INTRODUCTION

Climate changes can have fundamental impacts on the distribution patterns of montane species (Hewitt, 1999; Schweiger, Settele, Kudrna, Klotz, & Kühn, 2008). Many species underwent repeated distributional shifts in altitude and latitude in response to the climate change associated with the Pleistocene glaciations according to their ecological requirements (Avise, Walker, & Johns, 1998; Hewitt, 2000, 2004). The cold-adapted montane species potentially expanded their distribution ranges with the increase in suitable habitats during cold stages (Bannikova et al., 2010; de Lattin, 1967; Kropf, Kadereit, & Comes, 2003). They likely have experienced colonization involving a series of dispersal followed by vicariance and divergence (Knowles, 2001). Mountains act as naturally fragmented habitat islands and form barriers to population dispersal and gene flow, providing opportunities for long-term isolation and independent evolution of populations due to genetic drift and natural selection (Fjeldså, Bowie, & Rahbek, 2012). Suitable
climatic regions (e.g. refugia) allowed the persistence of isolated populations during unfavourable conditions, reinforcing allopatric differentiation, and later served as important sources of expansion (Bennett & Provan, 2008; Schmitt & Varga, 2012; Stewart, Lister, Barnes, & Dalen, 2010). For the inhabitants of mountain sky islands, the divergence process may not only be linked with the geographic isolation, but also related with the dynamic colonization during climate-induced distributional shifts (Knowles & Massatti, 2017). Exploring the historical process of persistence and divergence of montane species may provide insights into potential responses of endemic biodiversity to changing climatic conditions.

The significant impacts of recent glaciations on species divergence and distribution are frequently discussed within and between closely related montane species in Europe and North America (e.g. Knowles, 2001; Knowles & Massatti, 2017; Lohse, Nicholls, & Stone, 2011; Martínez-Freiría, Velo-Antón, & Brito, 2015; Muster & Berendonk, 2006; Wachter et al., 2016). Numerous phylogeographic studies on European and North American organisms have uncovered refugia in southern mountain areas during the glacial periods and northward colonizations during the postglacial periods (Hewitt, 2004; Shafer, Cullingham, Cote, & Coltman, 2010; Soltis, Morris, McLachlan, Manos, & Soltis, 2006; Taberlet, Fumagalli, Wust-Saucy, & Cosson, 1998). In contrast, eastern Asia with a mosaic of mountains experienced a relatively mild Pleistocene climate (Ju, Wang, & Jiang, 2007; Zhang, 1999), and probably provided multiple stable habitats and glacial refugia through the entire ranges (Qiu, Fu, & Comes, 2011). However, a significant gap is present in our knowledge regarding the climatic factors influencing past population dynamics (e.g. fragmentation, colonization) and present-day species divergence of eastern Asian montane species.

The Qinling-Bashan Mountains (QBMs) are a natural barrier between the southern subtropical and the northern warm temperate regions of central China, and also a boundary between the Palearctic and Oriental Regions in eastern Asia (Qu, Liu, Bao, & Wang, 2009; Zhang, 1999; Zhang, Qu, Wu, He, & Shi, 2004). QBMs were characterized by complicated

**FIGURE 1** Distributions of _Dicerapanorpa magna_, _Dicerapanorpa baiyunshana_ and _Dicerapanorpa shennongensis_. Locality codes for populations: BS: Bashan Mountains; BYS: Baiyunshan; HDT: Huoditang; HLS: Hualongshan; JLJ: Jialingjiang; LP: Liping Forest Park; MCS: Micangshan; MS: Minshan; NGS: Nangongshan; QL: Qinling Mountains; SNJ: Shennongjia; TB: Taibai Mountain; TTH: Tongtianhe Forest Park; XLS: Xiaolongshan; ZQ: Zhuque Forest Park. Arrows with solid lines indicate the possible postglacial colonization routes, while arrow with broken line shows the possible glacial migration.
topography and distinct environmental conditions between the northern and southern parts, and had been impacted by limited late Pleistocene ice-cover (Rost, 2000). Pleistocene climate fluctuations and historical factors played differing roles in promoting range fragmentation, vicariance and population divergence among taxa due to differences in life history characteristics. Previous studies have uncovered two dominant phylogeographic patterns of species in QBMs, a north–south genetic break (Qu et al., 2009; Tian et al., 2009; Yan, Wang, Chang, Ji, & Zhou, 2010) and an east–west genetic break (Fang et al., 2015; Huang et al., 2017; Liu, Yin, Liu, & Li, 2014; Wang, Jiang, Xie, & Li, 2012, 2013; Yang, Liu, Li, & Dyer, 2015; Yuan, Cheng, & Zhou, 2012).

The *Dicerapanorpa magna* group currently consists of *D. magna* (Chou), *Dicerapanorpa baiyunshana* Zhong & Hua, and *Dicerapanorpa shennongensis* Zhong & Hua. The scorpionfly *D. magna* is a typical cold-adapted montane species of Panorpidae (Mecoptera), and is currently distributed throughout the QBMs (Wang & Hua, 2017; Zhong & Hua, 2013), extending westward to the Minshan Mountain, the natural boundary between Sichuan and Gansu Provinces, and eastward to Shennongjia in Hubei Province (Figure 1). Its favoured habitats are mixed coniferous and broad-leaf forests above 1,000 m with *Anemone hupehensis* and *Artemisia* spp. as herbaceous groundcover. The adults, with relatively low dispersal ability, usually occur in moist microhabitats where they hide on plant stems in dense shade (Zhong & Hua, 2013). Species with narrow distributions, cold-adapted preference and weak dispersal capacity, tend to better reflect the influence of historical and climatic factors on present-day distribution than taxa with high vagility (Copilaş-Ciocianu & Petrusek, 2017; Lomolino, Riddle, Whittaker, & Brown, 2010). Therefore, *D. magna* provides an ideal model to examine the role of Pleistocene climate change on population distribution and divergence of eastern Asian montane species, although it has received little attention in genetic investigation to date.

The combination of coalescent-based phylogeographic and ecological niche modelling techniques provides insights into population differentiation and potential distribution ranges, helping better understand how demographic events coincide with changes in climatic histories (Alvarado-Serrano & Knowles, 2014; Carstens & Richards, 2007; Chan, Brown, & Yoder, 2011; Kozak, Graham, & Wiens, 2008). In this study, we examined the distribution pattern and evolutionary history of *D. magna* using phylogeographic and species distribution modelling analyses, with *D. baiyunshana* and *D. shennongensis* as outgroups. The main aims were as follows: (a) to reconstruct the demographic history of *D. magna* by examining its phylogeographic pattern and the level of genetic differentiation; (b) to elucidate the influence of Pleistocene climatic oscillations on divergence; and (c) to uncover the potential suitable climatic areas (e.g. refugia) and routes of colonization during the glacial periods.

**TABLE 1** Sample size (N), number of haplotypes (Nₙ), haplotype diversity (Hₜ) and nucleotide diversity (π) for *Dicerapanorpa magna*, *Dicerapanorpa baiyunshana* and *Dicerapanorpa shennongensis*

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographical group</th>
<th>Locality code</th>
<th>Number of samples</th>
<th>Nₙ</th>
<th>Hₜ</th>
<th>π (%)</th>
</tr>
</thead>
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<tr>
<td><em>Dicerapanorpa</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>baiyunshana</em></td>
<td>—</td>
<td>BYS</td>
<td>12♀2♂</td>
<td>3</td>
<td>0.275</td>
<td>0.110</td>
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<tr>
<td><em>Dicerapanorpa</em></td>
<td></td>
<td>—</td>
<td>SNJ</td>
<td>13♀4♂</td>
<td>12</td>
<td>0.956</td>
</tr>
<tr>
<td><em>shennongensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>magna</em></td>
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<td>JLJ</td>
<td>6♀4♂</td>
<td>3</td>
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</tr>
<tr>
<td></td>
<td>HDT</td>
<td>15♀4♂</td>
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<td>0.504</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TB</td>
<td>15♀1♂</td>
<td>10</td>
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<td>0.867</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TTH</td>
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<td>4</td>
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<tr>
<td></td>
<td>XLS</td>
<td>1♀</td>
<td>1</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>ZQ</td>
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<td>5</td>
<td>0.790</td>
<td>0.201</td>
<td></td>
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<tr>
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<td>24</td>
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<td>0.972</td>
<td></td>
</tr>
<tr>
<td><em>BS</em></td>
<td>HLS</td>
<td>4♀3♂</td>
<td>6</td>
<td>0.952</td>
<td>0.981</td>
<td></td>
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<td>LP</td>
<td>2♀4♂</td>
<td>5</td>
<td>0.933</td>
<td>1.595</td>
<td></td>
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<tr>
<td></td>
<td>MCS</td>
<td>11♀1♂</td>
<td>3</td>
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<td></td>
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<tr>
<td></td>
<td>NGS</td>
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<td>0.813</td>
<td>0.853</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SNJ</td>
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<td>2</td>
<td>0.667</td>
<td>1.955</td>
<td></td>
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<tr>
<td>Total</td>
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<td>42</td>
<td>20</td>
<td>0.928</td>
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</tr>
<tr>
<td><em>MS</em></td>
<td>MS</td>
<td>3♀2♂</td>
<td>6</td>
<td>0.952</td>
<td>0.485</td>
<td></td>
</tr>
</tbody>
</table>

Notes. BS: Bashan Mountains; BYS: Baiyunshan; HDT: Huoditang; HLS: Hualongshan; JLJ: Jialingjiang; LP: Liping Forest Park; MCS: Micangshan; MS: Minshan; NGS: Nangongshan; QL: Qinling Mountains; SNJ: Shennongjia; TB: Taibai Mountain; TTH: Tongtianhe Forest Park; XLS: Xiaolongshan; ZQ: Zhuque Forest Park.
2 | MATERIALS AND METHODS

2.1 | Specimen sampling

Adults of *D. magna* were collected in the Minshan Mountain (MS), the Qinling Mountains (QL), and the Bashan Mountains (BS) from 2007 to 2016, while the outgroups, *D. baiyun-shana* and *D. shennongensis*, were collected from the Baiyun shan Mountain (BYS) and Shennongjia (SNJ), respectively (Figure 1). In total, 125 females and 36 males were chosen for molecular analyses (Table 1). Sample IDs were created by coupling a two or three letter acronym corresponding to the locality and a number, with “m” to designate males. All the voucher specimens are preserved in 75%–100% ethanol at the Entomological Museum, Northwest A&F University, China (NWAU).

2.2 | DNA extraction, amplification and sequencing

Genomic DNA was extracted from three legs on one side of each specimen with the Genomic DNA Mini Preparation Kit (Beyotime, China). A mitochondrial gene fragment, cytochrome *c* oxidase subunit I (*COI*), was amplified for all samples, while *COII* and 28*S* rRNA were amplified for only a few samples (Supporting Information Table S1). The forward primer LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) and reverse primer HCO2198 (5′-TAA ACT TCA GGG TGAATA TGA TGA GCT CA-3′) (Whiting, 2002) were used to generate an extended *COI* barcode sequence of approximately 840 bp. In case of failure, the reverse primer was replaced by HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer et al., 1994) to amplify the standard 648 bp barcode region. *COII* was amplified with the primers COII-F-leu (5′-TCT AAT ATG GCA GAT TAG CCA AAA AAT CA-3′) (Folmer et al., 1994) and reverse primer C1-N-2329 (5′-ACT GTA AAT ATG TGA TGA TGA GCT CA-3′) (Simon et al., 1994) were used to generate an extended *COII* barcode sequence of approximately 840 bp. In case of failure, the reverse primer was replaced by LCO1490 (5′-TAA ACT TCA GGG TGAATA TGA TGA GCT CA-3′) and reverse primer COII-R-lys (5′-GAG ACC AGT ACT TGC TTT CAG TCA TC-3′), while 28*S* rRNA using the primers 28S rDNA 2.1.2a (5′-CCC SSG TAA TTT AAG CAT ATT A-3′) and 28S Rd4.2b (5′-CCT TGG TCC GTG TTT CAA GAC GG-3′) (Whiting, 2002).

PCR was carried out in 25 µl reactions, containing 12.5 µl 2× Taq MasterMix (CW BIO, China), 8.5 µl sterile distilled H2O, 1 µl 10 µM each primer and 2 µl DNA template. Thermocycling protocols for *COI* employed an initial denaturation at 94–95°C for 3 min; 35–40 cycles of denaturation at 94°C for 30 s, annealing at 50–53°C for 30 s, and extension at 72°C for 1 min; and a final extension at 72°C for 5 min. The annealing temperatures for *COII* and 28*S* were changed to 51 and 59°C, respectively, and other reaction conditions followed the above. PCR products were checked in a 1%–2% agarose gel stained with ethidium bromide, visualized under ultraviolet light, and sent to Shanghai Sangon Biotechnology Co. Ltd. (China) for Sanger sequencing in both directions. All sequences were uploaded to BOLD together with the trace files and specimen information, and cross-referred to GenBank (see Supporting Information Table S1 for details).

2.3 | Phylogenetic analyses

All the sequences were checked, assembled, and edited with SeqMan (Swindell & Plasterer, 1997), and were aligned using MAFFT 7.037 with the iterated refinement algorithm G-INS-I (Katoh & Standley, 2013). After multiple sequence alignment, ambiguous sites at both ends were manually trimmed with BioEdit 7.0.9.0 (Hall, 1999). DNA sequence data were translated into amino acids in MEGA 6.05 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013), and were used in the following network and demographic reconstruction. Haplotype data were generated in ALTER (Glez-Peña, Gomez-Blanco, Reboiro-Jato, Fdez-Riverola, & Posada, 2010) with the missing data considered, and then used in the subsequent phylogenetic analyses and divergence time estimation.

Phylogenetic reconstruction was inferred using maximum likelihood (ML) and Bayesian inference (BI). ML analysis was executed in raxmlGUI 1.3 (Silvestro & Michalak, 2012) with codon-specific partition scheme under GTR+GAMMA model. Bootstrap values were based on 1,000 rapid bootstrap replicates. The best-fitting substitution model for each codon position of *COI* was determined by jModelTest 2.1.4 (Darriba, Taboada, Doallo, & Posada, 2012) under Bayesian Information Criterion (BIC), and was applied in Bayesian inference. Partitioned BI analysis was performed in MrBayes 3.2.6, with JC, F81, and HKY+G for the 1st, 2nd and 3rd codon position of *COI*, respectively. Two parallel runs with four independent chains were conducted for 40 million generations, with sampling every 1,000 generations. An average standard deviation of split frequency below 0.01 means that the chain had converged. The first 25% of the samples were discarded as “burn-in,” and the remaining trees were used to generate a majority consensus tree. The program Tracer v1.7.1 (https://beast.bio.ed.ac.uk/software/tracer/) was used to evaluate the stationarity of the Markov chain by plotting log-likelihood values versus generation number.

The genealogical relationships were established using a minimum spanning network implemented in TCS v. 1.21 with the statistical parsimony analysis (Clement, Posada, & Crandall, 2000; Templeton, Crandall, & Sing, 1992). The network was constructed with a connection limit of 95%, and graphically plotted using tcsBU (Múrias dos Santos, Cabezas, Tavares, Xavier, & Branco, 2016).
2.4 Divergence time estimation

The divergence time among lineages in *D. magna* was estimated using BEAST 2 (Bouckaert et al., 2014) with the bModelTest package (Bouckaert & Drummond, 2017) under a Bayesian Model Averaging method. A lognormal relaxed clock was firstly conducted in BEAST v2.5.0 to assess the pertinence of a relaxed estimation. The coefficient of variation of the relaxed clock was checked in Tracer v1.7.1 (https://beast.bio.ed.ac.uk/software/tracer), and value <0.1 suggests that data are clock-like (Drummond & Bouckaert, 2015). The values were very close to 0.1 under constant and exponential tree prior; therefore, we proceeded with a strict molecular clock. To select for the best tree model, we compared three coalescent population processes (constant, exponential and Bayesian skyline) under the strict clock model. The marginal likelihoods of the three competing tree models were estimated with path sampling method (Lartillot & Philippe, 2006), implemented in the MODEL SELECTION package in BEAST2. All the path sampling analyses were run for 100 steps of 100,000 generations. Bayes factors for comparing models were estimated directly from the marginal run for 100 steps of 100,000 generations. Bayes factors for evaluating the level of genetic variation among and within groups (Excoffier, Smouse, & Quattro, 1992). The significance of variance components was tested with 1,023 permutations. The uncorrected genetic distances among lineages were calculated in MEGA 6.05 (Tamura et al., 2013) using the Kimura two-parameter (K2P) model (Kimura, 1980).

Because fossils in *Dicerapanorpa* are unavailable, we applied a molecular clock estimated at 2.3% Ma (1.15% site−1 Ma−1), a widely accepted substitution value for insect mitochondrial COI (Brower, 1994). Two independent chains were run for 20 million generations, with sampling every 2,000 generations. The log files from the two runs were imported into Tracer v1.7.1 to check the posterior distribution, ensuring the effective sample size (ESS) of each parameter in the combined trace file higher than 200. The first 25% of the samples were discarded as “burn-in,” and the remaining samples from the two runs were combined in Logcombiner (https://beast.bio.ed.ac.uk/logcombiner). A maximum clade credibility tree with common ancestor heights was generated using TreeAnnotator v2.5.0 (BEAST package), and visualized in FigTree v1.3.1 (https://beast.bio.ed.ac.uk/figtree).

Besides, we estimated the divergence time for the *D. magna* group with 28 more species from Hu, Yan, Xu, and Hua (2015) (Supporting Information Table S1), based on the combined COI, COII and 28S rRNA data. Fossil calibration point follows Hu and Hua (2016), with the node age of *Panorpa* constrained to a normal distribution of 52.9 ± 0.83 Ma. A lognormal relaxed clock model was selected (coefficient of variation greatly higher than 0.1) in BEAST v2.5.0 under the Yule model. The MCMC chain was run for 50 million generation, with sampling every 5,000 generations. The stationarity and ESS (>200) were checked in Tracer v1.7.1. TreeAnnotator v2.5.0 was used to summarize a maximum clade credibility tree with the first 25% of trees discarded as “burn-in.”

2.5 Molecular diversity and population structure

The genetic diversity for each population was estimated using DNASP, including the number of haplotypes (N_h), haplotype diversity (H_d) and nucleotide diversity (π) (Librado & Rozas, 2009). A spatial analysis of molecular variance (SAMOVA) was performed using SAMOVA 2.0 to define groups of populations and to detect genetic barriers, without prior structure parameters (Dupanloup, Schneider, & Excoffier, 2002). This approach maximizes the proportion of total genetic variance (F_CT) due to differences between population groups for a predefined number of groups (K). SAMOVA was run based on 10,000 simulated annealing steps with K from 2 to 7. AMOVA was also implemented in SAMOVA to evaluate the level of genetic variation among and within groups (Excoffier, Smouse, & Quattro, 1992). The significance of variance components was tested with 1,023 permutations. The uncorrected genetic distances among lineages were calculated in MEGA 6.05 (Tamura et al., 2013) using the Kimura two-parameter (K2P) model (Kimura, 1980).

2.6 Historical demography

Three methods were used to infer historical demography for each genetic cluster in *D. magna*. First, neutrality tests were conducted in ARLEQUIN v3.5 (Excoffier & Lischer, 2010) to estimate Tajima’s D and Fu’s Fs, with population expansion evidenced by the significant negative values. Second, mismatch distributions were analysed to detect past population expansion, as well as the sum of squared deviations (SSD) and Harpending’s raggedness index (Hrag). SSD and Hrag were used to test the goodness-of-fit, and the rapid expansion model was rejected by the significant values. Third, the coalescent-based Bayesian skyline plot (BSP) was used to estimate past population dynamics in BEAST v2.5.0 (Bouckaert et al., 2014). The coalescent tree prior was specified with Bayesian skyline process under the strict clock model. Two independent chains were run for 20 million generations, sampled every 2,000 generations.

2.7 Ecological niche modelling

Ecological niche modelling was performed to predict the potential distribution of *D. magna* during the Last Glacial Maximum (LGM; ~21 ka) and current periods using 12 sampling localities. Two models were simulated for LGM: the Community Climate System Model (CCSM) and the Model for Interdisciplinary Research on Climate (MIROC). Both models were originally developed from the Paleoclimate...
FIGURE 2  (a) Maximum likelihood tree of Dicerapanorpa magna based on mitochondrial cytochrome c oxidase subunit I (COI). Numbers at nodes indicate bootstrap support values for major clades. Numbers in parentheses indicate the number of individuals with a haplotype. (b) Minimum spanning network for all COI haplotypes in D. magna using TCS. The circle size is proportional to haplotype frequency. Each small circle denotes a mutational step. BS: Bashan Mountains; MS: Minshan Mountain; QL: Qinling Mountains
Ecological niche models were constructed in Maxent 3.3.3 k (Phillips, Anderson, & Schapire, 2006) with bioclimatic variables. Most bioclimatic variables were highly correlated, thereby leading to model over-fitting (Elith et al., 2011). To reduce over-fitting, we first ran Maxent 3.3.3 k with 19 variables to obtain the percentage contribution of each variable and then performed correlation analysis between variables using ENMTools 1.4.4 (Warren, Glor, & Turelli, 2010). The highly variables with correlation coefficient $r > 0.9$ were eliminated. All species distribution models were constructed with the following seven variables: BIO2 (mean diurnal range), BIO3 (isothermality), BIO7 (temperature annual range), BIO9 (mean temperature of the driest quarter), BIO10 (mean temperature of the warmest quarter), BIO14 (precipitation of the driest month) and BIO18 (precipitation of the warmest quarter). The final species distribution predictions were based on the median value of 10 replicates of cross-validation, with a maximum iteration of 2,000. The model performance was evaluated using the area under the curve (AUC) of the receiver operating characteristic. DIVA-GIS 7.5.0 (https://diva-gis.org/) was used to process all raster and generate distribution map.

3 | RESULTS

In total, 161 COI sequences were obtained—130 from *D. magna*, 14 from *D. baiyunshana* and 17 from *D. shennongensis*. The final alignment of COI contained 123 sequences of 777 bp and 38 sequences of 648 bp without gaps or stop codons. The subsequent analyses mainly focused on the 777 bp length with the incomplete sequences considered as missing data. Of the 777 nucleotide positions, 79 were variable and 60 were parsimony informative, with 77 haplotypes detected with the missing data considered.

The COI gene trees possess similar topologies between ML (Figure 2a) and BI (Supporting Information Figure S1) analyses, with three lineages (I–III) recovered for *D. magna*, although *D. shennongensis* is paraphyletic with *D. magna* and *D. baiyunshana* in both trees. Lineage I included all specimens from MS as well as several from TTH, LP and SNJ. Most members of Lineage III were collected from QL, but a few were from LP, MCS and SNJ. By contrast, all individuals of Lineage II were derived from BS. The haplotype network for *D. magna* (Figure 2b) exhibited the same geographical structure as the phylogenetic reconstruction with three well-defined haplogroups. The mean genetic distances among the three lineages of *D. magna* ranged from 0.019 to 0.024, slightly overlapping with the mean distance among the three lineages of *D. magna* and the two closely related species (from 0.022 to 0.028, Supporting Information Table S2).

The marginal likelihood estimation from path sampling analysis and Bayes factor are presented in Supporting Information Table S3, suggesting that the coalescent Bayesian skyline process is the best-fitting tree model for COI data. The divergence time estimated for the *D. magna* group from substitution rate is later than that from fossil calibration (Supporting Information Figure S2). The node age of the *D. magna* group was estimated at 1.73 Ma (95% highest posterior density, HPD, 1.15–2.39 Ma) and 4.58 Ma (95% HPD, 3.20–6.09 Ma), respectively. The time to the most recent common ancestor (TMRCA) of *D. magna* was estimated at 1.29 Ma (95% HPD, 0.81–1.88 Ma) and 3.60 Ma (95% HPD, 2.36–4.77 Ma), respectively.

The overall haplotype and nucleotide diversity (Table 1) were higher in BS (0.93% and 1.13%) than those in QL (0.88% and 0.97%). The *D. magna* populations had the highest haplotype diversity in HLS (0.95), but had the highest nucleotide diversity in SNJ (1.96%). SAMOVA indicated that $F_{CT}$ increased progressively as $K$ was increased from 2 to 7 (Supporting Information Table S4). Based on the phylogenetic topology and haplotype network, all the populations of *D. magna* can be categorized into three groups ($F_{CT} = 0.437$): (a) populations from MS and SNJ; (b) populations from HDT, JLJ, TB, TTH, XLS and ZQ; and (c) populations from HLS, LP, MCS and NGS. The AMOVA with $K = 3$ showed that 43.7% of the total genetic variation was explained by these three groups, while 21.0% was explained by variation among populations, and 35.3% by variation within populations (Supporting Information Table S5).

Lineages I and III of *D. magna* showed evidence of demographic expansion as indicated by significant negative Fu’s $Fs$ values (Table 2). However, the population expansion hypothesis was rejected for Lineage III due to the significant SSD value, but accepted for Lineages I and II owing to their non-significant SSD. The coalescent-based BSP (Figure 3b,d,f) suggested slight population increase or stability in Lineage I, long-term population stability followed by slight contraction in Lineage II, and population expansion in Lineage III.

The ecological niche model for *D. magna* had an average training AUC of 0.959, indicating a high predictive power. The suitable areas under the LGM condition were widespread and continuous in both the Qinling and Bashan Mountains, almost the same as that predicted under the current condition (Supporting Information Figure S3). The potential distribution ranges under the CCSM model were generally consistent with that under the MIROC model. Under the current condition, the model predicted moderate-to-high bioclimatic
suitability for \textit{D. magna} across the most distribution ranges except the Xiaolongshan and Minshan Mountains.

4 | DISCUSSION

This study reconstructed the population demographic dynamics and distribution range shifts of \textit{D. magna} during the Pleistocene by integrating the phylogeographic and ecological niche modelling analyses, a representative of cold-adapted montane insects in eastern Asia. The phylogeographic analyses revealed three lineages (I–III) that corresponded well with the geographically isolated Minshan, Bashan and Qinling Mountains, indicating a clear spatial genetic structure in \textit{D. magna}. Ecological niche modelling predicted that the most suitable distribution ranges were located in the Qinling and Bashan Mountains under the LGM and current conditions, implying potential glacial refugia and long-term climatic stability in these areas.
### 4.1 | Phylogeography of *Dicerapanorpa magna*

The phylogenetic reconstruction and haplotype network (Figure 2) reveal three haplotypes (TTH5, LP1 and SNJ1) from QL and BS were clustered with haplotypes from MS in Lineage I, suggesting a recent migration of *D. magna* from MS to QL and BS. In addition, one haplotype in Lineage I (SNJ1) has a disjunct distribution in the easternmost and western populations, SNJ and MS, which may result from the contemporary long-distance dispersal or the maintenance of ancestral haplotypes in large populations. The MS population possessed a low nucleotide diversity (Table 1), probably because the Minshan Mountain was a less suitable climatic region in LGM (Supporting Information Figure S3). The LP population with haplotypes scattered in all lineages (Figure 2) was located in the centre of the Qingling, Bashan and Minshan Mountain ranges and possessed the highest nucleotide diversity (Table 1), likely suggesting that it occupies a zone of secondary contact between the previously isolated lineages. Such populations like LP contained haplotypes that occurred in more than one regional phylogeographic lineage, reflecting the potential for gene flow between refugial populations, or at the least, insufficient time for sorting of ancestral variation by genetic drift (Knowles, 2001). The low genetic diversity in some populations (e.g. ZQ, Table 1) may reflect a postglacial expansion, as noted in certain European plant species (Comes & Kadereit, 1998) and many insect species (Drees, Matern, Oheimb, Reimann, & Assmann, 2010; Hill, Griffiths, & Thomas, 2011).

The node age estimated for *Dicerapanorpa* based on the combined molecular data (Supporting Information Figure S2b) is nearly consistent with that from Miao, Wang, and Hua (2018), but slightly inconsistent with that from Hu et al. (2015). The later divergence time estimated from substitution rate (Supporting Information Figure S2a) might suggest that the substitution rate at 2.3% Ma⁻¹ was relatively conservative for scorpionflies. Both dating strategies show that the divergence within *D. magna* predated LGM, implying that multiple refugia were maintained across the species’ distribution ranges rather than one single ancestral refugium. The initial divergence in *D. magna* appears to have occurred approximately 1.29 or 3.60 Ma (Supporting Information Figure S2), roughly corresponding to the Quaternary Qinghai-Tibet movement (3.60–1.40 Ma) (Li, 1999; Li & Fang, 1999; Shi et al., 1999). TMRCA is estimated at 0.65 and 0.83 Ma for Lineages II and III, respectively, and is 1.07 Ma for Lineage I, both roughly correlated with the Kunlun-Yellow River movement from 1.10 to 0.60 Ma (Cui et al., 1998). The population diversification and species distribution in *D. magna* have been deeply influenced by the Pleistocene tectonic movements and climatic fluctuations, as noted in a ring-necked pheasant (Qu et al., 2009), a toad-headed lizard gecko (Yan et al., 2010), frogs (Wang, Jiang, Xie, & Li, 2012; Yan et al., 2013), freshwater crabs (Fang et al., 2015, 2013), a geometrid moth (Cheng et al., 2016) and a salamander (Huang et al., 2017).

The three divergent lineages (I–III) of *D. magna* are highly structured geographically, corresponding closely with the northern (QL), western (MS) and southern (BS) mountain ranges (Figure 2). Besides, a clear spatial genetic structure among *D. magna* populations was confirmed by SAMOVA (K = 3; Supporting Information Table S5). Therefore, the Han River system and mountains have been important barriers to population dispersal and gene flow, generating a north–south genetic break in the QBMs.

### 4.2 | Pleistocene climate changes and divergence

Based on the phylogeographic and distribution modelling analyses, we infer that independent refugial origins for Lineages I–III were located in the Qinling, Bashan and perhaps Minshan Mountains, followed by vicariance and divergence among multiple ancestral refugial populations. The effects of the glaciations on genetic divergence among *D. magna* populations can be distinguished at different geographical and temporal scales: recent divergence associated with colonization of individual sky islands and historical divergence associated with displacement to multiple glacial refugia (Knowles, 2001; Muster & Berendonk, 2006). The divergence pattern driven by glaciation fluctuation was similar to that of other montane insects in Europe and North America (Knowles, 2001; Knowles & Massatti, 2017; Lohse et al., 2011; Muster & Berendonk, 2006; Wachter et al., 2016). In addition, some populations with extremely low diversity (e.g. ZQ in Table 1) may reflect that genetic drift, rather than gene flow, played a dominant role in structuring the population differentiation, as in the grasshopper and wolf spider (Knowles, 2001; Muster & Berendonk, 2006).

The Minshan Mountain was a less suitable area during glaciation (Supporting Information Figure S3); therefore, Lineage I probably had experienced a bottleneck, and some individuals migrated eastward to the Bashan Mountains (eg. LP, MCS) and northeastward to the Qinling Mountains (eg. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity (e.g. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity (eg. LP, MCS) and northeastward to the Qinling Mountains (eg. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity (eg. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity (eg. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity (eg. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity (eg. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity (eg. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity (eg. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity (eg. TTH; Figure 1). For the populations from the Bashan Mountains, there was an evident trend towards reduced genetic diversity.
Mountains. The multiple isolated microrefugia usually promote population differentiation, followed by short-distance colonization northward and recent divergences (Avise, 2000). The founder effects associated with postglacial colonizations had resulted in latitudinal gradients of genetic diversity across the species’ range of *D. magna*, from ice age refugia to high-latitude range margins, as in other insects (Drees et al., 2010; Hewitt, 2004).

Pleistocene climate fluctuations played a dominant role in shaping the demographic history, genetic structure and distribution of the world biota (Hewitt, 2003, 2004). Species migration is the main biological response to climate changes (Huntley & Webb, 1989). For the cold-adapted species such as *D. magna*, the latitudinal migration was usually accompanied by altitudinal migration (Nève & Verlaque, 2010). During the Pleistocene ice age, the *D. magna* populations likely migrated to the favourable conditions of lower elevation, or migrated southward to ice-free areas. As the climate warmed during the post-glacial period, they migrated back to higher elevation, or expanded the range northward and colonized much larger areas (Figure 1). Consequently, the *D. magna* group is now restricted to high mountain systems and high latitudes, as described in Varga and Schmitt (2008).

### 4.3 | Taxonomic implication

The three lineages of *D. magna* were highly divergent (Figure 2), and possibly in a process of incipient speciation. The mean K2P distances among the three lineages of *D. magna* (0.019–0.024) overlap with the interspecific distance (0.022–0.028) in the *D. magna* group (Supporting Information Table S2), reinforcing the inference of incipient speciation in *D. magna*. Speciation processes of many organisms were deeply influenced by the Pleistocene glaciations (Hewitt, 2000, 2004), and speciation is often an important evolutionary consequence of climate-induced range shifts and multiple refugia (Sánchez-Guillén, Córdoba-Aguilar, Hansson, Ott, & Wellenreuther, 2016; Shafer et al., 2010). During glacial advances, species differentiated into distinct genetic lineages across their extended geographic distributions, and repeated allopatric disjunctions accumulated genetic differences and accelerated speciation by selection for different adaptations (Habel, Schmitt, & Müller, 2005; Hewitt, 1996, 1999; Santucci, Emerson, & Hewitt, 1998).

The three divergent lineages (I–III) in *D. magna* likely warrant species status, and further morphological and biological studies are needed to confirm this inference.

### ACKNOWLEDGEMENTS

We are grateful to Lu Jiang, Shuang Xue, Ying Miao, Mei Liu and Li-Xuan Kou for assistance in field sampling. We thank Linda Lait and Jacopo D’Ercole for help with phylogeographic analyses. We also appreciate the anonymous reviewers for valuable comments on the revision of the manuscript. This research was supported by the National Natural Science Foundation of China (Grant nos. 31672341 and 31172125).

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### REFERENCES


**SUPPORTING INFORMATION**

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**How to cite this article:** Hu G-L, Hua Y, Hebert PDN, Hua B-Z. Evolutionary history of the scorpionfly Dicerapanorpa magna (Mecoptera, Panorpidae). Zool Scr. 2019;48:93–105. https://doi.org/10.1111/zsc.12326