Phylogenomic Analysis of Ultraconserved Elements Resolves the Evolutionary and Biogeographic History of Segmented Trapdoor Spiders

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Abstract.— The segmented trapdoor spiders (Liphistiidae) are the sole surviving family of the suborder Mesothelae, which forms the sister lineage to all other living spiders. Liphistiids have retained a number of plesiomorphic traits and their present-day distribution is limited to East and Southeast Asia. Studying this group has the potential to shed light on the deep evolutionary history of spiders, but the phylogeny and divergence times of the family have not been resolved with confidence. We performed phylogenomic and molecular dating analyses of 2,765 ultraconserved element loci from 185 liphistiid taxa. Our analyses show that the crown group of Liphistiidae appeared in the mid-Cretaceous at 102 Ma (95% credibility interval 92–113 Ma), but it was not until the Neogene that much of the diversification within the family occurred in mainland Southeast and East Asia. This diversification was coincident with tectonic events such as the extension of the East Asian continental margin, as well as geological upheavals in Indochina induced by the collision between India and Asia. Our study highlights the important role of major tectonic events in shaping the evolutionary history, present-day diversity, and geographical distribution of mesothele and liphistiid spiders.

Keywords: biogeography; Liphistiidae; UCEs; molecular dating; concatenation; summary coalescent.

There are about 50,000 extant spider species, divided between the two suborders Opisthothelae and Mesothelae. Almost all species belong to the suborder Opisthothelae, which lack external segmentation and are the archetypal spiders. This suborder comprises the groups Mygalomorphae (tarantulas, trap-door spiders, and their relatives) and Araneomorphae (true spiders). In contrast, Mesothelae Pocock, 1892 is represented by only a single extant family Liphistiidae Thorell, 1869, comprising at least 138 species with a distribution limited to East and Southeast Asia (World Spider Catalog 2020). Having retained plesiomorphic traits such as the abdominal tergites and appendage-like spinnerets projected from the middle of the ventral abdomen, liphistiids resemble the 299-million-year-old fossil mesothele *Palaeothele montceauensis* (Selden 1996) discovered in present-day France. Given the ancient origins of the liphistiid lineage and its retention of plesiomorphic traits, a clear phylogenetic picture of the group can shed light on the deep evolutionary history of spiders. However, the phylogeny and divergence times of Liphistiidae have not been adequately resolved.

The monophyly of Liphistiidae is well supported by morphological data (Platnick and Gertsch 1976; Coddington and Levi 1991), multilocus sequence data (Xu et al. 2015a; Wheeler et al. 2017), and transcriptomic and genomic data (Garrison et al. 2016; Fernández et al. 2018). The family comprises two subfamilies, Liphistiinae Thorell, 1869 and Heptathelinae Kishida, 1923, which consist of one and seven genera, respectively (Xu et al. 2015a, 2015b). The sole genus *Liphistius* Schiödte, 1849 of Liphistiinae is confined to Southeast Asia (Laos, Malaysia, Myanmar, Sumatra, and Thailand). Its species construct signal lines radiating from the burrow entrance (Fig. 1). The subfamily Heptathelinae is found only in China, Japan, and Vietnam, and its species produce burrows that lack signal lines around the entrance. The monophyly of each of the eight liphistiid genera was supported in phylogenetic analyses of two mitochondrial and three nuclear genes (Xu et al. 2015a).

However, the relationships among the heptatheline genera were not confidently resolved, leaving uncertainty in the intepretations of liphistiid evolution and biogeography. The low phylogenetic resolution might have been due to insufficient data (taxa and genes), incomplete lineage sorting, or gene flow between closely related species. These problems are likely to have been exacerbated by the occurrence of rapid divergences among lineages of crown Heptathelinae (Xu et al. 2015a).

The long evolutionary history of segmented trapdoor spiders has been strongly influenced by geological events in the region, particularly those that occurred in the Cenozoic (Xu et al. 2015a, 2016, 2018). The continental collision between India and Asia was the largest tectonic event of this era. Although the timing of this collision has not been resolved with precision (Ali and Aitchison 2008; van Hinsbergen et al. 2012; Hu et al. 2016), many geological processes are known to have been induced by this event. It caused the uplift of the Himalaya-Tibetan plateau and resulted in the eastward extension of the Asian continental margin. The collision also caused the southeast extrusion and clockwise rotation of the Indochinese peninsula through the Paleogene and well into the Miocene (Replumaz et al. 2010; Yin 2010; Zahirovic et al. 2014). These geological events are likely to have been a major driver of population fragmentation and speciation, sculpting the region's biodiversity (Lohman et al. 2011; de Bruyn et al. 2014; Favre et al. 2015). These processes would have had a particularly profound impact on short-range endemics (Harvey et al. 2011), such as liphistiid spiders. Liphistiid females, in contrast with vagile males, are sedentary and their lifestyles are restricted to long-lasting underground burrows. Thus, the biogeographic history of this group is likely to be predominantly vicariant with rare dispersal events (Xu et al. 2015a, 2016, 2018).

The fossil record has been silent on the evolutionary past of the liphistiid crown group, which aggravates the difficulty of studying the diversification of this family and calibrating estimates of its divergence times. Previous phylogenetic dating analyses of liphistiids were calibrated using the oldest mesothele fossil, from the late Carboniferous (Xu et al. 2015a). Given that previous divergence-time estimates have placed crown Liphistiidae in the Paleogene, there is an enormous time gap between the fossil evidence of mesotheles and the origin of Liphistiidae in Asia. Xu et al. (2015a) proposed that mesothele ancestors in Eurasia arrived in proto East and Southeast Asia via the northward-drifting Cimmerian continental strip, which connected Indochina, South China, and North China blocks in the Early Jurassic (Metcalfe 2013; Schwendinger 2017). Since that study, however, younger mesothele fossils have been discovered in the Middle Cretaceous amber forest of northern Myanmar (Wunderlich 2017, 2019). The impact of this discovery on estimates of the timescale and historical biogeography of Liphistiidae has remained untested.

Phylogenetic analyses of genome-scale data have been effective in resolving the relationships and evolutionary timescale of arachnids and spiders (Garrison et al. 2016; Fernández et al. 2018; Magalhaes et al. 2020). Although the cost and speed of genome sequencing have improved considerably over time, it remains impractical to sequence whole genomes for large numbers of related taxa. An alternative is to target a large sample of representative loci, for example, by using hybrid enrichment to sequence ultraconserved elements (UCEs) across the genome (Faircloth et al. 2012). Although a few UCE probe sets have been designed for arachnids and spiders, these enriched only a small number of loci from the liphistiid species, *Liphistius malayanus* (Starrett et al. 2017; Kulkarni et al. 2020).

Here, we generate and analyse a phylogenomic data set to infer the evolutionary relationships, divergence times, and historical biogeography of liphistiid spiders using a custom UCE probe set. Our data set consists of 2,765 UCE loci, including sequence data from all liphistiid genera and most of the known species. We analyse these data to evaluate the impacts of using different methods of data filtering, data partitioning, phylogenomic analysis, and molecular dating. Based on our dated phylogenetic tree, we reconstruct the biogeographic history of liphistiids and evaluate the impacts of the geological processes caused by the India-Asia collision. Our study provides new genomic resources and offers a comprehensive view of liphistiid evolution, diversification, and biogeography.

MATERIALS AND METHODS

Taxon Selection and Genomic Resources

We sampled 185 liphistiid specimens and selected 25 outgroup taxa, representing two infraorders (Mygalomorphae and Araneomorphae) of the spider order Araneae and three other arachnid orders (Amblypygi, Scorpiones, and Thelyphonida; Supplementary Table S1) (Starrett et al. 2017; Fernández et al. 2018; Lozano-Fernandez et al. 2019). Genomic DNA was extracted from leg tissues and sent with the probe sequences to Chiral Technologies, Inc. (West Chester, PA, USA) for library preparation and sequencing.

Since the available UCE probe sets target few loci from liphistiids, we designed a liphistiid-specific probe set. We began by generating low-coverage genomes for *Liphistius* aff. *malayanus* (Liphistiinae) and *Songthela xianningensis* (Heptathelinae). We removed the adapters and trimmed low-quality reads from the raw demultiplexed reads of the two genomes using Cutadapt v1.12 (Martin 2011). We quantified the trimmed reads using FastQC v0.11.5 (Andrews 2010), then *de novo* assembled the genomes using ABYSS v2.0.2 (Jackman et al. 2017). Using the PHYLUCE workflow (Faircloth 2016), we designed the probe set using the two liphistiid genomes as the ingroup, the mygalomorph *Acanthoscurria geniculata* genome as the outgroup, and the araneomorph *Stegodyphus mimosarum* genome (Sanggaard et al. 2014) as the base genome. To capture more conserved loci, we also extracted unique loci from an Arachnida probe set based on our ingroup and base genomes

(Faircloth 2017) and then combined these unique loci with our liphistiid-specific probes in the final probe-design step.

We removed adapters and low-quality bases from the raw demultiplexed UCE reads using illumiprocessor (Faircloth 2013), and assembled the trimmed reads using Trinity (Grabherr et al. 2011). We matched the contigs from all samples to the 'liphistiid-specific' probes. We also extracted UCE loci *in silico* from three other publicly available spider genomes, *Latrodectus hesperus*, *Loxosceles reclusa*, and *S. mimosarum*, as well as the arachnid UCE contigs from Starrett et al. (2017) (Supplementary Table S1).

We aligned UCE sequences using MAFFT (Katoh and Standley 2013) and internally trimmed the alignments with Gblocks (Castresana 2000; Talavera and Castresana 2007). We prepared two data sets, one comprising 2,765 UCE loci from 185 liphistiid taxa ('liphistiid' data set) and one comprising 2,801 UCE loci from the 25 outgroup taxa ('outgroup' data set). For the liphistiid data set, we produced subsets of the data based on 90% to 50% taxon coverage, to assess the effect of missing data on phylogenetic inference. For the outgroup data set, we produced only one subset based on 50% taxon coverage, in order to include a larger number of UCE loci because these selected outgroups from Starrett et al. (2017) only yielded an average of 177 UCE loci. Further details of taxon sampling and genomic data preparation are given in the Supplementary Material.

Phylogenomic Analyses and Molecular Dating

We performed phylogenomic analyses of the UCE data under a range of conditions and using two different methods to examine their effects on phylogenetic resolution. For the liphistiid and outgroup data sets, we inferred the species tree using concatenation and summary-coalescent methods. For the concatenation approach, we analysed the concatenated UCE alignments using IQ-TREE v1.6.12 (Nguyen et al. 2015). For the summary-coalescent approach, we inferred the individual gene trees using IQ-TREE, then inferred the species tree using ASTRAL-III (Zhang et al. 2018) based on these gene trees. Node support was estimated using ultrafast bootstrapping with 1000 replicates and SH-aLRT with 1000 replicates. To assess the concordance between the gene trees or nucleotide sites and the concatenation-based species tree, we used IQ-TREE to estimate gene concordance factors and site concordance factors. To compare the trees inferred by the different methods and data-filtering approaches, we calculated the normalized Robinson-Foulds pairwise distances using the R package phangorn (Schliep 2011).

Using Bayesian and likelihood-based methods, we estimated the evolutionary timescale for liphistiid spiders and explored the impacts of tree topology, data filtering, data partitioning, and clock models. For these analyses, we focused on the 185-taxon liphistiid data set based on 70% taxon coverage because we consider this to be the best of our phylogenetic estimates (see Results).

We used a two-step approach for Bayesian phylogenomic dating. In the first step, we estimated the substitution rate using the outgroup data set with 50% taxon coverage. We calibrated our dating analysis using two fossil-based age constraints (Magalhaes et al. 2020) and two secondary calibrations from previous molecular date estimates by Fernández et al. (2018). We analysed the data using MCMCTree (Yang 2007) using two different relaxed-clock models (correlated-rates and independent-rates). In the second step, we used MCMCTree to analyse the liphistiid data set with 70% taxon coverage, based on the two tree topologies inferred in our phylogenomic analyses above. Because no fossil can be reliably assigned to any groups within crown Liphistiidae, we placed a soft maximum constraint of 100 Ma on the age of crown Liphistiidae based on the mid-Cretaceous amber mesothele fossils recently described from Myanmar (Wunderlich 2017, 2019). We used three different clock-partitioning schemes (based on 1, 2, and 5 separate relaxed-clock models), with UCE loci assigned to the clock models on the basis of the mean root-to-tip distances of the midpoint-rooted gene trees.

We analysed the data using the correlated-rates and independent-rates relaxed-clock models, with gamma priors on the mean rate and rate-drift parameter being informed by the posterior estimates from our analysis of the outgroup data set. Formal comparison of the relaxed-clock models was not attempted, because estimation of marginal likelihoods was not computationally feasible. Instead, owing to evidence of low rate autocorrelation among lineages (see Results), we chose to focus on the divergence times inferred using the independent-rates relaxed-clock model for our subsequent diversification and biogeographic history analyses.

For comparison, we performed phylogenomic dating using penalized likelihood in treePL (Smith and O'Meara 2012) on the liphistiid data set with 70% taxon coverage. To obtain 95% credibility intervals for the estimates of node times, we also analysed 100 bootstrap trees with branch lengths inferred in IQ-TREE. We calibrated the crown node of Liphistiidae using the date estimate from our Bayesian analysis described above. Further details of the phylogenomic and molecular dating analyses are provided in the Supplementary Material.

Diversification Analysis and Biogeographic Reconstruction

We estimated the diversification rate of liphistiids in RevBayes v1.1.0 (Höhna et al. 2016) using an episodic model that allowed the diversification rate to vary between, but not within, time intervals. Our analysis was based on the dated phylogenomic tree inferred in our Bayesian analysis with five independent-rates relaxed-clock models. We specified 20 time intervals and assumed uniform taxon sampling with a sampling fraction of 166/213. The R package RevGadgets (Höhna et al. 2016) was used to plot the estimated episodic diversification rates.

To reconstruct the biogeographic history of liphistiids, we defined eight relevant, discrete biogeographic areas: (A) Sibumasu; (B) Indochina and east Peninsular Malaysia; (C) Hainan; (D) North China; (E) Southeast China; (F) Southwest China and north Vietnam; (G) Ryukyu Archipelago; and (H) Kyushu. We estimated the ancestral areas using BioGeoBEARS (Matzke 2013), based on the Bayesian dated phylogeny. We used the uncorrected and corrected Akaike information criterion to compare three models: dispersal, extinction, and cladogenesis model (DEC; Ree and Smith 2008), dispersal-vicariance model (DIVALIKE; Ronquist 1997), and BayArea-like model (BAYAREALIKE; Landis et al. 2013). These analyses were performed with or without an adjacency matrix for the biogeographic areas. To test the impact of model choice on biogeographic inference, we also reconstructed ancestral areas using dispersal, extinction, and cladogenesis analysis (DEC), statistical dispersal-vicariance analysis (S-DIVA), and Bayesian binary MCMC analysis (BBM) in RASP v4.2 (Yu et al. 2020). Further details are provided in the Supplementary Material.

RESULTS

Novel Genomic Data

We developed a liphistiid-specific probe set that comprises 19,740 baits targeting a total of 3,111 ultraconserved loci, including 2,900 loci that were targeted in this study and 211 loci that were targeted from the Arachnida probe set. Using the liphistiid-specific probe set, we obtained an average of 1,940 (range 896–2,204) ultraconserved loci and an average of 660 (range 95–1,298) nucleotides for the 192 samples in this study (Supplementary Table S1). A mean of 177 (126–210) ultraconserved loci were obtained for the selected 15 outgroup taxa from Starrett et al. (2017). Specifically, we obtained a mean of 3,331,490 pair-end reads, 17,337 contigs, and 1,959 UCEs per sample for the 185 liphistiid samples and 1,315,164 pair-end reads, 6,158 contigs, and 1,428 UCEs per sample for seven mygalomorphs. Our *in silico* tests of the probe set captured 2,416 conserved loci from *Stegodyphus mimosarum*, 1,684 from *Latrodectus hesperus*, and 986 from *Loxosceles reclusa*.

Phylogenomic Inferences

All of our analyses using concatenation and summary-coalescent methods, based on the different taxon coverages of the liphistiid data set, yielded congruent and well supported estimates of the phylogeny (Fig. 1; Supplementary Figs. S1–S2). Analyses of the liphistiid data sets with 50–90% taxon coverage strongly supported the monophyly of each of the two subfamilies, Liphistiinae and Heptathelinae, and each of the eight liphistiid genera. We also found strong support for the relationships among these genera.

Across our phylogenomic analyses, we found conflicting signals for the position of the genus *Qiongthela* Xu & Kuntner, 2015 (consisting of species endemic to Hainan and southern Vietnam). Concatenation and summary-coalescent methods both indicated that the liphistiid data sets with 50–70% taxon coverages strongly supported *Qiongthela* as a sister group to the two reciprocally monophyletic Japanese island genera, *Heptathela* Kishida, 1923 and *Ryuthela* Haupt, 1983 (Topology 1, Fig. 1c). However, the liphistiid data sets based on 80% and 90% taxon coverage consistently placed *Qiongthela* with the remaining East Asian mainland genera with high support, except in our summary-coalescent analysis of the data set with 80% taxon coverage (Topology 2, Fig. 1c; Supplementary Fig. S2). Low gene concordance factors and site concordance factors and the three quartet support values for the phylogenetic relationships of *Qiongthela* were similar for each of the data sets, regardless of the level of taxon coverage (Fig. 1b, 1c). For the different levels of taxon coverage, we found that differences between the estimates from the two phylogenomic approaches mainly occurred within genera (Supplementary Figs S1–S2). The concatenation analyses yielded identical tree topologies for the data sets with 50%, 60%, and 70% taxon coverage (RF distance = 0), whereas the summary-coalescent analyses yielded the same tree topologies only between the data sets with 50% and 70% taxon coverage.

The genus *Liphistius*, in our taxonomic samples represented by 35 examplars spanning almost its entire present-day distribution, is consistently split into two clades with high support values. One clade includes all species from Myanmar, one species from northwestern Thailand adjacent to Myanmar, and three species from southeast Peninsular Malaysia. The other clade contains the remaining species from Thailand and Peninsular Malaysia, Laos, and Sumatra (Indonesia). Among the four mainland heptatheline genera, *Vinathela* Ono, 2000, found in northern Vietnam and southern China, is the sister genus to the remaining genera. We found strong support for the southern China genus *Songthela* Ono, 2000 as the sister lineage to *Sinothela* Haupt, 2003 and *Ganthela* Xu & Kuntner, 2015. *Sinothela* is endemic to the north of the Yangtze River, whereas *Ganthela* is confined to southeastern China to the south of the Yangtze River.

The concatenation and the summary-coalescent analyses based on the outgroup data set with 50% taxon coverage yielded congruent estimates of the phylogeny. The nodes along the backbone of the tree were well supported. Although there was strong support for the monophyly of Mygalomorphae and of Araneomorphae, the two approaches inferred different species relationships within several clades of the two infraorders, and with poor support (Supplementary Fig. S3).

Evolutionary Timescale of Liphistiidae

The phylogenomic dating analyses based on the correlated-rates relaxed-clock model produced high estimates of the rate-drift parameter σ^2 for the 1-, 2-, and 5-clocks partition schemes (~0.6, Supplementary Fig. S4). This result suggests a very low degree of rate correlation between adjacent branches in the tree (Ho et al. 2015). Therefore, to simplify the presentation of divergence-time estimates from our various analyses, we focus here on the results from our Bayesian dating analysis using five independent-rates relaxed-clock models and Topology 1 (Fig. 2; Supplementary Table S2). Our phylogenomic dating analyses suggest a mid-Cretaceous age of 102 Ma (95% credibility interval, CI: 92–113 Ma) for crown Liphistiidae. This date slightly exceeds the soft maximum constraint that was specified as a prior in the Bayesian analysis, an outcome that has been observed in other phylogenomic studies that have used soft calibration bounds (e.g. Jarvis et al. 2014). We dated the crown group of Liphistiinae (*Liphistius*) to the late Oligocene at 28 Ma (95% CI: 25–31 Ma) and crown Heptathelinae to the late Eocene at 39 Ma (95% CI: 35–44 Ma). In the insular lineage of heptathelines, *Qiongthela* diverged from the two Japanese island genera, *Heptathela* and *Ryuthela*, in the late Eocene at 37 Ma (95% CI: 32–41 Ma). The split of *Vinathela* from the remaining three mainland genera, and the divergence of *Songthela* from the remaining two genera, occurred in the late Oligocene. All seven heptatheline genera began to diversify during the middle and late Miocene (7–16 Ma).

Divergence-time estimates were similar between the two different tree topologies (which differed in the placement of the genus *Qiongthela*) (Fig. 1). However, estimated divergence times for most nodes differed between the two relaxed-clock models. The independent-rates relaxed clock produced much younger estimates for almost all of the nodes than did the correlated-rates relaxed clock (Supplementary Table S2). The choice of clock-partitioning scheme had a modest impact on the estimates of divergence times, except for the age of the root. In general, most of the divergence times inferred using a 5-clock model were younger than those inferred using 1- or 2-clock models. Increasing the number of clocks also led to a reduction in the 95% CI widths of the divergence-time estimates, with this reduction being more pronounced for the independent-rates relaxed clock (Fig. 3; Supplementary Fig. S5; Supplementary Table S2).

The date estimates obtained using penalized likelihood for the major clades, except the root node, were slightly younger than those inferred in our Bayesian dating analysis with five independent-rates relaxed clocks (Supplementary Table S2). The widths of the 95% credibility intervals of the date estimates from penalized likelihood were less than one-tenth of the widths of the 95% credibility intervals of the Bayesian date estimates (Fig. 3).

Diversification and Biogeographic History

We found an increase in the net diversification rate starting from the Eocene, with a slight decrease in the Pliocene, a time period that corresponds to the radiation of the two liphistiid subfamilies (Fig. 2, Supplementary Fig. S6). Our biogeographic analysis in BioGeoBEARS favors the dispersal-extinction-cladogenesis model without an areas-adjacency matrix (Fig. 2, Supplementary Fig. S7; Supplementary Table S3). The ancestral-area analyses in RASP based on DEC, S-DIVA, and BBM models produced estimates for most of the ancestral location states similar to those inferred using BioGeoBEARS using the DEC model without the areas-adjacency matrix (Supplementary Fig. S7). The most likely ancestral area of Liphistiidae was the continental margin of Southeast and East Asia, during the mid-Cretaceous when Sibumasu, Indochina, and East Asia formed a single landmass (Fig. 4a). The liphistiine ancestors then occupied Sibumasu, Indochina, and east Peninsular Malaysia, whereas the heptatheline ancestors occupied a small part (southern Vietnam) of Indochina and the East Asian continental margin. We note that the DEC model with the areas-adjacency matrix yielded a smaller ancestral area for Liphistiidae, i.e., Indochina and

Hainan (Supplementary Fig. S7). This model produced estimates that were consistent with the liphistiine ancestral areas as described above, but suggested that the heptatheline ancestors occupied Hainan and Southeast China.

Within Heptathelinae, the ancestors of the present-day insular lineages underwent vicariant diversification before or when their ancestral areas became continental islands. The DEC model with the areas-adjacency matrix suggested that the ancestors of the insular lineage occupied the same ancestral areas as the heptatheline ancestors (Supplementary Fig. S7). Most of the analyses placed the ancestors of *Qiongthela* in both Hainan island and southern Vietnam. The ancestral area of the Japanese island genera, *Heptathela* and *Ryuthela*, was most likely on the Ryukyu archipelago, from where the ancestors of *Heptathela* dispersed to Kyushu.

The ancestors of the mainland heptatheline lineage (*Vinathela*, *Songthela*, *Sinothela*, and *Ganthela*) occupied Southeast China, before the genera arrived at their present-day distributions by a series of dispersal and vicariance events (Fig. 4b). The ancestors of *Vinathela* split from the mainland lineage and dispersed to southwestern China and northern Vietnam, with the same pattern found in the ancestors of *Songthela*. The ancestors of *Sinothela* and *Ganthela* diversified via a vicariant event, perhaps corresponding to the formation of the Yangtze River.

DISCUSSION

Our study supports a mid-Cretaceous date for crown Liphistiidae in the Southeast or East Asian continental margin, based on Bayesian relaxed-clock analyses of a novel phylogenomic data set with sampling from throughout the entire geographic range of the family. This date estimate is much earlier than the Paleogene origin inferred previously for crown Liphistiidae (Xu et al. 2015a). By tracing the history of the lineage to the

Carboniferous around 300 Ma, the age of the oldest mesothele fossil, *Palaeothele montceauensis* from present-day France (Selden 1996), we posit a series of extinctions of Mesothelae on its long eastward dispersal from Euramerica to the Asian ancestral location of the single extant Mesothelae lineage, Liphistiidae. The lineage is likely to have experienced further extinction events in the 60 Myr between the appearance of crown Liphistiidae and the crown nodes of the two subfamilies, Heptathelinae and Liphistinae. Our comprehensive phylogenomic dating and biogeographical analyses have produced the most comprehensive time-calibrated phylogeny of the extant mesothele family Liphistiidae by including 71% taxon coverage through the entire present-day distribution. Our analyses have revealed that the diversification of liphistiids was driven by both over-land dispersal and vicariant events.

Liphistiid Phylogeny

The liphistiid-specific probe set that we have developed here has targeted a larger number of UCE loci than any existing spider-specific or Arachnida probe set (Faircloth 2017; Starrett et al. 2017; Kulkarni et al. 2020). The novel probe set is also likely to be suitable for mygalomorphs, given the UCEs targeted for seven mygalomorphs in this study. Our phylogenetic analyses of the data set strongly support the monophyly of the family and each of its genera, as well as the reciprocally monophyletic relationship between the sole genus of the subfamily Liphistiinae, *Liphistius*, and the subfamily Heptathelinae. These results corroborate those from previous studies based on molecular data and morphology (Haupt 2003; Xu et al. 2015a, 2015b).

Our analyses resolve the relationships among the four heptatheline mainland genera, which were not confidently resolved in previous studies. Although the inferred relationships among heptatheline genera were consistent between our concatenation and summary-coalescent approaches, the position of *Qiongthela* remains uncertain. Our previous analysis of five genes was also unable to resolve the position of this genus (Xu et al. 2015a). The instability in the placement of this genus is probably due to the presence of a short internal branch, which has led to conflict among gene trees due to incomplete lineage sorting. Furthermore, the UCE alignments contain small numbers of informative sites, as indicated by our concordance analyses; the placement of *Qiongthela* is only supported by a third of gene trees and nucleotide sites. Other phylogenetic uncertainties are seen among rapidly diverging species in the liphistiid tree. As seen in other analyses of UCE loci from recently diverged species (e.g., Smith et al. 2014; Meiklejohn et al. 2016), these uncertainties are likely to be due to a combination of weak phylogenetic signal at shallow evolutionary scales and gene-tree incongruence resulting from incomplete lineage sorting.

Evolutionary Timescale and Biogeographic History of Liphistiids

Our comprehensive taxon sampling and genome-scale data set have enabled us to infer a detailed evolutionary timescale and biogeographic history for liphistiid spiders. All of the available evidence from fossils, palaeogeographical and palaeoclimatological data, along with the geographic distributions of the extant liphistiids, supports an origin of Mesothelae in Euramerica around 300 Ma (Selden 1996). We focus here on our Bayesian analysis using the independent-rates relaxed clock, which inferred a mid-Cretaceous origin of Liphistiidae (92–113 Ma) in Asia. The date estimates from this analysis are similar to those from previous analyses of smaller data sets (Xu et al. 2015a; 2016; 2018), but with narrower credibility intervals.

We infer that the mesothele ancestors spread through the Cimmerian continental strip, into Sibumasu, Indochina, and to the Southeast China margin, prior to the mid-Cretaceous. This would explain the recent finding of mid-Cretaceous mesothele amber fossils in northern Myanmar (Wunderlich 2017, 2019). Such a scenario suggests that mesothele spiders experienced a high extinction rate between their divergence from their common ancestor with opisthotheles and the emergence of crown Liphistiidae in Asia. Further discoveries of mesothele fossils from this time interval would shed new light on the eastward dispersal of this group of spiders. For example, any fossils discovered in Australia or on the other islands of the Sahul Shelf would be inconsistent with the hypothesis of a route through the Middle East, but would support an alternative biogeographic hypothesis (Xu et al. 2015a; Schwendinger 2017). Alternatively, mesotheles and liphistiids might have experienced a series of extinctions if they were already widespread in Asia hundreds of millions of years ago. We expect that further sampling efforts will uncover more mesothele fossils from the wide region between central Asia and Sibumasu.

Our biogeographic reconstructions indicate that the diversifications of the two liphistiid subfamilies in the late Eocene to Miocene coincided with the India-Asia collision. This major geological event commenced around 45 Ma and was probably complete by 35 Ma (Ali and Aitchison 2008) or 20–25 Ma (van Hinsbergen et al. 2012) (Fig. 4a). The strike-slip extrusion events affecting the landforms and the large-scale topography of mainland Southeast Asia might have driven the subsequent diversification of Liphistiinae. Nevertheless, *Liphistius* spiders have never crossed to the east of the Mekong River, which formed around the mid-Miocene or earlier (~17 Ma; Nie et al. 2018), with the exception of *L. laoticus*, found immediately on the east side of the river (Fig. 4b). Our results also suggest that the diversification of *Liphistius* lineages was largely shaped by vicariance events. For example, vicariance explains the splitting of the Myanmar lineage from the southeast Peninsular Malaysian lineage, and of the east Thailand lineage from the rest of the Sibumasu lineage. However, it seems that the Kra Isthmus between northern Thailand and Peninsular Malaysian did not act as an effective barrier for *Liphistius* lineages.

Our biogeographic reconstruction suggests that the diversification of Heptathelinae did not involve any over-water dispersal events, but was instead shaped by vicariant events caused by the extension of the eastern Asian margin. Heptathelines, whose ancestors occupied the margin of eastern Indochina and eastern Asia, diversified into seven genera in East Asia in the late Eocene through Miocene. The ancestors of heptathelines split into separate insular and mainland lineages through a vicariant event in the late Eocene, around 35–44 Ma. This evolutionary divergence coincided with the initial stage of the eastern Asian margin extension caused by the continued India-Asia collision at 35–45 Ma (Ren et al. 2002; Ali and Aitchison 2008).

The diversification of the insular lineage of heptathelines also occurred in the late Eocene. Our phylogenomic dating and biogeographical reconstruction suggested that the ancestors of the insular lineage exclusively occupied present-day Hainan island, southern Vietnam, and the Ryukyu archipelago, then split into two lineages, *Qiongthela* and two Japanese genera, around 32–41 Ma. The diversification of the insular lineage coincided with the collision-induced deformation of the continental-margin of eastern Asia at 36–17 Ma (Ren et al. 2002; Xu et al. 2012) and the opening of the South China Sea at 35–15 Ma (Hall 2002; Barckhausen et al. 2014; Li et al. 2014; Sibuet et al. 2016). The continued opening of the South China Sea during the Miocene might have led to the divergence between the *Qiongthela* lineages from Hainan island and from southern Vietnam. However, phylogenetic relationships between these two lineages, as well as the inferred age of the *Qiongthela* lineage from Hainan island (7 Ma, 95% CI: 6–8 Ma), are inconsistent with any geological hypotheses about the origin of the island (Zhu 2016; Ali 2018; Luo and Li 2018). Furthermore, the divergence between the two lineages predates the formation of the Qiongzhou Strait (2–2.5 Ma; Zhao et al. 2007). Within the Japanese lineages, our phylogenomic dating and biogeographic reconstruction suggest that the ancestors of

Heptathela colonized Kyushu through dispersal from the Ryukyus, and that the divergence within *Heptathela* was caused by a vicariant event that coincided with the formation of the Tokara Strait (Ota 1998). The diversification within *Ryuthela* coincides with the opening of the Kerama Gap, which is consistent with the findings of previous studies (Ota 1998; Xu et al. 2016).

The mainland heptatheline lineages diversified and reached their present-day geographic distributions from Southeast China via a combination of dispersal and vicariance. The ancestral areas of both *Vinathela* and *Songthela* overlapped, with both dispersing from Southeast China to southern China and northern Vietnam. However, *Vinathela* reached further south, and *Songthela* further west and north. *Vinathela* split from the other three mainland genera 24–30 Ma, whereas *Songthela* diverged from the remaining two genera 22–28 Ma. These evolutionary divergences coincided with some of the impacts of the India-Asia collision, including the extension of the East Asian continental margin and the clockwise rotation of Indochina at 32–17 Ma (Replumaz et al. 2010; Yin 2010; Zahirovic et al. 2014). The timing of the vicariant origin of the *Ganthela*+*Sinothela* lineage at 16–20 Ma overlaps with the timing inferred previously (Xu et al. 2018), but is estimated here with greater precision. The divergence between *Ganthela* and *Songthela* is, however, inconsistent with any hypothesized timing of the incision of the middle and lower Yangtze River (Xiang et al. 2007; Jia et al. 2010; Richardson et al. 2010; Zheng et al. 2013).

The timing and the extent of the eastward extension of the East Asian continental margin, and the southeast extrusion and clockwise rotation of Indochina, have been contentious (Yin 2010). Our results suggest that these tectonic events must have occurred simultaneously to shape the biogeographical patterns of liphistiids in the late Eocene to Miocene (39–7 Ma). Overall, our biogeographic reconstruction suggests that the present-day geographic distributions of liphistiids can be explained by both vicariance and dispersal events. Most importantly, liphistiids have always moved with the landmasses, and none of the dispersal events invoked in the biogeographic reconstruction involves the crossing of marine barriers.

CONCLUSIONS

Our phylogenomic analysis of a comprehensive genomic data set has enabled us to reconstruct the evolutionary and biogeographic history of liphistiids, the sister lineage to all other living spiders. This phylogeny sheds light upon the evolution of a highly distinctive lineage that diverged from other spiders 300 million years ago. However, the phylogeny of liphistiid spiders is characterized by an unusually long stem lineage spanning nearly 200 Myr, as well as a 60 Myr gap between the emergence of the crown group and the diversifications within its two subfamilies. We have found that the evolutionary radiations of Heptathelinae and Liphistiinae were driven by a series of tectonic events caused by the collision between India and Asia.

The reconstructed biogeographic history of liphistiids adds to the growing understanding of how geological processes have shaped biological diversity in East and Southeast Asia. Studies of organisms with poor dispersal capabilities can be particularly useful for examining the evolutionary impacts of tectonic events. Similar studies have been performed for cave spiders living in restricted habitats (Luo and Li 2018), stone frogs with poor over-land dispersal capabilities (Che et al. 2010), Asiatic salamanders with low vagility (Zhang et al. 2006), and freshwater fish constrained by drainage systems (Peng et al. 2006). These studies all highlight the tectonic events induced by the India-Asia collision as the driving forces in shaping the diversification, speciation, and evolution of a wide range of groups in the region.

SUPPLEMENTARY MATERIAL

Detailed description of the methods, along with figures and tables for all additional phylogenomic analyses discussed in this study, are available in the Supplementary Material. The probe set, filtered UCE data sets, alignment files, and tree files are provided in the Dryad repository (doi:10.5061/dryad.qv9s4mwcq).

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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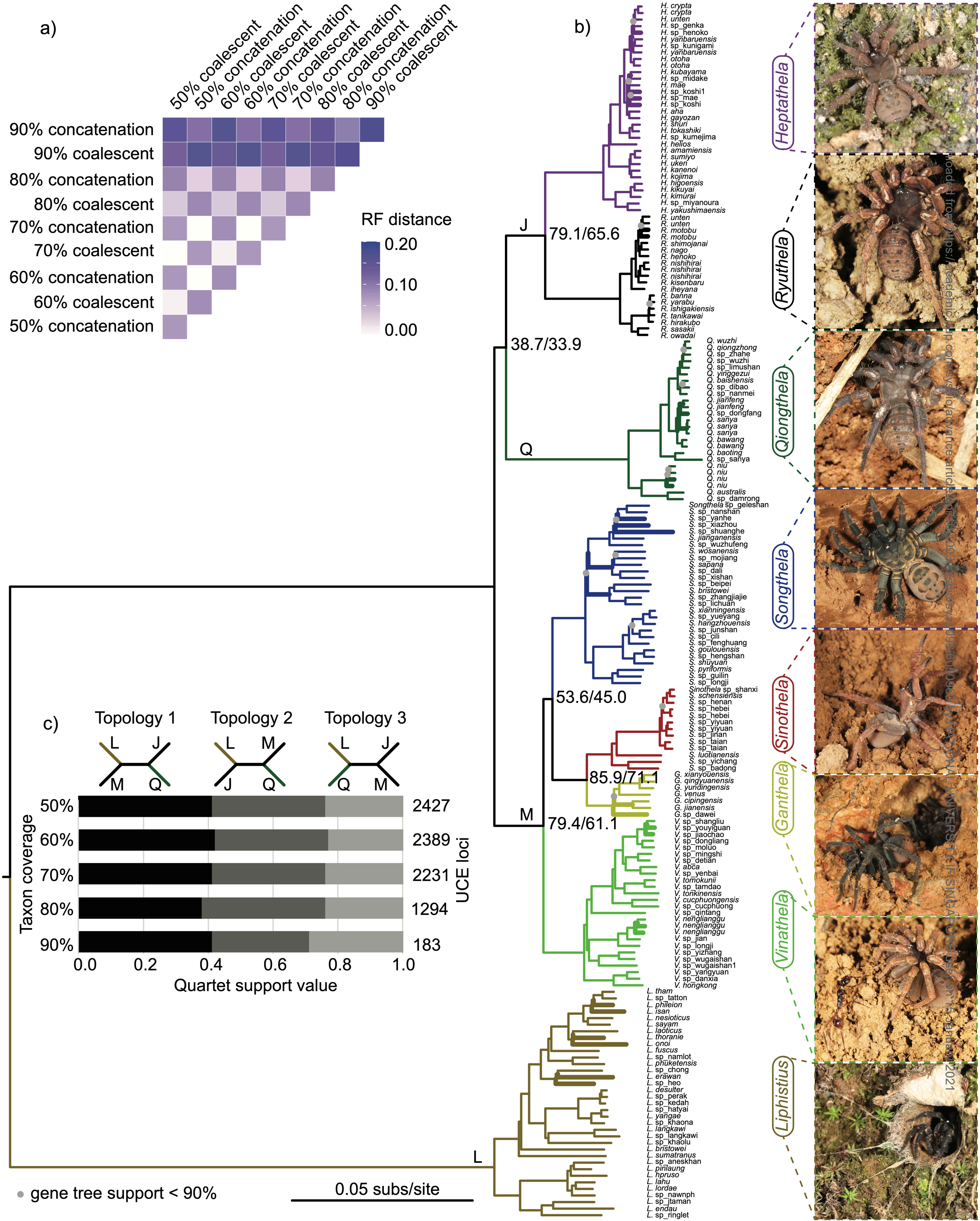
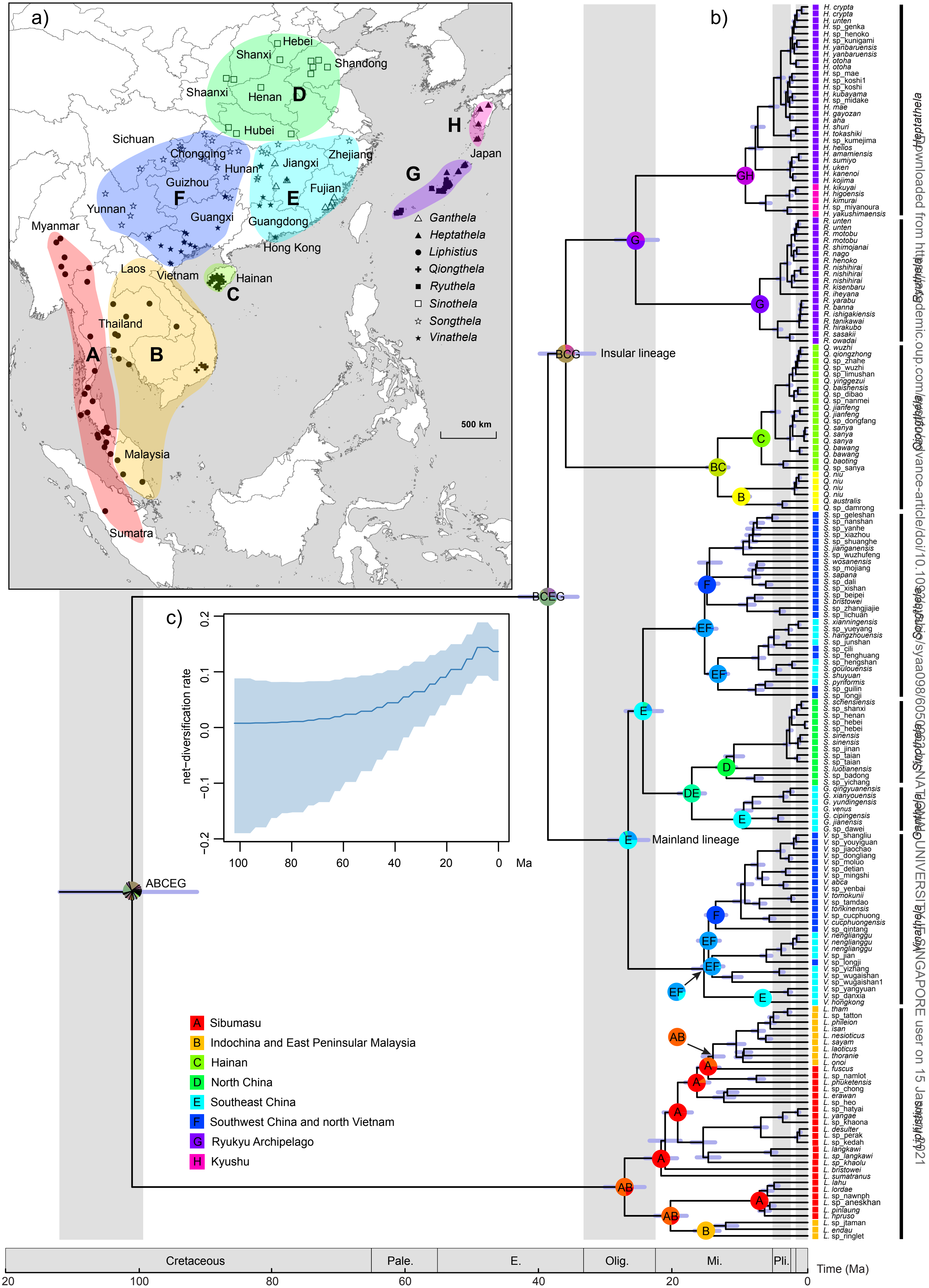
Figure 1. Phylogeny of liphistiid spiders inferred from ultraconserved elements. (a) Heatmap showing normalized Robinson-Foulds pairwise distances between species trees inferred from data sets with varying levels of taxon coverage, using concatenation and summary-coalescent methods. (b) Phylogenetic tree of the family Liphistiidae, inferred using a summary-coalescent method based on the 185-taxon liphistiid data set with 70% taxon coverage. Thick branches indicate the differences compared with the topology inferred using the concatenation method. Branch lengths have been estimated by maximum likelihood. The values next to each major node show the gene concordance factor and site concordance factor. Photographs of liphistiid species, from top to bottom, are: *Heptathela kimurai*, *Ryuthela nishihirai*, *Qiongthela baishensis*, *Songthela hangzhouensis*, *Sinothela* sp., *Ganthela yundingensis*, *Vinathela cucphuongensis*, and *Liphistius pinlaung*. All photographs were taken by X.X. (c) Support values for the three quartets showing different resolutions of the relationships among the four clades, J (Japanese clade), Q (*Qiongthela* clade), M (Mainland clade), and L (*Liphistius* clade) shown in the tree in (b).

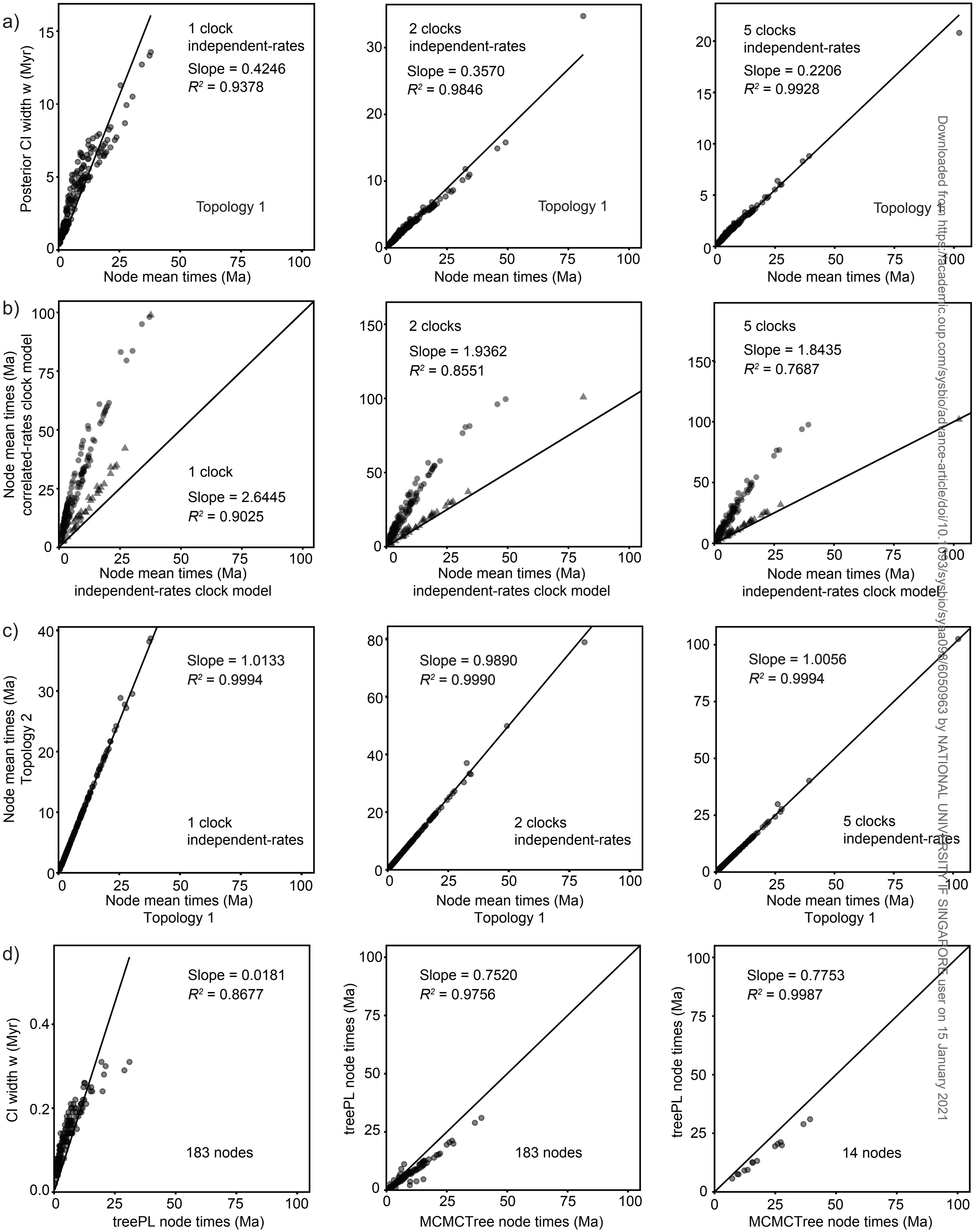
Figure 2. Evolutionary and biogeographic history of liphistiid spiders. (a) Geographic distributions of eight genera of liphistiid spiders in Southeast and East Asia, and the eight discrete geographical areas defined in this study. Symbols show the sampling locations for liphistiid spiders in the UCE data set. (b) Chronogram inferred using Bayesian phylogenomic analysis of the liphistiid data set with 70% taxon coverage. Colors at internal nodes show the ancestral areas estimated using the dispersal-extinction-cladogenesis model in BioGeoBEARS. The area colors correspond to the discrete geographical areas in the map. (c) Estimate of episodic diversification rate of liphistiids (blue line) and 95% credibility interval (light blue shading), obtained in RevBayes using the time-calibrated phylogeny of liphistiids.

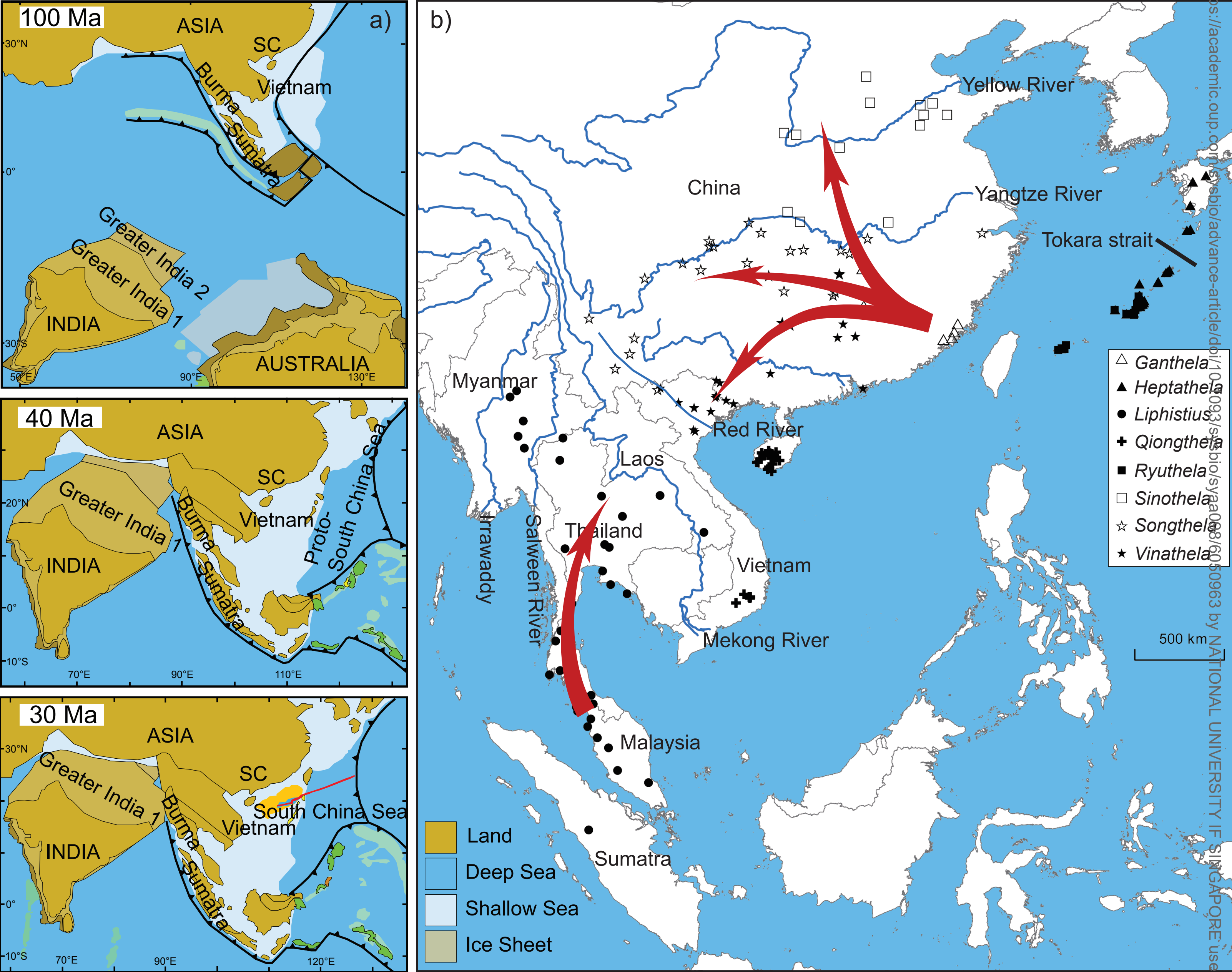
Figure 3. Comparisons of date estimates obtained using three different numbers of clock models, two models of among-lineage rate variation, two tree topologies, and two dating methods. (a) Plots of the 95% credibility interval (CI) widths against the mean age estimates of nodes in the liphistiid phylogeny based on 1, 2, and 5 separate relaxed-clock models, inferred using Bayesian phylogenomic dating in MCMCTree. The 95% CI widths decrease with increasing number of clocks (degree of clock partitioning). (b) Comparisons of the mean age estimates of nodes in the liphistiid phylogeny based on 1, 2, and 5 separate relaxed-clock models, under two different relaxed-clock models. Markers represent the node ages of Heptathelinae (circles) and of the root and Liphistiinae (triangles). (c) The mean age estimates of major nodes in the liphistiid phylogeny, based on 1, 2, and 5 separate relaxed-clock models, are similar between two different tree topologies (see Fig. 1c). (d) Plot of the 95% confidence interval widths (treePL) or 95% credibility interval widths (MCMCTree) against the mean age estimates of nodes in the liphistiid tree except for the root age, and comparisons of the mean age estimates of all and major nodes of liphistiid, except for the root age, from Bayesian analyses in MCMCTree and penalized-likelihood analyses in treePL.

Figure 4. Inferred historical dispersal routes for liphistiid spiders associated with tectonic events in East and Southeast Asia. (a) Reconstructed maps showing the distribution of land and tectonic history at 100 Ma, 40 Ma, and 30 Ma (modified from Hall 2012), and the tectonic events that might have driven the diversification of the family Liphistiidae and its two subfamilies, Heptathelinae and Liphistiinae. (b) Geographical distributions of eight extant liphistiid genera and the major river systems in East and Southeast Asia. Symbols show the sampling locations of liphistiid spiders for this study. Arrows show the inferred dispersal routes of liphistiids.


a)
50% coalescent
50% concatenation
60% coalescent
60% concatenation
70% coalescent
70% concatenation
80% coalescent
80% concatenation
90% coalescent
90% concatenation
90% coalescent
80% concatenation
80% coalescent
70% concatenation
70% coalescent
60% concatenation
60% coalescent
50% concatenation
RF distance
0.20
0.10
0.00
b)
J
79.1/65.6
38.7/33.9
Q
53.6/45.0
85.9/71.1
M
79.4/61.1
L
0.05 subs/site
gene tree support < 90%
Heptathela
Ryuthela
Qiongthela
Songthela
Sinothela
Ganthela
Vinathela
Liphistius
c)
Topology 1
Topology 2
Topology 3
L
J
M
Q
L
M
J
Q
L
J
Q
M
Taxon coverage
50%
60%
70%
80%
90%
2427
2389
2231
1294
183
UCE loci
0.0
0.2
0.4
0.6
0.8
1.0
Quartet support value


a)
Hebei
Shanxi
Shandong
Shaanxi
Henan
D
Hubei
Sichuan
Chongqing
Hunan
Jiangxi
Zhejiang
Guizhou
F
E
Fujian
Yunnan
Guangxi
Guangdong
Hong Kong
Myanmar
Laos
Vietnam
Hainan
C
Thailand
A
B
Malaysia
Sumatra
H
Japan
G
△ Ganthela
▲ Heptathela
● Liphistius
✚ Qiongthela
■ Ryuthela
□ Sinothela
☆ Songthela
★ Vinathela
500 km
b)
GH
G
G
BCG
Insular lineage
C
BC
B
BCEG
F
EF
EF
E
D
DE
E
E
Mainland lineage
F
EF
EF
EF
E
AB
A
A
A
A
AB
AB
A
B
ABCEG
c)
net−diversification rate
0.2
0.1
0.0
−0.1
−0.2
100
80
60
40
20
0
Ma
A Sibumasu
B Indochina and East Peninsular Malaysia
C Hainan
D North China
E Southeast China
F Southwest China and north Vietnam
G Ryukyu Archipelago
H Kyushu
H. crypta
H. crypta
H. unten
H. sp_genka
H. sp_henoko
H. sp_kunigami
H. yanbaruensis
H. yanbaruensis
H. otoha
H. otoha
H. sp_mae
H. sp_koshi1
H. sp_koshi
H. kubayama
H. sp_midake
H. mae
H. gayozan
H. aha
H. shuri
H. tokashiki
H. sp_kumejima
H. helios
H. amamiensis
H. sumiyo
H. uken
H. kanenoi
H. kojima
H. kikuyai
H. higoensis
H. kimurai
H. sp_miyanoura
H. yakushimaensis
R. unten
R. unten
R. motobu
R. motobu
R. shimojanai
R. nago
R. henoko
R. nishihirai
R. nishihirai
R. nishihirai
R. kisenbaru
R. iheyana
R. yarabu
R. banna
R. ishigakiensis
R. tanikawai
R. hirakubo
R. sasakii
R. owadai
Q. wuzhi
Q. qiongzhong
Q. sp_zhahe
Q. sp_wuzhi
Q. sp_limushan
Q. yinggezui
Q. baishensis
Q. sp_dibao
Q. sp_nanmei
Q. jianfeng
Q. jianfeng
Q. sp_dongfang
Q. sanya
Q. sanya
Q. sanya
Q. bawang
Q. bawang
Q. baoting
Q. sp_sanya
Q. niu
Q. niu
Q. niu
Q. niu
Q. australis
Q. sp_damrong
S. sp_geleshan
S. sp_nanshan
S. sp_yanhe
S. sp_xiazhou
S. sp_shuanghe
S. jianganensis
S. sp_wuzhufeng
S. wosanensis
S. sp_mojiang
S. sapana
S. sp_dali
S. sp_xishan
S. sp_beipei
S. bristowei
S. sp_zhangjiajie
S. sp_lichuan
S. xianningensis
S. sp_yueyang
S. hangzhouensis
S. sp_junshan
S. sp_cili
S. sp_fenghuang
S. sp_hengshan
S. goulouensis
S. shuyuan
S. pyriformis
S. sp_guilin
S. sp_longji
S. schensiensis
S. sp_shanxi
S. sp_henan
S. sp_hebei
S. sp_hebei
S. sinensis
S. sinensis
S. sp_jinan
S. sp_taian
S. sp_taian
S. luotianensis
S. sp_badong
S. sp_yichang
G. qingyuanensis
G. xianyouensis
G. yundingensis
G. venus
G. cipingensis
G. jianensis
G. sp_dawei
V. sp_shangliu
V. sp_youyiguan
V. sp_jiaochao
V. sp_dongliang
V. sp_moluo
V. sp_detian
V. sp_mingshi
V. abca
V. sp_yenbai
V. tomokunii
V. sp_tamdao
V. tonkinensis
V. sp_cucphuong
V. cucphuongensis
V. sp_qintang
V. nenglianggu
V. nenglianggu
V. nenglianggu
V. sp_jian
V. sp_longji
V. sp_yizhang
V. sp_wugaishan
V. sp_wugaishan1
V. sp_yangyuan
V. sp_danxia
V. hongkong
L. tham
L. sp_tatton
L. phileion
L. isan
L. nesioticus
L. sayam
L. laoticus
L. thoranie
L. onoi
L. fuscus
L. sp_namlot
L. phuketensis
L. sp_chong
L. erawan
L. sp_heo
L. sp_hatyai
L. yangae
L. sp_khaona
L. desulter
L. sp_perak
L. sp_kedah
L. langkawi
L. sp_langkawi
L. sp_khaolu
L. bristowei
L. sumatranus
L. lahu
L. lordae
L. sp_nawnph
L. sp_aneskhan
L. pinlaung
L. hpruso
L. sp_jtaman
L. endau
L. sp_ringlet
Heptathela
Ryuthela
Qiongthela
Songthela
Sinothela
Ganthela
Vinathela
Liphistius
Cretaceous
Pale.
E.
Olig.
Mi.
Pli.
120
100
80
60
40
20
0
Time (Ma)


a)
Posterior CI width w (Myr)
1 clock
independent-rates
Slope = 0.4246
R^2 = 0.9378
Topology 1
Node mean times (Ma)
2 clocks
independent-rates
Slope = 0.3570
R^2 = 0.9846
Topology 1
Node mean times (Ma)
5 clocks
independent-rates
Slope = 0.2206
R^2 = 0.9928
Topology 1
Node mean times (Ma)
b)
Node mean times (Ma)
correlated-rates clock model
1 clock
Slope = 2.6445
R^2 = 0.9025
Node mean times (Ma)
independent-rates clock model
2 clocks
Slope = 1.9362
R^2 = 0.8551
Node mean times (Ma)
independent-rates clock model
5 clocks
Slope = 1.8435
R^2 = 0.7687
Node mean times (Ma)
independent-rates clock model
c)
Node mean times (Ma)
Topology 2
Slope = 1.0133
R^2 = 0.9994
1 clock
independent-rates
Node mean times (Ma)
Topology 1
Slope = 0.9890
R^2 = 0.9990
2 clocks
independent-rates
Node mean times (Ma)
Topology 1
Slope = 1.0056
R^2 = 0.9994
5 clocks
independent-rates
Node mean times (Ma)
Topology 1
d)
CI width w (Myr)
Slope = 0.0181
R^2 = 0.8677
183 nodes
treePL node times (Ma)
treePL node times (Ma)
Slope = 0.7520
R^2 = 0.9756
183 nodes
MCMCTree node times (Ma)
treePL node times (Ma)
Slope = 0.7753
R^2 = 0.9987
14 nodes
MCMCTree node times (Ma)


100 Ma
a)
ASIA
SC
Vietnam
Burma
Sumatra
Greater India 2
Greater India 1
INDIA
AUSTRALIA
30°N
0°
30°S
50°E
90°E
130°E
40 Ma
ASIA
SC
Vietnam
Proto-South China Sea
Greater India 1
Burma
Sumatra
INDIA
20°N
0°
10°S
70°E
90°E
110°E
30 Ma
ASIA
SC
South China Sea
Vietnam
Greater India 1
Burma
Sumatra
INDIA
30°N
0°
10°S
70°E
90°E
120°E
b)
Yellow River
China
Yangtze River
Tokara strait
Myanmar
Red River
Laos
Thailand
Vietnam
Mekong River
Irrawaddy
Salween River
Malaysia
Sumatra
500 km
△ Ganthela
▲ Heptathela
● Liphistius
✚ Qiongthela
■ Ryuthela
□ Sinothela
☆ Songthela
★ Vinathela
Land
Deep Sea
Shallow Sea
Ice Sheet