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species, most geographical populations showed relatively high genetic diversity. Similar genetic diversity patterns (JTX, low; BYM, high) that were disclosed within these two species were also indicated by the habitat changes and population demography. Multiple clustering analyses were used to disclose differentiation among geographical population of these two amphibians species. The relatively shallow genetic differentiation within *Q. yei* was disclosed; while *P. shangchengensis* showed an opposite pattern. Under different historical climatic conditions, all ecological niche modelling disclosed a larger suitable habitats area for *Q. yei* than that for *P. shangchengensis*; these results indicated a wider environment tolerance or wider niche width of *Q. yei* than *P. shangchengensis*. Our approaches suggested that the synergistic effects of environmental niche variation and dispersal ability may help shape genetic structure across geographical topology, particularly for extreme narrowly distributed species.

Keywords: Comparative phylogeography; Genetic differentiation; Niche reconstruction; Range shifts; Sympatric distribution.

Running Head: Comparative phylogeography of stream species

Introduction

A key focus of biogeography is the exploration of how and why environmental gradients (e.g., elevation, latitude) affect global patterns biodiversity (Addo-Bediako *et al.* 2000, Gaston 2000). The evolutionary models, disclosed by phylogeographical studies, tended to best illustrate the population demography among related lineages in geographic landscapes (Avice 2000, Buckley 2009) based on multiple datasets (e.g., environmental, genetic and morphological information)(Hickerson *et al.* 2010). Due to the specificity of species traits, patterns disclosed by single species may be incomplete or misleading (Leaché *et al.* 2020, Wan *et al.* 2021). Simultaneous analysis of multiple species can clarify how the environment and heterogeneous landscapes might shape the phylogeographic pattern across their range or the population demographic patterns over time (Carstens, Richards 2007). How the

59 impact of ecological niche (promote or prevent) on the genetic differentiation and
60 distribution among populations or species are of particular interest under the
61 biodiversity conservation. Topographical or climatic barriers (e.g., a region's
62 climatic history, hydrology and geodynamics) are acted as the physiological barriers
63 for species dispersal, which direct correlation with species distribution patterns and
64 range (Ghalambor *et al.* 2006, Janzen 1967).

65 Global climate change is expected to have large negative impacts on the
66 biodiversity or species distribution (Barnosky *et al.* 2011, Dawson *et al.* 2011,
67 Parmesan 2006, Parmesan, Yohe 2003, Purvis *et al.* 2000, Thomas *et al.* 2004). In
68 the backdrop of global warming and increase in temperatures, some cold-adapted
69 species may shift their ranges to higher altitudes or higher latitudes due to the
70 sensitive to the high temperature for these mid-latitude species. However, for those
71 species with slow migration rates or the limited availability of new habitat, they will
72 experience a decrease or even extinction under the synergistic effects of narrow
73 range size and niche (Chen *et al.* 2011, Lenoir, Svenning 2015, Ohlemüller *et al.*
74 2008). The fragmentation and contraction of the species' geographical distribution
75 directly led to the biotic impoverishment, which increasing the threats to these
76 species (Devictor *et al.* 2008, Saupe *et al.* 2019, Thuiller *et al.* 2005). For the
77 mechanism of species distribution contraction, the associated climate conditions
78 changes may not meet species' climatic niche requirements, which may also led to
79 the local and/or global extinction (Jiguet *et al.* 2010, Thomas *et al.* 2004, Walther *et*
80 *al.* 2002, Wiens 2016). For those species, the climate change features (e.g., speed,
81 intensity) and biological variables (e.g., physiological tolerance, morpho-functional
82 traits, and life history) may also determine the responses of climate change (Araújo
83 *et al.* 2013).

84 Climate stability during geological period provided favorable conditions for the
85 surviving and evolving of more species (Graham *et al.* 2006, Jansson 2003,
86 Rosenzweig 1995), which provided the conditions for the formation of endemic and
87 small ranged size species (Jansson 2003). These species, endemic to climatically
88 stable regions, will form corresponding phylogenetic relationships along spatial and

environmental gradients, and will form branching changes related to phylogeny (Rosauer, Jetz 2015). In contrast, those species with strong dispersal capabilities may favored by dramatic climate changes, thus strongly affect biodiversity patterns and results in the persistence of generalist species with large range sizes (Sandel *et al.* 2011). Under the dramatic climate change, species will readjust their ranges and track suitable habitats due to the tendency of species to retain ancestral ecological features over time (i.e., niche conservatism)(Parmesan, Yohe 2003, Peterson *et al.* 1999, Thomas 2010), thereby leaving a marked imprint on future biogeographic patterns (Thuiller *et al.* 2011). For test niche conservatism hypotheses, the niche measurements (e.g., similarity, identity and overlap) of populations or species were identified and compare environmental niches (Broennimann *et al.* 2012). Under different climatic regimes, niche conservatism species often have relatively stable geographical distribution, because they cannot able to disperse into other adjacent to (or potentially reachable from) its original and current distribution. In contrast, species with strong dispersal ability are generally unlikely to have relatively conserved niche. Therefore, species dispersal ability may determine its range, although geographical or environmental barriers also limited dispersal (Wiens, Donoghue 2004). For widely distributed species, the heterogeneous environments may be their preferred type; but species with restricted distributions tend to prefer homogeneous environments (Slatyer *et al.* 2013).

The intrinsic factors for the coexistence of related species or similar niche species are the core of ecological and evolutionary research (Schoener 1974). For those coexistence species, the impact of abiotic/extrinsic factors (biogeographic barriers, geological events or past environmental change) will leave prints on the genetic variation, which play a key role in the formation of phylogeographic concordance (Avice 2000, Chase 2003). In classical ecological theory, two species with similar niches cannot be sympatric due to the interspecific competition caused by limited resource (Finstad *et al.* 2011, Schoener 1974, Violle *et al.* 2011). On the other hand, for some species with similar niche, differences in the use of habitats and their associated environmental variables (e.g. through character displacement) also

make sympatric distribution possible (Grant, Grant 2006). Additionally, habitat structure and resource availability may also play a key role for the sympatric distribution.

In nature, some closely related species have two completely different distribution ranges: highly restricted and widely distributed, which has attracted the attention of many researchers (Gaston 1996). Through this type of study, it is possible to distinguish the degree of similarity or difference among these climatic conditions in different regions of species (Quintero, Wiens 2013). For disclosing the intrinsic factors of geographical range sizes variation between species, several hypotheses have been proposed, such as niche breadth (Botts *et al.* 2013, Boulangeat *et al.* 2012, Slatyer *et al.* 2013, Vela Díaz *et al.* 2020), climatic variability (Boucher - Lalonde *et al.* 2016), climate tolerance (Pither 2003, Sheth, Angert 2014), evolution (Gaston *et al.* 1998), complex interactions (Brown *et al.* 1996), energy availability (Morin, Chuine 2006), glacial history (Jansson 2003) and the integration of habitat area and climate stability (Morueta - Holme *et al.* 2013). Considering the link between range sizes and global extinction risk (Botts *et al.* 2013, Purvis *et al.* 2000), the determinants of range sizes are more likely to influence their ability to alter ranges in response to global climate change (McCauley *et al.* 2014, Thomas *et al.* 2001). Generally, narrowly distributed species are more susceptible to climate change than widely distributed species (Ohlemüller *et al.* 2008, Slatyer *et al.* 2013, Thuiller *et al.* 2005), because they are usually untenable assumptions to the expected degree and velocity of climate change that they can adapt or adapt (Loarie *et al.* 2009, Thuiller *et al.* 2005). Additionally, if species with a narrower distribution are in fact more susceptible to climate change than those with a wider distribution, the outcome of global biodiversity will be unfavorable (Thomas *et al.* 2004), especially given that the narrowly geographical distribution of majority species (Gaston 1996). Compared with narrowly geographical distribution, species with wider geographical distribution is statistically more likely to cover a wider range of climatic conditions, indicating broader climatic tolerances and lower relative vulnerability for wider distributions species relative to narrowly distributions species (Birand *et al.* 2012).

Consequently, addressing species range determinants (e.g., the environmental factors) is crucial to understand which processes underlie species range at broad or narrow geographical distribution, how they affect threatened and even extinction factors and provide valuable perspectives for evolutionary and conservation biology in the context of expected climate change (Böhm *et al.* 2017, Brown *et al.* 1996, Faurby, Antonelli 2018, Newsome *et al.* 2020).

The Dabie Mountain is an ancient isolated mountain system with low and middle altitudes in East Asia, which had been listed as priority areas of biodiversity conservation in China (www.mee.gov.cn). The climate of this region is characterized by a transition from subtropical to temperate (Zheng *et al.* 2012). Ye's spiny-vented frog, *Quasipaa yei* (Anura, Dicroglossidae), is the unique anuran species endemic to Dabie mountain (Jiang *et al.* 2006), which normally restricted to montane rocky streams habitats at altitudes of approximately 500 m to 1000 m (Fei *et al.* 2012). As to the Shangcheng stout salamander (*Pachyhynobius shangchengensis*; Caudate, Hynobiidae), it is a cool and oxygen-rich streams salamander narrowly distributed above 500 meters in Dabie Mountains, slightly higher than that of *Q. yei* (Fei *et al.* 2012). Based on the survey data, only six isolated population of these two co-distributed species were founded, corresponding to Jiaoyuan-Tanghui-Xiaolongtan (JTX), Kangwangzhai-Huangbaishan-Jiufengjian (KHJ), Mazongling-Wochuan (MW), Tiantangzhai (TTZ), Baimajian-Yaoluoping-Mingtangshan (BYM) and Kujingyuan (KJY) (Fig. 1). In previous studies, sky island distribution and deep genetic divergence were disclosed among the *P. shangchengensis* populations (Pan *et al.* 2019, Pan *et al.* 2019). For spiny-vented frog, acted as another stream inhabited species, it shows relative slight larger distribution area than *P. shangchengensis*. Considering the distribution characteristics of the two species, together with existing phylogeographic pattern for *P. shangchengensis*, we predicted that these co-distributed species were the ideal model system to understand how climate change and species traits (niche breadth, dispersal ability) can impact phylogeographic structure in mid-latitude mountain systems, China.

Here, we used the two types of amphibians (frog and salamander) as a model to disclose the combined effects of climatic niche breadth (environmental factors) and dispersal ability on genetic structure and species geographical range sizes across different species in Dabie Mountains, East China. At the finer geographical scale, the divergence processes of different species can complement the relevant knowledge gaps. For example, do the consistency or inconsistency of the spatial structure of different species exists? Is there evidence of synchronous or asynchronous divergence of species? How about the intrinsic link between the species traits (niche breadth, dispersal ability) and the heterogeneity of spatial structure (habitat preference)? Do the combined of climatic niche breadth and dispersal ability influence the geographical range sizes difference? Therefore, based on these two ideal model species, we intend to disclosed the above scientific question within the mid-latitude mountain systems based on genetic and ecological data, in order to supplement the relevant knowledge system.

Methods

Sampling collection

For *Q. yei*, we collected 262 samples from 6 populations (JTX, KHJ, MW, TTZ, BYM and KJY) during 2012–2015 (Fig. 1). About 0.5 cm of toe tip of capturing adults was collected as sampling prior to release. In the field, absolute ethanol was used to preserve samples; while returning to the laboratory, immediately store in the refrigerator (−80°C). For each population, no fewer than 16 individuals were sampled. All the genetic data and geographic information of the Shangcheng salamander collected from the previous research (Pan *et al.* 2019).

DNA extraction, sequencing, and genotyping

Standard proteinase K/phenol–chloroform protocol was used to extract whole-genomic DNA (Sambrook *et al.* 1989), and then purified use an EasyPure PCR Purification Kit to purify the genome (TransGene Biotech, Beijing, China). Based on the mitogenome of *Q. boulengeri* (NC021937) and *Nanorana pleskei*

(NC016119), we designed specific primer sets of *ND2* gene (Table S1) by Primer v.5 (Clarke, Gorley 2001). The total volume of PCR amplification system was 30 μ L, which contained 1 μ L genomic DNA (concentration 5–25 ng/ μ L), 1.2 U *Taq* polymerase (TransGene Biotech), 2.4 μ L 1.2 mM dNTPs, 1.2 μ L 1.5 mM MgSO₄, 3 μ L 10 $\times$ buffer, 0.5 mM of each primer, and sufficient pure molecular biology grade water. The PCR amplification program includes an initial denaturation step at 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s, extension at 72°C for 80 s, and finally extension at 72°C for 10 seconds. PCR products, purifying by an EasyPure PCR Purification Kit (TransGene), were sequenced on an ABI Prism 3730 automated sequencer by the BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). For the nuclear genes, we selected eleven microsatellite loci (Table S1) from previous studies (Ding *et al.* 2013). The PCR amplification system and conditions were consistent with the above-mentioned *ND2* gene's system and conditions, except the DNA chain elongation time and temperature (30 s at 50°C–65°C; Table S1). The fluorescently (FAM, HEX or TAMRA) were added to the forward primers. For genotype the PCR products, ABI 3730 semiautomated sequencer (Applied Biosystems) with GS500 marker was applied. We analyzed data by GeneMarker v.1.85 (SoftGenetics LLC, State College, PA, USA) (Holland, Parson 2011).

Genetic diversity

SeqMan, embedded in DNASTar, was used to assemble *ND2* sequences (Burland 1999). DNA sequences were aligned and trimmed by Clustal X v.2.0 (Larkin *et al.* 2007). All the sequences were deposited into GenBank (XX–XX, provide after the article is received). DnaSP v.5.10 (Librado, Rozas 2009) were used to estimate population genetic indices, including nucleotide diversity (π) and haplotype diversity (*Hd*). For the microsatellite data, we checked the deviation of each locus and all loci for each population from Hardy-Weinberg equilibrium (HWE) by the exact probability tests in GenePop v.4.2.1 (Rousset 2008). The presence of null alleles and genotyping errors in microsatellite data was checked by

Micro-Checker (Van Oosterhout *et al.* 2004). Several population genetic diversity parameters were estimated using GenAlEx v.6.5 (Peakall, Smouse 2006) and GENETIX v.4.05 (Belkhir *et al.* 2001), including mean number of alleles (N_a), observed heterozygosities (H_o), expected heterozygosities (H_e), fixation index (F).

Phylogeography and population structure

Pairwise genetic differentiation (F_{ST}) among populations were calculated with 10,000 permutations using GenAlEx v.6.5 (Peakall, Smouse 2006) and Arlequin v.3.5.1.3 (Excoffier *et al.* 2005). For visualizing the mtDNA haplotypes relationships among localities, a minimum-spanning network was constructed by Network v.4.6.1 (Fluxus Technology, Suffolk, UK) based on the full median-joining algorithm (Bandelt *et al.* 1999). For probing the proportion of total genetic variation within and among populations or groups, analysis of molecular variance (AMOVA) was calculated by Arlequin.

The BEAST v.1.8 (Drummond *et al.*, 2012) was used to estimate the divergence time of *Q. yei*. The homologous sequences of 8 Dicroglossinae species were selected as outgroups. The best-fit model of evolution was selected by JMODELTEST.0.1 under the Bayesian information criterion (Darriba *et al.* 2012). Two calibration points were employed, of 22.9 Mya (with a 95% highest posterior density [HPD] between 15.60 and 30.20 Mya) from dated two genus (*Nanorana* and *Quasipaa*) and 18.4 Mya (with a 95% HPD between 12.09 and 24.71 Mya) from dated the genus *Quasipaa* (Che *et al.* 2010). The other parameters were set as follows: strict clock, 10 million MCMC generations, sampling every 1000th iteration, the initial 25% burn-in, the initial 25% burn-in, an uncorrelated lognormal model of lineage variation with an expansion growth tree prior. The convergence of the MCMC analyses was checked by the criteria (ESS values >200) by Tracer. TRACER v.1.6 (Rambaut *et al.* 2014) was used to do the model comparisons and check the convergence of model parameter values [effective sample size (ESS)] to ensure ESS values >200. The tree and posterior distribution were summarized with TREEANNOTATOR v.1.8 (Rambaut, Drummond 2010) and visualized with FIGTREE v.1.4.3 (Rambaut 2016).

For the microsatellite data, Bayesian analysis was used to infer the population structure, including the number of potential clusters and individuals to inferred clusters, by STRUCTURE (Pritchard *et al.* 2000). We set the inferred cluster number (K) from 1 to 8, using no prior information about individual location, and assuming admixture and correlated allele frequencies. Ten independent runs were conducted for each K value. The MCMC parameters were set as 1 million generations and the first 100,000 as burn-in. Based on the criterion that the highest log likelihood [Ln Pr(X/K)] of the posterior probability (Pritchard *et al.* 2000) and the ΔK statistic (Evanno *et al.* 2005), we determined the most likely K value to explain the variation in the data.

To further explore genetic structure of two species, two other Bayesian clustering algorithms were calculated in TESS v.2.3 (Chen *et al.* 2007) and the Geneland package (Guillot *et al.* 2005) in R 3.3.1 (R Development Core Team 2016). For the TESS analysis, we run 20 replicates of each K (K, 2 - 8) based on 50,000 sweeps with a burn-in period of 10,000. The deviance information criterion (DIC) of each K was calculated and plotted to probe the cluster number. For Geneland analyses, we set the parameters as K number (1-10), 100,000 MCMC repetitions, 100 thinning, 100 burn-in period and 20 different runs. The multilocus genotypes and spatial coordinates of each individual were also included. For further probing genetic structure of *Q. yei*, the factorial correspondence analysis (FCA) was also calculated by GENETIX (Belkhir *et al.* 2001).

The spatial autocorrelation analyses were calculated a mantel correlogram for mtDNA and microsatellite data to tests the genetic similarity of individuals within a distance class against the similarity of all other individuals in GenAlEx. Distance classes for our analysis were based on the nearest sampling distance (*Q. yei*, 10 km; *P. shangchengensis*, 5 km). Significance values across distance classes were adjusted using 999 random permutations. Significant positive r indicates that individuals within a particular distance class are genetically more similar than expected by random. In addition, to assess the significance of isolation by distance between populations, we conducted a Mantel test with 1000 random permutations on matrices of F_{ST} values and geographical distances in GenAlEx based on mtDNA and

microsatellite data.

Population demography

Mismatch distributions embedded in Arlequin were calculated to test the recent population growth. Rag (Harpending's raggedness index) and SSD (Sum of Squares Deviation) were used to test the goodness-of-fit: the smoothness of the observed mismatch distribution and the degree of fit between the observed and simulated data (Harpending 1994). The neutrality Tests, including *Tajima's D* (Tajima 1989) and *Fu's Fs* (Fu 1997), were also calculated the population demography by 10,000 coalescent simulations in Arlequin. For determining the past population dynamics of *Q. yei*, we conducted the Extended Bayesian Skyline Plot (EBSP) analysis of mtDNA data by BEAST. The above-mentioned divergence time was used to estimate the clock rate. The other parameters for the extended Bayesian skyline process were set as follows: 10 million MCMC generations, sampling every 1000th iterations, the initial 25% burn-in. The convergence of the MCMC analyses was checked by the criteria (ESS values >200) by Tracer.

The Msvar v.1.3 (Storz, Beaumont 2002), assuming that a current population (of size N_0) passed through a demographic change (a bottleneck or an expansion) to the history population size (N_1) at time T in the past, calculated the recent demographic history for each populations of *Q. yei* by microsatellite data. The various prior and hyperpriors used to do the coalescent simulations in Msvar were showed in Table S4. Four prior distributions models were conducted based on stable (i.e. N_0 and N_1 had the same prior upper bound), expansion (i.e. N_0 had a wider prior than N_1) and bottleneck models (i.e. N_1 had a wider prior than N_0). For the microsatellite mutation rate, the average vertebrate microsatellite mutation rate of 10^{-4} (range from 10^{-2} to 10^{-6} , Table S4) was set (Bulut *et al.* 2009). The other parameters were set as follows: generation time of *Q. yei* (2 years), 200 million MCMC generations, the initial 10% burn-in. The convergence of the MCMC analyses was checked by the criteria (ESS values >200) by Tracer. The convergence of the chains was checked based on the four runs of different priors for each population by Gelman and Rubin's diagnostic (Brooks, Gerlman 1998) using the

CODA library (Plummer *et al.* 2006) implemented in R.

Niche reconstruction

Ecological niche modelling (ENM) was used to simulate the distribution pattern of two species under different climate backgrounds based on climatic data and GPS coordinates of distribution localities by the maximum entropy algorithm in MAXENT v. 3.3.3k (Phillips *et al.* 2006). For ensuring that there is at most one species distribution point in each 1×1 km grid, all the selection criteria of distribution localities were the distance between all species distribution data greater than 1.42 km using ArcGis10.2 software. After screening, 22 and 34 occurrence records were selected for *Q. yei* and *P. shangchengensis*, respectively.

In this study, we collected 22 environmental variables, which can be roughly divided into two categories: bioclimate (Bio1-Bio19) and topography (Elevation, slope and aspect) at 30 arc-seconds resolution (~1 km²) (Data sources are shown in the Table S6). The entire Dabie Mountains's range (30 ~ 32°N, 114 ~ 117°E) were clipped from these climatic and topography coverages (Hijmans *et al.* 2005). To avoid model overfitting, all collected variables were processed by the band collection statistics function in ArcGIS10.2 and the environment variables with high correlation ($r > 0.75$) were removed (Table S6). After screening, 6 variables were selected, including Bio8 (Mean temperature of wettest quarter), Bio9 (Mean temperature of driest quarter), Bio13 (Precipitation of wettest month), Bio15 (Precipitation seasonality), Bio17 (Precipitation of driest quarter) and Elev (elevation). The correlation coefficient of selected environmental variables were shown in Table S7. Assuming niche conservatism of species (Wiens, Graham 2005), four climate data of different period (current environmental conditions; the mid-Holocene, 6,000 years ago; the last glacial maximum, LGM, about 22,000 years ago; the last inter-glacial LIG, 0.13 Mya ~ 0.12 Mya) (Otto-Bliesner *et al.* 2006), were used to obtain the predicted species distribution across time based on the species distribution model. The LIG, LGM and the mid-Holocene climate data (maximum resolution, 2.5 arc minutes) were downloaded from the WorldClim database. The paleodistribution was reconstructed by the community climate system

model (CCSM) (Collins *et al.* 2003).

The jackknife method was selected to evaluate the contribution of environmental variables. Then the distribution data of these two sympatric species was randomly divided into two groups: 25% was randomly selected as the test set, and the remaining 75% was used as the training set, then the analysis was performed by using MaxEnt.3.4.1 with Bootstrap replicates of 10 times. Analyses were run using default program parameter cumulative outputs, a 0.00001 convergence threshold, and a maximum of 5,000 iterations. Other parameters were selected with the default settings. Finally, for model evaluation, the predictive performance of the model was validated based on the area under the receiver-operating characteristic (ROC) curve (AUC) and the mean omission error (Phillips *et al.* 2006). The higher the AUC value, the more accurate the model performance. Normally, AUC value of > 0.9 indicates excellent prediction performance of the model. The ensemble threshold of model was calculated according to the Maximizing Sensitivity and Specificity (MSS) by using the dismo package in R. The MSS method is commonly used in the presence only kind of occurrence data (Hijmans *et al.* 2017, Liu *et al.* 2013). All models were post processed and visualized in ArcGIS 10.0 (Esri, Redlands, CA, USA). To further assess niche differences between the two species, we used the Statistics and Machine Learning Toolbox in Matlab 2021 (Mathworks Inc., USA) to fit the two-dimensional Gaussian mixture model based on the FAC1 and FAC2 collecting from dimension reduction analysis of climatic and ecological factors for describing the probabilities of niche parameters of two species.

Results

Genetic diversity

We sequenced 999 bp of the *ND2* gene from 180 individuals. Total *Hd* and π across all populations were 0.93 and 0.0073, respectively (Table 1). The MW population exhibited the highest haplotype diversity (*Hd*, 0.94) while the JTX population possessed the lowest value (0.64). The TTZ population exhibited the

highest nucleotide diversity (π , 0.0056) while JTX presented the lowest one (0.0009). For microsatellite data, 262 individuals were successfully genotyped at twelve loci (Table 1). No consistent departure from HWE or linkage disequilibrium was observed across the six populations. The nuclear genetic diversity varied between populations and summary statistic used with N_a ranging from 9.73 (MW) to 18.18 (BYM), H_o ranging from 0.75 (JTX) to 0.82 (MW), while H_e varied from 0.85 (MW) to 0.90 (BYM, KJY). Overall, among the six populations, BYM showed higher genetic diversity than the other five populations, meanwhile the JTX population own the relatively lower genetic diversity (Table 1). This pattern of genetic diversity was generally consistent with the results of *P. shangchengensis* (Pan *et al.* 2019).

Population structure

In this research, for comparing the genetic response of different types of amphibians under similar climatic and geographic background, we used multiple methods to calculate the genetic structure of these two species within Dabie Mountain. For *Q. yei*, the network analysis showed that two haplogroups exists, roughly corresponding to the North populations (JTX and KHJ) and Middle-South populations (MW, TTZ, BYM and KJY), which indicated the relative shallow genetic differentiation (Fig. 1). While for *P. shangchengensis*, there were five haplogroups with no shared haplotypes among different populations, especially for that between the North and South populations, which disclosed deep genetic differentiation among populations (Fig. 1).

In the TESS analysis, for *Q. yei*, the appropriate K value was K = 2, 3 or K = 4 due to the stabilize or slow vary of DIC values against Kmax (Fig. 1). When K = 2, 3, the JTX–KHJ–MW–TTZ and BYM–KJY populations formed into distinct genetic cluster, respectively; when K = 4, one population of KHJ forms an independent genetic cluster from JTX–KHJ–MW–TTZ (Fig. 1). While for *P. shangchengensis*, the relative distinct genetic structure within populations was disclosed (Pan *et al.* 2019). When K = 2, the JTX–KHJ and MW–TTZ–BYM–KJY populations formed into distinct genetic cluster, respectively. The results for northern populations (JTX and KHJ) showed the relative large genetic differentiation among populations (Fig. 1).

For southern populations, it also showed similar genetic pattern among populations (MW-TTZ, BYM-KJY). For the phylogenetic analyses, the chronogram of *Q. yei* didn't show distinct genetic cluster corresponding to the geographical population (Fig. S1), which was distinct from the phylogenetic structure of salamander (five distinct genetic cluster corresponding to geographical structure). As to the Structure results, significant genetic introgression among populations within *Q. yei* were disclosed (Fig. S2), while the relative distinct three genetic clusters corresponding to the North (JTX, KHJ), Middle (MW, TTZ) and South (BYM, KJY). In the results of Geneland spatial model, the geographical population of salamander disclosed six corresponded genetic groups. However, the genetic structure of frog displayed more complex results (Fig. S3 and S4). Additionally, the degree of genetic differentiation among genetic cluster also showed different pattern (*Q. yei*, low; *P. shangchengensis*, high). The FCA results of *Q. yei* (Fig. S5) show consistent results with that of Geneland.

For *Q. yei*, the F_{ST} values among populations ranged from -0.017 (MW and TTZ) to 0.813 (JTX and BYM) for mtDNA data and ranged from 0.009 (BYM and KJY) to 0.024 (MW and KJY) for microsatellite data, which indicated genetic differentiation among these *Q. yei* populations (Fig. 2). While for *P. shangchengensis*, the F_{ST} values among populations ranged from 0.494 (KJY and BYM) to 0.962 (MW and JTX) for mtDNA data and ranged from 0.087 (TTZ and MW) to 0.59 (KJY and JTX) for microsatellite data, which indicated the larger genetic differentiation among populations than that of *Q. yei*. The spatial autocorrelation analysis showed that *Q. yei* were significantly more related when they were separated by >10 km apart (Fig. S6a,b) and *P. shangchengensis* show more geographical genetic structure at closer distances (>5 km apart; more significant distance classes, ≥ 20 km based on Fig. S6c or ≥ 25 km based on Fig. S6d), which indicated that *Q. yei* showed the relative shallow isolation by distance (IBD) effect than *P. shangchengensis*. The AMOVA results disclosed different patterns within *Q. yei* depending on the marker type (mtDNA, [JTX,KHJ][MW,TTZ,BYM,KJY]; Microsatellite, [JTX,KHJ,MW,TT][BYM,KJY])

based on the maximum value of among groups (68.46; 0.87) within source of variance and Fixation index (0.723; 0.023) (Table S3), which indicated the relative shallow genetic differentiation comparing with that of *P. shangchengensis* (Pan et al. 2019). In addition, the Mantel test revealed a weak but significant positive correlation between genetic distance and geographical distance based on mtDNA and microsatellite data (Fig. S7).

Overall, the relative shallow genetic differentiation among the *Q. yei* populations, when compared that among the *P. shangchengensis* populations. In addition, the isolation by distance (IBD) phenomenon also disclosed among these two co-distributed species and the isolation of *Q. yei* was less than that of *P. shangchengensis*.

Historical demography

The neutrality test (Tajima's *D* and Fu's *F_s*), mismatch distribution analysis and BSPs were conducted on the mtDNA sequence data. Fu's *F_s* and Tajima's *D* were negative in all populations except the TTZ population, however, only BYM presented the significant *F_s* (Table 1). Additionally, the *F_s* of total population showed the significant negative value (Table 1), which indicated the statistically significant population expansion of *Q. yei*. For the two *Q. yei* mtDNA genetic clusters, we examined departures of the sequence mismatch distributions from those simulated under a population expansion model. We detected insignificant departures regardless of whether Rag or SSD measure was used (Fig. S8), which indicated fits to population expansions. For the Msva analysis, the convergence of the MCMC was checked by the Gelman and Rubin statistic (i.e., smaller than the upper threshold of 1.2). Compared with the results of *P. shangchengensis* (i.e., stable demography), the msvar's results of *Q. yei* showed distinct population expansions of most geographical population, except BYM population (Fig. 3 and S5, Table 2). In addition, the EBSP results revealed that *Q. yei* experienced an expansion of effective population size since 0.015 Mya, after the last glacial maximum (LGM, Fig. 4). This result was consistent with those from other mtDNA- or microsatellite-based demographic analyses, together pointing to expansions of the frog populations.

Ecological niche modeling

Based on ecological niche modeling, the predicted distribution of these two co-distributed species (*Q. yei* and *P. shangchengensis*) is generally consistent with their actual distribution (Fig. 5), differing significantly from random (*Q. yei*: AUC = 0.992; *P. shangchengensis*: AUC = 0.994; Fig. S9). Under different climatic conditions (LIG, LGM, Mid Holocene and current), habitats suitable for these two co-distributed species have been maintained and kept isolated from each other by unsuitable environment at low altitude in the Dabie Mountains, although the distribution area has changed (Fig. 5). Additionally, the predicted potential distribution area of *Q. yei* is relatively larger than that of the *P. shangchengensis* under different climatic conditions (Fig. 5 and Table 3).

The Jackknife analyses results showed that, for *Q. yei*, the variables with first three high contribution to the model were Elevation (81.4%), Bio13 (7.6%), and Bio 9 (5.5%), with a total contribution rate of 94.5%. For *P. shangchengensis*, the variables were Elevation (93.5%), Bio9 (3.2%) and Bio8 (1.3%), with a total contribution rate of 98% (Table S8). we compared 4 climatic (Bio13, Bio 9, Bio 17, Bio 15 and Bio 8) and 1 topography variables (Elevation) between *Q. yei* and *P. shangchengensis*, which indicated the relatively significant ecological factors differences these two species, except the Bio15 (Fig. 6). Overall, *Q. yei* have higher temperature adaptability and lower altitude distribution, while *P. shangchengensis* have relatively lower temperature adaptability and higher altitude distribution (Fig.6, 7).

Discussion

The inner causes of species range size difference

Range size is a fundamental unit of biogeography, reflecting differences in interspecies dispersal capacity, evolutionary history and ecological tolerance (Lester *et al.* 2007, Olalla - Tarraga *et al.* 2011, Thompson *et al.* 1999). The principal goal of biogeography and biodiversity conservation is to identify the mechanisms (e.g.,

species trait, dispersal ability, ecological tolerance) governing the distributions of species (Brown *et al.* 1996, Gaston 1996), which directly related to the global extinction risk (Böhm *et al.* 2017, Botts *et al.* 2013, Purvis *et al.* 2000) due to the dynamic changes between species distribution areas and climate change (McCauley *et al.* 2014, Thomas *et al.* 2001). To explain the differences in the geographic ranges size between species, various hypotheses have been proposed, such as organisms attribute (e.g., ecological, morphological and life-history) (Brown *et al.* 1996), climatic variability (Stevens 1989), niche breadth (Botts *et al.* 2013, Gaston *et al.* 1997, Slatyer *et al.* 2013), environmental tolerance (Pither 2003, Sheth, Angert 2014), adaptive capabilities (Brown 1984), population size (Gaston, Blackburn 1996), dispersal ability (Lester *et al.* 2007, McCauley *et al.* 2014, Thompson *et al.* 1999) and a combination of habitat area and climate stability (Morueta - Holme *et al.* 2013). Among these hypotheses, the niche breadth and environmental tolerance hypotheses, which has recently gained more support, suggests a positive correlation between niche breadth and geographical range size (Botts *et al.* 2013, Boulangeat *et al.* 2012, Pither 2003). Species with broad niches can persist in a variety of different habitat types, while species with narrow niches will be confined to places that meet the requirements of their particular niche (Brown 1984). Additionally, species with narrower environmental tolerance are expected to have narrower distributions than broad species, which means that larger geographic range sizes directly correlated to the larger environmental tolerances and habitat breadths (Slatyer *et al.* 2013).

In this study, *Q. yei* and *P. shangchengensis* were sympatric species. In an ecosystem, sympatric species evolve into co-existence through the division of habitat resources (Juarez, Marinho-Filho 2002, Soininen *et al.* 2015). According to field surveys, *Q. yei* have a relatively larger distribution area and lower altitude restrictions than *P. shangchengensis*. This results were also proved by the ENM results under different historical climate backgrounds (LIG, LGM, Mid Holocene, Current), which indicated that *Q. yei* have a relatively wider potential distribution area, lower altitude distribution and higher temperature tolerance than *P. shangchengensis* (Fig. 5, 6, 7 and Table 3). Additionally, the difference in the degree

of genetic differentiation, the degree of isolation by distance and population
 gemography of these two species also suggested this conclusion (Fig. 1, 2). The
 niche breadth-range size hypothesis states that the greater the breadth along one or
 more niche axes, the more a species can survive under a wider range of local
 conditions and thus spread over a larger geographic area (Botts *et al.* 2013, Slatyer *et*
al. 2013, Varzinczak *et al.* 2020). Therefore, we can inferred that *Q. yei* are expected
 to have broader niche breadth and environmental tolerances than *P. shangchengensis*,
 which maybe directly lead to the larger distributed of *Q. yei*. On the other hand,
 dispersal ability is another frequently cited potential key factor of the species
 distributed range size, involves various taxa within terrestrial and aquatic systems
 (Gaston 2003, Lester *et al.* 2007, McCauley *et al.* 2014, Rundle *et al.* 2007,
 Thompson *et al.* 1999, Waters *et al.* 2020). Species with high dispersal capability are
 expected to disperse more easily over long distances to colonize new areas and
 maintain viable populations (Böhning-Gaese *et al.* 2006, Gaston 2003, Lester *et al.*
 2007). Reduced dispersal may increase the sensitivity of lineages to
 landscape/environmental changes through reduced gene flow between populations,
 thereby driving the range size change and the phylogeographical structure formation
 (Gaston 2003), affecting the spatial scale of speciation (Kisel, Barraclough 2010).
 For *Q. yei*, it is more likely to inhabit in woodlands, grasses, rocks and other places
 along streams, while *P. shangchengensis* show strict aquatic attribute, reflecting that
 the frog has stronger dispersal ability than the salamander, which is also considered
 to be one of the main factors in shaping the range size of these two species.

The inner causes of phylogeographic pattern difference

For many ecological processes that influence population viability, population
 connectivity is critical (Curtis, Bevers 2002, Hayden 2006, Saastamoinen *et al.* 2018),
 which can be affected by dispersal behavior and the presence of natural barriers (e.g.,
 rivers and mountains), influencing the genetic variation and structure (Bowler, Benton
 2005, Clobert *et al.* 2001, Duellman, Trueb 1994, Olah *et al.* 2017), especially for
 amphibians (Lucati *et al.* 2020, Niwa *et al.* 2021, O Svinin *et al.* 2021, Pan *et al.* 2019,

Pan *et al.* 2019, Tominaga *et al.* 2021, Trevisan *et al.* 2020). For those species that inhabit streams, stream network structure and other natural landscape features may be effective barriers to disperse, providing opportunities for genetic differentiation (Niwa *et al.* 2021, Pan *et al.* 2019, Pan *et al.* 2019, Sunny *et al.* 2014). On the other hand, sky island is a mountainous region isolated from each other by valleys with different environmental conditions (McCormack *et al.* 2009). Species confined to montane sky island habitats, especially aquatic taxa, generally exhibit high levels of interpopulation genetic variation and unique genetic structure patterns (Atkins *et al.* 2020, de Oliveira *et al.* 2021, Shepard, Burbrink 2011), especially for species in mid - to high-latitude mountain ranges (Beniston 2016, Herman, Champagnac 2016). In such high-altitude habitats, populations are often separated by valley with unsuitable habitats due to the niche conservatism (He, Jiang 2014, McCormack *et al.* 2009, Pan *et al.* 2019, Pan *et al.* 2019, Shepard, Burbrink 2011). This segregation is usually exacerbated by the restriction of gene flow, habitat fragmentation, etc., caused by climate contrast between different elevation gradients or by elevation differentiation at lower altitudes brought about by orogeny (McCormack *et al.* 2009). Mountain systems tend to have more hidden clades or species, as the highly subdivided habitats and rugged terrain lead to the formation of sky island habitats (He, Jiang 2014, McCormack *et al.* 2009). Abiotic factors (e.g., climatic and tectonic events) and biological factors (e.g., interspecific or intraspecific interactions, competition and predation) may be the main driving forces of biological diversification and evolution temporally and geographically (Benton 2009). Generally, mountainous areas exhibit a variety of microhabitats with different ecological conditions from the surrounding landscape due to the interaction of multiple biological and abiotic factors, which urged the formation of unique and endemic species with relatively small populations and well-defined geographic boundaries (Huang *et al.* 2017, Shepard, Burbrink 2011).

Numerous scattered mountain ranges (e.g. Dabie mountains, Qinling Mountains, Hengduan Mountains) form potential sky islands in China's vast subtropical regions, showing spatial isolation within confined areas and considered ideal natural laboratories for studying endemic plant and animal species formation (Che *et al.* 2010,

Ye *et al.* 2016). During the Quaternary, violent climatic oscillations caused mid-latitudes species to experience the drastic population demography (contractions or expansions)(Hewitt 2004). During the LGM, RegCM2 simulation indicated that annual surface temperature decreases by 2 °C–4 °C over most land areas and the strongest cooling is up to 8 °C over coastal areas in East Asia (Ju *et al.* 2007), which maybe indicate that the annual temperature had impacted many species distributions (Cheng *et al.* 2021, Luo *et al.* 2021, Wei *et al.* 2022). The stream-dwelling frog (*Q. yei*) and salamander (*P. shangchengensis*), are typical stream inhabited species, endemic to the Dabie Mountains, and lives in or along the cool and oxygen-rich streams above 500 meters in elevation (Fei *et al.* 2012). Based on the ENMs prediction results, although the distribution area of these two co-distributed species (*Q. yei* and *P. shangchengensis*) had varied over different climatic conditions (LIG, LGM, Mid Holocene and current), suitable habitats for these two co-distributed species have been maintained and kept isolated from each other by unsuitable environment at low altitude in the Dabie Mountains (Fig. 5), even during the most drastic climate oscillations in Pleistocene (LGM) (Senczuk *et al.* 2017). Additionally, the existence of unsuitable habitats at low altitudes acts as a strict and effective isolation barrier for the two species due to the strict niche conservatism, although the different limitation degree of these two species (Fig. 5), which limited the dispersal among population and facilitates the allopatric divergence, directly correlated to the high levels of inter-population genetic divergence and unique patterns of genetic structure. This phylogeographic pattern caused by the sky island effect is also reflected in some other amphibians in mid-latitudes, such as three Plethodon species (*P. ouachitae*, *P. fourchensis* and *P. caddoensis*) within the Ouachita Mountains in western Arkansas (USA) (Shepard, Burbrink 2011), the stream salamander (*Batrachuperus tibetanus*) in the Qinling Mountains, East China (Huang *et al.* 2017).

The width of a species' climatic niche can be considered as a series of all the climatic conditions under where the species is distributed (Futuyma, Moreno 1988, Gaston 2003). Niche breadth has been used in various studies, for example, to build species' response models to global changes (e.g., species distribution models)

(Guisan, Thuiller 2005, Rolland, Salamin 2016, Slatyer *et al.* 2013, Varzinczak *et al.* 2020, Vela Díaz *et al.* 2020). As specialist species are less dispersal capacities than generalist species, the connection among population with narrow niche are less likely to be well (Birand *et al.* 2012, Kisdi 2002, Waters *et al.* 2020). Over time, higher spatial or ecological isolation may lead to the reproductive barriers that means the formation of new species (Birand *et al.* 2012, Kisel, Barraclough 2010, Pan *et al.* 2019, Ye *et al.* 2016).

In contrast, the range size of generalist species is larger than that of specialist species (Boucher - Lalonde *et al.* 2016, Colwell, Rangel 2009, Slatyer *et al.* 2013), and the dispersal rates may be probably higher (Gaston 2003). The increased connectivity among generalist species populations prevents speciation. In this study, the population genetics analyses of *Q. yei* disclosed the existing but relatively small genetic differentiation across the entire range, especially for that between north (JTX and KHJ) and south populations (BYM and KJY). Conversely, the deep genetic divergences (the MRCA of *Pachyhynobius* dates back to ~7.84 Ma) were disclosed among *P. shangchengensis* populations. Additionally, the distinct population demography status (*P. shangchengensis*, stable or decline; *Q. yei*, expansion) has an important contribution to the formation of biogeographical structure (Fig. 3, 4). Based on ENM results, *Q. yei* have higher temperature adaptability, lower altitude distribution, and larger distribution area, while *P. shangchengensis* have relatively lower temperature adaptability, higher altitude distribution, and smaller distribution area (Fig. 5, 6, 7 and Table 3), which indicated the relatively wider ecological niche breadths and environmental tolerances of *Q. yei* than that of *P. shangchengensis*. Differences in niche breadths and environmental tolerances are presumed to drive the differences in range size among species (Pither 2003). For these species affected by the sky island effect, range size shows a strong correlation with the population connectivity, while the connection level between populations directly affects the formation of genetic differentiation (Atkins *et al.* 2020, Beniston 2016, de Oliveira *et al.* 2021, Herman, Champagnac 2016, Shepard, Burbrink 2011). Therefore, due to the correlation of niche breadths, environmental tolerances and range size, we could

infer that the population connectivity within *Q. yei* would more frequently than that within *P. shangchengensis*, which played a key role to the formation of different genetic differentiation and structure of these two co-distributed species. Additionally, dispersal ability is another key factor that constraining the geographical range size and genetic variation (Gaston 2003), and thus influences the spatial scale of speciation (Lester *et al.* 2007, Lucati *et al.* 2020, McCauley *et al.* 2014, Saastamoinen *et al.* 2018, Waters *et al.* 2020). As mentioned in the previous paragraph, *Q. yei* has a stronger dispersal ability than *P. shangchengensis*, which also has been proposed to be one of the main factors shaping the genetic differentiation of these two species.

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Data availability statement

Data available from the GenBank (XX–XX, provide after the article is received).

Abbreviations

JTX, Jiaoyuan-Tanghui-Xiaolongtan; KHJ, Kangwangzhai-Huangbaishan-Jiufengjian; MW, Mazongling-Wochuan; TTZ, Tiantangzhai; BYM, Baimajian-Yaoluoping-Mingtangshan; KJY, Kujingyuan; ND2, NADH dehydrogenase 2; ENM, Ecological niche modelling; CSM, Community Climate

System Model; LIG, Last Inter-glacial; LGM, Last Glacial Maximum.

Consent for publication

Not applicable

Conflict of interest

The authors declare no conflict of interest.

Ethics approval and consent to participate

In the present study, our experimental procedures and sample collection complied with the current laws on animal welfare and research in China, and were specifically approved by the Animal Research Ethics Committee of Anhui Normal University.

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Titles and legends to figures

Fig. 1: The population genetic structure analysis of two endemic species (*Q. yei* and *P. shangchengensis*) in Dabie Mountains, China. All the genetic data about *P. shangchengensis* were collected from previous study (Pan et al., 2019). (a) Sampling sites of two endemic species. Sample sites are represented as green triangles (*Q. yei*) or yellow dots (*P. shangchengensis*). The blue lines represent the Rivers. The different colored values represent the altitude of the mountains. All the map and basic vector layers were downloaded from shared database (<https://glovis.usgs.gov/>; <http://www.resdc.cn/>). (b) Median-joining network results based on mitochondrial data. The node sizes indicated the frequencies of haplotypes. The mutation number between haplotypes are marked on the branches. The one-step mutation is omitted. The undetected haplotypes are marked by red diamond nodes. The node colors represent different populations: Red, JTX; origin, KHJ; darkBlue, MW; green, TTZ; blue, BYM and cyan, KJY. (c,d) TESS clustering results of *Q. yei* and *P. shangchengensis* based on microsatellite data. The boxplot of TESS DIC values based on 20 replicates. TESS output for $K = 2, 3, 4$, and 6.

Fig. 2 Heatmap visualization of pairwise F_{ST} among six populations in *Q. yei* and *P. shangchengensis*. (a) The F_{ST} values of mtDNA sequence based on haplotype frequency differences (*P. shangchengensis*, above diagonal; *Q. yei*, below diagonal). (b) The F_{ST} values based on microsatellite data (*P. shangchengensis*, above diagonal; *Q. yei*, below diagonal). The F_{ST} values were marked with different colors.

Fig. 3 Posterior distributions of the demographic parameters inferred with Msva of six populations *Q. yei* and *P. shangchengensis* based on microsatellite data (a–f). The results of *P. shangchengensis* collected from previous study (Pan et al., 2019). Estimated posterior distributions of current (N_0 , blue curve) and ancestral (N_1 , red curve) effective population sizes and time since population change (T, black curve) on a logarithmic scale. The generation time of *Q. yei* is 2 years.

Fig. 4 Population fluctuation inferred by Extended bayesian skyline plot (EBSP) of *Q. yei*. The x-axis indicates time in million years BP, and the y-axis indicates the effective population size divided by generation time in units of $N\tau$ (the product of effective population size and generation time in days). The blue areas represent 95 % highest posterior density. Time is expressed in million years.

Fig. 5 Ecological niche modeling of *Q. yei* (a, b, c, d) and *P. shangchengensis* (e, f, g, h) under different climate conditions. The results of *P. shangchengensis* collected from previous study (Pan et al., 2019). (a, e) Last Inter-glacial (LIG) conditions, (b, f) Last Glacial Maximum (LGM) conditions, (c, g) Mid Holocene, (d, h) Current conditions. The color scale from white to black indicates the habitat suitability value from 0 to 1.

Fig. 6 Boxplot of each factor under distribution probabilities greater than 0.5 for two species (*Q. yei* and *P. shangchengensis*). The significance of difference is indicated by an asterisk: $*P < 0.001$.

Fig. 7 The ecological response curves of ENMs and principal component analysis (PCA) results of climatic data extracted from the sampling site of *Q. yei* and *P. shangchengensis*. Response curves indicate the range that is more favorable for species distribution under environmental conditions. (a, c) The response curves of Bio 9; (b, d) The response curves of Elevation. (e, f) The Gaussian mixture model of PCA analyses of habitat niche of these two species (*Q. yei* and *P. shangchengensis*).

Titles and legends to tables

Table 1 Descriptive statistics of mitochondrial gene (ND2) and 11 microsatellite loci for the populations of *Quasipaa yei*. *N*, sample Size; *Hd*, haplotype diversity; π , nucleotide diversity; *Na*, number of Alleles; *Ne*, number of effective alleles; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *F*, fixation index. *P* values of Tajima's *D* test and Fu's *F_s* test were calculated with

1078 10,000 simulations, significant tests are indicated with an asterisk (* $P < 0.05$,
1079 ** $P < 0.02$).

1080 **Table 2 Parameters Msvar analysis of the total population in *Quasipaa yei* based**
1081 **on microsatellite data.** Posterior probabilities and 95% highest posterior
1082 intervals for the parameters inferred in Msvar. N_0 , Current effective population
1083 size; N_1 , Historical effective population size; T, Time of the bottleneck.

1084 **Table 3 Current habitat composition of *Q. yei* and *P. shangchengensis* in China**
1085 **under MaxEnt model.** HS: highly suitable habitat (0.75–1); MS: medium
1086 suitable habitat (0.5–0.75); PS: poorly suitable habitat (0.24–0.5); US:
1087 unsuitable habitat (<0.24).

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Supplement Figure S1-S9

Figure S1 Chronogram of *Q. yei* with outgroup species based on mitochondrial

data. The blue lines on nodes correspond to the 95% highest posterior density of the age of the node. The bottom axis is in millions of years.

Fig. S2 STRUCTURE analysis of *Q. yei* based on microsatellite data. (a) The

chart between the number of clusters (K) and LnP(D); (b) ΔK values of 5 runs; (c) The histogram of STRUCTURE clustering results (K = 2, 3 and 6).

Fig. S3 Spatial distribution of each group (K = 6) in *Q. yei*. (a-f) Cluster 1-6.

Clusters are represented by areas of different colors. Light-colored areas indicate a higher probability of an individual belonging to that cluster.

Fig. S4 Cluster membership showing geographic information at K = 6 including

sampling site of *Q. yei*.

Fig. S5 The factorial correspondence analysis (FCA) across populations of *Q.*

***yei*.** Six populations are indicated by different colors: red, JTX; blue, KHJ; White, MW; black, TTZ; pink, BYM; darkCyan, KJY.

Fig. S6 Spatial genetic autocorrelation analyses among individual pairs within

***Q. yei* and *P. shangchengensis*.** (a) individual pairs based on mtDNA (n =

18,264 pairs) in *Q. yei*, (b) individual pairs based on microsatellite data (n =

59,098 pairs) in *Q. yei*, (c) individual pairs based on mtDNA (n = 16,110 pairs)

in *P. shangchengensis*, (d) individual pairs based on microsatellite data (n =

34,191 pairs) in *P. shangchengensis*. r, genetic autocorrelation coefficient; L,

lower 95% confidence interval; U, upper 95% confidence interval. The

permuted 95% confidence intervals of r indicate the random spatial genetic

structure (dashed lines). The bootstrapped 95% confidence error bars are

marked around r. The smallest distance class sizes are set as 10 km (*Q. yei*) and

5 km (*P. shangchengensis*) respectively.

Fig. S7. Results of a Mantel test showing the correlation between genetic

distance values and the geographic distances of *Q. yei*. (a) The result based

on mitochondrial data . (b) The result based on microsatellite data.

Fig. S8 Mismatch distributions analyses for two clusters of *Q. yei* based on the

mtDNA sequence data. (a) The results of north cluster; (b) The results of south cluster. The x coordinate represents the number of differences between pairs of sequences and the y coordinate represents the frequencies of pairwise differences. The blue histograms are the observed frequencies of pairwise divergences among sequences and the green line refers to the expected shape of the distribution under the model of population expansion. SSD = sum of squared deviations; Rag= raggedness index.

Fig. S9 Receiver operating characteristic (ROC) curve and average test AUC for accuracy analysis of *Q. yei* and *P. shangchengensis* by MaxEnt model under the current situation.

Supplement Table S1-6

Table S1 Details on the primers for amplified the *ND2* gene and eleven microsatellite loci of *Q. yei*.

Table S2. Multiple runs for inferring the number of populations of *Q. yei* with Geneland. Bold indicates the highest average posterior probability.

Table S3 Hierarchical analysis of molecular variance (AMOVA) among *Q. yei* populations or groups.

Table S4 Parameters Msva analysis of the total population in *Quasipaa yei* based on microsatellite data. Posterior probabilities and 95% highest posterior intervals for the parameters inferred in Msva. N_0 , Current effective population size; N_1 , Historical effective population size; T, Time of the bottleneck.

Table S5 Environmental variables used for the distribution modeling of *Q. yei* and *P. shangchengensis* in Dabie Mountain, East China.

Table S6 The contribution and importance of each environment variable to the model.