

Research

Tectonic evolution of the Tethyan region created the Eurasian extratropical biodiversity hotspots: tracing *Pireneitega* spiders' diversification history

Zhe Zhao, Lili Shao, Fengyuan Li, Xiaoqing Zhang and Shuqiang Li

Z. Zhao (<https://orcid.org/0000-0002-0781-0204>), L. Shao, F. Li, X. Zhang and S. Li (<https://orcid.org/0000-0002-3290-5416>) ✉ (lisq@ioz.ac.cn), Key Laboratory of Zoological Systematics and Evolution, Inst. of Zoology, Chinese Academy of Sciences, Beijing, China.

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The withdrawal of the Tethys Sea and the formation of the Alpine-Himalayan orogenic belt profoundly impacted the distribution and composition of terrestrial biota in Eurasia. However, studies that have explicitly addressed the potential links between the series of tectonic activities in the Tethyan region and the formation of extratropical biodiversity hotspots in the Alpine-Himalayan belt are rare. Spiders in the genus *Pireneitega* (Agelenidae) are found throughout Eurasia and show high species richness in these hotspots. Thus, *Pireneitega* spiders may serve as a model group to shed light on how past tectonic events shaped Eurasian hotspots. To reconstruct the spatial and temporal evolution of *Pireneitega* spp., we conducted an integrative historical biogeographical analysis using thousands of novel DNA sequences and five novel transcriptome sequences from different species. Species distribution modelling based on complete geographical distribution information was used to assess the ecological preferences and the potential ecological interchangeability of *Pireneitega* species. Our study suggests that the rapid expansion of *Pireneitega* in Eurasia benefitted from regression of the Tethys Sea in the early Oligocene. Most *Pireneitega* species are distributed allopatrically, but in similar niches. The diversification of *Pireneitega* species relied on invading numerous new isolated habitats created by the uplift of Alpine-Himalayan mountains during the Miocene (wet valley model). These results imply that the formation of Alpine-Himalayan hotspots was driven by the series of tectonic events in the Tethyan region during the Oligocene–Miocene.

Keywords: biogeography, ecology, evolution, phylogenetics, speciation, spider

Introduction

The Alpine-Himalayan belt is a continuous orogen stretching from Spain to southeast Asia (Fig. 1). The area is famous not only for having the most spectacular mountains and landscapes on the planet, but for harboring extraordinarily high terrestrial biodiversity. In its entirety, it comprises six of the Earth's 36 biodiversity hotspots (Mittermeier et al. 2011, Noss et al. 2015). In the Mesozoic, however, most of the area was an ocean – the Tethys Sea. Since the Cenozoic, due to incremental convergence,





Figure 1. The Alpine-Himalayan belt supports six biodiversity hotspots (labeled with green text) and a diverse radiation of *Pireneitega* spiders. Indicated here are confirmed distributions of all 36 known species of *Pireneitega*. Species are indicated by number (which are used throughout the text) and all species names are provided in the figure.

collision and deformation of tectonic plates from Gondwana-derived fragments and the Eurasian continent (Rosenbaum and Lister 2002), the Tethys Sea has gradually regressed to the modern Mediterranean Sea (Sun et al. 2016). Surprisingly, despite increasingly detailed geological reconstructions and biogeographical studies, few studies have explicitly addressed the spatial and temporal relationships between the tectonic evolution of the Tethyan region and the development of Alpine-Himalayan hotspots. Because of the difficulty of sampling over large spatial scales, the biodiversity of Alpine-Himalayan hotspots often are studied by regions, such as in the Mediterranean (Bidegaray-Batista et al. 2014, Opatova et al. 2016), the Himalayas (Favre et al. 2015) and the mountains of southwest China (Xing and Ree 2017), respectively. Most studies concluded that the rich species diversity found in these areas was caused by the uplifts of the mountains since the Miocene. This concept has been questioned, though, because the geological history is often incongruous with the timing of the biological radiation. In some cases, the uplift obviously predates the radiation in plants or animals, while in others, speciation caused by recent (post-Miocene) uplift is often absent in young clades (Renner 2016). Spicer (2017) concluded that the rich biota of southern Asian biodiversity hotspots probably have an ancient origin (Paleogene), but this outcome is related more to the development of the Asian monsoon systems. Besides, as widely accepted mechanisms, the ‘sky islands’ and rate hypotheses are often used to explain the high species richness in montane hotspots (He and Jiang 2014, Xing and Ree 2017). The generality of these hypotheses are worth testing with additional taxa because montane environments vary regionally, and not all organisms are equally sensitive to altitude. Therefore, fascinating questions remain, such as: when were these Alpine-Himalayan hotspots formed? Is hotspot formation closely related to tectonic events? And, if so, are the mechanisms operated on diverse taxa the same?

Generally, by comparing time-calibrated phylogenies to areas of distribution, temporal congruence between cladogenic, geological and climatic events can be tested. Additionally, niche modeling and quantitative comparisons have contributed to understanding potential species diversification mechanisms (Price et al. 2014, Lee and Ho 2016, Manafzadeh et al. 2017). Therefore, the study of diversification mechanisms at a continental level is hindered only by the shortage of high-resolution, large-scale data resources in multiple taxa. Because these data are available for some well-documented birds, these have frequently been used as model organisms to address questions regarding biotic diversification and distribution (Price et al. 2014, Quintero and Jetz 2018). However, because of their migration capability and young origins, birds may be ideal model group for studies of biodiversity, but not for biogeography. In contrast, low vagility arthropods seem to have obvious advantages if sampling integrity is ensured (Wood et al. 2015). Common spiders of the genus *Pireneitega* (Agelenidae, Coelotinae) are distributed throughout the Eurasian mountains (e.g. the Pyrenees, Caucasus, Tian and Hengduan mountains) and lowland habitats (e.g. hills and flatlands in east Asia). There are 36 known species (World Spider Catalog 2018) in this monophyletic genus (Zhao and Li 2017), and about 80% of them are primarily distributed in the Alpine-Himalayan belt. Studying the diversification processes of *Pireneitega* spiders thus offers a unique opportunity to disentangle the origin of Eurasian extratropical hotspots.

Herein, we apply an integrative biogeographic method to reconstruct the diversification history of *Pireneitega* using 1115 novel DNA sequences of multiple genes (Sanger) from 201 local populations of 31 species (Fig. 1). Five more novel transcriptome sequences (HiSeq) from different species were sequenced and combined with a wider transcriptome dataset of spiders (Garrison et al. 2016) to enhance the divergence

dating for *Pireneitega* spp. Additionally, species distribution modeling based on 319 unduplicated geographical coordinates was used to assess the ecological preferences and the potential ecological interchangeability of *Pireneitega* species. We aim to reveal the potential links between tectonic activities of the Tethyan region and the formation of extratropical hotspots, and to propose a novel model that underlie current patterns of Eurasian biodiversity.

Material and methods

Taxon sampling and laboratory protocols

Our sampling included 201 local populations of 31 *Pireneitega* species that covered all major geographic regions across Eurasia (Supplementary material Appendix 1 Fig. A1). Samples from the type localities of endemic taxa were included, and multiple samples were used to represent widespread species. Species names, localities, geographical coordinates, DNA sequences and GenBank accession numbers of all *Pireneitega* samples are listed in the Supplementary material Appendix 1 Table A1. Fresh spider specimens for DNA extraction were collected, identified, preserved in 95% ethanol and stored at -20°C . For details of DNA extraction, amplification (PCR conditions and primer usage) and sequencing (Sanger) please see Zhao and Li (2017). Ultimately, 1115 de novo sequences were obtained from four nuclear genes (H3, wingless, 18S, 28S) and four mitochondrial genes (cox1, nad1, 12S, 16S). Live spiders belonging to five *Pireneitega* species were flash frozen in liquid nitrogen after starvation treatment for transcriptome sequencing. The mRNA was extracted using the TRIzol total RNA extraction method (Life Technologies). Purification of mRNA, library preparation, sequencing (Illumina HiSeq™ 2500) and quality control were done by Novogene Bioinformatics Technology Co. Next, clean reads were assembled using default parameters in Trinity v2.0.5 (Grabherr et al. 2011). Other detailed information and parameters followed Shao and Li (2018). All specimens used in this study were legally collected. All voucher specimens were deposited in the Inst. of Zoology, Chinese Academy of Sciences, Beijing, China.

Molecular datasets and sequence alignment

The transcriptome dataset was used to help calibrate the divergence times of *Pireneitega*. The 84-taxon dataset consisted of 79 available transcriptome sequences (including 78 spiders and one species of *Amblypygi* as an outgroup) which were published recently (Garrison et al. 2016), and five novel *Pireneitega* transcriptome sequences (Supplementary material Appendix 1 Table A2). The known transcriptome data were downloaded from GenBank and were quality-checked and trimmed using the FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/) and FASTXToolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html) before

assembly. Putative orthologs were determined for each species with HaMStR v13.2.3 (Ebersberger et al. 2009) using the Arthropoda core ortholog set. The resulting orthologous groups (OGs) were further processed by discarding amino acid sequences with lengths shorter than 75 bp. Only OGs that could be found in all taxa were selected and aligned with MAFFT v7.222 (Katoh and Standley 2016) using the L-INS-i method, followed by scoring and trimming with Aliscore (Kück et al. 2010) and Alicut (http://zfmk.de/web/ZFMK_Mitarbeiter/KckPatrick/Software/AliCUT/Download/index.en.html). The final 840 OGs were concatenated using FASconCAT v1.0 (Kück and Meusemann 2010) after alignment. The corresponding nucleotide sequences were concatenated as the final dataset. To minimize any negative effects of saturation, only the first and second positions of nucleotide data were used in the transcriptome dataset, with a final length of 466 912 bp.

The second dataset contained 248 taxa with eight genes used to help reconstruct the spatiotemporal evolution of *Pireneitega*. It comprised 201 *Pireneitega* taxa as the ingroup and 46 other species of Agelenidae plus one species of Amaurobiidae as outgroups. Protein-coding gene sequences were translated to amino acids before alignment with ClustalW embedded in BioEdit v7.0.9.0 (Hall 1999), they were then back-translated. To reduce misalignments, ribosomal RNA genes were first aligned in MAFFT with default parameters, then ambiguous regions were removed manually. Re-alignment used ClustalX v2.0.9 (Larkin et al. 2007) according to the guide tree generated by the protein-coding genes. Sequence assembly and minor manual adjustments were performed using BioEdit. Finally, the 248-taxon dataset contained 6267 aligned nucleotides (with ~13% missing data).

Phylogenetic analyses

PartitionFinder v2.1.1 (Lanfear et al. 2012) was used to select the optimal partitions and substitution models for both datasets. For the transcriptome dataset, maximum-likelihood (ML) topologies were inferred with RAxML v8.2.10 (Stamatakis 2014) using partitions as indicated, associated best-fit substitution models, and the GAMMA parameter to model rate-heterogeneity. Nodal support was measured with 1000 fast bootstrap pseudoreplicates. For the 248-taxon dataset, phylogenetic relationships were inferred using both ML and Bayesian inference (BI) methods. Model selection for ML analysis divided the dataset into eight partitions by gene, and the GTR+I+G model was favored for each partition. Similar results were obtained for BI analyses, except different models were selected for H3 (HKY+I+G), wingless (SYM+I+G) and 18S (K80+I+G). ML analyses were conducted in RAxML using the substitution model GTRGAMMAI for all partitions. A rapid bootstrap analysis of 1000 replicate ML inferences initiated with a random starting tree was performed for each dataset to determine the best-scoring ML tree and nodal support. BI analyses were conducted in MrBayes v3.2.1 (Ronquist and Huelsenbeck 2003) with posterior distributions estimated by Markov

chain Monte Carlo (MCMC) sampling. The corresponding model was selected for each partition. Two simultaneous runs with four MCMC chains were conducted for 20 million generations to ensure the average standard deviation of split frequency was below 0.01. The MCMC chains were sampled every 1000 generations. Tracer v1.6 (Rambaut et al. 2014) was employed to monitor the mixing of the MCMC chains, and the first 40% of sampled trees were discarded as burn-in before summarizing.

Molecular dating analysis

There are several fossil Agelenidae but none within *Pireneitega*, so the divergence times of *Pireneitega* species were calibrated based on the latest reliable dating framework for spiders (Shao and Li 2018) and coelotines (Zhao and Li 2017). The size of the transcriptome dataset precluded the use of some computationally intensive dating methods, such as BEAST (Drummond and Rambaut 2007), so the package mcmcree in conjunction with baseml of PAML v4.8 (Yang 2007) was used to estimate node ages and reduced the computational load by approximating the likelihood. The mcmcree analysis was conducted using the uncorrelated relaxed clock model and based on the topology inferred by RAxML using the transcriptome dataset. A total of seven calibration points was used for time estimation, and all calibrations were implemented as uniform priors with soft bounds, providing a very conservative estimate (Supplementary material Appendix 1 Table A3). The most general GTR+G model was chosen for the nucleotide dataset, and a gamma distribution with four rate categories was applied for among-site rate heterogeneity. Posterior distributions of divergence times were estimated using Markov chain Monte Carlo sampling, with samples drawn every 500 steps over a total of 2 000 000 steps, after a discarded burn-in of 200 000 steps. Each analysis was run in duplicate and results were visually inspected in Tracer to ensure that the effective sample sizes (ESS) of all parameters were above 200.

For the 248-taxa dataset, divergence times were estimated using BEAST v2.4. The four recalibration points (Supplementary material Appendix 1 Table A4) used in the analysis were assigned to the corresponding lineages based on fossils of *Tegenaria* (Agelenidae) and the results obtained from the transcriptome dataset (Supplementary material Appendix 1 Fig. A2). BEAST analysis used the uncorrelated relaxed lognormal clock with the branching prior set under both Yule and Birth–Death speciation processes. The GTR+I+G model was used for all eight partitions as in the BI analyses. To obtain a reliable result, the MCMC tree searches were run on the CIPRES web portal (Miller et al. 2010) for 60 million generations sampling every 2000 generations. Finally, the maximum clade credibility tree was calculated using the BEAST package TreeAnnotator based on 25 000 trees that were obtained after a discarded burn-in of the first 5000 samples. Length of analysis and burn-in proportion were inspected and confirmed by Tracer.

Historical biogeographical inference

To understand the historical spatiotemporal evolution of *Pireneitega*, seven areas (south Europe, Caucasus and Anatolian Plateau, the Pamir Plateau and northern Tibet, the mountains of central Asia, the mountains of southwest China, south China and surrounding areas and north China and surrounding areas) were delimited for ancestral area reconstruction based on climate, geographical divisions and the phylogeny and distributions of *Pireneitega* species (Fig. 2b, Supplementary material Appendix 1 Table A5). The analyses were performed based on a consensus species divergence time tree without outgroups. The 31 *Pireneitega* species were assigned to areas according to their distributional data (Supplementary material Appendix 1 Fig. A3). Biogeographic inferences were obtained by using the DEC and DEC+J models in BioGeoBEARS (Matzke 2014). Two time slices were used (33–17 Ma, 16–0 Ma) to reflect the descent of the dispersal probabilities of Eurasian animals caused by the increasing aridification of central Asia since the Mid-Miocene (Miao et al. 2012, Favre et al. 2015). The ancestral area at each node was limited to no more than two areas. The dispersal probabilities (0.01, 0.1, 0.5, 1.0) and limitations (0, 1) were assigned to all adjacent areas in each time slice (Supplementary material Appendix 1 Table A6, A7). The AICc was used as a reference to compare all models and determine the best-fitting model.

Diversity dynamics analysis

Changes in diversification-rates within *Pireneitega* by regions were evaluated using the packages ‘ape’ (Paradis et al. 2004), ‘GEIGER’ (Harmon et al. 2008), ‘TreePar’ (Stadler 2011) and ‘BAMM’ (Rabosky 2014) in the R environment (R Core Team). Based on the occurrence records of coelotines (Supplementary material Appendix 1 Table A8), a conservative estimation of species richness for each clade is shown in Supplementary material Appendix 1 Table A9. To monitor the accumulation of lineages over time, multiple lineage-through-time (LTT) plots were generated based on 1000 random BEAST trees under the Birth–Death model with mean node ages. The variation of the diversification rate shown on the LTT plots was calculated by TreePar. TreePar analyses were run based on the bd.shifts.optim function. The run is at first allowed one shift, and rates were estimated in 0.1 Ma steps between 0 and 40 Ma. Then, two shifts were allowed by fixing the first shift, and more shifts were added in this way, until the best value was obtained. For BAMM analyses, a prior block was automatically generated by the function setBAMMpriors, then run for 10 million generations and sampled every 1000 generations. Convergence of the run was assessed by the log-likelihood trace and effective sample sizes of the log-likelihood. The macroevolutionary cohort analysis was performed using the function getCohortMatrix. Evolutionary rate dynamics were analyzed and visualized using BAMMtools.

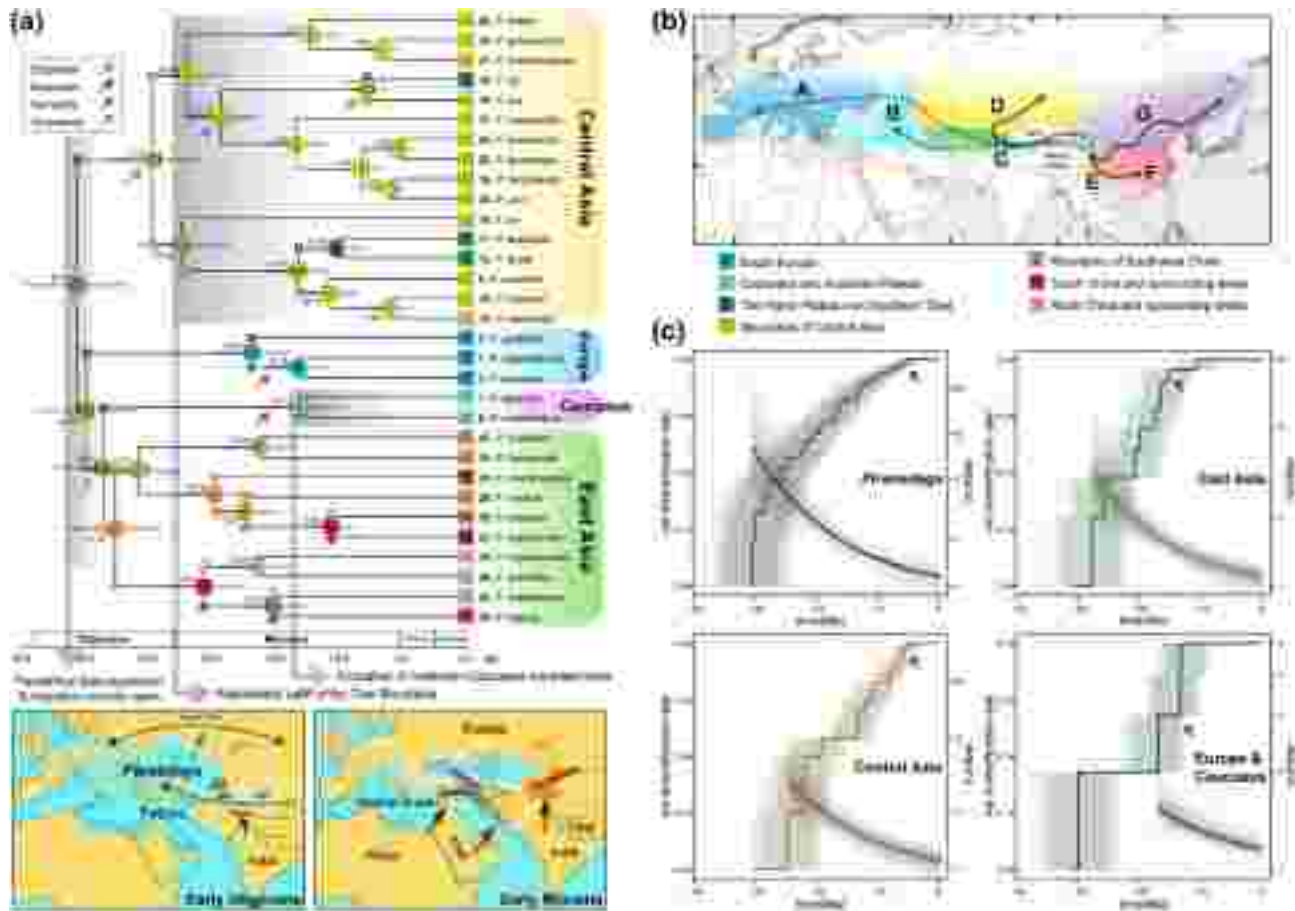


Figure 2. The diversification history of *Pireneitega*. (a) The simplified phylogenetic topology of *Pireneitega* species with molecular clock dating and biogeographic analysis results. The bars on nodes show the 95% probability distributions of the divergence time between clades. The squares on nodes show the most likely ancestral range reconstructed under the DEC model. The nodes indicated by arrows show when an anagenetic or cladogenetic event occurred. The maps under the tree show the main tectonic evolution in the Tethyan region during the Oligocene and the Miocene. The short arrows show the directions of continental movement (black) and withdrawal of the Tethys Sea (blue). The two dashed lines show the migration corridors for terrestrial animals. (b) Geographical areas used in the biogeographical analyses, and the origin and dispersal routes of *Pireneitega* species. (c) The diversification rates dynamics of *Pireneitega* lineages, including the lineage-through-time (LTT) plot with 95% confidence intervals, and the net diversification rates through time with 95% confidence intervals. The upper left panel is for the entire *Pireneitega* species analysis, whereas the other three panels pertain to taxa in geographic regions. The arrows show the rate shifts detected by TreePar.

Potential distribution modeling and niche comparisons

To examine the ecological variables responsible for niche modeling and the spatial distribution patterns of *Pireneitega*, in addition to the 201 sampling localities, we collected and confirmed 118 unduplicated occurrence records from the literature (Supplementary material Appendix 1 Fig. A1, Table A8). A total of 319 usable geographical coordinates of all 36 *Pireneitega* species were used in the niche and potential distribution modeling analyses with MaxEnt v3.4.0 (Phillips and Dudik 2008). Because the MaxEnt analyses were limited to each species having at least three unique occurrence points, a buffer of 1 decimal degree search radius was applied to point localities in place of modelling for the 14 species that only have one or two occurrence records. The MaxEnt analyses

for the other 22 species were implemented using the following parameters: random test percentage=25, regularization multiplier=1, maximum number of background points=10 000, replicates=10, replicated run type=cross validate, threshold=minimum training presence. To optimize the performance of MaxEnt and correct sampling biases (Elith et al. 2011), we used a bias file representing a Gaussian kernel density of all species occurrence localities sampled at a 10 decimal degree search radius (Brown et al. 2014). The habitat selections and potential distributions for the current climate of *Pireneitega* species were predicted using the 19 standard bioclimatic variables as well as non-climatic variables, such as altitude, aspect, slope, land cover, solar radiation and water vapor pressure (Supplementary material Appendix 1 Fig. A4). The bioclimatic layers were obtained from WorldClim 2 (Fick and Hijmans 2017), elevational layers were obtained

from SRTM 90m Digital Elevation Database v4.1 (<http://gisweb.ciat.cgiar.org/TRMM/SRTM_Resampled_250m/>), and the land cover layers were downloaded from the European Space Agency GlobCover Portal (<http://due.esrin.esa.int/page_globcover.php>). Here, all environmental grids were adjusted to a 2.5 arc-min resolution (roughly 5×5 km cells). For statistics and visualization, all distributed layers were projected to Asia Alber's Equal-Area Cylindrical projection in ArcMap at a resolution of 3025 km² (55×55 km) grid cells (Cai et al. 2018). Low probability (< 20%) predicted potential distributions were removed. A species was not included if its range covered less than 50% of the area of the grid. The real and potential distributions of all species were counted in grid cells.

Niche overlap was assessed with the relative rank (RR) metrics (Warren and Seifert 2011) to test the potential ecological interchangeability of *Pireneitega* species (Rader et al. 2005). Niche identity tests were conducted for 27 pairs species (RR value > 0.7) using 100 randomized pseudoreplicates to evaluate if the distribution modeling RR values were statistically different (one-tailed test) than expected under the null distribution. Both RR and niche identity tests were performed with ENMTools 1.4.4 (Warren et al. 2010). The matrices of niche overlap and of distributed overlap were visualized using the R package 'corrplot' (Wei and Simko 2017).

Isolation by regions

Isolation in different regions was evaluated by the potential relationship of geographic distance and divergence time. This was inspired by using the concept of isolation by distance to explain the correlation of genetic differences among populations and their spatial distributions (Ballesteros and Hormiga 2018). In *Pireneitega* spp., the lack of overlap in geographic ranges and their ground-based dispersal (*Pireneitega* spp. do not use their silk as sails to facilitate aerial dispersal (ballooning), unlike many other spiders) leads to a positive correlation between geographic and genetic distance. Thus, the varied correlation coefficients (ratios) can be used to reflect the different degrees of isolation by region. In this study, we replaced the genetic distance with divergence time because the latter are transformed from genetic distances by multiple genes which is a better method than considering genetic distances from a single gene. Moreover, this method avoids the problems of saturation associated with nucleotide mutation in genetic distance analyses and increases sampling in geographic distance analyses (some samples with coordinate records that lack molecular data can be used). Geographic distances (in kilometers) were calculated from the geographic coordinates with the aid of the R package 'geosphere' (Hijmans 2016), and divergence times (in million years) were extracted from the BEAST tree using the R package 'ape', followed by visualization via a scatter diagram (Supplementary material Appendix 1 Fig. A5). The trend line is based on the matrix of mean divergence times and mean geographic distances.

Results

The ML and Bayesian inference (BI) analyses of the 248-taxa (8 genes) datasets produced quite similar topologies (Supplementary material Appendix 1 Fig. A6). The genus *Pireneitega* is strongly supported as monophyletic in both analyses (ML bootstrap = 99, BI posterior probability = 1.00). The lineages of *Pireneitega* are divided into four major clades, consistent with geographic regions. Both analyses supported the relationships central Asia + (south Europe + (Caucasus + east Asia)), though the support values of the monophyletic group (south Europe + (Caucasus + east Asia)) are not strong (ML bootstrap = 68, BI posterior probability = 0.78). The four regional clades are separated by notably short branches. Among the four clades, the central Asian clade contains the richest species diversity, and the east Asian clade contains two widely distributed species, *P. spinivulva* and *P. luctuosa* (Fig. 1). The ML analyses of the 84-taxa transcriptome dataset yields a well-resolved phylogenetic tree (Supplementary material Appendix 1 Fig. A7) from which divergence times were estimated. The node ages estimated by the transcriptome dataset are provided in the Supplementary material Appendix 1 Fig. A2. The divergence times among *Pireneitega* were recalibrated using the 248-taxa dataset (Supplementary material Appendix 1 Fig. A8), and there is no obvious conflict between the results obtained under both Yule (lnL = -74 318.13) and Birth-Death (lnL = -74 309.75) speciation processes (Supplementary material Appendix 1 Table A10). The origin of the genus *Pireneitega* (27–35 Ma) already falls to early Oligocene, then quickly diverged into four major clades (Fig. 3a).

Our biogeographic reconstructions corroborate a central Asian origin for *Pireneitega*, but different speciation processes and dispersal routes were obtained respectively under the DEC (lnL = -59.23, AIC = 92.34) and DEC+J (lnL = -43.17, AIC = 122.45) models (Supplementary material Appendix 1 Fig. A3). Under the DEC model, the ancestral distributions of crown nodes are often two adjacent areas, species diversification is mainly the result of sympatry and vicariance events, and there are extinction events in the European and Caucasian clades (Fig. 2a). The DEC model shows *Pireneitega* spiders originated on the Pamir Plateau–northern Tibet (C), then spread northward to the central Asian mountains (D), westward to Europe (A) and the Caucasus (B) and eastward via the Hengduan Mountains (E) to east Asia (Fig. 2b). The 'Pamir Plateau–northern Tibet' area is not only the center of origin but also an important corridor for east–west dispersal. Using the DEC+J model, the ancestral distributions are mostly unique areas, and founder events occur frequently, replacing other cladogenetic events in the DEC model. The DEC+J model shows *Pireneitega* spiders originated in the central Asian mountains, then spread westward to the Caucasus and Europe and then from the Caucasus to south China and surrounding areas.

The diversification rates analyses show *Pireneitega* lineages experienced an initially high diversification in the early Oligocene, and the diversification rate gradually decreased

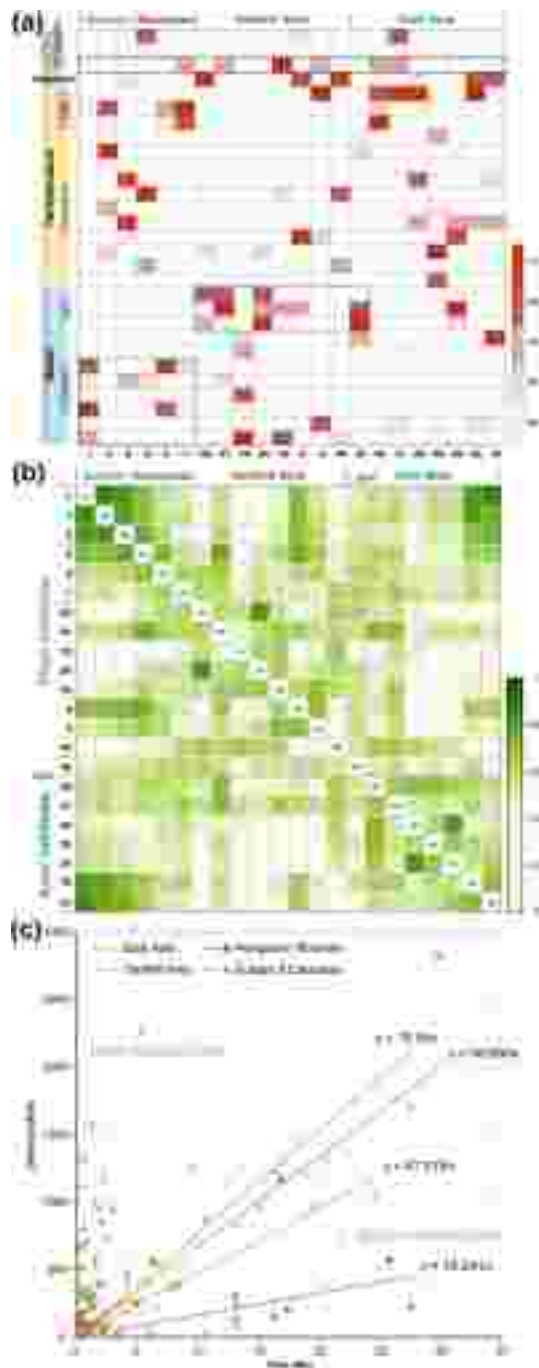


Figure 3. (a) The statistics of the top five environmental variables with the highest gain in the Jackknife test (MaxEnt modeling) for each *Pireneitega* species. The darker the color, the more important the environmental variable is to the species. The numbers along the horizontal axis represent different species. For details of each environmental variable, please see Supplementary material Appendix 1 Fig. A4. (b) The matrix shows pairwise comparisons of the niche of 22 *Pireneitega* spp. measured with the relative rank index (RR; Warren and Seifert 2011): values near 1.0 are considered identical or highly interchangeable. (c) The estimated relative geographical isolation *Pireneitega* spiders in different regions. Lower trend line slopes indicate stronger isolation.

through time (Fig. 2c and Supplementary material Appendix 1 Fig. A9a–b). The highest net diversification rate of each clade appeared in the early evolutionary stage. The macroevolutionary cohort analysis shows that all *Pireneitega* species share a common macroevolutionary rate dynamic (Supplementary material Appendix 1 Fig. A9c), and there is a 93% probability that no diversification rate shift was detected among different lineages. However, TreePar analyses supported that a single diversification rate shift occurred in each major clade (arrows, Fig. 2c) and rejected ($p < 0.05$) the models with a constant rate and more than one rate change (Supplementary material Appendix 1 Table A11). These shifts are related to the end of lineage accumulation in east Asian and central Asian clades and the start of lineage accumulation in the European and Caucasian clade (obviously lagging behind the other areas).

MaxEnt niche modeling was performed for 22 *Pireneitega* species, and AUC values of all species are close to 1.0. These models indicate that habitat suitability of *Pireneitega* species extends throughout Eurasia, which is wider than their actual distribution (Supplementary material Appendix 1 Fig. A10). Jackknife analyses show the regularized training gain of each variable and identify which variables contribute the most individually (Fig. 3a and Supplementary material Appendix 1 Fig. A4). Although the most important variables vary from species to species, there are consistencies between regions. Overall, the most important environmental factors are water and temperature, and these contribute more than vegetation and topography. Water is as important as temperature in the Mediterranean area but more important in central Asia and less important in southern east Asia. The water supply modes in mid-high latitude areas differ, with a strong reliance on rainfall in the Mediterranean (Fig. 3a, green dashed line) and a strong reliance on moisture in the air in the inland mid-high latitudes (Fig. 3a, blue dashed line). For the geographical factors, slope (Fig. 3a, grey dashed line) seems to contribute more than altitudes and aspects. The pairwise niche overlap of *Pireneitega* species are summarized in Fig. 3b and Supplementary material Appendix 1 Table A12. Although the probability of finding species in sympatry is rare, the environmental niches of most species are similar, even those that are separated by large distances (e.g. species from Europe and east Asia). Generally, the closer the geographic distribution of two species, the more their habitat suitability overlaps. Low overlap percentages appeared between the low latitudes and extratropical areas. The results of niche identity tests show some species from the same area or adjacent areas are even ecologically interchangeable (Supplementary material Appendix 1 Fig. A11). The geographic isolation analysis shows that the greatest geographical isolation occurs in the mountains of southwest China, followed by central Asia, and then the Mediterranean and east Asian lowlands (Fig. 3c).

Discussion

Our phylogenetic analyses and molecular dating results are consistent with previous studies on spiders (Garrison et al. 2016)

and coelotines (Zhao and Li 2017). The low support values of the monophyletic group (south European + (Caucasian + east Asian)) was probably caused by the short branches among the four major clades on the phylogenetic trees, which indicate a rapid expansion and diversification at the initial stage of *Pireneitega* evolution (Supplementary material Appendix 1 Fig. A6). This also is suggested by the high net diversification rate shown in the early evolutionary stage of *Pireneitega* (Fig. 2c). Though the likelihood value and AIC preferred the DEC + J model (J parameter modeled long-distance or 'jump' dispersal) in biogeographic reconstruction, the results inferred by the DEC model seem more reasonable for two reasons. 1) Founder-events (favored by the DEC + J model) likely are crucial to island ecosystems but less so in continental systems, and the wide use of DEC + J model has been questioned recently (Matzke 2014, Ree and Sanmartín 2018). 2) The absence of geographically distant sister species and no record of ballooning dispersal behavior show that ground-dwelling *Pireneitega* spiders lack the ability to disperse over long distances and 'jump' from the Caucasus to south China directly. Therefore, *Pireneitega* likely originated in northern Tibet and spread to Eurasia, as shown in Fig. 2b. The predicted shift of diversification rate points in the TreePar analyses occur either at the beginning or the end of species diversification, which does not alter the results of the BAMM analysis. Thus, species diversification rates in *Pireneitega* gradually decrease through time without obvious rate shifts.

Diversification is related to tectonic activities

The phylogenetic analyses show that geographical isolation seems to play a key role in speciation and the division of major clades within *Pireneitega*, as the four major clades of *Pireneitega* are distributed in four discontinuous regions divided by mountains, deserts and plateaus. Unexpectedly, species in Europe and the Caucasus are more closely related to the geographically distant east Asian species, which are sister to the central Asian species. This result indicates that there was once a barrier between central Asia and other regions. The Paratethys Sea was a large marine seaway that connected the Mediterranean Sea with central Asia during the Early Cenozoic. Due to the contraction and northward growth of the Pamir Plateau caused by the collision of India with Asia, the Paratethys gradually regressed westward from the Tarim Basin via the Tajik Basin beginning in the Middle–Late Eocene (47–39 Ma) (Carrapa et al. 2015, Sun et al. 2016). Since the Early Oligocene (33 Ma) the sea has gradually become a terrestrial habitat, opening a migration corridor for European and Asian terrestrial animals (Rögl 1998, Hou et al. 2011) (Fig. 2a). *Pireneitega* originated 27–35 Ma, and the main regional clades also formed rapidly during the same period. These results suggest that the rapid spread and colonization of *Pireneitega* in all of Eurasia benefitted from the Paratethys regression.

The diversification of Alpine-Himalayan *Pireneitega* species should be related to the orogenic activities there. The species of the central Asian clade are found throughout the Tian

Mountains area, and the sustained diversification of the clade lasted throughout the Miocene (24.6–5.2 Ma), consistent with the reactivated uplift of the Tian Mountains (Fig. 2a). Although the erosion of the Tian Mountain belt dates back to the Palaeozoic, the present-day Tian Mountains have risen significantly in elevation since the Miocene in response to the India–Asia collision (Manafzadeh et al. 2017). The split of the Caucasian clade (13.11 Ma) coincided with tectonic activities in the Caucasian area during the Miocene. The Arabia–Eurasia convergence began in the Late Oligocene and culminated in the Early Miocene. The collision drove the formation of the Iranian and eastern Anatolian plateaus and the accelerated uplift of the Caucasus Mountain belts during the Middle Miocene (Manafzadeh et al. 2017). The rapid uplift of the Hengduan Mountains began in the Late Miocene (Sun et al. 2011), whereas the diversification of *Pireneitega* in the area began 27.5 Ma (Late Oligocene). Though predating the orogenesis of the Hengduan Mountains, the diversification time is still included in the diachronous uplift of Tibetan Plateau (Favre et al. 2015). The only exception is the divergence of two Pyrenees species beginning during the Middle Miocene (16.84 Ma), significantly later than the formation of the Pyrenees, from the Late Cretaceous to the Late Oligocene (Vergés et al. 2002). Including the two Pyrenees species, only three *Pireneitega* species are widely distributed in south Europe (Italy and Pyrenees), but the colonization of *Pireneitega* spiders in Europe dates to the Early Oligocene (29.84 Ma). Our biogeographic reconstructions imply that the European clade probably suffered extinction events in its evolution process (Fig. 2a). The Quaternary glaciations had a stronger effect on European species than on those in central and east Asia (Kreihenwinkel et al. 2016). There are no studies suggesting that there was a continental ice sheet in central and east Asia, as occurred in Europe. In Asia, due to the arid inland climate and the barriers of plateaus, the areas between local glaciers will form potential shelters. Thus, the three extant south European species should be the relicts of former diverse communities, and the long evolutionary history with few species is a result of a high extinction rate. In conclusion, the diversification history of *Pireneitega* spiders implies that the current distribution of Alpine-Himalayan biodiversity is tied to a series of tectonic activities in the Tethyan realm, and the origins of these diversity hotspots perhaps could be traced back to the Oligocene–Miocene.

Mechanisms of diversification in Alpine-Himalayan hotspots

High speciation rate is a popular mechanism underlying the high species richness in the tropics and biodiversity hotspots (Rolland et al. 2014). For example, the uplift of Himalayas and the Hengduan Mountains has significantly increased the speciation rate of local animals and plants (Favre et al. 2015, Xing and Ree 2017). In our case, however, we detected no fluctuation in speciation rate due to Miocene uplift (Fig. 2c). The highest speciation rate occurred in the Early Oligocene, although this evidently was in response to rapid expansion

(occupation of new habitats) and spatial (distance) isolation caused by the withdrawal of the Paratethys rather than vicariant events caused by mountain uplifts. The gradually decreasing speciation rates throughout all lineages suggest that the evolution of *Pireneitega* spiders is similar to a density-dependent adaptive radiation, which means the appearance of new lineages likely is limited by niche space (Crisp and Cook 2009). In *Pireneitega*, the high speciation rate reflects the concentration of the appearance of new lineages, but seems not to be related to the final accumulation of species richness. Obviously, the high species diversity in the Alpine-Himalayan hotspots cannot be explained purely in terms of the rate hypothesis.

Isolation always plays a fundamental part in shaping diversity (Gillespie and Roderick 2014). Many studies indicate that montane hotspots, such as the Andes, are caused by sky islands isolating mechanisms (Fig. 4a). The drier valleys separating suitable habitat on mountainsides may act as speciation pumps in the presence of climatic fluctuations, resulting in abundant endemic species at higher elevations (Steinbauer et al. 2016, Hazzi et al. 2018). In our case, geographical isolation in the species-rich mountains of central Asia and southwest China is truly stronger than in other lowland areas. Although, inconsistently, *Pireneitega* species are often found in the valleys of the Alpine-Himalayan belts and do not show high endemism at higher elevations. Slope was a more influential variable among geographical factors, consistent with the observation that montane *Pireneitega* spiders

live mainly in valleys and are not affected by altitude and aspect. This may be due to two reasons: 1) as predators, the survival of spiders does not directly rely on plants or soil but rather on water and temperature. In fact, *Pireneitega* spiders are insensitive to altitude (some species can be distributed from 400 m to 3500 m); 2) in extratropical mountainous regions, especially in high latitude inland mountainous, wet valleys are often surrounded by dry mountainsides where the landforms and climatic conditions are different from the low latitudes and coastlands.

Studies from the Andes also indicate that orogenic events can drive biotic diversification through the development of ecological gradients and multiple types of habitats (Hoorn et al. 2010). Similarly, Spicer (2017) proposed that the close-proximity niche diversity caused by complex topographies and seasonally varying climates in mountainous regions turned areas such as southern Asia into biodiversity hotspots. Adaptation to different environments or resources cause niche isolation and drive sympatric ecological speciation. Our predicted richness map indicates a set of overlapping distributions of *Pireneitega* species in the Alpine-Himalayan belt, and some regions have a strong concentration of species richness (e.g. the Tian Mountains and the Pamirs), which seems to be the same pattern mentioned above (Fig. 4c). However, actual distributions of *Pireneitega* species are inconsistent with our predictions, as different species are allopatric with a low frequency of overlapping distributions, even within hotspots (Fig. 4d). MaxEnt modelling

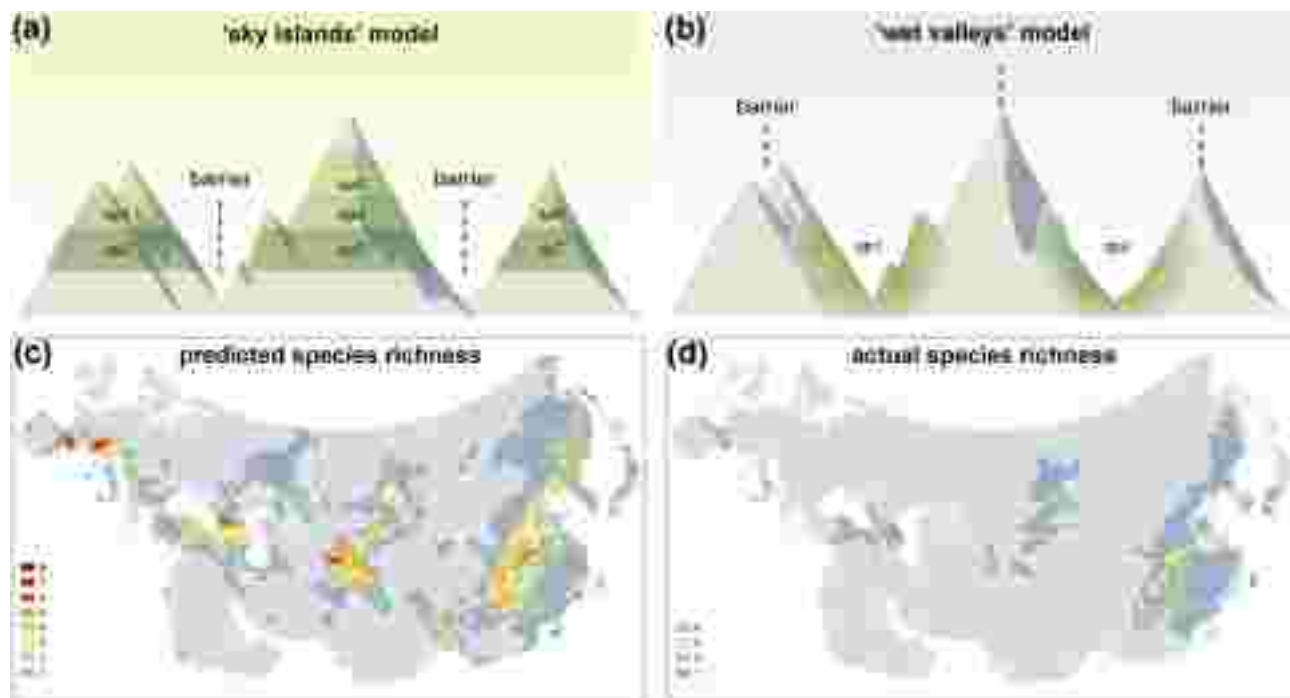


Figure 4. (a) Schematic for the 'sky islands' hypothesis. Different colors indicate various habitats and niches. (b) Schematic for the 'wet valleys' hypothesis, introduced here. (c) The predicted species richness map based on MaxEnt modeling showing an overlapping distribution pattern of *Pireneitega* species in the Alpine-Himalayan belt. (d) The actual species richness map showing a low overlapping distribution pattern.

assumes that species will appear in all areas with suitable habitat. Because the environmental niches of *Pireneitega* species are quite similar, the predicted hotspots in the mountains (Fig. 4c) only mean that mountains are suitable for the survival of most species, but not because of multiple niches. These observations suggest that the sky islands hypothesis may be only one of multiple potential mechanisms leading to high species diversity in montane hotspots. Additional mechanisms likely operated in the Alpine-Himalayan hotspots probably, especially at the mid-high latitudes.

Most *Pireneitega* sister species pairs are distributed allopatrically (Fig. 1) but differ little ecologically from each other (Fig. 3b), which implies that the dominant mode of genetic divergence and speciation in *Pireneitega* is via geographical isolation. This may be related to the extratropical seasonal climate, *Pireneitega* spiders in these areas must have a wide range of ecological amplitude and tolerance and do not benefit from niche specialization, which reduces the niche variability in mid-high latitude mountains. Species with similar niches are unlikely to appear in areas simultaneously due to competitive exclusion, or Gause's principle (Hardin 1960). Thus, in extratropical areas, speciation of organisms such as *Pireneitega* spiders seems independent of the variety of niches or habitats available (sympatric or parapatric), but rather depends on the appearance of new isolated habitats that can be occupied (allopatrically).

Extratropical mountain hotspots probably benefitted from the appearance of numerous isolated habitats caused by Tethyan sea-land changes and the orogenetic activities. The Eurasian mid-high latitudes and inland areas currently compose arid environments (e.g. desert, steppe, arid woodland, etc.), and water is the main limiting factor there for most organisms. With the regression of the Paratethys Sea, a restricted, evaporitic marine environment appeared, and desert-like environments have been established in central Asia since 39 Ma (Carrapa et al. 2015). During the Mid-Miocene, global cooling and the formation of plateau regions (e.g. Iranian and Tibetan plateaus) caused increasing aridification in Eurasian inland areas (Miao et al. 2012, Manafzadeh et al. 2017). On a regional scale, however, mountain ranges like the Tian and Pamir allow for the maintenance of 'wet valleys' in these vast and otherwise arid regions, through orographic precipitation on their windward side (Miao et al. 2012) (Fig. 4b). Such isolated wet habitats are not fragments (vicariance model) caused by mountain uplifts; rather, they are created by the uplifts. Price et al. (2014) noticed the effects of niche filling on mountain bird diversification and implied that biodiversity is limited by the appearance of new niches or habitats, rather than by the rate of reproductive isolation. If this is correct, then the appearance of a new niche or a relatively isolated habitat caused by uplift appears to be the direct cause of regionally high biodiversity formation but not of the uplift itself. Mountain uplift often promotes the appearance of new niches or isolated habitats that results in a high speciation rate, especially in the early stage of tectonic activities. However, interspecific competition within the

gradually saturated habitat subsequently leads to a decline or even stagnation of speciation when uplift fails to provide new niches or isolated habitats. This idea reconciles the contradiction that the geological history is often temporally incongruous with the biological radiation, and can reasonably explain why speciation caused by the recent uplift is often absent (Renner 2016). This happened even earlier in the lowlands of east Asia (no new *Pireneitega* species have appeared since the Middle Miocene) where geological activity is more ancient, and relatively low geographic isolation precludes the creation of independent habitats.

Conclusion

Plate tectonics have fundamental effects on today's biodiversity. The withdrawal of the Tethys Sea and the building of the Alpine-Himalayan mountain belts have promoted the expansion and diversification of terrestrial organisms, greatly impacting the distribution and composition of Eurasian biota, and forming biodiversity hotspots. In extratropical hotspots, in addition to the sky island mechanism, we propose that mountain systems promote speciation by providing many isolated habitats via a 'wet valleys' model. To cope with the rapid loss of biodiversity today, additional transnational biodiversity surveys and evolutionary studies involving more taxa are urgently needed.

Data availability statement

Data available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.g1jwstqnk>> (Zhao et al. 2020).

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Conflicts of interest – The authors declare no competing interests in this study.

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Supplementary material (available online as Appendix ecog-05044 at <www.ecography.org/appendix/ecog-05044>). Appendix 1.