

# The Nanling Mountains of southcentral China played a variable role as a barrier and refuge for birds depending upon landscape structure and timing of events

Zhengzhen Wang<sup>1</sup>, Min Zhang<sup>1</sup>, Xuebing Zhao<sup>2</sup>, Jiami Xie<sup>3</sup>, Yougui Peng<sup>3</sup>, F. H. Sheldon<sup>4</sup>, and Fasheng Zou<sup>1</sup>

<sup>1</sup>Guangdong Academy of Sciences

<sup>2</sup>Yunnan University

<sup>3</sup>South China Agricultural University

<sup>4</sup>Louisiana State University

February 7, 2023

## Abstract

The Nanling Mountains are an important mountain range and watershed in southcentral China. Because of the abundance in relictual plant species and geological stability throughout the Cenozoic, the mountains are considered a “museum” of subtropical biological diversity. With respect to birds, however, the roles of the Nanling Mountains in impeding the dispersal of the subtropical birds and, as a result, shaping their population and community structures have received little consideration. To examine these roles, we compiled and analyzed two datasets. (1) To test the mountains’ influence on gene flow, we undertook a population phylogeographic study comparing mitochondrial COI and Cytb DNA sequences of 5 sylvioid resident bird species of the mountains (Huet’s Fulvetta *Alcippe hueti*, Red-billed Leiothrix *Leiothrix lutea*, Greater Necklaced Laughingthrush *Pterorhinus pectoralis*, Indochinese Yuhina *Staphida torqueola*, and Mountain Bulbul *Ixos mccllellandii*). (2) To examine differentiated community development over the history of modern birds, we examined distributional data of all species of the Nanling region using public species occurrence records. For part (1), we sampled 327 individuals from 36 sites and conducted correlation analysis of genetic and geographic distances, taking into account the landscape of the mountains. We found that the mountains do not seriously impede gene flow among populations but influenced species differently. For part (2), comparative analysis of 446 species in 81 families indicated that family membership influenced the community composition of birds in Nanling region. Variation in family distributions is attributable to both environmental and evolutionary factors. Overall, we found that the Nanling Mountains are not currently a substantial barrier to gene flow among the species we studied but act as a corridor and refuge for these birds. However, analyses on higher ranked community data suggest the mountains acted as a barrier in older times, corresponding to the known diversification events in southeast Asian avifauna.

## INTRODUCTION

The variation in landscape configuration and dispersal ability of organisms could affect both the population structures (Hubbell 2001) and community structures (Soininen et al. 2007). Mountains, with heterogeneous landscape, play multiple roles in the evolutionary process (e.g., Hoorn et al. 2018, Rahbek et al. 2019a, b). The diverse habitats may provide refugia during periods of climatic change, and their elevational variation may provide both conduits and barriers to dispersal, connecting or disconnecting populations. In terms of dispersal ability of birds, the tropical and subtropical species tend to be sedentary despite that some taxa are considered highly vagile and capable of remarkable dispersal (Baker et al. 2001, Ramos et al. 2016). Variation in dispersal capabilities among species is substantial depending on their habitat requirements and migratory habits (Burney and Brumfield 2009), and tropical and subtropical forest species appear less mobile

than temperate species (Janzen 1967, Moore et al. 2008). It is now widely acknowledged that tropical birds tend to live sedentary lives to adapt to the tropical environment, while being sensitive to the heterogeneity and dynamics of the environment changes (e.g., Turcotte and Desrochers 2003, Stratford and Robinson 2005). Studies in tropical Amazonian forest, for example, have found that understory birds rarely cross roads (Laurance 2004, Develey and Stouffer 2008) and even avoid crossing forest gaps (Van Houtan et al. 2007).

The Nanling Range of subtropical southcentral China ( $c. 23.63^{\circ}$ - $27.23^{\circ}$ N and  $109.72^{\circ}$ - $116.68^{\circ}$ E) comprises low to medium elevation granitic hills and mountains (0-2000 m above sea level, m.a.s.l.; Supporting information). It is a biodiversity hotspot, renowned for its endemic plants and animals, whose conservation importance is growing because of its position at the center of an area of expansive human growth (Tang et al. 2006, Mi et al. 2021). The mountain range was formed in the late Cretaceous (Chen et al. 2002, Li et al. 2014) and subsequently served as a key refuge for animal and plant taxa, helping maintain biodiversity during the dramatic climatic shifts of the Quaternary ice ages (López-Pujol et al. 2011b, Dong et al. 2017). As a result, the mountains comprise a particularly rich area of paleo-endemism and are often regarded as an “evolutionary museum” (López-Pujol et al. 2011b, a; Hu et al. 2021, Mi et al. 2021). They are also thought to act as a barrier between the fauna of central China and southern China based on the composition of species in and around the range (Cheng and Chang 1956) and, as such, maybe important to fomenting current phylogeographic diversity. Although the Nanling Range’s historical role as a refuge is well studied and seems relatively certain (Cheng and Chang 1956, López-Pujol et al. 2011b, a; Hu et al. 2021, Mi et al. 2021), little is known about its role as a geographic barrier to birds.

One method of detecting and assessing the effectiveness of barriers to dispersal is to examine the degree of genetic connectivity between geographically separate populations. Many studies have examined genetic divergence in mountain birds (Spellman and Klicka 2006, Wu et al. 2012, Qu et al. 2015), but they have focused mostly on demographic history and rarely have considered the effect of landscape variation on the potential movement of birds. Great mountain range with large altitude span, such as in the Andes Mountains (e. g., Chaves et al. 2011, Hazzi et al. 2018) and the Himalayan Mountains (e.g., Päckert et al. 2012, White 2016), have led to significant divergence in avian lineages. While in the moderate elevation range of Nanling, the divergence, as opposed to the diversity, would be a more subtle question to address.

In this study, we take a two-pronged approach to investigate the extent to which the Nanling Mountains have acted in structuring bird populations in the Nanling region by examining in two different scales. (1) The phylogeography of five generalist forest bird species that reside in and around the mountains. With population genetic methods, studies could reveal the recent demographic changes caused by a barrier, e.g., the Nanling Mountains. (2) The distribution patterns of all bird species inhabiting the region. On the broad scale, it could illustrate how significant regional differences are and in which groups the differentiation nested. Together, the two approaches provide insight to the efficacy of the Nanling Mountains as an impediment to gene flow among populations and a causal factor in species distribution. They show that the Nanling mountains do, to some extent, act as a barrier. However, the extent of their influence is complicated by variations in the montane landscape and the idiosyncrasies of individual species’ dispersal characteristics. On the population level, the mountains have enhanced the expansion of generalist bird populations by serving as a forest buffer zone and corridor. But among higher taxa the mountains have acted as a significant north-south barrier to constituent species. The variable influence of the Nanling Mountains on the distribution of avian taxa at different taxonomic levels suggests the mountains’ role as a barrier is not only multidimensional now, but has changed—possibly many times—over their long history.

## MATERIALS AND METHODS

### Study species

The five species examined for phylogeographic structure were selected because they are common in the field and tissue collections and appropriately distributed across the Nanling region. They are residents (or at most local migrants) of the broadleaf forest habitats of southeastern China and are all members of the

superfamily Sylvioidea (Alström et al. 2013), a group representing substantial adaptive diversity. They are Huet’s Fulvetta *Alcippe hueti* (Pellorneidae), Red-billed Leiothrix *Leiothrix lutea* (Leiothrichidae), Greater Necklaced Laughingthrush *Pterorhinus pectoralis* (Leiothrichidae), Indochinese Yuhina *Staphida torqueola* (Zosteropidae), and Mountain Bulbul *Ixos mccllellandii* (Pycnonotidae), as classified by Gill et al. (2021). All five species reside in evergreen woody forests, but their precise habitats vary. *Ixos mccllellandii* inhabits mainly multilayered forest or woods from 800-2700 m near villages (Fishpool and Tobias 2020). *Alcippe hueti* resides between 400-2500 m in old growth and secondary forest and bamboo (Zhao 2001, Kirwan et al. 2021). *Leiothrix lutea* inhabits evergreen forest and forest edge at higher elevations, from 900-1200 m (Male et al. 2020). *Pterorhinus pectoralis* inhabits low elevation hills up to 1800 m (Collar and Robson 2021a) and almost never occurs in man-made habitats. *Staphida torqueola* resides in rainforest, usually from 350-2200 m (Collar and Robson 2021b) and is well-known as a reluctant flyer (Zhao 2001).

Outgroups were selected based largely on the availability of tissue samples in collections or DNA sequences in public datasets, as well as phylogenetic relatedness. For *A. hueti*, we used the subspecies *rufescentior*, because it is monophyletic and differs from *A. hueti* in the Nanling region by >15 base pairs (bp). For the same reasons, we selected the *P. pectoralis* subspecies *semitorquatus*. For *I. mccllellandii*, we used *H. flavala* as the outgroup because the two are relatively closely related (Shakya and Sheldon 2017). Similarly, we selected *Yuhina nigrimenta* (GenBank accession number: NC040991) for *S. torqueola* and *L. argentauris* (GenBank accession number: HQ690245) for *L. lutea* (Cai et al. 2019).

### Sampling, sequencing and genetic analysis

We sampled a total of 327 individuals of *A. hueti* (27), *I. mccllellandii* (52), *P. pectoralis* (60), *L. lutea* (149), and *S. torqueola* (39) from 36 sites (Supporting information, Figure 1). From each individual, blood or muscle samples were collected and stored at -80°C. Genomic DNA was extracted using a QIAGEN Blood/Tissue Extraction Kit following the manufacturer’s instructions. Two mitochondrial genes, cytochrome *b* (*Cytb*) and cytochrome *c* oxidase subunit 1 (*COI*), were selected for this part of experiments. The mitochondrial genome is known to experience higher substitution rate than nuclear genome (Ballard and Whitlock 2004). This is more evident in vertebrates, such as birds and mammals (Lynch et al. 2006) with substitution rate 25 folds higher in mitochondrial than in nuclear genome. Therefore, the mitochondrial marker serves as a suitable marker for the intraspecies study with rich informative polymorphism.

Both mitochondrial genes were amplified from all 327 samples using published primers (Supporting information; Amer et al. 2013). PCR was performed using High fidelity Superstar PCR Mix (GenStar, Beijing, China). The 30 µL PCR mixture contained 15 µL PCR Mix, 1.5 µL of each primer of 10µM, 3 µL of template DNA and 9 µL of deionized water. The PCR program was set as follows: denaturation at 94°C for 4 min; 30 cycles of 94°C for 10 s; annealing for 30 s (temperature in Supporting information); extension at 72°C for 90 s; and final extension at 72°C for 8 min. The amplicons were sent to Tian Yi Hui Yuan (Guangzhou, China) for Sanger sequencing from both ends. To rule out sequencing errors and produce an exact alignment, sequences were manually checked and the ends trimmed in MEGA X (Kumar et al. 2018).

We concatenated the 2 genes (*COI* + *Cytb*) into one sequence of 1681-1861 bp for further analysis (Table 1). Aligned sequences were imported to DNaSP 6.0 to calculate  $\theta$ ,  $\pi$  and Tajima’s D, and to produce a haplotype file (Rozas et al. 2017). Single nucleotide polymorphisms (SNPs) were retained and formatted into 5 matrices for further analysis of the 5 species, respectively. A haplotype network was calculated for each species with Network 10.0, using the median joining algorithm (Polzin and Daneshmand 2003). Because the uneven sample size was not suitable for regular estimates of diversity based on allele frequency, such as *Fst* (Nei and Miller 1990, Cruickshank and Hahn 2014), we calculated the number of segregating non-synonymous (na) and synonymous (ns) sites based on the JC69 model (Jukes and Cantor 1969). Pairwise distance, *N*, was approximated (using Python scrips) as the mean of among-individual ns+na values. Populations with only one individual were removed in calculations based on population genetic data. Genetic estimate based on species and genetic clustering, as mentioned in the following text, still involved all the sequences.

To determine population clustering, we performed genetic clustering on the time tree using *gmyc* function

in the R package *splits* (Ezard et al. 2021). The aligned sequences were tested for best substitution model in MEGA X (Kumar et al. 2018). A time tree was built using BEAST with substitution rate of  $10^{-8}$  site/year ((Nguyen and Ho 2016),  $10^7$  MCMC chain length, best fitted substitution model “HKY” for all species and other parameters as default. The time tree was imported to R for clustering analysis using *gmyc* .

### Calculation of geographic distance

Because exceptions in the correlation between genetic distance and geographic distance would indicate the existence of barriers to gene flow, we tested isolation-by-distance (IBD; Wright 1943) among populations of the five species. To estimate geographic distance across the varied landscape of the mountains, we examined the effects of the rugged montane terrain on dispersal patterns by hypothesizing scenarios of likely dispersal routes through, over, and around the mountains. Four dispersal scenarios for birds were hypothesized, resulting in four variants of dispersal (geographic) distance: (1) spheric distance, assuming that birds fly over mountains regardless of landscape; (2) optimized route cost-distance, with a penalty for traveling above or below the normal range of elevation for each species, assuming birds prefer to disperse within a specific elevational range; (3) optimized route cost-distance, with a penalty only for dispersing above a favored elevational range, assuming birds detour only to avoid high elevation; and (4) optimized route cost-distance, with a penalty only for dispersing below the lower threshold of a favored elevational range, assuming birds detour only to avoid low elevation.

Using the terrain distance function *costDistance* of the *gdistance* package in R (Van Etten 2017), we computed the geographic cost-distance. This was achieved first by calculating the three-dimensional distance among adjacent grid points using the Pythagorean theorem applied to two points—the elevational difference and the hypotenuse of the right triangle they formed—and second by using *transition* to transfer the raster matrix into a pairwise conductance distance matrix. In the case of  $n$  raster grids, the raster matrix transfers to an  $n \times n$  matrix in which only conductible grids have meaningful values. In our customized penalty function, the conductivity between two grids was recalculated by reassigning elevational differences with an extra penalty. The penalty was the sum of the out-of-bounds parts of the elevation of each point multiplied by a penalty factor. Calculation of the reciprocal of conductance,  $L$ , between two points was as follows:

$$L = \text{sqrt} (D^2 + p^2) \quad (1)$$

where  $p$  is the distance between two points, and  $D$  is the reassigned elevational difference. The reassignment was computed as:

$$D = d + f \times (\text{abs} (k1) + \text{abs} (k2)) \quad (2)$$

where  $d$  is the initial elevational difference between two points, and  $k1$  and  $k2$  are out-of-bounds elevations of the two points. *Abs* is the absolute value, and  $f$  is the factor used to decide the strength of the penalty for traveling out of the elevational bounds. The assignment of  $f$  is described below.

For scenarios with no penalties, spheric distances were calculated using pairwise latitude and longitude with the function *pointDistance* from the *raster* package in R (Hijmans, 2019, Karney, 2011). For those distances with applied penalties, the shortest distance across the landscape was calculated using an elevational raster map from the GMTED2010 database (Danielson and Gesch 2011). We set the preferred elevational range of each species based on empirical records from the literature (Table 1; Zhao 2001), then calculated the detour routes on the basis of this range. Because a direct route may not conform entirely to a species’ elevational range, we added a penalty to sections of the routes outside the species’ range. These are presented as the *ffactor* in Eq. (2). The  $f$  values were set to 0.001, 0.01, 0.1, 1, and 100 times the deviation from the upper or lower bound of the preferred elevation before searching for the least costly (most efficient) dispersal route. Least-cost routes were plotted to see their displacement as the  $f$  factor changes. It should be noted, however, that the penalized distance cannot be considered as an actual distance or be compared to values of other scenarios; the cost-distance was only penalized outside a certain range according to the hypothetical preference of the bird, which differs among scenarios, so it is not simply proportional to penalty level.

### Detecting isolation-by-distance versus barrier

To identify IBD patterns among populations, we analyzed the correlation between pairwise genetic distances and geographic locations of all populations using BARRIER 2.0 (Manni et al. 2004). BARRIER applies a geometric approach and produces the length and width of barriers. We also applied Mantel tests to determine concordance and statistical robustness between geographic and genetic distances. Mantel tests compared geographic distance matrices (produced according to the multiple dispersal scenarios described above) and the N-distance matrix. Mantel tests were performed on the 5 species with 4 scenarios of dispersal, respectively, while the BARRIER software accepts coordinate as input. We also calculated AIC for linear correlation models of genetic distance and geographic distance under different scenarios with the *AIC* function in R core functions (R Core Team 2020). Values were formatted into a  $\Delta$ AIC matrix to infer the best scenario for IBD pattern.

As noted by Wright (1943), an IBD pattern would be disrupted by non-random dispersal in reaction to specific environments. This observation led ultimately to the development of the idea of isolation-by-environment (IBE; Nosil et al. 2005). To test for IBE, we selected 20 environmental variables—including 19 bioclimatic variables from the public database CHELSA (Karger et al. 2017, Karger et al. 2018; Supporting information) and the GMTED2020 (Danielson and Gesch 2011) elevational dataset mentioned above—that are expected to be correlated with factors consequential to bird habitat. A negative correlation or no correlation between the climatic variables and genetic distance, therefore, would dispute IBE. To avoid overfitting in the environmental analyses, we first remove highly correlated variables. We randomly sampled 10000 grids to characterize each candidate variable and performed PCA analysis of their correlation with one another (Supporting information). Seven of the variables—Bio04 (temperature seasonality), Bio07 (annual range of air temperature), Bio12 (annual precipitation), Bio13 (precipitation of the wettest month), Bio16 (mean monthly precipitation of the wettest quarter), Bio18 (mean monthly precipitation of the warmest quarter), and elevation, were the least related to one another. Using these 7 variables, we tested for IBE by applying Mantel tests and linear regression on variable differences versus N-distances among genetic sample points using the *mantel* function in the *vegan* package (Oksanen et al. 2019) and *lm* function in R core functions (R Core Team 2020). A significant correlation would support IBE among populations.

To examine the effects of recent climate changes on the distribution of the 5 species, we performed environmental niche modeling (ENM), using the same 7 environmental variables and MAXENT 3.4.4 (Phillips et al. 2006) with default parameters. Variable values from the last glacial maximum (LGM, ~21,000 years before present), the final stages of the LGM (BAW, ~14,600 years ago at the maximum of the Bølling-Allerød warming; Karger et al. 2018), and present day (CHELSA; Karger et al. 2017) were used. The elevation above sea level and land contours for modeling and mapping were from Lambeck et al. (2014) (96 meters lower than present for the BAW), and Siddall et al. (2003) (125 meters lower than present for the LGM). Ten replicates were run, and cross-validation was used to test the model. To build the models, record data from the China Bird Report Center (CBRC, <http://www.birdreport.cn/>, retrieved on 2 February 2020) and eBird database (Sullivan et al. 2009; retrieved in May 2021) for the 5 species were used. Point occurrence records were reduced to one record per 15 km radius. Records with longer observation and higher count were selected during the thinning process. The final collection consisted of 3931 records in total (1480 for *I. maculosa*, 312 for *A. hueti*, 1079 for *L. lutea*, 663 for *P. pectoralis* and 397 for *S. torqueola*). For graphic illustration of the distribution of the species over time, we collected the logistic output raster. We also prepared a raster map of summed logistic output of all 5 species for an overview.

### Statistical tests of different scales in the Nanling Mountains

In addition to the phylogeography of the 5 target species, we examined the occurrence records of all other bird species in the Nanling region to determine the long-term effects of the mountains on their distribution patterns. Bird occurrence in the Nanling region was determined from records downloaded from the CBRC and the eBird database. The data included species' name, latitude and longitude and number of observations (Supporting information). Duration and distance of the observation were omitted in order to reconcile the formats of 2 datasets. Comparisons focused on the differences among three geographic subunits: north of the Nanling Mountains, south of the mountains, and the mountains themselves (Figure 1, Supporting infor-

mation). The Nanling region as a whole, and consequently its birds, was delimited manually by including administrative counties that overlapped with the mountains (Figure 1), while the north-end was decided so as to form comparable size of area (North Nanling 177534 km<sup>2</sup>, Nanling itself 162695 km<sup>2</sup> and south Nanling 175820 km<sup>2</sup>). The Nanling in the middle is rugged with low mountains, while in the north, there are plains with surrounding mountains. The south Nanling region comprises mountains, waters and coastlines. Before testing distribution patterns, we pruned wetland- and coastal birds based on the habitat data from AVONET (Tobias et al. 2022). The original datasets were based on observation events, which would lead to bias introduced by observers (Strimas-Mackey et al. 2020). We transformed the dataset by thinning the records omitting the time variance. For the 2 datasets from different databases, species were reduced to one record per 15 km radius. In the end, 27402 occurrence records for 446 species were used (Supporting information).

Two statistical tests from different taxonomic level were applied to examine differences in birds among the three geographic subunits. We first performed chi-square tests on the number of species in each family between each region pairs to locate significant differences in family composition. For this we used *chisq.test* in R core functions. Second, we performed one-tailed Wilcoxon’s rank tests in both directions number of each bird species records for each family to determine which family distributes differently among three geographic subunits. All tests were done using the *wilcox.test* in R core functions. To avoid abnormal estimates from very rare occurrence records, we omitted families with less than 10 occurrence records from the results.

## RESULTS

### Genetic variation among the five species

To assess genetic diversity within each species, we calculated nucleotide polymorphism, haplotype polymorphism (Hd), nucleotide diversity ( $\pi$ /kb), polymorphic sites ( $\vartheta$ /kb), and Tajima’s D (Table 2). Except for *S. torqueola*, four of the species have comparable  $\pi$  values, ranging from 2.73-4.52(/kb). *I. maclellandii*, *A. hueti* and *P. pectoralis* have significantly high  $\vartheta$  values (5.68 – 8.51/kb) compared to *L. lutea* (1.22/kb) and *S. torqueola* (1.8/kb). Hd values are similar in all five species (0.83 to 0.972). Tajima’s D is significantly negative in *I. maclellandii* and *L. lutea*, suggesting substantial recent population expansion or a selective sweep in these species (Tajima 1989). Population expansion is also supported by their radiating haplotype networks (Figure 2). *S. torqueola*, despite wide regional sampling and a relatively large number of individuals ( $n = 39$ ), appears to differ substantially from the other species in variation. It has a relatively low number of SNPs (single versus double digits) and low values of  $\pi$  and  $\vartheta$ . Its Tajima’s D is close to zero, signaling little recent population expansion. As a resident of moderate elevation (700-1500m), *S. torqueola* also presents a highly homogenous population composition suggestive of substantial gene flow (Figure 2E).

Except for *S. torqueola*, the species generally display comparable haplotype diversity, complexity, and patterns of recent population expansion. Most of their substitutions are synonymous, and their networks contain conspicuous patterns of haplotype radiation from highly diverse ancestral clusters (e.g., Figure 2A-D). In all 5 species’ networks, divergence among haplotypes of 2-5 bp is common between populations, strongly indicating rapid substitution combined with homogenous haplotype distribution in populations following isolation. Some of the species included a conspicuous core haplotype distinct from derived haplotypes, e.g., the blue haplotypes in *I. maclellandii* and *L. lutea* (Figure 2A and 2C). This pattern is less notable in other circumstances, e.g., the green cluster in *L. lutea* (Figure 2C). Under the conventional substitution rate of 10<sup>-8</sup>/site/year (Nguyen and Ho 2016), this pattern suggests divergence times of 0.1~0.3 Ma among diverged haplotypes in all species.

Interestingly, the haplotype network of *L. lutea* suggests this species went through a strong bottleneck, followed by rapid radiation of each of its haplotype groups (Figure 2C). The two star-like clusters are separated by a 2 bp substitution. One of these substitutions (at bp 1033) is non-synonymous, causing an amino acid change from Isoleucine (assumed to be the ancestral state by reference to the outgroup, *L. argentauris*, in the green cluster) to Valine (in the blue cluster). The high haplotype diversity in the ancestral cluster and the radiating haplotype network in the derived cluster suggest recent expansion of the new non-synonymous substitution, which is homogeneously distributed among the sampled (blue) populations.

Genetic clustering returned different results for the 5 species. Overall, all species were confidently clustered by the *gmyc* (Supporting information). *I. maclellandii* split into 3 maximum likelihood clusters, while *A. hueti* split into 4 clusters, *L. leiothrix* split into 6 clusters, *P. pectoralis* into 15 clusters, and *S. torqueola* into 5 clusters. None of the clusters show strong alias to populations except for *P. pectoralis*. In *P. pectoralis*, the clustering divided populations into smaller closely related groups (Supporting information). *Gmyc* also inferred the threshold time of the split. The threshold time of *I. maclellandii*, *A. hueti*, *L. lutea* and *S. torqueola* are in the approximate range of 0.2 MYA, while the inferred split time of *P. pectoralis* is later than 23 000 and 12 000 years ago (Supporting information). The clustering result is supported with significant likelihood ratio test. Homogeneous ancestry is an indication of simultaneous demographic changes. While for *P. pectoralis*, the short threshold time and fine arranged population clusters indicate that they probably were dispersed recently, e.g., in the last glacial cycle.

### Correlation analyses of isolation patterns

Cost distance was calculated while the route was plotted (Figure 3, Supporting information). A penalty of  $f = 1$  predicted that *A. hueti* and *P. pectoralis* could take “shortcuts” through the center of the mountain range (Figure 3, Supporting information). A better fit between cost distance and genetic distance for the species with IBD patterns was expected compared to spheric distance. IBE and IBD patterns with (Scenario 2) and without cost-distances were tested to determine patterns of isolation among populations within species. Mantel tests and linear correlations based on the 6 bioclimatic variables and elevational differences showed no significant relationship between genetic distance and environment difference (Supporting information). Mantel testing and linear regression of geographic distances and N-distances indicate different correlation patterns among the species (Table 3 and Figure 4, Supporting information), given penalties for dispersal routes outside each species’ preferred elevational range. Only one of the five species, *A. hueti*, is significantly isolated by distance. The linear correlation between genetic distance and cost distance is significant under penalized scenarios for *P. pectoralis*. While the Mantel test of this species is quasi significant ( $0.05 < p < 0.1$ ), with low linear correlation coefficient and low Mantel’s statistic  $r$  (Figure 4B, Supporting information). For these 2 species, the extent of obstruction caused by the montane landscape is variable. In *A. hueti*, a moderate dispersal penalty of  $f = 0.1$  leads to the highest correlation according to significance and AIC values (Supporting information). In *P. pectoralis*, the least cost route with no penalty outside preferred elevational range correlates with genetic distance, as supported by AIC values. The Mantel test results in the other 3 species, *I. maclellandii*, *L. lutea* and *S. torqueola*, indicated no significant correlation between geographic and genetic distance.

The location and length of potential barriers to dispersal among the sampled populations inferred by BARRIER are consistent with Mantel tests. Overall, none of the inferred barriers concur with the latitudinal layout of the mountain range. The main barriers within *A. hueti* (Figure 5B), which was significantly isolated by distance, separate populations with high diversity, suggesting the existence of substantial dispersal obstacles during historical expansion in high-diversity distribution centers. For example, the *A. hueti* populations NL and JXS occur in the center of the Nanling Mountains and are separated by three barriers (Figure 5B). In the other species, the layout and direction of the barriers were either longitudinal or around peripheral populations (Figure 5A, C, D and E). For *A. hueti*, when no penalty was applied, the least cost route distance was correlated with genetic distance and corresponded to direct distance (Supporting information). When the correlation was highest with  $f = 0.1$ , the route taken was constrained to more mountainous areas, compared to scenarios with no penalties (Supporting information).

To help explain genetic differences among populations, we performed ENM for each species using LGM, BAW and current environmental variables (Supporting information). Given likely environmental conditions for those time periods, *A. hueti* and *P. pectoralis* should have maintained a relatively stable distribution in the Nanling mountains and the mountainous region east of Nanling during all three time periods. The rest of the species should have experienced a major shift in distribution from the LGM and BAW to the current environmental situation. At all times, parts of the Nanling Mountains provided appropriate habitat for the five species and were disjunct or patchily distributed.

## Variation among bird families in the Nanling region

Comparisons of bird occurrence in the 3 Nanling regions—North Nanling, South Nanling, and the Nanling Mountains—indicated multiple differences in the species composition of families and the occurrence of individuals within families. Chi-square testing showed a significant difference in the occurrence numbers of birds in each family among the three subregions (Supporting information). One-tailed Wilcoxon’s tests among families based on the occurrence numbers of bird species showed a high probability ( $P < 0.3$ ) of a difference in numbers among the 3 subregions in multiple families (Supporting information). In the one-tailed tests, more species from Leiothrichidae, Laridae, Strigidae, Scolopacidae and Muscicapidae occur in South Nanling than North Nanling. Compared to North Nanling, the Nanling Mountains contain more individuals in Leiothrichidae, Phylloscopidae and Leiothrichidae. Compared to South Nanling, the Nanling Mountains hold more individuals in the Phasianidae family. Individuals in the Turididae, Sturnidae and Columbidae are more numerous in South Nanling than Nanling itself, while individuals in the Anatidae, Laridae, and Scolopacidae are more numerous North Nanling than in Nanling itself. All two-tailed test results with high probability ( $P < 0.3$ ) are included in one-tailed test results (Supporting information).

## DISCUSSION

### Phylogeography: the Nanling Range is not a simple barrier to gene flow

Although a modest mountain chain with respect to elevation (Körner et al. 2017), the Nanling Range is an effective barrier to dispersal of relatively sedentary taxa such as amphibians and many plant species (Chen et al. 2017, Wang et al. 2017). But, for vagile taxa like birds, the efficacy of the range as a barrier has until now yet been emphasized. Our phylogeographic examination indicates that the mountains have little effect on gene flow of populations of the 5 sylvioid bird species whose phylogeography we compared: *Alcippe hueti*, *Leiothrix lutea*, *Pterorhinus pectoralis*, *Staphida torqueola*, and *Ixos mcclellandii*. Indeed, the range may act more as a conduit than a barrier for these birds. This effect appears to have been the case even during the dramatic climatic (and consequent environmental) shifts from the LGM forward.

Although the 5 species move readily past the mountains, their population structures vary, suggesting differences in their dispersal pathways and histories of demographic changes. For example, only one species, *A. hueti*, display a significant correlation between genetic and geographic distances and, hence, IBD. For this species, genetic distance correlates best with penalized cost-distance rather than spheric or non-penalized distances (Supporting information). This bird more likely took routes flying under rather than over preferred elevation. Elevational niche gradient is known to have driven variable seasonal migration in birds (Williamson and Witt 2021, Céspedes Arias et al. 2022), and it could also shape birds’ behavior of activity range and direction of colonization. The closely related species to *A. hueti*, *A. fratercula* (Zou et al. 2007, Song et al. 2009) is distributed in the mountains in Yunnan, southwesten China. The specific preference for certain elevation range could be the echo of the its origination from the higher mountains. Another species, *P. pectoralis* showed correlation of genetic distance to geographic distance in part of the scenarios (Supporting information). Moreover, it was also the only species with clustered populations in the genetic clustering analysis (Supporting information), indicating its divergence was gradual over time as well as distance. Combined to the negative Tajima’ D (Table 2) which suggests a selective sweep or population expansion after a bottleneck or founder event, and threshold timing, this species likely went through population expansion recently, and most likely after the LGM period.

The 3 other species differ from *A. hueti* and *P. pectoralis* and from one another in their genetic patterns. *Leiothrix lutea* displays little population clustering and no IBD. This unusual pattern likely results from a history of anthropogenic transport. At one time, this species was a popular cage bird in China, and only recently has it been legally protected. As such, it was transported in unpredictable ways by pet traders and owners across the country. *Staphida torqueola* displays hardly any variation across its range. This species has a Tajima’s D close to zero, indicating a stable demographic history and no significant selection (Tajima 1989, Rozas et al. 2017), a pattern that may have resulted from redistribution of ancestral groups by population contraction from a continuous genetic pool, masking its gene flow patterns.

A general pattern in all 5 species is that no obvious shift in genotype is evident north-to-south across the mountains (Figure 2). This observation agrees with the BARRIER analysis (Figure 5), which identified relatively few barriers in the mountains and even fewer configured west-to-east, thus inhibiting north-south dispersal. Another general pattern is the apparently small effect imposed by differences in bioclimatic (or related) variables and elevation on bird divergence. In the three of the species with little or no IBD (*I. mcclllandii*, *L. lutea*, *S. torqueola*), dispersal may have been more strongly influenced by habitat (IBE) than distance, given that in the absence of IBD some other factors must be at play to differentiate populations (Wright 1943, Nosil et al. 2005). However, we found no correlation between genetic distances and environmental variable differences in these species (Supporting information). Although no significant habitat barrier was detected, the rugged terrain of the mountains (an environmental factor that is difficult to quantify) likely influenced the route taken by birds to some degree.

### Environmental niche modeling of the Nanling Range

ENM of the habitats of the 5 target species during the LGM (21,000 years ago) and the onset of the BAW (14,600 ybp) indicates that the range of all of them would have shifted largely to the eastern end of the Nanling Mountains, while a few discrete probability peaks remained within the rest of the range. During this time, the Nanling Mountains formed the approximate northern edge of warm forest vegetation in China (figure 4 in Tian et al. 2018a). As expected with global warming after ~14,600 years ago and a concomitant increase in suitable habitat, the species expanded their distributions westward across the mountains (Supporting information). The key conclusion of ENM is that the Nanling Mountains provided refugial habitat to sustain the 5 species during the LGM. No doubt, conditions in the mountains sustained many other bird species as well.

### More ancient influence of the Nanling Range on bird occurrence

Despite the mountains' current, limited effect on gene flow in some passerine species, our examination of the occurrence of all species of birds in the Nanling region indicates substantial differences between areas north, south, and in the range itself. These differences suggest the mountains have had a strong historical effect. The chi-square tests of family variation indicated the significant differences between South Nanling and North Nanling, which support Cheng and Chang's (1956) division of Chinese zoogeographic regions that used the Nanling Mountains as the boundary between the Central China and South China.

Statistical tests of family divergence based on occurrence records across the mountains indicate that pronounced taxonomic divergence exists among the three areas compared (Supporting information). For example, both South Nanling and the Nanling mountains themselves hold more members of the Leiothrichidae when compared to North Naling, and the Nanling Mountains showed exceeding abundance in species in Phasianidae, Phylloscopidae and Leiothrichidae compared to south and north of the mountain range. Phasianids are mainly terrestrial forest species (Tobias et al. 2020). Members of Phylloscopidae are small leaf warblers known to have experienced recent evolutionary radiation since c. 11.7MYA (Alström et al. 2018). Leiothrichidae is also a recent group, originating c. 14.73 MYA in the mid-Miocene (Cai et al. 2020). In the mid-Miocene, climatic conditions became humid and more tropical (Guo et al. 2008), giving rise to more broad-leafed vegetation worldwide and general diversification of tropical and subtropical bird groups (Jacques et al. 2011). In addition to the Leiothrichidae, multiple other babbler families originated in the mid-Miocene (Cai et al. 2020). Muscipapidae species are also more numerous in South Nanling compared to North Nanling. All The muscipapids are a diverse group of old-world flycatchers, assumed to originate c. 34 MYA, (Sangster et al. 2010, Kumar et al. 2017), at the end of Eocene. The end of the Eocene and beginning of the Oligocene was a major transition time for birds, as the extensive tropics in northern latitudes shifted southward and many older tropical lineages suffered extinction and were eventually replaced by more modern groups (Mayr 2013, Oliveros et al. 2019, Sheldon et al. 2015). Except for the the phasianids, all taxa are recently formed, indicating the current bird community has been shaped after this period. Habitat differences in north and south of the mountains clearly help maintain or accentuate those differences. South of the Nanling Range, there is more subtropical forest, where an enhancement of forest inhabitants would be expected.

The Nanling Range was formed in the Cretaceous and remained stable thereafter. Its long existence likely allowed ancestral montane lineages of birds to be conserved during the Oligocene extinction by virtue of the mountain's landscape heterogeneity in the face of changing climate. The uplift of mountains would also have led to differentiated climatic conditions between the coast and southcentral China, and stimulating adaptive and allopatric diversification (Hoorn et al. 2018). Being in the subtropical monsoon climate, Nanling could also drive climatic divergence by slowing down or block seasonal winds, forming differentiate climatic environment in southern China. The results of this study indicate that that the development of bird diversity in southern China had been directly or indirectly shaped by the existence of the Nanling Mountains.s

### Conservation implications and conclusions

Although the Nanling Range has long been recognized as a biodiversity and conservation hotspot (Spehn et al. 2010, Nieto Feliner 2014, Mi et al. 2021), most studies of the biogeographic and environmental importance of the Range have focused on its relictual plants, such as the endemic South China Five-needle Pine *Pinus armandii* (Tian et al. 2010) and the monotypic, tertiary, relictual tree *Eurycorymbus cavaleriei* (Wang et al. 2009), and the role of the mountains as a corridor for such plants (Tian et al. 2018). With respect to animals, studies are fewer, but initial work suggests there is little overlap between faunal and floral hotspots in the mountains (Xu et al. 2018). Nevertheless, ENM in this study indicates a likely relationship between plant and bird occurrence in the mountains over recent geologic history, from 21000 years to present (Supporting information). The mountains maintained appropriate habitat for the 5 target species at its eastern end during the LGM and then, with global warming, that habitat and its birds spread westward. Thus, the mountains provided a refuge during the harshest climate change and a conduit during more ameliorated times. The mountains also serve as a north-south boundary and watershed, separating open wetlands to the north from more subtropical forest habitat to the south.

### SUPPORTING INFORMATION

Supporting information is available as submitted.

### LITERATURE CITED

- Alström, P., U. Olsson, and F. Lei (2013). A review of the recent advances in the systematics of the avian superfamily Sylvioidea. *Chinese Birds* 4:99–131.
- Alström, P., F. E. Rheindt, R. Zhang, M. Zhao, J. Wang, X. Zhu, C. Y. Gwee, Y. Hao, J. Ohlson, C. Jia, D. M. Prawiradilaga, et al. (2018). Complete species-level phylogeny of the leaf warbler (Aves: Phylloscopidae) radiation. *Molecular Phylogenetics and Evolution* 126:141–152.
- Baker, A. M., P. B. Mather, and J. M. Hughes (2001). Evidence for long-distance dispersal in a sedentary passerine, *Gymnorhina tibicen* (Artamidae). *Biological Journal of the Linnean Society* 72:333–343.
- Ballard, J. W. O., and M. C. Whitlock (2004). The incomplete natural history of mitochondria. *Molecular Ecology* 13:729–744.
- Burney, CW, Brumfield, RT (2009) Ecology predicts levels of genetic differentiation in Neotropical birds. *American Naturalist* 174:358-368.
- Cai, T, Cibois, A, Alström, P, Moyle, RG, Kennedy, JD, Shao, S, Zhang, R, Irestedt, M, Ericson, PG, Gelang, M (2019) Near-complete phylogeny and taxonomic revision of the world's babblers (Aves: Passeriformes). *Molecular Phylogenetics and Evolution* 130:346-356.
- Cai, T., S. Shao, J. D. Kennedy, P. Alström, R. G. Moyle, Y. Qu, F. Lei, and J. Fjeldså (2020). The role of evolutionary time, diversification rates and dispersal in determining the global diversity of a large radiation of passerine birds. *Journal of Biogeography* 47:1612–1625.
- Céspedes Arias, L. N., S. Wilson, and N. J. Bayly (2022). Community modeling reveals the importance of elevation and land cover in shaping migratory bird abundance in the Andes. *Ecological Applications* 32.

- Chaves, J. A., J. T. Weir, and T. B. Smith (2011). Diversification in *Adelomyia* hummingbirds follows Andean uplift. *Molecular Ecology* 20:4564–4576.
- Chen, J.-M., W.-W. Zhou, N. A. Poyarkov, B. L. Stuart, R. M. Brown, A. Lathrop, Y.-Y. Wang, Z.-Y. Yuan, K. Jiang, M. Hou, H.-M. Chen, et al. (2017). A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asian horned toads, genus *Megophrys* sensu lato (Anura: Megophryidae). *Molecular Phylogenetics and Evolution* 106:28–43.
- Chen, P., R. Hua, B. Zhang, J. Lu, and C. Fan (2002). Early Yanshanian post-orogenic granitoids in the Nanling region - Petrological constraints and geodynamic settings. *Science in China, Series D: Earth Sciences* 45:755–768.
- Cheng, T., and Y. Chang (1956). On tentative scheme for dividing zoogeographical regions of China. *Acta Geologica Sinica* 22:93.
- Collar, N. and C. Robson (2021a). Greater Necklaced Laughingthrush (*Pterorhinus pectoralis*), version 1.1. In *Birds of the World* (J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, and E. de Juana, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. <https://doi.org/10.2173/bow.gnlthr.01.1>
- Collar, N. and C. Robson (2021b). Indochinese Yuhina (*Staphida torqueola*), version 1.1. In *Birds of the World* (J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, and E. de Juana, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. <https://doi.org/10.2173/bow.indyuh1.01.1>
- Cruickshank, T. E., and M. W. Hahn (2014). Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. *Molecular Ecology* 23:3133–3157.
- Danielson, J. J., and D. B. Gesch (2011). Global multi-resolution terrain elevation data 2010 (GMTED2010). OFR 2011–1073.
- Develey, P. F., and P. C. Stouffer (2008). Effects of roads on movements by understory birds in mixed-species flocks in central Amazonian Brazil. *Conservation Biology* 15:1416–1422.
- Dong, F., C. M. Hung, X. L. Li, J. Y. Gao, Q. Zhang, F. Wu, F. M. Lei, S. H. Li, and X. J. Yang (2017). Ice age unfrozen: severe effect of the last interglacial, not glacial, climate change on East Asian avifauna. *BMC evolutionary biology* 17:244.
- Van Etten, J. (2017). R Package gdistance : Distances and Routes on Geographical Grids. *Journal of Statistical Software* 76.
- Ezard, T., T Fujisawa, T. Barraclough (2021). splits: SPecies' LLimits by Threshold Statistics. R package version 1.0-20/r56, <<https://R-Forge.R-project.org/projects/splits/>>.
- Fishpool, L. and J. Tobias (2020). Mountain Bulbul (*Ixos mcclellandii*), version 1.0. In *Birds of the World* (J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, and E. de Juana, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. <https://doi.org/10.2173/bow.moubul2.01>.
- Gill, F, D Donsker, and P Rasmussen (Eds). 2021. IOC World Bird List (v 11.1). Doi 10.14344/IOC.ML.11.1. <http://www.worldbirdnames.org/>
- Guo, Z. T., B. Sun, Z. S. Zhang, S. Z. Peng, G. Q. Xiao, J. Y. Ge, Q. Z. Hao, Y. S. Qiao, M. Y. Liang, J. F. Liu, Q. Z. Yin, and J. J. Wei (2008). A major reorganization of Asian climate by the early Miocene. *Climate of the Past* 4:153–174.
- Hazzi, N. A., J. S. Moreno, C. Ortiz-Movliav, and R. D. Palacio (2018). Biogeographic regions and events of isolation and diversification of the endemic biota of the tropical Andes. *Proceedings of the National Academy of Sciences of the United States of America* 115:7985–7990.
- Hijmans, Robert J. (2019). raster: Geographic Data Analysis and Modeling. R package version 2.9-23.
- Hoorn, C., A. Perrigo, and A. Antonelli (2018). Mountains, climate and biodiversity. Wiley-Blackwell.

- Van Houtan, K. S., S. L. Pimm, J. M. Halley, R. O. Bierregaard, and T. E. Lovejoy (2007). Dispersal of Amazonian birds in continuous and fragmented forest. *Ecology Letters* 10:219–229.
- Hu, Y., H. Fan, Y. Chen, J. Chang, X. Zhan, H. Wu, B. Zhang, M. Wang, W. Zhang, L. Yang, X. Hou, X. Shen, T. Pan, W. Wu, J. Li, H. Hu, F. Wei,. (2021). Spatial patterns and conservation of genetic and phylogenetic diversity of wildlife in China. *Science Advances* 7:1–10.
- Hubble, S. P. (2001). The unified neutral theory of biodiversity and biogeography. Princeton University Press.
- Jacques, F. M. B., G. Shi, and W. Wang (2011). Reconstruction of Neogene zonal vegetation in South China using the Integrated Plant Record (IPR) analysis. *Palaeogeography, Palaeoclimatology, Palaeoecology* 307:272–284.
- Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American Naturalist* 101:233–249.
- Jukes, T. H., and C. R. Cantor (1969). Evolution of Protein Molecules. In *Mammalian Protein Metabolism*. Elsevier, pp. 21–132.
- Karger, D. N., O. Conrad, J. Bohner, T. Kawohl, H. Kreft, R. W. Soria-Auza, N. E. Zimmermann, H. P. Linder, and M. Kessler (2017). Climatologies at high resolution for the earth’s land surface areas. *Scientific Data* 4:170122.
- Karger, D. N., O. Conrad, J. Bohner, T. Kawohl, H. Kreft, R. W. Soria-Auza, N. E. Zimmermann, H. P. Linder, and M. Kessler (2018). Data from: Climatologies at high resolution for the earth’s land surface areas. Dryad Digital Repository. <http://dx.doi.org/doi:10.5061/dryad.kd1d4>
- Karney, C. F. F. (2011). Geodesics on an ellipsoid of revolution. <https://arxiv.org/abs/1102.1215>.
- Kirwan, G. M., N. Collar, C. Robson, and D. A. Christie (2021). Huet’s Fulvetta (*Alcippe hueti* ), version 2.0. In *Birds of the World* (B. K. Keeney, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. <https://doi.org/10.2173/bow.gycful5.02>
- Korner, C., W. Jetz, J. Paulsen, D. Payne, K. Rudmann-Maurer, and E. M. Spehn (2017). A global inventory of mountains for bio-geographical applications. *Alpine Botany* 127:1–15.
- Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution* 35:1547–1549.
- Kumar, S., G. Stecher, M. Suleski, and S. B. Hedges (2017). TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. *Molecular Biology and Evolution* 34:1812–1819.
- Lambeck, K., H. Rouby, A. Purcell, Y. Sun, and M. Sambridge (2014). Sea level and global ice volumes from the Last Glacial Maximum to the Holocene. *Proceedings of the National Academy of Sciences of the United States of America* 111:15296–15303.
- Laurance, S. G. W. (2004). Responses of understory rain forest birds to road edges in central Amazonia. *Ecological Applications* 14:1344–1357.
- Li, J., Y. Zhang, S. Dong, and S. T. Johnston (2014). Cretaceous tectonic evolution of South China: A preliminary synthesis. *Earth-Science Reviews* 134:98–136.
- Lopez-Pujol, J., F.-M. Zhang, H.-Q. Sun, T.-S. Ying, and S. Ge (2011a). Mountains of southern China as “Plant Museums” and “Plant Cradles”: Evolutionary and conservation insights. *Mountain Research and Development* 31:261–269.
- Lopez-Pujol, J., F. M. Zhang, H. Q. Sun, T. S. Ying, and S. Ge (2011b). Centres of plant endemism in China: Places for survival or for speciation? *Journal of Biogeography* 38:1267–1280.

- Lynch, M., B. Koskella, and S. Schaack (2006). Mutation Pressure and the Evolution of Organelle Genomic Architecture. *Science* 311:1727–1730.
- Male, T. D., S. G. Fancy, and C. J. Ralph (2020). Red-billed Leiothrix (*Leiothrix lutea*), version 1.0. In *Birds of the World* (S. M. Billerman, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. <https://doi.org/10.2173/bow.reblei.01>
- Manni, F., E. Guerard, and E. Heyer (2004). Geographic patterns of (genetic, morphologic, linguistic) variation: How barriers can be detected by using Monmonier’s algorithm. *Human Biology* 76:173–190.
- Mayr, G. (2013). The age of the crown group of passerine birds and its evolutionary significance - Molecular calibrations versus the fossil record. *Systematics and Biodiversity* 11:7–13.
- Mi, X., G. Feng, Y. Hu, J. Zhang, L. Chen, R. T. Corlett, A. C. Hughes, S. Pimm, B. Schmid, S. Shi, J.-C. Svenning, and K. Ma (2021). The global significance of biodiversity science in China: an overview. *National Science Review*. <https://doi.org/10.1093/nsr/nwab032>
- Moore, R. P., W. D. Robinson, I. J. Lovette, and T. R. Robinson (2008). Experimental evidence for extreme dispersal limitation in tropical forest birds. *Ecology Letters* 11:960–968.
- Nei, M., and J. C. Miller (1990). A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. *Genetics* 125:873–879.
- Nguyen, J. M. T., and S. Y. W. Ho (2016). Mitochondrial rate variation among lineages of passerine birds. *Journal of Avian Biology* 47:690–696.
- Nieto Feliner, G. (2014). Patterns and processes in plant phylogeography in the Mediterranean Basin. A review. *Perspectives in Plant Ecology, Evolution and Systematics* 16:265–278.
- Nosil, P., T. H. Vines, and D. J. Funk (2005). Perspective: Reproductive isolation caused by natural selection against immigration from divergent habitats. *Evolution* 59:705–719.
- Oksanen, A. J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn, P. R. Minchin, R. B. O. Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and E. Szoecs (2019). *Vegan*. *Encyclopedia of Food and Agricultural Ethics*:2395–2396.
- Oliveros, C. H., D. J. Field, D. T. Ksepka, F. Keith Barker, A. Aleixo, M. J. Andersen, P. Alstrom, B. W. Benz, E. L. Braun, M. J. Braun, G. A. Bravo, et al. (2019). Earth history and the passerine superradiation. *Proceedings of the National Academy of Sciences of the United States of America* 116:7916–7925.
- Packert, M., J. Martens, Y.-H. Sun, L. L. Severinghaus, A. A. Nazarenko, J. Ting, T. Topfer, and D. T. Tietze (2012). Horizontal and elevational phylogeographic patterns of Himalayan and Southeast Asian forest passerines (Aves: Passeriformes). *Journal of Biogeography* 39:556–573.
- Phillips, S. J., R. P. Anderson, and R. E. Schapire (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190:231–259.
- Polzin, T., and S. V. Daneshmand (2003). On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters* 31:12–20.
- Qu, Y., G. Song, B. Gao, Q. Quan, P. G. P. Ericson, and F. Lei (2015). The influence of geological events on the endemism of East Asian birds studied through comparative phylogeography. *Journal of Biogeography* 42:179–192.
- R Core Team (2020). *R: A language and environment for statistical computing*.
- Ramos, R., G. Song, J. Navarro, R. Zhang, C. T. Symes, M. G. Forero, and F. Lei (2016). Population genetic structure and long-distance dispersal of a recently expanding migratory bird. *Molecular Phylogenetics and Evolution* 99:194–203.

- Rahbek, C., M. K. Borregaard, A. Antonelli, R. K. Colwell, B. G. Holt, D. Nogues-Bravo, C. M. O. Rasmussen, K. Richardson, M. T. Rosing, R. J. Whittaker, and J. Fjeldsa (2019a). Building mountain biodiversity: Geological and evolutionary processes. *Science* 365:1114–1119.
- Rahbek, C., M. K. Borregaard, R. K. Colwell, B. Dalsgaard, B. G. Holt, N. Morueta-Holme, D. Nogues-Bravo, R. J. Whittaker, and J. Fjeldsa (2019b). Humboldt’s enigma: What causes global patterns of mountain biodiversity? *Science* 365:1108–1113.
- Rozas, J., A. Ferrer-Mata, J. C. Sanchez-DelBarrio, S. Guirao-Rico, P. Librado, S. E. Ramos-Onsins, and A. Sanchez-Gracia (2017). DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Molecular Biology and Evolution* 34:3299–3302.
- Sangster, G., P. Alstrom, E. Forsmark, and U. Olsson (2010). Multi-locus phylogenetic analysis of Old World chats and flycatchers reveals extensive paraphyly at family, subfamily and genus level (Aves: Muscicapidae). *Molecular Phylogenetics and Evolution* 57:380–392.
- Sheldon, FH, Lim, HC, Moyle, RG (2015) Return to the Malay Archipelago: the biogeography of Sundaic rainforest birds. *Journal of Ornithology* 156 (Supplement 1):S91–S113.
- Siddall, M., E. J. Rohling, A. Almogi-Labin, C. Hemleben, D. Meischner, I. Schmelzer, and D. A. Smeed (2003). Sea-level fluctuations during the last glacial cycle. *Nature* 423:853–858.
- Soininen, J., R. McDonald, and H. Hillebrand (2007). The distance decay of similarity in ecological communities. *Ecography* 30:3–12.
- Song, G., Y. Qu, Z. Yin, S. Li, N. Liu, and F. Lei (2009). Phylogeography of the *Alcippe morrisonia* (Aves: Timaliidae): Long population history beyond late Pleistocene glaciations. *BMC Evolutionary Biology* 9:1–11.
- Spehn, E., K. Rudmann-Maurer, C. Korner, and D. Maselli (2010). Mountain biodiversity and global change. *Global Mountain Biodiversity Assessment (GMBA) of DIVERSITAS*, Basel.
- Spellman, G. M., and J. Klicka (2006). Testing hypotheses of Pleistocene population history using coalescent simulations: Phylogeography of the pygmy nuthatch (*Sitta pygmaea*). *Proceedings of the Royal Society B: Biological Sciences* 273:3057–3063.
- Stratford, J. A., and W. D. Robinson (2005). Distribution of neotropical migratory bird species across an urbanizing landscape. *Urban Ecosystems* 8:59–77.
- Strimas-Mackey, M., W.M. Hochachka, V. Ruiz-Gutierrez, O.J. Robinson, E.T. Miller, T. Auer, S. Kelling, D. Fink, A. Johnston. 2020. Best Practices for Using eBird Data. Version 1.0. <https://cornelllabofornithology.github.io/ebird-best-practices/>. Cornell Lab of Ornithology, Ithaca, New York. <https://doi.org/10.5281/zenodo.3620739>
- Sullivan, B. L., C. L. Wood, M. J. Iliff, R. E. Bonney, D. Fink, and S. Kelling (2009). eBird: A citizen-based bird observation network in the biological sciences. *Biological Conservation* 142:2282–2292.
- Tang, Z., Z. Wang, C. Zheng, and J. Fang (2006). Biodiversity in China’s mountains. *Frontiers in Ecology and the Environment* 4:347–352.
- Tajima, F. (1989). The effect of change in population size on DNA polymorphism. *Genetics* 123:597 LP – 601.
- Tian, S., Y. Kou, Z. Zhang, L. Yuan, D. Li, J. Lopez-Pujol, D. Fan, and Z. Zhang (2018). Phylogeography of *Eomecon chionantha* in subtropical China: The dual roles of the Nanling Mountains as a glacial refugium and a dispersal corridor. *BMC Evolutionary Biology* 18:1–12.
- Tian, S., J. Lopez-Pujol, H. W. Wang, S. Ge, and Z. Y. Zhang (2010). Molecular evidence for glacial expansion and interglacial retreat during Quaternary climatic changes in a montane temperate pine (*Pinus*

kwangtungensis Chun ex Tsiang) in southern China. *Plant Systematics and Evolution* 284:219–229.

Tobias, J. A., J. Ottenburghs, and A. L. Pigot (2020). Avian Diversity: Speciation, Macroevolution, and Ecological Function. *Annual Review of Ecology, Evolution, and Systematics* 51:533–560.

Tobias, J. A., C. Sheard, A. L. Pigot, A. J. M. Devenish, J. Yang, F. Sayol, N. Alioravainen, T. L. Weeks, R. A. Barber, P. A. Walkden, H. E. A. Macgregor, et al. (2022). AVONET : morphological , ecological and geographical data for all birds. 581–597.

Turcotte, Y., and A. Desrochers (2003). Landscape-dependent response to predation risk by forest birds. *Oikos* 100:614–618.

Wang, J., C. Feng, T. Jiao, E. B. Von Wettberg, and M. Kang (2017). Genomic signature of adaptive divergence despite strong nonadaptive forces on edaphic islands: A case study of *Primulina juliae* . *Genome Biology and Evolution* 9:3495–3508.

Wang, J., P. Gao, M. Kang, A. J. Lowe, and H. Huang (2009). Refugia within refugia: the case study of a canopy tree ( *Eurycorymbus cavaleriei* ) in subtropical China. *Journal of Biogeography* 36:2156–2164.

White, A. E. (2016). Geographical Barriers and Dispersal Propensity Interact to Limit Range Expansions of Himalayan Birds. *The American Naturalist* 188:99–112.

Williamson, J. L., and C. C. Witt (2021). Elevational niche-shift migration: Why the degree of elevational change matters for the ecology, evolution, and physiology of migratory birds. *Ornithology* 138.

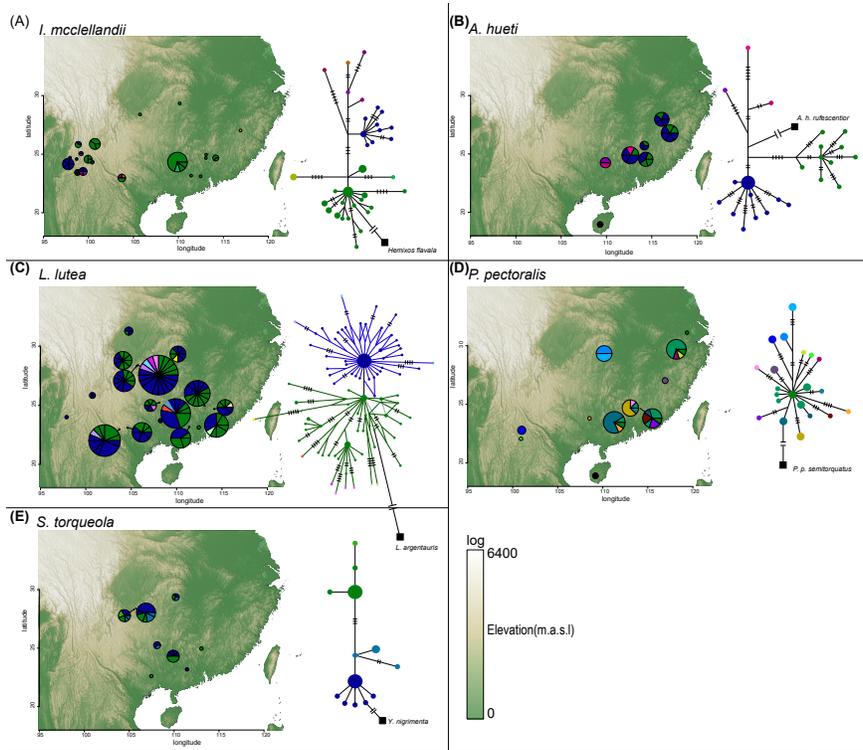
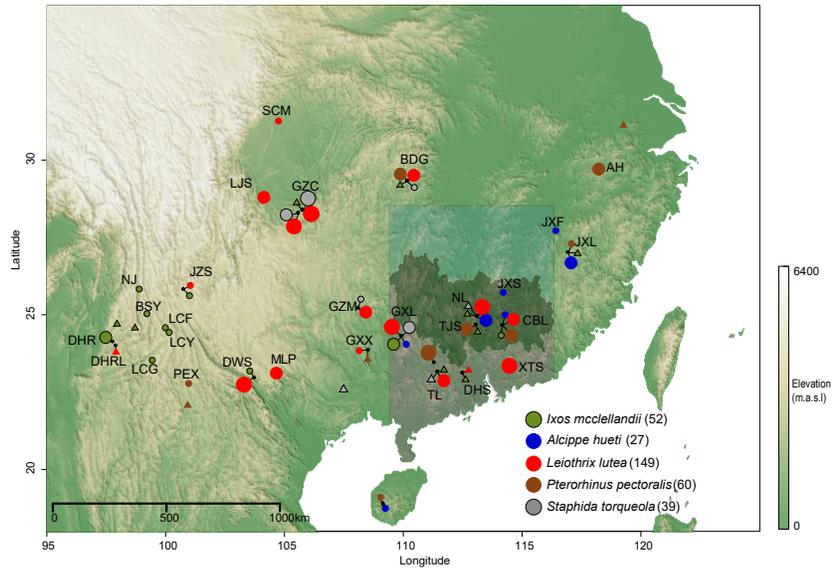
Wright, S. (1943). Isolation by distance. *Genetics* 28:114–138.

Wu, Y., J. Huang, M. Zhang, S. Luo, Y. Zhang, F. Lei, F. H. Sheldon, and F. Zou (2012). Genetic divergence and population demography of the Hainan endemic Black-throated Laughingthrush (Aves: Timaliidae, *Garrulax chinensis monachus*) and adjacent mainland subspecies. *Molecular Phylogenetics and Evolution* 65:482–489.

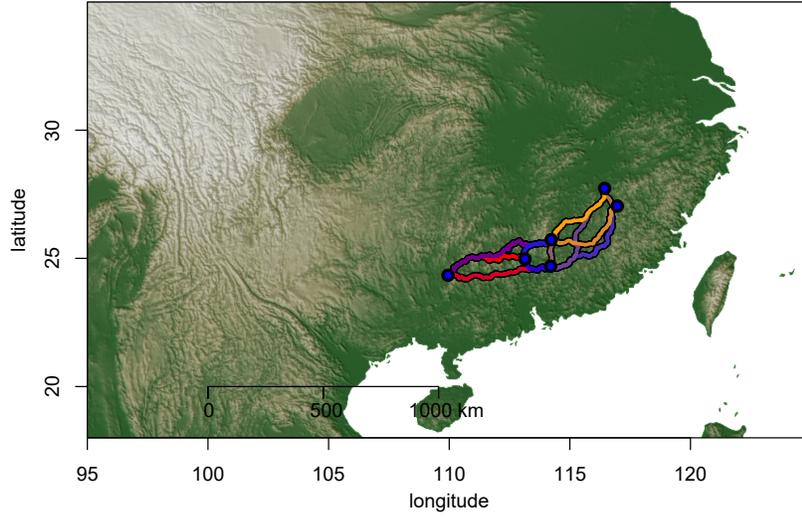
Xu, H., Y. Wu, Y. Cao, M. Cao, W. Tong, Z. Le, X. Lu, J. Li, F. Ma, L. Liu, F. Hu, et al. (2018). Low overlaps between hotspots and complementary sets of vertebrate and plant species in China. *Biodiversity and Conservation* 27:2713–2727.

Zhao, Z. (2001). *The handbook of the birds of China*. 2nd edition. Jilin Science and Technology Press, Jilin.

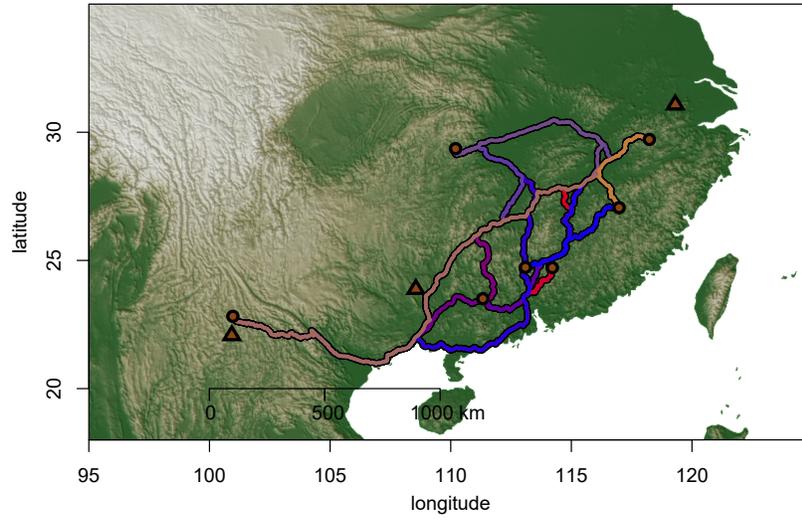
Zou, F., H. Chuan Lim, B. D. Marks, R. G. Moyle, and F. H. Sheldon (2007). Molecular phylogenetic analysis of the Grey-cheeked Fulvetta (*Alcippe morrisonia*) of China and Indochina: A case of remarkable genetic divergence in a “species”. *Molecular Phylogenetics and Evolution* 44:165–174.



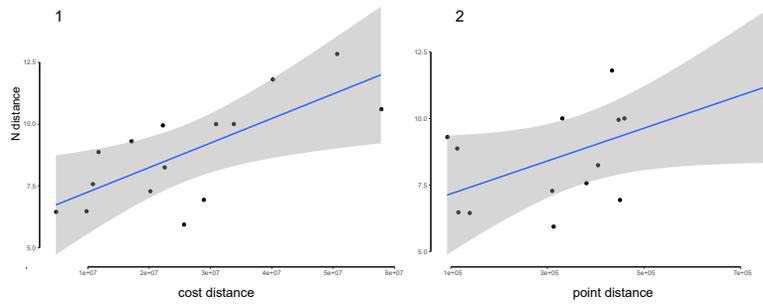
(A) *A. hueti*



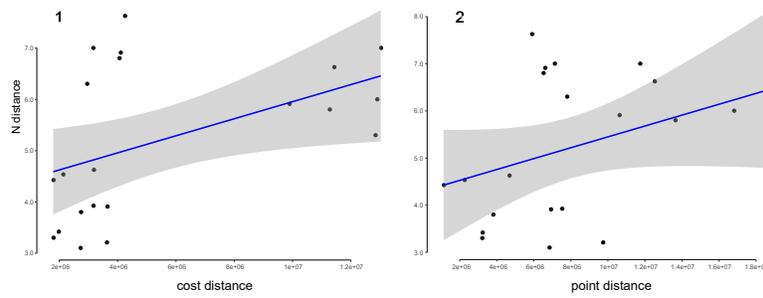
(B) *P. pectoralis*

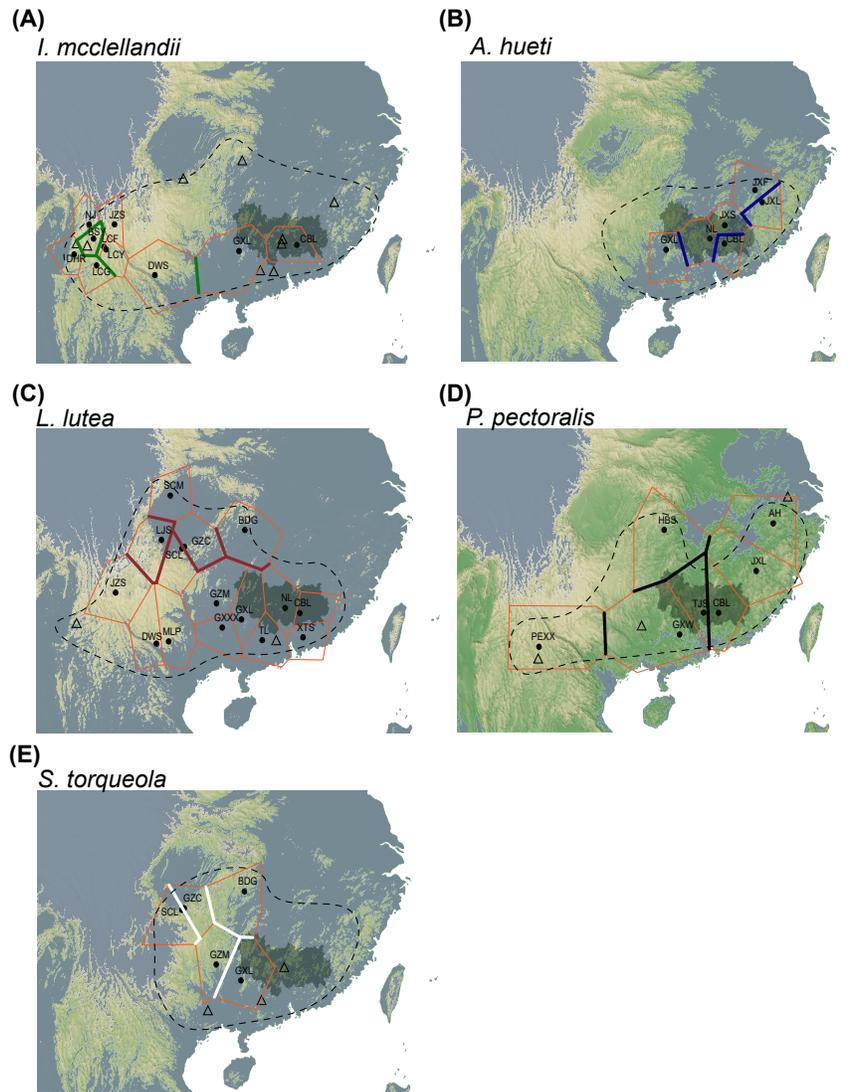


(A) *A. hueti*



(B) *P. pectoralis*





### Hosted file

hill landscape-230203-FigureLegends.docx available at <https://authorea.com/users/583446/articles/623070-the-nanling-mountains-of-southcentral-china-played-a-variable-role-as-a-barrier-and-refuge-for-birds-depending-upon-landscape-structure-and-timing-of-events>

### Hosted file

hill landscape-230203-Tables.docx available at <https://authorea.com/users/583446/articles/623070-the-nanling-mountains-of-southcentral-china-played-a-variable-role-as-a-barrier-and-refuge-for-birds-depending-upon-landscape-structure-and-timing-of-events>