Regional climates shape the biogeographic history of a broadly distributed freshwater crab species complex

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Abstract

Aim: The evolutionary importance of paleoclimate regimes has been noted in biogeographic studies. However, little is known about how paleoclimate differences shaped the biogeographic pattern and diversification history of the freshwater fauna in important zoogeographical boundary regions. Here, we aim to investigate how past regional climatic differences have shaped the biogeographic history of the inland aquatic fauna in China using an endemic freshwater crab species complex found on both sides of the Qinling Mountains–Huaihe River Line (QHL), a critical ecological boundary in eastern China, as a model system.

Location: Eastern China, the Qinling Mountains–Huaihe River Line.

Taxon: The Sinopotamon yangtsekiense species complex.

Methods: A total of 482 individuals of Sinopotamon yangtsekiense sensu lato were collected from 34 localities throughout its entire distributional range. The phylogeographic analyses of population structure, morphological and genetic variations, and demographic dynamics were made based on multiple mtDNA and nuDNA loci and on morphological traits. Fine-tuned ecological niche modeling was used to reconstruct the location of climatically suitable areas that existed during the Last Glacial Maximum.

Results: The divergence of two freshwater crab lineages across the QHL correlated with significant past variations in monsoon intensity and with the location of multiple refuges. The divergence time was broadly consistent with the timing of the critical paleoclimate transition event in the mid-Pleistocene (95% HPD, 0.48–1.06 Ma). Each freshwater crab lineage has evolved distinct male genital traits associated with their isolation in areas with different precipitation rates and temperatures in the past. The patterns of crab distribution observed today reflect past contractions of the two lineages in response to glacial and interglacial cycles during the Pleistocene, followed by their subsequent rapid expansion after the Last Glacial Maximum (~15 kya).

Main conclusions: Populations of the widespread species Sinopotamon yangtsekiense s.l. experienced a deep division in the past that led to the phylogeographical isolation observed today. The two main drivers of genetic isolation in this taxon were (a) differences in the intensity of the monsoons on each side of the QHL boundary during the...
1 | INTRODUCTION

The links between zoogeographic boundaries and evolutionary history have long been of interest to biogeographers (Avise, 2000; Barber et al., 2000; Burton, 1998; Nunez et al., 2015; Thiel-Egenter et al., 2009). The correspondence of the geographical distribution of genetic lineages with known biogeographic boundaries is one of the most important insights in biogeography (Avise, 2000; Burton, 1998; Mei et al., 2013). Research that focuses on species found near biogeographic boundaries can therefore provide many valuable insights into how biogeographic patterns have been shaped (Glor & Dan, 2015; Hu & Jiang, 2018; Mei et al., 2013). Zoogeographic boundaries affecting the phylogeographic patterns of species are the result of multiple synergistic effects of both abiotic (e.g., ecological changes, climatic oscillations, topographical and orogeny changes) and biotic factors (e.g., morphological variation, population expansion and/or contraction, species dispersal; Bouchenak-Khelladi et al., 2015; Ezard et al., 2011; Hewitt, 2004; Yan et al., 2013). The influence of these factors on biogeographic patterning, therefore, is best studied using multiple biogeographic perspectives (e.g., Poelstra et al., 2018; Pontarp & Petchey, 2018; Zhao et al., 2018).

This study focuses on the Qinling-Huaihe Line (QHL) which is a reference line used by geographers to distinguish between northern and southern China at a latitude of about 33° North (where Qinling refers to the Qinling Mountains, and Huaihe refers to the Huahe River). The QHL is also an important boundary between the Palearctic and the Oriental biogeographic areas and includes the Qinling-Dabie orogenic belt (QDB), the Yangtze River Plain, and the southern Anhui mountains (Chen, 2004; Cheng, 1987; He, 1994; Tan, 2011; Zhu et al., 2003). This region was significantly influenced by the uplift of the Qinghai-Tibet Plateau, while it had a profound impact on the development of the Great River Basin (e.g., the Yangtze and Yellow Rivers, and the Huahe River in the QHL; Dong et al., 2011, 2015; Meng & Zhang, 2000; Shi et al., 2013; Zhang et al., 2015). The climate in this region is heavily influenced by differences in the intensity of the East Asian monsoon that has created different ecological conditions in either side of the QHL. During the Pleistocene glacial and interglacial periods, the northern part of the QHL tended to be temperate with a cool dry climate, while the southern part of the QHL tended to be hot and wet with either a subtropical or tropical climate, and a temperature difference of about 6–7°C (An et al., 2001; Jin et al., 2010). The uplifted mountain range served to block the flow of the warm and humid southern air mass, resulting in a significantly different levels of precipitation in the south (which became wetter) than in the north (which became drier; Liu, 2005). The resulting ecological and geological complexity of the QHL today provides an ideal region for testing how ecological changes, Pleistocene climatic oscillations, and topographic variations have affected the biogeographic history of the species found there.

Recent research in the QHL has focused on lineage divergence in non-aquatic species on both sides of the QHL, and its role as a major geographical barrier (e.g., Liu et al., 2017; Song et al., 2016; Yan et al., 2010). In contrast, aquatic species might be expected to have responded differently to past climate fluctuations (i.e., show more genetic structuring) than their non-aquatic counterparts because aquatic habitats have experienced a different biogeographic history and are much less stable than terrestrial systems (Lam et al., 2018; Ribera & Valladares, 2011; Ribera & Vogler, 2000; Wang et al., 2013; Wang et al., 2012). The QHL and the surrounding regions are rich in freshwater systems, and the abundance and complexity of these habitats has driven the geographical divergence of a number of aquatic species. For example, this boundary has played a critical role in the geographical division of the fish fauna in the Palaearctic and Oriental zoogeographic regions (Shaanxi Institute of Zoology et al., 1987). However, little is actually known about the role that past climatic changes on both sides of QHL have played in promoting phylogeographic subdivisions and divergence. The present study focuses on the role of the QHL in shaping the historical diversification of aquatic invertebrates which have been largely overlooked to date.

Primary freshwater crabs (hereafter, referred to as ‘freshwater crabs’) are among the most ecologically important groups of freshwater benthic macroinvertebrates. Important taxonomic characters of freshwater crabs such as the male first gonopod (G1) show considerable morphological variation between species living in different ecological conditions (Cumberlidge, 1999; Daï, 1999; Guinot et al., 2013; Ng et al., 2008). The remarkably high morphological and genetic diversification of freshwater crabs is one of the most striking evolutionary radiations seen in any group of brachyurans (Cumberlidge & Ng, 2009; Dobson et al., 2010). Freshwater crabs, therefore, provide an ideal model for examining the effects of ecological changes (Daniels et al., 2015; Phiri & Daniels, 2014; Shih, Zhou, et al., 2011), paleoecological events (Fang et al., 2013; Klaus, 2010; Shih et al., 2010), and Pleistocene climate fluctuations (Daniels, 2011; Jesse et al., 2011; Klaus & Yeo, 2013; Parvizi et al., 2018; Shih, Ng, et al., 2011; Shih, Zhou, et al., 2011).

Sinopotamon Bott, 1967, is endemic to China and is the most speciose and widely distributed genus of freshwater crabs found anywhere in the world (see Chu et al., 2018; Dai, 1999; Ng et al., 2008;
Fang et al., 2013; Shih et al., 2016). For this study we focused on the widely distributed species Sinopotamon yangtsekiense sensu lato, which is found on both sides of the QHL, and exhibits a great deal of variation across its range, and may actually prove to be a species complex (Dai, 1999; Dai & Chen, 1981). For example, morphological variations of the G1 of S. yangtsekiense led Dai (1999) to recognize three subspecies, S. y. yangtsekiense, S. y. tongbaiense, and S. y. shanxianense, based on geographic differences between populations. However, this is not supported by the molecular evidence because these three subspecies do not form a monophyletic group, and the three taxa form two separate lineages (Zheng et al., 2006). In the present study, therefore, we refer to this taxon as S. yangtsekiense s.l. until the taxonomy of this species complex can be formally resolved. Interestingly, our morphological studies indicate that the differences in the morphology of the G1 of these three taxa may be related to past regional climate changes. For example, differences in the size and position of the female vulvae may be an adaptation to slow the evaporation rate of water in response to a drier climate, which has prompted corresponding co-evolutionary changes in the shape of the male genitalia necessary to ensure continued successful mating. The significant variation of the male G1 morphology in populations of S. yangtsekiense found to the north and south of the QHL, and the availability of a comprehensive genetic dataset for a wide range of specimens from this region, make this species complex an ideal research subject to study historical biogeography in these freshwater habitats.

Here, we reconstruct the biogeographical history of the S. yangtsekiense complex and provide insights into how biotic and abiotic factors may have driven interspecific phylogeography. We have employed a multi-level approach combining data from mtDNA (partial cytochrome oxidase subunit I gene [COX1] and partial nicotinamide adenine dinucleotide dehydrogenase subunit 5 gene [NADS1]), nuDNA (eight nuclear microsatellite loci; Sun et al., 2009), morphological measurements, and ecological niche modeling. We hypothesized that the complex phylogeographic patterns of the freshwater fauna in eastern China have been impacted by climatic changes on both sides of QHL since the Pleistocene. Our specific questions were as follows: (a) is there significant phylogeographical structure between the populations of the S. yangtsekiense complex found on different sides of the QHL boundary; (b) if so, what are the main factors that have driven the phylogeographical divergence of this species complex; and (c) have Pleistocene climatic fluctuations affected the evolutionary history of S. yangtsekiense s.l.?

2 | MATERIALS AND METHODS

2.1 | Population sampling, DNA extraction, and amplification

The taxonomy at the species level used in this study follows Dai (1999) and Ng et al. (2008). A total of 482 specimens of S. yangtsekiense s.l. (including the three subspecies assigned by Dai, 1999) were collected from 34 localities throughout its distributional range that includes the QHL, the Yangtze Plain, and the Southern Anhui mountains. All samples were collected between 2004 and 2015, stored in 95% ethanol and grouped according to morphological traits and locality (Figure 1; Table S1). The outgroup taxon, S. depressum, was collected from Kaihua County in Zhejiang Province, China. We sequenced mtDNA markers from 273 specimens, and microsatellite loci from 482 specimens.

The total genomic DNA was extracted from crab muscle or gill tissue using the DNeasy Blood & Tissue Extraction kit (Qiagen®). DNA quality and quantity were evaluated using agarose gel electrophoresis (TBE 1.0%) and the Qubit® 2.0 Fluorometer (Invitrogen®), and stored at –20°C.

Mitochondrial sequences were amplified by the polymerase chain reaction (PCR), COX1 was amplified with the primers HCC1 (5’-TAAACTTCAGGGTGACCGAAAAATCA-3’) and CCIL (5’-GCAC GTCTCAAATCATAAAGATTTG-3’), and NADS was amplified with the primers U-N5 (5’-GCCAGGGTTGAAAGCTAT-3’) and L-N5 (5’-AGAATCTCTGAGGTAGGTAG-3’). Each PCR amplification was carried out in a 50 μl volume, including 1.25 U of Taq DNA polymerase (TakaRa, Dalian), 5.0 μl 10 × PCR buffer, 2.0 mM MgCl₂, 0.4 mM of each dNTP, 2 μM of each primer, 1 μl (approximately 50–100 ng) of template DNA, and dd H₂O. The thermocycling program consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 55°C for 40 s, and 72°C for 40 s, plus a final extension at 72°C for 8 min. Two μl aliquots of the PCR amplifications were checked by 1.0% TBE agarose gel electrophoresis stained with Golden View.

Eight microsatellite loci primers developed by Sun et al. (2009) were used for PCR amplification: hxx3, hxx6, hxx7, hxx9, Ga2, Gaca12, Gagt21, and Gagt12 (see Table S2 in Supporting Information). Amplification conditions are described in Sun et al. (2009). PCR products were separated on 6.5% polyacrylamide gels using a LI-COR 4300 automated DNA sequencer and analyzed using LI-COR SAGATG software.

2.2 | Mitochondrial DNA data analyses

Mitochondrial sequences were assembled and edited by the DNASTar v.7.1 (DNASTAR Inc., Madison, WI, USA), separately aligned via MAFFT v.7 (Katoh et al., 2002). DnaSP v.5.0 was utilized to define the haplotypes (Librado & Rozas, 2009). Arlequin v.3.5 (Excoffier & Lischer, 2010) was used to evaluate the molecular diversity indices, including the haplotype diversity (h), nucleotide diversity (a), fixation index (Fst), and used hierarchical analysis of molecular variance (AMOVA) to quantify genetic diversity within and among groups with 10,000 permutations.

Haplotype networks were plotted first by the median-joining method (Bandelt et al., 1999) and then by the maximum parsimony Steiner method (Polzin & Daneshmand, 2003) using Network v.5.0.0.1 (www.fluxus-engineering.com). The significance of each pairwise comparison was tested with 1000 random replicates, and a standard Bonferroni correction was applied for multiple tests. The relationship between genetic distance and geographical distance was tested using
isolation-by-distance (IBD) analysis for all sampling sites using Mantel tests in IBD v.1.53 (Jensen et al., 2005). To verify whether there was significant gene flow between populations, we used the software package Migrate v.4.4.3 (Beerli, 2006, 2009) to estimate the effective number of migrants ($M = m/\mu$, where $m$ is the migration rate and $\mu$ is the mutation rate) and effective migrants per generation ($N_{e,m}$).

We generated a relative period for the lineage divergence by COX1 and NAD5. Molecular dating was performed in BEAST v.1.10.4 (Uchard et al., 2018). We conducted two runs of 10,000,000 generations (sample freq = 1,000). After checking for convergence in Tracer v.1.6 (Rambaut & Drummond, 2013), 25% of MCMC were discarded as burn-in. Divergence time was estimated with an uncorrelated log-normal model and a speciation Yule tree priority with a mutation rate substitution rate of $(2.0 \pm 0.6 \text{ stdev}) \times 10^{-9}$ substitutions per neutral site per year, which is commonly used for the COX1 gene and NAD5 in crustaceans (Jesse et al., 2009). We set the common ancestor of S. yangtsekiense as 1 Ma $\pm$ 0.2 stdev based on the high probability density (HPD) of the analysis performed in Ji et al. (2016).

### 2.3 Microsatellites data analyses

The software Micro-Checker v.2.2.3 (Van Oosterhout et al., 2004) was used to detect possible null alleles and potential errors in genotypic. Genetix v.4.05 (Belkhir, 2004) and GenePop v.3.4 (Raymond & Rousset, 1995) were used to evaluate the genetic diversity indices, including number of alleles (N), effective number of alleles (Ne), observed heterozygosity (Ho), and expected heterozygosity (He) and to test for Hardy–Weinberg equilibrium (HWE). The significance of the deviation of genotype frequencies from the HWE within populations is expressed by the deviation of $F_{IS}$ from zero based on estimates of multilocus genotypes. These statistics were assessed using FSTAT v.2.9.3 (Goudet, 2001).

Microsatellite data were unambiguously assigned to particular mtDNA lineages by neighbor-joining (NJ) analysis in MEGA v.7.0.26 (Kumar et al., 2016). Genetix v.4.0.5 was used to analyze the genetic variation patterns of eight microsatellite loci by Factorial Correspondence Analysis (FCA) and the genetic diversity values of individual multilocus were calculated, and displayed in three-dimensional spatial patterns. The NJ tree of multilocus data was constructed by individual shared allele distances (Du & Lin, 2006). The population genetic structure without an outgroup was evaluated under the Bayesian framework via STRUCTURE v.2.3.4 (Pritchard et al., 2000) with the mixed model and the correlated allele frequency model. The tested $K$ ranged from 1 to 10 independent runs of 1,000,000 MCMC iterations each, the first 100,000 iterations were discarded, and the average operation was performed 10 times. The most appropriate value of $K$ was determined by the $\Delta K$ using StructureHarvester v.0.6.94 (Earl & von Holdt, 2012). The results were visualized with Distruct v.1.1 (Rosenberg, 2004).
2.4 Morphological and statistical analysis

The first (G1) and second (G2) gonopods of male brachyurans are typically complex and exhibit considerable morphological variation among taxa (Cumberlidge, 1999; Dai, 1999; Davie et al., 2015; Guinot et al., 2013). This is especially true in freshwater crabs where the unique shape of the G1 is used as a high-weight species-level character in taxonomic identification, along with characters of the G2, and the sixth pleonan segment (A6; Chen et al., 2007; Dai, 1999; Ng, 2008). In this study, we focused on the morphology of the G1, G2, and A6 of males because of difficulties quantifying the characters of the female genitalia.

The terminology used follows Cumberlidge (1999) and the classification follows Dai (1999) and Ng et al. (2008). Measurements of the subterminal segments (SS) of the G1 and G2 were made along a straight line beginning at the midpoint of the basal margin and ending at the midpoint of the distal margin (at the junction between the two parts). Measurements of the terminal articles (TAs) of the G1 were made on the ventral side along the midline beginning at the midpoint of the junction between the TA basal margin and the distal SS, and ending at the TA tip. The length of the TA of G1 and G2 relative to the length of the SS of each of these structures is presented as the ratio of the terminal article/subterminal segment (TA/SS).

We measured five morphological traits from 147 adult male specimens belonging to 27 populations as follows: (a) the G1 SS and G1 TA (see Figure S1a); (b) the G2 SS and G2 TA (see Figure S1b); (c) the height of the G1TA ventral (V) and dorsal (D) lobes (see Figure S1a); (d) the width (W) and length (L) of the sixth pleonan segment (A6; see Figure S1c); (e) and the angle of the G1TA with respect to the longitudinal axis of the G1 and SS (G1 TA angle; see Figure S1a). G1 TA used the average of the V and D. These values were converted into ratios (except the G1 TA angle) to exclude errors due to age and size as follows: G1 TA/SS, G2 TA/SS, G1 TAV/D, and A6 W/L. Coefficients of variation (CV%) were determined to indicate morphological variability (see Table S3 in Supporting Information).

Morphological measurements would be expected to be correlated with the present climate conditions (Begliomini et al., 2017; Sun et al., 2017). We obtained, therefore, 64 climate factors for the sampling sites from 1981 to 2010 from the China Meteorological Data Sharing Service System (http://data.cma.gov.cn/site/index.html; see Table S4 and Appendix S1 for details of climatic factors).

We conducted a one-sample Shapiro–Wilks test and a Kolmogorov–Smirnov test to determine whether data conformed to a normal distribution and the homogeneity of variances, and one-way ANOVAs to compare each trait and factor among populations and groups. In an effort to test whether these morphological characters support distinct differences among populations, we standardized morphological traits and conducted a principal component analysis (PCA) to indicate differences among populations; the linear discriminant function analysis (DFA) was conducted using the lda function in the MASS package v7.3 (Venables & Ripley, 2002); and the clustering algorithm based on Euclidean distance was conducted via R package cluster v.2.1.0 and factoextra v.1.0.5. The Pearson correlation coefficient was calculated to determine the significant relationships between the morphological variables and climatic factors. All statistical analyses were performed in Program R v. 3.6.1 (R Core Team, 2016).

2.5 Demographic history and ecological niche modeling

To investigate the demographic history of S. yangtsekiense s.l., we used Arlequin to calculate Tajima’s D, and Fu’s Fs, and significance was evaluated through 10,000 merge simulations. The distribution of all nucleotide mismatches between DNA sequences was accessed using the mismatch distribution analysis, whereby a unimodal distribution indicated that the population had recently experienced rapid growth, and a multimodal distribution indicated a balanced population size (Rogers & Harpending, 1992). The bootstrap method was used to statistically test the validity of the extended model and to calculate the sum of squares (SSD) between the observed and expected distributions (Excoffier & Lischer, 2010). Finally, the demographic history of the population size over time was calculated using the Extended Bayesian Skyline Plot (EBSP; Ho & Shapiro, 2011) implemented in BEAST by assessing the time variation of effective population size. We performed two independent runs for 100,000,000 generations, sampling one tree every 10,000 generations. We applied a unique model of evolution (GTR) and an identical strict substitution rate for all partitions (mean substitution rate value, see above).

Ecological niche modeling (ENM) was performed for the Last Glacial Maximum (LGM) and presented for all populations. Bioclimatic variables of 2.5 arc-minutes resolution were downloaded from the World-Clim database website (http://www.worldclim.org/; Fick & Hijmans, 2017; Hijmans et al., 2005). The ENM analysis was based on maximum entropy modeling in MaxEnt v.3.3.3k (Phillips et al., 2006) to reconstruct the geographical distribution of the species today and during the Last Glacial Maximum (LGM, 22 kya). The results were imported into ArcGIS v.10.5 to edit and display. The effectiveness of the model was assessed using the area under the curve value (AUC; Araujo & Guisan, 2006).

Other abbreviations used: ENM, Ecological niche modeling; EBSP, Extended Bayesian Skyline Plot; HMR, Huang Mountain Region; LGM, Last Glacial Maximum; LHR, Lower Huaihe River; MHR, Middle Huaihe River; QDB, Qinling–Dabie Orogenic Belt; SEHM, Southeast Huang Mountains; SWHM, Southwest Huang Mountains; TBM, Tongbai Mountain; QML, Qinling Mountains–Huaihe River Line; DBM, Dabie Mountains; YR, Yellow River; YTR, Yangtze River.

3 RESULTS

3.1 Genetic variation

The concatenated dataset consisted of 273 individuals from 34 populations. Ambiguously aligned nucleotide sites were removed, leaving a total of 1,206 nucleotides of the mitochondrial genes COX1
(657 bp) and NAD5 (549 bp). All haplotype sequences were deposited in GenBank (accession numbers COX1: AY896797–AY896825, JF918762–JF918850; NAD5: AY896828–AY896862, JF918851–JF918925). A total of 171 unique haplotypes were identified from the COX1 and NAD5 sequences obtained (117 and 110 haplotypes, respectively), with 200 parsimony informative sites, 4 singleton mutations, and no gaps.

Haplotype diversity (H) within 34 populations ranged from 0.250 (pop. 33) to 1.000 (pop. 5–8, 12, 14, 18, 20, 21, 31–34) and nucleotide diversity ranged from 0.001 (pops. 1, 4) to 0.022 (pop. 6; Table S1). Within the nine groups, haplotype diversity (h) ranged from 0.901 (YR) to 0.990 (LHR), nucleotide diversity (s) from 0.004 (SEHM) to 0.034 (HMR), and the overall haplotype diversity and nucleotide diversity were 0.992 and 0.043, respectively (Table S1). AMOVA analysis showed that 54.19% and 48.71% of the total genetic diversity was partitioned among populations (Table 1).

The eight microsatellite loci from 482 individuals showed that all population combinations deviated significantly from HWE. The effective number of alleles (Ne) ranged from 1.93 (pop. 19) to 4.59 (pop. 2). Within the nine populations the observed heterozygosity (Ho) ranged from 0.548 (MHR) to 0.818 (TBM) and the overall observed heterozygosity was 0.694. The expected heterozygosity (He) ranged from 0.547 (LHR) to 0.745 (YR), with an overall expected heterozygosity of 0.828 (Table S1).

### 3.2 Phylogenetic analyses and population genetic structure

The network relationship (Figure 2a) and phylogenetic tree (Figure 2b) both showed that the populations of this species were split into two distinct clades (lineages A and B) which had significant genetic differentiation (mtDNA: FstA–B = 0.937, p < 0.001; microsatellite loci: FstA–B = 0.946, p < 0.001). Lineage A (green in Figures 1–3) encompassed individuals from populations 1–9, while lineage B (red in Figures 1–3) contained individuals from populations 10–34. The haplotype network reconstruction revealed that there were at least five genotype deletions between lineages A and B. The PCA analysis indicated that the first principal coordinate axis (which distinguished individuals of lineage A from all remaining populations) explained 26.75% of the total variance in the association between alleles and individuals, while the second and third axes explained 17.30% and 15.90%, respectively; Figure 2c. The STRUCTURE analysis based on Bayesian clustering indicated the highest support (Figure 2d; Table S5; K = 2 with the highest mean probability) for two clusters among the sampled populations using microsatellites. Fst results showed that populations were genetically highly structured (Figure S2). Estimates of gene flow calculated with Migrate v.4.4.3 indicated that the levels of gene flow between lineages A and B are low and restricted (mtDNA: M_A→B = 31.1, NemA→B = 0.10, M_B→A = 17.4, NemB→A = 0.07; microsatellite loci: M_A→B = 20.0, NemA→B = 0.94, M_B→A = 4.0, NemB→A = 0.82). The IBD analysis revealed a significant correlation between the genetic and geographic distances for the 34 populations (Figure S3). An uncorrelated lognormal model was expected for the samples (Figure 3). The divergence time estimate for the split between lineages A and B was 0.78 Ma (95% HPD, 0.48–1.06 Ma).

### 3.3 Morphological variation

Current morphological changes to determine the likely factors that contribute to these variations (the G1 characteristics of lineages are shown in Figure 1). The ANOVA results determined that the five morphological traits and CVs were statistically significant (p ≤ 0.01) among the lineages and populations. Correlation analysis confirmed that morphological traits do not vary with the sample size (p = 0.097–0.945) and provides strong support for the results of the morphological analysis. Two significant PCs found the total variance of the morphological measures to be 87.94% and 12.02%, respectively (see Figure S4 in Supporting Information). Discriminant function analysis showed that individuals were split into two distinct clades with strong support (membership probability >0.95). Overall,

| Source of variation | df | Sum of squares | Variance components | Percentage of variation | Fixation indices | p value |
|---------------------|----|----------------|---------------------|------------------------|-----------------|--------|
| mtDNA               |    |                |                     |                        |                 |        |
| Among groups        | 1  | 2370.099       | 20.70754 Va         | 54.19                  | F_ST = 0.70357  | <0.001 |
| Within populations  |   | 3436.272       | 12.31658 Va         | 32.23                  | F_SC = 0.86420  | <0.001 |
| Total               | 238| 7088.54        | 5.18927 Vb          | 13.58                  | F_CT = 0.54189  | <0.001 |
| SSR                 |    |                |                     |                        |                 |        |
| Among groups        | 6  | 384.740        | 0.37336 Va          | 48.71                  | F_ST = 0.24724  | <0.001 |
| Within populations  | 27 | 317.876        | 0.21307 Vb          | 33.01                  | F_SC = 0.16763  | <0.001 |
| Total               | 448| 873.345        | -0.15506 Vc         | 18.28                  | F_CT = 0.10710  | <0.001 |
PCA, DFA, and cluster analysis indicated that the partitioning was basically consistent with genetic analysis (Figure S4). However, the finding that population 8 clustered with Lineage B is doubtful because it is likely that it is an artifact arising from using data from a single individual rather than from a larger sample. All the morphological data exhibited a normal distribution and were, therefore, used for subsequent correlation analyses.

The influence of precipitation and temperature on morphological changes was studied by comparing differences among the traits of present-day populations. We linked climate factors to changes in form using the Pearson correlation analysis (Figure 4). The precipitation treatments indicated that the G2TA/SS value was significantly positively correlated with average precipitation (AAP20/AAP8/AAAP/AAIP/ADP/ACP/MNLP/MDAP/SDAP/AAPM) and humidity (AAH) and significantly negatively correlated with coefficient of variation (CVA/CVP); G1TA was significantly negatively correlated with average precipitation (AAP20/AAP8/AAAP/AAIP/ADP/ACP/MNLP/MDAP/SDAP/AAPM)/AAH), and significantly negatively correlated with the coefficient of variation (CVA/CVP). The results showed that high humidity and rainfall levels were associated with a more curved G1TA and a higher G2TA/SS value. The negative correlation with the variation coefficient of precipitation showed that the more stable the climate, the lower the morphological variation.

The temperature treatments indicated that G1TA/SS and G2TA/SS were significantly positively correlated with the mean

**FIGURE 2** The phylogeographic relationships of the Sinopotamonyangtsekiense complex (green, lineage A; red, lineage B): (a) rooted median-joining networks based on COX1 + NAD5, each circle represents a haplotype, its size being proportional to the frequency of occurrence of a certain haplotype. Small plain circles represent haplotypes that were not sampled or extinct; (b) unrooted NJ tree based on eight microsatellite loci via distance shared allele distance; (c) factorial correspondence analysis computed based on eight microsatellite loci using Genetix showing relationships among the multilocus genotypes; and (d) Bayesian clustering results of STRUCTURE (K = 2) based on eight microsatellite loci [Colour figure can be viewed at wileyonlinelibrary.com]
temperature (AAT/MAT/MIT/MEAT/ELT/DAT), and negatively correlated with the days when the temperature was <2°C (ADT2/DIT2/ADT0/DIT0/ADTN2/DITN2). G1TA was significantly negatively correlated with the mean temperature (AAT/MIT/MEAT), and negatively correlated with the days when the temperature was <2°C (ADT2/DIT2/ADT0/DIT0/ADTN2/DITN2). Increases in the annual average temperature were associated with a more curved G1TA and a higher G2TA/SS value.

3.4 | Historical demographic inferences and ecological niche modeling

The mtDNA data for three different datasets (lineages A and B, and all samples) resulted in neutrality tests that indicated demographic and spatial expansion in both lineages (by significant positive values of Fu’s Fs, and not significant positive values of Tajima’s D statistics, Table 2). Similar results were manifested in the mismatch distribution
analyses and the Extended Bayesian skyline plots (Figure 5). EBSP suggested that demographic expansion occurred approximately 15 kya, which corresponded to the end of the LGM occurring in continental China (Anhuf et al., 2006).

The ecological niche modeling reconstructed the geographic distribution of the S. yangtsekiense complex both during the LGM and at present, and yielded AUC scores of >0.95, indicating strong support for the model. Of the 19 bio-climatic factors used, we found that the main factors affecting species distribution were Bio1, Bio11, Bio6, Bio9, and Bio18. The predicted distribution pattern for the present-day populations closely matched the actual occurrences of this species (Figure 6). In addition, the distribution of lineage A during the LGM indicated that the populations then had a significantly smaller range compared to present day levels. For example, during the LGM, the distributional range of the S. yangtsekiense complex was limited mostly to regions of low elevation in the eastern part of its range.

4 | DISCUSSION

Our results show that the most basal split within the S. yangtsekiense complex was between populations in the northwest (lineage A) and those in the southeast (lineage B) that are found today on both sides of the QHL. The most recent common ancestor of Sinopotamon most likely originated in the Sichuan Basin and surrounding mountains and subsequently dispersed throughout central and eastern China. The dispersal of populations of the S. yangtsekiense complex has happened relatively recently (~1 Ma; Ji et al., 2016), spreading from northwest to southeast along the QHL. The restricted gene flow and morphological/genetic divergence between lineages A and B may be due to paleoclimatic differences on both sides of the QHL caused by...

TABLE 2 Neutrality tests and mismatch analysis (Spatial expansion and Demographic expansion)

|                      | Lineage A | Lineage B | Lineage A + B |
|----------------------|-----------|-----------|--------------|
| Neutrality tests     |           |           |              |
| Fu’s Fs              | -14.094   | -23.796   | -23.778      |
| p-value              | 0.011     | 0.004     | 0.001        |
| Tajima’s D           | 0.112     | 0.52      | 1.069        |
| p-value              | 0.580     | 0.717     | 0.813        |
| Spatial expansion    |           |           |              |
| SSD                  | 0.004     | 0.005     | 0.003        |
| Model (SSD) p-value  | 0.96      | 0.33      | 0.36         |
| Raggedness index     | 0.005     | 0.001     | 0.001        |
| Raggedness p-value   | 0.92      | 0.91      | 1            |
| Demographic expansion|           |           |              |
| Theta 0              | 22.05     | 3.98      | 37.517       |
| Theta 1              | 82.99     | 273.504   | 271.895      |
| Tau                  | 39.638    | 84.431    | 89.352       |

East Asian monsoon intensity. The species complex contracted into multiple isolated refuges during the glacial periods, where local climatic differences resulted in the evolution of the observed morphological divergence of the male genitalia, resulting in phylogeographic divergence. The high genetic divergence between these two lineages indicates a high probability that the S. yangtsekiense s.l. actually comprises one or more cryptic species.

4.1 | Interspecific relationship and splitting

The QHL represents a natural zoogeographic boundary in China dividing the moist and warm region southeast of the line from the dry and cooler region northwest of the line. This division has increased following a critical paleoclimatic transition event—the intensification of the East Asian monsoon (0.85 Ma; Guo et al., 1993; Zhao et al., 2020). Interestingly, the divergence time between the two lineages of the S. yangtsekiense complex was estimated to be 0.78 Ma (95% HPD: 0.48–1.06 Ma), which is largely consistent with sharp climatic changes. This, taken together with our historical demography analyses, infers that the climatic differences on both sides of the QHL may have acted as a phylogeographic barrier, splitting and genetically isolating the existing population, in an event that subsequently led to the evolution of two independent lineages. Interestingly, this dividing line broadly coincides with the Palearctic / Oriental boundary along the QHL (Cheng, 1987; Zhang, 1999).

The unexpectedly close relationship of populations within lineage A that are nevertheless separated by a distributional gap between TBM and YR (Figure 1) may be attributed to these areas serving as common refugia during the glacial period. This gap between population in TBM and YR might be due to the competitive displacement of S. yangtsekiense by S. honanense which is also found there (Dai & Chen, 1981). It is possible that S. honanense may have caused the removal of the formerly established Nanyang basin habitat of S. yangtsekiense through direct or indirect competitive interactions (Altschuler, 2006; Cheng et al., 2009; Douglas et al., 1994; Kfir, 1997). This phenomenon of competitive exclusion is also found between other closely related species (Altschuler, 2006; Cheng et al., 2009; Douglas et al., 1994; Kfir, 1997) especially where the ranges of two species overlap in hybrid zones (Rieseberg et al., 2000).

The historical river capture events in eastern China could explain the close relationship within lineage B that Li et al., 2005; Ren et al., 1959), where the shared haplotypes and strong gene flow indicate that there were no obvious gene barriers. Historically, the Huaihe and Yangtze Rivers were connected and flowed southward into the Guhung River (Clark et al., 2004; Yang, 2006). Although the exact timing of the individual river connections is controversial, it seems to have occurred at some point in the late Pleistocene and produced significant changes to the drainage network in eastern China (which is consistent with this study). In addition, during the Pleistocene in eastern China numerous freshwater lakes formed, such as Lakes Luoma, Hongze, Gaoyou, and Taihu (Cao, 1989; Zhan, 2008; Zhu et al., 2003). The intermittent connectivity of hydrological features...
and summer floods produced homogenization that allowed more migration opportunities for populations of *S. yangtsekiense* in the eastern region of China (Yan et al., 2013; Yang et al., 2009; Yu et al., 2014).

Long distance dispersal of freshwater crabs in the past has resulted in the reproductive isolation of populations through reduced gene flow leading eventually to the fixation of novel adaptations and allopatric speciation. In addition, the isolation of small populations of crabs may have led to the fixation of characters that may not be adaptive due to genetic drift and the founder effect (Chung et al., 2014; Wang et al., 2017; Yong et al., 2015). It is possible that the dispersal of the *S. yangtsekiense* complex has led to significant population fragmentation in parts of its range that were associated with numerous founder events. If this were the case, then the allelic diversity and the number of heterozygotes in the population would both significantly decrease as the number of founder events separating populations increased (Clegg et al., 2002; Cun & Wang, 2010). We inferred that Qinling–Dabie orogenic belt acted as a dispersal corridor for the *S. yangtsekiense* complex, and that strong founder effects had occurred in lineage B, and these colonization routes

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**FIGURE 5** Pairwise mismatch distributions (left) and Extended Bayesian skyline plots (right) based on COX1 + NAD5 gene sequences. Pairwise mismatch distributions: The X-axis shows the observed distribution of pairwise genetic variation, and the Y-axis represents the frequencies; Extended Bayesian skyline plots: the bold line indicates LGM [Colour figure can be viewed at wileyonlinelibrary.com]

**FIGURE 6** Results from the ecological niche modeling reconstruction showing potential distribution range of the *Sinopotamonyangtsekiense* complex at the Last Glacial Maximum (LGM, 22 kya) and its distribution today. Black dots indicate the sampled populations [Colour figure can be viewed at wileyonlinelibrary.com]
are likely to be driven by the Yangtze River. The inference for the founder effect is supported by the finding that the genetic diversity of lineage A is greater than lineage B, and by the gradual increase in $N_e$, $H_o$, and $H_e$ along the QHL from west to east (Table S1).

### 4.2 The morphological divergence driven by climatic change

The morphological analysis infers that the S. yangtsekiense complex has been affected by changes in temperature and humidity / precipitation (Figure 4). High humidity and high rainfall are associated with an elongated G1TA, while lower humidity and less rainfall are associated with a curved G1TA and an accompanying shortened G2SS. Freshwater crabs are semi-terrestrial and are often found out of water where they dig burrows in river banks and forage on nearby land (Dai, 1999; Dai & Chen, 1981). When the habitat of these crabs is subjected to low levels of humidity and precipitation and even prolonged drought, reducing the evaporation rate from the female openings by altering their size and position, is a selective pressure that could lead to morphological variation not only in females, but also in the variation of the G1 shape of the males that must mate with them.

Compared with other non-sexual traits, the variation of genital traits caused by climate changes can lead to reproductive isolation and can promote lineage divergence. We inferred, therefore, that climate change on both sides of the QHL is likely the intrinsic cause of genital divergence in these crabs, which in turn has reduced gene flow and has led to phylogeographic divergence. The genital morphology of males and females has co-evolved, at least in marine crabs (Guinot et al., 2013), where variation in genital traits has prevented hybridization and enhanced reproductive isolation (Brennan & Prum, 2015; Eberhard, 2011). Notably, we inferred that genital co-evolution in the S. yangtsekiense complex is consistent with Yao et al. (2020), and appeared to have been subjected to rapid divergence by relation to historical and climatic drive. We proposed that the changes in the genital of the S. yangtsekiense complex is geographically characterized as the northwest is more curved than the southeast. We speculate that the gradual drying of the environment in the northwest of the QHL has resulted in a shift of the size and position of the female genital vulvae towards the midline of the sternum, presumably to reduce the evaporation of water during egg production. These changes in the size of the opening and the position of the female genital vulvae were accompanied by the co-evolution of the shape of the male genitalia (the G1 TA) which became more angled (bent) to facilitate alignment with the female openings necessary for successful mating.

### 4.3 Historical demography and glacial refugia

ENM showed that the distributional range of S. yangtsekiense s.l. was significantly constricted during the last glacial maximum (0.78 Ma) and then gradually recovered over time to occupy its present distribution (Figure 6). The neutrality tests and mismatched distributions indicated that lineages A and B have experienced a recent expansion (Table 2). The EBSP analysis also showed that S. yangtsekiense s.l. has undergone a rapid demographic expansion after the last glacial maximum (~15 kya; Figure 5), similar to that described for other freshwater species found in this region (Horreo et al., 2018; Li et al., 2014; Lopes-Lima et al., 2018; Salvi et al., 2014; Ling et al., 2012). Tajima’s D, however, is not significantly negative, indicating that the population has undergone recent expansion after which it was subdivided, undergoing either an extensive migration and expansion and/or contraction (De Queiroz, 2007; Sharma et al., 2014; Wang & Ge, 2010). Local genetic diversity changes with increasing geographic latitude might be, in part, due to historical contractions or subdivisions of the original population (Yang et al., 2016). Similarly, the relatively low genetic diversity found in several distinct populations (1, 4, 10, 23, and 24) suggests that these may have experienced a bottleneck effect in the past.

Freshwater crabs grow and reproduce best in warm and humid environments, and this provides two explanations for why Pleistocene climate fluctuations have been major factors affecting crab populations in the past. For example, S. yangtsekiense s.l. grows fastest in warm temperatures (between 20 and 28°C), and slowest in cooler temperatures (between 10 and 20°C; Chen et al., 1994). The climate during the last glacial maximum 18–21 kya contrasts sharply with the climate of the past 17,000 years and with the most recent glacial period (Cullen, 1981). According to a series reports, the temperature during the last glacial period in eastern China was between 4 and 12°C (Shi et al., 1997; Xu et al., 2010; Zhen et al., 2014), which is cold enough to reduce the growth rate and increase the mortality rate. In addition, cold temperatures reduce the fertility rate in freshwater crabs because females lay fewer eggs than normal (only 30–100), and females have even been observed to eat their own eggs when subjected to cold conditions and food shortages (Chen et al., 1994; Dai, 1999).

According to the EBSP and mismatch distribution analyses, populations of S. yangtsekiense s.l. could have undergone a recent population expansion across the QDB from west to east (Figure 5), followed by bottleneck events associated with population contractions (Rogers & Harpending, 1992). Our study provides corroborative evidence for cumulative founder effect events during the eastward expansion.

The QDB mountain range provided numerous glacial refuges for freshwater species during the past ice ages (Hewitt, 2000; Shih, Ng, et al., 2011; Zhang et al., 2008; Zhao et al., 2020). The results of our genetic diversity analysis confirmed the presence of at least three glacial refugia (YR, TBM, and HMR) within the QDB during the LGM. The population expansion of S. yangtsekiense s.l. from refugia in the QDB during the late Pleistocene is evidenced by intensified genetic divergence during this time. This scenario was similar to that reported for the recent colonization of the QBD by S. acutum (Fang et al., 2015). The climatic impact of the glacial period on the QHL in China was different from its impact on Western Europe and North America. In the latter two regions, significant areas were covered
by ice sheets during the glacial period, forcing many species to migrate to warmer areas to the south. When temperatures warmed up during the interglacial periods, species recolonized the northern areas (Hewitt, 2004). In contrast, during the glacial period, the QHL in China was not significantly glaciated, but the change in climate altered the ecosystems there dramatically, leaving only a few sheltered pockets (refugia) within the QDB and the Huangshan region (HMR) that remained environmentally suitable for some species where more tolerable conditions persisted. Interestingly, we found that these refugia (e.g. TBM, YR) either coincided with, or were adjacent to, the existing distributional range of populations of freshwater crabs. The high annual precipitation rates in the highlands of the QDB (such as the Qinling and Tongbai mountains) during the glacial period established local humid conditions where populations of S. yangtsekiense s.l. could survive (despite their inland location; Ju et al., 2007; Knutti et al., 2004). A similar pattern of species distributions coinciding with refugia in the QBD was also found in studies of amphibians (Zhang et al., 2008) and reptiles (Ding et al., 2011).

4.4 Taxonomic consideration of the S. yangtsekiense complex

Dai and Chen (1981) classified S. y. tongbaiense as a subspecies based on morphological characteristics and its geographical distribution (the TBM and the Huaihe River). The morphology of this subspecies (e.g. the degree of bending of the G1 TA) is intermediate to that of the other two subspecies (S. y. shanxianense and S. y. yangtsekiense). Our molecular and morphological evidence, however, found S. y. shanxianense (distributed in the YR basin), and S. y. tongbaiense (distributed in the TBM) to be closely related, indicating a high probability of the presence of a second cryptic species (lineage A). Furthermore, the S. y. tongbaiense populations in the middle reaches of the Huaihe River clustered into lineage B. We considered, therefore, that the populations distributed in the middle reaches of the Huaihe River should be classified as S. y. yangtsekiense (which was formerly S. y. tongbaiense), and the individuals of lineage A should be classified as a new species (which was formerly S. y. shanxianense and S. y. tongbaiense).

The high divergence of the lineages A and B in the S. yangtsekiense complex, and the congruence of genetic and morphological data, suggest that this complex probably contains a cryptic species. It is likely that the existence of cryptic diversity in the S. yangtsekiense complex has been underestimated, given the rapid diversification within Sinopotamon (since 0.8 Ma; Ji et al., 2016). We have not revised the taxonomic status of the S. yangtsekiense complex here because this is beyond the scope of the present work whose focus is on the biogeography of this taxon in relation to historical climate changes. Clearly, further studies are needed to clarify the unstable taxonomy of the S. yangtsekiense complex.

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DATA AVAILABILITY STATEMENT

The COX1 and NAD5 sequences have been submitted to GenBank under the accession numbers AY896797–AY896862 and JF918762–JF918925 (www.ncbi.nlm.nih.gov/genbank). Microsatellite loci, primer sequences, and morphological data are listed in online Supporting Information Table S2 and Table S3 (The data are openly available in Dryad at DOI https://doi.org/10.5061/dryad.1vhmgqps4).

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REFERENCES

Altschuler, D. L. (2006). Flight performance and competitive displacement of hummingbirds across elevational gradients. The American Naturalist, 167(2), 216–229.
An, Z., Kutzbach, J. E., Prell, W. L., & Porter, S. C. (2001). Evolution of Asian monsoons and phased uplift of the Himalaya-Tibetan plateau since Late Miocene times. Nature, 411(6833), 62–66.
Anhuf, D., Ledru, M.-P., Behling, H., Da Cruz, F. W., Cordeiro, R. C., Van der Hammen, T., Karmann, I., Marengo, J. A., De Oliveira, P. E., Pessenda, L., Siffedine, A., Albuquerque, A. L., & Da Silva Dias, P. L. (2006). Paleo-environmental change in Amazonian and African rainforest during the LGM. Palaeogeography, Palaeoclimatology, Palaeoecology, 239(3–4), 510–527.
Araujo, M. B., & Guisan, A. (2006). Five (or so) challenges for species distribution modelling. Journal of Biogeography, 33(10), 1677–1688.
Avise, J. C. (2000). Phylogeography: The history and formation of species. Harvard University Press.
Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. Molecular Biology & Evolution, 16(1), 37–48.
Barber, P. H., Palumbi, S. R., Erdmann, M. V., & Moosa, M. K. (2000). Biogeography. A marine Wallace’s line? Nature, 406(6877), 692–693.
Beerli, P. (2006). Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics, 22, 341–345.
Beerli, P. (2009). How to use MIGRATE or why are Markov chain Monte Carlo programs difficult to use? In G. Bertorelle, M. W. Bruford, H. C. Hauffe, A. Rizzoli, & C. Vernesi (Eds.), Population Genetics for Animal Conservation (vol. 17 of Conservation Biology, pp. 42–79). Cambridge University Press.
Begliomini, F. N., Maciel, D. C., de Almeida, S. M., Abessa, D. M., Maranhão, L. A., Pereira, C. S., Yogui, G. T., Zanardi-Lamardo, E.,
& Castro, I. B. (2017). Shell alterations in limpets as putative biomarkers for multi-impacted coastal areas. *Environmental Pollution*, 226, 494–503.

Belkhir, K. (2004). *Genetix 4.05, logiciel sous Windows TM pour la génétique des populations*. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II.

Bouchenak-Khelladi, Y., Onstein, R. E., Xing, Y., Schwery, O., & Linder, H. P. (2015). On the complexity of triggering evolutionary radiations. *New Phytologist*, 207(2), 313–326.

Brennan, P. L. R., & Prum, R. O. (2015). Mechanisms and evidence of genital coevolution: The roles of natural selection, mate choice, and sexual conflict. *Cold Spring Harbor Perspectives in Biology*, 7(7), a017749.

Burton, R. S. (1998). Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution*, 52(3), 734–745.

Chao, Y. (1989). River-lake system and climatic change in east China. In *China Environmental Science*, 9(4), [In Chinese with English abstract.]

Chen, L. (2004). The precise biogeographic division of palaearctic and congeneric species. *Zoological Research*, 25(5), 369–377.[In Chinese with English abstract.]

Chen, T., Wei, L., & Du, N. (1994). Growth, reproduction & population structure of the freshwater crab *Sinoptomon yangtsekiense* bott, 1967, from Zhejiang, China. *Chinese Journal of Oceanology & Limnology*, 12(1), 84–90.

Chen, W. J., Cheng, J. H., Chen, T. I., & Hsu, M. J. (2007). A comparison of the micromorphology of the G1 of freshwater crabs of the genus *Geothelphusa* (Brachyura, Potamidae) from Taiwan. *Crustaceana*, 80(7), 861–889.

Cheng, T. (1987). A synopsis to the *avifauna of China*. Science Press.

Cheng, X., Xie, P., Cheng, F., Xu, R., & Xie, B. (2009). Competitive displacement of the native species *Bursaphelenchus murrayi* from an alien species *Bursaphelenchus xylophilus* (Nematoda: Aphelenchida: Aphelenchoididae): a case of successful invasion. *Biological Invasions*, 11(2), 205–213.

Chu, K., Ma, X., Zhang, Z., Wang, P., Lv, L., Zhao, Q., & Sun, H. (2018). A checklist for the classification and distribution of China's freshwater crabs. *Biodiversity Science*, 26(3), 274–282.[In Chinese with English abstract.]

Chung, K., Leong, W., Rubite, R. R., Repin, R., Kiew, R., Liu, Y., & Peng, C. (2014). Phylogenetic analyses of Begonia sect. Coelocentrum and allied limestone species of China shed light on the evolution of Sino-Vietnamese karst flora. *Botanical Studies*, 55(1), 1.

Clark, M. K., Schoenbohm, M. L., Royden, L. H., Whipple, K. X., Burchfiel, B. C., Zhang, X., Tang, W., Wang, E., & Chen, L. (2004). Surface uplift, tectonics, and erosion of eastern Tibet from large-scale drainage patterns. *Tectonics*, 23, TC1006.

Clegg, S. M., Degnan, S. M., Jiro, K., Craig, M., Arnaud, E., & Owens, I. P. F. (2002). Genetic consequences of sequential founder events by an island-colonizing bird. *Proceedings of the National Academy of Sciences of the United States of America*, 99(12), 8127–8132.

Cullen, J. L. (1981). Microfossil evidence for changing salinity patterns in the Bay of Bengal over the last 20,000 years. *Palaeoecography, Palaeoclimatology, Palaeoecology*, 35(81), 315–356.

Cumberlidge, N. (1999). The freshwater crabs of West Africa: family *Potamonautidae* (Vol. 36). Collection Faune et Flore Tropicales. IRD.

Cumberlidge, N., & Ng, P. K. L. (2009). Systematics, evolution, and biogeography of freshwater crabs. In J. W. Martin, K. A. Crandall, & P. K. L. Ng (Eds.), *Decapod crustacean phylogenetics* (pp. 503–520). CRC Press.

Cun, Y., & Wang, X. (2010). Plant recolonization in the Himalaya from the southeastern Qinghai-Tibetan Plateau: Geographical isolation contributed to high population differentiation. *Molecular Phylogenetics and Evolution*, 56(3), 972–982.

Dai, A. (1999). *FAUNA SINICA - Crustacea - malacostraca: decapoda*. The Science Publishing Company. [In Chinese with English abstract.]

Dai, A., & Chen, G. (1981). On two new subspecies of *Sinoptomon yangtsekiense* Bott, 1967. *Acta Zoologica Sinica*, 27, 172–180.

Daniels, S. R. (2011). Reconstructing the colonisation and diversification history of the endemic freshwater crab *Seychellum alluaudi* in the granitic and volcanic Seychelles Archipelago. *Molecular Phylogenetics and Evolution*, 61(2), 534–542.

Daniels, R. S., Phiri, E. E., Klaus, S., Albrecht, C., & Cumberlidge, N. (2015). Multilocus Phylogeny of the Afrotropical Freshwater Crab Fauna Reveals Historical Drainage Connectivity and Transoceanic Dispersal Since the Eocene. *Systematic biology*, 64(4), 549–567.

Davie, P. J. F., Guinot, D., & Ng, P. K. L. (2015). Anatomy and functional morphology of Brachyura. In *Treatise on Zoology-Anatomy, Taxonomy, Biology. The Crustacea, Volume 9 Part C (2 vols)* (pp. 11–163). Brill.

De Queiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*, 56(6), 879–886.

Ding, L., Gan, X. N., He, S. P., & Zhao, E. M. (2011). A phylogeographic, demographic and historical analysis of the short-tailed pit viper (*Gloydius brevicaudus*): evidence for early divergence and late expansion during the Pleistocene. *Molecular Ecology*, 20(9), 1905–1922.

Dobson, M., Magana, A. M., Lancaster, J., & Mathooko, J. M. (2010). Aseasonality in the abundance and life history of an ecologically dominant freshwater crab in the Rift Valley, Kenya. *Freshwater Biology*, 52(2), 215–225.

Dong, Y., Zhang, G., Neubauer, F., Liu, X., Genser, J., & Hauenberger, C. (2011). Tectonic evolution of the Qinling orogen, China: Review and synthesis. *Journal of Asian Earth Sciences*, 41(3), 213–237.

Dong, Y., Zhang, X., Liu, X., Li, W., Chen, Q., Zhang, G., Zhang, H., Yang, Z., Sun, S., & Zhang, F. (2015). Propagation tectonics and multiple accretionary processes of the Qinling Orogen. *Journal of Asian Earth Sciences*, 104, 84–98.

Douglas, M. E., Marsh, P. C., & Mckinley, W. L. (1994). Indigenous fishes of Western North America and the hypothesis of competitive displacement: *Meda fulgida* (*Cyprinidae*) as a case study. *Copeia*, 1994(1), 9–19.

Du, Z., & Lin, F. (2006). pNJTree: A parallel program for reconstruction of neighbor-joining tree and its application in ClustalW. *Parallel Computing*, 32(5), 441–446.

Earl, D. A., & von Holdt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359–361.

Eberhard, W. G. (2011). Experiments with genitalia: A commentary. *Trends in Ecology & Evolution*, 26(1), 17–21.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564–567.

Ezard, T. H. G., Tracy, A., Pearson, P. N., & Andy, P. (2011). Interplay between changing climate and species’ ecology drives macroevolutionary dynamics. *Science*, 332(6027), 349–351.

Fang, F., Ji, Y., Zhao, Q., Wang, Y., Gao, W., Chu, K., & Sun, H. (2015). Phylogeography of the Chinese endemic freshwater crab *Sinoptomon acutum* (Brachyura, Potamidae). *Zoologica Scripta*, 44(6), 653–666.

Fang, S., Sun, H., Zhao, Q., Lin, C., Sun, Y., Gao, W., Xu, J., Zhou, J., Ge, F., & Liu, N. (2013). Patterns of diversity, areas of endemism, and multiple glacial refuges for freshwater crabs of the genus *Sinoptomon* in China (*Decapoda: Brachyura: Potamidae*). *PLOS One*, 8(1), e53143.

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37, 4302–4315.

Glor, R. E., & Dan, W. (2015). Testing ecological explanations for biogeographic boundaries. *Evolution*, 69(3), 673–683.

Goudet, J. (2001). FSTAT (Version 2.9.3): A program to estimate and test gene diversities and fixation indices, Retrieved from http://www2.unil.ch/popgen/softwares/fstat.htm
fluctuations in driving the genetic structure of Quasipaa boulangeri (Anura: Dicroglossidae). *Molecular Ecology*, 22(4), 1120–1133.

Yan, J., Wang, Q., Chang, Q., Ji, X., & Zhou, K. (2010). The divergence of two independent lineages of an endemic chinese gecko, *Gekko swinhonis*, launched by the Qinling orogenic belt. *Molecular Ecology*, 19(12), 2490–2500.

Yang, J.-Q., Hsu, K.-C., Liu, Z.-Z., Su, L.-W., Kuo, P.-H., Tang, W.-Q., Zhou, Z.-C., Liu, D., Bao, B.-L., & Lin, H.-D. (2016). The population history of *Garra orientalis* (Teleostei: Cyprinidae) using mitochondrial DNA and microsatellite data with approximate Bayesian computation. *BMC Evolutionary Biology*, 16(1), 1–15.

Yang, L., Mayden, R. L., & He, S. (2009). Population genetic structure and geographical differentiation of the Chinese catfish *Herminagrus macropterus* (Siluriformes, Bagridae): Evidence for altered drainage patterns. *Molecular Phylogenetics and Evolution*, 51(2), 405–411.

Yang, D. (2006). The formation of Yangtze River geomorphology, (78–209). Beijing: Geological Publishing House. [In Chinese with English abstract.]

Yao, F., Shi, B., Wang, X., Cheng, J., Bai, M., Yan, J., Cumberlidge, N., & Sun, H. (2020). Rapid divergent co-evolution of freshwater crab genitalia facilitates a burst of species diversification. *Integrative Zoology*, 15(3), 174–186.

Yong, G., Ai, B., Kong, H., Ming, K., & Huang, H. (2015). Geographical pattern of isolation and diversification in karst habitat islands: a case study in the *Primulina eburnea* complex. *Journal of Biogeography*, 42(11), 2131–2144.

Yu, D., Chen, M., Tang, Q., Li, X., & Liu, H. (2014). Geological events and Pliocene climate fluctuations explain the phyleogeographical pattern of the cold-water fish *Rhynchocypris oxycephalus* (Cypriniformes: Cyprinidae) in China. *BMC Evolutionary Biology*, 14(1), 225.

Zhan, J. (2008). A brief account of the formation and evolution of the drainage system in the Pearl River Basin. *Pearl River Modern Construction*, 8(5), 9–10.

Zhang, H., Yan, J., Zhang, G., & Zhou, K. (2008). Phylegeography and demographic history of Chinese black-spotted frog populations (*Pelophylax nigromaculata*): Evidence for independent refugia expansion and secondary contact. *BMC Evolutionary Biology*, 8(1), 1–16.

Zhang, H., Yu, H., Zhou, D., Zhang, J., Dong, Y., & Zhang, G. (2015). The meta-gabbroic complex of Fushui in north Qinling orogen: A case of syn-subduction mafic magmatism. *Gondwana Research*, 28(1), 262–275.

Zhang, R. (1999). *Zoogeography of China*. Science Press. [In Chinese].

Zhao, Y., Yin, G., Pan, Y., & Gong, X. (2018). Ecological and genetic divergences with gene flow of two sister species (*Leucomeris decora* and *Nouelia insignis*) driving by climatic transition in Southwest China. *Frontiers in Plant Science*, 9, 31–35.

Zhao, H., Qiang, X., Xu, X., & Sun, Y. (2020). Iron oxide characteristics of the Chinese loess-red clay sequences and their implications for the evolution of the East Asian summer monsoon since the Late Oligocene. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 543, 109604.

Zhen, S., Zhao, J., & Zheng, B. (2014). Distribution and features of the glaciers’ ELAs and the decrease of ELAs during the Last Glaciation in China. *Journal of Glaciology & Geocryology*, 36(1), 9–19.

Zheng, F., Lu, X., Sun, H., & Zhao, Q. (2006). Genetic differentiation of *Sinopotamon yangtsekiense* based on mitochondrial cytochrome oxidase subunit I gene. *Journal of Nanjing Normal University (Natural Science Edition)*, 29(2), 103–105. [In Chinese with English abstract.]

Zhu, X., Cai, Y., & Wang, J. (2003). Spatial dimensions of mountains, faults and river basins of continental China. *Journal of Mountain Science*, 21(3), 311–317. [In Chinese with English abstract.]

**BIOSKETCH**

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Author Contributions: H.S., B.S., D.P., Y.S., and N.C. conceived the study; Y.S. and H.S. collected the samples, and Y.S. and X.Lv prepared them for DNA sequencing; Y.S. prepared the microsatellite data and carried out the analyses; X.Liu carried out the morphological trait measurements; B.S. and Y.S. carried out the genetic analyses, demographic inferences, and ecological niche modeling; B.S. and X.Liu carried out the morphological analyses; B.S., D.P., Y.S., N.C., and H.S. wrote and edited the manuscript; and all authors read and approved the final manuscript.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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