Colonization and diversification of the white-browed shortwing (Aves: Muscicapidae: *Brachypteryx montana*) in the Philippines

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ABSTRACT

Molecular phylogenetic approaches have greatly improved our knowledge of the pattern and process of biological diversification across the globe; however, many regions remain poorly documented, even for well-studied vertebrate taxa. The Philippine archipelago, one of the least-studied 'biodiversity hotspots', is an ideal natural laboratory for investigating the factors driving diversification in an insular and geologically dynamic setting. We investigated the history and geography of diversification of the Philippine populations of a widespread montane bird, the White-browed Shortwing (*Brachypteryx montana*). Leveraging dense archipelago-wide sampling, we generated a multi-locus genetic dataset (one nuclear and two mtDNA markers), which we analyzed using phylogenetic, population genetic, and coalescent-based methods. Our results demonstrate that Philippine shortwings (1) likely colonized the Philippines from the Sunda Shelf to Mindanao in the late Miocene or Pliocene, (2) diversified across inter-island barriers into three divergent lineages during the Pliocene and early Pleistocene, (3) have not diversified within the largest island, Luzon, contrary to patterns observed in other montane taxa, and (4) colonized Palawan from the oceanic Philippines rather than from Borneo, challenging the assumption of Palawan functioning exclusively as a biogeographic extension of the Sunda Shelf. Additionally, our finding that divergent (c. 4.0 mya) lineages are coexisting in secondary sympatry on Mindanao without apparent gene flow suggests that the speciation process is likely complete for these shortwing lineages. Overall, these investigations provide insight into how topography and island boundaries influence diversification within remote oceanic archipelagos and echo the results of many other studies in demonstrating that taxonomic diversity continues to be underestimated in the Philippines.

1. Introduction

The Philippines has long served as a model island archipelago, playing a crucial role in informing our understanding of the mechanisms of evolution in an insular and geologically dynamic setting (Dickerson, 1928; Diamond & Gilpin, 1983; Heaney, 1986; Brown et al., 2013). However, despite the Philippines' importance as a natural laboratory and status as a 'biodiversity hotspot' (Myers et al., 2000), knowledge of the pattern and process of diversification across the archipelago remains largely incomplete. Even within well-studied vertebrate groups, new species are regularly described from the Philippines, largely resulting from both increased sampling efforts and the widespread application of molecular phylogenetic tools in taxonomic

research (Posa et al., 2008; Welton et al., 2010; Heaney et al., 2009; Heaney et al., 2011; Heaney et al., 2016; Hosner et al., 2013a). Consequently, estimates of endemism for Philippine taxa have risen substantially, and currently range from 56 to 80% for terrestrial vertebrate taxa (Brown et al., 2013). These figures will likely continue to rise as more cryptic taxa are uncovered by molecular approaches (Lohman et al., 2010; Hosner et al., 2014; Campbell et al., 2016). Such research underscores the importance of intensive field and taxonomic research in informing conservation priorities, especially in poorly studied regions of the world (Mace et al., 2004; Posa et al., 2008).

The proliferation of well-sampled molecular phylogenies for Philippine taxa has also greatly increased our knowledge about the historical processes of colonization and diversification in the
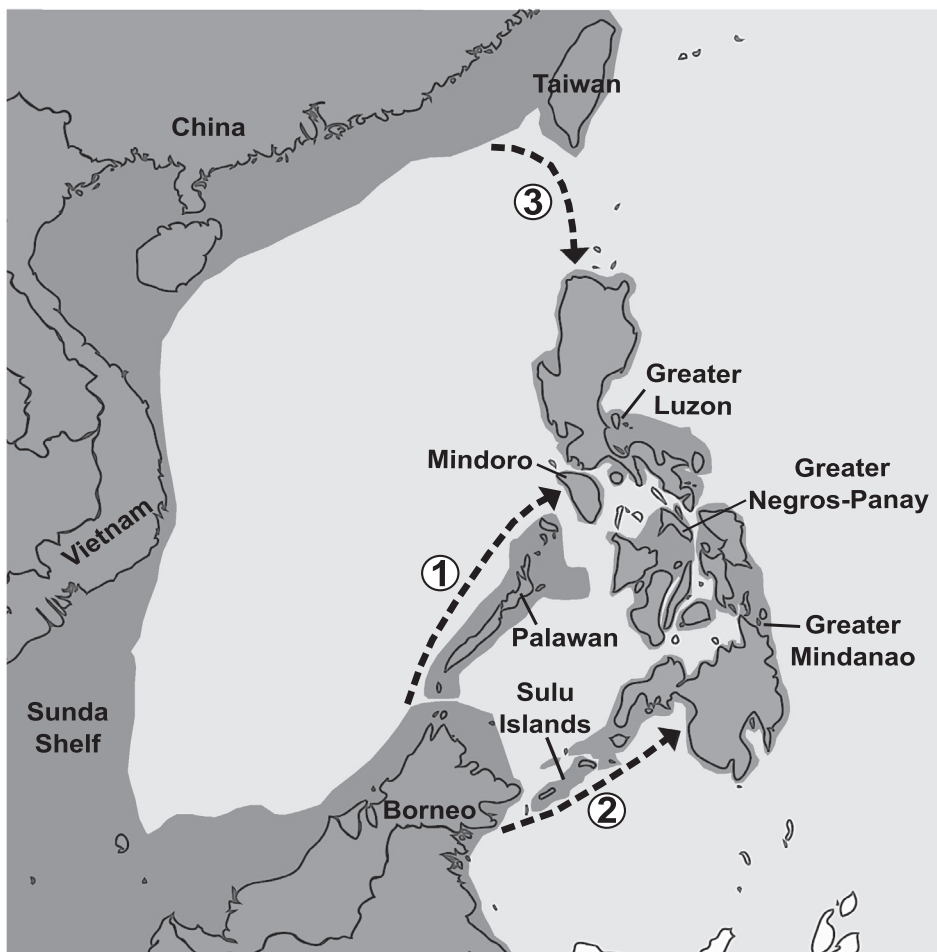

Fig. 1. Map of Southeast Asia depicting three potential colonization routes into the Philippines tested for *Brachypteryx montana,* including: (1) a northern route from Borneo to Mindoro via Palawan, (2) a southern route from Borneo to Mindanao via the Sulu Islands, and (3) a direct route from mainland Asia. Shading depicts late Pleistocene coastlines based on 120 m submarine bathymetric contour.

Philippines. Largely due to the Philippines' high degree of isolation from mainland Asia (Heaney, 1986; Heaney et al., 2016; Voris, 2000), colonization into the Philippines has been relatively limited for most terrestrial groups (Diamond & Gilpin, 1983; Heaney, 1985). For lineages that have colonized the oceanic Philippines (i.e., excluding Palawan, which may have formed land-bridge connections with the Sunda Shelf at times during the Pleistocene), numerous routes of arrival have been documented (Dickerson, 1928; Brown et al., 2013), including western or northern origin from mainland Asia or Taiwan (Jansa et al., 2006; Esselstyn & Oliveros, 2010), southern origin from Wallacea (Evans et al., 2003; Schweizer et al., 2012), and eastern origin from the Sunda Shelf (Diamond & Gilpin, 1983; Oliveros & Moyle, 2010; Brown & Siler, 2014). In particular, a large number of studies have focused on testing two potential routes originating from the Sunda Shelf, including: (1) a northern route characterized by colonization from Borneo to the northern Philippines via Palawan and Mindoro, and (2) a southern route consisting of colonization from Borneo to Mindanao via the Sulu archipelago, followed by expansion northward (Fig. 1; Diamond & Gilpin, 1983; Jones & Kennedy, 2008; Oliveros & Moyle, 2010; Brown et al., 2013; Brown & Siler, 2014; Hosner et al., 2013b). Although these routes were initially proposed based on avian distributional data (Diamond & Gilpin, 1983), molecular phylogenetic studies have found surprisingly little support for colonization of the oceanic Philippines along the northern route in birds (Jones & Kennedy, 2008; Oliveros & Moyle, 2010; Sheldon et al., 2012; Hosner et al., 2013b). Instead, some research suggests that Palawan may represent a biogeographic 'dead end' for many taxa (Oliveros & Moyle, 2010; Esselstyn et al., 2010; Brown et al., 2013), with few examples of widespread Philippine radiations originating from Palawan.

Regardless of their geographic origin, the lineages that have

colonized the oceanic Philippines have often diversified substantially throughout the archipelago (Jansa et al., 2006; Esselstyn et al., 2009; Oliveros & Moyle, 2010; Siler et al., 2011; Hosner et al., 2013b). For many organisms, phylogeographic structure is coincident with present-day island coastlines, implicating water barriers as important barriers to dispersal and drivers of diversification (e.g., Steppan et al., 2003; Jones & Kennedy, 2008; Siler et al., 2011). In addition, numerous studies have documented the importance of Pleistocene sea-level fluctuations as determinants of genetic structure, due to their role in periodically connecting present-day islands by dry land (Fig. 2; Inger, 1954; Heaney, 1985; Voris, 2000; Brown & Diesmos, 2002; Roberts, 2006). Finally, a growing body of research has identified the importance of topographic features within islands as drivers of diversification (Esselstyn et al., 2009; Sanguila et al., 2011; Hosner et al., 2013a, b; Justiniano et al., 2015; Heaney et al., 2016; Kyriazis et al., 2017). In particular, these studies have highlighted the presence of numerous locally endemic species on the isolated mountain ranges of Luzon and Mindanao, thus implicating these 'sky islands' as drivers of diversification.

In this study, we combine extensive geographic sampling and multi-locus genetic analyses to study the patterns of colonization and diversification of the White-browed Shortwing (*Brachypteryx montana*) in the Philippines. *Brachypteryx montana* is a widespread montane forest species (family: Muscicapidae; Zuccon & Ericson, 2010) occurring through much of tropical Asia, ranging from the eastern Himalayas, to southeastern China, throughout much of the Sunda Shelf, and into the Philippines, where it is present on Luzon, Mindanao, Mindoro, Negros, Palawan, and Panay islands (Fig. 2). In total, 14 subspecies of *B. montana* have been described, 7 of which are endemic to the Philippines (Gill & Donsker, 2017). These subspecies tend to be distinctive in both
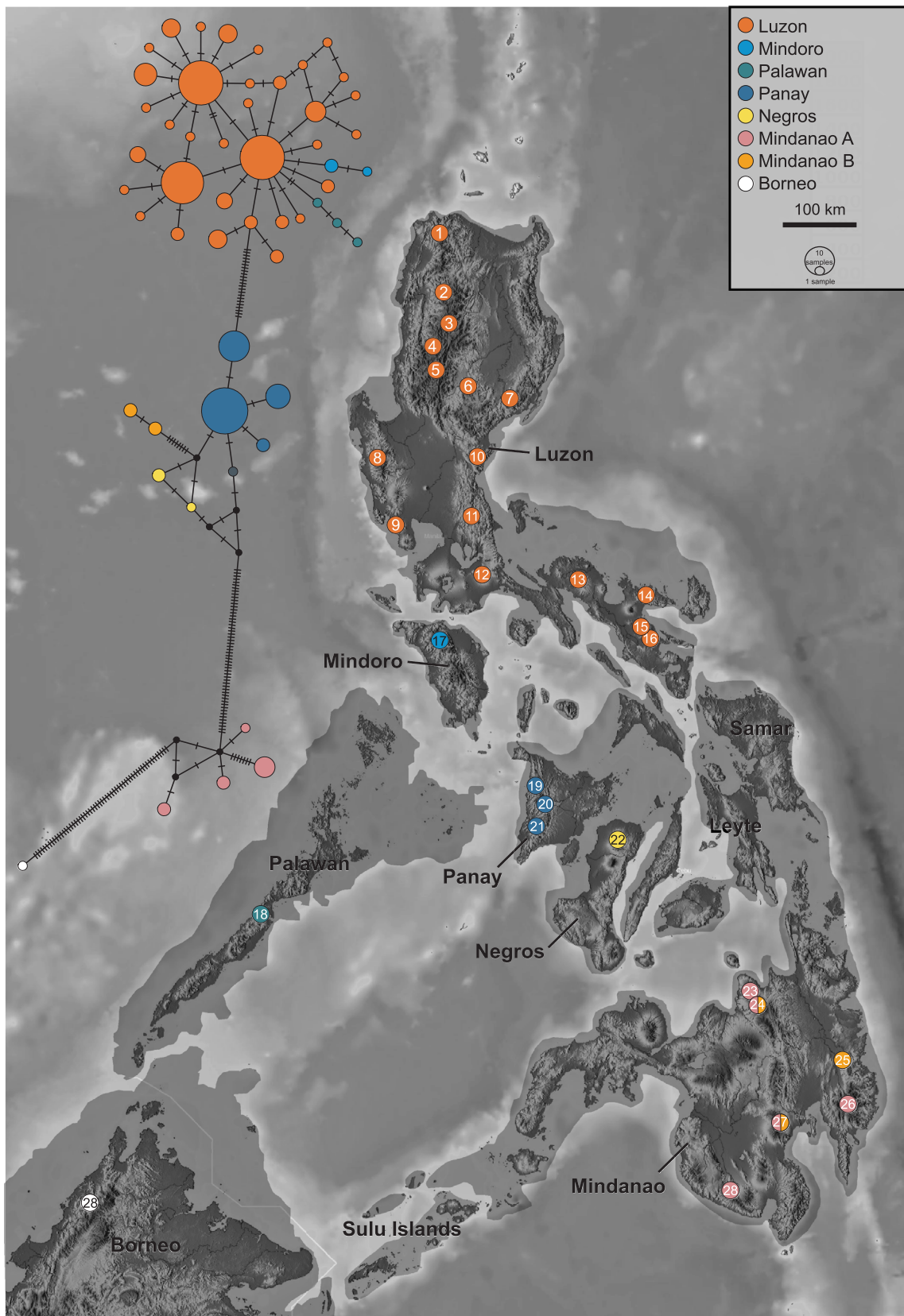
Fig. 2. Map of the Philippines with median joining network of 195 *B. montana* mtDNA haplotypes showing genetic differentiation across the Philippine archipelago. Late Pleistocene island coastlines, as determined by 120 m submarine bathymetric contour, are shaded surrounding each major island complex. Sampling areas are represented with color-coded dots that correspond to major haplotype groups, and are numbered as followed: (1) Mt. Pao, (2) Balbalasang, (3) Mt. Amuyao, (4) Mt. Data, (5) Mt. Pulag, (6) Mt. Palali, (7) Mt. Anacuao, (8) Mt. Tapulao, (9) Mt. Natib, (10) Mt. Mingan, (11) Mt. Irid., (12) Mt. Banahaw, (13) Mt. Labo, (14) Saddle Peak, (15) Mt. Malinao, (16) Mt. Mayon, (17) Mt. Halcon, (18) Mt. Mantalingahan, (19) Mt. Madja-as, (20) Mt. Baloy, (21) Barangay Aningalan, (22) Bungol, (23) Mt. Balatukan, (24) Mt. Lumot, (25) Mt. Paisan, (26) Mt. Kampalili, (27) Mt. Talomo, (28) Mt. Busa. Note that Mindanao clades A and B both sampled at Mt. Talomo and Mt. Lumot. Color-coding scheme selected to accommodate colorblind viewers.

plumage and vocalizations, though in general shortwing males have a dull but slightly iridescent grayish-blue plumage with a white supercilium and females tend to be a dull olive or rusty-brown. Shortwings are present in the Philippines in montane and mossy forest habitat from c. 600–2700 m (Supplemental Table 1), but are most abundant above 1500 m, where they tend to be found on or near the ground foraging for worms and small arthropods (Goodman & Gonzales, 1990). Restriction to montane forest understory, along with high subspecies diversity, imply that shortwings may be poor dispersers over open water and/or lowland habitat, suggesting that substantial genetic differentiation may have occurred both between and within Philippine islands. Additionally, the widespread distribution of *B. montana* within the Philippines and across mainland Asia and the Sunda Shelf makes it an ideal system for further investigating avian colonization routes into the Philippines. Here, we examine phylogeographic patterns in *B. montana* within the Philippines, and ask: (1) how and when did *B. montana* colonize the Philippines, (2) to what extent has *B. montana* diversified genetically within the Philippines, and (3) what are the geographic features that have structured its diversification?

2. Material and methods

2.1. Sampling and sequence data

We collected shortwing specimens throughout the Philippines, totaling 196 samples from Luzon (n = 126), Mindoro (n = 3), Negros (n = 3), Panay (n = 46), Mindanao (n = 15), and Palawan (n = 3; Supplemental Table 1). Our sampling included representation of 6 out 7 Philippine subspecies, as we lacked samples of *B. m. malindangensis* from western Mindanao. Our outgroup sampling included *B. montana erythrogyna* from Borneo (1), *B. montana cruralis* from Myanmar (n = 1), *B. montana sinensis* from China (n = 1), and *B. leucophrys* from China (n = 2) and Vietnam (n = 1). We extracted genomic DNA from these 200 tissues using the Qiagen DNeasy kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol. Using standard PCR protocols, we amplified two mitochondrial gene regions (nicotinamide adenine dinucleotide dehydrogenase subunit 2 [ND2] and subunit 3 [ND3]) and one Z-linked region (Aconitase 1 intron 9 [ACO1]) for each specimen (see Supplemental Table 2 for details). We sequenced each PCR product using an ABI 3730 DNA analyzer (Life Technologies, Carlsbad, CA, USA). We assembled and edited reads, called heterozygous sites, aligned sequences, and trimmed final alignments using GENEIOUS v.9.1.2 (Kearse et al., 2012). For *B. leucophrys* and Philippine *B. montana* samples, we resolved ACO1 haplotype phases using PHASE (Stephens et al., 2001) implemented in DNASP v5.10.1 (Librado & Rozas, 2009). We used the resulting phased alignment for our haplotype networks and skyline plots (see below).

2.2. Haplotype networks, phylogenetic analyses, and molecular dating

We used POPART (Leigh & Bryant, 2015) to create median joining networks (Bandelt et al., 1999) for the concatenated mtDNA and the complete phased ACO1 data set. Using RAxML v.8.2.3 (Stamatakis, 2014), we estimated a phylogenetic tree for a concatenated ND2 + ND3 + ACO1 data set, as well as for the mtDNA and ACO1 data sets separately. For the concatenated ND2 + ND3 + ACO1 data set, we used PARTITIONFINDER2 (Lanfear et al., 2012, 2016) to determine the optimal partitioning scheme from a possible 7 partitions (three codon positions for each of ND2 and ND3 and one partition for ACO1) using AICc and a greedy search algorithm. We applied a separate GTRCAT substitution model to each of the resulting five partitions (ND3_pos1 + ND2_pos1, ND3_pos2 + ND2_pos2, ND3_pos3, ND2_pos3, ACO1) and allowed RAxML to automatically halt bootstrapping when it reached stationarity. We used the same strategy to examine gene trees by conducing separated mtDNA and ACO1 RAXML analyses.

We used BEAST2 (Bouckaert et al., 2014) to estimate a time-calibrated

phylogeny for *B. montana* using the concatenated ND2 + ND3 + ACO1 data set. We included one randomly-chosen representative for each Philippine population (see Section 3) as well as outgroup *B. montana* samples from Borneo, China, Myanmar, and *B. leucophrys*. We calibrated the tree using estimates of the ND2 and ND3 clock rate mean and range from Lerner et al. (2011), which were applied using a relaxed log-normal clock model to each gene partition with log-normal prior distributions reflecting the values shown in Supplemental Table 2. We estimated substitution models for each partition using JMODELTESTV2.1.7 (Guindon & Gascuel, 2003; Darriba et al., 2012; see Supplemental Table 2 for results), and used a 'coalescent constant population' tree model to account for shallow divergences among some Philippine lineages. We ran the analysis for 40 million generations sampling every 5,000, repeating once to confirm the consistency of our results. All above phylogenetic analyses were run using the Cipres Science Gateway (Miller et al., 2010) and all trees were viewed using FIGTREE v1.4.2 (Rambaut, 2009). For both BEAST analyses, we prepared XML files using BEAUTI (Bouckaert et al., 2014), examined trace files using TRACER v1.6 to ensure all ESS values were greater than 200 (Rambaut & Drummond, 2007), and constructed maximum clade credibility trees with a 20% burn in using TREEANNOTATOR v2.2.1 (Bouckaert et al., 2014).

2.3. Population genetic analyses

To more closely examine the geographical features structuring shortwing diversification in the Philippines, we ran a two-level Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992) testing the role of present-day islands and variation within islands in explaining the observed patterns of genetic structure. We also ran an AMOVA on Luzon comparing the role of 11 mountain ranges/areas of endemism, sampling areas within mountain ranges, and variation within sampling areas in determining the observed patterns of genetic variation. All AMOVA were conducted using ARLEQUIN v3.5 (Excoffier & Lischer, 2010) with a concatenated mtDNA dataset.

We used our complete ND2 + ND3 + ACO1 dataset to test for evidence of population size fluctuations in Philippine *B. montana* lineages by constructing Extended Bayesian Skyline Plots (Heled & Drummond, 2008). We limited our analysis to islands/clades that had at least 10 samples (i.e., Luzon, Mindanao clade A, and Panay). To avoid over-parameterizing our analysis of these smaller intra-island data sets, we used an HKY substitution model for each gene partition, as other authors have done (e.g., Amaral et al., 2013). We linked tree models for the mtDNA partitions and placed a prior on the substitution rates of the ND2 and ND3 partitions using the same strategy described above (Supplemental Table 2). We ran one chain for 100 million generations sampling every 10,000. We examined the trace files for convergence using TRACER v1.6 (Rambaut & Drummond, 2007).

3. Results

3.1. Sequence data

We obtained 200 ND2 sequences, 200 ND3 sequences, and 168 ACO1 sequences, combining for a total of 2233 aligned bases (Supplemental Tables 1 and 2). The ACO1 alignment included numerous indels, including three 1 bp indels shared by *B. leucophrys* relative to *B. montana*, two 1 bp indels shared by *B. leucophrys* and *B. montana* from China relative to the rest of *B. montana*, and two 1 bp indels that were variable within Philippine *B. montana* though did not correspond to any apparent phylogeographic groupings.

3.2. Phylogenetic analyses

Philippine shortwings were consistently supported as monophyletic in our phylogenetic analyses (bootstrap [BS] = 94, posterior probability [PP] = 0.99; Figs. 3 and 4). Our concatenated RAXML and BEAST2 analyses
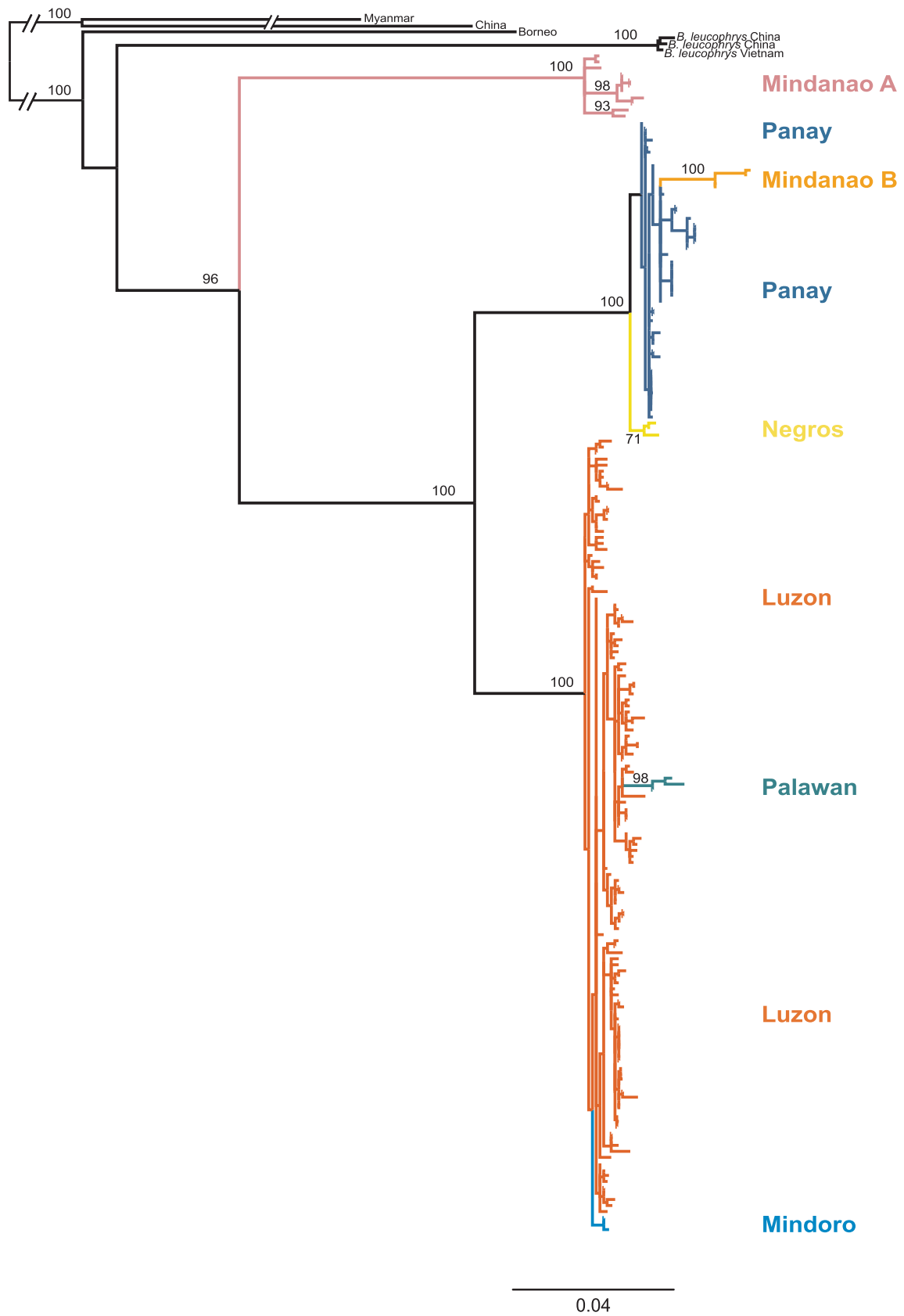
Fig. 3. Maximum likelihood tree estimated from a concatenated dataset depicting relationships among Philippine shortwing lineages. Major lineages, each corresponding to a Philippine island or Borneo, are labeled and color-coded following Fig. 2. Bootstrap values > 70 are shown above key nodes. Refer to Supplemental Fig. 6 for tip labels. Color-coding scheme selected to accommodate colorblind viewers.
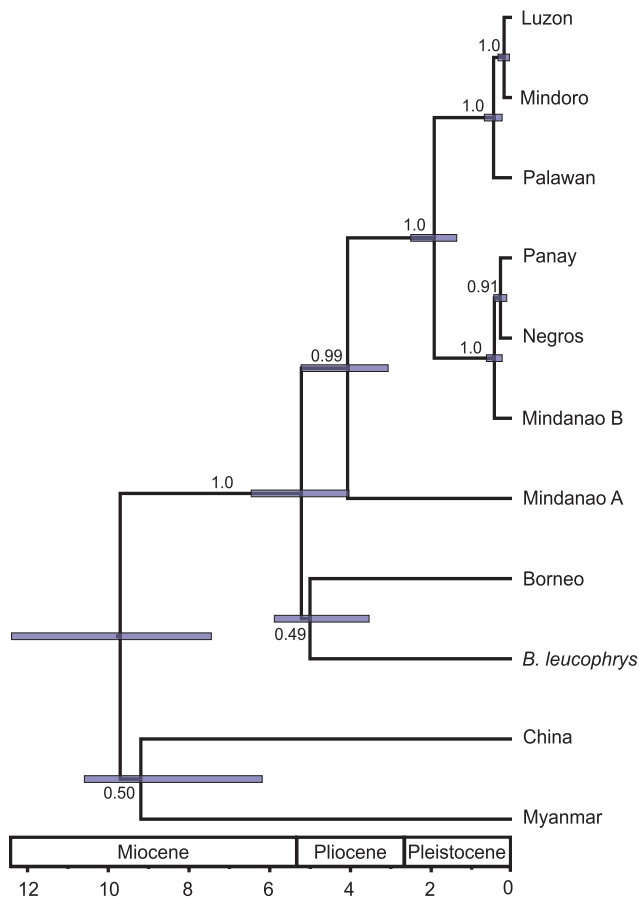
Fig. 4. Time-calibrated tree estimated using BEAST2. Nodes are labeled with Bayesian posterior probability estimates and node bars indicate 95% HPD intervals for divergence dates. All tip labels refer to *B. montana* lineages unless otherwise noted.

both found strong support for *B. montana erythrogyna* from Borneo and *B. leucophrys* as sister to Philippine *B. montana*; however, the branching patterns among these three lineages were unresolved (Figs. 3 and 4). Our mtDNA-only RAXML analysis (Supplemental Fig. 4) was nearly identical to the concatenated RAXML, whereas the ACO1 RAXML analysis (Supplemental Fig. 5) generally had low support for most nodes.

Within the Philippines, our mtDNA and concatenated analyses recovered strong support for the presence of three major clades: (1) a clade comprising 11 Mindanao samples (henceforth, Mindanao clade A) that is sister to the remainder of Philippine shortwings, (2) a clade including four Mindanao samples (henceforth, Mindanao clade B) and all samples from Negros and Panay, and (3) a clade including all samples from Luzon, Mindoro, and Palawan (Figs. 3 and 4). Within these clades, we found evidence for shallow, and often poorly-supported, divergences among islands in our concatenated RAXML analysis: Negros and Mindanao clade B each formed monophyletic clades, though with Mindanao clade B nested within the Panay clade, and Palawan and Mindoro samples similarly formed monophyletic clades that were nested within the Luzon clade (Fig. 3). Our BEAST2 analysis found moderate support for Negros and Panay as sister (PP = 0.91) and strong support for Luzon and Mindoro as sister (PP = 1.0; Fig. 4).

3.3. Divergence dating

Molecular dating analysis using BEAST2 (Fig. 4) found that Philippine *B. montana* diverged from a sister clade including *B. leucophrys* and *B. m. erythrogyna* from Borneo 5.17 mya (95% HPD: 4.04–6.46 mya) and that diversification among Philippine lineages began 4.02 mya (95% HPD: 3.07–5.22 mya). Additionally, BEAST2 estimated the split between

the Luzon-Mindoro-Palawan clade and Negros-Panay-Mindanao clade at 1.91 mya (95% HPD: 1.37–2.51 mya) and placed divergences within these clades during the last c. 440 ky (combined 95% HPD: 60–660 kya).

3.4. Population genetic analyses

Median joining networks constructed using concatenated mtDNA sequences indicated that no haplotypes were shared among islands and that samples from each island generally clustered closely together, with the exception of Mindanao clades A and B (Fig. 2). Similarly, a median joining network for the phased ACO1 dataset found few instances of haplotype sharing among islands, though without clearly defined structure (Supplemental Fig. 1). Finally, a median joining network examining differentiation on Luzon found high levels of haplotype sharing among populations across different mountain ranges (Fig. 5), whereas a median joining network for Mindanao clade A found evidence for genetic structure partitioned by the montane regions of Mindanao (Supplemental Fig. 2).

Our archipelago-wide AMOVA indicated that 90.0% of mtDNA variation was explained by island coastlines, whereas 10.0% was explained by variation within islands (Table 1). Our Luzon-only AMOVA found that 12.5% of mtDNA variation was explained by 11 isolated mountain ranges on the island, with 7.0% attributed to sampling areas within mountain ranges, and 80.5% attributed to variation within sampling areas (Table 2).

Extended Bayesian skyline plot analysis found evidence for demographic stability on Panay and Mindanao and expansion on Luzon (Fig. 6). By calibrating these analyses using estimates of avian mtDNA substitution rates, we found that expansion on Luzon began c. 90,000 years ago and has continued at a constant rate to the present. Estimates of present-day effective population size suggested relatively comparable values for Panay and Mindanao, and a much a larger (~4×) effective population size on Luzon (Fig. 6).

4. Discussion

4.1. Pattern and timing of colonization and diversification

Our results suggest that Philippine shortwings originated on Mindanao, implying a southern route of colonization into the Philippines from the Sunda Shelf (Fig. 1; Dickerson, 1928; Diamond & Gilpin, 1983; Brown et al., 2013). Evidence for colonization in the southern Philippines followed by expansion northward comes from our phylogenetic results, which recovered a clade from Mindanao as sister to the rest of Philippine shortwings (Figs. 3 and 4), as well as from our demographic analysis, which found evidence for long-term stability in populations from Mindanao and Panay, contrasting the pattern of demographic expansion we recovered on Luzon (Fig. 6). This pattern of south-to-north colonization across the Philippines mirrors results documented for numerous other avian lineages in the Philippines (Jones & Kennedy, 2008; Oliveros & Moyle, 2010; Hosner et al., 2013b), many of which arrived initially on Mindanao from the Sunda Shelf. However, we note that the lack of support for our Bornean shortwing sample as sister to Philippine populations leaves open the possibility of colonization from other sources, such as mainland Asia. Further genetic and population sampling across mainland Asia are necessary to fully resolve the source of shortwing colonization to the Philippines.

In addition to providing insight into the route of shortwing colonization into the Philippines, our results constrain the timing of initial colonization within the late Miocene or Pliocene (Fig. 4). Following arrival in the southern Philippines, our results are consistent with a sequential pattern of expansion and diversification north through the archipelago. This process likely began with colonization of Negros and Panay c. 4.0 mya, followed by expansion to Luzon, Mindoro, and
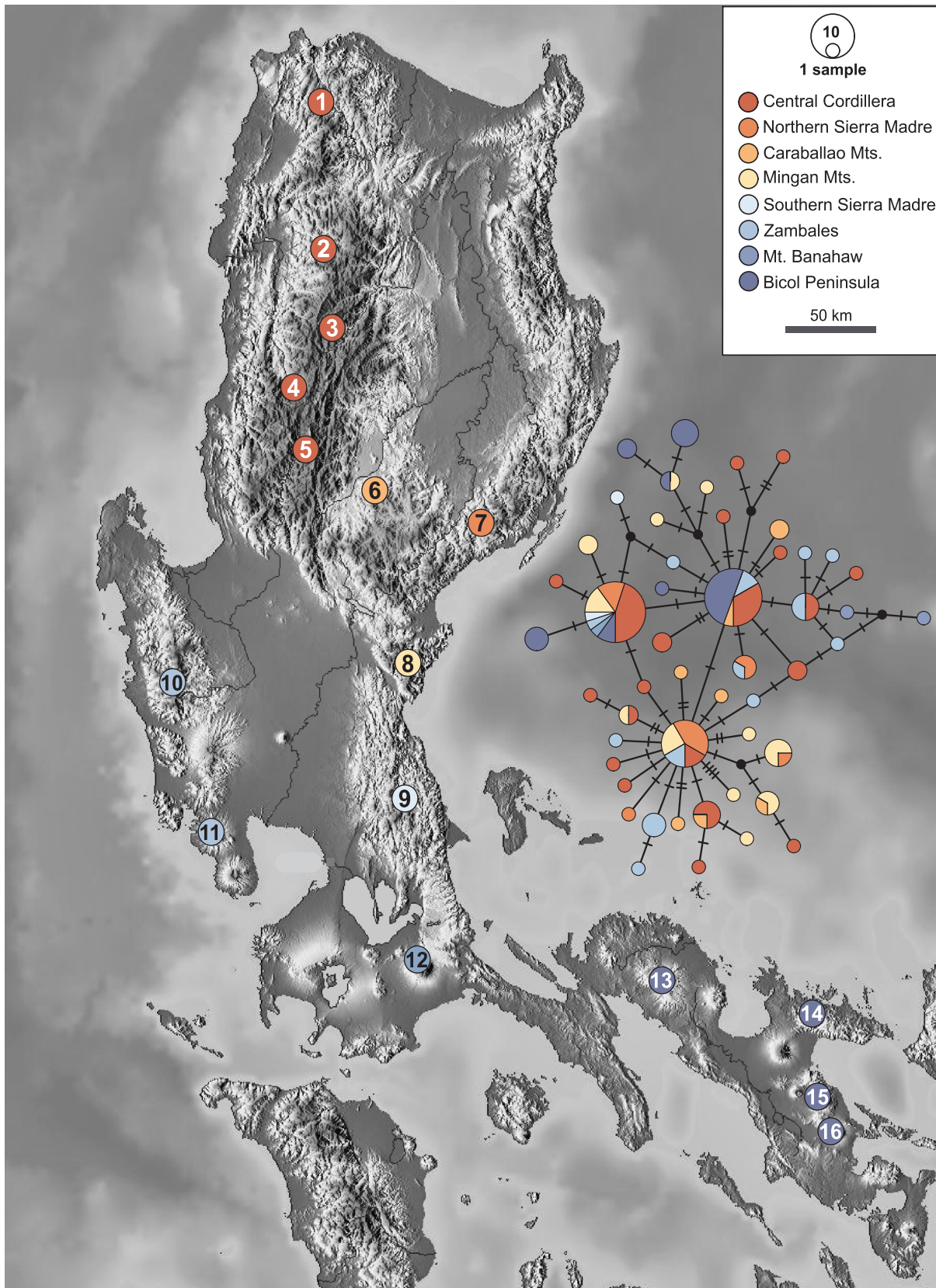
Fig. 5. Median joining network for 124 *B. montana* mtDNA haplotypes accompanied by map of Luzon showing lack of intra-island structure. Sampling areas are represented with color-coded dots that correspond to major haplotype groups, and are numbered as followed: (1) Mt. Pao, (2) Balbalasang, (3) Mt. Amuyao, (4) Mt. Data, (5) Mt. Pulag, (6) Mt. Palali, (7) Mt. Anacuao, (8) Mt. Mingan, (9) Mt. Irid, (10) Mt. Tapulao, (11) Mt. Natib, (12) Mt. Banahaw, (13) Mt. Labo, (14) Saddle Peak, (15) Mt. Malinao, (16) Mt. Mayon. To simplify presentation of results, Bicol Peninsula localities are grouped together, though were tested as separate montane biogeographic regions in AMOVA. Color-coding scheme selected to accommodate colorblind viewers.

Table 1
Archipelago-wide AMOVA results.

Source of Variation	Sum of Squares	Variance Components	% Variation	P
Among islands	2688.54	27.31	90.0	< .0001
Within Islands	567.88	3.03	10.0	< .0001
Total	3256.41	30.34		

Table 2
Luzon AMOVA results.

Source of variation	Sum of squares	Variance components	% Variation	P
Among mountain ranges	40.91	0.18	11.62	< .0001
Among sampling areas within mountain ranges	15.78	0.12	7.72	0.0117
Within sampling areas	136.49	1.28	80.66	0.0078
Total	193.18	1.59		

Palawan within the last c. 1.9 Myr (Fig. 4). Additionally, our results suggest that shortwings back-colonized Mindanao from Negros-Panay c. 430 kya, and place the divergence between a Luzon/Mindoro clade and Palawan at c. 440 kya (Fig. 4). We interpret the slightly deeper divergences between Luzon/Mindoro and Palawan as evidence of recent arrival on Palawan, whereas we interpret the more shallow divergences (c. 190 kya) between Luzon and Mindoro as evidence of ongoing gene flow between these two nearby islands (see below for further discussion).

### 4.2. Implications for Palawan biogeography

Our result of Palawan *B. montana* samples being nested within populations from the oceanic Philippines, and not closely related to *B. m. erythrogyna* from Borneo, represents one of the more unexpected patterns in this study, and adds to our growing understanding of Palawan's complex biogeographic history. Perhaps the majority of studies examining Palawan's native biota have emphasized both its distinctiveness and its close affinity with the Sunda Shelf, as exemplified by the large number of species shared by Palawan and the Sunda Shelf as well as the presence of numerous endemic species on Palawan that are sister to Sunda Shelf taxa (e.g., Heaney, 1986; Esselstyn et al., 2004, 2010; Piper et al., 2011; Oliveros & Moyle, 2010; Lim et al., 2014). Additionally, due to Palawan's close affinity with the Sunda Shelf and proximity to the oceanic Philippines, researchers have long hypothesized that Palawan may serve as a stepping stone island into the

Philippines (Dickerson, 1928; Inger, 1954; Diamond & Gilpin, 1983; Oliveros & Moyle, 2010; Brown & Siler, 2014).

The patterns we observe in *B. montana*, by characterizing Palawan populations as being more closely affiliated with those from the oceanic Philippines than with those from the Sunda Shelf, offer yet another perspective on Palawan's complex biogeography. Although this pattern has been observed in avian lineages that colonized the Philippines from sources other than the Sunda Shelf (Esselstyn et al., 2010), we suggest that it is especially noteworthy and unexpected for *B. montana* due to its long-term presence on Borneo (c. 5 Myr) and the proximity of Borneo and Palawan (with several possible land-bridge connections during this period). This result parallels patterns documented for another montane forest bird, *Phylloscopus trivirgatus*, which also colonized the oceanic Philippines from the Sunda Shelf and subsequently colonized Palawan (Jones & Kennedy, 2008). We suggest that the apparent inability of these montane forest species to colonize Palawan directly from Borneo may be related to the expansion of dry grassland/savannah habitats across Palawan and the Sunda Shelf at various times throughout the Pleistocene, which likely limited potential dispersal corridors for montane forest species (Heaney et al., 1991; Bird et al., 2005, 2007). Indeed, several studies have concluded that the late Pleistocene climatic and environmental conditions on Palawan probably differed substantially from present-day conditions (Bird et al., 2007; Piper et al., 2011), possibly linking recent environmental change on Palawan with the eventual colonization of *B. montana* from the oceanic Philippines. Overall, we suggest that these results highlight the importance of considering factors other than the width of water barriers as potential determinants of inter-island dispersal.

4.3. Geographical features structuring diversification

Our results strongly support the importance of present-day island coastlines as drivers of diversification in the Philippines. Each island formed a distinctive, though not always reciprocally monophyletic, genetic cluster, with no haplotypes shared between islands (Figs. 2 and 3). Additionally, our archipelago-wide AMOVA found overwhelming support for islands as determinants of mtDNA structure (Table 1), corroborating their importance as drivers of diversification in Philippine shortwings. However, we note two instances in which our results appear to be consistent with ongoing or recent gene flow between islands. First, we found especially shallow divergences between Luzon and Mindoro (Figs. 2–4), perhaps unsurprising given the proximity of these islands (ca. 15 km). We note that this pattern has been observed in several other avian lineages in the Philippines (Jones & Kennedy, 2008), suggesting that the deep but narrow water channel separating these two islands may not represent a substantial barrier to gene flow. Second, we interpret the shallow and poorly-supported divergences between Negros and Panay as potential evidence for recent gene flow
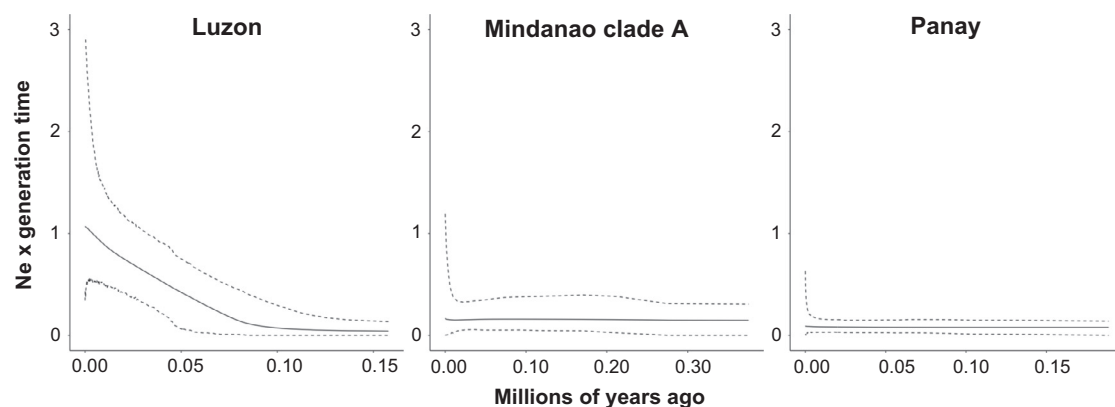
Fig. 6. Extended Bayesian skyline plots for Luzon, Mindanao clade A, and Panay Islands. Median effective population size estimate denoted by solid line with 95% HPD intervals shown with dashed lines.

between these populations, perhaps facilitated by periods of low sea level and dry land connections between these islands during the late Pleistocene (Fig. 2).

Contrary to our expectations, we found little support for diversification within Philippine islands. Our archipelago-wide AMOVA attributed minimal support to variation within islands (Table 1); this was reflected in our AMOVA on Luzon, which similarly found little evidence for mountain regions as determinants of genetic structure (Table 2), despite a dense sample (n = 126) across numerous distinct montane regions of the island (Fig. 5). Given the high-elevation distribution of *B. montana* on Luzon (the absence of this species in lower elevations [< 600 m] has been corroborated by numerous elevational transects [L. Heaney, pers. obs.]), and the emerging pattern that Luzon's mountain ranges often influence genetic structure in other high-elevation taxa (e.g., Jones & Kennedy, 2008; Balete et al., 2012; Justiniano et al., 2015; Kyriazis et al., 2017), this relative lack of differentiation in *B. montana* on Luzon was unexpected. These results contrast the patterns documented for *Robsonius* ground-warblers on Luzon, which diversified into three species across lowland river-valley barriers despite being present in lowland habitat ranging from sea level to 1300 m (Hosner et al., 2013b). We anticipate that this relative lack of differentiation between shortwing populations on Luzon may reflect their recent expansion across the island (Fig. 6), providing insufficient time for genetic differentiation and lineage sorting to occur between these montane populations.

Contrasting these patterns on Luzon, our limited sampling of *B. montana* Mindanao clade A did exhibit signals of genetic structure suggestive of differentiation driven by montane regions. Specifically, our phylogenetic analysis (Fig. 3; Supplemental Fig. 6) and haplotype networks (Supplemental Fig. 2) for Mindanao clade A recovered divergent and well-supported clades from eastern (Mt. Kampalili) and central Mindanao (Mts. Talomo, Lumot, and Balatukan), and more limited evidence for differentiation on Mt. Busa in southwestern Mindanao. This pattern of greater differentiation on Mindanao relative to Luzon is consistent with the hypothesis that diversification on these islands is limited by the time since colonization, given our result of more recent colonization of Luzon relative to Mindoro (Fig. 4). Additionally, we note that similar patterns of differentiation on Mindanao have recently been documented for a range of taxa (Jones & Kennedy, 2008; Sánchez-González et al., 2011; Sanguila et al., 2011; Hosner et al., 2013b), suggesting that intra-island diversification on Mindanao may be more common than previously recognized. Additional genetic and geographic sampling is needed to further examine the degree to which shortwings have diversified on Mindanao.

4.4. Secondary sympatry on Mindanao

Our finding that two divergent and paraphyletic shortwing lineages are present on Mindanao contrasts the predominantly allopatric distribution observed for all other Philippine lineages and offers additional insight into the dynamics of diversification in *B. montana*. Specifically, we recovered four samples across three localities on Mindanao (clade B) that were nested within a clade from Negros and Panay and genetically distinct from 11 other Mindanao samples (clade A; Figs. 2 and 3). Furthermore, samples of these two clades were collected in proximity on both Mt. Lumot in north-central Mindanao and Mt. Talomo in south-central Mindanao, suggesting that these lineages are coexisting in secondary sympatry across much of the island. Although our limited sampling does not permit a rigorous test of genetic isolation between these lineages, we did not find any evidence of introgression (i.e., genetic results were consistent for each gene/sample). Consistent with this result, our molecular dating analyses indicate that these lineages have been diverging for c. 4.0 mya (Fig. 4), a period of time that is thought to be sufficient for establishing reproductive isolation for birds (Weir & Price, 2011; Pigot & Tobias, 2014). Additionally, we note that field observers have recognized the presence of two distinct shortwing

songs in this region of Mindanao (Morris, 1996), supporting the conclusion that these lineages are reproductively isolated. Unfortunately, recordings of a second song type on Mindanao apparently do not exist at this time (P. Hosner, pers. obs., http://xeno-cano.org/, http://macaulaylibrary.org/), although vocal differences between the Mindanao, Negros, and Luzon populations are readily apparent (Boesman, 2016). We anticipate that additional research on these *B. montana* lineages will provide valuable insights into the dynamics of speciation and community assembly in the Philippines, as well as into the factors maintaining reproductive isolation in secondary sympatry more broadly.

4.5. Taxonomic implications

Numerous studies have demonstrated that biological diversity is frequently underestimated even in comparatively well-studied groups in the Philippines (e.g., Lohman et al., 2010; Sánchez-González et al., 2011; Barley et al., 2013; Heaney et al., 2016). Our results suggest that this is likely the case in *B. montana*. Specifically, we found that Philippine *B. montana* was highly divergent from mainland populations and that substantial diversification had taken place among shortwing lineages within the Philippines. These lineages include Mindanao clade A (separated c. 4.0 mya from the rest of Philippine *B. montana*), a Negros/Panay/Mindanao clade, and a Luzon/Palawan/Mindoro clade (latter two clades diverged c. 1.9 mya). These clades differ substantially from the subspecies delineations proposed for the Philippine shortwings (Gill & Donsker, 2017; Supplemental Fig. 3), and highlight the need for a more detailed examination of morphometric, ecological, and vocal variation to better characterize species limits in *B. montana* in the Philippines. Finally, we note that our results indicate that mainland *B. montana* lineages are highly divergent and may not be monophyletic relative to *B. leucophrys* (Figs. 3 and 4). Due to our limited sampling of these lineages, the implications of these results are beyond the scope of this study.

4.6. Conclusions

To date, our study incorporates one of the largest datasets to examine phylogeographic structure across the Philippines. Leveraging this sampling, we found that shortwings colonized the Philippines during the late Miocene or Pliocene, and have since expanded across much of the oceanic Philippines and Palawan during the Pleistocene. During this process, Philippine shortwings have diversified largely across inter-island barriers into three divergent lineages, with surprisingly little evidence for intra-island diversification. Our extensive sampling on Luzon found no clear patterns of 'sky island' differentiation, despite the montane distribution of these birds and a growing body of evidence highlighting the prevalence of intra-island diversification on Luzon in other animals. However, our more limited sampling on Mindanao recovered patterns which were consistent with intra-island diversification, although additional research is needed to more thoroughly investigate this result. Additionally, our results documented numerous instances of recent colonization and/or incipient diversification in Philippine shortwings, including evidence for recent colonization of Palawan and back-colonization of Mindanao both dated at c. 440 kya, and recent demographic expansion on Luzon over the last c. 90 kyr. We interpret these patterns of recent expansion and incipient diversification to suggest that shortwing diversification in the Philippines is an ongoing process, and one that has been perpetually stimulated by the dynamic geography of the Philippines during the Pleistocene. Additional research is needed to further investigate the role of the Philippines' dynamic climatic and geological history in shaping the diversification of its rich and highly endemic native biota.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2017.12.025.

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