuliginosa ANWC 50360
P. pectoralis fuliginosa KUNHM 6118
P. pectoralis fuliginosa KUNHM 6093
P. pectoralis fuliginosa KUNHM 6175
P. pectoralis fuliginosa ANWC 31781
P. pectoralis fuliginosa UWBM 60858
P. pectoralis fuliginosa ANWC 31704
P. pectoralis fuliginosa KUNHM 6132
P. pectoralis fuliginosa MV 2658 (GB)
Western Australia
P. m. melanura ANWC 33262
P. m. melanura ANWC 34474
P. m. melanura ANWC 50720
P. m. melanura ANWC 34428
P. m. melanura ANWC 33097
P. m. melanura ANWC 33207
Kimberley & Pilbara, Australia
P. melanura robusta ANWC 51358
P. melanura robusta ANWC 51359
Queensland, Australia
P. melanura robusta ANWC 48664
P. melanura robusta MV 1248 (GB)
P. melanura robusta ANWC 33754
P. melanura robusta ANWC 50901
Northern Territory, Australia
P. melanura robusta ANWC 29385
P. melanura robusta ANWC 29432
P. melanura robusta ANWC 29433
P. melanura robusta ANWC 54440
P. melanura robusta ANWC 54449
P. melanura robusta ANWC 54550
P. melanura robusta ANWC 54522
P. melanura robusta ANWC 54441
P. melanura robusta ANWC 52425
Gulf of Carpentaria, Australia
P. melanura dahli CAS 96840
P. melanura robusta ANWC 43800
P. melanura dahli CAS 96793
P. melanura dahli CAS 96795
P. melanura dahli CAS 96839
P. melanura dahli CAS 96844
P. melanura dahli CAS 96845
P. melanura dahli CAS 96846
P. melanura dahli SNZP TKP2003069
P. melanura dahli SNZP TKP2003070
P. melanura dahli CAS 96850
P. melanura dahli CAS 96851
P. melanura dahli CAS 96787
P. melanura dahli CAS 96794
P. melanura dahli CAS 96838
P. melanura dahli KUNHM 27666
P. melanura dahli KUNHM 27797
P. melanura dahli UWBM 67949
P. melanura dahli ZMUC 95283 (GB)
P. melanura dahli KUNHM 27795
P. melanura dahli KUNHM 27798
P. melanura dahli KUNHM 27799
P. melanura dahli KUNHM 27800
P. melanura dahli UWBM 68054
P. melanura dahli ZMUC 95284 (GB)
P. melanura dahli ZMUC 95285 (GB)
Papua New Guinea & Australia (8)
P. feminina AMNH DOT6801
P. feminina ZMUC 95292 (GB)
Rennell
P. flavifrons KUNHM 104114
P. flavifrons KUNHM 104115
Upolu
P. flavifrons KUNHM 104123
P. flavifrons KUNHM 107854
P. flavifrons KUNHM 104126
P. flavifrons KUNHM 104129
Savai'i
Samoa (9)
P. jacquinoti DMNH 11331
P. jacquinoti DMNH 11332
P. jacquinoti AMNH 250556
P. jacquinoti AMNH 250567
Tonga
P. graeffii torquata KUNHM 24297
P. graeffii torquata KUNHM 24299
P. graeffii torquata KUNHM 24323
P. graeffii torquata KUNHM 24349
Taveuni (10)
P. vitiensis kandavensis KUNHM 24411
P. vitiensis kandavensis KUNHM 24412
P. vitiensis kandavensis KUNHM 24405
P. vitiensis kandavensis KUNHM 25220
Kadavu (11)
P. vitiensis lauana KUNHM 26412
P. vitiensis lauana KUNHM 26324
P. vitiensis lauana KUNHM 26326
P. vitiensis lauana KUNHM 26330
P. vitiensis lauana KUNHM 26337
Lau Arch. (12)
P. graeffii graeffii KUNHM 24366
P. graeffii graeffii KUNHM 22537
P. graeffii graeffii KUNHM 22555
P. graeffii graeffii KUNHM 22502
P. graeffii optata KUNHM 30491
P. graeffii graeffii KUNHM 22567
P. graeffii optata KUNHM 30505
P. graeffii optata KUNHM 30506
Viti Levu & Ovalau (13)
P. graeffii aurantiiventris KUNHM 24277
P. graeffii ambigua KUNHM 26487
P. graeffii aurantiiventris KUNHM 24257
P. graeffii aurantiiventris KUNHM 24288
P. graeffii ambigua KUNHM 26469
P. graeffii aurantiiventris KUNHM 24229
P. graeffii ambigua KUNHM 26493
P. graeffii aurantiiventris KUNHM 26513
P. graeffii aurantiiventris KUNHM 24245
P. graeffii aurantiiventris KUNHM 26520
P. graeffii aurantiiventris KUNHM 26523
P. graeffii aurantiiventris KUNHM 24265
P. graeffii aurantiiventris KUNHM 24281
P. graeffii ambigua KUNHM 26458
P. graeffii ambigua KUNHM 26462
P. graeffii ambigua KUNHM 26479
P. graeffii ambigua KUNHM 26449
P. graeffii aurantiiventris KUNHM 26510
Vanua Levu, Rabi, & Kioa (14–15)
Fiji
0.02 substitutions/site
A
B
C
D
E
F
G
H
I
J
K
L
M
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Figure 2.2

*Pachycephala graeffii graeffii* and *P. g. optata* of Viti Levu and Ovalau, respectively, were strongly-supported as sister to a clade comprising *P. g. aurantiiventris* from Vanua Levu and *P. g. ambigua* of Rabi and Kioa Islands. Finally, *P. flavifrons* comprised two well-supported clades from Savai'i and Upolu Islands, Samoa.

Overall, the species tree topology differed from the concatenated analyses in several important ways. First, the species tree found strong support for the placement of *P. feminina* as sister to *P. orioloides* (PP=1.0). This clade was sister to *P. citreogaster*, albeit with lower support (PP=0.94) in the species tree. Second, the species tree found strong support for the sister relationship of *P. p. pectoralis* + *P. p. fuliginosa* (PP=0.95), which was equivocal with respect to *P. melanura* in the concatenated analyses. Finally, the posterior distribution of trees as viewed in DensiTree suggests several alternative topologies for Polynesian lineages (Fiji, Vanuatu, Santa Cruz group), with resulting low posterior probabilities for these clades.

## Discussion

This study represents the most robust and densely sampled molecular phylogeny of arguably the world's most polytypic bird species complex, *Pachycephala pectoralis*, to date. Emphasizing the Australasian and Polynesian lineages, we present a detailed view of the evolutionary history in this classically polytypic group of Pacific island birds. The dense and widespread sampling scheme dramatically improves upon existing phylogenetic hypotheses (Smith and Filardi 2007; Jonsson et al. 2008a) and provides much greater phylogeographic resolution for populations in Australia and the Solomon Islands, including highland Bougainville and Guadalcanal, and the New Georgia and Santa Cruz groups. Additionally, this study includes

the first molecular data on *Pachycephala* lineages from the Louisiade Archipelago of Papua New Guinea, the Santa Cruz group of Solomon Islands, Vanuatu, Fiji, Samoa, and Tonga.

### *Australia, New Guinea, and Bismarck Archipelago*

Australian populations are divided into three well-supported clades, two of *P. pectoralis* (clades I and J) and one of *P. melanura* (clade K). The clade from south-western Australia corresponds to *P. p. fuliginosa*, a subspecies that Schodde and Mason (1999) considered to have a disjunct range in south-eastern and south-western Australia. Our samples of *P. pectoralis* from eastern Australia including Tasmania correspond to *P. p. glaucura* (Tasmania and Deal Island) and *P. p. youngi* and *P. p. pectoralis* (mainland southeast Australia). Further sampling is necessary in south-eastern Australia including its putative populations of *P. p. fuliginosa*, which we have not sampled, to disentangle the genetic signatures of migratory and non-migratory populations of *P. p. youngi* and *P. p. fuliginosa*, respectively (Schodde and Mason 1999; Higgins and Peter 2003), and any patterns of present or past gene flow among subspecies. The third Australian clade corresponds to *P. melanura*, which contains substantial geographic structure. Samples from Kimberley and Pilbara in Western Australia form a clade corresponding to nominotypical *P. m. melanura*. The subspecies *P. m. robusta* is paraphyletic and divided into three well-supported phylogroups: one in the Northern Territory west of the Gulf of Carpentaria, one from the southeast of the Gulf of Carpentaria in Queensland and the Northern Territory, and one from near Ayr, Queensland. The Gulf of Carpentaria clade was sister to *P. m. dahli*, which is broadly distributed throughout coastal eastern New Guinea. Notably, one *P. m. robusta* sample (ANWC 43800) from near Rockhampton, Queensland possessed a *P. m. dahli* mitochondrial

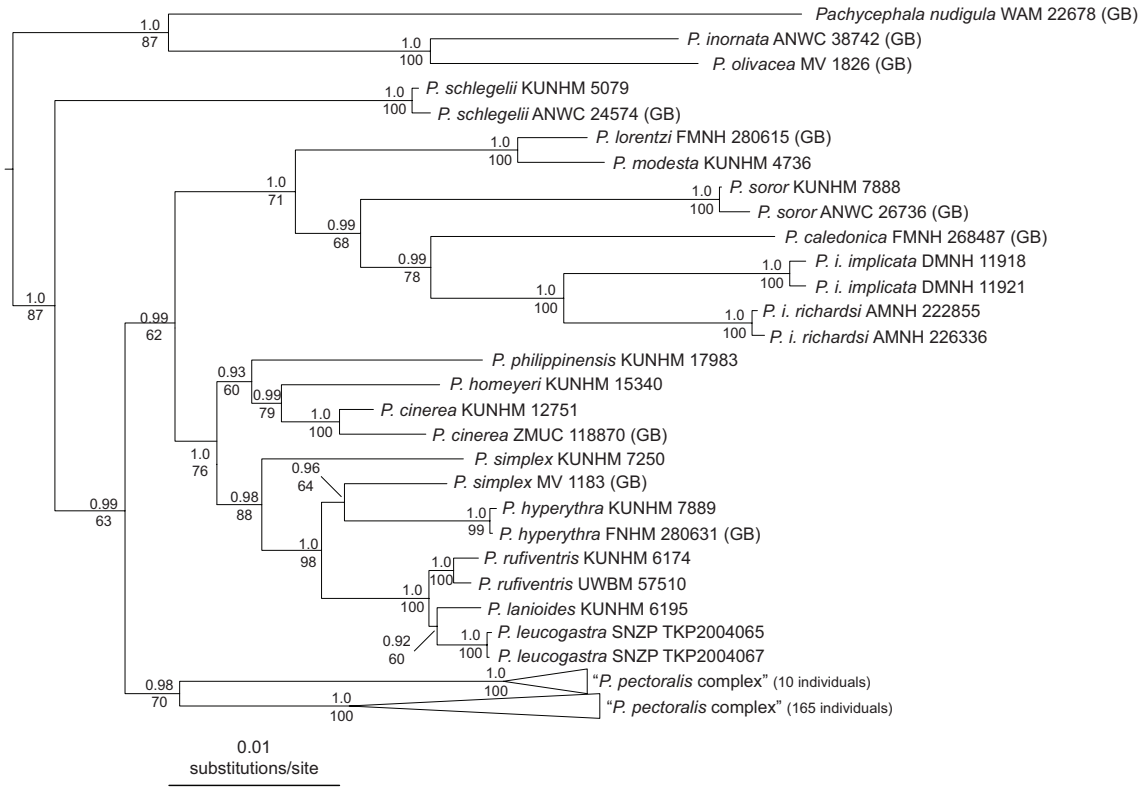



Figure 2.3. Molecular phylogeny of outgroup *Pachycephala* species. The tree is the Bayesian maximum consensus tree from the concatenated, partitioned analysis. Node support is denoted as Bayesian posterior probabilities (above) and maximum likelihood bootstrap support (below). Sequences of taxa labeled with "(GB)" were downloaded from GenBank. The ingroup is collapsed into two triangles, represented here by clades B and C. The ingroup phylogeny is depicted in Fig 2.2.

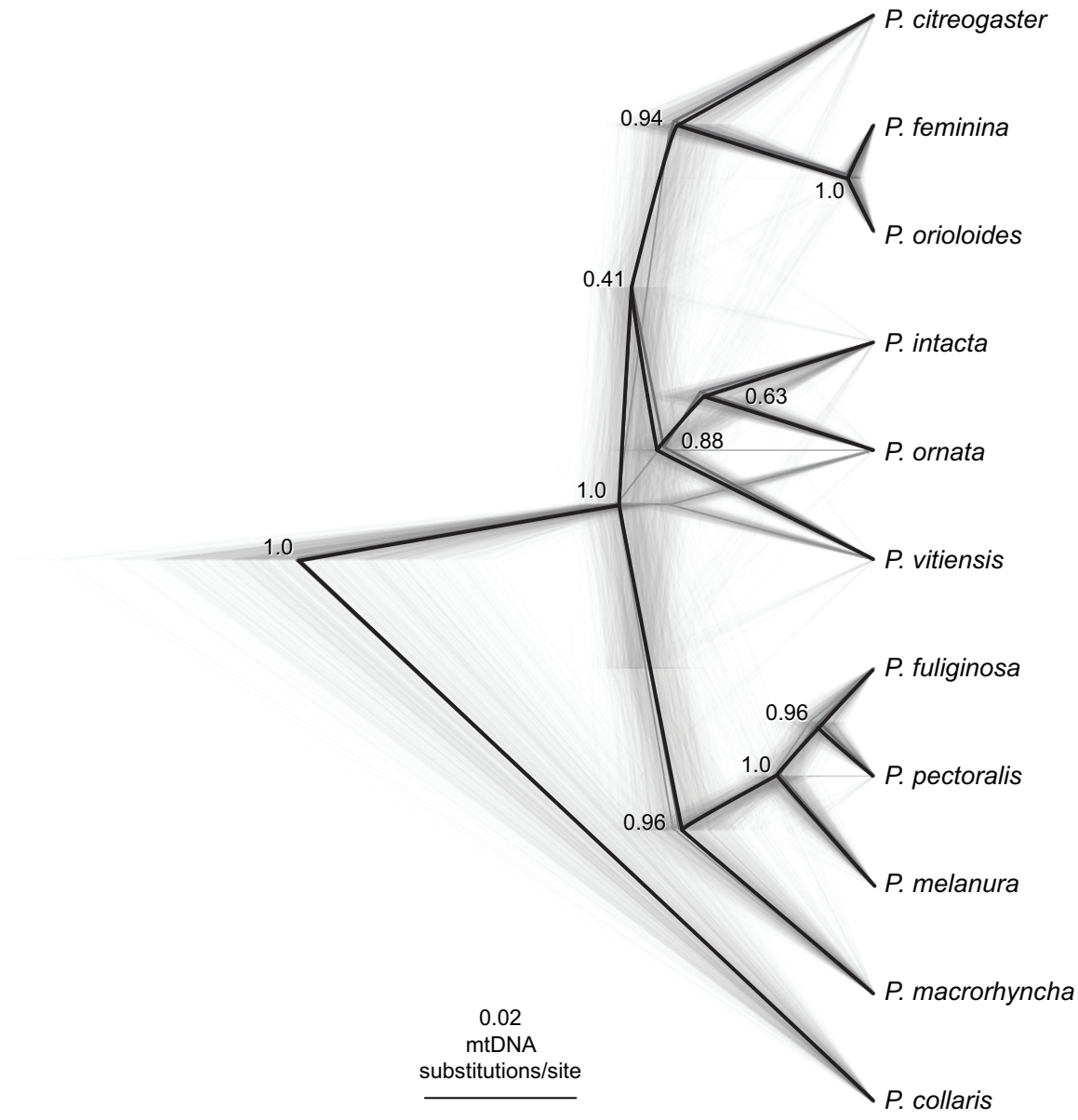



Figure 2.4. Coalescent species tree from *BEAST analysis of nuclear and mitochondrial DNA. The maximum clade credibility tree is superimposed on the cloudogram of the posterior tree distribution, visualized with DensiTree. Node support is denoted as Bayesian posterior probabilities.

haplotype, suggesting either the presence of gene flow between New Guinea and Queensland or incomplete lineage sorting between these clades. Further investigation of this issue should include samples from *P. m. spinicaudus*, which is distributed on islands in the Torres Strait and along the south coast of New Guinea from Merauke to Hall Sound, to determine the extent—if any—of gene flow between Australia and New Guinea.

The Bismarck Archipelago clearly has experienced multiple independent colonizations of *Pachycephala* populations from within the species complex. *Pachycephala melanura dahli* occurs on small islets that surround many of the major islands throughout the archipelago, whereas *P. citreogaster* is confined to the large islands of New Britain, New Ireland, and New Hanover, plus smaller islands such as Dyaul, Feni, Mussau, and Manus. Superficially, male plumage of *P. melanura dahli* and *P. citreogaster* is quite similar; both are white-throated, but small differences in tail color exist. Female plumage differs in head color and overall brightness of the yellow belly. Despite their similar appearance and similar distribution throughout the Bismarck Archipelago, they occupy different habitats: *P. melanura* inhabits coastal scrub forest on small islands and *P. citreogaster* occurs in mature forest, mostly on larger islands. We found little geographic structure within each of these clades, but our results suggest that samples from Manus (*P. c. goodsoni*) and Mussau Islands (*P. c. sexuvaria*) are genetically distinct from the rest of *P. citreogaster* (0.018 ND2 *p*-distance), and their classification as distinct subspecies is warranted. Interestingly, a coincident pattern of peripheral isolates in the Bismarck Archipelago is found also in *Todiramphus* kingfishers (*T. saurophagus* with respect to *T. chloris*) and *Monarcha* flycatchers (*M. cinerascens* with respect to *M. castaneiventris*). This pattern suggests that islets play an important role in the diversification of avian lineages in archipelagos such as the Bismarcks.

Three *Pachycephala* subspecies in the Louisiade Archipelago sometimes are lumped with *P. citreogaster* (P. citreogaster collaris, P. c. rosseliana, and P. c. misimae; Dickinson 2003; Dutson et al. 2011; Clements et al. 2013), based on morphological (white-throated) and geographic similarities. We sampled two of these subspecies (*P. citreogaster collaris* and *P. c. rosseliana*) and found them to form a highly divergent clade that was sister to the rest of the ingroup. The average sequence divergence between these clades was 0.087 in ND2 *p*-distance. The Louisiade Archipelago has many endemic avian subspecies (Clements et al. 2013), suggesting that birds in this archipelago may not share a close evolutionary history with those from the Bismarcks and mainland New Guinea. To our knowledge, this high degree of genetic distinctiveness for a Louisiades population is rare in avian lineages; see Kearns et al. (2013) for an example of a distinct Louisiade lineage of butcherbirds (Aves: Cracticidae). Additional sampling in the region is necessary, especially of *P. citreogaster misimae*, but our results suggest the presence of an overlooked species-level taxon in the region, *P. collaris*.

Indonesian sampling was not a focus of this study, and it remains a major obstacle to a full understanding of the evolutionary history of the *P. pectoralis*/*melanura* species complex; however, we did sequence toepads from museum study skins of two individuals of *P. pectoralis balim*, an enigmatic taxon restricted to the Balim and Bele Valleys on the north slopes of Mount Wilhelmina in the Snow Mountains of New Guinea. We found a well-supported sister relationship (PP=0.99, BS=75) between *P. macrorhyncha* of Tanimbar Island, Indonesia and *P. p. balim*. Although these represent the only two Indonesian taxa sampled in this study, this result does suggest an affinity of *P. p. balim* to other Indonesian taxa as opposed to species distributed throughout New Guinea (e.g., *P. soror*, *P. schlegelii*, *P. citreogaster*) or Australian *P. pectoralis* lineages (i.e., clades I–K). Additionally, the placement of this clade as sister to clade H hints at

the possibility that Indonesian members of the *P. pectoralis*/*melanura* species complex are more closely related to Australian taxa than they are to the more diverged Melanesian and Polynesian lineages.

### *Solomon Islands*

The topology of the Solomon Islands clade (*P. orioloides*; clade E) is characterized by well-diverged lineages. Relationships within *P. orioloides* are coincident with other Solomon Islands lineages. For example, a sister relationship between populations on Bougainville Island (*P. o. bougainvillei*) and Choiseul + Isabel Islands (*P. o. orioloides*) has been found in other species complexes, including *Monarcha castaneiventris* and *Ceyx lepidus* (Uy et al. 2009a; Andersen et al. 2013). Indeed, this is an expected relationship because Bougainville, Choiseul, and Isabel were connected as a single island, Greater Bukida, during the last glacial maximum (Mayr and Diamond 2001). We suspect this pattern is more pervasive than the literature suggests owing to poor sampling of Bougainville taxa in other studies (e.g., Smith and Filardi 2007). Our results placed the "Greater Bukida" clade sister to samples from the New Georgia group, which is an unusual pattern in the Solomon Islands. Most studies suggest a closer relationship of Guadalcanal to the "Greater Bukida" clade (Smith and Filardi 2007; Uy et al. 2009a). Within the New Georgia group, we sampled two of the three described subspecies from three islands and found a well-supported split between *P. o. melanonota* from Vella Lavella Island and *P. o. centralis* from New Georgia and Kolombangara Islands. Additional sampling is necessary from islands such as Ranongga (*P. o. melanonota*), Rendova and Tetepare (*P. o. melanoptera*), and Vangunu and Nggatokae (*P. o. centralis*) to better understand the phylogeographic history of whistlers in the New Georgia group. The two basal branches of the *P. orioloides* group are *P. o.*

*cinnamomea* (Guadalcanal) and *P. o. christophori* (Makira), but we lacked samples of *P. o. sanfordi* from Malaita. Taken as a whole, we found a well-resolved topology in the Solomon Islands that suggests an east to west biogeographic history, starting with *P. feminina* and *P. o. christophori* on Rennell and Makira Islands, respectively and working west to Bougainville. Uy et al. (2009a) reported the best-resolved topology of Solomon Islands birds to date (the polytypic *Monarcha castaneiventris* Verreaux, J, 1858). Their results showed that basal divergences divided populations from eastern islands such as Malaita and Makira from all others. We lacked samples from Malaita and the aforementioned New Georgia group islands, thus, a complete biogeographic reconstruction of the Solomon Islands taxa is not yet possible.

*Pachycephala implicata* is an enigmatic taxon distributed in the highlands of Bougainville and Guadalcanal. Two subspecies are described with distinctive male plumages: (1) *P. i. richardsi* on Bougainville is yellow below with an olive back and black hood, and (2) nominate *P. i. implicata* on Guadalcanal is overall greenish-olive with a gray hood. These taxa are sexually dimorphic, but female plumages are similar to each other. Our results represent the first molecular phylogenetic hypothesis for this taxon (Fig. 2.3), which was well-supported as sister to *P. c. caledonica* from New Caledonia. Furthermore, *P. i. richardsi* and *P. i. implicata* were 7.9% diverged in ND2 sequences. This high degree of genetic differentiation combined with plumage differences and substantial allopatry suggest they are best treated as separate species, *P. richardsi* and *P. implicata*, a decision that was adopted recently by Dutson et al. (2011).

### *Polynesia*

Phylogenetic relationships of Polynesian taxa were equivocal. Overall, the most striking aspect of these lineages is that each *Pachycephala* taxon from Rennell Island in the Solomon

Islands to Tonga is monophyletic and substantially diverged from all other taxa (e.g., mean divergence between Fijian *Pachycephala* and *P. feminina* from Rennell Island = 6.1%; *P. flavifrons* and *P. jacquinoti* are 4.5% and 4.0% diverged from Fijian *P. graeffii*, respectively. We interpret this pattern of shallow internodes at the base, long stem lineages, and shallow divergences within each taxon as support for a scenario in which *Pachycephala* achieved its full geographic distribution in Polynesia rapidly followed by little or no subsequent gene flow among most island populations. This biogeographic pattern of rapid and widespread colonization across Southeast Asia and the Pacific islands is thought to have occurred in other widespread polytypic species complexes such as *Todiramphus chloris* and *Turdus poliocephalus* (Mayr and Diamond 2001). Densely-sampled phylogenetic hypotheses are not available to test this hypothesis in these species complexes; however, this pattern has been documented at multiple taxonomic scales, including the *Ceyx lepidus* radiation (Andersen et al. 2013), a genus of Pacific ground doves (*Alopecoenas*; Moyle et al. 2013); and a family-level lineage with dozens of species (e.g., Zosteropidae; Moyle et al. 2009). It seems likely that the *Pachycephala pectoralis* species complex fits into this broader pattern of geographic expansion and speciation in Pacific island birds.

We achieved dense sampling from Fiji, including six of the 10 described subspecies from eight islands across the archipelago, and found them to form a well-supported clade (PP=0.98, BS=77). Several interesting patterns emerged in Fiji including the presence of two white-throated lineages (*P. vitiensis lauana* from the Lau Archipelago and *P. v. kandavensis* from Kadavu Island). Mayr (1932b) hypothesized that Fiji was colonized by a single white-throated lineage, but our results suggest two independent colonizations of white-throated forms into Fiji. Additional sampling of the third white-throated subspecies from Gau, Fiji (*P. v. vitiensis*) plus

samples from additional populations of *P. v. kandavensis* (e.g., Beqa Island) are necessary to disentangle the apparent complex biogeographic history of white-throated *P. vitiensis* in Fiji. Secondly, *P. graeffii torquata* was found to be a distinct lineage, but its phylogenetic position within Fiji is equivocal. Individuals of this taxon are substantially larger than other Fijian populations and they have prominent yellow nape patches and lack yellow lores, features unique in Fiji. Finally, *P. g. graeffii* of Viti Levu received strong support as being sister to a clade comprised of *P. g. aurantiiventris* of Vanua Levu and *P. g. ambigua* from Kioa and Rabi Islands. Mayr (1932b) hypothesized a scenario in which *P. g. torquata* and *P. g. aurantiiventris* + *P. g. ambigua* were closely related and distant from *P. g. graeffii*. Indeed, we found the opposite to be true, and we did not detect geographic structure between *P. g. aurantiiventris* and *P. g. ambigua*, despite noticeable morphological variation between these two subspecies. We recommend synonymizing these subspecies as one until a fine-scale study of gene flow is undertaken.

Samoa and Tonga represent the easternmost islands inhabited by *Pachycephala*, thus, the genus does not extend east of the Andesite Line. Each archipelago has a distinct species-level taxon: *P. jacquinoti* of Tonga is uniquely black-throated, whereas *P. flavifrons* of Samoa is entirely gray-backed with a variably mottled gray throat and thin yellow lores. Clearly, both have disparate plumage patterns from the 'standard' *P. pectoralis* complex; *P. flavifrons* has never been included in the complex, whereas *P. jacquinoti* was placed within the complex by Galbraith (1956), who synonymized it with *P. p. melanops*. Our results show that these species are nested within the *P. pectoralis*/*melanura* complex, but their exact relationships are unresolved. The phylogenetic placement of these two species, nested well within the ingroup, supports Galbraith (1956) overall treatment of dividing the complex into numerous species, a treatment that we recommend, as well. This pattern of species or genera embedded phylogenetically within a

radiation is not novel in Pacific bird lineages, and it adds to a growing body of literature suggesting there is still much to be learned about the phylogenetic relationships of Pacific island birds. For example, Filardi and Moyle (2005) found several aberrant genera of monarch flycatchers to be nested within the *Monarcha* radiation (e.g., *Metabolus*, *Clytorhynchus*, *Mayrornis*, and *Neolalage*) and Moyle et al. (2009) found several genera of white-eyes to be nested within *Zosterops* (e.g., *Chlorocharis*, *Speirops*, *Woodfordia*, and *Rukia*).

### *Taxonomy*

A full taxonomic revision is beyond the scope of this study due to incomplete sampling of nominal taxa. Based on our phylogeny, we offer a review and critique of three widely-used avian taxonomic classifications (Dickinson 2003; Gill and Donsker 2012; Clements et al. 2013; summarized in Table 2.1), and where possible, we make suggestions for more phylogenetically appropriate circumscription of species limits.

As noted above, Tonga and Samoa represent examples of archipelago-specific lineages that are each recognized as species, but the scenario appears far more complex in the rest of Polynesia. Phylogenetic relationships of taxa from New Caledonia, the Santa Cruz group, and Vanuatu remain uncertain owing in part to poor sampling and muddled taxonomy. This region, including Fiji, has been the most difficult for taxonomists to circumscribe geographically- and morphologically-cohesive species in this complex. Clements et al. (2013) and Gill and Donsker (2012) divided the region into three polytypic species, each with 5–7 subspecies: (1) *P. caledonica* (New Caledonia, Loyalty Islands, Vanuatu, and Vanikoro Island in the Santa Cruz group), (2) *P. vitiensis* (Nendo and Utupua Islands, Santa Cruz group, and southern and eastern Fiji), and (3) *P. graeffii* (northern and western Fiji); Dickinson (2003) subsumed *P. graeffii* into

an expanded *P. vitiensis* with 12 total subspecies (Table 2.1). We outline a more phylogenetically consistent taxonomic treatment below.

Although our results do not complete the picture in Polynesia, they do support several instances where current taxonomy does not reflect phylogeny. First, our single sample of *P. c. caledonica* (downloaded from GenBank) is not part of the ingroup species complex, a result first reported by Jonsson et al. (2008a). We found it to be well-supported as sister to *P. implicata* (Fig. 2.3; PP=99, BS=76), whereas Jonsson et al. (2008a) did not place it with certainty. We sampled only one other taxon from the *P. caledonica* group, *P. c. intacta* from Espiritu Santo, Vanuatu. This subspecies was found to be the basal lineage of clade G (Fig. 2.2), thus rendering *P. caledonic*a paraphyletic. Clements et al. (2013) split *P. caledonica* into two geographically cohesive groups (i.e., New Caledonia and Vanuatu), but maintained their single-species status. Whether these groups pertain to phylogenetic lineages remains to be seen when better sampling is achieved, but these groups are not each other's closest relatives and their placement in linear classifications such as Clements et al. (2013) should be changed. Second, we sampled three of five subspecies in the *P. vitiensis* species group: *P. v. ornata* (Nendo Island, Santa Cruz group), *P. v. kandavensis* (Kadavu, Fiji), and *P. v. lauana* (Lau Archipelago, Fiji). The English name of this species, White-throated Whistler, reflects their unifying morphological character. We found support for these three subspecies as independent lineages (Fig. 2.2), but their relationships to other Polynesian taxa were equivocal. The two Fijian subspecies were closely related (clade M), but relationships within this clade also were unresolved. Finally, the remaining Fijian taxa and one from Vanua Lava, Vanuatu have been ascribed to *P. graeffii*, which lacks a white throat (Clements et al. 2013). We sampled four of seven subspecies in this group (all from Fiji), and found them to be part of clade M, which also includes *P. v. kandavensis* and *P. v. lauana*. Given

that white-throated birds in Polynesia do not form a monophyletic group, we advise not recognizing *P. vitiensis,* the so-called white-throated whistler (*sensu* Gill and Donsker 2012; Clements et al. 2013) as different from *P. graeffii*. At this time, we advocate the conservative taxonomic treatment of Dickinson (2003) who lumped *P. graeffii* and *P. vitiensis*.

Despite relatively dense sampling in this study, the treatment of Australian lineages is still equivocal. Our concatenated analysis did not resolve the basal polytomy (clade H) of Australian populations, however, the species tree did. Jonsson et al. (2008a) found greater resolution of this node, with *P. pectoralis youngi* sister to *P. melanura*, and *P. pectoralis fuliginosa* sister to them, thus, our species tree is in conflict with the tree presented in Jonsson et al. (2008a). Additionally, questions are left unanswered with regards to gene flow between *P. p. pectoralis*, *P. p. youngi*, and *P. p. fuliginosa* across southern Australia. Our sampling was not adequate to address the apparent high-degree of gene flow between these population boundaries suggested by Higgins and Peter (2003). Further work is also needed in the *P. melanura* clade, in which there is complex geographic structure, including paraphyly of at least two subspecies (*P. m. robusta* and *P. m. dahli*). The population from Ayr, Queensland is geographically associated with *P. m. robusta*, but it groups genetically with *P. m. dahli* from the Bismarck Archipelago, a result highlighted by Nyári and Joseph (2013). We believe fine-scale studies of gene flow including all populations of Australasian *P. pectoralis* and *P. melanura* are necessary before a comprehensive reworking of taxonomy can be undertaken.

Our results emphasize the disconnect between traditional, morphology-based taxonomy and molecular phylogeny-based evolutionary histories in Pacific bird lineages, a topic recently reviewed by (Pratt 2010). Although this study represents the most densely-sampled phylogeny of the *Pachycephala pectoralis*/*melanura* species complex to date many questions remain

unanswered. A thorough taxonomic overhaul is needed, along with detailed analyses of biogeography and character evolution. Significant additional geographic sampling is needed from Polynesia and throughout Indonesia, and additional genomic sampling is warranted before such analyses can achieve statistical rigor.

# Chapter 3*

## A molecular phylogeny of Pacific honeyeaters (Aves: Meliphagidae) reveals extensive paraphyly and an isolated Polynesian radiation

* Andersen, M. J., Naikatini, A., and R. G. Moyle. In press. A molecular phylogeny of Pacific honeyeaters (Aves: Meliphagidae) reveals extensive paraphyly and an isolated Polynesian radiation. Molecular Phylogenetics and Evolution.

Abstract

We investigated the molecular phylogenetic placement of 14 species of Pacific island honeyeaters (Aves: Meliphagidae) in the broader context of an existing family-level phylogeny. We examined the evolutionary history of Pacific honeyeater lineages to assess the accuracy of current taxonomies and to evaluate their biogeographic history. We compare these biogeographic patterns to other Pacific birds to identify emergent patterns across lineages. We found strong support for a previously unknown endemic radiation in central Polynesia, which comprises five genera: *Meliarchus*, *Guadalcanaria, Gymnomyza*, *Xanthotis*, and *Foulehaio*. Conversely, other Pacific lineages were found to be strongly allied with continental radiations (e.g., *Philemon eichhorni, P. cockerelli*, and *Lichmera incana*). Our results necessitated taxonomic changes, both at the generic level (e.g., *Xanthotis, Melidectes*/*Vosea,* and *Glycifohia*/*Gliciphila*) and regarding species limits within polytypic species. Here, we discuss species limits in *Foulehaio* and *Gymnomyza* and recommend elevating three nominal subspecies of *Foulehaio* to species status, each of which forms well-differentiated clades.

## Introduction

The avian family Meliphagidae, or honeyeaters, is a diverse lineage whose center of diversity is Australasia. The 184 species in the family are distributed from Polynesia, through New Guinea and Australia, across Wallacea, and one species extends west of Wallace's Line to Bali (Higgins et al. 2008). The diversity in this family is multifaceted and includes not only large numbers of species, but also great disparity in eco-morphology (e.g., bill size and shape), body size, habitat, behavior, and plumage. Despite its species richness and diversity, the Meliphagidae has received surprisingly little attention from molecular systematists. In the most comprehensive work to date, Driskell and Christidis (2004) published a higher-level phylogeny that established a framework for the family. Generally, their sampling within genera was sparse, but they did uncover several paraphyletic genera (e.g., *Phylidonyris* and *Certhionyx*). Other efforts have focused on denser sampling of some larger genera within the family; some genera were found to be massively paraphyletic (e.g., *Lichenostomus*; Nyári and Joseph 2011), whereas others were monophyletic (e.g., *Meliphaga*; Norman et al. 2007).

Pacific island species represent an almost entirely unsampled geographic component of the Melihpagidae. The Pacific meliphagids comprise two groups: the genus *Myzomela* contains ~ 30 species that are small, morphologically similar, and almost continuously distributed on most islands from Fiji through Indonesia. In contrast, all other Pacific meliphagids (i.e., non-*Myzomela*) are larger-bodied, morphologically diverse, and more sparsely distributed, with species often restricted to single islands within archipelagos. Taxonomically, these species are placed in several genera, some of which are endemic to the Pacific, although others also include Australopapuan representatives. Some of these Australopapuan genera, such as *Philemon*, occur on oceanic archipelagos near continents (i.e., the Bismarcks, Admiralties, and Lesser Sundas).

However, other genera, such as *Xanthotis*, have more anomalous Pacific representatives. *Xanthotis provocator* is endemic to Kadavu, Fiji, whereas the rest of the genus occurs in Australia and New Guinea. *Meliarchus sclateri*, endemic to the island of Makira in the southeast Solomon Islands, has been placed in *Melidectes* (Sibley and Monroe 1990; Schodde and Mason 1999) or with *Acanthagenys* (Parkes 1980), genera endemic to New Guinea and Australia, respectively. The possibility of such geographically disjunct relationships is afforded some support from published molecular phylogenies. Driskell and Christidis (2004) and Nyári and Joseph (2011) included a single Pacific representative in their analyses: *Foulehaio carunculatus*, endemic to central Polynesia. Possibly because of taxon sampling differences, the exact placement of this species differed between the two studies, but each placed it sister to Australian genera.

Recent work on molecular systematics of endemic Pacific bird groups (Filardi and Moyle 2005; Filardi and Smith 2005; Moyle et al. 2009; Gibb and Penny 2010; Cibois et al. In press) has revealed that many morphologically aberrant, island-endemic genera are embedded within more morphologically homogeneous, widespread genera. Other unexpected patterns, such as upstream colonization of Australasia from Pacific archipelagos have also been supported (Filardi and Moyle 2005). It is unknown if the large-bodied honeyeaters represent one or more colonizations of the Pacific, and thus, it is of taxonomic and biogeographic interest to decipher the evolutionary relationships among Pacific meliphagids.

Here we present a molecular phylogenetic hypothesis of the Meliphagidae that focuses on relationships of non-*Myzomela* Pacific species. Using the framework produced by Driskell and Christidis (2004) and specimens from recent field work in the Pacific, we examine evolutionary history of the group to 1) assess the accuracy of current taxonomies, 2) evaluate the

biogeographic history of the group, and 3) compare these patterns to other published phylogenetic work of other avian lineages to look for emergent biogeographic patterns in the Pacific.

## Methods

### *Taxon sampling*

The framework for phylogenetic placement of the Pacific meliphagids relied on data produced by Driskell and Christidis (2004) and Nyári and Joseph (2011). Both studies used a mitochondrial gene (NADH dehydrogenase subunit 2; ND2) and a nuclear intron (Beta-Fibrinogen intron 5; Fib5) and sampled broadly from the Australian and Papuan members of the family. To this matrix we added sequences of 46 individuals from 16 additional species (Table 3.1). Fourteen of these species are endemic to Pacific islands, from New Britain to Samoa; the other two occur in Australia and New Guinea (*Xanthotis flaviventer* and *X. polygrammus*). Dense geographic sampling of all three subspecies was included for *Foulehaio carunculatus*, which is broadly distributed across Fiji, Samoa, and Tonga. Both subspecies of *Gymnomyza viridis* were included. We lacked material from only one Pacific meliphagid species: *Gymnomyza aubryana* of New Caledonia, of which we know of only one museum study skin in North America, from which we could not obtain viable DNA sequence data. Of 46 newly sequenced samples, 34 were derived from fresh muscle tissue; the remaining twelve samples were obtained from toepads clipped from museum study skins. Outgroup sampling (Appendix III) was the same as Driskell and Christidis (2004), but these results are not presented here. We follow the taxonomy of Gill and Donsker (2013) until taxonomic changes are proposed. For the spelling of *Sugomel niger*, we follow Schodde and Mason (1999).

Table 3.1. List of newly sequenced samples used in this study. Ancient DNA samples derived from museum specimens (i.e., toepads) are noted with *. Genus and species names in brackets indicates where taxonomy from (Gill and Donsker 2013) differs from our proposed changes. Institutional abbreviations: AMNH, American Museum of Natural History; DMNH, Delaware Museum of Natural History; KUNHM, University of Kansas Natural History Museum; LSUMNS, Louisiana State University Museum of Natural Science; UMMZ, University of Michigan Museum of Zoology; NMNH, Smithsonian Institution, National Museum of Natural History; UWBM, University of Washington Burke Museum; YPM, Yale Peabody Museum.

| Genus | Species | Subspecies | Institution | Sample | Locality |
|---|---|---|---|---|---|
| *Foulehaio* | *carunculatus* | *carunculatus* | KUNHM | 104023* | SAMOA: Upolu Is. |
| *Foulehaio* | *carunculatus* | *carunculatus* | KUNHM | 104025* | SAMOA: Upolu Is. |
| *Foulehaio* | *carunculatus* | *carunculatus* | KUNHM | 104041* | SAMOA: Savai'i Is. |
| *Foulehaio* | *carunculatus* | *carunculatus* | KUNHM | 104050* | AMERICAN SAMOA: Tutuila Is. |
| *Foulehaio* | *carunculatus* | *carunculatus* | KUNHM | 107639* | AMERICAN SAMOA: Tau Is. |
| *Foulehaio* | *carunculatus* | *carunculatus* | UWBM | Bu42872 | TONGA: 'Eua |
| *Foulehaio* | *carunculatus* | *carunculatus* | UWBM | Bu42885 | TONGA: 'Eua |
| *Foulehaio* | *carunculatus* | *carunculatus* | KUNHM | 26303 | FIJI: Lau Archipelago; Ono-I-Lau Is. |
| *Foulehaio* | *carunculatus* | *carunculatus* | KUNHM | 26344 | FIJI: Lau Archipelago; Ogea Levu Is. |
| *Foulehaio* | *carunculatus* | *carunculatus* | KUNHM | 26386 | FIJI: Lau Archipelago; Fulaga Is. |
| *Foulehaio* | *carunculatus* | *carunculatus* | KUNHM | 26425 | FIJI: Matuku Is. |
| *Foulehaio* | [*carunculatus*] | *procerior* | KUNHM | 24378 | FIJI: Viti Levu Is. |
| *Foulehaio* | [*carunculatus*] | *procerior* | KUNHM | 24382 | FIJI: Viti Levu Is. |
| *Foulehaio* | [*carunculatus*] | *procerior* | KUNHM | 30509 | FIJI: Ovalau Is. |
| *Foulehaio* | [*carunculatus*] | *procerior* | KUNHM | 30524 | FIJI: Ovalau Is. |
| *Foulehaio* | [*carunculatus*] | *taviuensis* | KUNHM | 24220 | FIJI: Vanua Levu Is. |
| *Foulehaio* | [*carunculatus*] | *taviuensis* | KUNHM | 26536 | FIJI: Vanua Levu Is. |
| *Foulehaio* | [*carunculatus*] | *taviuensis* | KUNHM | 24307 | FIJI: Taveuni Is. |
| *Foulehaio* | [*carunculatus*] | *taviuensis* | KUNHM | 24351 | FIJI: Taveuni Is. |
| *Foulehaio* | [*carunculatus*] | *taviuensis* | KUNHM | 26495 | FIJI: Kioa Is. |
| *Glycifohia* | *notabilis* | | LSUMNS | B45775 | VANUATU: Espiritu Santo |
| *Glycifohia* | *notabilis* | | LSUMNS | B45807 | VANUATU: Espiritu Santo |
| *Glycifohia* | *undulata* | | YPM | 71297* | NEW CALEDONIA |
| *Guadalcanaria* | *inexpectata* | | DMNH | 11854* | SOLOMON ISLANDS: Guadalcanal Is. |
| *Gymnomyza* | *samoensis* | | KUNHM | 104021* | SAMOA: Upolu Is. |
| *Gymnomyza* | *samoensis* | | KUNHM | 107665* | SAMOA: Upolu Is. |
| *Gymnomyza* | *viridis* | *brunneirostris* | KUNHM | 30461 | FIJI: Viti Levu Is. |
| *Gymnomyza* | *viridis* | *viridis* | KUNHM | 24318 | FIJI: Taveuni Is. |
| *Lichmera* | *incana* | | UMMZ | 221981* | NEW CALEDONIA: Balabio Is. |
| *Meliarchus* | *sclateri* | | KUNHM | 13544 | SOLOMON ISLANDS: Makira Is. |
| *Meliarchus* | *sclateri* | | KUNHM | 13546 | SOLOMON ISLANDS: Makira Is. |
| *Myzomela* | *jugularis* | | KUNHM | 22536 | FIJI: Viti Levu Is. |
| *Philemon* | *cockerelli* | | KUNHM | 27644 | PAPUA NEW GUINEA: New Britain Is. |
| *Philemon* | *eichhorni* | | KUNHM | 27770 | PAPUA NEW GUINEA: New Ireland Is. |

| Genus | Species | Subspecies | Institution | Sample | Locality |
|---|---|---|---|---|---|
| *Philemon* | *eichhorni* | | NMNH | B4027 | PAPUA NEW GUINEA: New Ireland Is. |
| *Stresemannia* | *bougainvillei* | | KUNHM | 5280 | PAPUA NEW GUINEA: Bougainville Is. |
| *Stresemannia* | *bougainvillei* | | KUNHM | 5281 | PAPUA NEW GUINEA: Bougainville Is. |
| *Vosea* [*Melidectes*] | *whitemanensis* | | AMNH | 778167* | PAPUA NEW GUINEA: New Britain Is. |
| *Vosea* [*Melidectes*] | *whitemanensis* | | AMNH | 778172* | PAPUA NEW GUINEA: New Britain Is. |
| *Xanthotis* | *flaviventer* | | KUNHM | 5588 | PAPUA NEW GUINEA: Gulf Prov. |
| *Xanthotis* | *flaviventer* | | KUNHM | 7571 | PAPUA NEW GUINEA: East Sepik Prov. |
| *Xanthotis* | *flaviventer* | | KUNHM | 9557 | PAPUA NEW GUINEA: Western Prov. |
| *Xanthotis* | *polygrammus* | | KUNHM | 5133 | PAPUA NEW GUINEA: Simbu Prov. |
| *Xanthotis* | *polygrammus* | | KUNHM | 9640 | PAPUA NEW GUINEA |
| *Xanthotis* | *provocator* | | KUNHM | 24416 | FIJI: Kadavu Is. |
| *Xanthotis* | *provocator* | | KUNHM | 25211 | FIJI: Kadavu Is. |

### *DNA sequencing*

Total genomic DNA was extracted from frozen or alcohol-preserved muscle tissue using a Qiagen tissue extraction protocol (Qiagen, Valencia, California). All muscle tissue samples have associated museum study-skin vouchers. For taxa with no available tissue samples, DNA was extracted from toepads of museum study skins (Table 3.1). We sequenced the entire second subunit of mitochondrial nicotinamide adenine dinucleotide dehydrogenase (hereafter ND2) and the fifth intron of the Beta-fibrinogen gene (Fib5). Target DNA fragments were amplified using polymerase chain reaction (PCR) with the following external primers for ND2 (L5215; Hackett 1996) and (H6313; Johnson and Sorenson 1998) and Fib5 (Fib5 and Fib6; Marini and Hackett 2002). Additionally, we used internal primers designed for this study to amplify 200–250 bp fragments of toepad samples (Table 3.2).

PCR amplifications were performed in 13 µl reactions using Promega GoTaq DNA polymerase. A touchdown protocol was used in PCR for ND2 with annealing temperatures of 58, 54, and 50 °C. Annealing temperatures were held at 54 °C for Fib5 following recommendations by (Kimball et al. 2009). Amplified PCR products were screened on high-melt, 1% agarose gels

Table 3.2. Newly designed primers to sequence ND2 and Fib5 from samples derived from museum specimen toepads.

| Primer name | 5' to 3' sequence | Primer name | 5' to 3' sequence |
|---|---|---|---|
| ND2 | | ND2 (cont.) | |
| FoulH190 | AGGACTAGTGTTGAGGCAGCTG | GymL170 | GTGCCATTGAAGCAGCAAC |
| FoulH411 | AGGWAGAGCAAGGTGATGGG | GymL347 | CCATTYCACTTCTGATTCCC |
| FoulH551 | GGCTATTCAACCTAAGTGTGAG | GymL367 | GAAGTCCTCCAAGGCACCT |
| FoulH709 | ATAGAGTTTAGGGGTGGGG | GymL506 | GCCTTAGGAGGATGAATAGG |
| FoulH864 | CGGAGATAGAAGAATAGGCTG | GymL513 | GGAGGGTGARTAGGCCTTAA |
| FoulL142 | GCCCTTAATCTCAAAATCCC | GymL644 | GCCTAATAACYGCARCYG |
| FoulL644 | GCCTAATAACCGCAGCCG | GymL829 | GARCTCACYAAACAAGACATAGCC |
| FoulL829 | GAACTCACTAAACAAGACATAGCC | LichmeraH242 | GTAATGTCCCACTGTCCGG |
| GlycH202 | GCTGGAGAATAGAACTAAGGC | LichmeraH380 | CTGTTGATAGGAGGAGGC |
| GlycH341 | GGAGGACTTCTGGGAATC | LichmeraL20 | GAACCCCCAGGCAAAACT |
| GlycH500 | GTCTGGTTAAGTCCCATTC | LichmeraHL196 | CTTTCTAGTACAAGCAGCCG |
| GlycH671 | CAGCGTTGGGAGACTCAGCA | Vosea660F | GCCGTATTTCTCACCCTAAAC |
| GlycL174 | GCCATCGAAGCAGCAACCAAG | Vosea841F | CATGGCCCCTACAGCAATCG |
| GlycL319 | GCGGCTATTGCAATAAAACTAG | Vosea866R | CGGAGGTAGAAGAACAGGC |
| GlycL478 | GCTAATCACTATAGCCATCC | | |
| Gym.extH | GGCCTTCGGTTTAAGGTAATCC | Fib5 | |
| Gym.extL | GATGGTTTAACTCCTTCCCCTAT | GymFib5-ext | GCCATACAGAGTATACTGTGAC |
| GymH190 | AGGACTAGTGYKGAGGCAGCTG | GymFib5-227F | CAGGAAAGTCTTGTTGAGGTC |
| GymH362 | CCGGTAGTTAGAGAGGTGC | GymFib5-383F | GTGCCAGACAAAAGACCAGG |
| GymH406 | CAAGGTAATGGGTGGGAA | GymFib6-246R | CCAGTTTCACATTAGAAGTATCC |
| GymH411 | AGGAAGAGYARGGTRATGGG | GymFib6-408R | CCTTGCTTCATAAGGAAAGGTGC |
| GymH538 | CGAGATGGAAGAAAAGGC | GymFib6-ext | CTGCAGGAGCAAGAGTATC |
| GymH551 | CTATTCAGCCCAAATGCGAG | MyzFib5-ext | CAGATAATGGAGGTTAGTGTG |
| GymH709 | ATGGTGTTGAGAGATGGGG | MyzFib5-270F | GACCAGCATGGACAATGAATAGG |
| GymH723 | CAGTGTGAGAAGGAATATGGAG | MyzFib6-262R | CCTATTCATTGTCCATGCTGGTC |
| GymH861 | CGGAGRTARAAGAAYAGGCTGAG | MyzFib6-328R | CTTGAAGGAYGGCCCTGGTCT |
| GymL142 | GCCCCTAATCTCAAAATCCC | MyzFib6-ext | CAAAGTCCAGCCTGCAGGA |

stained with GelRed, and purified with 10% Exo-SAP-IT™ (GE Healthcare Bio-Sciences Corp.). We cycle-sequenced purified PCR products in both directions with the same primers used in PCR for 25 cycles using the ABI Big Dye Terminator Cycle-Sequencing Kit version 3.1 (Applied Biosystems Inc., Foster City, CA). Sequencing was performed on an ABI Prism 3730 high-throughput capillary electrophoresis DNA analyzer.

### *Model selection and phylogenetic analysis*

Sequence contigs were assembled in Geneious 6.1 and individual nuclear intron alignments were constructed by hand and checked against an automated alignment in MUSCLE (Edgar 2004). Additionally, we aligned by hand a particularly difficult indel in Fib5 according to

(Driskell and Christidis 2004)Driskell and Christidis (2004). Appropriate models of sequence evolution for each of the four partitions were identified using Akaike's Information Criterion (AIC), as implemented in MrModelTest 2.3 (Nylander 2004)(Nylander, 2004). A GTR+I+G model was implemented in all phylogenetic analyses for each of three codon positions in ND2, whereas, GTR+G was used for Fib5.

Phylogenetic reconstruction was performed on the partitioned, concatenated data and separately on each individual locus. Maximum likelihood (ML) heuristic tree searches were performed using GARLI 2.0 (Zwickl 2006). To avoid local optima, 1000 independent searches were performed, each starting from a random tree. GARLI's default parameters were adjusted to terminate searches when no topological improvements were found after 50,000 generations (genthreshfortopoterm = 50000); otherwise, default settings were used. We selected the topology with the best likelihood as our ML estimate. Statistical support for this topology was obtained by running 1,000 non-parametric bootstrap replicates (Felsenstein 1985) in GARLI to assess clade credibility and SumTrees 3.3.1, part of the DendroPy 3.12.0 package (Sukumaran and Holder 2010), was used to create a 50% majority-rule consensus tree. Nodes with >70% bootstrap support were considered well-supported (Hillis and Bull 1993; Wilcox et al. 2002). Bayesian analysis (BA) was conducted using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003; Altekar et al. 2004; Ronquist et al. 2012) implemented with BEAGLE (Ayres et al. 2012). Two independent MCMC runs of 50 million generations were conducted using four chains per run (nchains=4) and incremental heating of chains (temp=0.1), sampling every 2,000 generations.

For all Bayesian analyses, TRACER 1.5 (Drummond and Rambaut 2007) and Are We There Yet? (AWTY; Wilgenbusch et al. 2004; Nylander et al. 2008) were used to assess convergence of parameter estimates and tree splits, respectively. For MrBayes analyses, the

average standard deviation of split frequencies (ASDSF) and the potential scale reduction factor (PSRF) were used to determine topology convergence between runs. The appropriate burn-in generations (25% for all analyses) were discarded based on convergence assessments of the ASDSF passing below 0.01. The remaining trees were summarized in a 50% majority-rule consensus tree.

## Results

The combination of newly sequenced samples and those downloaded from GenBank (Appendix III) produced a final data alignment of 131 individuals and 1714 bp. New sequences are deposited in GenBank. Monophyly of the Meliphagidae received unequivocal support, but major sub-clades within the family were separated by short internodes and many received low support. In general, Bayesian analysis produced significant posterior probabilities (> 0.95) for many basal nodes that had low (< 70%) bootstrap support. When analyzed separately, we found discordance between the mitochondrial ND2 gene tree (Fig. 3.1) and the nuclear Fib5 gene tree (Fig. 3.2), similar to that found by Driskell and Christidis (2004). We noted that each tree supported a different placement of *Myzomela* and *Philemon*, with strong, but conflicting support in both cases: the ND2 tree supported a closer relationship of these two genera than did the Fib5 tree. The concatenated phylogeny (Fig. 3.3) reflects the more distant relationship between *Myzomela* and *Philemon* supported by Fib5 (i.e., these two genera as members of clades A and E). Other studies with more loci, but not Fib5, have shown a closer relationship between *Myzomela* and *Philemon* (Gardner et al. 2010). Furthermore, Fib5 has been shown to support alternative topologies in birds (Hackett et al. 2008); therefore, we view the Fib5 results with caution, particularly in light of the many unresolved nodes in the base of our tree.

Figure 3.1. Molecular phylogeny of the Meliphagidae; Bayesian maximum consensus tree from analysis of the mitochondrial gene NADH dehydrogenase subunit 2 (ND2). Node support is denoted as Bayesian posterior probabilities.


Outgroups
Stresemannia bougainvillei KUNHM 5280
Stresemannia bougainvillei KUNHM 5281
Gliciphila melanops
Glycifohia undulata YPM 71297
Glycifohia notabilis LSUMNS B45775
Glycifohia notabilis LSUMNS B45807
Glycichaera fallax
Ptiloprora guisei
Ptiloprora plumbea
Conopophila albogularis
Conopophila rufogularis
Ramsayornis fasciatus
Ramsayornis modestus
Melipotes fumigatus
Melilestes megarhynchus
Timeliopsis fulvigula
Timeliopsis griseigula
Ashbyia lovensis
Epthianura albifrons
Epthianura aurifrons
Epthianura crocea
Epthianura tricolor
Acanthorhynchus superciliosus
Acanthorhynchus tenuirostris
Certhionyx variegatus
Prosthemadera novaeseelandiae
Pycnopygius cinereus
Pycnopygius stictocephalus
Stomiopera flava
Meliphaga albonotata
Meliphaga gracilis
Lichenostomus melanops
Purnella albifrons
Manorina flavigula
Manorina melanophrys
Melidectes torquatus
Melidectes belfordi
Melidectes ochromelas
Bolemoreus frenatus
Gavicalis virescens
Ptilotula penicillata
Ptilotula flavescens
Ptilotula flavescens
Acanthagenys rufogularis
Caligavis obscura
Anthochaera chrysoptera
Anthochaera lunulata
Anthochaera phrygia
Anthochaera carunculata
Anthochaera paradoxa
Sugomel niger
Vosea [Melidectes] whitemanensis AMNH 778167
Vosea [Melidectes] whitemanensis AMNH 778172
Myzomela obscura
Myzomela rosenbergii
Myzomela erythrocephala
Myzomela sanguinolenta
Myzomela cardinalis
Myzomela jugularis KUNHM 22536
Grantiella picta
Plectorhyncha lanceolata
Xanthotis polygrammus KUNHM 5133
Xanthotis polygrammus KUNHM 9640
Xanthotis flaviventer KUNHM 9557
Xanthotis flaviventer
Xanthotis flaviventer KUNHM 5588
Xanthotis flaviventer KUNHM 7571
Philemon citreogularis
Philemon meyeri
Philemon cockerelli KUNHM 27644
Philemon eichhorni KUNHM 27770
Philemon eichhorni NMNH 4027
Philemon buceroides
Philemon argenticeps
Philemon corniculatus
Nesoptilotis flavicollis
Nesoptilotis leucotis
Entomyzon cyanotis
Melithreptus albogularis
Melithreptus brevirostris
Cissomela pectoralis
Lichmera alboauricularis
Lichmera incana UMMZ 221981
Lichmera indistincta
Trichodere cockerelli
Phylidonyris pyrrhopterus
Phylidonyris niger
Phylidonyris novaehollandiae
Meliarchus sclateri KUNHM 13544
Meliarchus sclateri KUNHM 13546
Guadalcanaria inexpectata DMNH 13546
Gymnomyza viridis viridis KUNHM 24318
Gymnomyza viridis brunneirostris KUNHM 30461
Xanthotis provocator KUNHM 24416
Xanthotis provocator KUNHM 25211
Gymnomyza samoensis KUNHM 104021
Gymnomyza samoensis KUNHM 107665
Foulehaio procerior KUNHM 24378
Foulehaio procerior KUNHM 24382
Foulehaio procerior KUNHM 30509
Foulehaio procerior KUNHM 30524
Foulehaio taviuensis KUNHM 24307
Foulehaio taviuensis KUNHM 24351
Foulehaio taviuensis KUNHM 26495
Foulehaio taviuensis KUNHM 24220
Foulehaio taviuensis KUNHM 26536
Foulehaio carunculatus KUNHM 26386
Foulehaio carunculatus KUNHM 26425
Foulehaio carunculatus KUNHM 26303
Foulehaio carunculatus KUNHM 26344
Foulehaio carunculatus KUNHM 104041
Foulehaio carunculatus KUNHM 104050
Foulehaio carunculatus KUNHM 107639
Foulehaio carunculatus
Foulehaio carunculatus KUNHM 104023
Foulehaio carunculatus KUNHM 104025
Foulehaio carunculatus UWBM 42872
Foulehaio carunculatus UWBM 42885
ND2
0.04 substitutions/site

Figure 3.1

Figure 3.2. Molecular phylogeny of the Meliphagidae; Bayesian maximum consensus tree from analysis of the fifth intron of the nuclear Beta-Fibrinogen gene (Fib5). Node support is denoted as Bayesian posterior probabilities.

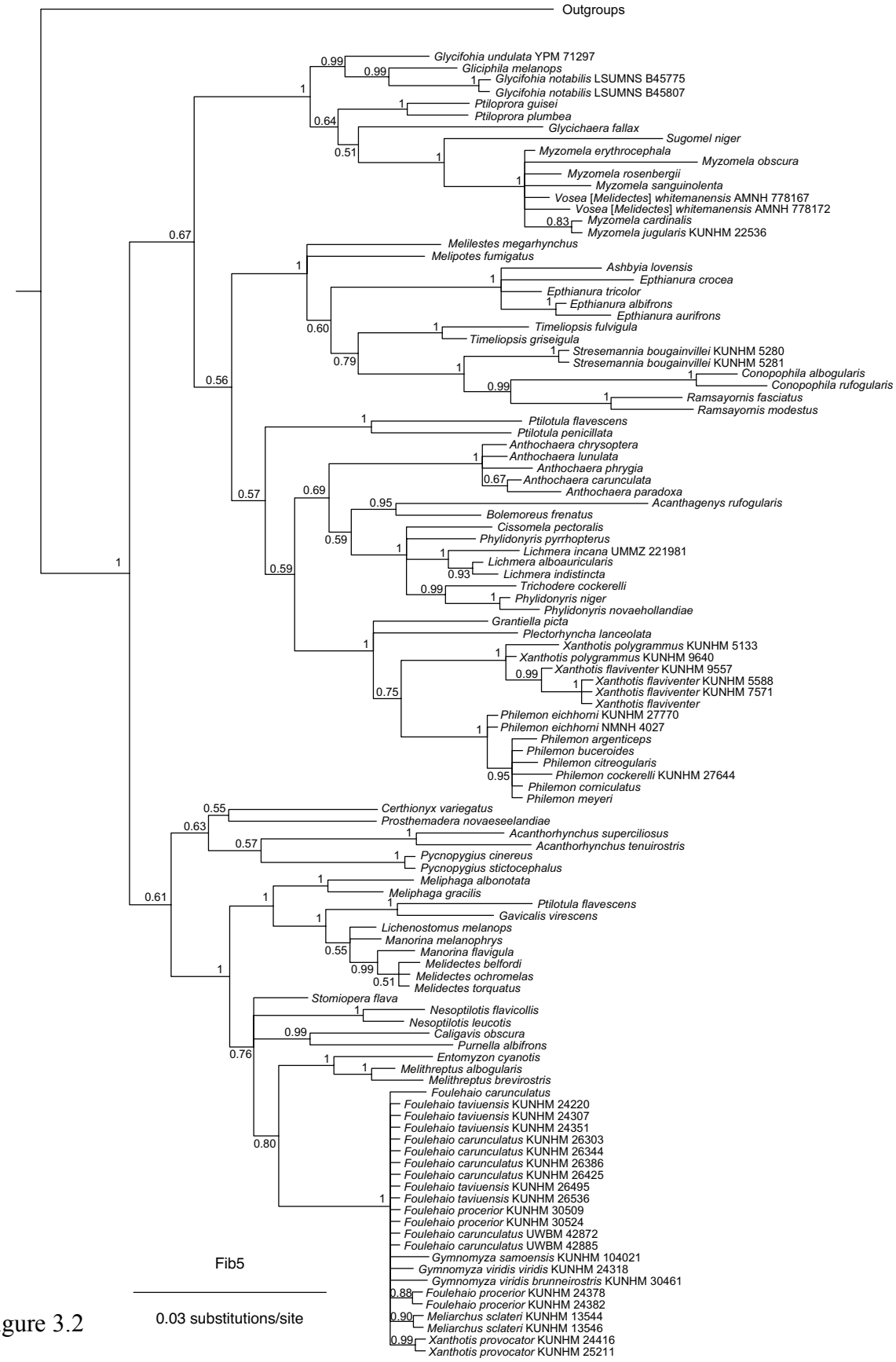

Outgroups
Glycifohia undulata YPM 71297
Gliciphila melanops
Glycifohia notabilis LSUMNS B45775
Glycifohia notabilis LSUMNS B45807
Ptiloprora guisei
Ptiloprora plumbea
Glycichaera fallax
Sugomel niger
Myzomela erythrocephala
Myzomela obscura
Myzomela rosenbergii
Myzomela sanguinolenta
Vosea [Melidectes] whitemanensis AMNH 778167
Vosea [Melidectes] whitemanensis AMNH 778172
Myzomela cardinalis
Myzomela jugularis KUNHM 22536
Melilestes megarhynchus
Melipotes fumigatus
Ashbyia lovensis
Epthianura crocea
Epthianura tricolor
Epthianura albifrons
Epthianura aurifrons
Timeliopsis fulvigula
Timeliopsis griseigula
Stresemannia bougainvillei KUNHM 5280
Stresemannia bougainvillei KUNHM 5281
Conopophila albogularis
Conopophila rufogularis
Ramsayornis fasciatus
Ramsayornis modestus
Ptilotula flavescens
Ptilotula penicillata
Anthochaera chrysoptera
Anthochaera lunulata
Anthochaera phrygia
Anthochaera carunculata
Anthochaera paradoxa
Acanthagenys rufogularis
Bolemoreus frenatus
Cissomela pectoralis
Phylidonyris pyrrhopterus
Lichmera incana UMMZ 221981
Lichmera alboauricularis
Lichmera indistincta
Trichodere cockerelli
Phylidonyris niger
Phylidonyris novaehollandiae
Grantiella picta
Plectorhyncha lanceolata
Xanthotis polygrammus KUNHM 5133
Xanthotis polygrammus KUNHM 9640
Xanthotis flaviventer KUNHM 9557
Xanthotis flaviventer KUNHM 5588
Xanthotis flaviventer KUNHM 7571
Xanthotis flaviventer
Philemon eichhorni KUNHM 27770
Philemon eichhorni NMNH 4027
Philemon argenticeps
Philemon buceroides
Philemon citreogularis
Philemon cockerelli KUNHM 27644
Philemon corniculatus
Philemon meyeri
Certhionyx variegatus
Prosthemadera novaeseelandiae
Acanthorhynchus superciliosus
Acanthorhynchus tenuirostris
Pycnopygius cinereus
Pycnopygius stictocephalus
Meliphaga albonotata
Meliphaga gracilis
Ptilotula flavescens
Gavicalis virescens
Lichenostomus melanops
Manorina melanophrys
Manorina flavigula
Melidectes belfordi
Melidectes ochromelas
Melidectes torquatus
Stomiopera flava
Nesoptilotis flavicollis
Nesoptilotis leucotis
Caligavis obscura
Purnella albifrons
Entomyzon cyanotis
Melithreptus albogularis
Melithreptus brevirostris
Foulehaio carunculatus
Foulehaio taviuensis KUNHM 24220
Foulehaio taviuensis KUNHM 24307
Foulehaio taviuensis KUNHM 24351
Foulehaio carunculatus KUNHM 26303
Foulehaio carunculatus KUNHM 26344
Foulehaio carunculatus KUNHM 26386
Foulehaio carunculatus KUNHM 26425
Foulehaio taviuensis KUNHM 26495
Foulehaio taviuensis KUNHM 26536
Foulehaio procerior KUNHM 30509
Foulehaio procerior KUNHM 30524
Foulehaio carunculatus UWBM 42872
Foulehaio carunculatus UWBM 42885
Gymnomyza samoensis KUNHM 104021
Gymnomyza viridis viridis KUNHM 24318
Gymnomyza viridis brunneirostris KUNHM 30461
Foulehaio procerior KUNHM 24378
Foulehaio procerior KUNHM 24382
Meliarchus sclateri KUNHM 13544
Meliarchus sclateri KUNHM 13546
Xanthotis provocator KUNHM 24416
Xanthotis provocator KUNHM 25211
Fib5
0.03 substitutions/site

Figure 3.2

Figure 3.3. Molecular phylogeny of the Meliphagidae; Bayesian maximum consensus tree from the concatenated, partitioned analysis. Node support is denoted as Bayesian posterior probabilities and maximum likelihood bootstrap support (PP/BS). Newly sequenced taxa are labeled with corresponding sample numbers; all others were downloaded from GenBank (see Supplementary Table S1). Clades A–H are discussed in the text. Gray boxes highlight Pacific lineages in the phylogeny. Outgroup sequences (not shown) were the same as Driskell and Christidis (2004) and included representatives from four families: Dasyornithidae, Pardalotidae, Acanthizidae, and Maluridae.


Outgroups
A
Sugomel niger
Vosea [Melidectes] whitemanensis AMNH 778167
Vosea [Melidectes] whitemanensis AMNH 778172
New Britain, PNG
Myzomela rosenbergii
Myzomela obscura
Myzomela erythrocephala
Myzomela sanguinolenta
Myzomela cardinalis
Myzomela jugularis KUNHM 22536 Fiji
B
Glycichaera fallax
Ptiloprora guisei
Ptiloprora plumbea
Glycifohia undulata YPM 71297 New Caledonia
Gliciphila melanops
Glycifohia notabilis LSUMNS B45775
Glycifohia notabilis LSUMNS B45807
Espiritu Santo, Vanuatu
Stresemannia bougainvillei KUNHM 5280
Stresemannia bougainvillei KUNHM 5281
Bougainville, PNG
C
Conopophila albogularis
Conopophila rufogularis
Ramsayornis fasciatus
Ramsayornis modestus
Melipotes fumigatus
Melilestes megarhynchus
Timeliopsis fulvigula
Timeliopsis griseigula
Ashbyia lovensis
Epthianura crocea
Epthianura tricolor
Epthianura albifrons
Epthianura aurifrons
D
Cissomela pectoralis
Lichmera alboauricularis
Lichmera incana UMMZ 221981 Balabio, New Caledonia
Lichmera indistincta
Trichodere cockerelli
Phylidonyris pyrrhopterus
Phylidonyris niger
Phylidonyris novaehollandiae
Grantiella picta
Plectorhyncha lanceolata
E
Xanthotis polygrammus KUNHM 5133
Xanthotis polygrammus KUNHM 9640
Xanthotis flaviventer KUNHM 9557
Xanthotis flaviventer
Xanthotis flaviventer KUNHM 5588
Xanthotis flaviventer KUNHM 7571
Philemon citreogularis
Philemon meyeri
Philemon eichhorni KUNHM 27770 New Ireland, PNG
Philemon eichhorni NMNH 4027
Philemon cockerelli KUNHM 27644 New Britain, PNG
Philemon buceroides
Philemon argenticeps
Philemon corniculatus
F
Acanthorhynchus superciliosus
Acanthorhynchus tenuirostris
Certhionyx variegatus
Prosthemadera novaeseelandiae New Zealand
Pycnopygius cinereus
Pycnopygius stictocephalus
G
Meliphaga albonotata
Meliphaga gracilis
Stomiopera flava
Caligavis obscura
Purnella albifrons
Lichenostomus melanops
Manorina flavigula
Manorina melanophrys
Melidectes torquatus
Melidectes belfordi
Melidectes ochromelas
Gavicalis virescens
Ptilotula penicillata
Ptilotula flavescens
Ptilotula flavescens
Bolemoreus frenatus
Acanthagenys rufogularis
Anthochaera chrysoptera
Anthochaera lunulata
Anthochaera phrygia
Anthochaera carunculata
Anthochaera paradoxa
H
Nesoptilotis flavicollis
Nesoptilotis leucotis
Entomyzon cyanotis
Melithreptus albogularis
Melithreptus brevirostris
Meliarchus sclateri KUNHM 13544
Meliarchus sclateri KUNHM 13546
Makira, Solomon Is.
Guadalcanaria inexpectata DMNH 11854 Guadalcanal, Solomon Is.
Gymnomyza viridis viridis KUNHM 24318 Taveuni, Fiji
Gymnomyza viridis brunneirostris KUNHM 30461 Viti Levu, Fiji
Xanthotis provocator KUNHM 24416
Xanthotis provocator KUNHM 25211
Kadavu, Fiji
Gymnomyza samoensis KUNHM 104021
Gymnomyza samoensis KUNHM 107665
Samoa
Foulehaio procerior KUNHM 30509
Foulehaio procerior KUNHM 30524
Foulehaio procerior KUNHM 24378
Foulehaio procerior KUNHM 24382
Viti Levu & Ovalau, Fiji
Foulehaio taviuensis KUNHM 24307
Foulehaio taviuensis KUNHM 24351
Foulehaio taviuensis KUNHM 26495
Foulehaio taviuensis KUNHM 24220
Foulehaio taviuensis KUNHM 26536
Vanua Levu, Rabi, & Taveuni, Fiji
Foulehaio carunculatus KUNHM 26425
Foulehaio carunculatus KUNHM 26386
Foulehaio carunculatus KUNHM 26303
Foulehaio carunculatus KUNHM 26344
Foulehaio carunculatus UWBM 42872
Foulehaio carunculatus UWBM 42885
Foulehaio carunculatus KUNHM 104050
Foulehaio carunculatus KUNHM 104023
Foulehaio carunculatus KUNHM 104025
Foulehaio carunculatus KUNHM 107639
Foulehaio carunculatus KUNHM 104041
Foulehaio carunculatus
American Samoa, Samoa, Tonga, & Lau Arch., Fiji
0.04 substitutions/site

Figure 3.3

Overall, eight large clades (clades A–H, Fig. 3.3) diverged from near the base of the tree, two of which (B, F) lacked any node support and should be considered unresolved but are useful for discussion. Pacific island species were found in seven of the eight major clades. Because much of our matrix was comprised of sequences from Driskell and Christidis (2004), we restrict our reporting of results to the newly added taxa. The gene tree discordance mentioned above does not affect the overall interpretation of Pacific island lineages.

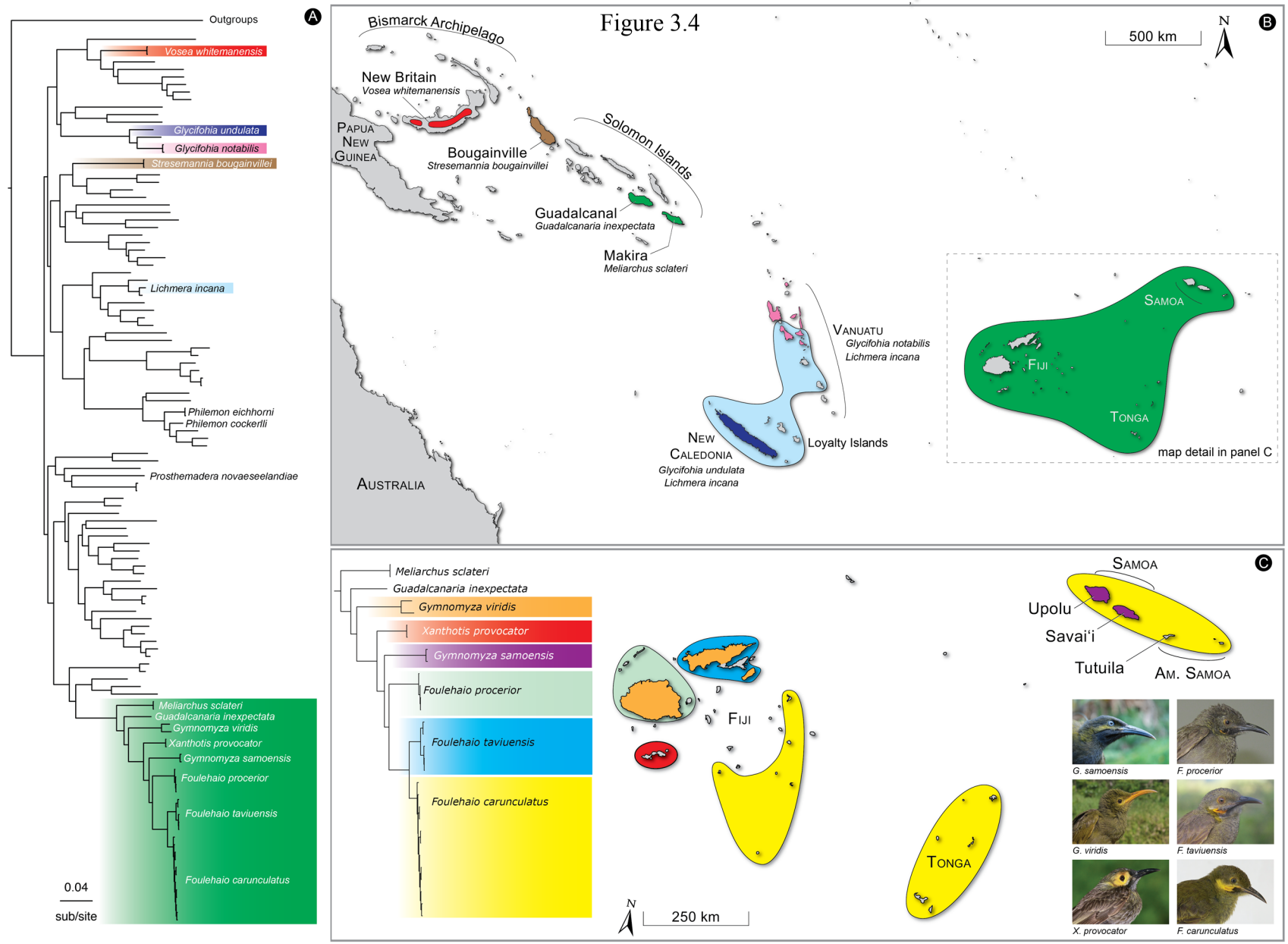
Clade A contained the monotypic genus *Sugomel* and the large and widespread genus *Myzomela*. Substantially better taxon sampling will be required to decipher relationships among the ~30 species in this genus, which includes many Pacific island taxa. A surprising result was the placement of *Melidectes whitemanensis*—a montane New Britain endemic—between *Sugomel* and *Myzomela*. Sister to clade A was a collection of genera comprising clade B (Fig. 3.3). Two Pacific island species, *Glycifohia undulata* from New Caledonia and *Glycifohia notabilis* from Vanuatu were part of this clade, but were rendered paraphyletic by *Gliciphila melanops* of Australia. The Bougainville Island endemic genus *Stresemannia* was part of clade C (Fig. 3.3), but separate from other species and on a long branch from near the base of the clade.

*Lichmera incana* was placed among other *Lichmera* species in clade D (Fig. 3.3), separated from *Lichmera indistincta* by only 1.6% ND2 divergence. Within clade E, all *Philemon* species included in the study formed a clade, with the two Pacific species, *P. eichhorni* of New Ireland and *P. cockerelli* of New Britain, embedded in the genus. Although not sister to one another in the phylogeny, this relationship received low support. Also included in clade E were two of the three species of *Xanthotis* in the study. The two species distributed in Australia and New Guinea, *X. polygrammus* and *X. flaviventer*, formed a clade but the Fiji endemic *X. provocator* was strongly supported as a member of clade H (see below).

Clade F was poorly supported and likely comprises multiple independent lineages, but it did contain one Pacific lineage: *Prosthemadera novaeseelandiae* of New Zealand. The large and diverse clade G contained no Pacific island species. Six of the fourteen sampled Pacific species outside of *Myzomela* were contained in clade H. Branching basally from the clade was the sister pair of *Nesoptilotis leucotis* and *N. flavicollis*, endemic to Australia and Tasmania, respectively. The rest of the clade comprised two Australian genera (*Entomyzon* and *Melithreptus*) sister to a large and well-supported Pacific assemblage (Fig. 3.3). Branching sequentially from the base of the Pacific clade were *Meliarchus sclateri* of Makira Island, *Guadalcanaria inexpectata* of Guadalcanal Island, and *Gymnomyza viridis* of Fiji, but support for the specific branching order was equivocal. Above these three species, *Xanthotis provocator* of Kadavu Island, Fiji; *Gymnomyza samoensis* of Samoa; and the widespread *Foulehaio carunculatus* of Fiji, Tonga, and Samoa formed a well-supported clade. Posterior probability supporting *X. provocator* as sister to the other two species fell short of significant (0.90), but ML bootstrap support was more persuasive (87%). Samples from throughout the range of *F. carunculatus* were grouped into three strongly supported clades corresponding to nominal subspecies. Samples from Viti Levu and Ovalau Islands, Fiji, comprising *F. c. procerior*, formed a clade that was ~10% diverged in ND2 (uncorrected P) from *F. c. taviuensis* (Vanua Levu, Taveuni, and Rabi Islands, Fiji) and *F. c. carunculatus*. Within *F. c. carunculatus*, samples from Samoa, Tonga, and the Lau Archipelago of Fiji— separated from one another by hundreds of kilometers of open ocean (Fig. 3.4)—showed no geographic structure and less than 1% mitochondrial divergence.

Figure 3.4. Map of the southwest Pacific illustrating distributions of most of the non-*Myzomela* honeyeater lineages sampled in this study. (A) Bayesian phylogeny reproduced from Fig. 1 with Pacific lineages labeled. Colored boxes pertain to distribution colors in map panel B. (B) Map of the southwest Pacific with distributions of *Vosea whitemanensis* (red; New Britain), *Stresemannia bougainvillei* (brown; Bougainville), *Glycifohia undulata* (dark blue; New Caledonia), *Glycifohia notabilis* (pink; northern Vanuatu), and *Lichmera incana* (light blue; New Caledonia, Loyalty Islands, and central Vanuatu). The Polynesian radiation is labeled green and includes: *Meliarchus sclateri*, *Guadalcanaria inexpectata*, *Gymnomyza viridis*, *G. samoensis*, *Xanthotis provocator*, and three species of *Foulehaio*. The dotted line around central Polynesia marks the area depicted in panel C. (C) Map of central Polynesia including Fiji, Samoa, and Tonga. The Polynesian clade is reproduced from the phylogeny in panel A, with each lineage colored to match its corresponding distribution on map panel C as follows: *Gymnomyza viridis* (orange; Viti Levu, Vanua Levu [to the exclusion of the Natewa Peninsula], and Taveuni, Fiji), *Xanthotis provocator* (red; Kadavu, Fiji), *Gymnomyza samoensis* (purple; Samoa), *Foulehaio procerior* (light green; Viti Levu, Fiji), *Foulehaio taviuensis* (blue; Vanua Levu, Kioa, and Taveuni, Fiji), *Foulehaio carunculatus* (yellow; Lau Archipelago, Fiji; Samoa; and Tonga). Note: colors correspond between panels A and B, but are unique in panel C. Photographs of six lineages in this radiation are depicted at right. Names throughout this figure follow the taxonomy proposed in the text.

# Figure 3.4


A
Outgroups
Vosea whitemanensis
Glycifohia undulata
Glycifohia notabilis
Stresemannia bougainvillei
Lichmera incana
Philemon eichhorni
Philemon cockerlli
Prosthemadera novaeseelandiae
Meliarchus sclateri
Guadalcanaria inexpectata
Gymnomyza viridis
Xanthotis provocator
Gymnomyza samoensis
Foulehaio procerior
Foulehaio taviuensis
Foulehaio carunculatus
0.04
sub/site
B
Bismarck Archipelago
New Britain
Vosea whitemanensis
Papua New Guinea
Bougainville
Stresemannia bougainvillei
Solomon Islands
Guadalcanal
Guadalcanaria inexpectata
Makira
Meliarchus sclateri
Australia
Vanuatu
Glycifohia notabilis
Lichmera incana
New Caledonia
Glycifohia undulata
Lichmera incana
Loyalty Islands
500 km
N
Samoa
Fiji
Tonga
map detail in panel C
C
Meliarchus sclateri
Guadalcanaria inexpectata
Gymnomyza viridis
Xanthotis provocator
Gymnomyza samoensis
Foulehaio procerior
Foulehaio taviuensis
Foulehaio carunculatus
Fiji
Tonga
N
250 km
Samoa
Upolu
Savai'i
Tutuila
Am. Samoa
G. samoensis
F. procerior
G. viridis
F. taviuensis
X. provocator
F. carunculatus

## Discussion

### *Overview*

Our phylogenetic results continue the recent trend of DNA sequences producing major discrepancies compared to traditional honeyeater taxonomy (e.g., Driskell and Christidis 2004; Nyári and Joseph 2011). The Pacific island taxa added to the meliphagid phylogenetic framework were reconstructed in several clades within the family, some corresponding to expected relationships but others were far from congeners. Below we discuss how our results impact taxonomy in the family and our understanding of evolution in the Pacific.

In addition to taxonomic discrepancies, our phylogeny supported topological differences from those reported by Driskell and Christidis (2004) and Gardner et al. (2010). For example, both studies found *Myzomela* to be more closely related to the clade containing *Philemon* than we did in this study (Fig. 3.3; clades A and E). We found this curious given that the backbone of our phylogeny was based on data from Driskell and Christidis (2004). One explanation could be the relative ease of conducting rigorous Bayesian analyses today than in 2004. Driskell and Christidis (2004) reported sampling their MrBayes MCMC chain for 1 million generations, whereas we accomplished 50 times as many generations in two days using modern computers. An alternative explanation is the discordance we noted in the mitochondrial ND2 gene tree versus the nuclear Fib5 gene tree. The former supported a closer relationship of *Myzomela* and *Philemon*, than did Fib5. Gardner et al. (2010) had sparse sampling because they focused more on higher-level relationships in the Meliphagoidea, an Australian clade containing Meliphagidae and related families. This could be one reason why their phylogeny is not in agreement with ours. Regardless, it is clear that much greater taxonomic sampling and genomic coverage would help to achieve the most robust estimate of meliphagid phylogeny.

*Taxonomy*

Our results suggest some taxonomic changes are needed. For example, *Melidectes whitemanensis* was found to be sister to *Myzomela*—an entirely unexpected placement in the tree—and completely unrelated to other *Melidectes* (clade G, Fig. 3.3). *Melidectes whitemanensis* is restricted to montane regions of New Britain; it is the only *Melidectes* out of ten species in the genus to be found outside of mainland New Guinea. We recommend resurrecting *Vosea* Gilliard, 1960, to which it was originally described. This monotypic genus reflects its unique placement within the phylogeny and the species' substantial morphological and ecological differentiation from *Myzomela* (a speciose genus of small, eco-morphologically conserved, sunbird-like honeyeaters). Despite the strong node support for *Vosea whitemanensis*, we are cautious with this recommendation because our results are based on incomplete data (602 bp of ND2 and 165 bp of Fib5).

Another taxon in need of nomenclatural revision is *Glycifohia*, which was found to be paraphyletic with respect to *Gliciphila melanops* of Australia. *Glycifohia* comprises two taxa endemic to Vanuatu and New Caledonia: *G. notabilis* and *G. undulata*, respectively. *Gliciphila* Swainson, 1837 has priority over *Glycifohia* Matthews, 1929, thus, we recommend treating all three species in *Gliciphila.* Alternatively, these taxa could be split into three monotypic genera. This option would maintain *Glycifohia undulata*, but necessitate a new genus for *notabilis*, which was originally described as *Glyciphila notabilis* Sharpe, 1899 (note the emended genus name with 'y' instead of 'i'). Clearly, treating all three species in one genus—*Gliciphila*—would help avoid such nomenclatural confusion and still correspond to the phylogeny.

*Foulehaio carunculatus* was found to contain three genetically well-differentiated clades, each pertaining to nominal subspecies (*carunculatus*, *procerior*, and *taviuensis*). These clades are allopatrically distributed across central Polynesia, and each clade is morphologically distinct and easily diagnosable. We suggest that *Foulehaio* is best treated as three species: *F. carunculatus*, *F. procerior*, and *F. taviuensis.* Further, *Xanthotis provocator* is only distantly related to the other *Xanthotis* species and should be removed from the genus. However, placing it in another genus is not without complication. It might be returned to *Foulehaio*, where it was occasionally placed (Salomonsen 1967), but the phylogeny (Fig. 3.3) indicates that *Gymnomyza samoensis* would render an expanded *Foulehaio* paraphyletic. Of the three *Gymnomyza* species, *G. samoensis* was described first, thus it holds priority; however, it was not the first to be placed in the genus. *Gymnomyza aubryana* was the first to be ascribed to the genus, whereas *G. samoensis* and *G. viridis* were in *Amoromyza* (Mayr 1944). Because we lack samples of *G. aubryana*, the type species of the genus, any changes to expand *Gymnomyza* to incorporate *X. provocator* and three species of *Foulehaio* are premature. An argument could be made to include the entire clade including *Meliarchus*, *Guadalcanaria, Gymnomyza*, *Xanthotis provocator*, and *Foulehaio* in *Amoromyza*, but for now, we recommend maintaining current taxonomy (e.g., Gill and Donsker 2013) until complete taxon sampling of this clade is achieved (e.g., *Gymnomyza aubryana*).

The Fijian endemic, *Gymnomyza viridis,* shows marked geographic variation between two nominal subspecies distributed across Fiji's largest three islands. *Gymnomyza v. viridis* is distributed on Vanua Levu and Taveuni Islands, whereas, *G. v. brunneirostris* occurs on Viti Levu. These subspecies are 4.2% diverged in ND2 (uncorrected P) and are phenotypically and behaviorally distinct, as well. For example, bill and leg color differs between them, and there are stark differences in vocalizations. Male-female pairs of *G. v. brunneirostris* sing loud duets that

are characteristic sounds of Viti Levu forests (see Macaulay Library 166522, http://macaulaylibrary.org/audio/166522), whereas the vocal repertoire of *G. v. viridis* is generally more reserved (ML 181332, http://macaulaylibrary.org/audio/181332), including soft chattering notes quite unlike individuals on Viti Levu (Watling 2004). It is worth noting that the level of genetic differentiation between *Gymnomyza viridis* subspecies matches closely that observed between *Foulehaio procerior* and *F. taviuensis* across the same distribution (4%). Clearly, *Gymnomyza viridis* deserves more attention in order to better delimit species in this group. At the very least, a genetic sample from Vanua Levu is necessary, and ideally, a detailed analysis of vocal differences across all populations in Fiji would help characterize differences. We suspect *G. viridis* represents two species, but refrain from making a formal recommendation at this time.

### *Biogeography and evolution*

Explanations for the large geographic disjunctions between some Pacific meliphagid clades and their continental sister taxa necessarily require overwater dispersal because these land areas have never been in contact. However, long distance dispersal directly between the regions is not required, and is unlikely. A series of stepping-stone dispersal events along the archipelagoes of Melanesia could produce the current distributions if intervening populations subsequently became extinct. The long stem lineages that connect *Vosea*, *Stresemannia*, *Guadalcanaria*, and *Meliarchus* to their respective sister taxa indicate relatively ancient separations from their respective common ancestors and are congruent with the hypothesis that these Pacific meliphagid lineages represent relictual distributions. All four lineages are single-island, montane endemics that are the result of at least three independent colonizations of the

Pacific. Bougainville and Guadalcanal share several montane conspecifics, including *Actenoides bougainvillei bougainvillei* and *A. b. excelsus*; *Pachycephala implicata richardsi* and *P. i implicata*; and *Rhipidura drownei drownei* and *R. d. ocularis*. To date, only the *Pachycephala* have been sequenced (Andersen et al., in press), which showed them to be sister, albeit old (7.9% diverged in ND2 uncorrected P). It is uncertain whether the *Actenoides* and *Rhipidura* from the highlands of Bougainville and Guadalcanal are sisters and to what level of divergence they have achieved, but our result—that *Stresemannia* and *Guadalcanaria* are not sister taxa—differs from the deeply diverged, but sister *Pachycephala* lineages.

In contrast, other meliphagid lineages colonized the Pacific relatively recently. Sequences from our sample of *Lichmera incana* from Vanuatu were only slightly diverged from those of *Lichmera indistincta* from Australia (1.6% ND2 divergence). Five described subspecies of *L. incana* are distributed throughout New Caledonia, the Loyalty Islands, and Vanuatu, of which we sampled only one, *L. i. incana*. Additional geographic sampling is needed to be certain, but the colonization of *L. incana* in the Pacific and subsequent differentiation appears to be quite recent. Furthermore, *Lichmera incana* forms a superspecies with *L. indistincta* of Australia and *L. limbata* of the Lesser Sunda Islands (Higgins et al. 2008). Together, these three species have 10 described subspecies, and although *L. limbata* was unsampled in this study, we suspect it to be minimally divergent, as well. A detailed phylogeographic study of *Lichmera* is warranted to better understand the colonization history of this clade in and out of Australia.

A striking contrast between old, restricted range species and young, dispersive species exists in the Polynesian endemic clade. Three forest-dwelling species branching sequentially from the base of the clade are restricted to one (*Meliarchus sclateri* and *Guadalcanaria inexpectata*) and three (*Gymnomyza viridis*) large islands in the Solomon Islands and Fiji,

respectively. Also in clade H, *Foulehaio* inhabits numerous islands of all sizes across three widely separated archipelagos (Fig. 3.4). This genus is divided into three clades separated by substantial genetic distance (ND2; 10%, 4%), and we argue these clades represent three distinct species. Two clades (*F. procerior* and *F. taviuensis*) are restricted to large islands in Fiji, whereas the third (*F. carunculatus*) spans the small remote islands of eastern Fiji and Tonga to the large islands of Samoa. Almost no genetic divergence separates individuals from Samoa, Tonga, and the remote Lau Archipelago of Fiji, indicating very recent colonization of these areas and/or ongoing gene flow. *Gymnomyza viridis* co-occurs with *Foulehaio procerior* on Viti Levu, Fiji and with *F. taviuensis* on Vanua Levu and Taveuni, Fiji. On these islands, *Foulehaio* can be found in all forested habitats, from mature primary forest to coastal mangroves, villages, and even city parks; whereas *Gymnomyza viridis* is restricted to large, mature forest blocks, which are generally found in the islands' montane interior. The occurrence of species with strikingly different ecology and apparent dispersal ability in the same clade is thought to require rapid shifts in these attributes over evolutionary time. Similar rapid shifts in other Pacific bird groups have been implicated in allowing rapid diversification in spite of apparent dispersal ability (Moyle et al. 2009). If viewed through the lens of taxon cycle-mediated shifts in dispersal and specialization (Wilson 1959, 1961; Ricklefs and Cox 1978), it is interesting to note that the Polynesian clade comprises multiple distinct phases.

Our results add to a growing list of studies demonstrating that avian diversity in the Pacific is poorly understood. A common theme among these studies is that divergent morphology has obscured true evolutionary relationships (Filardi and Moyle 2005; Moyle et al. 2009; Andersen et al. In press-b). In some groups, morphological divergence of island taxa obscured their close relationships to widespread and continental relatives (e.g., *Clytorhynchus*

and *Mayrornis*; Filardi and Moyle 2005). In contrast, the central Polynesian meliphagid radiation was not recognized because morphologically divergent island endemics were linked to similar genera in Australia and New Guinea (e.g., *Xanthotis* and *Glycifohia* [formerly *Phylidonyris*]), rather than to one another. Finally, many insular species are polytypic across their range and under-sampling in these lineages will often lead to under-representation of their true diversity (e.g., *Foulehaio* [this study] and *Ceyx*; Andersen et al. 2013). These conflicting diversification patterns highlight the need to sample insular species as broadly as possible across their distributions, rather than relying solely on single exemplars—a far too common practice in systematics today. Additionally, we caution against sampling biases towards continental radiations to the exclusion of Pacific island relatives. Doing so is likely to confound, not rectify, our understanding of phylogeny across both continental and insular lineages.

In the context of meliphagid systematics, our results demonstrate the dire state of our understanding of this group. The current phylogenetic framework, based on two molecular markers, is not sufficient to decipher many evolutionary relationships in the family; thus, a more comprehensive suite of markers, especially those offered by high-throughput sequencing (Faircloth et al. 2012) will be necessary to resolve many of these relationships. Because of remote island locales and permitting difficulties across numerous governments, many meliphagid species and distinct populations lack modern, vouchered tissue samples. However, these species will be necessary to produce a complete phylogenetic hypothesis of this enigmatic Australo-Pacific radiation. We advocate for renewed interest in field-based ornithology on Pacific islands to obtain these specimens and associated natural history data, which is vital for understanding honeyeater evolution, biogeography, and systematics.

# Chapter 4

## Rapid diversification of an insular kingfisher spans 13,000 km of the Pacific

Abstract

*Todiramphus chloris* (Aves: Alcedinidae) is the most widely distributed of the "great speciators" in the Pacific. Nearly 50 described subspecies of this kingfisher are distributed from the Red Sea to Polynesia, a distance of > 19,000 km. We reconstructed a molecular phylogeny of this enigmatic avian radiation from six genes and 157 individuals that spanned the entire Pacific distribution from the Marquesas to Singapore. The resulting phylogeny offers strong support that *T. chloris* radiated rapidly over an immense region of the Pacific. Genetic distances across the phylogeny were remarkably low, and molecular dating suggests that this radiation underwent extensive range expansion and diversification less than 1 Ma. Incredibly, several instances of sympatry have accumulated in this group on Australia, as well as far-flung oceanic islands, including Palau, Vanuatu, and the Solomon Islands. In each case of sympatry, significant eco-morphological and behavioral differences exist, suggesting that pre-mating isolating mechanisms were achieved rapidly during diversification. Our analyses found good node support cross the entire phylogeny, despite shallow internode distances. We revealed several complex radiations within the ingroup, as well as numerous novel relationships, which require major taxonomic revision throughout the entire species complex. Of the 22 species in the genus *Todiramphus*, ten were embedded within or closely related to *T. chloris*, including five species that radiated in the remote islands of Eastern Polynesian. Complex biogeographic patterns were inferred from the topology. A major phylogeographic break in the eastern Solomon Islands separates a Northern Melanesian clade from Polynesian taxa. Otherwise, the biogeographic origin of the *T. chloris* complex was equivocal, likely owing to the radiation's rapid origin. Systematic relationships within each clade are discussed in detail, and an updated taxonomy is proposed for *Todiramphus chloris* and its allies. This study makes a significant contribution to the study of diversification

on island systems and to the systematics of a classically polytypic avian species complex in the Pacific.

## Introduction

Islands have long been recognized as ideal natural laboratories for the study of evolution (Darwin 1859; Wallace 1881). Their isolation, discrete geographic boundaries, and relatively well-known geologic histories make them especially well-suited for studies on the tempo and mode of biological diversification. Indeed, islands spawned more than a quarter-century of intensive research on the ecology and evolution of insular species' distributions following seminal works by MacArthur and Wilson (1963, 1967). The islands of the southwest Pacific, in particular those of Melanesia, inspired major ideas on the processes of insular diversification such as taxon cycles (Wilson 1959, 1961), community assembly rules (MacArthur and Wilson 1967; Diamond 1975), the supertramp strategy hypothesis (Diamond 1974), and the paradox of the great speciators (Diamond et al. 1976; Diamond and Mayr 1976).

A conspicuous element of island bird faunas in the southwest Pacific is the profusion of widespread 'polytypic' species complexes (Mayr and Diamond 2001). These taxa occur on many islands—often across multiple archipelagos—and although apparently closely related, each island population may differ markedly in plumage pattern or coloration. Examples of these geographic radiations include the Variable Dwarf-Kingfisher complex (*Ceyx lepidus* sensu lato; Woodall 2001), the Golden Whistler complex (*Pachycephala pectoralis* sensu lato; Boles 2007), Island Thrush (*Turdus poliocephalus* sensu lato; Collar 2005), and *Monarcha* and *Symposiachrus* monarch-flycatchers (Coates et al. 2006). Classification of these distinct allopatric populations has hindered taxonomists, and under the Biological Species Concept (Mayr 1963) distinctive populations were merged into single 'species complexes' that include upwards of several dozen subspecies. Although a frustration for taxonomists, these broadly-distributed but well-differentiated populations have proved excellent study systems for the development of classic

concepts in evolutionary biology (Mayr 1942; Diamond 1974, 1975; Diamond et al. 1976), and more recently, using modern phylogenetic methods (Moyle et al. 2009; Uy et al. 2009a; Uy et al. 2009b; Andersen et al. 2013; Irestedt et al. 2013; Andersen et al. In press-b).

The most widespread example of a polytypic species complex is the Collared Kingfisher (*Todiramphus chloris*), which comprises 50 subspecies spanning a distance > 15,000 km from the Red Sea to Samoa, including India, mainland southeast Asia, the Sunda Shelf, and Australasia (Fry et al. 1992; Woodall 2001; Gill and Donsker 2013). The full geographic extent of the genus extends a further 3,000 km east to the Marquesas Islands in French Polynesia. Most subspecies correspond to single-island populations that are distinct in appearance, but some islands, including Palau, Vanuatu, several islands in the Solomon Islands, and Australia have multiple sympatric *Todiramphus* species. Most sympatric scenarios involve one *T. chloris* taxon and one non-*chloris* species, with even more in Australia. In situations of co-occuring congeners, the *T. chloris* subspecies is generally more disparate in plumage than its allopatric conspecifics. Additionally, these sympatric taxa exhibit ecological, morphological, and behavioral differences suggesting a high degree of reproductive isolation between each pair. Generally, Collared Kingfishers have turquoise blue-green backs with varying amounts of white or rufous below. Their eponymous white collar extends across the upper back and divides the blue back from the crown, which is variably blue or white. Also, a supercilium is variable both in extent and color, ranging from white to cinnamon. Subspecies differ not only in complex combinations of plumage, but in size, as well; there is nearly a two-fold difference in mass across all subspecies (Fry et al. 1992; Woodall 2001).

Traditionally, taxonomy of this group has been relegated to one polytypic ‘superspecies’ (Mayr 1931a). Little progress has been made on circumscribing geographically or phenotypically

cohesive groups, unlike similar species complexes that have received more attention recently: *Monarcha castaneiventris* (Uy et al. 2009a), *Ceyx lepidus* (Andersen et al. 2013), *Pachycephala pectoralis* (Jonsson et al. 2008a; Andersen et al. In press-b), *Erythropitta erythrogaster* (Irestedt et al. 2013), and *Turdus poliocephalus* (Peterson 2007; Jones and Kennedy 2008b). One previous study examined the higher-level phylogenetic relationships of kingfishers using molecular sequence data (Moyle 2006) and found *Todiramphus* to be a distinct clade separate from *Halcyon*. This first molecular result substantiated the long-held view that *Todiramphus* was unique and warranted its own genus (Mayr 1931a), however, nothing further could be said of the *T. chloris* species complex because only one such sample was included (Moyle 2006).

Here, we present the first phylogenetic analysis of the *Todiramphus chloris* species complex. We sampled both widely and densely across the distribution with a focus on Pacific lineages, and we included 15 additional *Todiramphus* species to investigate the monophyly of *T. chloris*. Additionally, we investigated species limits to propose an updated taxonomy of this widespread radiation.

## Methods

### *Taxon sampling*

Sampling included 157 individuals (Table 4.1, Fig. 4.1), including two *Syma* and 155 *Todiramphus* samples. Of the 155 *Todiramphus* samples, 93 were *T. chloris* and 62 were composed of 15 additional *Todiramphus* species: *T. cinnamominus*, *T. farquhari*, *T. gambieri*, *T. godeffroyi*, *T. leucopygius*, *T. macleayii*, *T. nigrocyaneus*, *T. pyrrhopygius*, *T. recurvirostris*, *T.*

Table 4.1. List of samples used in the study following the taxonomy of (Gill and Donsker 2013). Ancient DNA samples derived from museum specimens (i.e., toepads) and unvouchered blood samples are noted. Institutional abbreviations: AMNH, American Museum of Natural History; ANWC, Australian National Wildlife Collection; FMNH, Field Museum of Natural History; KUNHM, University of Kansas Natural History Museum; LSUMNS, Louisiana State University Museum of Natural Science; MHNG, Muséum d'histoire naturelle de la Ville de Genève; MNHN, Le Muséum National d'Histoire Naturelle; SNZP, Smithsonian National Zoological Park; UWBM, University of Washington Burke Museum.

| Genus | Species | Subspecies | Institution | Sample | Locality |
|---|---|---|---|---|---|
| Ingroup | | | | | |
| *Todiramphus* | *chloris* | *alberti* | UWBM | Bu60188 | SOLOMON ISLANDS: Isabel Is. |
| *Todiramphus* | *chloris* | *alberti* | UWBM | Bu60266 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Todiramphus* | *chloris* | *alberti* | UWBM | Bu60296 | SOLOMON ISLANDS: Kiaba Is. (north coast Isabel Is.) |
| *Todiramphus* | *chloris* | *alberti* | UWBM | Bu60320 | SOLOMON ISLANDS: Fera Is. (north coast Isabel Is.) |
| *Todiramphus* | *chloris* | *alberti* | UWBM | Bu60362 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Todiramphus* | *chloris* | *alberti* | UWBM | Bu63065 | SOLOMON ISLANDS: Choiseul Is. |
| *Todiramphus* | *chloris* | *alberti* | UWBM | Bu63233 | SOLOMON ISLANDS: Choiseul Is. |
| *Todiramphus* | *chloris* | *alberti* | UWBM | Bu66007 | SOLOMON ISLANDS: New Georgia Is. |
| *Todiramphus* | *chloris* | *alberti* | UWBM | Bu66038 | SOLOMON ISLANDS: New Georgia Is. |
| *Todiramphus* | *chloris* | *alberti* | AMNH | DOT6704 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Todiramphus* | *chloris* | *albicilla* | KUNHM | 22581 | NORTHERN MARIANA ISLANDS: Saipan Is. |
| *Todiramphus* | *chloris* | *albicilla* | KUNHM | 22591 | NORTHERN MARIANA ISLANDS: Saipan Is. |
| *Todiramphus* | *chloris* | *albicilla* | KUNHM | 22592 | NORHTERN MARIANA ISLANDS: Saipan Is. |
| *Todiramphus* | *chloris* | *albicilla* | KUNHM | 22603 | NORTHERN MARIANA ISLANDS: Saipan Is. |
| *Todiramphus* | *chloris* | *albicilla* | KUNHM | 22611 | NORTHERN MARIANA ISLANDS: Saipan Is. |
| *Todiramphus* | *chloris* | *amoenus* | UWBM | Bu58741 | SOLOMON ISLANDS: Rennell Is. |
| *Todiramphus* | *chloris* | *amoenus* | UWBM | Bu58743 | SOLOMON ISLANDS: Rennell Is. |
| *Todiramphus* | *chloris* | *amoenus* | AMNH | DOT6588 | SOLOMON ISLANDS: Rennell Is. |
| *Todiramphus* | *chloris* | *chloris* | AMNH | DOT12606 | INDONESIA: Sulawesi Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 13960 | PHILIPPINES: Camiguin Sur Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 13971 | PHILIPPINES: Camiguin Sur Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 14010 | PHILIPPINES: Camiguin Sur Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 14446 | PHILIPPINES: Tablas Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 14447 | PHILIPPINES: Tablas Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 17938 | PHILIPPINES: Batan Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 18130 | PHILIPPINES: Mindanao Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 18134 | PHILIPPINES: Mindanao Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 28455 | PHILIPPINES: Mindanao Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 28674 | PHILIPPINES: Mindanao Is. |
| *Todiramphus* | *chloris* | *collaris* | KUNHM | 20983 | PHILIPPINES: Bohol Is. |
| *Todiramphus* | *chloris* | *collaris* | UWBM | F358326 | PHILIPPINES: Sibuyan Is. |
| *Todiramphus* | *chloris* | *colonus* | SNZP | TKP2003070 | PNG: Louisiade Archipelago; Rossel Is. |

| Genus | Species | Subspecies | Institution | Sample | Locality |
|---|---|---|---|---|---|
| *Todiramphus* | *chloris* | *colonus* | SNZP | TKP2003071 | PNG: D'Entrecasteaux Archipelago; Duchess Is. |
| *Todiramphus* | *chloris* | *colonus* | SNZP | TKP2003089 | PNG: D'Entrecasteaux Archipelago; Tobwoiama Is. |
| *Todiramphus* | *chloris* | *colonus* | SNZP | TKP2003092 | PNG: D'Entrecasteaux Archipelago; Tobwoiama Is. |
| *Todiramphus* | *chloris* | *colonus* | SNZP | TKP2003097 | PNG: D'Entrecasteaux Archipelago; Tobwoiama Is. |
| *Todiramphus* | *chloris* | *eximius* | KUNHM | 25219 | FIJI: Kadavu Is. |
| *Todiramphus* | *chloris* | *eximius* | KUNHM | 25227 | FIJI: Kadavu Is. |
| *Todiramphus* | *chloris* | *humii* | UWBM | Bu67535 | SINGAPORE |
| *Todiramphus* | *chloris* | *humii* | UWBM | Bu76183 | SINGAPORE |
| *Todiramphus* | *chloris* | *humii* | UWBM | Bu76211 | SINGAPORE |
| *Todiramphus* | *chloris* | *laubmannianus* | UWBM | Bu81948 | MALAYSIA: Borneo; Sarawak |
| *Todiramphus* | *chloris* | *manuae* † | KUNHM | 104154 | AMERICAN SAMOA: Ta'ū Is. |
| *Todiramphus* | *chloris* | *manuae* † | KUNHM | 104156 | AMERICAN SAMOA: Ofu Is. |
| *Todiramphus* | *chloris* | *manuae* † | KUNHM | 104157 | AMERICAN SAMOA: Ta'ū Is. |
| *Todiramphus* | *chloris* | *manuae* † | KUNHM | 107630 | AMERICAN SAMOA: Ofu Is. |
| *Todirampus* | *chloris* | *marinus* | KUNHM | 26338 | FIJI: Lau Archipelago; Ogea Levu Is. |
| *Todiramphus* | *chloris* | *marinus* | KUNHM | 26342 | FIJI: Lau Archipelago; Ogea Driki Is. |
| *Todiramphus* | *chloris* | *marinus* | KUNHM | 26348 | FIJI: Lau Archipelago; Ogea Levu Is. |
| *Todiramphus* | *chloris* | *marinus* | KUNHM | 26369 | FIJI: Lau Archipelago; Namuka-i-Lau Is. |
| *Todiraphus* | *chloris* | *marinus* | KUNHM | 26383 | FIJI: Lau Archipelago; Fulaga Is. |
| *Todirampus* | *chloris* | *marinus* | KUNHM | 26393 | FIJI: Lau Archipelago; Fulaga Is. |
| *Todiramphu* | *chloris* | *marinus* | KUNHM | 26408 | FIJI: Lau Archipelago; Kabara Is. |
| *Todiramphus* | *chloris* | *marinus* | KUNHM | 26410 | FIJI: Lau Archipelago; Kabara Is. |
| *Todiramphus* | *chloris* | *marinus* | KUNHM | 26411 | FIJI: Lau Archipelago; Vuagava Is. |
| *Todiramphus* | *chloris* | *marinus* | KUNHM | 26439 | FIJI: Lau Archipelago; Vanua Vatu Is. |
| *Todiramphus* | *chloris* | *nusae* | KUNHM | 27723 | PNG: Bismarck Archipelago; New Ireland Is. |
| *Todiramphus* | *chloris* | *nusae* | KUNHM | 27753 | PNG: Bismarck Archipelago; New Ireland Is. |
| *Todirapmhus* | *chloris* | *nusae* | KUNHM | 27792 | PNG: Bismarck Archipelago; Nusalaman Is. |
| *Todiramphus* | *chloris* | *nusae* | KUNHM | 27793 | PNG: Bismarck Archipelago; Nusalaman Is. |
| *Todiramphus* | *chloris* | *nusae* | KUNHM | 27812 | PNG: Bismarck Archipelago; Nusalaman Is. |
| *Todirapmhus* | *chloris* | *nusae* | KUNHM | 27857 | PNG: Bismarck Archipelago; Dyaul Is. |
| *Todiramphus* | *chloris* | *orii* | UWBM | Bu85102 | NORTHERN MARIANA ISLANDS: Rota Is. |
| *Todiramphus* | *chloris* | *orii* | UWBM | Bu85104 | NORTHERN MARIANA ISLANDS: Rota Is. |
| *Todirampus* | *chloris* | *orii* | UWBM | Bu85105 | NORTHERN MARIANA ISLANDS: Rota Is. |
| *Todiramphus* | *chloris* | *ornatus* | KUNHM | 19404 | SOLOMON ISLANDS: Santa Cruz Group; Nendo Is. |
| *Todiramphus* | *chloris* | *pealei* † | KUNHM | 104160 | AMERICAN SAMOA: Tutuila Is. |
| *Todiramphus* | *chloris* | *pealei* † | KUNHM | 104164 | AMERICAN SAMOA: Tutuila Is. |
| *Todiramphus* | *chloris* | *pealei* | UWBM | Bu89771 | AMERICAN SAMOA: Tutuila Is. |
| *Todiramphus* | *chloris* | *sacer* | UWBM | Bu42835 | TONGA: 'Eua Is. |
| *Todiramphus* | *chloris* | *sacer* | UWBM | Bu42841 | TONGA: 'Eua Is. |
| *Todiramphus* | *chloris* | *sacer* | UWBM | Bu42904 | TONGA: 'Eua Is. |
| *Todiramphus* | *chloris* | *santoensis* | LSUMNS | B45831 | VANUATU: Espiritu Santo Is. |
| *Todiramphus* | *chloris* | *solomonis* | KUNHM | 12834 | SOLOMON ISLANDS: Makira Is. |
| *Todiramphus* | *chloris* | *solomonis* | KUNHM | 15921 | SOLOMON ISLANDS: Ugi Is. (north coast Makira Is.) |

| Genus | Species | Subspecies | Institution | Sample | Locality |
|---|---|---|---|---|---|
| *Todiramphus* | *chloris* | *solomonis* | KUNHM | 15922 | SOLOMON ISLANDS: Ugi Is. (north coast Makira Is.) |
| *Todiramphus* | *chloris* | *solomonis* | KUNHM | 15926 | SOLOMON ISLANDS: Ugi Is. (north coast Makira Is.) |
| *Todiramphus* | *chloris* | *sordidus* | ANWC | 33719 | AUSTRALIA: Northern Territory, NE Darwin |
| *Todiramphus* | *chloris* | *sordidus* | ANWC | 33720 | AUSTRALIA: Northern Territory, NE Darwin |
| *Todiramphus* | *chloris* | *colcloughi* | ANWC | 44296 | AUSTRALIA: Queensland; N Rockhampton |
| *Todiramphus* | *chloris* | *sordidus* | ANWC | 51462 | AUSTRALIA: Queensland; Cape York Peninsula |
| *Todiramphus* | *chloris* | *sordidus* † | KUNHM | 8589 | AUSTRALIA: Northern Territory, NE Darwin |
| *Todiramphus* | *chloris* | *teraokai* | KUNHM | 23630 | PALAU: Babeldaob Is. |
| *Todiramphus* | *chloris* | *teraokai* | KUNHM | 23631 | PALAU: Babeldaob Is. |
| *Todiramphus* | *chloris* | *teraokai* | KUNHM | 23690 | PALAU: Peleliu Is. |
| *Todiramphus* | *chloris* | *vitiensis* | KUNHM | 24247 | FIJI: Vanua Levu Is. |
| *Todiramphus* | *chloris* | *vitiensis* | KUNHM | 24248 | FIJI: Vanua Levu Is. |
| *Todiramphus* | *chloris* | *vitiensis* | KUNHM | 26496 | FIJI: Kioa Is. |
| *Todiramphus* | *chloris* | *vitiensis* | KUNHM | 26529 | FIJI: Vanua Levu Is. |
| *Todiramphus* | *chloris* | *vitiensis* | KUNHM | 30462 | FIJI: Viti Levu Is. |
| *Todiramphus* | *chloris* | *vitiensis* | KUNHM | 30469 | FIJI: Lomaiviti Group; Koro Is. |
| *Todiramphus* | *chloris* | *vitiensis* | KUNHM | 30489 | FIJI: Lomaiviti Group; Ovalau Is. |
| *Todiramphus* | *chloris* | *vitiensis* | KUNHM | 30504 | FIJI: Lomaiviti Group; Ovalau Is. |
| *Todiramphus* | *cinnamominus* | *cinnamominus*† | KUNHM | 47548 | MARIANA ISLANDS: Guam Is. |
| *Todiramphus* | *cinnamominus* | *pelewensis* | KUNHM | 23651 | PALAU: Babeldaob Is. |
| *Todiramphus* | *cinnamominus* | *pelewensis* | KUNHM | 23662 | PALAU: Babeldaob Is. |
| *Todiramphus* | *cinnamominus* | *pelewensis* | KUNHM | 23674 | PALAU: Peleliu Is. |
| *Todiramphus* | *cinnamominus* | *reichenbachii* † | KUNHM | 40147 | MICRONESIA: Pohnpei Is. |
| *Todiramphus* | *farquhari* | | LSUMNS | B45388 | VANUATU: Espiritu Santo Is. |
| *Todiramphus* | *farquhari* | | LSUMNS | B45401 | VANUATU: Espiritu Santo Is. |
| *Todiramphus* | *gambieri* | *gertrudae** | MHNG | PO3-43 | FRENCH POLYNESIA: Tuamotu Archipelago; Niau Is. |
| *Todiramphus* | *godeffroyi* † | | MNHN | 1822 | FRENCH POLYNESIA: Marquesas Archipelago; Tahuata Is. |
| *Todiramphus* | *godeffroyi* † | | MNHN | 1823 | FRENCH POLYNESIA: Marquesas Archipelago; Tahuata Is. |
| *Todiramphus* | *recurvirostris*† | | KUNHM | 104171 | SAMOA: Upolu Is. |
| *Todiramphus* | *recurvirostris*† | | KUNHM | 104172 | SAMOA: Upolu Is. |
| *Todiramphus* | *recurvirostris*† | | KUNHM | 104178 | SAMOA: Savai‘i Is. |
| *Todiramphus* | *recurvirostris*† | | KUNHM | 104181 | SAMOA: Savai‘i Is. |
| *Todiramphus* | *ruficollaris* | | UWBM | Bu42791 | COOK ISLANDS: Mangaia Is. |
| *Todiramphus* | *ruficollaris* | | UWBM | Bu42806 | COOK ISLANDS: Mangaia Is. |
| *Todiramphus* | *sanctus* | *canacorum* * | MNHN | NC10 | NEW CALEDONIA: xxxxx |
| *Todiramphus* | *sanctus* | *canacorum* * | MNHN | NC83 | NEW CALEDONIA: xxxxx |
| *Todiramphus* | *sanctus* | *sanctus* | ANWC | 34636 | AUSTRALIA: Northern Territory; SE Darwin |
| *Todiramphus* | *sanctus* | *sanctus* | ANWC | 34659 | AUSTRALIA: Western Australia; N Albany |
| *Todiramphus* | *sanctus* | *sanctus* | ANWC | 50292 | AUSTRALIA: Western Australia; NW Mt. Barker |
| *Todiramphus* | *sanctus* | *sanctus* | ANWC | 54622 | AUSTRALIA: Northern Territory; Roper River |
| *Todiramphus* | *sanctus* | *sanctus* | KUNHM | 7557 | PNG: Western Province |

| Genus | Species | Subspecies | Institution | Sample | Locality |
|---|---|---|---|---|---|
| *Todiramphus* | *sanctus* | *sanctus* | KUNHM | 7567 | PNG: xxxxx |
| *Todiramphus* | *sanctus* | *sanctus* | KUNHM | 19403 | SOLOMON ISLANDS: Santa Cruz Group; Nendo Is. |
| *Todiramphus* | *sanctus* | *sanctus* | LSUMNS | B45812 | VANUATU: Espiritu Santo Is. |
| *Todiramphus* | *sanctus* | *sanctus* | UWBM | Bu57468 | AUSTRALIA: New South Wales |
| *Todiramphus* | *sanctus* | *sanctus* | UWBM | Bu58750 | SOLOMON ISLANDS: Santa Isabel Is. |
| *Todiramphus* | *sanctus* | *sanctus* | UWBM | Bu62818 | AUSTRALIA: New South Wales |
| *Todiramphus* | *sanctus* | *sanctus* | UWBM | Bu63200 | SOLOMON ISLANDS: Choiseul Is. |
| *Todiramphus* | *sanctus* | *sanctus* | UWBM | Bu68059 | PNG: Bismarck Archipelago; Schumann Is. (north coast New Britain Is.) |
| *Todiramphus* | *sanctus* | *sanctus* | UWBM | Bu68062 | PNG: Bismarck Archipelago; Schumann Is. (north coast New Britain Is.) |
| *Todiramphus* | *sanctus* | *sanctus* | UWBM | Bu72545 | AUSTRALIA: Queensland |
| *Todiramphus* | *sanctus* | *sanctus* | UWBM | Bu76296 | SOLOMON ISLANDS: New Georgia Is. |
| *Todiramphus* | *sanctus* | *sanctus* | AMNH | DOT12594 | INDONESIA: Sulawesi Is. |
| *Todiramphus* | *sanctus* | *vagans* | KUNHM | 14877 | NEW ZEALAND: Aukland; Warkworth |
| *Todiramphus* | *sanctus* | *vagans* | KUNHM | 14879 | NEW ZEALAND: Auckland; Waiheke Is. |
| *Todiramphus* | *saurophagus* | *saurophagus* | KUNHM | 27804 | PNG: Bismarck Archipelago; Nusalaman Is. |
| *Todiramphus* | *saurophagus* | *saurophagus* | UWBM | Bu60204 | SOLOMON ISLANDS: Kiaba Is. (north coast Isabel Is.) |
| *Todiramphus* | *saurophagus* | *saurophagus* | UWBM | Bu60326 | SOLOMON ISLANDS: Hekelake Is. (north coast Isabel Is.) |
| *Todiramphus* | *saurophagus* | *saurophagus* | UWBM | Bu69666 | SOLOMON ISLANDS: Hekelake Is. (north coast Isabel Is.) |
| *Todiramphus* | *tutus* | *atiu* | UWBM | Bu42503 | COOK ISLANDS: Atiu Is. |
| *Todiramphus* | *tutus* | *atiu* | UWBM | Bu42504 | COOK ISLANDS: Atiu Is. |
| *Todiramphus* | *tutus* | *mauke* | UWBM | Bu42603 | COOK ISLANDS: Mauke Is. |
| *Todiramphus* | *tutus* | *mauke* | UWBM | Bu42604 | COOK ISLANDS: Mauke Is. |
| *Todiramphus* | *tutus* | *tutus* * | MHNG | HH7-60 | FRENCH POLYNESIA: Society Islands; Ra‘iatea Is. |
| *Todiramphus* | *tutus* | *tutus* * | MHNG | HH7-62 | FRENCH POLYNESIA: Society Islands; Ra‘iatea Is. |
| *Todiramphus* | *veneratus* | *veneratus* * | MHNG | PO2-88 | FRENCH POLYNESIA: Society Islands; Tahiti Is. |
| *Todiramphus* | *veneratus* | *youngi* * | MHNG | HH7-75 | FRENCH POLYNESIA: Society Islands; Mo‘orea Is. |
| *Todiramphus* | *veneratus* | *youngi* * | MHNG | HH7-77 | FRENCH POLYNESIA: Society Islands; Mo‘orea Is. |
| Outgroup | | | | | |
| *Syma* | *megarhyncha* | | KUNHM | 7143 | PNG: Morobe Province |
| *Syma* | *torotoro* | | KUNHM | 5215 | PNG |
| *Todiramphus* | *leucopygius* | | KUNHM | 15882 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Todiramphus* | *leucopygius* | | KUNHM | 15901 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Todiramphus* | *leucopygius* | | KUNHM | 15902 | SOLOMON ISLANDS: Guadalcanal Is. |
| *Todiramphus* | *leucopygius* | | AMNH | DOT6654 | SOLOMON ISLANDS: Isabel Is. |
| *Todiramphus* | *macleayii* | | ANWC | Au33585 | AUSTRALIA |
| *Todiramphus* | *nigrocyaneus* | | KUNHM | 5294 | PNG: Gulf Province |
| *Todiramphus* | *pyrrhopygius* | | ANWC | Au32904 | AUSTRALIA |
| *Todiramphus* | *winchelli* | *nesydrionetes* | KUNHM | 14453 | PHILIPPINES: Tablas Is. |
| *Todiramphus* | *winchelli* | *nesydrionetes* | KUNHM | 14490 | PHILIPPINES: Tablas Is. |
| *Todiramphus* | *winchelli* | *nesydrionetes* | FMNH | F358323 | PHILIPPINES: Sibuyan Is. |
| *Todiramphus* | *winchelli* | *nigroroum* | KUNHM | 14302 | PHILIPPINES: Leyte Is. |

| Genus | Species | Subspecies | Institution | Sample | Locality |
|---|---|---|---|---|---|
| *Todiramphus* | *winchelli* | *nigroroum* | KUNHM | 28186 | PHILIPPINES: Bohol Is. |

† Samples from museum toepads.
* Samples from unvouchered blood.

*ruficollis*, *T. sanctus*, *T. saurophagus*, *T. tutus*, *T. veneratus*, and *T. winchelli*. Only six *Todiramphus* species were lacking (*T. diops*, *T. lazuli*, *T. albonotatus*, *T. funebris*, *T. enigma*), owing to their distribution in areas where collecting fresh genetic source material is difficult. Our *T. chloris* sampling included 22 of 50 described subspecies (Gill and Donsker 2013). The phylogenetic placement of *Todiramphus* was shown to be a clade distinct from *Halcyon* and sister to *Syma* (Moyle 2006), therefore, we used *S. megarhyncha* and *S. torotoro* as outgroups to root trees. Whenever possible we sequenced multiple individuals per population (i.e., per island) to guard against errors of misidentification, mislabeling, or sample contamination.

### *DNA sequencing*

Total genomic DNA was extracted from frozen or alcohol-preserved muscle tissue using a noncommercial guanidine thiocyanate method (Esselstyn et al. 2008). All muscle tissue samples have associated museum study-skin vouchers. For taxa with no available tissue samples, DNA was extracted from toepads of museum study skins (Table 4.1) using a QIAamp DNA mini extraction kit (Qiagen) in lab space separate from other *Todiramphus* pre-PCR products to minimize contamination risk (Mundy et al. 1997). Several unvouchered blood samples were used from remote islands in French Polynesia where collection of vouchered specimen material was not possible owing to small population sizes of endangered taxa (e.g., *T. gambieri*; Table 4.1).

We sequenced the entire second and third subunits of mitochondrial nicotinamide adenine dinucleotide dehydrogenase (hereafter ND2 and ND3, respectively) and four nuclear gene regions: the coiled-coil domain containing protein 132 (CCDC132), the high mobility group protein B2 (HMGB2), the second intron of the nuclear myoglobin gene (Myo2), and the fifth intron of the transforming growth factor β2 (TGFβ2). Target DNA fragments were

amplified using polymerase chain reaction (PCR) with external and internal primers. External primers were as follows: L5215 (ND2, Hackett 1996) and H6313 (ND2, Johnson and Sorenson 1998), L10755 and H11151 (ND3, Chesser 1999), CDC132L and CDC132H (Backström et al. 2008), HMG2L and HMG2H (Backström et al. 2008), MUSK-I3F and MUSK-I3R (Kimball et al. 2009), and TGF5 and TGF6 (Primmer et al. 2002). We modified external primers for CCDC and HMGB2 to better suit *Todiramphus* and designed internal primers to amplify 200–250 bp fragments of toepad samples (Table 4.2).

PCR amplifications were performed in 13 μl reactions using Promega Go*Taq* DNA polymerase. A touchdown protocol was used in PCR for ND2, ND3, CCDC132, and HMGB2 with annealing temperatures of 58, 54, and 50° C. Annealing temperatures were held constant for MUSK (50° C) and TGF (58° C). Amplified PCR products were screened on high-melt, 1% agarose gels stained with GelRed, and purified with 10% Exo-SAP-IT™ (GE Healthcare Bio-Sciences Corp.). We cycle-sequenced purified PCR products in both directions with the same primers used in PCR for 25 cycles using the ABI Big Dye Terminator Cycle-Sequencing Kit version 3.1 (Applied Biosystems Inc., Foster City, CA). Sequencing was performed on an ABI Prism 3730 high-throughput capillary electrophoresis DNA analyzer.

### *Model selection and phylogenetic analysis*

Sequence contigs were assembled in Geneious 6.1 and individual nuclear intron alignments were constructed by hand and checked against an automated alignment in MUSCLE (Edgar 2004). Intron alignments were trimmed using the external sequencing primers (CDC132L.Todi, CDC132H.Todi; HMG2L.Todi, HMG2H.Todi; MUSK-I3F, MUSK-I3R; TGF5, TGF6, respectively). Appropriate models of sequence evolution for each of the seven

Table 4.2. Newly-designed primers to sequence samples derived from museum specimen toe pads.

| Locus | Primer name | 5’ to 3’ sequence |
|---|---|---|
| CDC132 | CDC132H.Todi | CTCCAACTTGCATCAGCCTG |
| | CDC132L.Todi | CTGTCTAACTTCAAATACGACGAC |
| | CDC132H.Todi.int | GAGACCTCATTAGGCAGG |
| | CDC132L.Todi.int | AGTGCCGGTCTCTCTTTCTT |
| HMG2 | HMG2H.Todi | GCTCTTGGCACGATATGCCG |
| | HMG2L.Todi | GGTCTGAACAGTCGGCAAAAG |
| | HMG2H.Todi.int | GGGATTTCCATGCTTACAGC |
| | HMG2L.Todi.int | AGTGTTTGTCAGCCTTTTCCA |
| MUSK | MUSK.Todi.IntF | GTCCAGATGCTGCTGAATG |
| | MUSK.Todi.IntR | TGACACACTCACTCATCCCTGT |
| ND2 | Todi190L | AATTAAATACTTCCTGGTCCAAG |
| | Todi410L | ATCAACAATAATAAAATTTCC |
| | Todi452L | AACATCTCACTCCCTAAACCC |
| | Todi625L | ACCCTATTAACTTTCTACCTGTAC |
| | Todi822L | CAAGAACTAACTAAACAAGA |
| | Todi897L | ACCTACGTCTCGCATACTAC |
| | Todi230H | GTCCTGTCTGYCAGGCAT |
| | Todi232H | CTCATTGTCCTGTCTGTCAGGC |
| | Todi465H | TGCTGATATTAAGGCTATTAGG |
| | Todi618H | CGGTTATTAGGGAGTACAGG |
| | Todi648H | ATTTTGTTGTGTTAAGTGAGAGG |
| | Todi890H | GGTGATTGTTGAGTAGTATG |
| ND3 | 160L.ND3.Todi | AATCCGATTCTTCCTCAGTAG |
| | 218L.ND3.Todi | GACCTAGAAATCGCCCTCC |
| | 227H.ND3.Todi | TAGTTGGATGGCTCAGGGGAG |
| TGF5 | TGF5.Todi.int | CTCTGGGATGATTACCAGACCC |
| | TGF6.Todi.int | CTCTCTGAGTAGGTGAGCACAT |

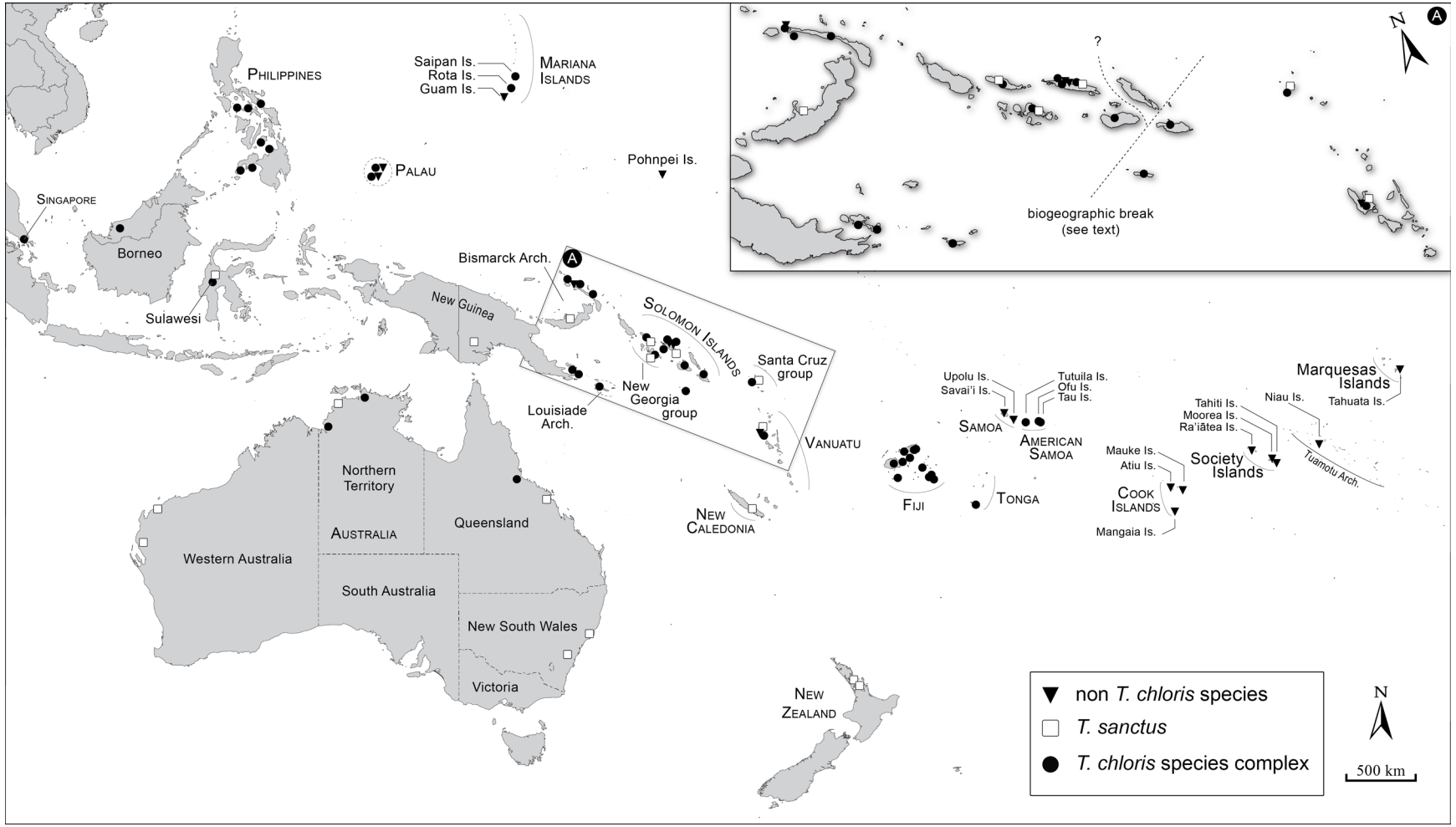



Figure 4.1. Sampling sites for ingroup *Todiramphus* used in this study. Color-coded circles, squares, and triangles represent sampling points for *T. chloris*, *T. sanctus*, and other ingroup taxa, respectively, with colors to match the clades in Figure 2. Points are not scaled to the number of sampled individuals per locality (the reader is referred to Table 4.1 for sampling numbers). Inset A shows greater detail in Melanesia, from the Bismarck Archipelago to New Caledonia.

Table 4.3. Summary statistics of the six gene regions sequenced in this study.

| Locus | Aligned length | Category, chromosome # | Substitution model | A, C, G, T frequency | Variable sites | Informative sites | Source |
|---|---|---|---|---|---|---|---|
| CCDC132 | 730 | intron, 2 | HKY+I+G | 0.260, 0.159, 0.237, 0.344 | 66 | 39 | (Backström et al. 2008) |
| HMGB2 | 533 | intron, 4 | HKY+I+G | 0.25, 0.25, 0.25, 0.25 | 64 | 56 | (Backström et al. 2008) |
| MUSK | 600 | intron, Z | HKY+G | 0.284, 0.198, 0.210, 0.309 | 55 | 37 | (Kimball et al. 2009) |
| TGFβ2 | 552 | intron, 3 | HKY+I | 0.25, 0.25, 0.25, 0.25 | 43 | 28 | (Primmer et al. 2002) |
| ND2+ND3 | 1041+351 | mitochondrial | | | | | |
| | | codon pos. 1: | HKY+G | 0.351, 0.315, 0.152, 0.182 | 108 | 89 | (Sorenson et al. 1999) |
| | | codon pos. 2: | HKY+I | 0.181, 0.332, 0.115, 0.372 | 48 | 31 | |
| | | codon pos. 3: | GTR+I+G | 0.467, 0.360, 0.069, 0.104 | 271 | 219 | |

partitions were identified (Table 4.3) using Akaike's Information Criterion (AIC), as implemented in MrModelTest 2.3 (Nylander 2004).

Phylogenetic reconstruction was performed on the total concatenated data, on separate concatenated mtDNA and nDNA, and separately on each individual locus. Maximum Likelihood (ML) heuristic tree searches were performed using GARLI 2.0 (Zwickl 2006). To avoid local optima, 250 independent searches were performed, each starting from a random tree. GARLI's default parameters were adjusted to terminate searches when no topological improvements were found after 100,000 generations (genthreshfortopoterm = 100000); otherwise, default settings were used. We selected the topology with the best likelihood as our maximum-likelihood estimate. Statistical support for this topology was obtained by running 1000 non-parametric bootstrap replicates (Felsenstein 1985) in GARLI to assess clade credibility and SumTrees 3.3.1, part of the DendroPy 3.12.0 package (Sukumaran and Holder 2010), was used to create a 50% majority-rule consensus tree. Nodes with >70% bootstrap support were considered well-supported (Hillis and Bull 1993; Wilcox et al. 2002).

Bayesian analysis (BA) was conducted using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003; Altekar et al. 2004; Ronquist et al. 2012) implemented with BEAGLE (Ayres et al. 2012). The data were partitioned by codon position for mtDNA and by gene for the nuclear introns. Four independent Markov chain Monte Carlo (MCMC) runs of 25 million generations were conducted using four chains per run (nchains=4) and incremental heating of chains (temp=0.1), sampling every 2,500 generations. TRACER 1.5 (Rambaut and Drummond 2007) and Are We There Yet? (AWTY?; Wilgenbusch et al. 2004; Nylander et al. 2008) were used to assess convergence of parameter estimates and tree splits, respectively. The average standard deviation of split frequencies (ASDSF) and the potential scale reduction factor (PSRF) were used to determine topology convergence between runs. The appropriate burn-in generations (25% for all analyses) were discarded based on convergence assessments of the ASDSF passing below 0.01. The remaining trees were summarized in a 50% majority-rule consensus tree.

*Molecular dating and species delimitation*

Divergence time estimation of *Todiramphus* was conducted in BEAST v1.7.5 (Drummond et al. 2002; Drummond et al. 2012) implemented with BEAGLE (Ayres et al. 2012). We pruned the dataset (n=81) to include two individuals per nominal subspecies for all *Todiramphus* taxa except *T. sanctus*, which we pruned samples from non-breeding localities (e.g., New Guinea, southeast Asia; Table 1). Partitioning schemes were identical to the MrBayes run described above with the addition of a birth-death speciation process for the tree prior. We tested for clock-like evolution by comparing likelihoods of runs with a strict clock to those with a relaxed lognormal clock, and failed to reject a strict molecular clock using likelihood ratio test (p=1.0). Ten independent MCMC chains were run for 100 million generations and sampled

every 20,000$^{th}$ generation. Burn-in and convergence diagnostics of each run were examined in TRACER 1.5 (Rambaut and Drummond 2007). Burn-in values were set specific to each run; at least 25% of samples were discarded, with some runs requiring up to 40% burn-in. Lacking fossil calibration data for this group, we relied on published rates of mitochondrial DNA sequence evolution to date our phylogeny. Specifically, we used the range of ND2 sequence divergence published for Hawaiian honeycreepers (2.4–3.3% per lineage Myr-1; Lerner et al. 2011).

We delimited species based on mtDNA sequences of all *Todiramphus* samples using a Bayesian implementation of the general mixed Yule-coalescent model as implemented in the R package bGMYC (Reid and Carstens 2012). This extension of the original GMYC species delimitation model (Pons et al. 2006) uses a distribution of trees instead of a single point estimate, thereby accounting for phylogenetic uncertainty. The GMYC model is beneficial for single-locus datasets such as those generated by DNA barcodes. We employed the model to *Todiramphus* because the majority of the phylogenetic signal in our dataset exists in the mtDNA. Other species delimitation models that use the coalescent rely on far greater numbers of independent loci than we sequenced, thus, these methods were not viable here (Carstens et al. 2013).

## Results

### *Sequence attributes*

The aligned dataset was 3,807 bp and included 157 samples (summary statistics presented in Table 4.3). All new sequences are deposited in GenBank. We obtained complete DNA sequences for all genes for all fresh samples. For samples from museum skins, or for those

downloaded from GenBank, it was not possible to obtain complete sequences for certain genes. Alignment lengths were 1,041 bp (ND2), 352 bp (ND3), 730 bp (CCDC132), 533 bp (HMGB2), 600 bp (MUSK), and 552 bp (TGF). The ND3 gene sequence contained a single cytosine insertion at position 174 in all samples, an insertion reported in several other bird groups and turtles (Mindell et al. 1998). This insertion does not disrupt the reading frame because it is not translated. Apart from this insertion in ND3, the mitochondrial data showed no other insertions, deletions, or anomalous stop-codons; thus, there was no evidence that mtDNA sequences were of nuclear origin (i.e., pseudogenes; Sorenson and Quinn 1998). The relative divergence among codon positions was typical for mtDNA (3 > 1 > 2). Numerous short indels were noted throughout the nuclear dataset (Table 4.4). The aligned dataset contained 655 variable sites (17.2%) and 499 (13.1%) parsimony-informative sites. Pairwise distances in ND2 (uncorrected P) between different nominal subspecies ranged from 0.01 % (*T. c. orii* and *T. c. albicilla*) to 2.3 % (*T. c. humii* and *T. c. vitiensis*). The basal split between *Syma* + *T. nigrocyaneus* and the rest of *Todiramphus* was 12.6 % diverged in ND2 (uncorrected P).

### *Phylogenetic relationships*

The topologies inferred from multiple independent ML and BA runs were highly concordant. Stationarity was achieved in MrBayes (i.e., the ASDSF remained < 0.01) after 8.15 million generations. The PSRF values for all parameters were 1.0. We report well-supported nodes as defined by Bayesian posterior probability (PP) > 0.95 and ML bootstrap (BS) > 70. Individual gene trees were largely uniformative, except for mtDNA. No conflicting topologies were strongly supported between indivdiual gene tree analyses (results not shown).

The ingroup was defined by a well-supported clade that included all *T. chloris* samples

Figure 4.2. Molecular phylogeny of the *Todiramphus chloris* species complex. The tree is the Bayesian maximum consensus tree from the concatenated, partitioned analysis. Node support is denoted as Bayesian posterior probabilities and maximum likelihood bootstrap support, above and below the nodes, respectively. Lettered clades (A–I) are discussed in the text.

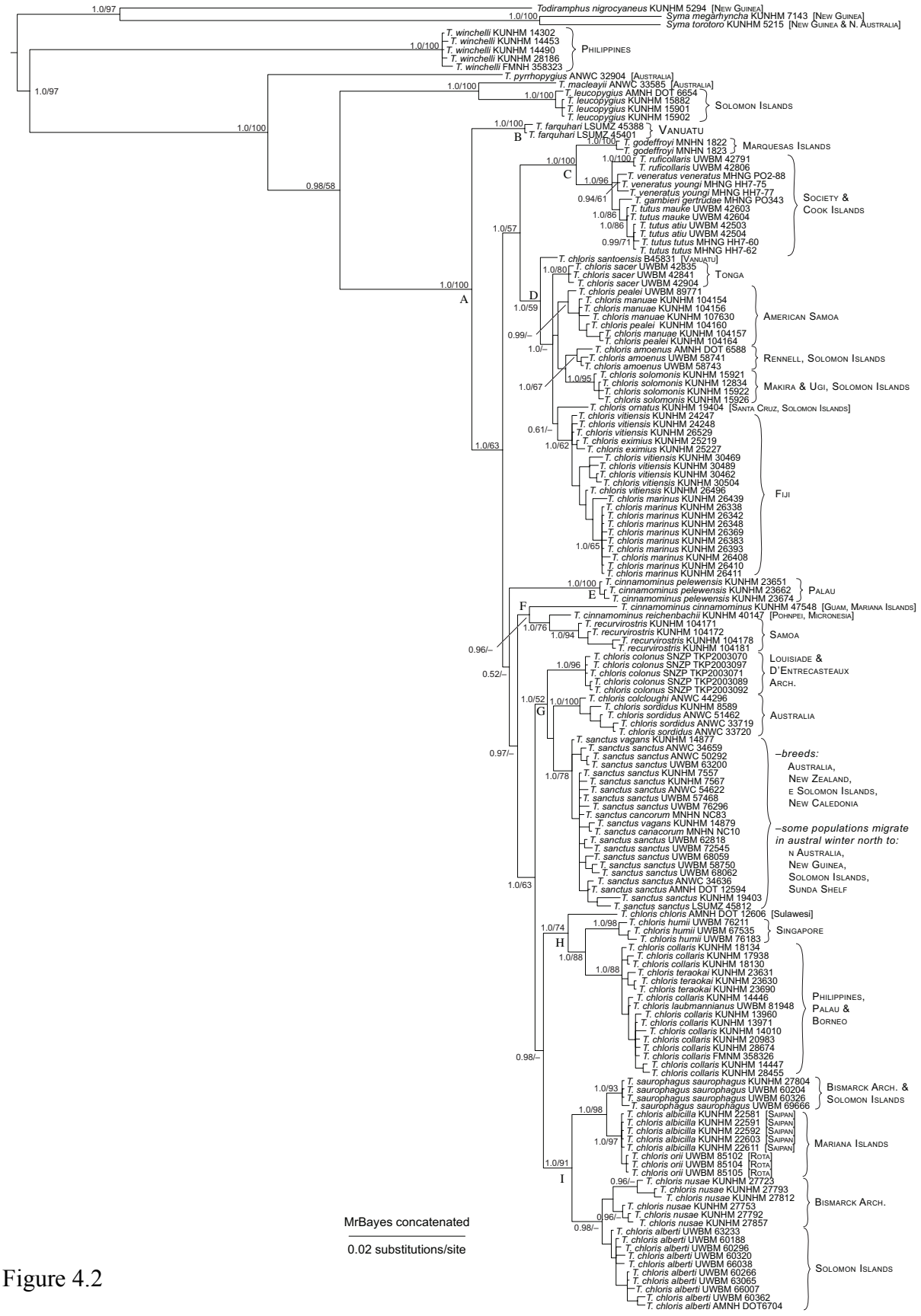

Todiramphus nigrocyaneus KUNHM 5294 [New Guinea]
Syma megarhyncha KUNHM 7143 [New Guinea]
Syma torotoro KUNHM 5215 [New Guinea & N. Australia]
T. winchelli KUNHM 14302
T. winchelli KUNHM 14453
T. winchelli KUNHM 14490
T. winchelli KUNHM 28186
T. winchelli FMNH 358323
Philippines
T. pyrrhopygius ANWC 32904 [Australia]
T. macleayii ANWC 33585 [Australia]
T. leucopygius AMNH DOT 6654
T. leucopygius KUNHM 15882
T. leucopygius KUNHM 15901
T. leucopygius KUNHM 15902
Solomon Islands
T. farquhari LSUMZ 45388
T. farquhari LSUMZ 45401
Vanuatu
B
T. godeffroyi MNHN 1822
T. godeffroyi MNHN 1823
Marquesas Islands
C
T. ruficollaris UWBM 42791
T. ruficollaris UWBM 42806
T. veneratus veneratus MHNG PO2-88
T. veneratus youngi MHNG HH7-75
T. veneratus youngi MHNG HH7-77
T. gambieri gertrudae MHNG PO343
T. tutus mauke UWBM 42603
T. tutus mauke UWBM 42604
T. tutus atiu UWBM 42503
T. tutus atiu UWBM 42504
T. tutus tutus MHNG HH7-60
T. tutus tutus MHNG HH7-62
Society & Cook Islands
T. chloris santoensis B45831 [Vanuatu]
T. chloris sacer UWBM 42835
T. chloris sacer UWBM 42841
T. chloris sacer UWBM 42904
Tonga
A
D
T. chloris pealei UWBM 89771
T. chloris manuae KUNHM 104154
T. chloris manuae KUNHM 104156
T. chloris manuae KUNHM 107630
T. chloris pealei KUNHM 104160
T. chloris manuae KUNHM 104157
T. chloris pealei KUNHM 104164
American Samoa
T. chloris amoenus AMNH DOT 6588
T. chloris amoenus UWBM 58741
T. chloris amoenus UWBM 58743
Rennell, Solomon Islands
T. chloris solomonis KUNHM 15921
T. chloris solomonis KUNHM 12834
T. chloris solomonis KUNHM 15922
T. chloris solomonis KUNHM 15926
Makira & Ugi, Solomon Islands
T. chloris ornatus KUNHM 19404 [Santa Cruz, Solomon Islands]
T. chloris vitiensis KUNHM 24247
T. chloris vitiensis KUNHM 24248
T. chloris vitiensis KUNHM 26529
T. chloris eximius KUNHM 25219
T. chloris eximius KUNHM 25227
T. chloris vitiensis KUNHM 30469
T. chloris vitiensis KUNHM 30489
T. chloris vitiensis KUNHM 30462
T. chloris vitiensis KUNHM 30504
T. chloris vitiensis KUNHM 26496
T. chloris marinus KUNHM 26439
T. chloris marinus KUNHM 26338
T. chloris marinus KUNHM 26342
T. chloris marinus KUNHM 26348
T. chloris marinus KUNHM 26369
T. chloris marinus KUNHM 26383
T. chloris marinus KUNHM 26393
T. chloris marinus KUNHM 26408
T. chloris marinus KUNHM 26410
T. chloris marinus KUNHM 26411
Fiji
E
T. cinnamominus pelewensis KUNHM 23651
T. cinnamominus pelewensis KUNHM 23662
T. cinnamominus pelewensis KUNHM 23674
Palau
F
T. cinnamominus cinnamominus KUNHM 47548 [Guam, Mariana Islands]
T. cinnamominus reichenbachii KUNHM 40147 [Pohnpei, Micronesia]
T. recurvirostris KUNHM 104171
T. recurvirostris KUNHM 104172
T. recurvirostris KUNHM 104178
T. recurvirostris KUNHM 104181
Samoa
T. chloris colonus SNZP TKP2003070
T. chloris colonus SNZP TKP2003097
T. chloris colonus SNZP TKP2003071
T. chloris colonus SNZP TKP2003089
T. chloris colonus SNZP TKP2003092
Louisiade & D'Entrecasteaux Arch.
G
T. chloris colcloughi ANWC 44296
T. chloris sordidus KUNHM 8589
T. chloris sordidus ANWC 51462
T. chloris sordidus ANWC 33719
T. chloris sordidus ANWC 33720
Australia
T. sanctus vagans KUNHM 14877
T. sanctus sanctus ANWC 34659
T. sanctus sanctus ANWC 50292
T. sanctus sanctus UWBM 63200
T. sanctus sanctus KUNHM 7557
T. sanctus sanctus KUNHM 7567
T. sanctus sanctus ANWC 54622
T. sanctus sanctus UWBM 57468
T. sanctus sanctus UWBM 76296
T. sanctus cancorum MNHN NC83
T. sanctus vagans KUNHM 14879
T. sanctus canacorum MNHN NC10
T. sanctus sanctus UWBM 62818
T. sanctus sanctus UWBM 72545
T. sanctus sanctus UWBM 68059
T. sanctus sanctus UWBM 58750
T. sanctus sanctus UWBM 68062
T. sanctus sanctus ANWC 34636
T. sanctus sanctus AMNH DOT 12594
T. sanctus sanctus KUNHM 19403
T. sanctus sanctus LSUMZ 45812
–breeds:
Australia,
New Zealand,
e Solomon Islands,
New Caledonia
–some populations migrate in austral winter north to:
n Australia,
New Guinea,
Solomon Islands,
Sunda Shelf
T. chloris chloris AMNH DOT 12606 [Sulawesi]
T. chloris humii UWBM 76211
T. chloris humii UWBM 67535
T. chloris humii UWBM 76183
Singapore
H
T. chloris collaris KUNHM 18134
T. chloris collaris KUNHM 17938
T. chloris collaris KUNHM 18130
T. chloris teraokai KUNHM 23631
T. chloris teraokai KUNHM 23630
T. chloris teraokai KUNHM 23690
T. chloris collaris KUNHM 14446
T. chloris laubmannianus UWBM 81948
T. chloris collaris KUNHM 13960
T. chloris collaris KUNHM 13971
T. chloris collaris KUNHM 14010
T. chloris collaris KUNHM 20983
T. chloris collaris KUNHM 28674
T. chloris collaris FMNH 358326
T. chloris collaris KUNHM 14447
T. chloris collaris KUNHM 28455
Philippines, Palau & Borneo
T. saurophagus saurophagus KUNHM 27804
T. saurophagus saurophagus UWBM 60204
T. saurophagus saurophagus UWBM 60326
T. saurophagus saurophagus UWBM 69666
Bismarck Arch. & Solomon Islands
T. chloris albicilla KUNHM 22581 [Saipan]
T. chloris albicilla KUNHM 22591 [Saipan]
T. chloris albicilla KUNHM 22592 [Saipan]
T. chloris albicilla KUNHM 22603 [Saipan]
T. chloris albicilla KUNHM 22611 [Saipan]
T. chloris orii UWBM 85102 [Rota]
T. chloris orii UWBM 85104 [Rota]
T. chloris orii UWBM 85105 [Rota]
Mariana Islands
I
T. chloris nusae KUNHM 27723
T. chloris nusae KUNHM 27793
T. chloris nusae KUNHM 27812
T. chloris nusae KUNHM 27753
T. chloris nusae KUNHM 27792
T. chloris nusae KUNHM 27857
Bismarck Arch.
T. chloris alberti UWBM 63233
T. chloris alberti UWBM 60188
T. chloris alberti UWBM 60296
T. chloris alberti UWBM 60320
T. chloris alberti UWBM 66038
T. chloris alberti UWBM 60266
T. chloris alberti UWBM 63065
T. chloris alberti UWBM 66007
T. chloris alberti UWBM 60362
T. chloris alberti AMNH DOT6704
Solomon Islands
MrBayes concatenated
0.02 substitutions/site

Figure 4.2

Table 4.4. Description of indels in nuclear sequence data. Sample numbers are listed if an indel was not shared by all members of a taxon, otherwise only taxon names are given. The sequence position is numbered from the 5' end of the alignment. The relative type depends on whether the insertion or deletion is most common in the alignment (e.g., deletion is the relative type if fewer taxa possess it).

| Locus | Position | # bp | Relative type | Taxa |
|---|---|---|---|---|
| CCDC132 | 160 | 1 | deletion | *Syma megarhyncha*, *S. torotoro*, *T. leucopygius*, *T. macleayii*, *T. nigrocyaneus*, *T. pyrrhopygius*, *Todiramphus chloris albicilla* (KUNHM 22581, 22591, 22592, 22603, 22611*), *T. chloris nusae* (KUNHM 27792, 27857), *T. chloris alberti* (UWBM 60266, 60296, 60320, 60362, 63233, 66007, AMNH DOT6704), *T. chloris orii*, *T. chloris colonus* (SNZP TKP2003070), *T. saurophagus*, *T. winchelli* |
| HMGB2 | 79–83 | 5 | deletion | *Syma megarhyncha*, *S. torotoro*, *T. nigrocyaneus* |
| HMGB2 | 144 | 1 | insertion | *T. nigrocyaneus* |
| HMGB2 | 314–315 | 2 | deletion | *Syma megarhyncha*, *S. torotoro* |
| HMGB2 | 398 | 1 | insertion | *T. nigrocyaneus* |
| HMGB2 | 446 | 1 | insertion | *Syma megarhyncha*, *S. torotoro* |
| HMGB2 | 457 | 1 | deletion | *Syma megarhyncha*, *S. torotoro* |
| HMGB2 | 507 | 1 | deletion | *Syma megarhyncha*, *S. torotoro*, *T. nigrocyaneus*, *T. winchellii* |
| MUSK | 148–151 | 4 | insertion | *Syma megarhyncha*, *S. torotoro* |
| MUSK | 554 | 1 | deletion | *T. nigrocyaneus* |
| TGF5 | 539 | 1 | deletion | *Syma megarhyncha*, *S. torotoro* |

* Data were missing from the CCDC132 intron for this sample.

plus ten additional *Todiramphus* species (Fig. 4.2, clade A: PP=1.0, BS=100). We defined the ingroup inclusive of *Todiramphus farquhari* because this circumscribed a suite of eleven closely related species that were significantly differentiated from all other *Todiramphus* taxa (i.e., ougroups). Clade A contained seven subclades (Fig. 4.2, clades B–I), each with PP=1.0, except clade F (PP=0.96). Of the ten non-*T. chloris* species in the ingroup, clade C comprised five species endemic to French Polynesia and the Cook Islands: *T. godeffroyi*, *T. ruficollaris*, *T. veneratus*, *T. gambieri*, *T. tutus*. Clade D was sister to clade C and comprised *T. chloris* lineages from Central Polynesia, inclusive of American Samoa, Tonga, Fiji, Vanuatu, and the eastern Solomon Islands including Makira and Ugi Islands, Rennell Island, and the Santa Cruz group.

The placement of clades E and F was equivocal. The three subspecies of *T.*

*cinnamominus* were split between these clades. The Palau endemic, *T. c. pelewensis*, was the sole member of clade E, whereas *T. c. cinnamominus* and *T. c.* reichenbachii, island endemics of Guam and Pohnpei, respectively, were sequentially sister to *T. recurvirostris*, itself an endemic of American Samoa.

Clade G comprised *T. chloris* lineages from Australia and Papua New Guinea plus *T. sanctus*, which was embedded inside this clade. Clade H comprised three genetically distinct lineages: nominotypical *T. c. chloris* from Sulawesi, Indonesia; *T. c. humii* from Singapore; and a clade that comprised multiple subspecies from Borneo to the Philippines and Palau. Finally, clade I included lineages from such geographically disparate regions as Melanesia and the Mariana Islands. *Todiramphus saurophagus* was sister to *T. chloris albicilla* + *T. c. orii* from Saipan and Rota, Mariana Islands. The other half of clade I included *T. c. nusae* and *T. c. alberti* of the Bismarck Archipelago and Solomon Islands, respectively, to the exclusion of the eastern Solomon Islands (Makira, Ugi, and Rennell; clade D).

### *Divergence time estimation and species limits*

The explosive diversification of *Todiramphus* appears to have occurred recently. We used two rates of sequence divergence for ND2 derived from Hawaiian honeycreeper mitogenomes (3.3% or 2.4% per lineage $Myr^{-1}$; Lerner et al. 2011), which places the start of diversification of clade A between 0.6–0.3 Ma (Fig. 4.3). This time frame in the early- to mid-Pleistocene is coincident with the diversification of the pitta *Erythropitta erythrogaster* throughout the Philippines, Wallacea, and New Guinea (Irestedt et al. 2013).

Threshold species delimitation with bGMYC suggested that current species diversity is vastly underestimated in *Todiramphus*. Currently, the most liberal taxonomies treat 11 biological

Figure 4.3. Maximum clade credibility tree with 95% highest posterior density bars from BEAST analysis. Node support is given as Bayesian posterior probability (PP): black circles at nodes denote PP=1.0, gray circles denote $0.95 \leq PP \leq 0.99$. Unlabeled nodes denote $PP < 0.95$. Divergence time estimated based on two calibrations for the rate of mtDNA sequence evolution in ND2 (2.4% and 3.3%; Lerner et al. 2011). Colored lineages denote taxa with sympatric distributions as denoted in the legend. The red Solomon Islands refer to the four large islands of Bougainville, Choiseul, Isabel, and Guadalcanal.

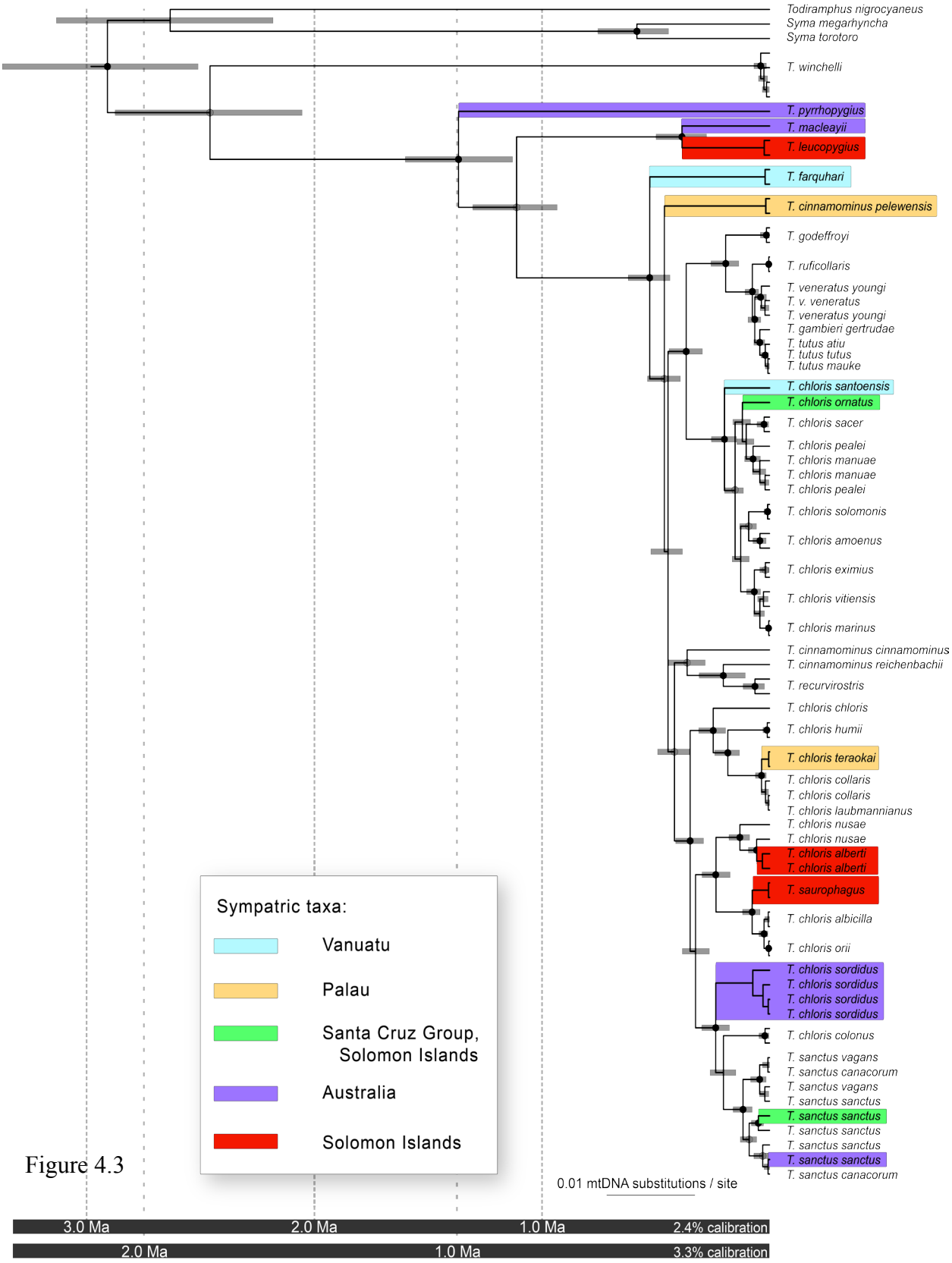

Todiramphus nigrocyaneus
Syma megarhyncha
Syma torotoro
T. winchelli
T. pyrrhopygius
T. macleayii
T. leucopygius
T. farquhari
T. cinnamominus pelewensis
T. godeffroyi
T. ruficollaris
T. veneratus youngi
T. v. veneratus
T. veneratus youngi
T. gambieri gertrudae
T. tutus atiu
T. tutus tutus
T. tutus mauke
T. chloris santoensis
T. chloris ornatus
T. chloris sacer
T. chloris pealei
T. chloris manuae
T. chloris manuae
T. chloris pealei
T. chloris solomonis
T. chloris amoenus
T. chloris eximius
T. chloris vitiensis
T. chloris marinus
T. cinnamominus cinnamominus
T. cinnamominus reichenbachii
T. recurvirostris
T. chloris chloris
T. chloris humii
T. chloris teraokai
T. chloris collaris
T. chloris collaris
T. chloris laubmannianus
T. chloris nusae
T. chloris nusae
T. chloris alberti
T. chloris alberti
T. saurophagus
T. chloris albicilla
T. chloris orii
T. chloris sordidus
T. chloris sordidus
T. chloris sordidus
T. chloris sordidus
T. chloris colonus
T. sanctus vagans
T. sanctus canacorum
T. sanctus vagans
T. sanctus sanctus
T. sanctus sanctus
T. sanctus sanctus
T. sanctus sanctus
T. sanctus sanctus
T. sanctus canacorum
Sympatric taxa:
Vanuatu
Palau
Santa Cruz Group, Solomon Islands
Australia
Solomon Islands
0.01 mtDNA substitutions / site
3.0 Ma
2.0 Ma
1.0 Ma
2.4% calibration
2.0 Ma
1.0 Ma
3.3% calibration

Figure 4.3

species in clade A. The bGMYC estimate found strong support for 26 species in this clade, plus the seven species outside clade A (Fig. 4.4). This estimate of 26 ingroup species is conservative given we lacked 28/50 nominal subspecies of *T. chloris*, plus 6 additional Wallacean and Bismarck *Todiramphus* species.

## Discussion

### *Overview*

This study represents a robust and densely sampled molecular phylogeny of the *Todiramphus chloris* complex—the first phylogenetic hypothesis of this group to date. Emphasizing the Pacific lineages, with representative sampling spanning the 13,000 km from Singapore to the Marquesas Islands, we present a detailed view of the evolutionary and biogeographic history in this widespread and rapid radiation of Pacific island birds.

Three noteworthy patterns were revealed from this study. First, the timing of diversification in *T. chloris* was apparently rapid and recent. Internode distances within the ingroup were found to be incredibly shallow. The maximum divergence across the entire ingroup was only 2.3% (ND2 uncorrected P). We found the timing of diversification to have occurred relatively recently (0.65–0.3 Ma), using two calibrations for the rates of molecular evolution of ND2 (Lerner et al. 2011). We caution against drawing firm conclusions based on this estimate owing to the myriad shortcomings of molecular clock calibrations for divergence time estimation (Arbogast et al. 2002; Lovette 2004; Lanfear et al. 2010). Overall, we interpret the striking pattern of shallow internodes at the base and relatively shallow divergences between ingroup taxa as support for a scenario in which *Todiramphus* achieved its full geographic distribution—from the Sunda Shelf to French Polynesia—rapidly and recently. Similar patterns have been

noted in other Pacific bird lineages, including *Zosterops* white-eyes (Moyle et al. 2009), *Ceyx* kingfishers (Andersen et al. 2013), *Alopecoenas* doves (Jønsson et al. 2011; Moyle et al. 2013), *Erythropitta* pittas (Irestedt et al. 2013), and *Pachycephala* whistlers (Andersen et al. In press-b), which suggests there is an emerging paradigm for the tempo and mode of avian diversification in the Pacific.

Second, the incidence of sympatry across the Pacific distribution of the *Todiramphus* ingroup is remarkably high given the recency of the radiation. Sympatry occurs on Australia, as well as remote oceanic islands like Palau (see below). This finding has important implications on the ability of a rapid insular radiation to attain reproductive isolation. From the perspective of species limits, the level of sympatry of the ingroup suggests the BSC-influenced taxonomy that was prevalent in the 20$^{th}$ Century (Mayr 1942),—and still maintains a strong grip on *Todiramphus*—likely is misguided. Based on divergence time estimates, the speed at which reproductive isolation was achieved is notable, as is the remote nature of the islands upon which it is evident.

Third, despite shallow divergences, *Todiramphus chloris* showed extensive geographic structure across the Pacific with numerous instances of paraphyly. Ten species were found to be embedded within or minimally divergent from the ingroup, rendering *T. chloris* massively paraphyletic. From the perspective of lineage-based or phylogenetic-based species concepts (Simpson 1961; Wiley 1978; Cracraft 1983; de Queiroz 1998), this level of paraphyly, including long-recognized species-level taxa, necessitates massive taxonomic revision of species limits in *Todiramphus*. Our results from bGMYC species delimitation suggest a liberal reinterpretation of species limits, including 26 ingroup species (Fig. 4.4). Of these 26 mitochondrial lineages, 16 were formerly treated as part of the *T. chloris* species complex. With improved taxon sampling

Figure 4.4. Summary of bGMYC species delimitation. The tree is the maximum clade credibility tree from BEAST with ingroup clade A labeled. The red vertical line represents the Maximum Likelihood threshold of species limits as determined by bGMYC. The heatmap is a sequence-by-sequence matrix in which cells are colored by the posterior probability that the corresponding sequences are conspecific, with increasing probability represented by light yellow colors. Thirty-three species are delimited across the entire tree, with 26 species inside the ingroup (clade A). Species names are those assigned by bGMYC. The reader is referred to the text for discussion of our more conservative proposed taxonomy of 17 ingroup species.

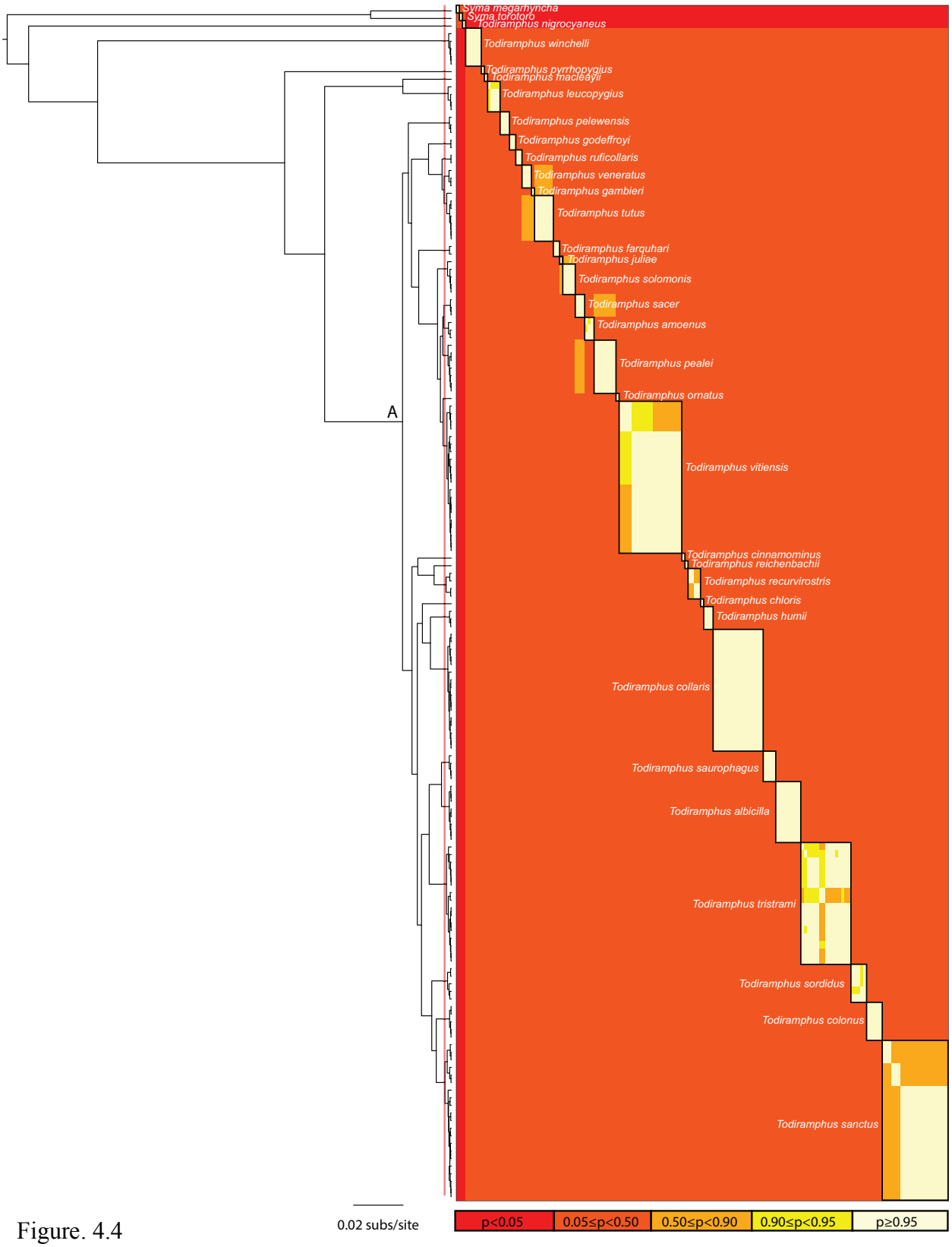

Syma megarhyncha
Syma torotoro
Todiramphus nigrocyaneus
Todiramphus winchelli
Todiramphus pyrrhopygius
Todiramphus macleayii
Todiramphus leucopygius
Todiramphus pelewensis
Todiramphus godeffroyi
Todiramphus ruficollaris
Todiramphus veneratus
Todiramphus gambieri
Todiramphus tutus
Todiramphus farquhari
Todiramphus juliae
Todiramphus solomonis
Todiramphus sacer
Todiramphus amoenus
Todiramphus pealei
Todiramphus ornatus
A
Todiramphus vitiensis
Todiramphus cinnamominus
Todiramphus reichenbachii
Todiramphus recurvirostris
Todiramphus chloris
Todiramphus humii
Todiramphus collaris
Todiramphus saurophagus
Todiramphus albicilla
Todiramphus tristrami
Todiramphus sordidus
Todiramphus colonus
Todiramphus sanctus
0.02 subs/site
p<0.05
0.05≤p<0.50
0.50≤p<0.90
0.90≤p<0.95
p≥0.95

Figure. 4.4

from Wallacea, the Indian Ocean, and the Bismarck Archipelago, this number likely will increase.

### *Assembly of sympatric kingfishers across the Pacific and the paradox of the "great speciators"*

Multiple instances of sympatric *Todiramphus* occur throughout the Pacific. In every case, the sympatric lineages diverged substantially in terms of phenotype, morphology, ecology, and behavior. For example, Palau holds two *Todiramphus* species: *T. cinnamominus pelewensis* and *T. chloris teraokai*. These taxa have diverged morphologically and in habitat preference, such that *T. c. pelewensis* is ca. 50% smaller in body mass and inhabits forest interior, whereas *T. chloris teraokai* is large and prefers coconut groves and beaches (Fry 1980). They differ in phenotype as well: *T. c. pelewensis* has an orange crown, whereas *T. chloris teraokai* has a blue-green crown typical of many *T. chloris* forms. The remote Santa Cruz Group in the eastern Solomon Islands, as well as the Vanuatu archipelago host multiple sympatric taxa, including *T. farquhari*, *T. chloris ornatus*, *T. chloris santoensis*, and *T. sanctus*. The latter taxon is discussed in detail below.

The pattern of eco-morphological differentiation between sympatric lineages continues with the beach kingfisher *Todiramphus saurophagus*, which is broadly sympatric with the *T. chloris* clade from the Bismarck Archipelago and Solomon Islands. *Todiramphus saurophagus* is the largest species in the genus; it is twice the size of the sympatric *T. chloris* forms, and it differs phenotypically from most other *Todiramphus* in having a completely white head (save a blue post-ocular stripe). It strictly inhabits beaches, coastal forest, reefs, islets, and occasionally

mangroves. Throughout its distribution from the northern Moluccas to the Solomon Islands, it is sympatric with 1–2 species of *Todiramphus*, including representative *T. chloris* forms. For example, *T. chloris alberti* and *T. chloris nusae* occur in the Solomon Islands and Bismarck Archipelago, respectively, where they inhabit secondary forest and open areas away from the coast. They are smaller than *T. saurophagus* and have plumage typical of the majority of *T. chloris* forms—blue crown, not white. On Halmahera, New Britain, and the main Solomon Island chain, *T. saurophagus* is also sympatric with three additional congeners, albeit ones not thought to be closely related (*T. funebris*, *T. albonotatus*, and *T. leucopygius*, respectively). These kingfishers occupy the interior of primary lowland forest, thus, they represent another instance of sympatry with eco-morphological differentiation between more divergent congeners.

Perhaps the most complex example of sympatric *Todiramphus* is centered in Australia with clade G (Fig. 4.2). This clade comprises all *T. chloris* from Australia and New Guinea, which are split in two lineages: (1) an endemic to the Milne Bay Province islands of southeast Papua New Guinea, *T. c. colonus,* and (2) the Australian taxon, *T. c. sordidus* (Australian *T. c. colcloughi* and *T. c. pilbara* were not sampled, but are presumed to be allied with *sordidus*). The distribution of *T. chloris* in this clade is coastal Australia (mangroves and coastal estuaries) and on small islands such as those of the D'Entrecasteaux and Louisiade Archipelagos, PNG. Interestingly, *Todiramphus sanctus* is the third lineage in clade G. This species is widespread and highly migratory. Its breeding range occurs throughout Australia, New Zealand, New Caledonia, and some islands in the eastern Solomon Islands (e.g., Santa Cruz Group), and possibly Vanuatu. Many populations migrate north in the austral winter to the Sunda Shelf, New Guinea, and Northern Melanesia. We sampled three of the five nominal subspecies (Gill and Donsker 2013) and despite the geographic complexity of this species' distribution, there was no genetic

differentiation of *T. sanctus*. Together, the two *T. chloris* clades and *T. sanctus* root to a polytomy at the base of clade G. In this scenario, the sympatric forms differ eco-morphologically and behaviorally. *Todiramphus sanctus* is smaller and contains more rufous plumage than any sympatric *T. chloris* throughout its range, including those in the Santa Cruz Group and Vanuatu, which are outside clade G. Behaviorally, the migratory nature of *T. sanctus* is novel in *Todiramphus* kingfishers. Only *T. chloris* in southern Australia are thought to be nomadic (Pizzey and Knight 2012). This discussion is relevant in light of the "great speciators" paradox (Diamond et al. 1976). The paradox poses the question: why are the species most capable of long-distance dispersal also the most geographically well-differentiated from island-to-island in an archipelago? Diamond et al. (1976) suggest that some of the "great speciators" underwent colonization cycles in which they had past phases of higher immigration rates and dispersal abilities followed by a loss of dispersal ability with subsequent differentiation on newfound islands. They count *Todiramphus* [*Halcyon*] *chloris* among the several lineages as evidence for this idea. If this idea of colonization cycles is true, the migratory nature of *T. sanctus*—especially given its placement deeply embedded in the phylogeny—is intriguing, indeed. Birds can acquire migratory ability quickly in response to selective pressure (Berthold et al. 1992; Helbig 1994), and this trait is thought to be evolutionarily labile (Pulido 2007). A prevailing paradigm is that extant migratory species evolved from sedentary tropical ancestors, however, recent evidence in parulid wood-warblers suggests otherwise. Winger et al. (2012) showed that losses of migration are as common as gains, and that extant sedentary tropical radiations (e.g., some *Geothlypis*, and a clade containing *Myiothlypis*, *Basileuterus*, and *Myioborus*) represent at least two losses of latitudinal migration with possible colonization of the tropics from temperate region.

Is it possible that *T. sanctus* is the vestigial *Todiramphus* lineage still experiencing the

colonization phase of Diamond et al. (1976), whereas all others have since lost their propensity for long-distance disperal? If so, this is perhaps the most intriguing evidence to date in support of this component of the paradox. Following this logic, it's possible that the *T. chloris* radiation originated in temperate/subtropical Australia, expanded far and wide into the tropical Pacific, southeast Asia, and parts of the Indian Ocean, then became sedentary with subsequent diversification in allopatry.

### *Colonization of the eastern Pacific*

The rapid and widespread nature of *Todiramphus* diversification across the Pacific precludes a simple stepping-stone model of colonization. There is a major biogeographic break in the Solomon Islands that separates clades C and D from the rest of the ingroup (see below for further discussion). This biogeographic break implies a single, long-distance colonization event from the ancestral *T. chloris* lineage, with subsequent diversification in two broadly distributed radiations from the eastern Solomon Islands to French Polynesia. Clade C comprised a radiation of five species endemic to some of the most remote islands in the world. Interestingly, *T. godeffroyi,* an endemic of the Marquesas Islands—the most remote archipelago within the distribution of *Todiramphus*—was sister to the other four species in the clade: *T. ruficollaris*, *T. veneratus*, *T. gambieri*, and *T. tutus*. Each species was monophyletic, but relationships among them were equivocal. The taxonomic history of this group is confused, likely owing to the great reluctance of BSC-influenced taxonomists to delimit allopatric insular taxa—despite the existence of fixed morphological and behavioral differences—as species. (To their credit, such an endeavor is seemingly easier today with evidence from molecular phylogenetics.) For example, *T. ruficollaris* has been treated by various authors as a nominal subspecies of *T. sanctus*

or *T. tutus*, or as a full species-level taxon (Fry 1980; Pratt 1987; Woodall 2001). Our results show it is phylogenetically unrelated to *T. sanctus* (Fig. 4.2). Instead, it is part of a geographically cohesive radiation in the Cook and Society Islands that comprises *T. tutus* and *T. veneratus*, plus the critically endangered *T. gambieri* of Niau Atoll, Tuamotu. Each of these lineages are minimally divergent (0.8% ND2 uncorrected P), but are allopatric with fixed plumage differences. Sister to this clade is the critically endangered *T. godeffroyi* from the Marquesas Islands, the easternmost *Todiramphus* in the world.

Sister to the eastern Polynesian clade C, is a large radiation from central Polynesia (clade D). This radiation is geographically centered on Fiji, but extends west to Makira and Rennell Islands in the Solomon Islands and east to Tonga and American Samoa, to the exclusion of "Western" Samoa. Numerous island- or archipelago-specific lineages were monophyletic, but many basal relationships were equivocal in clade D. Geographic differentiation was noted with clades from Vanuatu, Tonga, American Samoa, the eastern Solomon Islands, and Fiji. Perhaps the most novel finding in this clade involved a biogeographic break in the eastern Solomon Islands between Guadalcanal and Makira Islands. Thus, clade D was defined as lineages occuring east of this line (e.g., *T. c. solomonis*, Makira and Ugi; *T. c. amoenus*, Rennell; and *T. c. ornatus*, Santa Cruz group). Lineages to the west (i.e., the main Solomon Islands chain plus the New Georgia group) formed a completely unrelated monophyletic group in clade I (Fig. 4.2). The placement of this break relative to Malaita is uncertain because we did not sample *T. c. mala* from that island. Malaita has a unique geologic history different from the Pleistocene aggregate island, Greater Bukida (Mayr and Diamond 2001); therefore, we suspect *T. c. mala* will be found to be part of clade D when this taxon is sampled. This biogeographic break in the eastern Solomon Islands is not novel—several other taxa exhibit breaks there including the *Monarcha*

*castaneiventris* complex and *Pachycephala orioloides* (Uy et al. 2009a; Andersen et al. In press-b); however, this break generally splits taxa into sister groups. We are not aware of examples where this break is so profound such that taxa on either side are as divergent as possible in the phylogeny. Several additional taxa in clade D are incorrectly treated as members of an expanded *T. sanctus* (*T. c. vitiensis* and *T. c. eximius*; Pratt 1987; Clements et al. 2013). Pratt (1987) also considered the possibility that *T. chloris* forms from Fiji (*T. c. vitiensis, T. c. eximius*, and *T. c. marinus*), Tonga (*T. c. sacer*), and American Samoa (*T. c. pealei* and *T. c. manuae*) were closely allied to *T. tutus*, which our results show not to be true.

Three long-distance biogeographical connections are noteworthy and provide additional support for the lack of a stepping-stone colonization model in this group. First, clade F unites the Samoan taxon *T. recurvirostris* with two sequentially sister lineages of *T. cinnamominus* (*reichenbachii* of Pohnpei and *cinnamominus* of Guam). A similar pattern linking Micronesia with Polynesia was found in *Acrocephalus* reed-warblers (Cibois et al. 2011), though eastern Polynesia was integral to this example, not Samoa. Second, *T. saurophagus* of coastal Solomon Islands, New Guinea, and Halmahera was sister to Northern Mariana forms of *T. chloris* (*albicilla* on Saipan and Tinian and *orii* on Rota). To our knowledge, this biogeographic link has not been noted in birds, but it makes sense in light of the phenotype of these taxa. All are exceptionally large *Todiramphus* with extensive white heads and large bills. Finally, *T. c. tera*okai of Palau is embedded within the Philippines + Borneo clade (clade F, Fig. 4.2). This implicates an intriguing biogeographic link between the Sunda Shelf (Borneo), a near oceanic archipelago (Philippines) and a distant oceanic island (Palau).

*Species limits and a proposed updated taxonomy*

Species limits in polytypic species complexes such as *Todiramphus chloris* are debated by systematic ornithologists. Much of the debate centers on criteria for delimiting species, and inevitably, discussions of the merits and utility of subspecies as a meaningful taxonomic rank persist (Fitzpatrick 2010; Pratt 2010; Remsen 2010). Here, we follow a lineage-based species concept to evaluate species limits in the *Todiramphus chloris* species complex. We draw upon multiple lines of evidence including 1. the molecular phylogeny of this study, 2. the results of bGMYC species delimitation of the mtDNA data, 3. patterns of sympatry between multiple pairs of ingroup taxa (discussed above), and 4. knowledge of fixed plumage and/or ecological differences. It is worth recalling that despite our robust sampling, we still lacked 6 *Todiramphus* species and 28 of 50 nominal subspecies of *T. chloris*. Most species we lacked are Wallacean endemics, plus *T. albonotatus* from New Britain, and most subspecific diversity we lacked was from Indonesia, the Indian Ocean, and Vanuatu. Thus, we suggest that this taxonomic treatment be considered with caution. We suspect that with complete sampling our estimates of species limits would include even more species than those listed below.

*Todiramphus nigrocyaneus* and two *Syma* species were found to be sister lineages and deeply diverged from each other (11–11.9% ND2 uncorrected P). Together, they comprised the first branch in the phylogeny. Three phenotypically distinct populations of *T. nigrocyaneus* are distributed across New Guinea: *T. n. nigrocyaneus*, *T. n. quadricolor*, and *T. n. stictolaemus*, of which we sampled the latter from southern Papua New Guinea. This group warrants further phylogeographic study to include all three nominal subspecies of *T. nigrocyaneus* plus the myriad forms of *Syma* from New Guinea and Australia, and outgroup taxa in *Actenoides*. Until this study is undertaken, we refrain from recommending taxonomic change to the generic

placement of *T. nigrocyaneus*.

*Todiramphus winchelli*, *T. pyrrhopygius*, *T. macleayii*, *and T. leucopygius* are unequivocally considered valid species by taxonomists, and our study supports this treatment. All but *T. pyrrhopygius* are thought to form a group of morphologically cohesive species defined by deep blue colors, different from the blue-green typical of other *Todiramphus* species. Some authors have included *T. diops*, *T. lazuli*, *T. funebris*, *T. albonotatus* and *T. farquhari* in this morpho-group (Woodall 2001), but our results suggest there is no phylogenetic basis for such a grouping. Indeed, *T. farquhari* of Vanuatu appears to be closely allied with the *T. chloris* ingroup, whereas the other species' affinities and genetic distinctiveness remain uncertain. Two additional species, *T. australasia* and *T. enigma* are thought to be closely allied with *T. sanctus* and *T. chloris*, respectively, based on phenotypic similarities; however, these were unsampled in this study, so little can be said of their relationships. Continued efforts to collect specimens with associated genetic material will be necessary to include these six species in an expanded *Todiramphus* phylogeny. Until then, any speculation as to their placement should be treated cautiously.

Species limits of ingroup clade A (Figs. 4.2–4.4) are complex and in need of major revision. Our phylogenetic results highlight numerous clades that warrant species status. Results of a bGMYC species delimitation analysis suggested the presence of 26 species in clade A (Fig. 4.4). This liberal interpretation is based on population-level sampling of mtDNA sequences only. The method has limitations, but it provides one metric for comparison. Below we provide an annotated list of a slightly more conservative approach to species delimitation with comments on their relative divergence, fixed phenotypic and ecological characters, and patterns of sympatry between congeners.

- ***Todiramphus farquhari* Sharpe, 1899 (Vanuatu Kingfisher).** Unequivocally considered a valid biological species by all authors. It is as morphologically distinct as any ingroup lineage. Thought by some to be part of the dark blue-and-white morpho-group (see above; Woodall 2001), but our results support a close affinity with the ingroup.

The Micronesian endemic *T. cinnamominus* has three extant nominal subspecies that are distributed on Palau, Guam, and Pohnpei. A fourth hypothetical taxon on the Ryukyu Islands, Japan, *T cinnamominus miyakoensis*, is thought to be extinct. All three extant *T. cinnamominus* taxa differ substantially in plumage and size, and are highly allopatric from each other. Our molecular data show that *T. cinnamominus pelewensis* of Palau is well differentiated from the other *T. cinnamominus*, but it's phylogenetic placement is equivocal. The MrBayes analysis placed it inside the ingroup, whereas the BEAST analysis placed it just outside. Neither case was well supported, and both analyses recovered short internode distances, suggesting an uncertain evolutionary history of this taxon. The remaining two taxa, *T. cinnamominus cinnamominus* of Guam and *T. cinnamominus reichenbachii* of Pohnpei appear to be closely related, albeit paraphyletic with respect to *T. recurvirostris*, a Samoan endemic species. This clade represents a biogeographic enigma with three geographically disparate island distributions spanning Micronesia and Central Polynesia.

- ***Todiramphus pelewensis* Wiglesworth, 1891 (Palau Kingfisher).** This taxon is sympatric with *T. chloris teraokai* and differs substantially from it and other *T. cinnamominus* forms in size, plumage, and habitat preference.
- ***Todiramphus cinnamominus* Swainson, 1821 (Guam Kingfisher).** The nominotypical *T. cinnamominus* from Guam is extirpated in the wild and survives only in captive breeding programs. It differs morphologically from *T. pelewensis* and *T. reichenbachii* in being

entirely rufous below, whereas rufous is confined to the crown of the other two species. Genetically, it is 2% diverged (ND2 uncorrected P) from *P. pelewensis*, but only 0.01% diverged from *T. reichenbachii*. We recommend species status for the three *T. cinnamominus* taxa because they are not each others' closest relatives and there are fixed phenotypic differences, as well as vast distances of open ocean between their respective Micronesian ranges.

- ***Todiramphus reichenbachii* Hartlaub, 1852 (Pohnpei Kingfisher).** Endemic to Pohnpei, Caroline Islands.
- ***Todiramphus recurvirostris* Lafresnaye, 1842 (Flat-billed Kingfisher).** This species is sister to *T. reichenbachii*. Authors have variously treated *T. recurvirostris* as its own species or as part of *T. sanctus* (Fry 1980; Woodall 2001). Our results warrant species status based on its phylogenetic differentiation from *T. sanctus*, and morphological differences including small size and bill morphology. It is endemic to Samoa; the only *Todiramphus* found there.

The following two species form a clade centered on Australia and New Guinea. Three clades were recovered in our analysis, and bGMYC supported all three as species; however, we recommend a more conservative approach because of insufficiently dense sampling in New Guinea and lack of topological resolution amongst the three clades.

- ***Todiramphus sordidus* Gould, 1842 (Mangrove Collared Kingfisher).** Breeds in coastal Australia and New Guinea. This species likely includes nominal subspecies *sordidus*, *pilbara*, *colcloughi*, and *colonus*. Further sampling is recommended to better understand the phylogeographic history of these forms in Australo-Papua.
- ***Todiramphus sanctus* Vigors & Horsfield, 1827 (Sacred Kingfisher).** Breeds throughout Australia, plus New Zealand and some islands in the eastern Solomons and possibly

Vanuatu. Extent of breeding range not full understood in the east. Some authors expanded the taxonomic scope of *T. sanctus* with respect to *T. chloris* and *T. recurvirostris* to include as many as nine nominal subspecies (Fry 1980; Pratt 1987; Woodall 2001; Clements et al. 2013). Pratt (1987) attributed the Fijian populations, *T. c. vitiensis* and *T. c. eximius*, as part of *T. sanctus* based on plumage and voice. *Todiramphus recurvirostris* from Upolu and Savai'i, Samoa is sometimes lumped as part of *T. sanctus* because differences in bill morphology are minimal (Fry 1980; Woodall 2001). Our results support a more restricted circumscription of *T. sanctus*. Furthermore, we found no evidence for geographic differentiation between the three nominal subspecies sampled, suggesting ongoing gene flow—possibly aided by their migratory nature.

A large radiation across southeast Asia is represented by Clade H. The basal lineage is the nominotypical subspecies *T. c. chloris*, which is widespread throughout Wallacea (sampled here from Sulawesi). Samples from Singapore comprise another lineage (*T. c. humii*), which is, in turn, sister to a large clade from the Philippines, Borneo, and Palau. Interestingly, despite the geographic complexity of the Philippines, no genetic structure was found across the entire archipelago. This is a rare biogeographic pattern in Philippine birds, in part because systematists tend to study the most differentiated taxa first. However, lack of biogeographic structure in Philippines birds has been found in *Rhipidura javanica* (Sánchez-González and Moyle 2011) and *Copsychus saularis* (Sheldon et al. 2009). Both examples, however, were distinct from Bornean taxa. The Palau result is completely novel in birds and further investigation should be taken to determine the origins of Palau's avifauna. Despite the relative proximity between Palau and the Mariana Islands, this results highlights their different geologic histories in belonging to different island arc systems.

- ***Todiramphus chloris* Boddaert, 1783 (Collared Kingfisher).** We support a conservative approach by treating the large Asian clade H as one species. We recognize that there is genetic structure in this clade worthy of further species delimitation (i.e., Sulawesi; mainland southeast Asia; and Borneo, Philippines, and Palau), but there are too many gaps in our sampling to say definitively.

In clade I, we recommend recognizing three species. Further sampling is necessary in the Bismarck Archipelago (*T. albonotatus* of New Britain, and several nominal subspecies of the *T. chloris* complex from Musau (*matthiae*) to Nissan (*bennetti*).

- ***Todiramphus saurophagus* Gould, 1843 (Beach Kingfisher).** The largest *Todiramphus* species. This coastal specialist is distinctive morphologically with a massive bill and white head.
- ***Todiramphus albicilla*, Dumont, 1823 (Mariana Kingfisher).** The sister species to *T. saurophagus*. Our sampling is incomplete, so we treat this recommendation with caution. We sampled birds from Saipan (*albicilla*) and Rota (*orii*), but lacked samples from Asuncion, Agrihan, Pagan, and Almagan in the northern part of the archipelago (*owstoni*). All forms are large, like *T. saurophagus*, but only *albicilla* from Saipan is white-headed. The other forms are variably white or blue-crowned suggesting this trait is phenotypically plastic in this clade. Interestingly, birds from Mussau Island in the St. Matthias Islands, the northernmost island in the greater Bismarck Archipelago is similarly plumaged to *orii* and *owstoni*. Furthermore, about 40% of individuals of *T. saurophagus admiralitatis* from the Admiralty Islands show blue-green crowns (Dutson et al. 2011). It is highly speculative, but this pattern is suggestive of ancestral polymorphisms of crown plumage within the broader clade of *T. saurophagus* + *T. albicilla*.

- ***Todiramphus tristrami* E. L. Layard, 1880 (Melanesian Kingfisher).** This species corresponds to a geographically cohesive clade from the Bismarck Archipelago and the main Solomon Islands chain (Bougainville to Guadalcanal). We sampled only two nominal subspecies (*nusae* and *alberti*), but *tristrami* (New Britain) has priority. Much denser sampling is needed including the following taxa: *mathiae*, *stresemanni*, *novaehiberniae*, *bennetti*, *tristrami*, and *pavuvu*.

Clade D contains numerous lineages endemic to relatively small geographic areas. The bGMYC species delimitation results supports seven species (Fig. 4.4). We caution against this interpretation given the large number of sampling gaps from this region. Mayr examined the kingfishers of central Polynesia, which resulted in his naming 15 nominal subspecies of *T. chloris* (Mayr 1931a, 1935b, 1938, 1941).

- ***Todiramphus sacer* J. F. Gmelin, 1788 (Pacific Kingfisher).**

Clade C comprises a radiation of eastern Polynesian kingfishers that have long-been treated as 5–6 species (Fry 1980; Fry et al. 1992; Woodall 2001). Our phylogenetic and species delimitation results support this with surprisingly strong support at most nodes. We recommend maintaining current taxonomy of the following five species (Gill and Donsker 2013).

- ***Todiramphus godeffroyi* Finsch, 1877 (Marquesan Kingfisher).**
- ***Todiramphus ruficollaris* Holyoak, 1974 (Mewing Kingfisher).**
- ***Todiramphus veneratus* J. F. Gmelin, 1788 (Society Kingfisher).**
- ***Todiramphus gambieri* Oustalet, 1895 (Tuamotu Kingfisher).**
- ***Todiramphus tutus* J. F. Gmelin, 1788 (Chattering Kingfisher).**

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# Appendix I

Phenotypic characters of the 15 subspecies of *Ceyx lepidus*, based on examination of specimens at the University of Kansas Biodiversity Institute and from Fry et al. (1992). Reprinted from (Andersen et al. 2013).

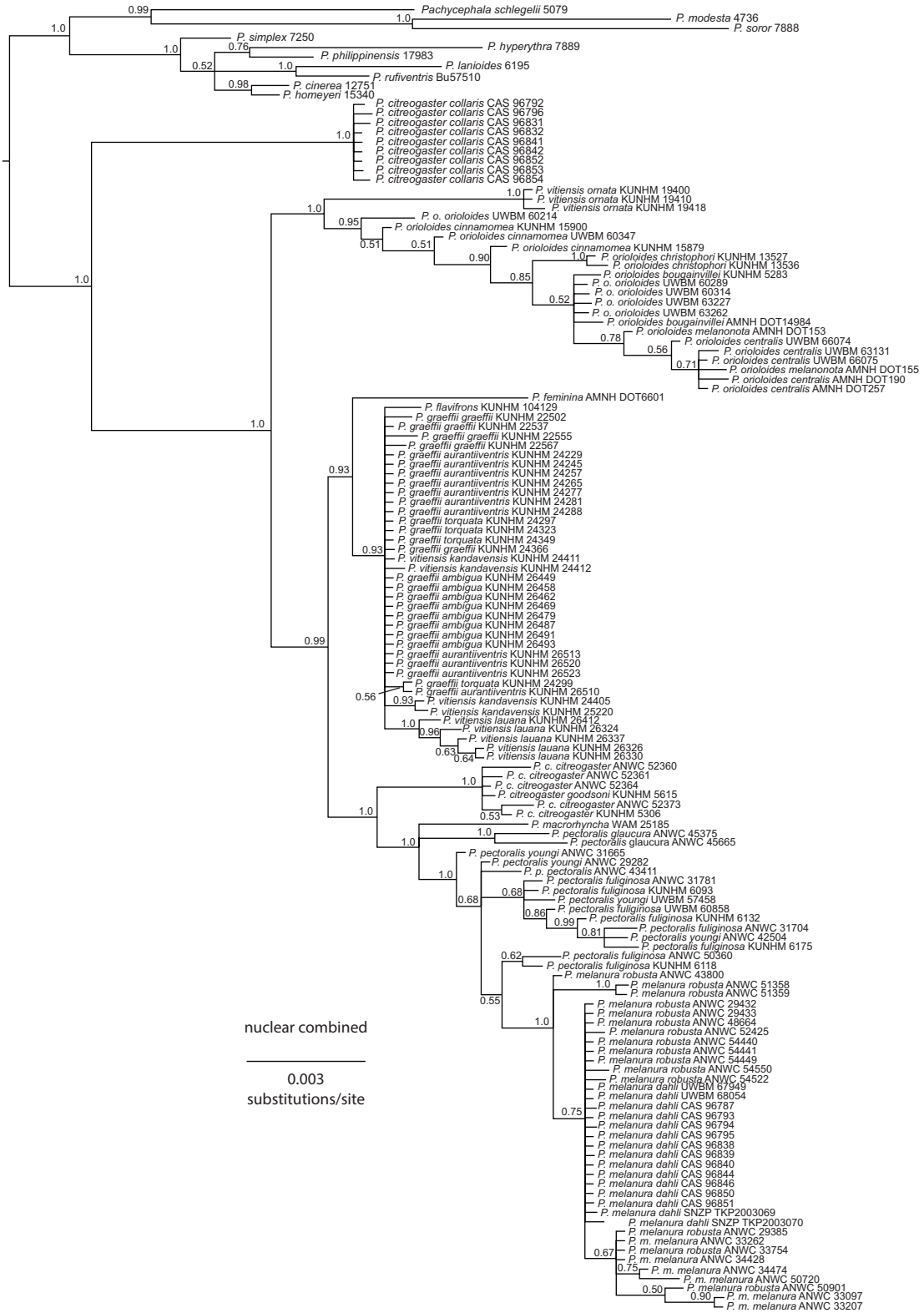
| Subspecies | Bill | Head | Wings | Frontal spot | Malar stripe | Neck blaze | Back | Rump | Uppertail coverts | Chin and throat | Breast and belly | Legs and feet |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. l. cajeli* | Red but stouter | Blackish, almost without dark blue | Blackish, almost without dark blue | Yellow-orange | | | Brilliant silvery-blue | Brilliant silvery-blue | Brilliant silvery-blue | White | Yellow-orange | Orange |
| *C. l. collectoris* | Red but stouter | Black, washed and spotted with very dark blue | Black, washed and spotted with very dark blue | Orange | | | Brilliant purple-blue | Brilliant purple-blue | Brilliant purple-blue | Yellowish-white | Rich orange | Orange |
| *C. l. dispar* | Red | Male: black, washed and spotted with dark blue; Female: orange, with blue-black restricted to a stripe on the hindcrown and an area in front of neck blaze | Black, spotted and washed with ultramarine | Male: orange; female: lore and small area in front of eye black | | White | Brilliant pale silvery-blue | Brilliant pale silvery-blue | Brilliant dark ultramarine–blue | Male: yellowish-white; female: white | Breast dark rufous, belly rich orange | Red |
| *C. l. gentianus* | Black | Black, washed and spotted with dark blue | Black, washed and spotted with dark blue | White | | White | Brilliant ultra-marine-blue | Silvery-blue | Brilliant ultramarine-blue | White | White | Orange |
| *C. l. lepidus* | Red | Black, washed and spotted with dark blue | Black, washed and spotted with dark blue | Orange | Blue-black | | Brilliant ultra-marine-blue | Silvery-blue | Brilliant ultramarine-blue | Yellowish-white | Rich orange | Orange |
| *C. l. malaitae* | Upper man-dible mainly black, lower man-dible yellow | Black, washed and spotted with very dark blue | Black, washed and spotted with very dark blue | Orange | | | Brilliant purple-blue | Brilliant purple-blue | Brilliant purple-blue | Yellowish-white | Rich orange | Orange |
| *C. l. margarethae* | Red | Pale morph: pale cobalt blue; dark morph: ultramarine blue | Pale morph: pale cobalt blue; dark morph: ultrama-rine blue | Dark chestnut | Orange | | Pale morph: pale cobalt blue; dark morph: ultra-marine blue | Pale morph: pale silvery cobalt blue; dark morph: ultramarine blue | Pale morph: pale cobalt blue; dark morph: ultrama-rine blue | Yellowish-white | Breast rich orange, belly pale yellow | Orange |

| Subspecies | Bill | Head | Wings | Frontal spot | Malar stripe | Neck blaze | Back | Rump | Uppertail coverts | Chin and throat | Breast and belly | Legs and feet |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. l. meeki* | Black | Blue-black, spangled with pale blue | Blue-black, spangled with pale blue | Pale yellowish-buff on male, more orange-yellow on female | | Male: pale yellowish-buff; Female: pale orange-yellow | Lower mantle pale blue | Silvery | Pale blue | Pale yellowish-buff on male, more orange-yellow on female | Pale yellowish-buff on male, more orange-yellow on female | Flesh-pink |
| *C. l. mulcatus* | Black | Black, washed and spotted with dark blue | Black, washed and spotted with dark blue | Orange | | | Brilliant ultramarine-blue | Silvery-blue | Brilliant ultramarine-blue | Yellowish-white | Rich orange | Orange |
| *C. l. nigromaxilla* | Upper mandible mainly black, lower mandible mainly orange-red | Black, washed and spotted with very dark blue | Black, washed and spotted with very dark blue | Orange | | | Brilliant purple-blue | Brilliant purple-blue | Brilliant purple-blue | Yellowish-white | Rich orange | Orange |
| *C. l. pallidus* | Black | Blue-black, spangled with pale ultramarine blue | Blue-black, spangled with pale ultramarine blue | Pale yellowish-buff | | Pale yellowish buff | Brilliant ultramarine-blue | Brilliant ultramarine-blue | Cobalt blue | Pale yellow | Pale orange-yellow | Yellow |
| *C. l. sacerdotis* | Upper mandible dusky red, lower mandible red | Black, washed and spotted with dark blue | Black, washed and spotted with dark blue | Orange | | | Brilliant ultramarine-blue | Brilliant ultramarine-blue | Brilliant ultramarine-blue | Yellowish-white | Breast rich orange, belly and undertail coverts very pale orange-yellow | Orange |
| *C. l. solitarius* | Black | Black, washed and spotted with dark blue | Black, washed and spotted with dark blue | Yellowish-white | | | Brilliant ultramarine-blue | Brilliant ultramarine-blue | Brilliant ultramarine-blue | Yellowish-white | Rich orange | Orange |
| *C. l. uropygialis* | Red, more slender | Black, softly washed and spotted with dark blue | Black, softly washed and spotted with dark blue | Orange | | | Brilliant ultramarine-blue | Silvery-blue | Brilliant ultramarine-blue | Rich yellowish-white | Dark rich orange | Orange |
| *C. l. wallacii* | Red | Black, washed and spotted with blue (paler) | Black, washed and spotted with blue (paler) | Orange | | | Brilliant pale cobalt-blue | Brilliant pale cobalt-blue | Ultramarine-blue | Yellowish-white | Rich orange | Orange |

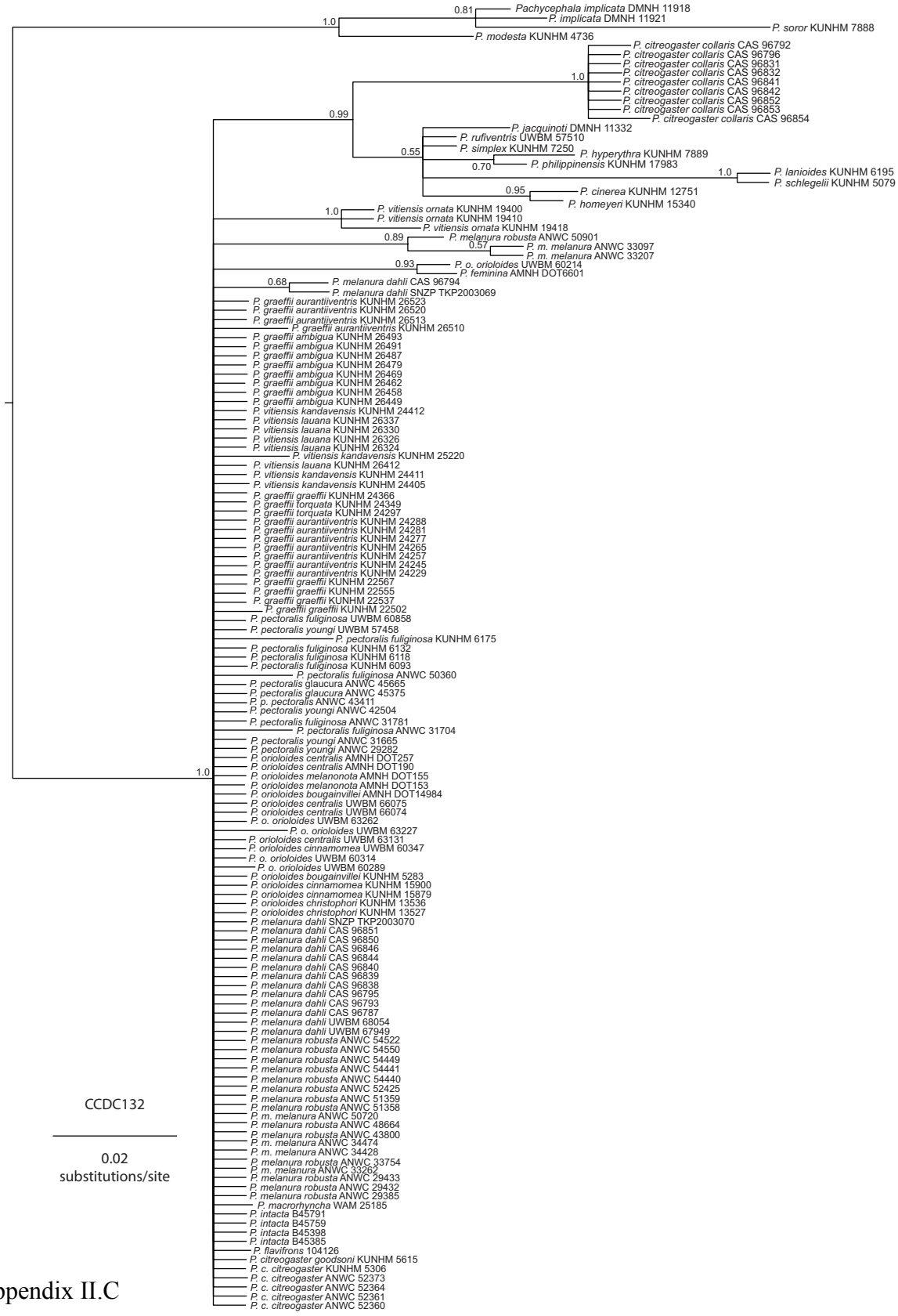
# Appendix II

Supplementary tree figures from (Chapter 2; Andersen et al. In press-b). All trees are Bayesian maximum consensus trees of reduced datasets from MrBayes. Node support is denoted as Bayesian posterior probabilities. Each figure is labeled with the corresponding data partition (e.g., gene) in the following order: A) combined, partitioned mitochondrial DNA (ND2 and ND3; concatenated and partitioned by codon position); B) combined, partitioned analysis of the nuclear sequence data (n=8 introns); C) CCDC132 intron; D) Fib5 intron; E) GAPDH intron; F) HMGB2 intron; G) MUSK intron; H) Myo2 intron; I) ODC intron; and J) TGF intron.

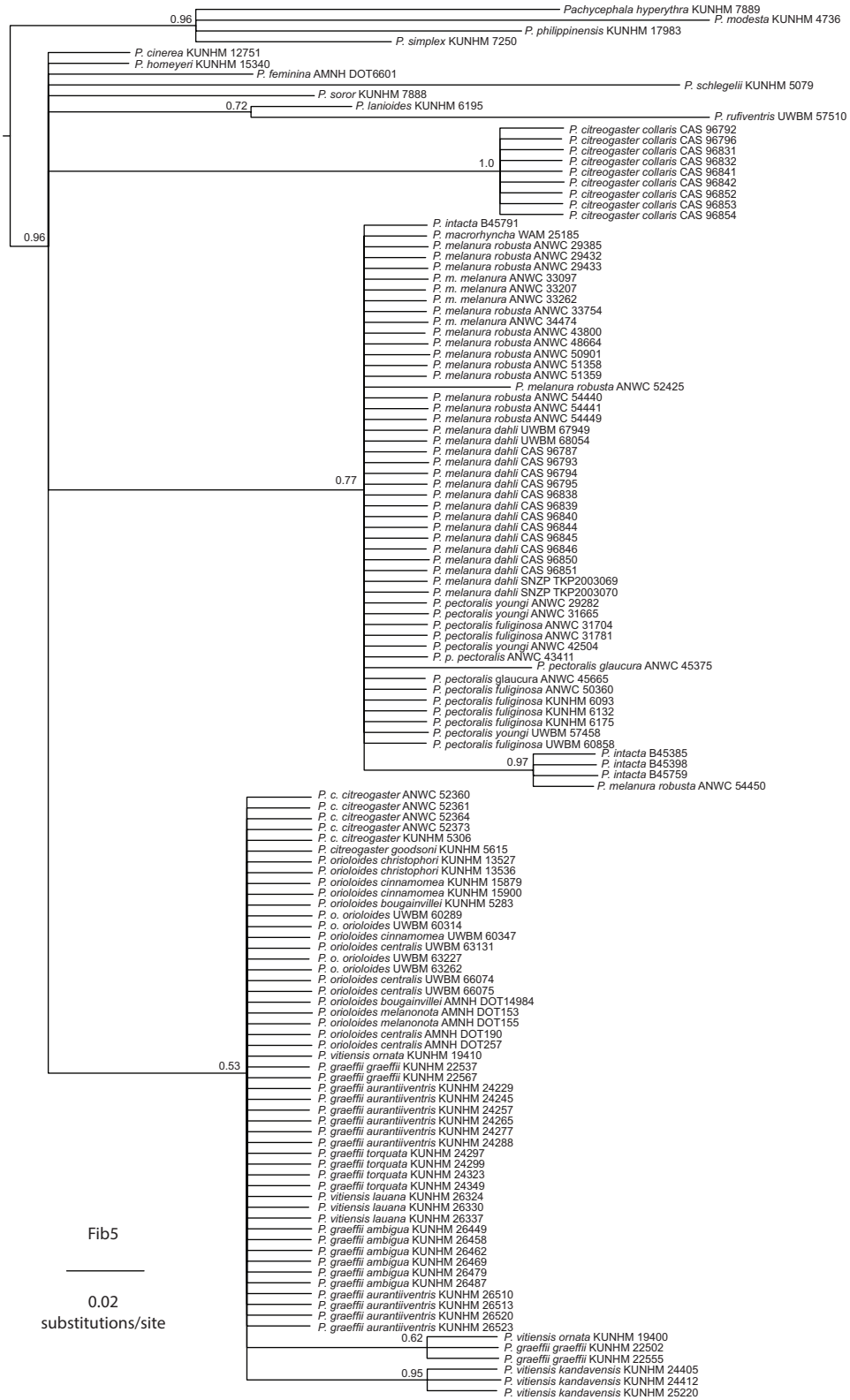

Appendix II.A



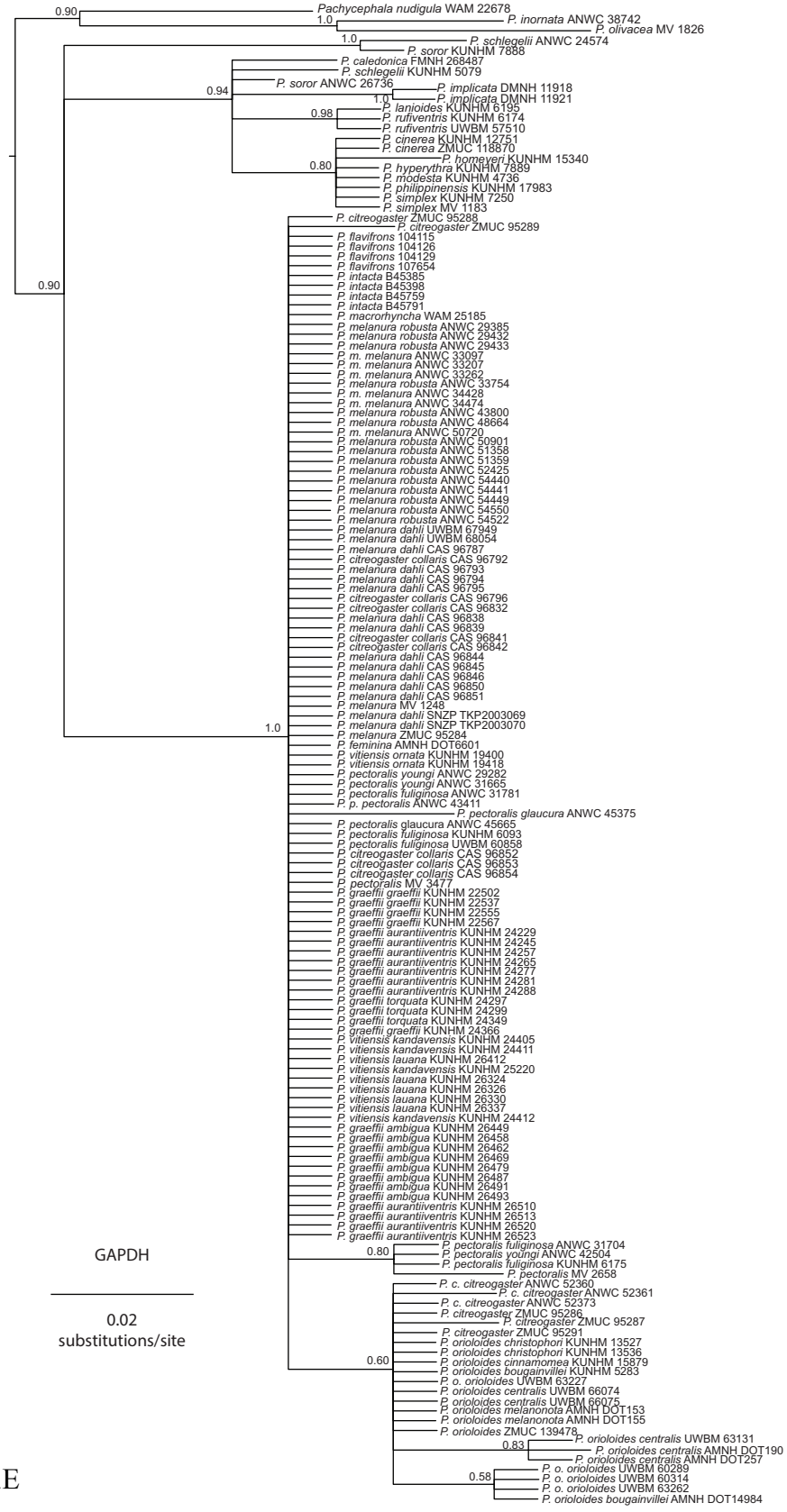

Appendix II.B



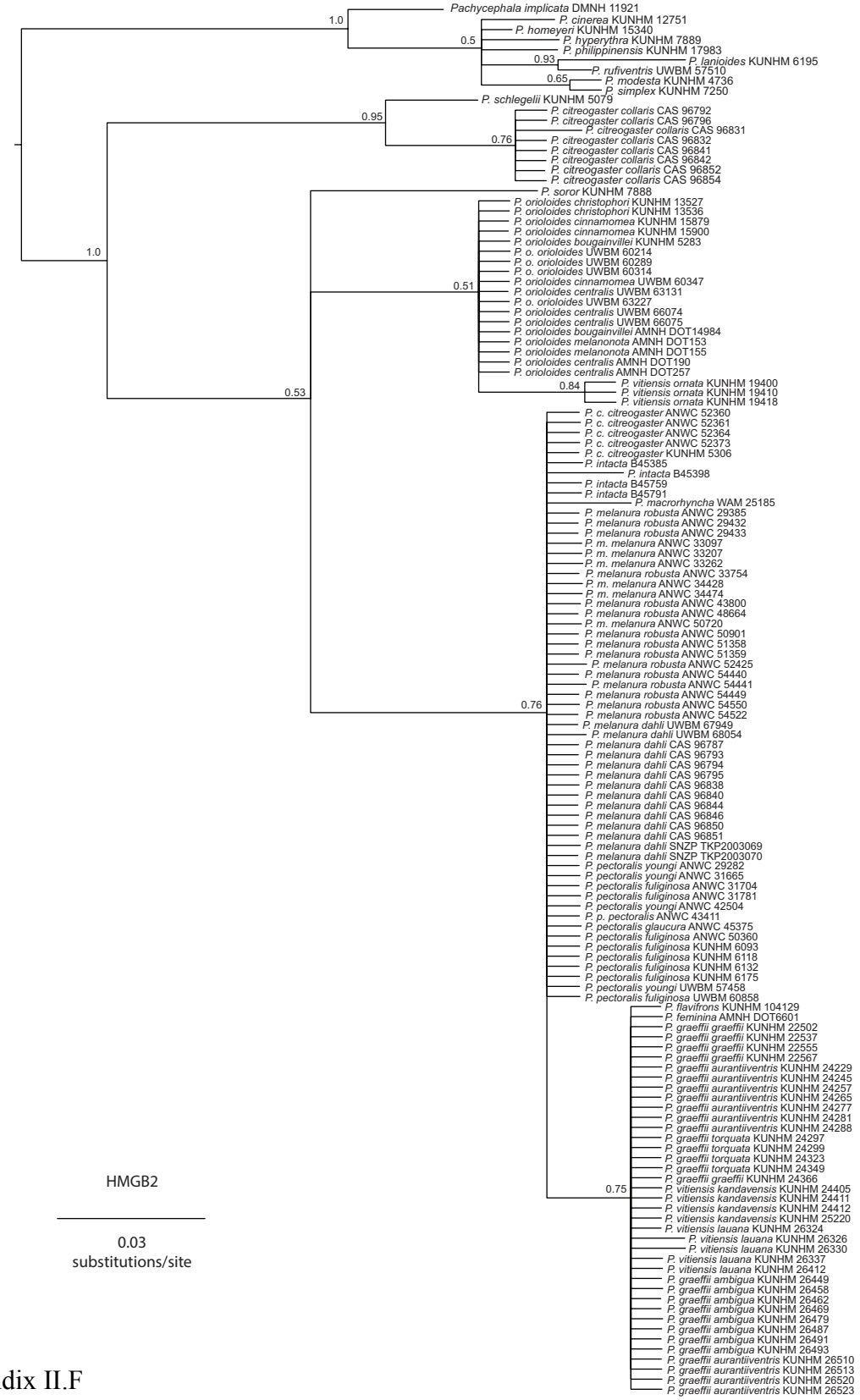

Appendix II.C



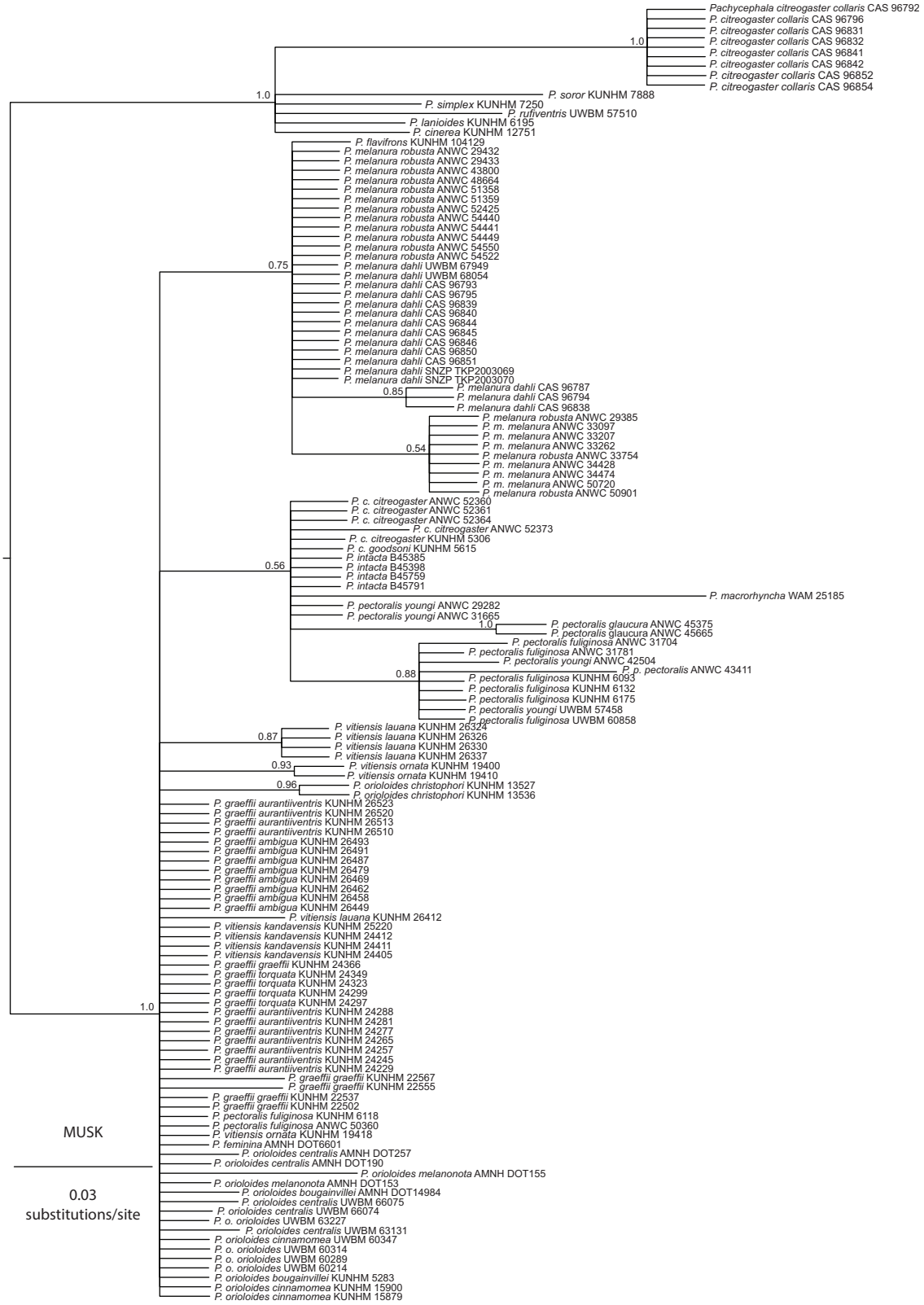

Appendix II.D



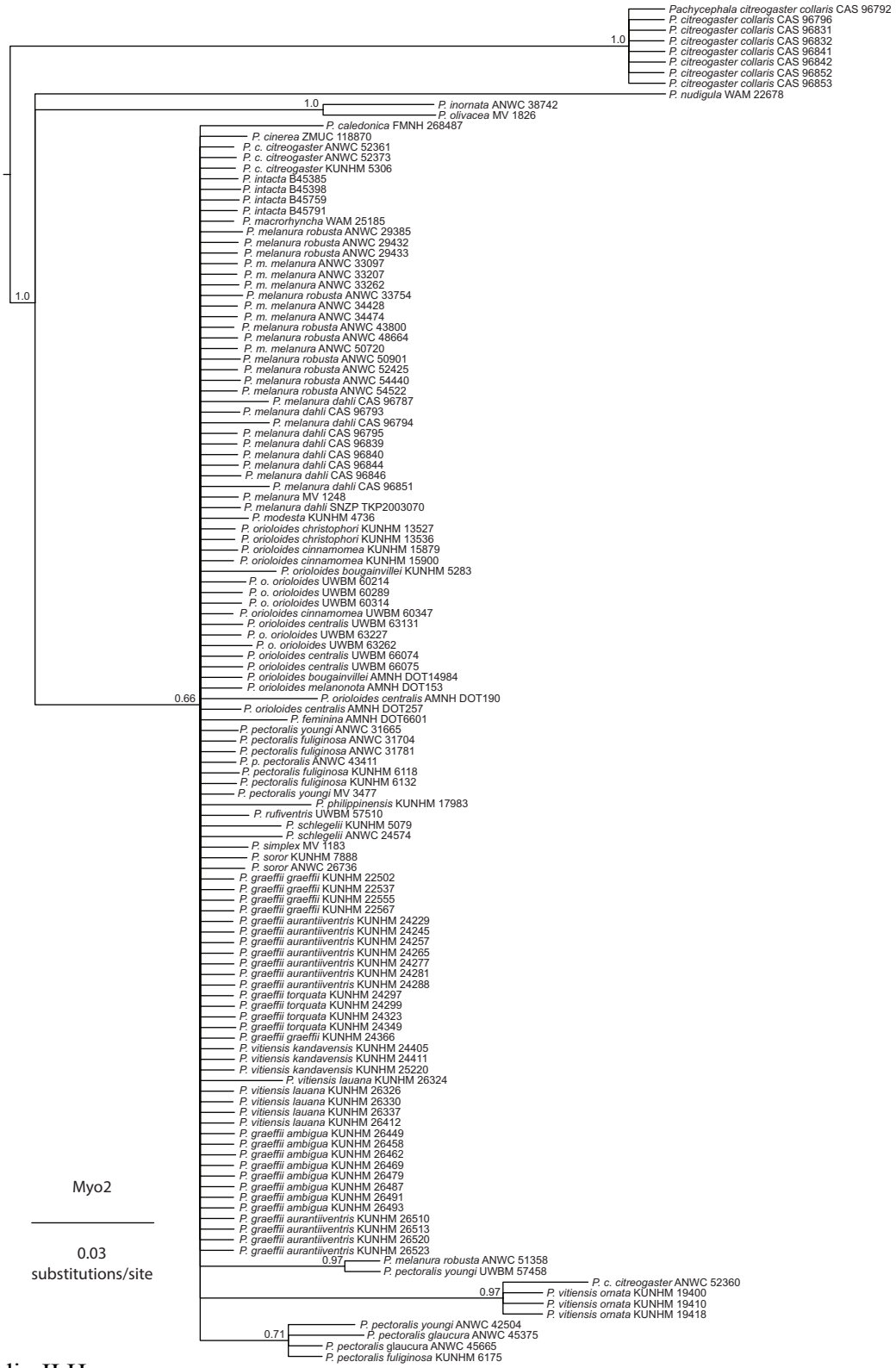

Appendix II.E




Appendix II.F

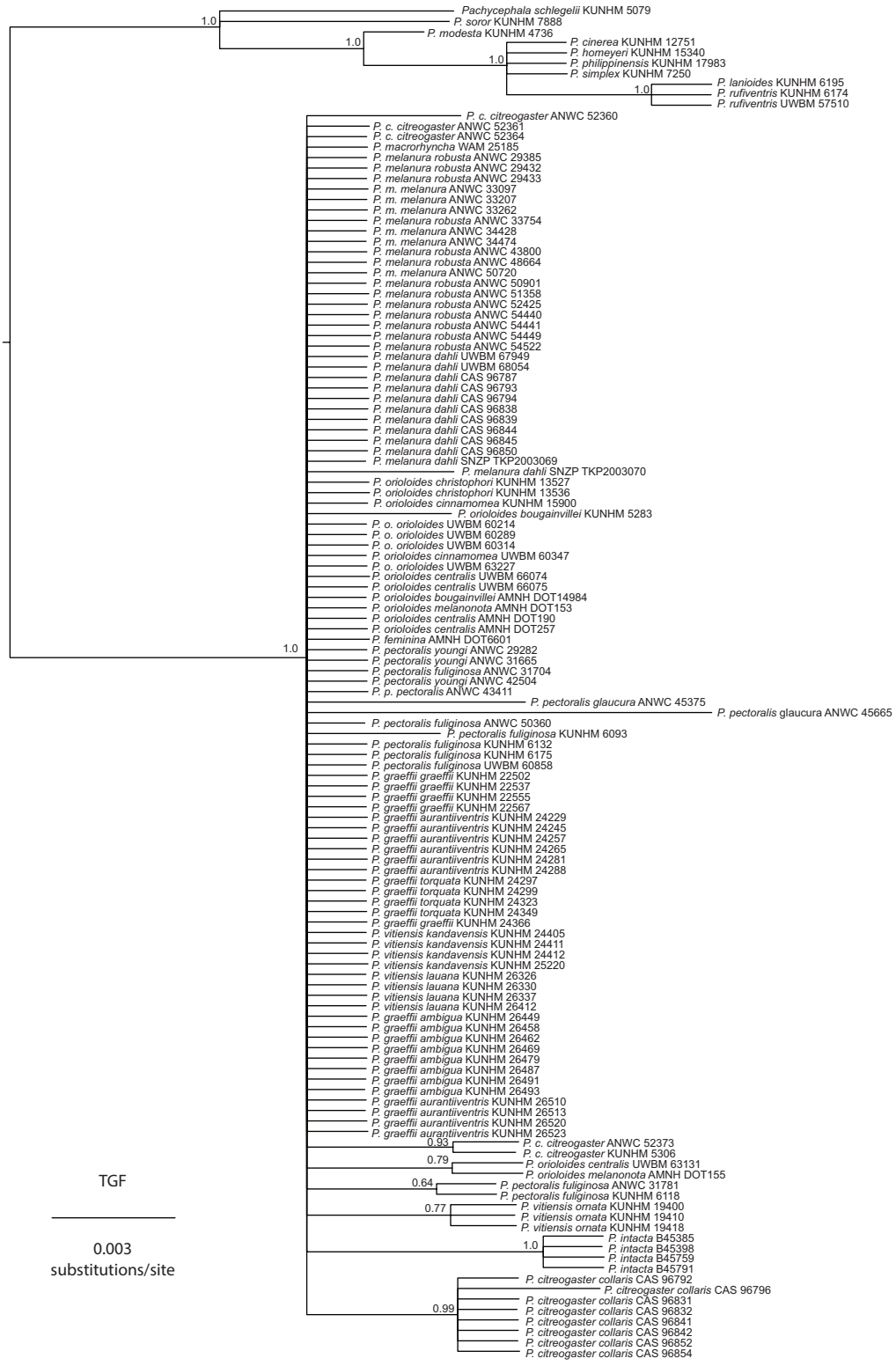



Appendix II.G




Appendix II.H


Appendix II.I




Appendix II.J

# Appendix III

Complete list of samples used in (Chapter 3; Andersen et al. In press-a). Samples in bold are newly sequenced for this study. Institutional abbreviations: AMNH, American Museum of Natural History; DMNH, Delaware Museum of Natural History; KUNHM, University of Kansas Natural History Museum; LSUMNS, Louisiana State University Museum of Natural Science; UMMZ, University of Michigan Museum of Zoology; NMNH, Smithsonian Institution National Museum of Natural History; UWBM, University of Washington Burke Museum; YPM, Yale Peabody Museum.

| Genus | Species | Voucher | Genus (Driskell and Christidis 2004) | GenBank Accession (ND2) | (Fib5) |
|---|---|---|---|---|---|
| Ingroup | | | | | |
| *Acanthagenys* | *rufogularis* | MV1122 | | AY488259 | AY488410 |
| *Acanthorhynchus* | *superciliosus* | MV248 | | AY488260 | AY488411 |
| *Acanthorhynchus* | *tenuirostris* | B873 | | AY488261 | AY488412 |
| *Anthochaera* | *carunculata* | C257 | | AY488262 | AY488413 |
| *Anthochaera* | *chrysoptera* | B792 | | AY488263 | AY488414 |
| *Anthochaera* | *lunulata* | MV175 | | AY488264 | AY488415 |
| *Anthochaera* | *paradoxa* | B736 | | AY488265 | AY488416 |
| *Ashbyia* | *lovensis* | D173 | | AY488266 | AY488417 |
| *Bolemoreus* | *frenatus* | ANWC 41565 | *Lichenostomus* | HQ267669 | HQ267689 |
| *Caligavis* | *obscurus* | KUNHM 7379 | *Lichenostomus* | HQ267675 | HQ267695 |
| *Certhionyx* | *variegatus* | W036 | | AY488269 | AY488420 |
| *Cissomela* | *pectoralis* | C912 | *Certhionyx* | AY488268 | AY488419 |
| *Conopophila* | *albogularis* | MV1216 | | AY488270 | AY488421 |
| *Conopophila* | *rufogularis* | MV1300 | | AY488271 | AY488422 |
| *Entomyzon* | *cyanotis* | F274 | | AY488272 | AY488423 |
| *Epthianura* | *albifrons* | D328 | | AY488273 | AY488424 |
| *Epthianura* | *aurifrons* | D156 | | AY488274 | AY488425 |
| *Epthianura* | *crocea* | D175 | | AY488329 | AY488426 |
| *Epthianura* | *tricolor* | D229 | | AY488405 | AY488427 |
| *Foulehaio* | *carunculatus* | 2077 | | AY488275 | AY488428 |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 24220** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 24307** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 24351** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 24378** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 24382** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26303** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26306** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26321** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26332** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26344** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26386** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26387** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26425** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26495** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 26536** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 30509** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 30524** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 104023** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 104025** | | | |

| Genus | Species | Voucher | Genus (Driskell and Christidis 2004) | GenBank Accession (ND2) | (Fib5) |
|---|---|---|---|---|---|
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 104041** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 104050** | | | |
| ***Foulehaio*** | ***carunculatus*** | **KUNHM 107639** | | | |
| ***Foulehaio*** | ***carunculatus*** | **UWBM 42872** | | | |
| ***Foulehaio*** | ***carunculatus*** | **UWBM 42885** | | | |
| *Gavicalis* | *virescens* | KUNHM 6160 | *Lichenostomus* | HQ267682 | HQ267702 |
| *Gliciphila* | *melanops* | D451 | *Phylidonyris* | AY488407 | AY488456 |
| *Glycichaera* | *fallax* | E663 | | AY488276 | AY488429 |
| ***Glycifohia*** | ***notabilis*** | **LSUMNS B45775** | | | |
| ***Glycifohia*** | ***notabilis*** | **LSUMNS B45807** | | | |
| ***Glycifohia*** | ***undulata*** | **YPM 71297** | | | |
| *Grantiella* | *picta* | MV2673 | | AY488277 | AY488430 |
| ***Guadalcanaria*** | ***inexpectata*** | **DMNH 11854** | | | |
| ***Gymnomyza*** | ***samoensis*** | **KUNHM 104021** | | | |
| ***Gymnomyza*** | ***samoensis*** | **KUNHM 107665** | | | |
| ***Gymnomyza*** | ***viridis*** | **KUNHM 24318** | | | |
| ***Gymnomyza*** | ***viridis*** | **KUNHM 30461** | | | |
| *Lichenostomus* | *melanops* | ANSP 22940 | | HQ267674 | HQ267694 |
| *Lichmera* | *alboauricularis* | E629 | | AY488279 | AY488432 |
| ***Lichmera*** | ***incana*** | **UMMZ 221981** | | | |
| *Lichmera* | *indistincta* | C271 | | AY488280 | AY488433 |
| *Manorina* | *flavigula* | 42856 | | AY488281 | AY488434 |
| *Manorina* | *melanophrys* | 42737 | | AY488282 | AY488435 |
| ***Meliarchus*** | ***sclateri*** | **KUNHM 13544** | | | |
| ***Meliarchus*** | ***sclateri*** | **KUNHM 13546** | | | |
| *Melidectes* | *belfordi* | E168 | | AY488283 | AY488436 |
| *Melidectes* | *ochromelas* | E360 | | AY488284 | AY488437 |
| *Melidectes* | *torquatus* | E389 | | AY488285 | AY488438 |
| *Melilestes* | *megarhynchus* | E557 | | AY488286 | AY488439 |
| *Meliphaga* | *albonotata* | E471 | | AY488287 | AY488440 |
| *Meliphaga* | *gracilis* | C753 | | AY488288 | AY488441 |
| *Melipotes* | *fumigatus* | E332 | | AY488289 | AY488442 |
| *Melithreptus* | *albogularis* | JC100 | | AY488290 | AY488443 |
| *Melithreptus* | *brevirostris* | MV371 | | AY488291 | AY488444 |
| *Myzomela* | *cardinalis* | 2494 | | AY488292 | AY488445 |
| *Myzomela* | *erythrocephala* | MV1198 | | AY488406 | AY488446 |
| *Myzomela* | *obscura* | C531 | | AY488293 | AY488447 |
| *Myzomela* | *rosenbergii* | E240 | | AY488294 | AY488448 |

| Genus | Species | Voucher | Genus (Driskell and Christidis 2004) | GenBank Accession (ND2) | (Fib5) |
|---|---|---|---|---|---|
| *Myzomela* | *sanguinolenta* | C402 | | AY488295 | AY488449 |
| *Nesoptilotis* | *flavicollis* | ANWC 45751 | *Lichenostomus* | HQ267667 | HQ267687 |
| *Nesoptilotis* | *leucotis* | KUNHM 8763 | *Lichenostomus* | HQ267673 | HQ267693 |
| *Philemon* | *argenticeps* | JCW095n | | AY488296 | AY488450 |
| *Philemon* | *buceroides* | C863n | | AY488297 | AY488451 |
| *Philemon* | *citreogularis* | D008n | | AY488298 | AY488452 |
| ***Philemon*** | ***cockerelli*** | **KUNHM 27644** | | | |
| *Philemon* | *corniculatus* | C720 | | AY488299 | AY488453 |
| ***Philemon*** | ***eichhorni*** | **KUNHM 27770** | | | |
| ***Philemon*** | ***eichhorni*** | **NMNH B4027** | | | |
| *Philemon* | *meyeri* | E683 | | AY488300 | AY488454 |
| *Phylidonyris* | *niger* | MV198 | | AY488302 | AY488457 |
| *Phylidonyris* | *novaehollandiae* | B685 | | AY488303 | AY488458 |
| *Phylidonyris* | *pyrrhopterus* | B615 | | AY488408 | AY488459 |
| *Plectorhyncha* | *lanceolata* | C379 | | AY488304 | AY488460 |
| *Prosthemadera* | *novaeseelandiae* | 111996 | | AY488305 | AY488461 |
| *Ptiloprora* | *guisei* | E173 | | AY488306 | AY488462 |
| *Ptiloprora* | *plumbea* | C173 | | AY488409 | AY488463 |
| *Ptilotula* | *flavescens* | ANSP 25785 | *Lichenostomus* | HQ267666 | HQ267686 |
| *Ptilotula* | *flavescens* | D029 | *Lichenostomus* | AY488278 | AY488431 |
| *Ptilotula* | *penicillata* | KUNHM 6179 | *Lichenostomus* | HQ267677 | HQ267697 |
| *Purnella* | *albifrons* | D361 | *Phylidonyris* | AY488301 | AY488455 |
| *Pycnopygius* | *cinereus* | C057 | | AY488307 | AY488464 |
| *Pycnopygius* | *stictocephalus* | C035 | | AY488308 | AY488465 |
| *Ramsayornis* | *fasciatus* | MV1230 | | AY488309 | AY488466 |
| *Ramsayornis* | *modestus* | C900 | | AY488310 | AY488467 |
| *Sugomel* | *niger* | C954 | *Certhionyx* | AY488267 | AY488418 |
| *Stomiopera* | *flava* | ANSP 25088 | *Lichenostomus* | HQ267668 | HQ267688 |
| ***Stresemannia*** | ***bougainvillei*** | **KUNHM 5280** | | | |
| ***Stresemannia*** | ***bougainvillei*** | **KUNHM 5281** | | | |
| *Timeliopsis* | *fulvigula* | E233 | | AY488311 | AY488468 |
| *Timeliopsis* | *griseigula* | E714 | | AY488312 | AY488469 |
| *Trichodere* | *cockerelli* | 42941 | | AY488313 | AY488470 |
| ***Vosea* [*Melidectes*]** | ***whitemanensis*** | **AMNH 778167** | | | |
| ***Vosea* [*Melidectes*]** | ***whitemanensis*** | **AMNH 778172** | | | |
| *Xanthomyza* | *phrygia* | F724 | | AY488314 | AY488471 |
| ***Xanthotis*** | ***flaviventer*** | **KUNHM 5588** | | | |
| ***Xanthotis*** | ***flaviventer*** | **KUNHM 7571** | | | |
| ***Xanthotis*** | ***flaviventer*** | **KUNHM 9557** | | | |
| *Xanthotis* | *flaviventer* | E594 | | AY488315 | AY488472 |
| ***Xanthotis*** | ***polygrammus*** | **KUNHM 5133** | | | |
| ***Xanthotis*** | ***polygrammus*** | **KUNHM 9640** | | | |

| Genus | Species | Voucher | Genus (Driskell and Christidis 2004) | GenBank Accession (ND2) | (Fib5) |
|---|---|---|---|---|---|
| ***Xanthotis*** | ***provocator*** | **KUNHM 24416** | | | |
| ***Xanthotis*** | ***provocator*** | **KUNHM 25211** | | | |
| Outgroup | | | Family | | |
| *Acanthiza* | *apicalis* | MV158 | Acanthizidae | AY488316 | AY488473 |
| *Acanthiza* | *chrysorrhoa* | MV116 | Acanthizidae | AY488317 | AY488474 |
| *Dasyornis* | *broadbenti* | MV2172 | Dasyornithidae | AY488318 | AY488475 |
| *Gerygone* | *chloronotus* | E122 | Acanthizidae | AY488319 | AY488476 |
| *Gerygone* | *chrysogaster* | E670 | Acanthizidae | AY488320 | AY488477 |
| *Pardalotus* | *punctatus* | B479 | Pardalotidae | AY488321 | AY488478 |
| *Pardalotus* | *striatus* | B471 | Pardalotidae | AY488322 | AY488479 |
| *Sericornis* | *frontalis* | MV228 | Acanthizidae | AY488323 | AY488480 |
| *Sericornis* | *perspicillatus* | E313 | Acanthizidae | AY488324 | AY488481 |
| *Amytornis* | *striatus* | SGW1 | Maluridae | AY488325 | AY488482 |
| *Malurus* | *lamberti* | VW104 | Maluridae | AY488326 | AY488483 |
| *Malurus* | *splendens* | SW683 | Maluridae | AY488327 | AY488484 |
| *Stipiturus* | *mallee* | MEW1 | Maluridae | AY488328 | AY488485 |