DeCicco, L. H., DeRaad, D. A., Holland, P., Pikacha, D., Gumo Tigulu, I., Tako, R., Boseto, D., Sweet, P. R., & Moyle, R. G. (2024). Phylogeography of the Island Leaf Warbler (Aves: *Phylloscopus poliocephalus*) in Northern Melanesia Reveals Rapid Secondary Sympatry or Ecological Speciation on Kolombangara Island, Solomon Islands. *Bulletin of the Society of Systematic Biologists*. https://doi.org/10.18061/bssb.v3i2.9439

Investigations

Phylogeography of the Island Leaf Warbler (Aves: *Phylloscopus Poliocephalus*) in Northern Melanesia Reveals Rapid Secondary Sympatry or Ecological Speciation on Kolombangara Island, Solomon Islands

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Keywords: gene flow, character displacement, mitochondrial-nuclear discordance, Phylloscopus amoenus, Phylloscopus poliocephalus, Phylloscopidae

https://doi.org/10.18061/bssb.v3i2.9439

Bulletin of the Society of Systematic Biologists

Abstract

Island archipelagos are well known for promoting geographic and adaptive radiations in terrestrial animals. Sympatry of closely related species in island systems has been thought to occur primarily through double-invasions from the same continental source population. Alternatively, this process can occur on a smaller geographic scale where divergent populations isolated from one another within an archipelago may come back into contact via intra-archipelago dispersal. Depending on degree of divergence and the development of reproductive isolating barriers, the result of this secondary contact could be gene flow between isolated populations, exclusion or extirpation of one population via competition, or the establishment of reproductively isolated sympatric species. Here, we provide strong evidence for intra-archipelago secondary sympatry within a radiation of *Phylloscopus* warblers in the Solomon and Bismarck archipelagos by presenting the first well-sampled phylogeny of *Phylloscopus* warbler populations in the region. By using genome-wide genetic data and complete mitochondrial genomes we also present evidence for a complex history of mitochondrial-nuclear discordance within this radiation, particularly in the only pair of sympatric populations east of Wallace's Line, located on Kolombangara Island in the Solomon Islands. Our genomic data suggest that the divergent single-island endemic species on Kolombangara (*Phylloscopus amoenus*) is not a relic resulting from a double invasion from outside the Bismarck and Solomon archipelagos as previously thought. Rather, the sympatry on Kolombangara Island between the widespread *P. poliocephalus* and the endemic *P. amoenus* appears to result from recent intra-archipelago diversification, either via sympatric speciation or the rapid establishment of secondary sympatry. These novel genomic data provide insights into the evolutionary history of *Phylloscopus* warblers in Melanesia and improve our general understanding of how biodiversity accumulates in island systems.

1 INTRODUCTION

1.1 Synthesis

Allopatric divergence (i.e., the differentiation of populations due to spatial isolation) is generally recognized as the dominant force in the accumulation of biodiversity (Coyne & Price, 2000; Dobzhansky, 1937; Mayr, 1963), particularly for organisms that easily colonize disjunct geographic regions via dispersal. Dispersive organisms such as birds readily form large radiations across island archipelagos and are considered exemplars of this allopatric divergence and speciation model (Moyle et al., 2009). These avian radiations have played an integral role in the development of speciation theory (e.g., Mayr, 1942), a key component of which states that, via either dispersal or range shifts, allopatric populations may eventually come into secondary contact. In island systems secondary contact falls under two similar categories that differ on geographic and temporal scales: 1) multiple invasions of an archipelago from a

single continental source population, or 2) secondary contact of divergent populations within an archipelago after original colonization. This intra-archipelago secondary contact can occur under multiple scenarios, but most commonly occurs via the dispersal of individuals from one isolated population to another. How individuals from two populations interact upon secondary contact varies and is related to how much genomic, phenotypic, and ecological divergence has accumulated between the populations. At one extreme, individuals from two populations may freely interbreed upon contact producing signals of gene flow between populations due to the dispersal of individuals between geographically isolated populations. At the other extreme, upon secondary contact, individuals from divergent populations may not successfully interbreed due to reproductive isolating mechanisms, which may be pre-zygotic, post-zygotic, or both (e.g., Orr, 1996). Although a continuum exists between these two extremes of interaction upon secondary contact, the eventual outcomes on small islands are generally categorical: population fusion, sympatric co-existence of two species with or without gene flow, or extirpation via competition.

The sympatric co-existence of two closely related species due to secondary contact can occur without gene flow between populations (i.e., complete reproductive isolation) or with some level of historical or ongoing gene flow. This latter scenario can occur under processes such as reinforcement (e.g., Dobzhansky, 1940; Howard, 1993) where hybridization is selected against post-zygotically due to low hybrid fitness, creating positive selection pressure for the development of pre-zygotic isolating mechanisms. Gene flow between populations can generate discordant signals of relatedness among genomic regions or between nuclear and mitochondrial genomes (i.e., mito-nuclear discordance). The extreme example of this phenomenon is the complete sweep or capture of mitochondrial genomes from one population into another, which has been documented in island systems (e.g., Andersen et al., 2021 and references therein). The presence of mito-nuclear discordance among closely related taxa can be evidence of historical or on-going gene flow (e.g., Mikkelsen & Weir, 2022), incomplete lineage sorting (e.g., DeRaad et al., 2023), and generally interesting and complex evolutionary histories.

1.2 Focal system

A phenotypically and ecologically divergent *Phylloscopus* warbler (*P. amoenus*) is endemic to the mountains of Kolombangara Island in the Solomon Islands, where it occurs alongside its sympatric congener, *P. poliocephalus pallescens*—the only case of multiple *Phylloscopus* warbler populations occurring on the same island anywhere east of Wallace's Line. The endemic *P. amoenus* differs from typical *Phylloscopus* warblers in being adapted to a more terrestrial lifestyle and exhibiting larger feet and legs, a shorter tail, larger bill, and darker plumage. In habits, instead of being a canopy dweller and foliage gleaner like most *Phylloscopus* warblers, it has adapted to foraging along mossy tree trunks, limbs, and often on the ground (Dutson 2011, DeCicco pers. obs.). Due to the ecological and phenotypic uniqueness of the endemic *P. amoenus* (e.g., it was originally described under a monotypic genus *Mochthopoeus*; Hartert, 1929), it has been suggested that it is only distantly related to populations of *P. poliocephalus* throughout Northern Melanesia which all share a similar phenotype and ecology (Mayr, 1944, 1955; Mayr & Diamond, 2001). This scenario was proposed as evidence for a "double invasion" of *Phylloscopus* warblers into Northern Melanesia from a single source population in New Guinea or farther west. Under this scenario, it was proposed that the endemic *P. amoenus* represented the last remaining population from a previous invasion and the widespread populations of *P. poliocephalus* resulted from a more recent invasion from the same source population that previously produced the relict *P. amoenus* (Mayr, 1944, 1955; Mayr & Diamond, 2001). Despite the phenotypic distinctiveness of *P. amoenus*, recent molecular data have suggested that it is closely related to the Solomon Islands clade of *P. poliocephalus* (Alström et al., 2018; Ng et al., 2018; Reeve et al., 2023), an interesting but inconclusive result as all previously published genetic research lacked complete population-level sampling within the Solomon Islands. This incomplete sampling prevented confident phylogenetic placement of *P. amoenus* among the populations of *P. poliocephalus* distributed broadly throughout Northern Melanesia and the inference of an evolutionary history of the unique case of sympatric occurrence of two species on Kolombangara Island.

Due to complex taxonomy among the allopatric populations of the widespread *P. poliocephalus*, we refer to all allopatric populations by their subspecific epithet (e.g., *P. p. ssp.*). Names of allopatric *P. poliocephalus* populations follow Gill et al. (2024), with the exception that we here treat the allopatric Makira population as *P. p. makirensis* rather than *P. makirensis*. The single-island endemic species occurring on Kolombangara Island (*P. amoenus*) in sympatry with *P. p. pallescens* has never been considered conspecific with the widespread *P. poliocephalus* radiation and was in fact described under its own genus *Mochthopoeus* (Hartert, 1929). Given the results presented here the current taxonomy is not representative of the evolutionary history in this group, however, we lack complete population-level sampling of *P. poliocephalus* outside of Northern Melanesia, and therefore refrain from making taxonomic suggestions as part of this study.

1.3 System background

The *Phylloscopus* warblers within the Bismarck and Solomon archipelagos are represented by 10 allopatric populations (Mayr & Diamond, 2001), all but one of which are restricted to montane cloud forest habitat which typically occurs above 1,000 m elevation, but varies by island, e.g., montane cloud forest starts as low as 500 m on Makira (Dutson, 2011; Clement et al., 2021). These 10 populations include: *P. p. moorhousei* in the mountains of New Britain and Umboi islands; *P. p. leletensis* in the mountains of New Ireland Island (this population was not sampled in this study); *P. p. matthiae* in all elevations on Mussau Island (the only lowland population); *P. p. bougainvillei* on Bougainville Island (Mayr, 1945); *P. p. becki* on Isabel (highly restricted,

see DeCicco et al., 2019), Malaita, and Guadalcanal islands (Mayr, 1945); *P. p. makirensis* on Makira Island (Mayr, 1945; currently recognized as a separate species due to the yellower ventral plumage; Gill et al., 2024); and *P. p. pallescens* being the typical *Phylloscopus* warbler on Kolombangara Island (Mayr, 1945) occurring in sympatry with the divergent *P. amoenus*. All populations of *P. poliocephalus* share a relatively similar ecology and phenotype, having an olive-green dorsum, pale eye-line, pale central crown stripe, and varying in ventral coloration from lemon yellow (e.g., *P. p. makirensis*) to pale grayish white in *P. p. pallescens*. They all share similar advertisement songs and a similar ecology, as active canopy or mid-story foliage gleaners (Clement et al., 2021). In stark contrast, the Kolombangara endemic *P. amoenus* exhibits a very dark olive-brown plumage with little counter shading, a different morphology (longer bill, longer legs, larger feet, shorter tail), and a very different ecology—that of foraging primarily among lower mossy trunks of trees (Clement, 2020; DeCicco pers. obs.).

1.4 Objectives and summary

Here we present the first comprehensively sampled phylogeny of Northern Melanesian *Phylloscopus* warblers and compare genome-wide nuclear DNA sequence data to mitochondrial genome DNA sequence data. We test the hypothesis that the endemic *P. amoenus* represents the last remaining relict population from the first colonists of a double-invasion of *Phylloscopus* warblers into the Solomon Islands from a source population in New Guinea or farther west. We provide two well supported hypotheses for the evolutionary history of the group both of which consistently contradict the traditionally hypothesized "double invasion" origin of *P. amoenus* on Kolombangara (Mayr & Diamond, 2001). Despite our inability to distinguish the exact details of the complex speciation history of the two sympatric species on Kolombangara Island, we speculate on the potential evolutionary scenarios that could have generated the patterns we observe and discuss their implications for understanding the general process of biodiversity accumulation in island archipelagos.

2 METHODS

2.1 Sampling, laboratory procedures, and sequencing

We obtained specimen-vouchered genetic samples from 27 individuals representing nine of the 10 extant populations of *Phylloscopus* warblers in the Solomon and the Bismarck archipelagos (Table 1, Fig. 1a). We included samples from New Guinea and Borneo as outgroups. Due to limited modern sampling, we extracted genetic material for five of the 10 sampled populations from degraded toepad samples of historical museum specimens collected before 1930. Samples of the remaining populations came from modern specimen-vouchered and ethanol-preserved muscle tissue (Table 1).

We digested tissue subsamples in a 7% proteinase K and tissue lysis buffer solution for 24 hrs at 57°C and extracted genomic DNA using a manual bead-based method (https://github.com/phyletica/lab-protocols/blob/master/extraction-spri.md) based on Rohland and Reich (2012) and eluted DNA from beads using buffer EB. We washed degraded toepad samples in a 1× STE buffer solution to remove surface contamination (McCormack et al., 2016), and handled all toepad material, when not in a sealed vial, in a UV sterilized hood with laminar airflow to reduce risk of contamination. Toepad samples were extracted using a similar bead-based method but in a Maxwell RSC extraction robot.

We followed established ultraconserved elements (UCEs) library preparation protocols to produce libraries enriched for DNA fragments that included UCEs (Faircloth et al., 2012; McCormack et al., 2016). In brief, we sonicated all fresh tissue extracts to 300 base-pairs (bp) of length using a Covaris M220 focused-ultrasonicator at the University of Kansas Genome Sequencing Core. Toepad samples were not sonicated because DNA strands were already degraded and "naturally" shortened. We then used the Kapa Biosystems Library Prep kit to end repair, A-tail, and ligate iTru stub adapters to the ends of DNA fragments and dual-indexed samples using iTru i5 and i7 indexes (Glenn et al., 2019). We then PCR amplified these indexed samples and used a 1× AMPure XP bead cleanup post-amplification (a 3× bead cleanup was used for degraded samples). We standardized concentrations and combined indexed samples into pools of eight (for fresh tissues) or five (for degraded toepads) and broadly followed the Mybaits protocol v. 3.01 for baiting amplified DNA fragments using the Arbor Biosciences MYbaits kit for Tetrapods UCE-5Kv1, targeting 5060 UCE loci (www.ultraconserved.org). Using Dynabeads we then performed another bead cleanup of this baited hybridization reaction and PCR amplified this cleaned reaction while baited DNA fragments were still attached to the Dynabeads. We removed the amplified reaction from the Dynabeads and did another 1× AMPure XP bead cleanup of this amplified reaction. For degraded toepad samples, we did a size-selection step after this to remove fragments <150 bp in length using QIAGEN's GeneRead Size Selection kit.

We pooled individually barcoded libraries from each sample for sequencing at the Oklahoma Medical Research Foundation, generating paired-end 150 bp reads from an Illumina NovaSeq 6000 machine. We adjusted pooling concentrations to aim for ca. 2.2 million reads per sample for fresh tissue samples and ca. 5 million reads per sample for toepads due to lower input quality of these samples.

2.2 Data assembly and filtering

We trimmed demultiplexed reads using *Fastp* (Chen et al., 2018) to remove adapters, remove four base pairs (bp) from the start and end of each read, discard reads with an average phred score of <30, discard reads in which >10 % of bases were unqualified, and conduct overlap correction. We then ran a standard reference-aligned assembly for resequencing data using *GATK* (v3; Van der Auwera & O'Connor, 2020) and the reference genome of a male *Phylloscopus*

Table 1. *Phylloscopus* warbler samples used in this research, *P. poliocephalus* is abbreviated to "*P. p.*" and the subspecies epithet is provided. Taxon nomenclature follows Gill et al., (2024) with the exception of treating the population on Makira Island as an allopatric subspecies, not species. In the locality column, Solomon Islands are abbreviated to "S.I." and the Bismarck Archipelago is abbreviated to "B.A.". Institution abbreviations are as follows: the University of Kansas (KU), American Museum of Natural History (AMNH).

Taxon	Locality	Material	Institution	Catalog No.	Tissue No.
P. amoenus	S.I., Kolombangara Is.	Tissue	KU	134986	36181
P. amoenus	S.I., Kolombangara Is.	Tissue	KU	134989	36184
P. amoenus	S.I., Kolombangara Is.	Tissue	KU	135013	36208
P. p. becki	S.I., Isabel Is.	Toepad	AMNH	218146	—
P. p. becki	S.I., Malaita Is.	Toepad	AMNH	227294	—
P. p. becki	S.I., Malaita Is.	Toepad	AMNH	227295	—
P. p. becki	S.I., Guadalcanal Is.	Tissue	KU	132032	32812
P. p. becki	S.I., Guadalcanal Is.	Tissue	KU	132031	32828
P. p. bougainvillei	S.I., Bougainville Is.	Toepad	AMNH	225170	—
P. p. bougainvillei	S.I., Bougainville Is.	Toepad	AMNH	225177	—
P. p. bougainvillei	S.I., Bougainville Is.	Toepad	AMNH	225186	—
P. p. giulianettii	Papua New Guinea	Tissue	KU	113282	16583
P. p. giulianettii	Papua New Guinea	Tissue	KU	114919	16584
P. p. giulianettii	Papua New Guinea,	Tissue	KU	122745	27869
P. p. giulianettii	Papua New Guinea	Tissue	KU	123581	27874
P. p. makirensis	S.I., Makira Is.	Tissue	KU	133532	34962
P. p. makirensis	S.I., Makira Is.	Tissue	KU	133706	35004
P. p. makirensis	S.I., Makira Is.	Tissue	KU	133561	35012
P. p. matthiae	B.A., Mussau Is.	Toepad	AMNH	450354	—
P. p. moorhousei	B.A., New Britain Is.	Toepad	AMNH	777977	—
P. p. moorhousei	B.A., New Britain Is.	Toepad	AMNH	777983	—
P. p. moorhousei	B.A., New Britain Is.	Toepad	AMNH	777987	—
P. p. pallescens	S.I., Kolombangara Is.	Tissue	KU	134996	36191
P. p. pallescens	S.I., Kolombangara Is.	Tissue	KU	134997	36192
P. p. pallescens	S.I., Kolombangara Is.	Tissue	KU	135005	36200
P. trivirgatus	Borneo, Sabah	Tissue	KU	112953	17722
P. trivirgatus	Borneo, Sabah	Tissue	KU	112952	17759

whistleri (Zhang et al., 2021; GenBank assembly accession GCA_017589585.1). We indexed the reference genome using *Bowtie2* (v. 2.3.5.1; Langmead & Salzberg, 2012), then mapped our trimmed reads to this reference genome using *Bowtie2* with the "very sensitive" setting. This produced a SAM file that we then converted to a BAM and sorted using SAMtools v1.16.1 (Danecek et al., 2021). Using the *Picard Toolkit* (2019), we formatted our reference genome for use by *GATK*, and formatted our BAM files by adding readgroup information, marking duplicates, and running *FixMateInformation*. We then called biallelic variable sites (i.e., biallelic single nucleotide polymorphisms, SNPs) using *GATK HaplotypeCaller* and output variable sites in Variant Call Format (vcf; Danecek et al., 2011). Using *GATK*'s *VariantFiltration* program, we then flagged sites that did not pass the following quality filtering: QD < 2.0, FS > 60.0, MQ < 40.0, MQRankSum <- 12.5, ReadPosRankSum <- 8.0, and SOR > 3.0. We removed these flagged sites with *VCFtools* (Danecek et al., 2011) and set a further 50% completeness by site threshold to retain SNPs. We then filtered this vcf file using the R packages *SNPfiltR* v1.0.0 (DeRaad, 2022) and *vcfR* v1.12.0 (Knaus & Grünwald, 2017) following the suggested settings. Specifically, we required SNPs to have a depth of coverage ⩾ 5 and < 150 and a genotype quality score ⩾ 30. We also removed heterozygous sites where one of the alleles was called by either < 25% of reads or > 75% of reads, as each allele in a "real" heterozygous site should be called by ca. 50% of the reads. Finally, we removed invariant sites and produced four biallelic nuclear SNP datasets varying in completeness and inclusion of singleton variable sites (i.e., a minor allele count of 2 was enforced; Linck & Battey, 2019): 1) 80% complete by site with singleton variable sites included, 2) 80% complete by site with singleton variable sites removed, 3) 90% complete by site with singleton variable sites included, and 4) 90% complete by site with singleton variable sites removed.

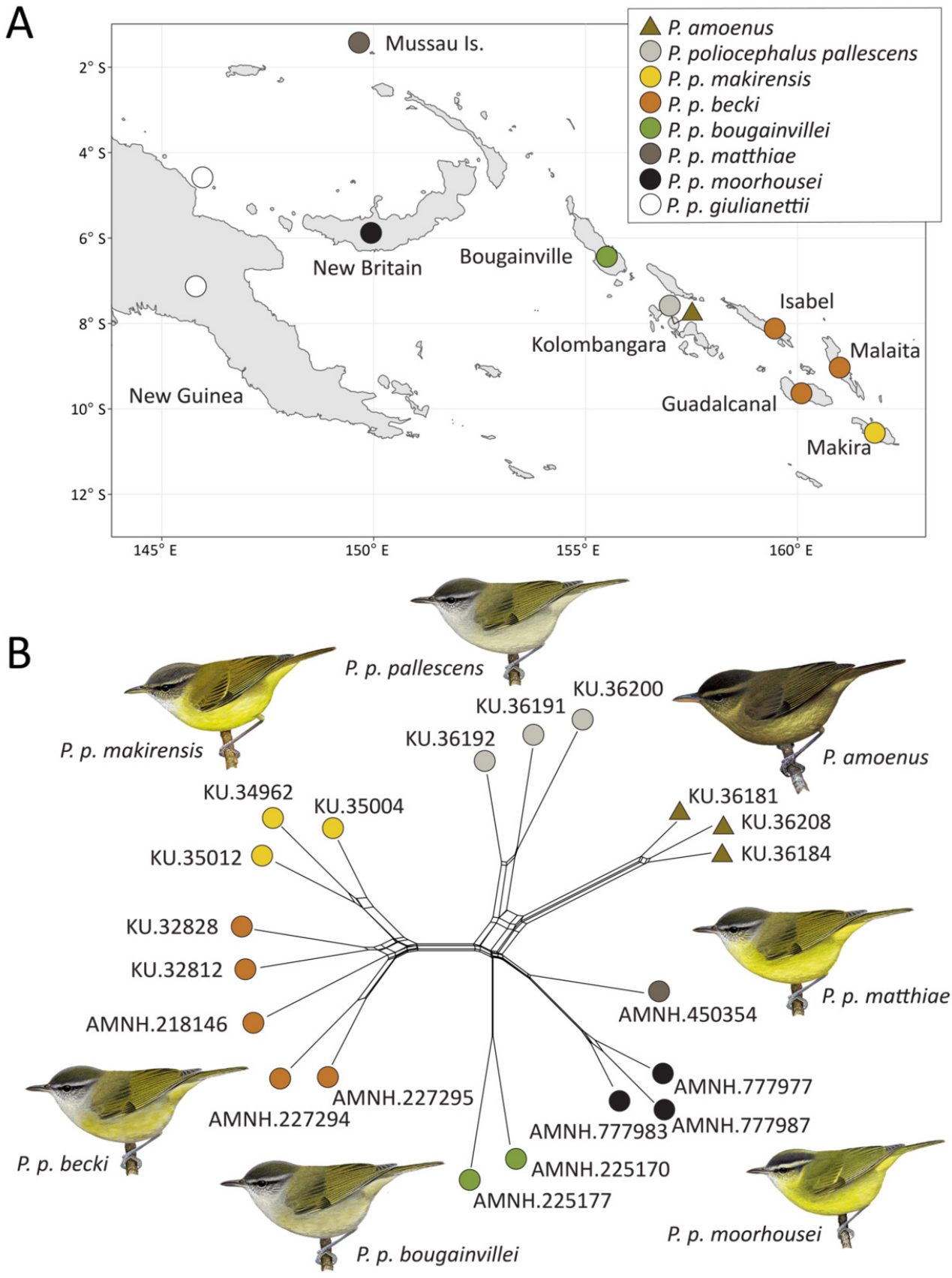

Figure 1. (a) Sampling map showing locations of all sequenced populations, except for outgroup samples from Borneo. Note that the occurrence of the Kolombangara-endemic *P. amoenus* with its sympatric congener *P. p. pallescens* is the only case of sympatry in *Phylloscopus* warblers east of Wallace's Line. (b) Unrooted network visualization of Nei's genetic distances using our 90% complete nuclear SNP dataset as input, after removing the outgroup samples from Borneo and New Guinea. Of interest is the apparent sister relationship between the sympatric populations on Kolombangara. Original illustrations by David Quinn updated digitally by Rene P. Martin, Copyright Cornell Lab of Ornithology.

2.3 Mitochondrial genome assembly and alignment

Mitochondrial sequences are a common byproduct of UCE library preparation, despite not being explicitly targeted (e.g., Do Amaral et al., 2015). We used an assembled mitochondrial genome from *P. borealoides* (MN125373; Sun et al., 2019), a species that is ca. 9 Ma divergent from our ingroup taxa (Alström et al., 2018) to call mitochondrial genomes from our raw UCE-enriched reads. We used the programs *MITObim* (C. Hahn et al., 2013) and *MIRA* (Chevreux et al., 1999) to iteratively map these reads to the *P. borealoides* mitochondrial reference genome. We discarded mitochondrial genomes that were < 75% complete, i.e., had > 25% missing data. We aligned the remaining mitochondrial genomes to the reference from *P. borealoides* in *Geneious Basic* (Kearse et al., 2012) and manually trimmed these alignments to remove all missing data to produce a concatenated complete mitochondrial genome alignment. Using the annotated *P. borealoides* mitochondrial genome, we manually parsed a 100% complete, 1,040 bp alignment of the ND2 gene sequence from this complete mitochondrial genome alignment and calculated raw pairwise distances for this gene using *Geneious*. This process produced two mitochondrial DNA datasets: 1) a complete mitochondrial genome alignment and 2) an ND2 alignment parsed from the complete mitochondrial genome.

2.4 Phylogenetic analyses

We produced phylogenetic trees for each of our four nuclear SNP datasets and our complete mitochondrial genome alignment using a concatenated maximum-likelihood method implemented in *IQ-TREE 2* v2.2.0 (Minh et al., 2020) and automatic model finder (Kalyaanamoorthy et al., 2017). Input for *IQ-TREE 2* was a concatenated phylip file output by *Geneious* (for mitochondrial genomes) or converted from a vcf using the *Stacks Populations* program (Rochette et al., 2019). For the SNP datasets, an ascertainment bias correction was included in all models considered during the model selection process by specifying the flag '-m MFP+ASC'. Node support was obtained using 10,000 ultrafast bootstrap replicates in *IQ-TREE 2* (Hoang, 2018). We also produced a pairwise distance matrix (Nei's D; Nei, 1972) for all nuclear SNP datasets using the R package *StAMPP* v1.6.3 (Pembleton et al., 2013) to visualize an unrooted Neighbor-Net phylogenetic network in *SplitsTree4* (v4.15.1; Bryant & Moulton, 2004; Huson and Bryant, 2006). Finally, we used a quartet-based approach implemented in *SVDquartets* (Chifman & Kubatko, 2014) to estimate a species tree from each of the four nuclear SNP datasets using 1,000 bootstrap replicates to estimate node support values. Each named taxon was used to assign individual samples to pre-defined population tips for species tree reconstruction.

3 RESULTS

3.1 Sequencing and data retention

Our sequencing produced on average 4.3 million reads (paired-end 150 bp) per tissue sample and 23.4 million reads per toepad sample, with an average of 3.0 million and 18.8 million reads retained respectively after filtering and trimming (supplemental Table S1). Tissue samples had an average insert size of 204 bp while toepads had an average insert size of just 95 bp (supplemental Table S3), as expected due to the degraded nature of these approximately century-old toepads. Compared with fresh tissue samples, the degraded toepad samples consistently had a larger proportion of missing genotypes in SNP datasets despite receiving roughly five times the sequencing effort. Because of this excess missing data, degraded samples strongly affected SNP retention when implementing dataset-wide completeness cutoffs. For example, at a 50% per-SNP genotype completeness threshold, toepad samples had nearly 75% fewer called genotypes (~10,000 per sample; range 3,236 – 14,299) than tissue samples (~37,500 per sample; range 34,364 – 39,089; supplemental Table S1). To improve the overall missing data proportion among these degraded samples, we removed a single outlier toepad sample (AMNH.225186) which received proportionately lower sequencing coverage (7.8 million reads post filtering, compared to an average of 19.9 million reads across all other toepad samples). Additionally, to assess the effect of missing data and the inclusion of rare alleles on our downstream phylogenetic inferences, we performed each phylogenetic analysis on four quality filtered SNP datasets: 80% complete (8,093 SNPs retained), 80% with singletons removed (3,391 SNPs retained), 90% complete (5,668 SNPs retained), and 90% with singletons removed (2,339 SNPs retained).

Our mitogenome baiting and iterative mapping produced a mitochondrial genome alignment averaging 17,069 bp per sample (supplemental Table S1). We removed a single sample, AMNH.225186, due to ca. 15% missing sites (268 missing out of 16,946) compared to zero missing sites in all other samples (supplemental Table S1). Aligning and manually trimming produced a 100% complete matrix of 16,692 sites, ca. 99% of the length of the reference mitochondrial genome (16,904 bp). Our extracted ND2 gene alignment was 100% complete and 1,040 bp in length.

3.2 Nuclear phylogenetic relationships

Our four nuclear SNP datasets (80% and 90% complete, with and without singletons removed) all produced nearly identical, well-supported phylogenetic hypotheses using distance-based, quartet-based, and maximum likelihood approaches (Figs. 1, 2, and 3 respectively; supplemental Figs. S1.1–S3.4). Both the outgroup *P. trivirgatus* and the New Guinea samples (*P. p. giulianettii*) were consistently recovered as sister to all sampled populations from the Bismarck and Solomon archipelagos (from here on referred to as the "ingroup"). The endemic Kolombangara species, *P.*

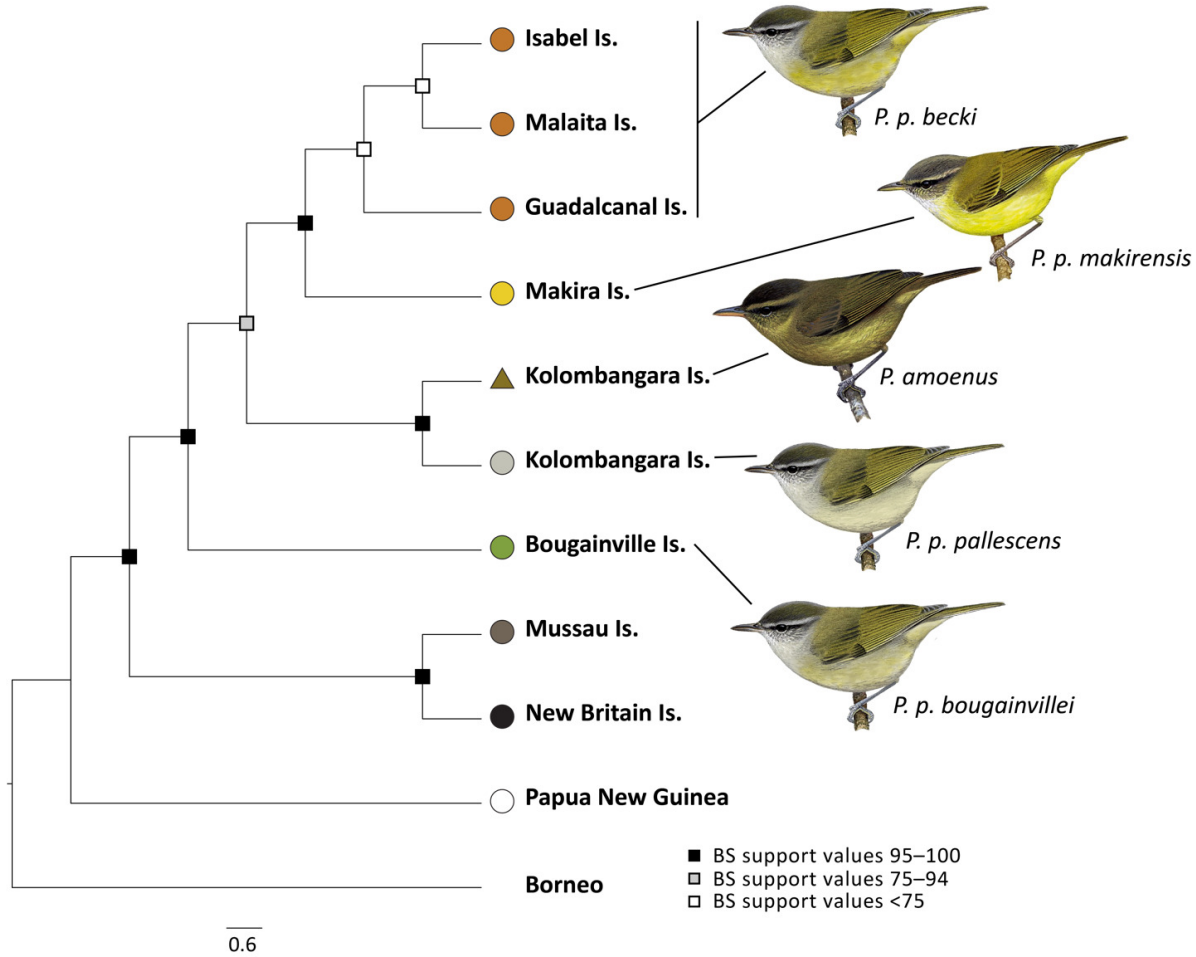

Figure 2. Quartet-based species tree reconstruction using the 90% complete matrix of 5,668 SNPs as input. Note that identical nuclear topologies are recovered by this species-tree method and a maximum likelihood approach (Fig. 3b). Subspecies assignments, node support and species illustrations all follow the same conventions as in Figure 1. Original illustrations by David Quinn updated digitally by Rene P. Martin, Copyright Cornell Lab of Ornithology.

amoenus, was embedded within this ingroup in all analyses. All four nuclear SNP datasets recovered samples from the Bismarck Archipelago as a unique clade, sister to all Solomon Island populations (Figs. 1–3). Within the Solomon Islands, the two samples from Bougainville were recovered as first branching, and a southern Solomons clade including all samples from Makira, Guadalcanal, Malaita, and Isabel islands was consistently recovered as sister to a northern clade of the two Kolombangara species (Figs. 2 and 3b). All distance-based phylogenetic network analyses recovered a concordant pattern, with the longest internal branch separating the southern Solomons clade from a clade containing all samples from the northern Solomons and Bismarck archipelago (Figs. 1b, supplemental Figs. S2.1–S2.4). In every phylogeny reconstructed using nuclear SNPs as input, regardless of filtering scheme or methodological approach, the two species occurring in sympatry on Kolombangara were recovered as sister with high bootstrap support (Figs. 1, 2, and 3b; supplemental Figs. S1.1–S3.4).

3.3 Mitochondrial phylogenetic relationships and divergence

Phylogenetic reconstructions using our mitochondrial genome alignment (100% complete and 16,692 bp) as input found strong support for a discordant set of relationships, compared to the topology consistently recovered from nuclear SNP datasets. While a southern Solomons clade was again recovered with high support, it does not include the sample from Isabel Island (Fig. 3a). Additionally, samples from the Bismarck Archipelago were recovered as sister to a northern Solomons group including both Kolombangara species, plus samples from Bougainville and Isabel islands. Importantly, the two species occurring on Kolombangara were not recovered as sister in reconstructions based on mitochondrial DNA. Instead, the Kolombangara endemic *P. amoenus* was sister to a clade containing the sympatric *P. p. pallescens* and samples from Bougainville and Isabel islands. Uncorrected pairwise distances among populations based on the mitochondrial gene ND2 ranged from 0.34% to 4.33% among Bismarck and Solomons populations (Table 2). ND2 divergence between the two sympatric Kolombangara species was 3.14%, indicating an extended period of

Table 2. Uncorrected pairwise distances among populations of *Phylloscopus* warblers in Northern Melanesia, including outgroup taxa from Borneo and New Guinea, based on a complete mitochondrial alignment of the ND2 gene. The only sympatric occurrence of populations is between the two species on Kolombangara.

	New Guinea	New Britain	Mussau	Bougainville	Isabel	Malaita	Guadalcanal	Makira	Kolombangara—*pallescens*	Kolombangara—*amoenus*
Borneo	4.81	4.98	5.24	5.19	5.05	4.86	4.95	4.57	5.46	6.36
New Guinea	—	2.97	2.84	3.56	3.41	3.32	3.41	3.64	3.80	4.81
New Britain		—	2.24	2.97	2.82	3.21	3.59	3.53	3.30	3.17
Mussau			—	2.84	2.69	3.17	3.46	3.59	3.17	3.05
Bougainville				—	0.34	3.89	3.99	3.92	1.01	2.80
Isabel					—	3.75	3.85	3.78	0.87	2.66
Malaita						—	0.87	1.63	4.23	3.91
Guadalcanal							—	1.92	4.33	4.20
Makira								—	4.26	3.88
Kolombangara—*pallescens*									—	3.14

historical isolation between these taxa, contradicting the results from the nuclear SNP datasets.

4 DISCUSSION

4.1 Summary

All results, from complete mitochondrial genomes and four quality-filtered nuclear SNP datasets, each with complete population level sampling within the Solomon Islands, supported the monophyly of *Phylloscopus* warbler populations in the Bismarck Archipelago and Solomon Islands. Further, all reconstructions based on genome-wide nuclear SNP data recovered the Kolombangara-endemic species *P. amoenus* as embedded within the Solomon Island *Phylloscopus* radiation. If we follow the conventions of Mayr and Diamond in considering New Guinea a continental source population (Mayr, 1944, 1955; Mayr & Diamond, 2001), our results demonstrate the first intra-archipelago secondary sympatry (i.e., "completed speciation") among the avifauna of Northern Melanesia. Our results confidently reject the previously hypothesized double-invasion from New Guinea or farther west as a valid explanation for the establishment of *P. amoenus* on Kolombangara (Mayr, 1944, 1955; Mayr & Diamond, 2001), regardless of whether our recovered nuclear or mitochondrial topology reflects the true bifurcating species history. We identified strongly supported mito-nuclear discordance across the tree, including in the recovered relationship between the Kolombangara endemic *P. amoenus* and the sympatric *P. p. pallescens*. This widespread mito-nuclear discordance suggests a complex and potentially reticulate evolutionary history among the closely related and rapidly radiating *Phylloscopus* warbler lineages of Northern Melanesia. Herein, we explicitly discuss the limitations of our current dataset and cautiously interpret the biogeographic implications of the patterns of relatedness we observed.

4.2 Extinction in biogeographic inference

Unknown patterns of extinction remain an issue when inferring relationships among sampled extant taxa (Louca & Pennell, 2020; Sanmartín & Meseguer, 2016). In our *Phylloscopus* dataset, for example, the sister relationship that we infer in the nuclear genome between the two sympatric species on Kolombangara is a robust conclusion given our sampling of all closely related extant taxa. However, this relationship among extant tips could be misleading if there are extinct or unsampled populations that were previously embedded within this clade. The probability of having unknown extinct lineages embedded within a clade increases with the crown age of the group. The only way to limit the unknowable influence of lineage extinction is to focus biogeographic inferences on recent radiations, where the young crown age of the clade reduces the chance of being misled by the absence of unsampled extinct lineages. Although it is impossible to be fully certain that the relationships we recovered among *Phylloscopus* warblers in this study were not misled by extinction, the crown age of the Solomon Island *Phylloscopus* clade is recent among avian radiations at ca. 1.0 to 1.5 Ma (dated node of *P. amoenus* to *P. makirensis*; Alström et al., 2018), making this an ideal group for studying biogeography while limiting the confounding effects of extinction. Additionally, no montane bird community in the Solomon Islands lacks a population of *Phylloscopus* warblers, including the tiny area of montane habitat on Isabel Island (DeCicco et al., 2019). This biogeographic pattern suggests that there are no "missing" populations based on available islands of suitable habitat, providing further evidence for a lack of extinction among this recently diverged clade. Although biogeographic inferences must always be interpreted with caution, we conclude based on the available evidence that we have compiled complete sampling to produce robust phylogenetic inferences for the *Phylloscopus* warblers within the Solomon Islands.

4.3 Mitochondrial-nuclear discordance

Our results showed strongly supported mito-nuclear discordance across the Northern Melanesia radiation of *Phylloscopus* warblers, an increasingly acknowledged pattern in systems with complex evolutionary histories (e.g., DeRaad et al., 2023). Discordance, be it mito-nuclear or among nuclear gene trees, can be caused by incomplete lineage sorting (deep coalescence) or gene flow (M. W. Hahn, 2018). However, these processes are not mutually exclusive and can result in similar signals, making disentangling the causes of mito-nuclear discordance exceedingly difficult (Toews & Brelsford, 2012). Because our reconstructed mitochondrial and nuclear topologies differ markedly, yet each reconstruction is strongly statistically supported, we conclude that the *Phylloscopus* warblers of Northern Melanesia present a true case of mito-nuclear discordance, rather than a failure to accurately reconstruct either tree (i.e., mitochondrial or nuclear; Kimball et al., 2021). Future work using more extensive genomic sampling will be required to disentangle the exact roles of gene flow versus incomplete lineage sorting, in driving the phylogeny-wide mito-nuclear discordance we observed here. Despite our inability to parse the roles of specific evolutionary processes, our current dataset makes it clear that the rapid diversification of this group of *Phylloscopus* warblers in Northern Melanesia has produced conflicting gene tree histories between the nuclear and mitochondrial genomes, making it difficult to support a single bifurcating evolutionary history for this group (Degnan & Rosenberg, 2006).

4.4 On the evolutionary history of *P. amoenus*

The challenge of inferring an evolutionary history among extensive gene tree discordance is exemplified by the only sympatric taxa in this clade, *P. amoenus* and *P. p. pallescens*, which co-occur in the mountains of Kolombangara Island. Based on morphology and ecology, *P. amoenus* is a unique endemic species restricted to the mossy montane forests of Kolombangara Island. The morphology and ecology of this lineage, specifically its adaptation to tree-trunk and ground-dwelling, are unique among both the Northern

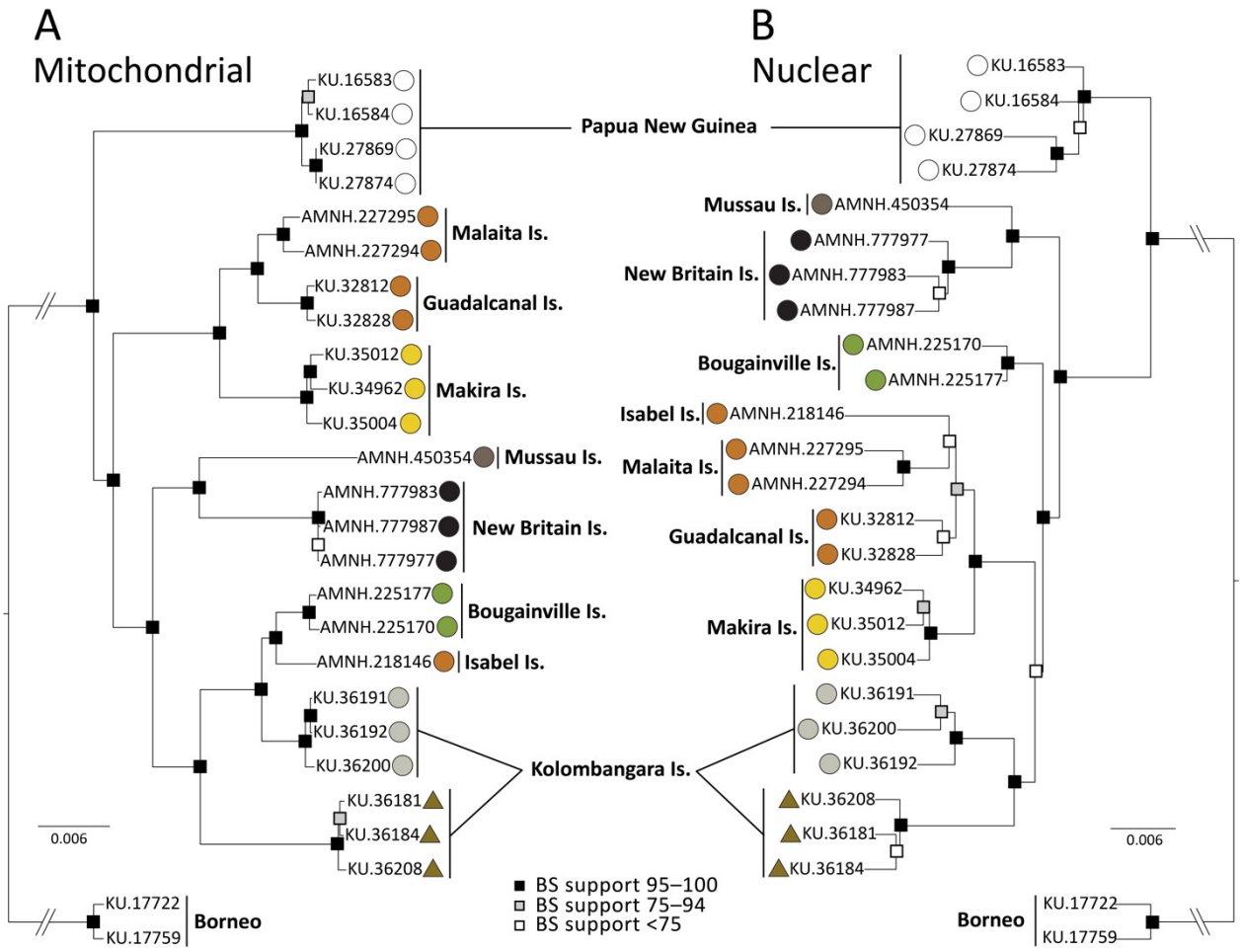

Figure 3. Maximum-likelihood phylogenetic relationships among all Northern Melanesian (save for New Ireland Island) populations of *Phylloscopus* warblers based on (A) complete mitochondrial genomes and (B) a 90% complete nuclear SNP dataset of 5,668 SNPs. Note highly supported mito-nuclear discordance throughout the tree, and the conflicting topological relationship between the two sympatric species on Kolombangara Island (olive triangles represent the endemic *P. amoenus* and gray circles represent the sympatric typical *P. p. pallescens*). Subspecies assignments follow Figures 1 and 2.

Melanesian radiation of *Phylloscopus* warblers and *Phylloscopus* warblers overall. Meanwhile, *P. p. pallescens* shares many morphological and ecological traits with all other allopatric populations of *Phylloscopus* warblers across Northern Melanesia and should be considered typical of the genus overall. Based on this pattern, the sympatry of these two lineages on Kolombangara has long been hypothesized to have resulted from a "double invasion" from a source population of *Phylloscopus* warblers in New Guinea or farther west; *P. amoenus* being the last remaining population of the first invasion, and the rest of the extant *Phylloscopus* radiation in the Bismarck and Solomon archipelagos being a result of the second invasion (Mayr, 1944, 1955; Mayr & Diamond, 2001). This hypothesis carries the expectation that *P. amoenus* descended from a lineage distantly related to the entire Solomon and Bismarck radiation, and therefore should be recovered as sister to these populations in all phylogenetic reconstructions. Despite the conflicting signals presented by our nuclear and mitochondrial data, all reconstructions recovered *P. amoenus* embedded within a monophyletic clade containing all extant lineages sampled from the Solomon and Bismarck archipelagos, contradicting the expected pattern of a double invasion from outside these archipelagos. This pattern allows us to confidently reject the long-standing double-invasion hypothesis. In birds, this is the first documented case of secondary sympatry (i.e., "completed speciation") occurring entirely within the Solomon Islands. All other known cases of secondary sympatry within Northern Melanesia, i.e., sympatric occurrence of two lineages originating from the same source population, (18 known cases; Mayr & Diamond, 2001, p. 3) appear to be examples of double-invasions from areas outside of Northern Melanesia.

4.5 Secondary sympatry *vs.* sympatric speciation

Instead of supporting the previously hypothesized distant relationship between *P. amoenus* and *P. p. pallescens*, all four nuclear SNP datasets strongly supported a sister relationship between these two sympatric taxa on Kolombangara. Meanwhile, our phylogenetic reconstruction from the

mitochondrial genome supported *P. amoenus* as embedded within the rest of the Solomon Island radiation, sister to samples of allopatric populations of *P. poliocephalus* from Bougainville, Isabel, and Kolombangara islands. Here we present two evolutionary scenarios that could plausibly explain the discordant topologies we recovered for the sympatric species pair on Kolombangara: 1) rapid secondary sympatry including a period of gene flow in which the nuclear genome became admixed between the two species but the mitochondrial genomes did not, or 2) sympatric ecological speciation on Kolombangara with deep coalescence (incomplete lineage sorting) of the mitochondrial genome. Differentiating between these two evolutionary scenarios is highly challenging, because both scenarios could plausibly produce the conflicting topological patterns of relatedness we observed.

If the mitochondrial branching pattern reflects the true evolutionary history of these species, then the discordant nuclear genome pattern could be explained by ongoing or historical gene flow between *P. amoenus* and *P. p. pallescens* on Kolombangara (Stervander et al., 2022), without the capture or sweep of mitochondrial genomes in either direction between these species. This scenario is plausible as low levels of gene flow have been shown to alter phylogenetic inference under both empirical (Zhang et al., 2021) and simulated conditions (Hibbins & Hahn, 2024). Meanwhile, the sister relationship between *P. amoenus* and *P. pallescens* recovered by the nuclear genome data could also reflect the true evolutionary history. This scenario would invoke sympatric ecological speciation on Kolombangara, which has been documented only rarely in birds (Bolnick & Fitzpatrick, 2007; Coyne & Price, 2000). This scenario would also require deep coalescence in the mitochondrial genome, which is plausible considering the rapid branching (producing short waiting times between speciation events) and broader mito-nuclear discordance we observe across Northern Melanesian *Phylloscopus* radiation. Ultimately, future investigations incorporating greater sample size and genomic coverage will be needed to distinguish between the two divergent evolutionary scenarios presented here. Regardless, both of these evolutionary scenarios imply a complex and highly unexpected origin for the divergent Kolombangara endemic species *P. amoenus*.

5 CONCLUSIONS

Here we demonstrate that the only sympatric pair of *Phylloscopus* warblers east of Wallace's Line resulted from recent ecological sympatric speciation or rapid intra-archipelago secondary sympatry with severe character displacement in the Kolombangara endemic *P. amoenus*. Based on the evidence presented here, we can confidently reject the long-held hypothesis that *P. amoenus* is a relict from the first of two invasions of the Solomon and Bismarck archipelagos from a source population in New Guinea or further west (Mayr, 1944, 1955; Mayr & Diamond, 2001). This case study adds to the growing body of genomic datasets supporting the ubiquity of complex and non-bifurcating evolutionary histories among recent vertebrate radiations (e.g., Barley et al., 2022). While the traditional model of dispersal and isolation (Mayr, 1942) may sufficiently explain the rapid accumulation of allopatric taxa in many of the "great speciator" (Moyle et al., 2009) taxa of the Pacific Islands, this study suggests that secondary sympatry may often result from more complex evolutionary scenarios. The Northern Melanesian radiation of *Phylloscopus* warblers provides an excellent case study for understanding the processes that can produce rapid sympatry of two closely related species, e.g., secondary contact or ecological speciation. Ultimately, understanding the evolutionary mechanisms that mediate the coexistence of closely related taxa and determine the maintenance or erosion of species boundaries upon secondary contact is crucial for understanding how biodiversity has accumulated in island systems across the globe.

Funding

This research was funded by the NSF grants to Moyle (DEB-1557053) and Andersen (DEB-1557051), with support to DeCicco by the University of Kansas Biodiversity Institute Panorama Grant and the University of Kansas Genome Sequencing Core Student Research Grant (supported by National Institutes of Health grant 5P20GM103638 to E.A. Lundquist).

Acknowledgements

We thank the Solomon Islands Ministry of Environment, Climate Change, Disaster Management, and Meteorology for permissions and the provincial governments and the local community members throughout the Solomon Islands for their support of our fieldwork. We thank the American Museum of Natural History for loaning samples. Reviews and comments by Herman Lee Mays, Sean Reilly, Ryan Garrick, and Bryan Carstens are appreciated and greatly improved this manuscript. We thank Rene P. Martin for kindly editing illustrations to accurately represent the appropriate taxa and Brian L. Sullivan for working with us to make these illustrations available.

Supporting Information

Additional phylogenetic tree inferences and data tables are in supplemental file 1, code used for the data analysis presented here is in supplemental file 2, and output from the filtering steps conducted in *SNPfiltR* is included in supplemental file 3. Raw sequencing reads for each sample used in this manuscript are archived on DRYAD (https://orcid.org/0000-0001-9477-3124)

Submitted: March 01, 2023 EDT, Accepted: February 21, 2024 EDT

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