


Pleistocene glaciation explains the disjunct distribution of the Chestnut-vented Nuthatch (Aves, Sittidae)

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Abstract

Pleistocene climatic oscillations have played an important role in shaping many species' current distributions. In recent years, there has been increasing interest in studying the effects of glacial periods on East Asian birds. Integrated approaches allow us to study past distribution range changes due to Pleistocene glaciation, and how these changes have affected current population genetic structure, especially for species with unusual distribution patterns. The Wuyi disjunction is the disjunct distribution of birds between the Wuyi Mountains in south-eastern China and south-western China. Although several species exhibit the Wuyi disjunction, the process behind this unusual distribution pattern has remained relatively unstudied. Therefore, we used the Chestnut-vented Nuthatch *Sitta nagaensis* as a model species to investigate the possible causes of the Wuyi disjunction. Based on phylogenetic analyses with three mitochondrial and six nuclear regions, the Wuyi population of the Chestnut-vented Nuthatch was closely related to populations in mid-Sichuan, from which it diverged approximately 0.1 million years ago, despite the long geographical distance between them (over 1,300 km). In contrast, geographically close populations in mid- and southern Sichuan were genetically divergent from each other (more than half a million years). Ecological niche modelling suggested that the Chestnut-vented Nuthatch has experienced dramatic range expansions from Last Interglacial period to Last Glacial Maximum, with some range retraction following the Last Glacial period. We propose that the Wuyi disjunction of the Chestnut-vented Nuthatch was most likely due to recent range expansion from south-western China during the glacial period, followed by postglacial range retraction.

1 | INTRODUCTION

Species distributions have received much attention, although the factors that determine a species distribution and that might cause it to shift are not yet fully elucidated (Brown et al., 1996; Lester et al., 2007). Disjunct distributions, where one species occurs in two or more widely separated regions, can provide insights into the determinants of species' distribution. Previous work on disjunct distributions have been performed on various taxa including birds (Fok et al., 2002;

Li et al., 2010; Wang et al., 2013a), mammals (Lv et al., 2018; Walker et al., 2008), amphibians (Thesing et al., 2016), insects (Schmitt et al., 2006), gastropods (Schultheiß et al., 2014) and plants (Beatty & Provan, 2013; Honnay et al., 2002) and have demonstrated that disjunctions arise due to different factors including human activities, habitat fragmentation due to natural vicariant events, recent climate change, glaciation and tectonic events.

A common explanation for disjunct distributions within a species is Pleistocene climate fluctuations. There are two

primary scenarios that are often invoked to explain glacial-induced disjunction. First, as the glacial ice expanded and climate cooled down in the Pleistocene, some species survived the severe temperatures by retreating to refugia (Hewitt, 1999, 2000). Following glacial retreat, if a species failed to disperse back to its former range, there might be disjunct distribution between refugia (Hewitt, 1999), as has been suggested to explain the disjunction of the Azure-winged Magpie *Cyanopica cyanus* between East Asia and Iberia in Spain (Fok et al., 2002; Zhang et al., 2012). In this case, we would expect the species to have a broader distribution and population size growth in warm periods, and the recent common ancestor of the isolated population and its sister population to date back to an interglacial period. In contrast to lowland species such as the magpie, montane species could survive the climatic oscillations during glacial periods by descending and expanding to lowlands (Hewitt, 2004). As the climate warmed during interglacial periods, they may have retreated to different higher regions as geological barriers reappear (e.g., bodies of water) or unsuitable habitat intervenes, thus causing disjunct distribution. For example, the isolated Taiwan Island population of the Green-backed Tit *Parus monticolus* likely resulted from marine transgression in warm period, after its glacial range expansion from the continent to the island (Wang et al., 2013a). For species like this, we would expect to see broader distribution and population size growth in glacial periods, and the recent common ancestor of the isolated population and its sister population to date to a glacial period.

The Wuyi Mountains contain the highest peaks in south-eastern mainland China (2,160 m above sea level, a.s.l.), with humid subtropical climates and mixed broad-leaf evergreen and deciduous forests (Cheng & Lin, 2011). The “Wuyi phenomenon” was first described by He et al., (2006), to refer to the disjunct distribution pattern of several bird species (Blyth’s Kingfisher *Alcedo hercules*, Chestnut-vented Nuthatch *Sitta nagaensis*, Yellow-browed Tit *Sylviparus modestus*, and Green Shrike-babbler *Pteruthius xanthochlorus*) between the Wuyi Mountains in south-eastern China and their main ranges in south-western China and adjacent areas (He et al., 2006). Based on distribution maps from BirdLife International, Handbook of the Birds of the World (2017), observations from BirdTalker Bird Records Center (www.szbird.org.cn/birdtalker.net), eBird (www.ebird.org) and Cheng and Lin (2011), we suggest this disjunction is also shared by montane birds like Spotted Elachura *Elachura formosa*, White-spectacled Warbler *Seicercus affinis*, Yellow-bellied Bush-warbler *Horonias acanthizoides* and Pale-headed Woodpecker *Gecinulus grantia* (Supporting Information Figure S1). Unlike the intensively studied phylogeographic divides in south-western China (Lei et al., 2014; Päckert et al., 2012; Qu et al., 2011, 2015; Song et al., 2009; Wang, McKay, et al., 2013a), or the distribution gap between Europe and

East Asia (Haring et al., 2007; Tritsch et al., 2017; Zhang et al., 2012), the Wuyi disjunction remains largely unstudied.

The Chestnut-vented Nuthatch is an arboreal-dwelling generalist, occurring from 1,100 to 4,570 m throughout its range (Harrap, 2008), and 1,100–1,900 m in Wuyi Mountains (Cheng & Lin, 2011). It is resident in the montane mixed forests of south-western China, south-eastern China and Indochina Peninsula, presumably only with altitudinal migration (Dickinson & Christidis, 2014; Harrap & Quinn, 1996; Harrap et al., 2008). A distance of over 1,300 km, primarily lowlands and some scattered lower mountain ranges, separates the isolated Wuyi population of the Chestnut-vented Nuthatch and its nearest occurrence in south-western China (Figure 1), making it an ideal model species to understand factors that may have led to the Wuyi disjunction.

In this study, we addressed the following questions: (a) What is the overall genetic structure among Chestnut-vented Nuthatch populations across Asia? (b) If there is structure, is the timing of divergences among populations consistent with Pleistocene glaciation events? and (c) If the Wuyi population could be due to glaciation-induced disjunction, is it due to glacial retraction or glacial expansion? To answer these questions, we reconstructed the phylogeographic structure and estimated the divergence time between the Wuyi population and other populations of the Chestnut-vented Nuthatch, investigated the potentially suitable habitats in different times using ecological niche modelling, and examined genetic structure and demographic history of the Chestnut-vented Nuthatch. Our results suggested recent glacial range expansion, and postglacial range retraction could explain the present disjunction of the Chestnut-vented Nuthatch, and that in some cases, geographically distant populations were genetically very similar, while geographically close populations were genetically distinct.

2 | MATERIALS AND METHODS

2.1 | Sampling and sequencing

Thirty-one individuals of Chestnut-vented Nuthatch were used in our study: 14 fresh samples from the Bird Research Group in Institute of Zoology (IOZ), Chinese Academy of Sciences (CAS); 16 toepads from the National Zoological Museum of China, IOZ, CAS and the American Museum of Natural History (AMNH); and one sample retrieved from GenBank. These individuals were collected from 26 different localities (Supporting Information Table S1, Figure 1), basically covering the entire distribution range of the species, including the isolated populations in west Myanmar and southern Vietnam. The Eurasian Nuthatch is sister to the group comprising the Kashmir Nuthatch *Sitta cashmirensis* and our focal species (Pasquet et al., 2014). As we were unable to obtain a Kashmir Nuthatch sample, we used two

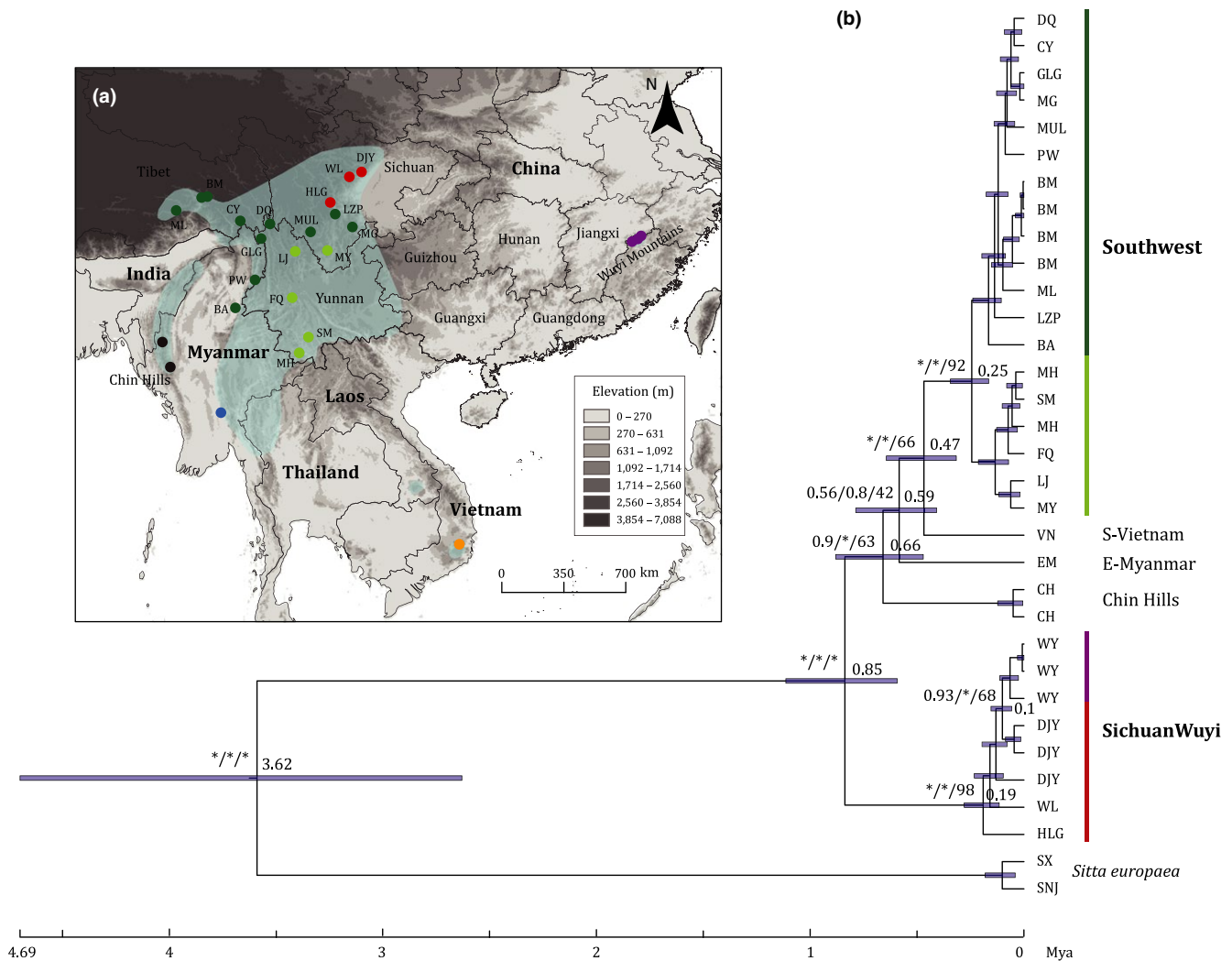


FIGURE 1 (a) Sample localities (circles) and current distribution (light blue shapefile) of the Chestnut-vented Nuthatch *Sitta nagaensis*. (b) Phylogeny based on three mitochondrial and six nuclear regions estimated using BEAST. Values at nodes are posterior probabilities or bootstrap values from BEAST/MrBayes/RAXML. *Posterior probability 1.00 or 100% bootstrap support [Colour figure can be viewed at wileyonlinelibrary.com]

individuals of the Eurasian Nuthatch *Sitta europaea sinensis* (samples from IOZ, CAS) as outgroups.

DNA of the fresh tissue samples was extracted using Aidlab Biotech DN07-Blood and Tissue DNA Fast Kit, following the manufacturer's protocols. For the toepad samples, DNA was extracted with Qiagen DNeasy Blood & Tissue Kit, following the manufacturer's protocols, except that 20 μ l of 1% Dithiothreitol (DTT) was added into each extraction to reduce the formation of DNA dimers.

Three mitochondrial regions (cytochrome c oxidase subunit 1 [CO1], control region [CR], cytochrome b [Cytb]) and six nuclear introns (aconitase 1 [ACO1], aryl hydrocarbon receptor nuclear translocator-like [ARNTL], beta-fibrinogen intron 5 [Fib5], Myoglobin [Myo], muscle-specific tyrosine kinase [MUSK], transforming growth factor beta-2 [TGFB2]) were used in this study. We sequenced all nine regions (6,657 bp in total) for all fresh tissue samples including the outgroups using primer pairs BirdF1/

BirdR1 (CO1), L14990/H16065 (Cytb), L437/H1248 (CR), ACO1-19F/ACO1-19R, ARNTL12F/ARNTL13R, Fib5/Fib6, Fib.5F2/Fib.6R2, MUSK-13F2/MUSK-13R2, MUSK-13F/MUSK-13R, TGFB2.5F/TGFB2.6R, and Myo3F/Myo2 (Hebert et al., 2004; Helm-Bychowski & Cracraft, 1993; Kimball et al., 2009; Kornegay et al., 1993; Tarr, 1995), except that we designed an additional primer pair, MyoF/MyoR, for two individuals from Wuyi Mountains and Bomi, Tibet (Supporting Information Table S2) using Oligo 7 (Rychlik, 2007). Only the three mitochondrial genes (2,430 bp) were amplified and sequenced for the toepad samples. This was accomplished using small overlapping segments (170–250 bp) due to DNA degradation, requiring us to design 5–7 pairs of primers for each mitochondrial gene (Supporting Information Table S2). Only two mitochondrial genes (CO1, Cytb) are available in GenBank for the individual from Gaoligong Mountains, Yunnan.

TransStart KD Plus DNA Polymerase Kit was used for DNA amplification. Two to four microlitres of DNA eluate was added for amplifying toepad samples, and all the other procedures were followed manufacturer's protocols. For annealing temperatures, see Supporting Information Table S2.

Sequences were assembled in DNASTar Lasergene SeqMan (Burland, 2000). Segments for toepad samples were assembled into single sequences in MEGA 6 (Tamura et al., 2013), using fresh tissue sequences as references. Sequences were aligned using Clustal W (Larkin et al., 2007) as implemented in MEGA 6, with some manual adjustments. SequenceMatrix (Vaidya et al., 2011) was used to concatenate multiple genes.

2.2 | Genetic structure

We used DnaSP 5 (Librado & Rozas, 2009), which phases nuclear regions, to generate haplotypes for concatenated mitochondrial and each nuclear region. Median Joining in Network 4.6 (Bandelt et al., 1999) was used to draw haplotype networks. The McDonald–Kreitman test (McDonald & Kreitman, 1991) in DnaSP was used to test for neutral evolution for three mitochondrial coding regions. PhiTest in Splitstree 4 (Huson & Bryant, 2005) was used to verify if there was recombination in nuclear regions. In addition to haplotype networks, we also used BAPS 5.3 (Corander & Marttinen, 2006) to infer population structure for mitochondrial regions, and STRUCTURE 2.3.4 (Pritchard et al., 2000) for nuclear regions (just including individuals with nuclear data). For BAPS analysis, five replicates were run for each *K* value (2–7). Heterozygous positions in six nuclear regions were phased by the program PHASE 2.1 (Stephens & Donnelly, 2003; Stephens et al., 2001) before further analysis in STRUCTURE. Ten replicates were run for each *K* value (2–7) in STRUCTURE.

To examine whether the genetic structure resulted from the reduced gene flow due to isolation by distance (IBD), we performed a mantel test between the genetic and geographical distances of all 31 individuals. Pairwise genetic distances were calculated in MEGA under a maximum composite likelihood model with gamma distributed rate variation among sites, following the procedure used in Wang et al., 2013b. Pairwise geographical distances were calculated as the shortest distance over the earth's surface between two collecting localities under the GCS_WGS_1984 projection system in MATLAB, without consideration of elevations. The mantel test was then performed between these two matrices using the ade4 package (Dray & Dufour, 2007) in R, with 1,000 permutations. Heat maps of two matrices were made in Excel.

2.3 | Phylogenetic analyses

We used PartitionFinder 1.1.1 (Lanfear et al., 2012) to identify partitions and substitution models for MrBayes analyses

of two datasets: concatenated mitochondrial and concatenated all genes. Mitochondrial genes were separated into 1st, 2nd and 3rd codon positions, and the six nuclear regions were set as separate partitions for consideration by PartitionFinder. jModelTest 2.1.10 (Darriba et al., 2012) was used to estimate substitution models for all nine genes, concatenated mitochondrial and concatenated all genes. In both cases, we used the corrected Akaike Information Criteria (AICc).

MrBayes 3.2 (Ronquist & Huelsenbeck, 2003) on CIPRES Science Gateway (Miller et al., 2010) was used to reconstruct phylogenetic trees with three datasets: partitioned mitochondrial dataset, partitioned all genes concatenated dataset and partitioned six nuclear regions. Markov chains were run for 50 million generations and sampled every 1,000 generations, with first 25% of the generations discarded as burn-in. Tracer 1.6 (Rambaut et al., 2014) was used to inspect the convergence of chains, with a threshold of minimum effective sample sizes (ESSs >200).

We also used RAXML 8 as implemented in raxmlGUI 1.5 (Silvestro & Michalak, 2012; Stamatakis, 2014) to estimate the phylogenies for the concatenated mitochondrial, concatenated all genes and all nuclear regions unpartitioned datasets, using substitution models estimated by jModelTest, with the ML + Rapid Bootstrap method and 1,000 replicates.

We used BEAST 1.8.2 (Drummond et al., 2012) to estimate the divergence time of the Chestnut-vented Nuthatch. Two initial analyses were run with either a strict clock or an uncorrelated lognormal relaxed clock; the divergence rate of 2.07% (substitution rate of 0.01035 per site per million year) was set for *Cytb* (Weir & Schluter, 2008), and used to estimate rates for mitochondrial CO1-CR concatenated and six nuclear regions (eight partitions all together). MCMC chains were run for 50 million generations, and sampled every 1,000 generations. Log files were imported into Tracer 1.6 (Rambaut et al., 2014) for inspection. Likelihood ratio test was performed to examine the goodness of fit of the two clock models (Felsenstein, 1981), and using the strict clock for all regions was rejected. We followed the guidelines recommended in the BEAST manual and examined the *ucl.d.stdev* and *coefficient of variation* parameters for each gene region in Tracer, and failed to reject a strict clock for *Cytb*, CO1-CR concatenated region, ARNTL, MUSK and TGFB2 (details in Supporting Information Table S3). A final BEAST analysis was then run using different clock models for each gene region, and estimating rates for each gene region based on the rate of *Cytb*.

*BEAST in BEAST 1.8.2 (Drummond et al., 2012) was used to reconstruct a coalescent species tree with *Cytb*, CO1-CR concatenated and six nuclear regions (running two analyses, one using unphased and one phased data). We used five groups preset based on the Bayesian phylogeny: Yunnan, Tibet, mid-Sichuan, Wuyi and the outgroup. Only the individuals with available nuclear regions were used. We chose the Yule Process as the species tree prior.

Clock and substitution models were determined in the same process as in the BEAST analysis (Supporting Information Table S3). The substitution rate of 0.01035 per site per million year was set for *Cytb* and used to estimate rates for the other gene regions. MCMC chains were run for 50 million generations, and sampled every 1,000 generations, with first 25% of generations discarded as burn-in.

2.4 | Demographic dynamics

In order to reconstruct the demographic dynamics and estimate effective population sizes through time, we conducted extended Bayesian Skyline Plot (EBSP) analysis with mitochondrial regions (since not all individuals were sampled for nuclear regions) in BEAST 1.8 (Drummond et al., 2012) on two clades with more than three individuals (Southwest: 19, SichuanWuyi: 8), based on the clade information from the Bayesian phylogeny using all genes. Fu's F (Fu, 1997) and Tajima's D (Tajima, 1989) were also estimated in DnaSP from mitochondrial regions to infer population expansion for all samples and the two well-sampled clades.

2.5 | Inferring evolutionary scenarios

To test whether the current population structure of the Chestnut-vented Nuthatch was due to glacial expansion or retraction, we used approximate Bayesian computation in DIYABC 2.1.0 (Cornuet et al., 2014) to verify the goodness

of fit of six most plausible scenarios by comparing the summary statistics between observed and simulated data sets. As DIYABC requires a minimum of one genotyped individual per population for each locus to calculate the summary statistics, only mitochondrial regions were used in the simulations. An un-sampled ancestral population was defined for four clades (Wuyi, mid-Sichuan, Southwest and Indochina). In the glacial expansion scenarios (scenarios 1–3; Figure 2), the ancestral population was assumed to locate somewhere near or in south-western China, and eventually expanded to Wuyi Mountains. In the glacial retraction scenarios (scenarios 4–6; Figure 2), we assumed the ancestral population was widespread in south China and then dispersed to Wuyi Mountains and south-western China (or Indochina) separately. The commonly used summary statistics, number of haplotypes, Tajima's D (Tajima, 1989) and F_{st} (Hudson et al., 1992), were selected, and mutation model was set as HKY. Six million simulated data sets, one million for each scenario, were simulated to get computationally and statistically robust results, as suggested by Cornuet et al. (2014). Logistic regression in DIYABC was performed on the linear discriminant analysis components of the summary statistics to estimate the posterior probabilities for all scenarios (Estoup et al., 2012).

2.6 | Ecological niche modelling

To estimate potentially suitable habitats through time, we obtained 560 occurrences of the Chestnut-vented Nuthatch

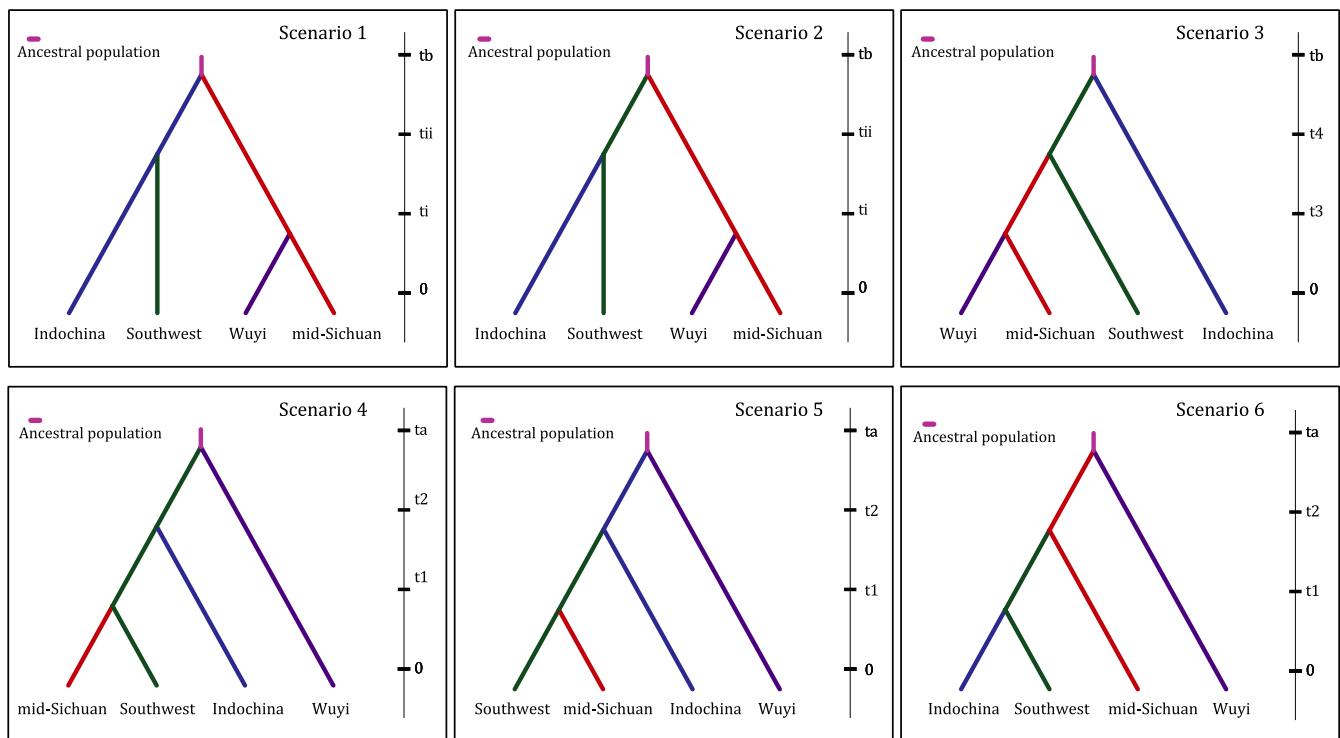


FIGURE 2 Historical scenarios constructed with mitochondrial data for analysis in DIYABC, based on the glacial expansion (scenarios 1–3) and glacial retraction (scenarios 4–6) hypotheses. In the graphic, the time axis is not to scale [Colour figure can be viewed at wileyonlinelibrary.com]

from eBird, GBIF (www.gbif.org), BirdTalker Bird Records Center and National Zoological Museum of China. We selected 159 spots from all the confirmed occurrences (Supporting Information Figure S2), with at least one of the longitude and latitude differences larger than 0.1° between every two spots, to reduce sampling bias and spatial auto-correlation.

We downloaded 19 bio-climatic variables (2.5' grid, Supporting Information Table S4) simulated with Community Climate System Model 4 (CCSM4) from WorldClim 1.4 (Hijmans et al., 2005) for four different time periods: Last Interglacial (LIG, 120–140 thousand years ago [Ka]), Last Glacial Maximum (LGM, 22 Ka), Middle Holocene (MIH, 6 Ka) and Current (1960–1990 AD). All climatic layers were trimmed to our focal research area ($E76^\circ$, $E123^\circ$, $N39^\circ$, $N8^\circ$) using ArcGIS. We first performed ecological niche modelling with all 19 variables. Fifty bootstrap replicates were run in Maxent (Phillips & Dudík, 2008), with 5,000 maximum iterations, and random assignment of the occurrences as training (80%) and testing (20%) data sets. To reduce the correlation between the bio-climatic variables, we used ENMtools (Warren et al., 2010) to calculate the pairwise correlation of 19 variables. If the correlation coefficient of two variables was higher than 0.8, we omitted the one with lower contribution based on the average contribution from the first ENM analysis. After this process, we retained bio2, bio3, bio7, bio10, bio12, bio14, bio15, bio18 and bio19 (Supporting Information Table S4) and used these to estimate the potential distribution ranges of the Chestnut-vented Nuthatch at each of the four different historical times. Program settings in Maxent were the same as in the first analysis. The area under the ROC curve (AUC) was used to measure the performance of the species modelling. A modelling with AUC above 0.75 is considered potentially useful (Phillips & Dudík, 2008). The final output in logistic format (ranging from 0 to 1) was the average of the 50 independent analyses. Only the regions with suitability greater than the 10 percentile training presence (logistic value >0.2918 in our case) were kept for further analysis. Areas and average elevations were calculated for the potentially suitable habitats of all four time periods using Spatial Analyst in ArcGIS.

3 | RESULTS

3.1 | Genetic structure

The mitochondrial haplotype network resulted in five groups (Figure 3a). The three Wuyi samples were placed within the SichuanWuyi group, while the rest of the samples from south-western China formed two subgroups that were highly divergent from each other. The three isolated populations from Indochina Peninsula formed their own groups. Population genetic structure inferred by BAPS resulted in three clusters (Figure 3b), with all

individuals from Indochina Peninsula in one cluster, individuals from Wuyi Mountains and those from middle Sichuan in one cluster, and rest of the individuals from south-western China in the final cluster ($K = 3$). STRUCTURE could not resolve different populations, likely due to the limited variation in the nuclear regions (Supporting Information Figure S3). Although there was less signal in the haplotypes of nuclear regions, the Wuyi and mid-Sichuan samples shared haplotypes in ACO1, ARNTL and MUSK, but also had their own private haplotypes in all nuclear haplotype networks except ARNTL and MUSK (Supporting Information Figure S4). The McDonald–Kreitman test found no significant deviation from neutrality for the three mitochondrial coding regions (Supporting Information Table S5). The PhiTest did not find statistically significant evidence for recombination for all nuclear regions.

The phylogenetic tree based on mitochondrial and nuclear data inferred five major clades, and strongly supported placing the three Wuyi individuals within the SichuanWuyi clade (Figure 1), in agreement with the haplotype network. A similar clustering of Wuyi and mid-Sichuan individuals was found in the species tree generated with mitochondrial and unphased nuclear data, but not in the species tree with mitochondrial and phased nuclear data, although using phased data had lower support (Supporting Information Figure S5). The remainder of the individuals from south-western China and adjacent north-eastern Myanmar formed a large clade (Southwest clade). Three isolated populations from southern Vietnam, eastern and western Myanmar formed their own clades. The mitochondrial tree estimated by MrBayes produced a well-supported and well-resolved phylogeny (Supporting Information Figure S6). It had the same topology as the BEAST tree (Figure 1), possibly reflecting that the mitochondrial genes contributed most of the variation in the phylogenetic analysis. The phylogeny estimated just from the combined nuclear regions was generally unresolved and poorly supported (Supporting Information Figure S7), consistent with the lack of resolution seen in STRUCTURE using the nuclear regions. We also observed pattern of incomplete lineage sorting, which is more obvious in nuclear regions than in the mitochondrial ones.

There was a positive correlation between the genetic and geographical distances of all 31 individuals ($r_m = 0.442$, $p = 0.001$) based on the 1,000 permutations with Mantel test. About 19.5% (i.e. 0.442^2) of the genetic divergence was explained by the geographical distance. However, comparing the SichuanWuyi clade with the Southwest clade in the matrices heat map (Supporting Information Figure S8), we found a negative association between genetic and geographical distances of Southwest clade and individuals from mid-Sichuan (short distances in geo matrix, long distances in genetic matrix), and positive association for Southwest clade and individuals from Wuyi Mountains (long distances in both matrices).

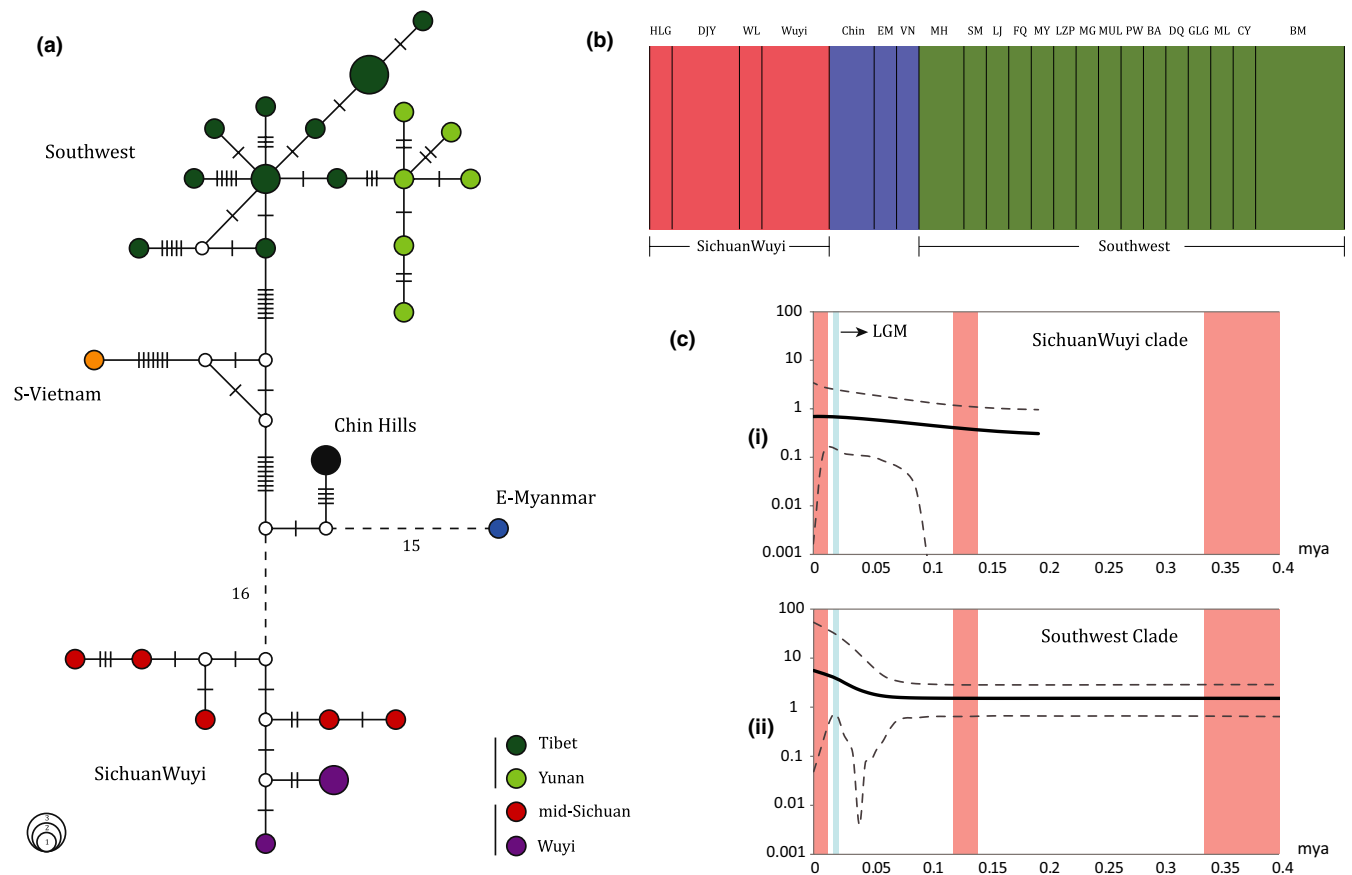


FIGURE 3 (a) Mitochondrial haplotype network constructed using DnaSP and Network. Each circle represents a haplotype, while its size indicates the haplotype frequencies. White circles represent un-sampled haplotypes. Each short dash represents one mutation position, and dashed line with a number labelled aside represents a certain number of mutation positions. Haplotypes of different clades or groups are indicated in different colours, in consistency to the colours of sample localities in Figure 1a. (b) Population structure constructed using BAPS with mitochondrial regions ($K = 3$). (c) Extended Bayesian Skyline Plots based on concatenated mitochondrial genes of the Southwest clade and SichuanWuyi clade of the Chestnut-vented Nuthatch *Sitta nagaensis*. Black solid lines represent the median values of the effective sample sizes. Grey dashed lines represent the 95% highest posterior densities of the effective sample sizes. Glacial periods are indicated in white, interglacial periods in red, and Last Glacial Maximum in blue [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Divergence times & demographic history

The Chestnut-vented Nuthatch was estimated to have diverged from the Eurasian Nuthatch (subspecies *sinensis*) around 3.62 mya using a mitochondrial molecular clock of 2.07% per million year (Figure 1). The SichuanWuyi clade diverged from the remainder of the clades 0.85 mya (95% HPD: [0.59, 1.11]), while the divergence between Wuyi and mid-Sichuan populations occurred only 0.1 mya (95% HPD: [0.06, 0.15]; Figure 1). Divergence times among Chestnut-vented Nuthatch populations were overall shallower in the *BEAST analyses although with much lower support (Supporting Information Figure S5).

Based on the extended BSP analysis, the Southwest clade of the Chestnut-vented Nuthatch underwent rapid population growth around 70 Ka during the Last Glacial period (c. 12–120 Ka; Figure 3c). For SichuanWuyi clade, it has grown

gradually since around 0.19 mya. Population expansions were also detected for Southwest and SichuanWuyi clades based on the results of two neutrality tests: the Southwest clade (Fu's F : -9.10^{**} , Tajima's D : -1.26) was estimated to have a much stronger population expansion than SichuanWuyi (Fu's F : -1.21 , Tajima's D : -0.33 ; Supporting Information Table S5).

3.3 | Evolutionary scenarios

The posterior probabilities calculated for all six scenarios using DIYABC showed that scenario 1, which reflected the topology of the Bayesian phylogeny, was most highly supported with our data (scenario 1 posterior probability: 0.6108, scenario 2: 0.3865, scenario 3: 0.0015, scenario 4: 0, scenario 5: 0, and scenario 6: 0.0012; Supporting Information Table S6). Posterior probabilities of the three scenarios based on glacial retraction were close to zero.

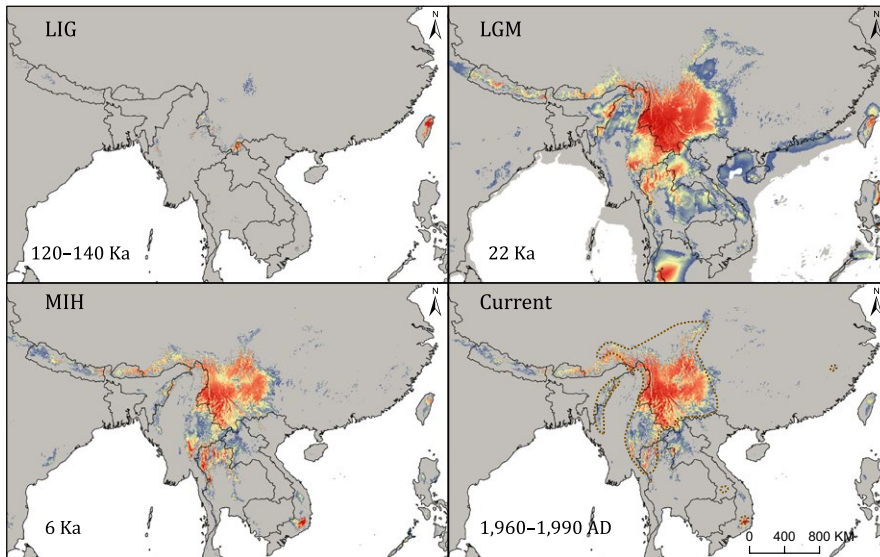


FIGURE 4 Potentially suitable habitats of the Chestnut-vented Nuthatch *Sitta nagaensis* in four different times (LIG, LGM, MIH and Current) estimated by ecological niche modelling in Maxent. Habitat suitability ranges from 0.2918 to 1 (blue to red). Dotted lines in Current (right bottom) indicate the current distribution of the species [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | Ecological niche modelling

Our results showed that the Chestnut-vented Nuthatch had a very limited distribution in south-western China during LIG (88,695 km²), but there were great expansions in suitable habitat from LIG to LGM (2,564,774 km²), although this retracted to a certain degree after LGM (Figure 4, Supporting Information Table S7). Current suitable range (1,133,387 km²) remained similar to that of six thousand years ago (MIH, 1,328,832 km²), and more similar to LGM than to LIG. The average elevation of suitable habitats was the lowest in LGM (1,128 m), and the highest in Current (1,889 m). The area under the ROC curve in the ENM analysis was 0.956, which indicates an overall good estimation of the potentially suitable habitats.

4 | DISCUSSION

4.1 | Divergence of the Chestnut-vented Nuthatch

Our phylogenetic analyses revealed lineage diversification in the populations of the Chestnut-vented Nuthatch, and that most divergences likely occurred during the Pleistocene. Results from phylogeny inference, haplotype reconstruction and population structure congruently showed that the three Wuyi samples were firmly nested within the SichuanWuyi clade, with a divergence time around 0.1 mya, which suggests that this disjunction occurred very recently during the Last Glacial period in late Pleistocene. The Chestnut-vented Nuthatch from mid-Sichuan were genetically very similar to the geographically distant Wuyi population, but deeply divergent from the southern Sichuan individuals that were geographically very close. This discordance between genetic and geographical distances indicated that mid-Sichuan and Wuyi

populations may share a recent common ancestor, but mid- and southern Sichuan populations have different origins. A similar discordance was observed in the Yellow-bellied Bush-warbler, with Sichuan populations sister to the south-eastern populations in Wuyi Mountains and Taiwan Island (0.2–0.6 mya), but more genetically and vocally diverged from nearby Himalayan populations (Alström et al., 2007).

On the other hand, our results also showed relatively deep divergence of the Chestnut-vented Nuthatch in south-western China over very short distances. For example, our samples from Hailuogou and Liziping (HLG and LZP in Figures 1 and 3b) in Sichuan are located on the east and south slopes of the Mount Gongga (7,514 m a.s.l. at highest peak) but only 80 km apart, and they belong to two very distinct clades (Southwest and SichuanWuyi clades) that diverged from each other more than half a million years ago. While such divergence might be expected for less motile species, such as the South China Field Mouse *Apodemus draco* (Fan et al., 2012) and the Chinese Mole Shrew *Anourosorex squamipes* (He et al., 2016), that it is also found in birds, which are less likely to be affected by biogeographic barriers such as steep mountains and wide rivers, suggests that Mount Gongga may have been serving as an important biogeographic barrier which limits the gene flow within multiple taxa. We see a second example of the deep genetic divergence across short distances in the divergence between the individuals from Tibet-Yunnan-Myanmar border and those from inland Yunnan (marked as dark green and light green within the Southwest clade, Figures 1 and 3a), which are distributed on the west and east sides of the Mekong-Salween Divide. This divide also appears to have been an important biogeographic barrier for population differentiation in several other passerines (Päckert et al., 2012), as well as in both less motile or immotile taxa like mammals and plants (Geissmann et

al., 2011; Li et al., 2011; Qiu et al., 2011; Ward, 1921). During the Pleistocene climatic oscillations, the mountains in south-western China not only provided in situ refugia for species to survive, but also highly complex geological structures that promoted diversification (Fan et al., 2012; Fan et al., 2011; He et al., 2016; Li et al., 2009; Qu et al., 2014, 2011, 2015).

The three sampled Indochinese populations diverged from the Southwest clade much earlier in the Pleistocene. The two isolated populations in Chin Hills and south-eastern Vietnam, which are currently included in the subspecies *grisiventris*, do not form a monophyletic group based on our phylogenetic results, although they cluster together in the population structure using mitochondrial regions. This suggests the current subspecies circumscription will need to be revisited with more samples.

In contrast to these observations, the Mantel test suggested a moderate, positive correlation when considering all Chestnut-vented Nuthatch individuals. It is widely accepted that genetic distances among populations can be strongly influenced by geographical distance, that is isolation by distance, as populations in adjacent areas can have more substantial gene flow than physically separated populations (Lee & Mitchell-Olds, 2011; Wang et al., 2013b). IBD can explain a part of the genetic variation pattern, as the disjunct Wuyi population is both genetically divergent and geographically distant from most of the rest populations, as are the three Indochinese populations. Additionally, most the individuals are highly genetically similar to their neighbours. However, an alternative explanation is needed for what may have led to the current genetic pattern regarding mid-Sichuan and the isolated Wuyi populations.

4.2 | Glacial range expansion and postglacial retraction

Given the timing of the divergence, evolutionary scenario simulations and the results of the niche modelling, it seems most likely that Wuyi disjunction of the Chestnut-vented Nuthatch was due to very recent dispersal events during glaciation rather than the isolation in refugia such as performed by the Azure-winged Magpie (Fok et al., 2002; Zhang et al., 2012). The most highly supported evolutionary scenario by DIYABC results suggested that the isolated Wuyi population split from the mid-Sichuan population rather than originate from a widespread ancestral population. Potentially suitable habitats estimated by ENM analysis suggested possible range expansions during the Last Glacial period, which would have been followed by range retractions after glacial retreat. Result from ENM was also corroborated by neutrality tests and EBSP analysis (although large confidence intervals

were observed with EBSP and using only mitochondrial regions in coalescent demographic analysis may have introduced bias), which collectively suggested that the Southwest clade underwent rapid population growth in Last Glacial period, although there was no obvious growth in the Sichuan Wuyi clade. Last Glacial period has shifted the potential distribution of the Chestnut-vented Nuthatch greatly: the total area of suitable habitats increased more than 12-fold from LIG to Current. After the Last Glacial period, potential distributions in the MIH and Current retracted to only a half of what was in LGM, but still remained much greater than LIG. As an exclusively arboreal montane species, the Chestnut-vented Nuthatch could have expanded to lower mountain areas when the mountain tops in the south-western China were covered by glacial ice. These range expansions to lowlands in LGM thus resulted in a much lower average elevation and a much broader area than in the interglacial periods (LIG, MIH and Current). It may also have dispersed further to south-eastern China, possibly via the lower mountain ranges around Sichuan Basin, scattered mountain ranges in southern China or along the emerged continental shelf (Figure 4, LGM). Following glacial retreat, there was no suitable habitat in mid-southern China for our focal species, populations in that area may have been locally extirpated or ascended to higher regions, such as Wuyi Mountains where they have remained.

In addition to the isolated Wuyi population, some of the Wuyi disjunction species also have disjunct populations in Indochina (Supporting Information Figure S1, Chin Hills in western Myanmar, Annamite Range in southern Laos and Da Lat Plateau in south-eastern Vietnam). Based on the ENM estimation for LGM, the suitable habitats of the Chestnut-vented Nuthatch in Indochina were somewhat continuous along the mountain ranges. It is likely that the populations in Chin Hills and south-eastern Vietnam became isolated in a similar process as the Wuyi population but in more ancient times.

5 | CONCLUSIONS

We used the Chestnut-vented Nuthatch as a model species to explore the possible causes of the Wuyi disjunction. Our results from phylogenetic and ecological niche modelling analyses suggested that the Wuyi population of the Chestnut-vented Nuthatch became isolated due to recent range expansion from south-western China during glacial period and postglacial range retraction as the habitats in between were no longer suitable. Collectively, our study shows that the current pattern of genetic variation in the Chestnut-vented Nuthatch has been affected by multiple different processes (e.g., glaciation,

isolation by geographical barriers and distances) acting at different time and spatial scales. As our results revealed interesting biogeographic patterns for our focal species, it would be worthy to investigate these in other Wuyi disjunction species. Our study also provides the first phylogenetic analysis of the Chestnut-vented Nuthatch, which is a further step towards fully understanding its evolutionary history.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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