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Evolutionary history of Old World cellar spiders in the context of Neo-Tethyan sea-land transformations

Zhi-Yuan Yao^{1,2}, Shu-Qiang Li^{1,3,*}

¹ College of Life Sciences, Anhui Normal University, Wuhu, Anhui 241003, China

² College of Life Science, Shenyang Normal University, Shenyang, Liaoning 110034, China

³ Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

ABSTRACT

The ancient Neo-Tethyan region underwent profound tectonic transformations, including the orogenesis of the Xizang Plateau and the westward retreat and ultimate closure of the Neo-Tethyan Ocean. These events significantly influenced the diversification and biogeography of aquatic animals. However, the impact of these large-scale sea-land shifts on the long-range evolutionary history of terrestrial fauna remains unclear. This study investigated how geological changes in the Neo-Tethyan region shaped the evolutionary trajectories and dispersal patterns of *Pholcus sensu lato* cellar spiders across the Old World. Molecular dating, ancestral area reconstructions, and diversity analyses were conducted using sequences from seven genes across 234 samples representing 209 species. Results indicated that these spiders originated in the eastern Neo-Tethyan region during the Early Eocene, with their subsequent diversification driven by a series of concomitant geological events. The Eurasian (ER) Group dispersed into Europe following the westward retreat of the Neo-Tethyan Ocean in the Early Miocene, while the African (AF) Group migrated into Africa via the Arabian Plate land bridge following the closure of the Neo-Tethyan Ocean in the Oligocene. The East Asian (EA) Group, which expanded along the southeastern margin of the Himalayas, experienced explosive diversification in response to sustained orogenesis at the Eocene-Oligocene boundary. These findings illustrate how large-scale geological processes and sea-land changes shaped the evolutionary history of terrestrial fauna in the Neo-Tethyan region.

Keywords: Biogeography; Diversification; Distribution pattern; Molecular dating; Pholcidae

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INTRODUCTION

The early Cenozoic Era was marked by extensive geodynamic transformations in the Neo-Tethyan region, including subduction, continental drift, seafloor movement, and the eventual ocean closure in the Neo-Tethyan Ocean. These processes represent one of the most remarkable ancient sea-land transformations on a vast geographic scale, culminating in the formation of the present-day Alps-Himalayas orogenic belt (Pan et al., 1997). The Neo-Tethyan Ocean once hosted a diverse assemblage of aquatic species (Maisey, 2012), and many regions formerly inundated beneath its waters, such as the Caribbean Islands, Mediterranean Sea, eastern Himalayas, and Indo-West Pacific Islands, now serve as biodiversity hotspots (Cowman et al., 2017; Dornburg et al., 2015; Huyse et al., 2004). The extensive sea-land transformations of the Neo-Tethyan region have long attracted the attention of evolutionary biologists (Zhang et al., 2023; Zhao et al., 2020, 2022), with biogeographic and phylogenetic analyses revealing that the breakup of the Neo-Tethyan Ocean played a crucial role in shaping the diversification and distribution patterns of many aquatic lineages (Hou & Li, 2018). For instance, the retreat of the Neo-Tethyan Ocean drove the dispersal of amphipods from the western Neo-Tethys to the rivers of East Asia, where they underwent rapid diversification in freshwater habitats (Hou et al., 2011; Mamos et al., 2016). Similarly, fossil and extant reef fish experienced a major shift in diversity from the western Neo-Tethys to the Indo-West Pacific over the past 50 million years (Ma) (Leprieur et al., 2016; Renema et al., 2008). However, the extent to which Neo-Tethyan sea-land changes shaped the evolutionary history of terrestrial fauna on a broad geographic scale has not yet been studied. As such, this study explored the phylogenetic and zoogeographic patterns of *Pholcus sensu lato* cellar spiders, a diverse and widely distributed group that persists in regions corresponding to remnants of the ancient Neo-Tethyan landscape.

The assemblage referred to as *Pholcus sensu lato* includes the true genus *Pholcus*, as well as recently recognized genera

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*Corresponding author, E-mail: lisq@ioz.ac.cn

such as *Meraha*, *Kintaqa*, and *Cantikus* (Huber et al., 2018). Most species exhibit limited dispersal capabilities, with the exception of several synanthropic taxa that have been inadvertently transported by humans (Huber, 2011; Schäfer et al., 2001). This group, comprising more than 400 described species (World Spider Catalog, 2024), is predominantly distributed across the Old World, occupying nearly the entire Neo-Tethyan region. Its highest diversity is concentrated along

the southeastern margin of the Himalayas, a region uplifted from the eastern Neo-Tethyan domain (Figure 1B). Early phylogenetic analyses of the family Pholcidae proposed an Early Cretaceous origin for *Pholcus sensu lato* (Dimitrov et al., 2013). However, this estimate appears doubtful, as it predates the oldest known fossil evidence for Pholcidae (Dunlop et al., 2023) and was derived from a limited dataset comprising only a few dozen samples. The present distribution of *Pholcus*

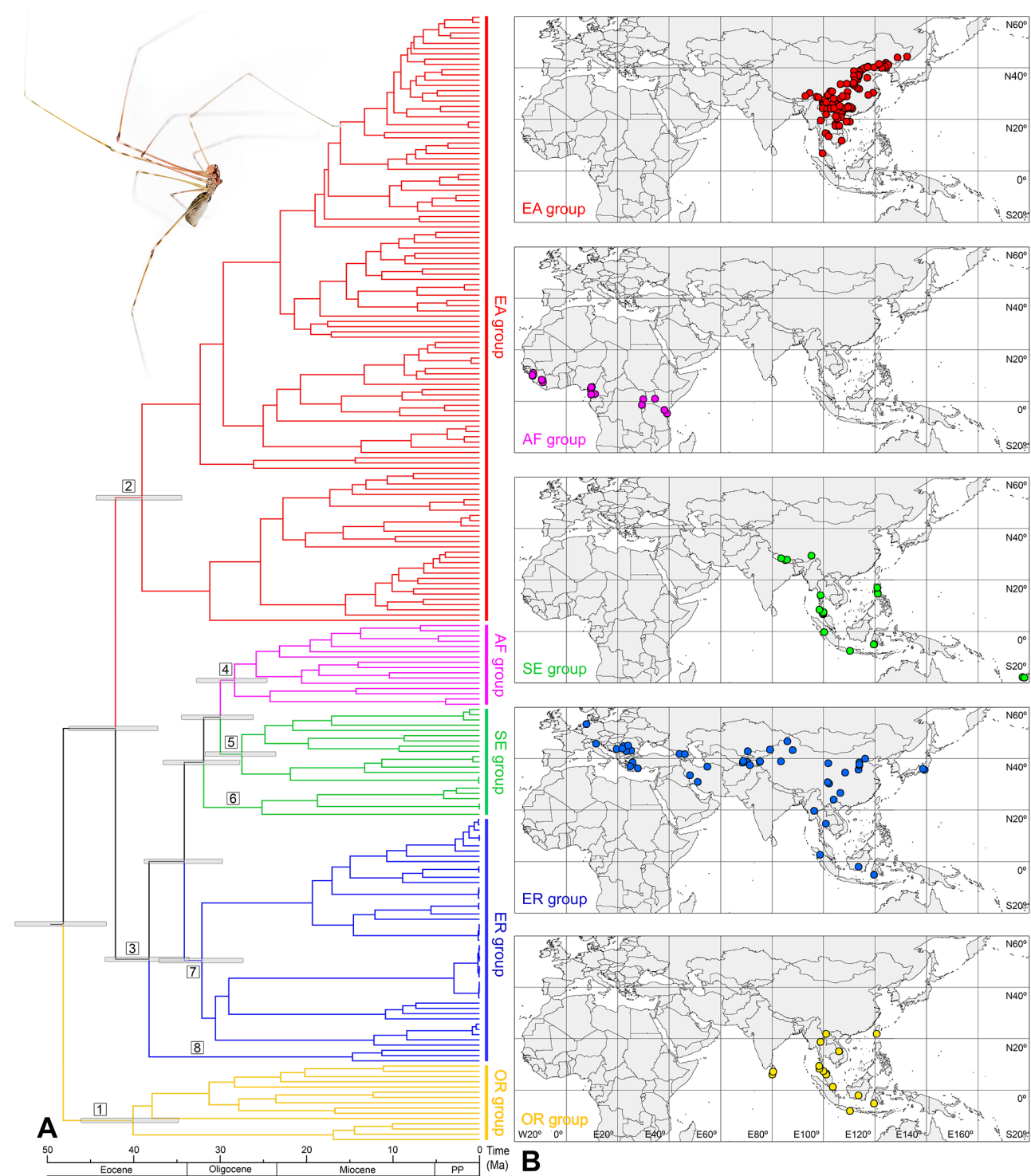


Figure 1 Time-calibrated phylogenetic tree and geographic distribution of *Pholcus sensu lato*
A: Time-calibrated phylogenetic tree. Gray bars represent 95% credibility intervals for divergence time estimates for clades of interest. Numbers with a black box indicate clades of interest. B: Maps of sampling sites of five *Pholcus sensu lato* groups. Dot colors on maps correspond to major clades on the tree. Maps were generated with ArcGIS v.10.2 (ESRI Inc.).

sensu lato suggests that its evolutionary history was likely shaped by Neo-Tethyan sea-land changes during the Cenozoic Era.

The Neo-Tethyan sea-land changes involved a series of significant geological and tectonic events. During the Middle Paleogene, India initially collided with Asia at a low latitude in the Northern Hemisphere (Hu et al., 2016). This was followed by intracontinental convergence in the Late Eocene, which led to the closure of the Neo-Tethys north of the Indian Plate and triggered continuous tectonic deformation and uplift of the Xizang Plateau (Royden et al., 2008; van Hinsbergen et al., 2012). The Paratethys Seaway, which extended as far east as Tajikistan and covered the Tarim Basin from the Cretaceous to the Late Eocene, represented a significant paleogeographic feature of the region (Carrapa et al., 2015; Sun & Jiang, 2013). Continued uplift of the Xizang Plateau during the Oligocene drove the retreat of the Neo-Tethyan Ocean from present-day Xizang, leading to the emergence of the Pamir Mountains and the Turgai Strait as terrestrial corridors that may have facilitated faunal migrations between continental Europe and Asia (Carrapa et al., 2015; Miller et al., 2005; Rögl, 1998). At the Eocene-Oligocene boundary, the Arabian promontory initially collided with the southern accreted margin of Eurasia along the Bitlis and Zagros sutures (Robertson et al., 2006; Vincent et al., 2007). The subsequent counterclockwise rotation of the Arabian Plate in the Early Miocene resulted in the complete closure of the Neo-Tethyan Seaway (Rögl, 1998; Steininger & Rögl, 1984), forming the Arabian Plate land bridge, which permitted terrestrial exchanges between Eurasia and Africa. These geological events may have profoundly shaped the evolutionary history of *Pholcus sensu lato*.

This study hypothesized that *Pholcus sensu lato* cellar spiders originated in the eastern Neo-Tethys, where many extant species are concentrated, and that their diversification and distribution patterns were shaped by successive phases of Neo-Tethyan sea-land transitions. DNA sequence data were used to reconstruct the phylogenetic relationships of these spiders and estimate divergence times for major lineages. Combined with zoogeographic analyses, these data provide a comprehensive framework for understanding the relationship between the evolutionary history of *Pholcus sensu lato* and the closure of the Neo-Tethyan Ocean, as well as broader tectonic plate convergence in the ancient Neo-Tethyan region.

MATERIALS AND METHODS

Taxon sampling

Study sampling covered all major geographic regions, including Eurasia, Southeast Asia, the South Pacific (exemplified by Fiji), and Africa (Supplementary Table S1; Figure 1B). Samples were mainly collected from the type localities of nominal taxa, and multiple samples were used to represent several widespread species. In total, 234 samples representing 209 species of *Pholcus sensu lato* constituted the ingroup. Based on previously published phylogenetic trees (Dimitrov et al., 2013; Wheeler et al., 2017), the outgroup included 22 species of *Micropholcus*, *Leptopholcus*, and *Quamtana* of the family Pholcidae (all closely related to *Pholcus sensu lato*) and 34 species of the Haplogynae families, viz., Tetrablemmidae, Oonopidae, Ochyroceratidae, and Filistatidae (all closely related to Pholcidae). For

biogeographic reconstruction and diversity analysis, one sample was used for each species. The reduced matrix consisted of 192 taxa.

Laboratory protocols and phylogenetic analyses

Genomic DNA extraction and amplification were performed following the protocols outlined in Yao et al. (2016). Seven gene regions were targeted for sequencing, including three mitochondrial gene fragments encoding cytochrome oxidase I (*COI*), 12S rRNA (12S), 16S rRNA (16S), and four nuclear gene fragments encoding histone 3 (*H3*), wingless (*wnt*), 18S rRNA (18S), and 28S rRNA (28S). The primers used are listed in Supplementary Table S2. DNA sequences were checked and edited using SeqMan II (Burland, 2000) and BioEdit v.7.2.2 (Hall, 1999). Protein-coding sequences were aligned with CLUSTAL W (Thompson et al., 1994), while other sequences were aligned with MAFFT v.7.0 (<https://mafft.cbrc.jp/alignment/server/>). Visual inspection, translation, and alteration were conducted to minimize alignment errors. Ambiguous regions were processed using the Gblocks Server v.0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). The final dataset comprised 290 taxa and a total alignment length of 5 326 bp, including 1 201 bp for *COI*, 256 bp for 12S, 343 bp for 16S, 1 721 bp for 18S, 1 155 bp for 28S, 330 bp for *H3*, and 320 bp for *wnt*.

Maximum-likelihood (ML) and Bayesian inference (BI) methods were used to reconstruct phylogenetic trees. ML analysis was conducted using RAXML v.8.2.9 (Stamatakis, 2014) under a GTRCAT model across all partitions, with 1 000 rapid bootstrap pseudoreplicates followed by a thorough ML tree search. Separate ML analyses of individual genes found no conflicts among well-supported nodes (bootstrap support > 70%), allowing the concatenation of all seven loci for subsequent analyses. BI analysis was performed with MrBayes v.3.2.4 (Ronquist et al., 2012). The best-fitting DNA substitution models were selected based on the Akaike Information Criterion (AIC) using MrModeltest v.2.3 (Nylander, 2004). The GTR+I+G model was recommended for all partitions. Two simultaneous runs of four Monte Carlo Markov chains (MCMCs) with default heating parameters were executed for 30 million generations. Trees were sampled every 1 000 generations, with the first 25% of sampled trees discarded as burn-in. The results were checked using Tracer v.1.6 (Rambaut & Drummond, 2014) to ensure stationarity.

Divergence time analysis

Five fossil calibration points were selected for molecular clock analyses. The amber fossil of *Leptopholcus* sp. (Pholcidae) from the Dominican Republic (20 Ma) (Huber & Wunderlich, 2006) was used to constrain extant *Micropholcus*, following the reassignment of Dominican *Leptopholcus* species to *Micropholcus* (Huber et al., 2014). The amber fossil of *Quamtana* sp. (Pholcidae) from the Paris Basin (lowermost Eocene) served to constrain extant *Quamtana* from Africa (Penney, 2006). The amber Myanmar fossil *Leclercera* sp. (Ochyroceratidae) from the Late Cretaceous, Cenomanian (unpublished data) was used to constrain extant representatives of the genus. The amber fossil of extinct *Electroblemma* sp. (Tetrablemmidae) from Myanmar (Late Cretaceous, Cenomanian) was applied to constrain its subfamily Tetrablemminae (Selden et al., 2016). Additionally, the oldest known amber fossil of *Orchestina* sp., considered a basal member of Oonopidae, from the early Albian of Cantabria, Spain, was used to constrain the Oonopidae family

(Saupe et al., 2012). All fossil calibrations were applied as minimum age constraints on the stem lineage to which the fossil was assigned (Donoghue & Benton, 2007; Renner, 2005). Parameter settings are listed in Supplementary Table S3.

Divergence time estimates were obtained using the Bayesian MCMC framework implemented in BEAST v.1.8.2 (Drummond et al., 2012), assuming a lognormal relaxed clock and a Yule tree prior. The GTR+I+G model was used for nuclear gene fragments and the HKY+I+G model was used for mitochondrial gene fragments. MCMCs were run for 60 million generations with a sampling frequency of 1 000 generations. Two independent runs were performed using the CIPRES web portal (Miller et al., 2010) to confirm convergence. Stationarity of each run was examined using Tracer v.1.6 (Rambaut & Drummond, 2014). The last 45 million generations were used to construct the maximum clade credibility tree and estimate the 95% highest posterior density distributions for node ages.

Biogeographic analysis

The historical distribution of ancestral lineages was reconstructed using BioGeoBEARS (Matzke, 2013). The present-day distributions of *Pholcus sensu lato* were categorized into nine biogeographic regions: Europe, Central Asia, northern China, southern China, northeastern Asia (exemplified by Japan), Indochina, Southeast Asian Islands, Pacific Islands (exemplified by Fiji), and Africa (Figure 2A). The chronogram produced in BEAST v.1.8.2 (Figure 2B) was used for analysis after excluding outgroups and one lineage (*Cantikus*), which clustered with outgroups. Dispersal probabilities between geographic regions were defined by dispersal matrices (Supplementary Table S4) and adjusted across two time slices (60–32 Ma, 32–0 Ma). Beginning in the Late Eocene, the regression of the Paratethys Sea and the emergence of the Turgai Strait as a terrestrial corridor facilitated faunal exchanges between Europe and Asia (Carrapa et al., 2015; Miller et al., 2005; Rögl, 1998). Dispersal probabilities were assigned values of 0.01, 0.5, and 1.0, ranging from “unlikely” to “likely”. A maximum of four ancestral areas was allowed at each node. The DEC and DEC+J models were compared to determine the influence of founder-event dispersal on biogeographic patterns. Based on AIC, DEC+J was selected as the best-fitting model (Supplementary Table S5) and was subsequently used to infer the most likely biogeographic history of *Pholcus sensu lato*.

Diversification patterns

Diversification dynamics were analysed across *Pholcus sensu lato* (excluding *Cantikus*) and within key biogeographic groups: Original Group (OR, clade 1), primarily consisting of Southeast Asian species; Eurasian Group (ER, clades 7+8, representing independent dispersals into Eurasia; Southeast Asian Group (SE, clades 5+6, a second Southeast Asian lineage resulting from independent dispersals; African Group (AF, clade 4), encompassing species from Africa; and East Asian Group (EA, clade 2), predominantly including species from East Asia (Figure 1). To assess diversification rate variation across phylogenetic branches, Bayesian Analysis of Macroevolutionary Mixtures v.2.5 (BAMM) (Rabosky, 2014) was applied. To account for potential biases introduced by incomplete sampling, a nonrandom taxon-sampling correction was implemented. Analysis was run for 5 million generations, with sampling conducted every 250 generations and event data sampled every 250 generations. Convergence was

assessed using BAMMtools v.2.0.5 (Rabosky et al., 2013; Rabosky, 2014), with the first 10% of sampled generations discarded as burn-in. Effective sample sizes (ESS) were evaluated using the CODA package (Plummer et al., 2006), ensuring a target value of 500. BAMMtools v.2.0.5 was used to generate rate-through-time plots. Modeling Evolutionary Diversification Using Stepwise AIC (MEDUSA) (Alfaro et al., 2009) was applied to detect significant rate shifts along the branches using the Geiger package (Harmon et al., 2008) with default settings, including corrected AIC and a mixed model approach.

Regional diversification patterns within *Pholcus sensu lato* were further explored using custom scripts from Mahler et al. (2010) and Yuan et al. (2016). Lineage-through-time (LTT) analysis (Paradis et al., 2004) was employed to visualize the accumulation of lineages across biogeographic regions (EA, AF, SE, ER, and OR). While LTT analysis provides insights into the temporal accumulation of species, it does not explicitly model speciation or extinction rates.

RESULTS

Phylogenetic relationships

A total of seven gene regions, including *COI*, *12S*, *16S*, *H3*, *wnt*, *18S*, and *28S*, were sequenced for 253 samples representing 196 species of *Pholcus sensu lato* and 19 outgroup taxa (Supplementary Table S1). To increase coverage, publicly available sequences from an additional 13 species of *Pholcus* and 45 closely related outgroups were incorporated from GenBank. Both ML and BI methods produced similar topologies, with the BI tree presented in Supplementary Figure S1. *Cantikus* formed a sister group to *Micropholcus* and *Leptopholcus* (Supplementary Figure S1). The remaining *Pholcus sensu lato* species were clustered into three major clades (Figure 1A). Clade 1, the Original (OR) Group, occupied the basal position in the tree and was primarily composed of Southeast Asian species. Clade 2, the East Asian (EA) Group, consisted mainly of taxa from East Asia. Clade 3 exhibited further subdivision into three well-supported groups: Clade 4, the African (AF) Group, comprising species from Africa; Clades 5+6, a second Southeast (SE) Asian Group, including species mainly from Southeast Asia; and clades 7+8, the Eurasian (ER) Group, primarily composed of taxa from Eurasia. Given that Clades 5+6 and Clades 7+8 represented independent dispersal events into Southeast Asia and Eurasia, respectively, subsequent analyses treated these groups separately.

Divergence time estimates

Divergence time estimates inferred using BEAST are shown in Figure 1A, with detailed estimates provided in Supplementary Figure S2. The origin of *Pholcus sensu lato* was dated to approximately 48.4 Ma in the Early Eocene. The initial diversification into three major clades occurred nearly simultaneously in the Late Eocene, with estimated divergence times of 40.3 Ma for the OR group, 39.3 Ma for the EA group, and 38.4 Ma for the ER group. Subsequent diversification within the SE group was inferred at 32.1 Ma, while the AF lineage diverged at 30.1 Ma in the Early Oligocene.

Biogeographic analysis

Biogeographic reconstructions using BioGeoBEARS indicated that the DEC+J model provided a significantly better fit than the DEC model (Supplementary Table S5). The most recent

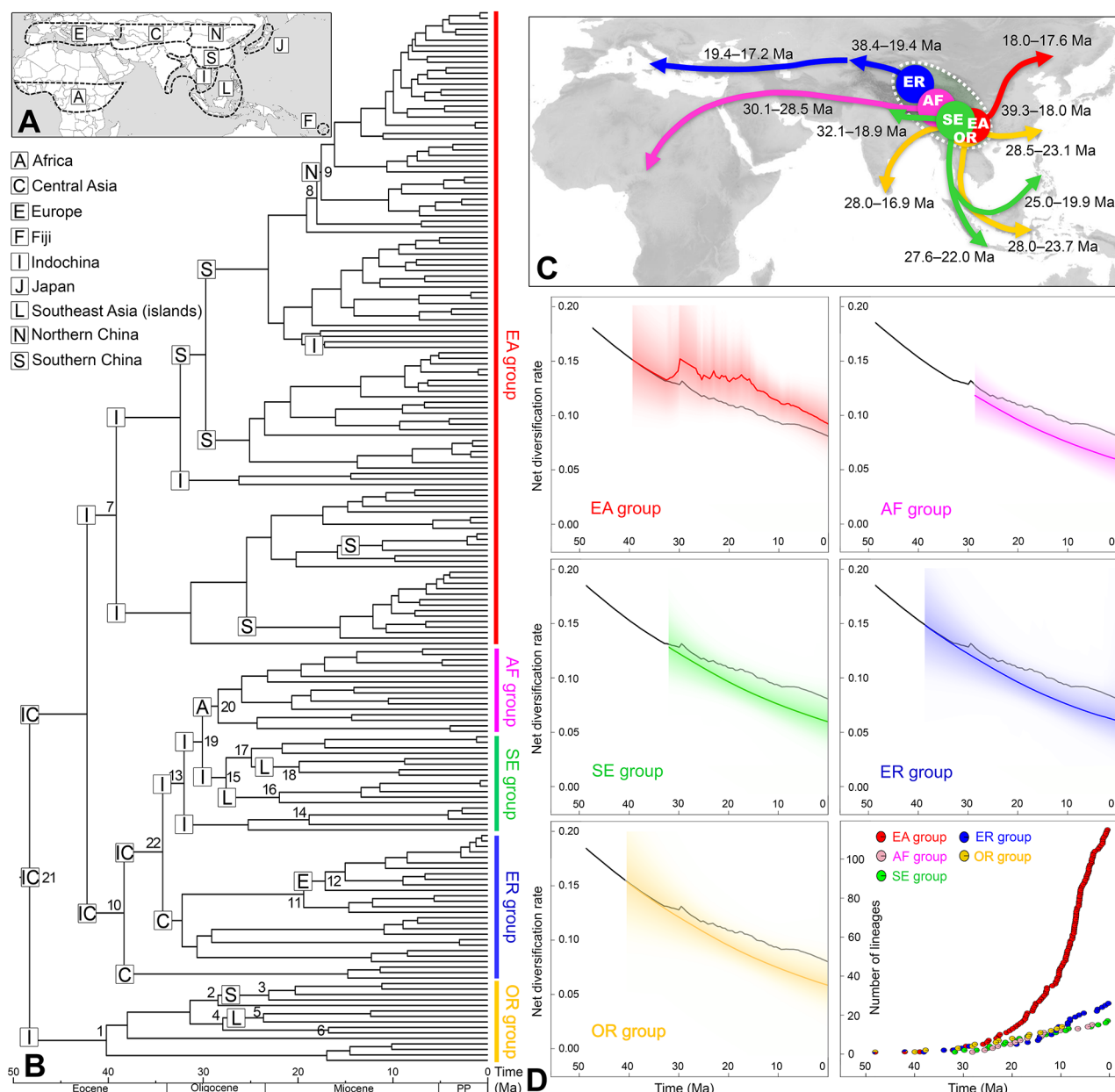


Figure 2 Evolutionary history of *Pholcus sensu lato*

A: Dashed line denotes nine major geographic regions. B: Ancestral area reconstructions. Letters in boxes indicate the most likely ancestral range. Numbers represent nodes of interest. C: Map depicting the most probable origin area of *Pholcus sensu lato* (white dotted circle) and major dispersal routes (colored arrows) of five major groups. D: Rate-through-time curves showing group-specific net diversification trajectories (first five squares). Black line denotes curve for *Pholcus sensu lato*. Colored lines denote curves for five *Pholcus sensu lato* groups. Last square shows species accumulation curves. Maps were generated with ArcGIS v.10.2 (ESRI Inc.).

common ancestor (MRCA) of *Pholcus sensu lato* was estimated to have originated in northern Indochina and southeastern Central Asia (Figure 2C; Supplementary Figure S3), representing an eastern Neo-Tethyan origin. The initial diversification occurred nearly synchronously (Figure 2B, C). The OR group gradually expanded across Indochina between 40.3 and 28.5 Ma (nodes 1–2), before nearly simultaneous dispersals into Taiwan, China (28.5–23.1 Ma, nodes 2–3), Southeast Asian islands (28.0–23.7 Ma, nodes 4–5), and Sri Lanka (28.0–16.9 Ma, nodes 4–6). The EA group was largely restricted to northern Indochina and southwestern China between 39.3 and 18.0 Ma (nodes 7–8), with a later expansion into northern China at 18.0–17.6 Ma (nodes 8–9). The ER group remained primarily in Central Asia between 38.4 and

19.4 Ma (nodes 10–11), except for one lineage that dispersed into Indochina at 30.7 Ma, with subsequent expansion of the ER group into Europe at 19.4–17.2 Ma (nodes 11–12). Later dispersal events involved two major colonization pathways (Figure 2B, C). First, the SE group dispersed to the Himalayas, Indonesia, and the Philippines from Indochina approximately 32.1–18.9 Ma (nodes 13–14), 27.6–22.0 Ma (nodes 15–16), and 25.0–19.9 Ma (nodes 17–18), respectively. Second, the AF Group, originating from Indochina, established populations in Africa between 30.1 and 28.5 Ma (nodes 19–20).

Diversification patterns

Diversification rate analysis using BAMM revealed an initially

high net diversification rate in *Pholcus sensu lato*, which gradually declined over time (Figure 2D; Supplementary Figure S4). Analyses further supported strong heterogeneity in diversification dynamics, particularly between EA and the other four groups. While the OR, ER, SE, and AF groups exhibited similar diversity-dependent speciation, characterized by early bursts of diversification followed by a gradual decline, their net diversification rates remained consistently lower than the overall background rate for *Pholcus sensu lato* (Figure 2D). In contrast, both BAMM and MEDUSA analyses identified a distinct rate shift within the EA group, marking a deviation from the diversification patterns observed in other groups. BAMM analysis indicated that the net diversification rate initially decreased but experienced a sharp increase around 32 Ma, peaking at approximately 0.15 species/Ma. Thereafter, the diversification rate in the EA group remained elevated relative to background levels, although it gradually declined over time (Figure 2D). Similarly, MEDUSA analysis identified a rate shift at approximately 32 Ma as the most likely explanation for the high species diversity within the EA group (Supplementary Figure S5), with the average speciation rate increasing significantly from 0.08 to 0.16 species/Ma.

Regional LTT analyses (Figure 2D, bottom right) further supported distinct evolutionary trajectories across groups. The EA lineage began accumulating species at 39.3 Ma at a relatively slow rate until about 32 Ma, when it underwent rapid cladogenesis consistent with an exponential diversification process. In contrast, lineage accumulation within the OR, ER, SE, and AF groups remained relatively slow and constant throughout their evolutionary history.

DISCUSSION

Early Eocene origin in the eastern Neo-Tethyan region

Our analyses strongly supported an Early Eocene origin of *Pholcus sensu lato* in the eastern Neo-Tethyan region. Previous molecular clock analyses of Pholcidae suggested a *Pholcus sensu lato* origin in the Early Cretaceous (Aptian) (Dimitrov et al., 2013), a considerably earlier estimate than our findings. Several factors may account for this difference. First, our phylogenetic estimate was based on a substantially larger dataset, incorporating a broader taxon sampling than previous studies. Second, recent discoveries of Late Cretaceous amber fossils from Myanmar have yielded numerous spider specimens, particularly from Haplogynae families such as Ochyroceratidae, Tetrablemmidae, and Oonopidae (Dunlop et al., 2023). If *Pholcus sensu lato* had originated in the Early Cretaceous, fossil evidence of the group would be expected in these deposits. However, despite extensive collections of Burmese amber spiders, no fossils attributable to *Pholcus sensu lato* or other pholcid fossils have been identified. Additionally, transcriptomic analyses incorporating 10 fossil calibration points have estimated the divergence of *Pholcus sensu lato* between 65.1 and 38.3 Ma (Li et al., 2020), a timeframe that aligns well with the present estimate of 53.9–43.3 Ma. Collectively, these findings provide robust support for an Early Eocene origin of *Pholcus sensu lato*, consistent with emerging molecular and fossil evidence.

Correlations between global distribution patterns and Neo-Tethyan sea-land transformations

The evolutionary history of *Pholcus sensu lato* appears to have been shaped by four major divergence events, each

corresponding to key tectonic events in the Neo-Tethyan region (Figure 2B).

The first divergence event involved the separation of the OR group from the other lineages in northern Indochina (node 21; 53.9–43.3 Ma). This occurred during the Early Eocene, a period marked by the northward movement of the Indian plate and its eventual collision with Asia (Hu et al., 2016). The resulting geotectonic shifts may have triggered the initial divergence of *Pholcus sensu lato*.

The second major divergence event occurred in the Late Eocene, coinciding with a series of tectonic events that led to the nearly simultaneous divergence of three groups. Notably, the OR group began diversifying in northern Indochina around 46.1–34.8 Ma (node 1), the EA group diverged in the same region approximately 44.3–34.5 Ma (node 7), and the ER group split in southeastern Central Asia (modern-day Xinjiang) around 43.4–33.6 Ma (node 10). During this period, intracontinental convergence resulted in the closure of the Neo-Tethys Seaway and extensive tectonic deformation associated with the uplift of the Xizang Plateau (Royden et al., 2008; van Hinsbergen et al., 2012). The timing and geographic patterns of the divergences are congruent with these geological changes. Additionally, high-elevation orographic features were already established in the Xizang Plateau between 40 and 35 Ma (Favre et al., 2015; Lippert et al., 2014; Zhao & Li, 2017), potentially driving vicariance between AF+SE and ER (38.9–29.8 Ma, node 22), which occupied southeastern and northwestern regions of the Xizang Plateau, respectively.

The third divergence event involved dispersal of the ER group from eastern Central Asia to Europe approximately 23.2–16.0 Ma (node 11). During the Early Oligocene (34–32 Ma), the Neo-Tethyan Ocean retreated, and continentalization progressed in central and western Europe (Miller et al., 2005; Popov et al., 2004). By the Early Miocene (23–19 Ma), a rapid regression of the eastern Paratethys from Tajikistan expanded terrestrial habitats, establishing a migration pathway for European and Asian continental animals (Carrapa et al., 2015; Rögl, 1998). This likely explains why ER remained confined to eastern Central Asia until the Early Miocene (23.2–16.0 Ma), when it began to disperse from Central Asia to Europe.

The fourth divergence event involved the SE and AF lineages, which began to differentiate around 36.5–27.8 Ma (node 13) and 32.8–24.6 Ma (node 20), respectively, following their split from ER. Unlike ER, which remained geographically constrained until the Early Miocene, the SE group rapidly expanded into the Himalayas, Indonesia, and the Philippines. Although our results provide precise dating for the AF group, its dispersal history remains unclear, as *Pholcus sensu lato* is absent from potential intermediate regions. Nevertheless, two key lines of evidence support a dispersal route from northern Indochina to Africa via the western Arabian Plate land bridge. First, *Pholcus sensu lato*, like other members of Pholcidae, lacks the ability to disperse by ballooning (Schäfer et al., 2001), suggesting that the AF lineage could only have reached Africa through a terrestrial corridor. Second, the estimated timing of the initial AF dispersal coincides with the emergence of the western Arabian Plate land bridge. A suture between the western Arabian Plate and Eurasia initiated at the Eocene-Oligocene boundary induced the uplift of the Western Greater Caucasus (Robertson et al., 2006; Vincent et al., 2007), with complete closure of the Neo-Tethyan Ocean by the Arabian Plate occurring in the Middle Miocene (Rögl,

1998; Steininger & Rögl, 1984). Additional evidence supporting this dispersal route comes from fossil records of embriothopods, which were widely distributed from the Late Paleocene to Late Eocene in Turkey and Romania, and later at the Eocene-Oligocene boundary in the Arabian Peninsula and Africa (Sen, 2013). A similar land bridge-mediated dispersal pattern has been proposed to explain the biogeographic distribution of embriothopods on both sides of the Neo-Tethyan Seaway.

Explosive diversification in the eastern Neo-Tethyan region is linked to rapid Eocene-Oligocene orogenesis of the Xizang Plateau

The exceptional diversity of *Pholcus sensu lato* was most pronounced in the EA group, particularly along the southeastern margin of the Himalayas. Diversification analyses revealed distinct evolutionary trajectories between EA and the other four groups (Figure 2D; Supplementary Figure S4). Lineage accumulation in the OR, ER, SE, and AF groups proceeded very slowly, with initially high net diversification rates that subsequently declined, consistent with a diversity-dependent speciation process. This pattern may result from an initial availability of new ecological opportunities, followed by subsequent niche saturation as these groups dispersed into new habitats in the wake of sea-land transformations in the eastern Neo-Tethyan region (Rabosky et al., 2007; Rabosky & Lovette, 2008). In contrast, the EA group exhibited a markedly different diversification pattern. Although lineage accumulation began slowly, diversification rates increased sharply around 32 Ma, initiating a phase of rapid cladogenesis under an exponential model. Despite a gradual decrease over time, diversification rates in EA have remained higher than those observed in the other groups. This explosive diversification coincided with the rapid uplift of the Xizang Plateau during the Eocene-Oligocene transition (Favre et al., 2015; Lippert et al., 2014; Zhao & Li, 2017). At that time, almost all EA species were restricted to southwestern China and northern Indochina. Uplift of the

southeastern Xizang Plateau region became a significant orographic barrier by the Late Eocene, obstructing westward dispersal and confining EA taxa to this region, with the exception of a single lineage in northern China during the Early Miocene. Prolonged orogenesis appears to have generated a series of localized vicariant events, ultimately driving the rapid diversification of the EA lineage.

Different responses of terrestrial and aquatic fauna to Neo-Tethyan sea-land transformations

Terrestrial and aquatic animals exhibited divergent responses to the large-scale Neo-Tethyan sea-land changes. Over the past 50 Ma, the orogenesis of the Xizang Plateau and the westward retreat of the Neo-Tethyan Ocean fundamentally altered dispersal patterns. Aquatic animals dispersed from the western Neo-Tethys to the eastern Neo-Tethys in response to shifting marine environments (Hou et al., 2011; Hou & Li, 2018) (Figure 3B). Similarly, our findings indicated that Neo-Tethyan sea-land changes within the same timeframe shaped the distribution patterns of terrestrial animals, dispersing from the eastern Neo-Tethys to the western Neo-Tethys following the westward retreat of the Neo-Tethyan Ocean and exposure of new land (Figure 3A). Both groups underwent rapid diversification in the eastern Neo-Tethys region, with many marine animals evolving to occupy freshwater habitats within a relatively short period. In contrast, terrestrial taxa diversified rapidly in response to the extensive orogenesis of the Xizang Plateau during the Eocene-Oligocene. Despite these contrasting evolutionary pathways, Neo-Tethyan sea-land changes played a significant role in shaping the diversification and biogeographic distribution of both terrestrial and aquatic lineages.

CONCLUSIONS

This study establishes a direct link between the phylogeny and zoogeography of Old World cellar spiders and the history of orogeny and ocean closure in the ancient Neo-Tethyan region.

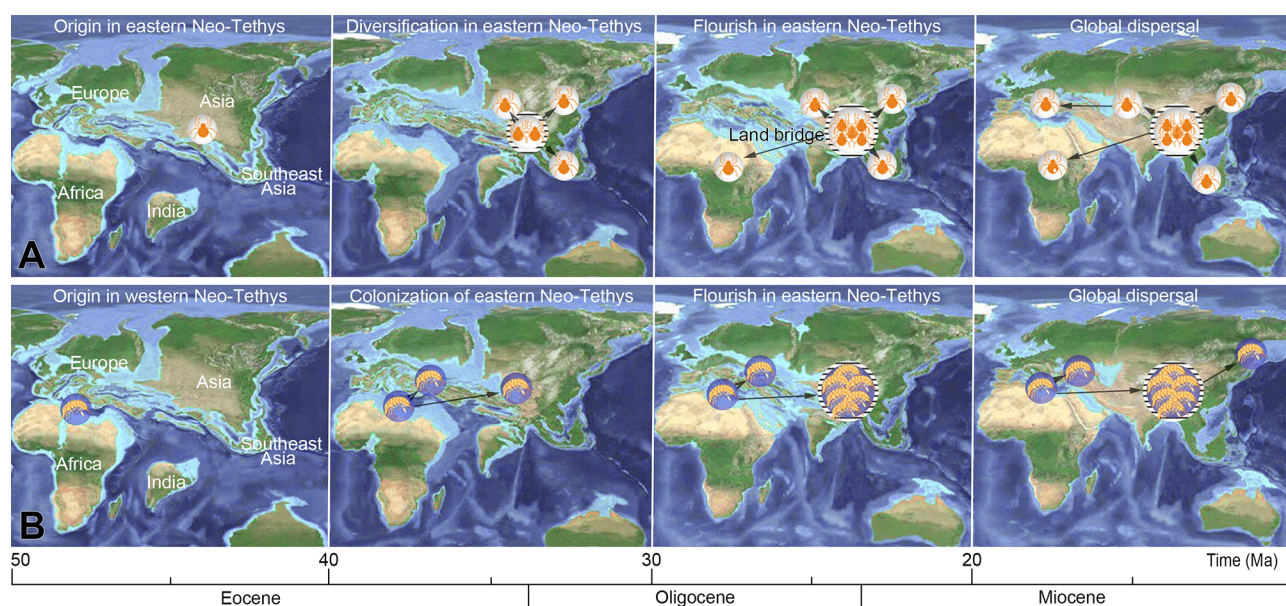


Figure 3 Evolutionary models for terrestrial and aquatic animals during Neo-Tethyan sea-land changes

A: Evolutionary model for terrestrial animals, where eastern Neo-Tethys acted as both an origin center and cradle of evolutionary novelty. B: Evolutionary model for aquatic animals, where western Neo-Tethys acted as an origin center and eastern Neo-Tethys acted as cradle of evolutionary novelty. Maps were modified from Popov et al. (2004) and Ron Blakey, NAU Geology (<http://jan.ucc.nau.edu/~rcb7/globaltext2.html>).

By examining patterns across a vast geographic scale, these findings offer broader insights into how large-scale geological events have shaped the diversification and distribution of terrestrial fauna.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Permission for field surveys was granted by the External Cooperation Program of BIC, Chinese Academy of Sciences and the Kenya Wildlife Service (KWS).

DATA AVAILABILITY

All datasets generated in this study were deposited in GenBank (accession numbers: PV031101–PV031324, PV060551–PV060765, PV060766–PV060988, PV073029–PV073217, PV073228–PV073392, PV073393–PV073575, and PV073582–PV073796) and the Science Data Bank (<https://www.scidb.cn/en/anonymous/YjYzSU5u>; doi: 10.57760/sciencedb.j00139.00166).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

S.Q.L. designed the study. Z.Y.Y. and S.Q.L. contributed to fieldwork. Z.Y.Y. performed the molecular experiments and analytical work. Z.Y.Y. and S.Q.L. drafted and revised the manuscript. All authors read and approved the final version of the manuscript.

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REFERENCES

Alfaro ME, Santini F, Brock C, et al. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proceedings of the National Academy of Sciences of the United States of America*, **106**(32): 13410–13414.

Burland TG. 2000. DNASTAR's Lasergene sequence analysis software. *Methods in Molecular Biology*, **132**: 71–91.

Carrapa B, Decelles PG, Wang X, et al. 2015. Tectono-climatic implications of Eocene Paratethys regression in the Tajik basin of central Asia. *Earth and Planetary Science Letters*, **424**: 168–178.

Cowman PF, Parravicini V, Kulbicki M, et al. 2017. The biogeography of tropical reef fishes: endemism and provinciality through time. *Biological Reviews*, **92**(4): 2112–2130.

Dimitrov D, Astrin JJ, Huber BA. 2013. Pholcid spider molecular systematics revisited, with new insights into the biogeography and the evolution of the group. *Cladistics*, **29**(2): 132–146.

Donoghue PCJ, Benton MJ. 2007. Rocks and clocks: calibrating the Tree of Life using fossils and molecules. *Trends in Ecology & Evolution*, **22**(8): 424–431.

Dornburg A, Moore J, Beaulieu JM, et al. 2015. The impact of shifts in marine biodiversity hotspots on patterns of range evolution: evidence from the Holocentridae (squirrelfishes and soldierfishes). *Evolution*, **69**(1): 146–161.

Drummond AJ, Suchard MA, Xie D, et al. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**(8): 1969–1973.

Dunlop JA, Penney D, Jekel D. 2023. A summary list of fossil spiders and their relatives. In: World Spider Catalog. Natural History Museum Bern.

Favre A, Päckert M, Pauls SU, et al. 2015. The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. *Biological Reviews*, **90**: 236–253.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**: 95–98.

Harmon LJ, Weir JT, Brock CD, et al. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics*, **24**(1): 129–131.

Hou ZE, Li SQ. 2018. Tethyan changes shaped aquatic diversification. *Biological Reviews*, **93**(2): 874–896.

Hou ZE, Sket B, Fišer C, et al. 2011. Eocene habitat shift from saline to freshwater promoted Tethyan amphipod diversification. *Proceedings of the National Academy of Sciences of the United States of America*, **108**(35): 14533–14538.

Hu XM, Garzanti E, Wang JG, et al. 2016. The timing of India-Asia collision onset - facts, theories, controversies. *Earth-Science Reviews*, **160**: 264–299.

Huber BA. 2011. Revision and cladistic analysis of *Pholcus* and closely related taxa (Araneae, Pholcidae). *Bonner Zoologische Monographien*, **58**: 1–509.

Huber BA, Carvalho LS, Benjamin SP. 2014. On the New World spiders previously misplaced in *Leptopholcus*: molecular and morphological analyses and descriptions of four new species (Araneae : Pholcidae). *Invertebrate Systematics*, **28**(4): 432–450.

Huber BA, Eberle J, Dimitrov D. 2018. The phylogeny of pholcid spiders: a critical evaluation of relationships suggested by molecular data (Araneae, Pholcidae). *ZooKeys*, **789**: 51–101.

Huber BA, Wunderlich J. 2006. Fossil and extant species of the genus *Leptopholcus* in the Dominican Republic, with the first cases of egg-parasitism in pholcid spiders (Araneae : Pholcidae). *Journal of Natural History*, **40**(41-43): 2341–2360.

Huysse T, Van Houdt J, Volckaert FAM. 2004. Paleoclimatic history and vicariant speciation in the “sand goby” group (Gobiidae, Teleostei). *Molecular Phylogenetics and Evolution*, **32**(1): 324–336.

Leprieux F, Descombes P, Gaboriau T, et al. 2016. Plate tectonics drive tropical reef biodiversity dynamics. *Nature Communications*, **7**: 11461.

Li FY, Shao LL, Li SQ. 2020. Tropical niche conservatism explains the Eocene migration from India to Southeast Asia in Ochyroceratid spiders. *Systematic Biology*, **69**(5): 987–998.

Lippert PC, Van Hinsbergen DJJ, Dupont-Nivet G. 2014. Early Cretaceous to present latitude of the central proto-Tibetan Plateau: a paleomagnetic synthesis with implications for Cenozoic tectonics, paleogeography, and climate of Asia. In: Nie JS, Horton BK, Hoke GD. Toward an Improved Understanding of Uplift Mechanisms and the Elevation History of the Tibetan Plateau. Boulder: Geological Society of America, 1–21.

Mahler DL, Revell LJ, Glor RE, et al. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. *Evolution*, **64**(9): 2731–2745.

Maisey JG. 2012. What is an ‘elasmobranch’? The impact of palaeontology in understanding elasmobranch phylogeny and evolution. *Journal of Fish Biology*, **80**(5): 918–951.

Mamos T, Wattier R, Burzyński A, et al. 2016. The legacy of a vanished sea: a high level of diversification within a European freshwater amphipod species complex driven by 15 My of Paratethys regression. *Molecular Ecology*, **25**(3): 795–810.

Matzke NJ. 2013. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography*, **5**(4): 242–248.

Miller KG, Kominz MA, Browning JV, et al. 2005. The phanerozoic record of

- global sea-level change. *Science*, **310**(5752): 1293–1298.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of 2010 Gateway Computing Environments Workshop. New Orleans: IEEE, 1–8.
- Nylander JAA. 2004. MrModeltest v2. Uppsala: Evolutionary Biology Centre, Uppsala University.
- Pan GT, Chen ZL, Li XZ. 1997. Geological-Tectonic Evolution in the Eastern Tethys. Beijing: Geological Publishing House. (in Chinese)
- Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**(2): 289–290.
- Penney D. 2006. The oldest fossil pholcid and selenopid spiders (Araneae) in lowermost eocene amber from the Paris Basin, France. *Journal of Arachnology*, **34**(3): 592–598.
- Plummer M, Best N, Cowles K, et al. 2006. CODA: convergence diagnosis and output analysis for MCMC. *R News*, **6**: 7–11.
- Popov SV, Rögl F, Rozanov AY, et al. 2004. Lithological-paleogeographic maps of Paratethys: 10 maps Late Eocene to Pliocene. *Courier Forschungs-Institut Senckenberg*, **250**: 1–46.
- Rabosky DL. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS One*, **9**(2): e89543.
- Rabosky DL, Donnellan SC, Talaba AL, et al. 2007. Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. *Proceedings of the Royal Society B: Biological Sciences*, **274**(1628): 2915–2923.
- Rabosky DL, Lovette IJ. 2008. Density-dependent diversification in North American wood warblers. *Proceedings of the Royal Society B: Biological Sciences*, **275**(1649): 2363–2371.
- Rabosky DL, Santini F, Eastman J, et al. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nature Communications*, **4**: 1958.
- Rambaut A, Drummond AJ. 2014. Tracer v. 1.6. <http://beast.bio.ed.ac.uk/Tracer>.
- Renema W, Bellwood DR, Braga JC, et al. 2008. Hopping hotspots: global shifts in marine Biodiversity. *Science*, **321**(5889): 654–657.
- Renner SS. 2005. Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science*, **10**(11): 550–558.
- Robertson AHF, Ustaömer T, Parlak O, et al. 2006. The Berit transect of the Tauride thrust belt, S Turkey: Late Cretaceous-Early Cenozoic accretionary/collisional processes related to closure of the Southern Neotethys. *Journal of Asian Earth Sciences*, **27**(1): 108–145.
- Rögl F. 1998. Palaeogeographic considerations for mediterranean and paratethys seaways (Oligocene to Miocene). *Annalen des Naturhistorischen Museums in Wien*, **99**: 279–310.
- Ronquist F, Teslenko M, Van der Mark P, et al. 2012. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**(3): 539–542.
- Royden LH, Burchfiel BC, Van der Hilst RD. 2008. The geological evolution of the Tibetan plateau. *Science*, **321**(5892): 1054–1058.
- Saupe EE, Pérez-de La Fuente R, Selden PA, et al. 2012. New *Orchestina* Simon, 1882 (Araneae: Oonopidae) from Cretaceous ambers of Spain and France: first spiders described using phase-contrast X-ray synchrotron microtomography. *Palaeontology*, **55**(1): 127–143.
- Schäfer MA, Hille A, Uhl GB. 2001. Geographical patterns of genetic subdivision in the cellar spider *Pholcus phalangioides* (Araneae). *Heredity*, **86**(Pt 1): 94–102.
- Selden PA, Zhang WW, Ren D. 2016. A bizarre armoured spider (Araneae: Tetrablemmidae) from Upper Cretaceous Myanmar amber. *Cretaceous Research*, **66**: 129–135.
- Sen S. 2013. Dispersal of African mammals in Eurasia during the Cenozoic: Ways and whys. *Geobios*, **46**(1-2): 159–172.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**(9): 1312–1313.
- Steininger FF, Rögl F. 1984. Paleogeography and palinspastic reconstruction of the Neogene of the Mediterranean and Paratethys. *Geological Society, London, Special Publications*, **17**: 659–668.
- Sun JM, Jiang MS. 2013. Eocene seawater retreat from the southwest Tarim Basin and implications for early Cenozoic tectonic evolution in the Pamir Plateau. *Tectonophysics*, **588**: 27–38.
- Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**(22): 4673–4680.
- Van Hinsbergen DJJ, Lippert PC, Dupont-Nivet G, et al. 2012. Greater India Basin hypothesis and a two-stage Cenozoic collision between India and Asia. *Proceedings of the National Academy of Sciences of the United States of America*, **109**(20): 7659–7664.
- Vincent SJ, Morton AC, Carter A, et al. 2007. Oligocene uplift of the Western Greater Caucasus: an effect of initial Arabia-Eurasia collision. *Terra Nova*, **19**(2): 160–166.
- Wheeler WC, Coddington JA, Crowley LM, et al. 2017. The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. *Cladistics*, **33**(6): 574–616.
- World Spider Catalog. 2024. World Spider Catalog, version 26. Natural History Museum Bern. <http://wsc.nmbe.ch>.
- Yao ZY, Dong TT, Zheng G, et al. 2016. High endemism at cave entrances: a case study of spiders of the genus *Uthina*. *Scientific Reports*, **6**: 35757.
- Yuan ZY, Zhou WW, Chen X, et al. 2016. Spatiotemporal diversification of the true frogs (Genus *Rana*): a historical framework for a widely studied group of model organisms. *Systematic Biology*, **65**(5): 824–842.
- Zhang QQ, Li Y, Lin YC, et al. 2023. Regression of East Tethys resulted in a center of biodiversity: a study of Mysmenidae spiders from the Gaoligong Mountains, China. *Zoological Research*, **44**(4): 737–738.
- Zhao Z, Hou ZE, Li SQ. 2022. Cenozoic Tethyan changes dominated Eurasian animal evolution and diversity patterns. *Zoological Research*, **43**(1): 3–13.
- Zhao Z, Li SQ. 2017. Extinction vs. rapid radiation: the juxtaposed evolutionary histories of coelotine spiders support the eocene-oligocene orogenesis of the Tibetan Plateau. *Systematic Biology*, **66**(6): 988–1006.
- Zhao Z, Shao LL, Li FY, et al. 2020. Tectonic evolution of the Tethyan region created the Eurasian extratropical biodiversity hotspots: tracing *Pireneitega* spiders' diversification history. *Ecography*, **43**(9): 1400–1411.