

## Research



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# Differences in Quaternary co-divergence reveals community-wide diversification in the mountains of southwest China varied among species

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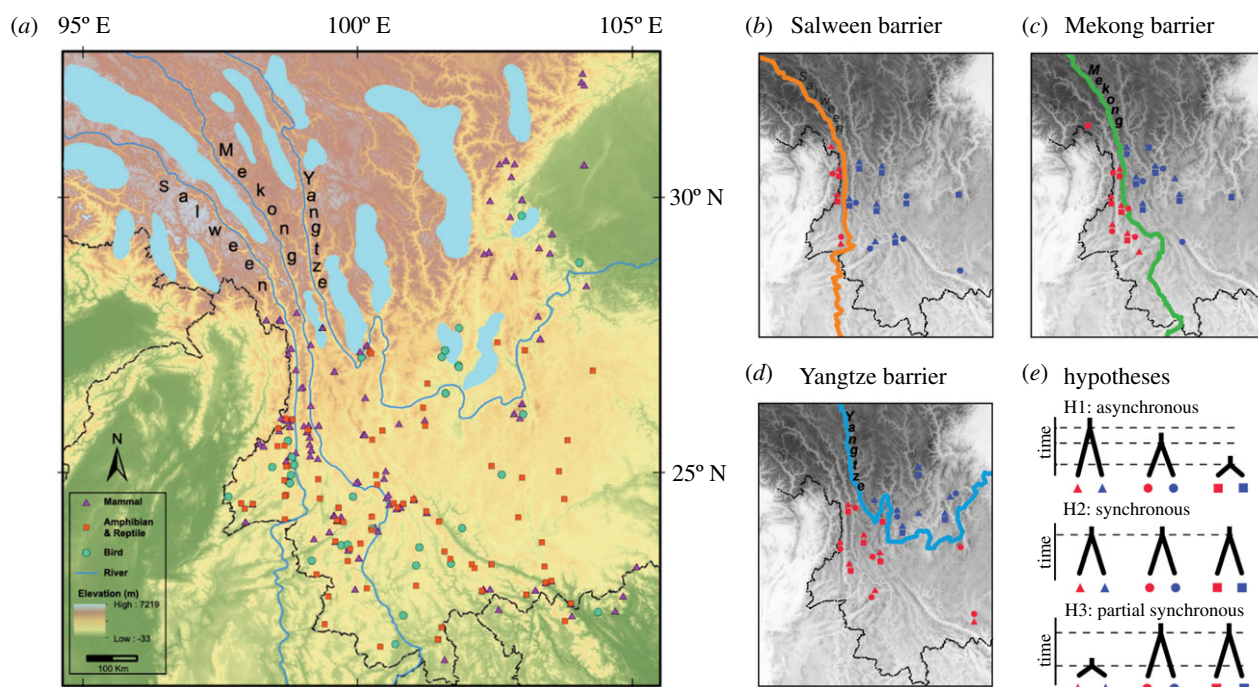
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The mountains of southwest China (MSWC) is a biodiversity hotspot with highly complex and unusual terrain. However, with the majority of studies focusing on the biogeographic consequences of massive mountain building, the Quaternary legacy of biodiversity for the MSWC has long been overlooked. Here, we took a statistical comparative phylogeography approach to examine factors that shaped community-wide diversification. With data from 30 vertebrate species, the results reveal spatially concordant genetic structure, and temporally clustered co-divergence events associated with river barriers during severe glacial cycles. This indicates the importance of riverine barriers in the phylogeographic history of the MSWC vertebrate community. We conclude that the repeated glacial cycles are associated with co-divergences that are themselves structured by the heterogeneity of the montane landscape of the MSWC. This orderly process of diversification has profound implications for conservation by highlighting the relative independence of different geographical areas in which some, but not all species in communities have responded similarly to climate change and calls for further comparative phylogeographic investigations to reveal the connection between biological traits and divergence pulses in this biodiversity hotspot.

## 1. Introduction

Although the uplift of mountains and the associated impact on diversification is widely recognized, including for the mountains of southwest China (MSWC), the processes associated with this complex topography and associated barriers as recent drivers of divergence remain relatively less studied. This is especially the case for the MSWC, one of the most topographically complex and species-rich biodiversity hotspots [1]. In the past two decades, researchers conducted hundreds of biogeographic and phylogeographic studies in the MSWC to study the vicariance events associated with the uplifting of Tibetan Plateau [2,3], such as the separation between Asian catfish genera [4], and between Senecioneae genera [5]. Yet, many cryptic species and recent speciation events have been discovered and documented in this region [6–10], with divergence dates that clearly occur after major orogenesis events in this region [9,10], suggesting recent divergence may play a role in generating some of the biodiversity in this region, and that mechanisms other than geographical events in the distant past should be considered.

We currently have limited information on recent diversification events and the mechanisms—those responsible for population differentiations and cryptic



**Figure 1.** (a) Map of the main rivers and topography of the mountains of southwest China. Coloured symbols represent the geographical location of the samples; purple triangle, orange square and green circle denote samples of mammals, amphibians and reptiles, birds, respectively. Cyan patches show the maximum extent of glaciation during the Last Glacial. (b–d) The pairs of clades with a phylogeographic break at each river (the Salween, Mekong and Yangtze) were identified at first. (e) Comparative phylogeographic hypotheses among species with spatially concordant phylogeographic structure were tested using the full-likelihood Bayesian computation method. Different symbol shape represents different species; the within-species clades bounded by rivers are represented as contrasting colours. (Online version in colour.)

speciation—in the MSWC. On this temporal scale, these events are typically concentrated in the Pleistocene [11] (e.g. 2.0 Ma–150 ka). Spatially, species often exhibit discrete genetic patterns, including those that correspond to geographical or ecological barriers to dispersal, such as arid-hot valleys, basins or icy mountain tops [12]. However, the geographical scope of such studies is fairly limited. For example, most studies only recognize the whole MSWC region as an east-west dispersal barrier. Yet, the MSWC is characterized by a complex of extremely rugged terrain that is divided by three major rivers (the Salween, Mekong and Yangtze), forming deep-cutting canyons that are at least 2000 m lower than the mountain ridges on the two sides (figure 1). A pronounced knowledge gap is associated with the divergence processes at this finer geographical scale. For example, do populations of different species have concordant spatial structure? If so, is there evidence of synchronous divergence at this finer temporal scale (i.e. recent history)? To what extent are community spatio-temporal divergence patterns similar, as opposed to being associated with species-specific characteristics (e.g. habitat preference)? Do the rivers have similar effects on divergence, or does the effect of the river on community divergence vary (i.e. river-specific effects on species divergence)? It is these questions that motivate our research, and the answers of which, are arguably an important component to understanding the accumulation of diversity in the MSWC.

Here, we adopted a community-wide approach to decipher the phylogeographic pattern across multiple co-distributed species in MSWC. The concordance of spatial structures and the synchronization in divergence time in a biological community are used to test hypotheses about mechanisms that generated biodiversity [13–16]. Specifically, we analyse a new dataset generated for some mammal species (electronic supplementary material, table S1) along with data from previous phylogeographic studies conducted in the MSWC (electronic

supplementary material, table S2) to increase the representation of taxa for tests of concordance of species phylogeographic structure using a full-likelihood Bayesian computation method (FBC; given differences in performance [17], as well as more general theoretical arguments, we focus exclusively on tests based on FBC, as opposed to relying on approximations in hierarchical approximate Bayesian computation, hABC) [18]. For all non-flying terrestrial animals in this study, genetic data reveal that at least one of the rivers functioned as a dispersal barrier, with many species exhibiting spatially concordant phylogeographic structure. Hence, we more specifically tested three hypotheses with different levels of temporal synchronization among spatially concordant species: asynchronous hypothesis (H1) in which taxa have independent divergence histories; synchronous hypothesis (H2) in which all taxa ‘share’ one co-divergent event; and partially synchronous hypothesis (H3) in which taxa are grouped into more than one co-divergence event (figure 1e). With this set of hypotheses our work aims to address (i) whether there are prevailing phylogeographic histories among co-distributed species in the MSWC, and (ii) what factors contribute to community-wide phylogeographic diversity in the MSWC by comparing the results of species grouping by river versus taxonomy.

Since the building of the MSWC has been suggested as a prolonged process of orogenesis (i.e. 20–5 Ma) [19–21], species created through vicariance in the process (e.g. river capture, mountain uplift) simply have idiosyncratic history. Thus, because of the unique evolutionary history and biological features (e.g. vagility, niche breadth), different species from the same community should have asynchronous phylogeographic divergence when their populations disperse on complex landscape (H1, asynchronous hypothesis). On the other hand, owing to the drastic climate changes during the Pleistocene (i.e. glacial expansion from the Tibetan Plateau), there might be one predominant event that isolates the

whole community into separate refugia (H2, synchronous hypothesis). Alternatively, the distributional shift of species could be affected by several drastic glaciations, which result in divergence times of different species concentrated into limited time windows (H3, partial-synchronous hypothesis).

## 2. Material and methods

Please see the electronic supplementary material for full material and methods.

## 3. Results

### (a) Data acquisition

We took the *sensu lato* concept of the MSWC, which is geographically broader than the biodiversity hotspot MSWC [12]. In addition to the sequence data generated specifically for this study, we surveyed the Web of Science using the key-word ‘phylogeog\*’ and ‘China’ keeping only studies with representative sampling across one of the four focal areas (figure 1a), and for which, there was georeferenced genetic data. In addition to the published data, we generated data for six mammal species, thus the compiled dataset consisted of 2278 mitochondrial sequences from 30 terrestrial species (14 small mammals, 10 birds and six amphibians or reptiles; electronic supplementary material, table S2). All these species are typical members of the MSWC animal community.

### (b) Examining the spatial phylogeographic structure

We examined whether river barriers correspond to monophyletic clades on the mitochondrial gene tree estimated by BEAST 2 [22] (electronic supplementary material, figures S1–S3), or clades delimited by the generalized mixed yule-coalescent [23] (electronic supplementary material, table S1). Rivers correspond to phylogeographic breaks in all examined mammal species—that is, few clades were distributed across a river (see details in the electronic supplementary material, figures S1–S3). Depending on the whole species range, the number of phylogeographic divisions ranges from one to three (figure 2). All amphibian/reptilian taxa show conspicuous within-species genetic divergence as well—that is, species have at least one primary phylogeographic break, and three genetic clusters are apparent in two species (figure 2). Mammals are characterized by more monophyletic clades whose divisions coincide with the rivers, however, populations of amphibian/reptile taxa were generally less geographically structured. None of the bird species showed population structure associated with rivers. No genealogical structure was apparent in two bird species. While the other four species have two clades in their mitochondrial gene tree, the clades overlap geographically (i.e. there is no correspondence between the genealogical structure in the bird species with geographical barriers in this region).

With regards to phylogeographic structure associated with specific rivers, the Mekong river contributes more than the other two in shaping the phylogeographic diversity of the MSWC vertebrate community studied here (figure 2). Specifically, 14 phylogeographic divisions are associated with the Mekong (12 of which are in mammals), seven with the Salween (four in mammals) and eight with the Yangtze (six in mammals). However, there is not a significant

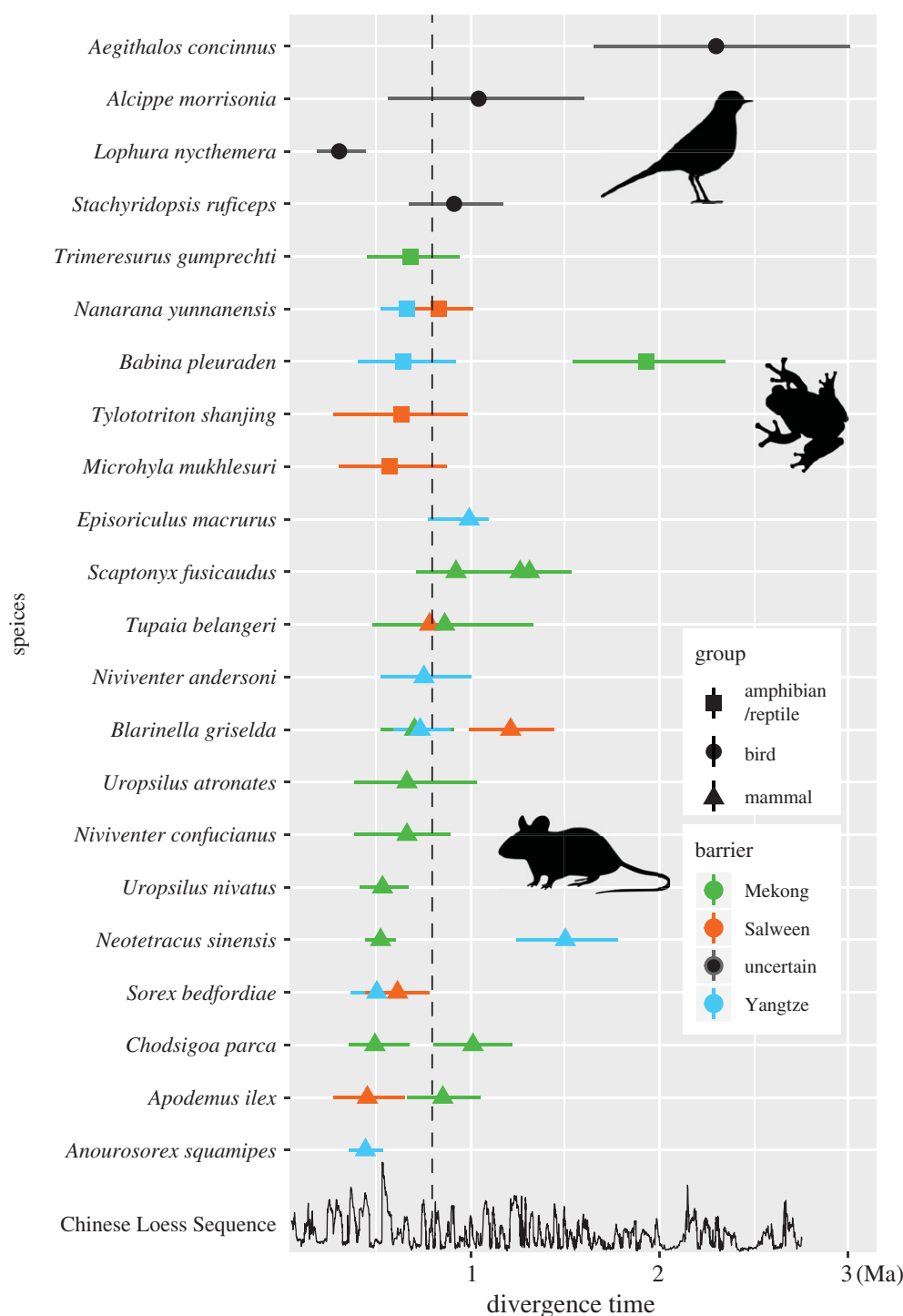
association between particular taxonomic groups and specific river barriers (paired *t*-test,  $p > 0.05$ ).

### (c) Testing community-wide co-divergence in time

Using the estimated gene trees to define clades, in some cases, the clades correspond to proposed subspecies, or even potential species boundaries that are under debate. Given the taxonomic status of lineages defined by the clades does not have a direct effect on the time estimates of divergence events, we hereafter refer to all of them as clades, and which results in a total of 33 clade pairs (i.e. divergence events) in our study (figure 2). All clades of terrestrial non-flying vertebrates have the majority of individuals coming from one side of a river barrier, except a few individuals seem to ‘cross’ the river (electronic supplementary material, figures S1–S3). These individuals might represent recent migration after the separation of geographical allineages, but their existence does not affect the timing of clade divergence (electronic supplementary material, figures S1–S3).

For each individual clade pair, we applied BPP v.3 [24] to estimate the divergence time (electronic supplementary material, table S1). The estimated divergence times of mammalian clades span from 0.44 to 1.50 Ma, compared with 0.57 and 0.83 Ma in amphibians and reptiles, with the single exception of an older split at 1.93 Ma in the Yunnan pond frog (*Babina pleuraden*; figure 2). By contrast, the divergence times in birds were much more varied, ranging from 0.30 to 2.30 Ma (figure 2; electronic supplementary material, table S1). From the perspective of each of the river barriers, the timing of divergence events largely overlap in range, but clearly do not support a single divergence event for all species in the community (Salween: 0.45–1.21 Ma; Mekong: 0.49–1.93 Ma; Yangtze: 0.44–1.50 Ma; figure 2).

We chose the recently developed FBC method implemented in *ecoevolity* [18] (<http://phyletica.org/ecoevolity/>; see the electronic supplementary material, Methods) to test co-divergence across species. This method combines genetic data from multiple clade pairs to test whether their divergences are clustered in time. Briefly, it considers divergences occurred at the same time as one co-divergence event, and performs full-likelihood Bayesian model choice among models that assume different numbers of co-divergence events and assign different clade pairs to different events. We first ran FBC with all the taxa pairs multiple times with different priors on the concentration parameter of the Dirichlet process. Because of the lack of clear correspondence of divergence with geographical barriers, we excluded the bird species from this comparative analysis, leaving 29 mammalian, amphibian and reptilian clade pairs. Tests of concordant divergence were initially conducted on all the clade pairs, including multiple runs using different concentration priors on the divergence events. There is no posterior support for total synchronous divergence (Bayes factor  $< 1$ ). Instead, a more complex history of divergence with varying degrees of synchrony is suggested. Specifically, regarding the number of co-divergent events, the FBC analyses suggests a model with 14–19 co-divergence events; this model has the highest posterior probability, but with considerable uncertainty (i.e. the posterior distribution is very flat; electronic supplementary material, table S3). Given this lack of temporal synchrony when considering the entire dataset, we conducted separate FBC runs for each taxonomic group (i.e. mammal or amphibian/reptile) and for each river to examine whether there was

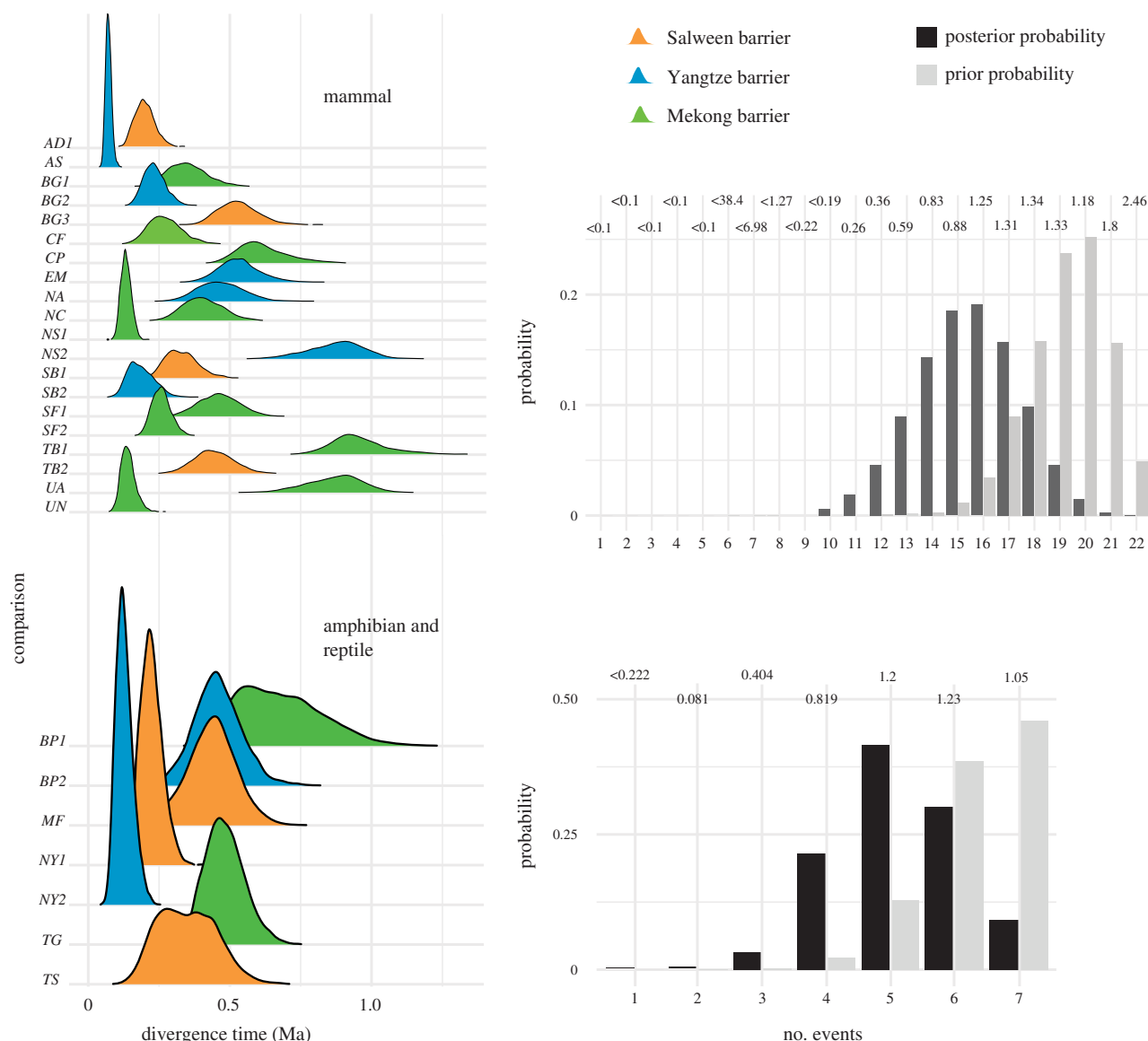


**Figure 2.** Divergence times (with mean and 95% credible intervals) of 33 phylogeographic clades cross riverine barriers; branch lengths estimated in BPP were divided by the mutation rate to express time in years. Divergence times of amphibians and reptiles, birds and mammals, are represented by different shapes, whereas divergences associated with the different rivers are shown in differing colours. The dashed line marks the boundary of Early and Mid-Pleistocene; the bottom plot shows the climatic cycles of the Chinese Loess Sequence, where peaks represent glaciations. (Online version in colour.)

any clustering of the divergence events. Taxonomic specific tests showed no clear evidence for concordant divergence. Specifically, multiple divergence events were supported, with 16 co-divergence events inferred for the 22 mammal clade pairs, and five co-divergence events inferred for the seven amphibian/reptile clade pairs (figure 3). That is, very few co-divergence events were identified in each taxonomic group.

In striking contrast, analyses run on each river separately clearly show support for clustered co-divergence (i.e. the partial-synchronous hypothesis in figure 1e; figure 4 and electronic supplementary material, table S4). Specifically, three co-divergence events for the Yangtze river (posterior

probability (PP) = 0.55,  $2\ln\text{BF} = 18.28$ ; figure 4), and two co-divergence events for the Mekong (PP = 0.52,  $2\ln\text{BF} > 27.82$ ) and Salween (PP = 0.91,  $2\ln\text{BF} = 23.55$ ) rivers, were supported (figure 4), although there is no posterior support for synchronous divergence for any given river (Bayes factor  $< 1$ ; figure 4). For any given river, we note that all the divergence events are relatively recent. For example, divergence events around 0.5 Ma for the Salween and Yangtze, and 0.85 Ma for the Mekong river, are coupled with very recent diversification pulses between 0.1–0.2 Ma for each river. This combination of a very recent and relatively older divergence explains why the overall correlation between the temporal



**Figure 3.** Testing phylogeographic synchronization of different groups of taxa. Mammal species ( $n = 22$ ) were tested in one group, while the amphibians and reptiles ( $n = 7$ ) were grouped together for the other test. The numbers above each bar denote the Bayes factor. (Online version in colour.)

and spatial scale is not significant. For example, despite sharing the same geographical barrier (i.e. river), the overlap of the posterior distribution of estimated divergence times among taxa for any particular river is not greater than the overlap of the posterior of divergence times for pairs across different river barriers. The mean overlap of the posterior for divergence times estimates between two clade pairs at the same river is 12%, 18% and 7% for the Mekong, Salween and Yangtze, respectively, compared with an average overlap of 18% in the posterior of divergence times across different rivers (electronic supplementary material, figure S4 versus electronic supplementary material, table S3). That is, two species sharing a geographical barrier are not more likely to have similar divergence time than those from different geographical barriers. However, the FBC analyses per river clearly show that species in the MSWC community do not have completely idiosyncratic histories (figure 4).

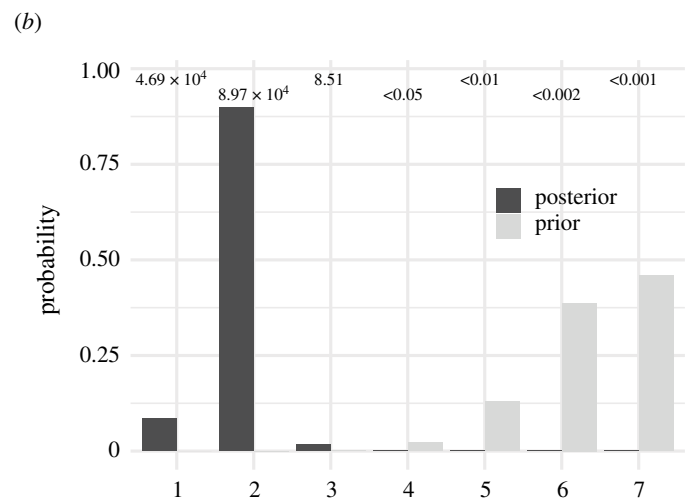
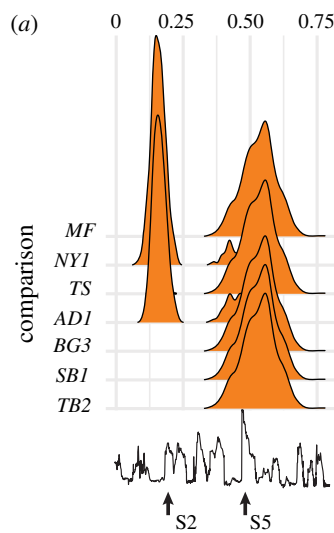
## 4. Discussion

Phylogeographic concordance, the common structuring of genetic variation across co-distributed taxa, is key evidence

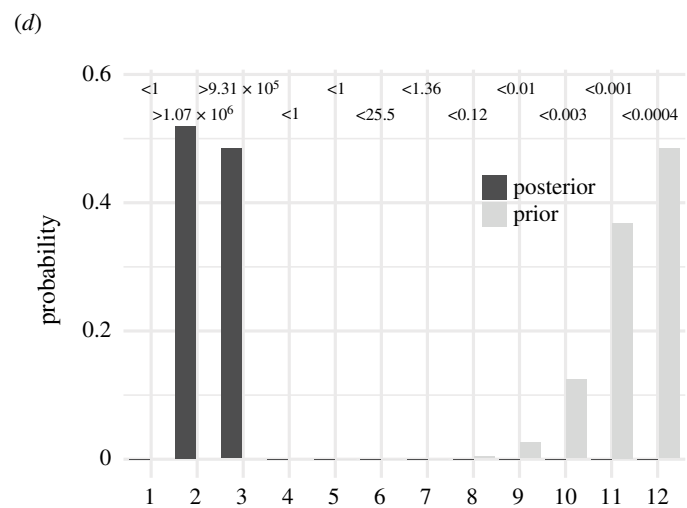
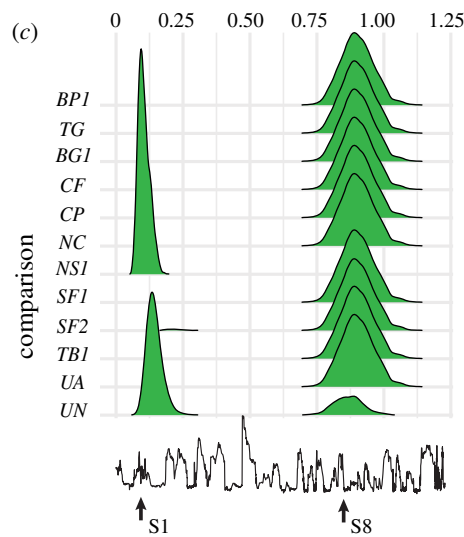
of the impact of abiotic/extrinsic factors (biogeographic barriers, geological events or past environmental change) on genetic variation [25]. However, despite more than two hundred molecular phylogenetic/phylogeographic studies on the evolution of biodiversity in the MSWC over the past two decades [26], our study is, to our knowledge, the first to assess community-wide shared phylogeographic patterns in MSWC terrestrial animals. By applying statistical testing for shared evolutionary histories in the community based on the recently developed FBC method (as opposed to relying on approximation approaches), we are better able to extract information from the genealogical histories across taxa [18]. Despite the limited availability of published data (mitochondrial loci only), our study provides insights into the MSWC's less studied Quaternary diversification process. In particular, our results reveal concordant spatial genetic structure supporting the hypothesized role of rivers as barriers structuring communities in the MSWC (electronic supplementary material, figures S1–S3; except birds). Moreover, tests of temporal synchrony showed evidence of clustered co-divergence for each river barrier (hypothesis H3 in figure 1e). Specifically, each river supported a model with

## Salween barrier

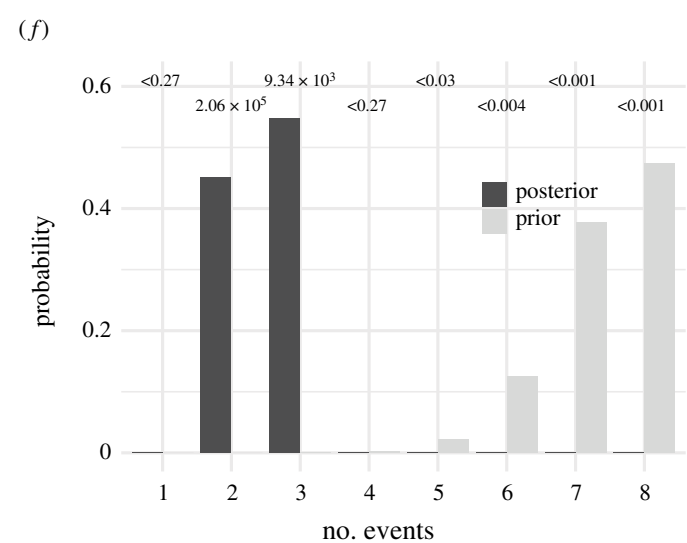
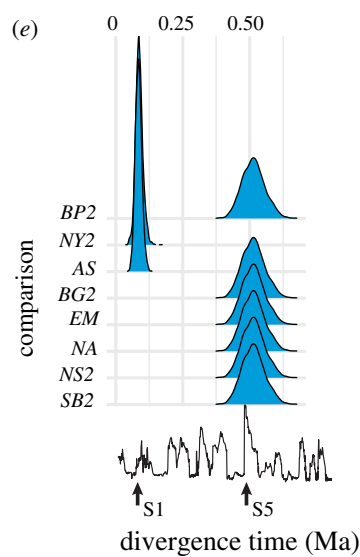
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## Mekong barrier



## Yangtze barrier



**Figure 4.** Testing phylogeographic synchronization at each river. (a), (c), (e) show the approximate marginal posterior densities of divergence time for each clade pair. (b), (d), (f) show the prior (light bars) and the posterior (dark bars) probabilities of the numbers of co-divergence events. (Online version in colour.)

multiple pulses of co-divergence events, all of which, however, occurred during the mid to late Pleistocene (i.e. 67% of the divergence events occurred between 0.5 and 1.0 Ma;

figure 2). Below we discuss the implications of what this spatial concordance, but temporal dissonance, implies about diversification in the MSWC.

## (a) Diversification in the mountains of southwest China as a result of geographical features and Pleistocene climate history

All the divergent events in this study are during the Pleistocene, by which the prolonged process of mountain building and river formation are considered to be finished. This pattern of partially synchronized co-divergence is consistent with the Pleistocene ‘species pump’ effect [27], where cycles of interconnections and isolation associated with climatic cycles promote divergence. Given that this study relied upon published datasets, we did not select species depending on particular biological traits. Likewise, the taxonomical scope is large within the taxonomic groups, spanning five different families of mammal, and six families of amphibians and reptiles. This speaks to the predominance of the climate oscillations’ effect (i.e. co-divergence was observed despite the taxonomic breadth of the species studied).

Climate oscillations have long been known as an effective speciation mechanism in mountainous regions [28,29]. Climate-induced species range shifts could lead to isolation (e.g. isolation between different refugia) or gene flow (e.g. sky island dwellers moved down to the valley forming one population) [30]. Hence it is not surprising that phylogeographic studies on organisms in other mountains systems (e.g. the Alps in Europe and Madrean Sky Island mountain ranges in North America) usually reveal genealogical splits possibly representing different refugia populations, and the estimated divergence times often fall within the Pleistocene [31–33]. In the MSWC, any climate-induced distributional shifts (and associated refugial areas) are structured by the rivers (figure 2; i.e. there is a general spatial correspondence between the genealogical splits and the distribution of clades on either side of the river barriers in the non-flying vertebrates).

Despite the spatial concordance of divergence patterns for vertebrates in the MSWC, partially synchronized divergences on each river suggests the effectiveness of river barriers varies across time. We propose that a potential explanation lies in the mountains themselves. Specifically, the mountains have a north-south orientation and all have altitudinal zonation spanning from tundra to tropical forests. This terrain with parallel north-south oriented high mountains/deep canyons is very unique in the world and has no doubt contributed to the substantial stability of ecosystems in the MSWC. For example, for moderate glacial cycles, the majority of the MSWC species only have to migrate vertically or slightly southward to track climate change [34], given that the species richness concentrates at mid-elevations [35,36] (figure 5). Even if these animals reach the lower altitudes of the mountains, the rivers can still prevent gene flow between different populations. That is, the environmental gradients in the MSWC act as a buffer against extinction in the face of climate change and the rivers as barriers to secondary contact [11]. During dramatic glaciations in which community’s distributions would have been displaced to the lowest altitudes, as well as latitudinally where the southern mountains are lower (figure 5), there were opportunities for connectivity across rivers (i.e. the river barriers become permeable [37]). We think that large distribution shifts of the whole community caused by severe glacial cycles may be the reason why the divergence times between contemporary populations

are clustered (i.e. in some degree of synchrony). Yet, the confidence intervals of divergent time estimates are broader than a single glacial cycle (figure 5), so we could not narrow down to specific glacial cycles and less extreme climate changes may have also contributed to the co-divergence. Future datasets with more species and more loci would help reduce the uncertainty in time estimates.

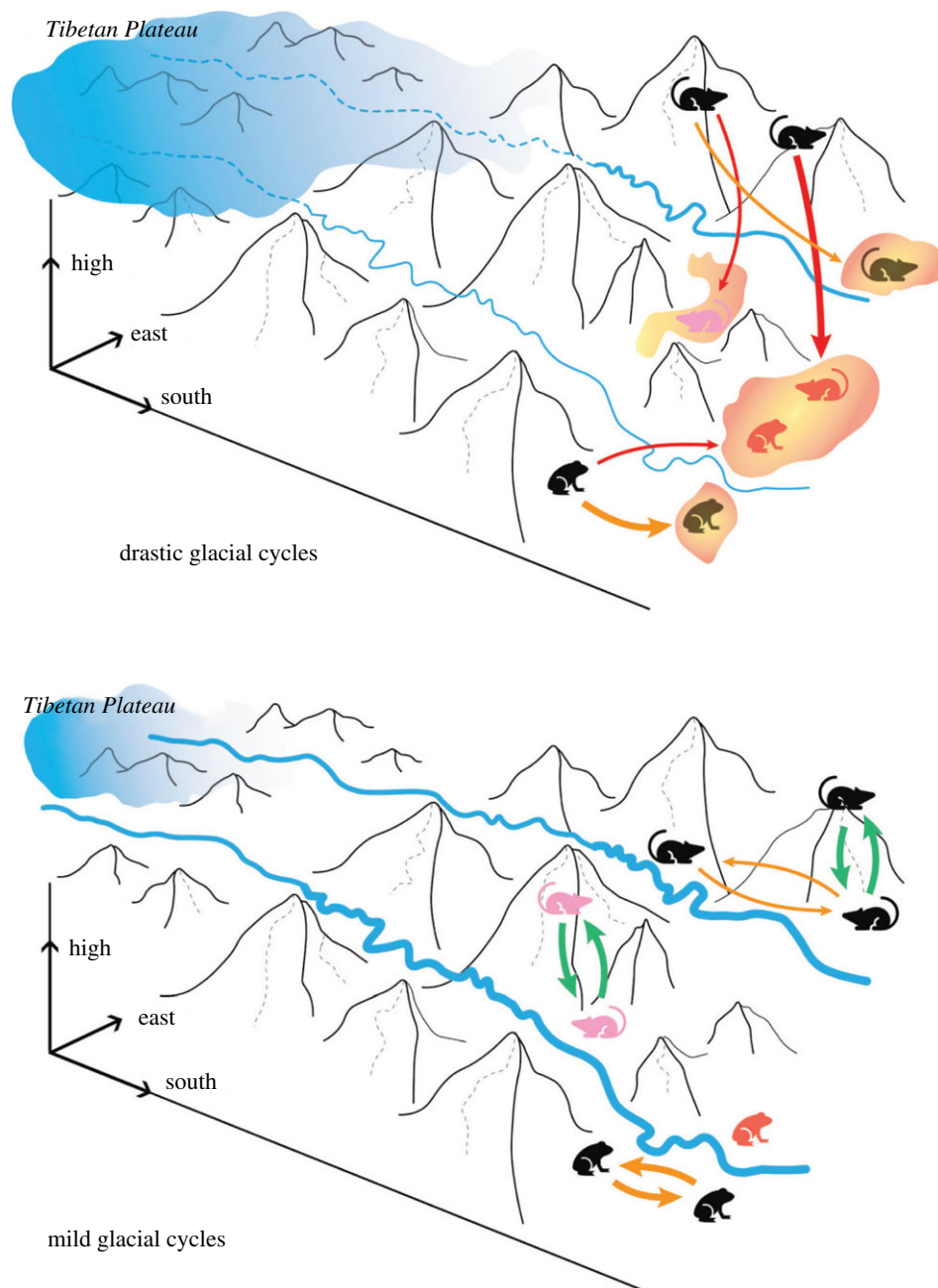
This diversification process in pulses contrasts with examples from the Amazon, another famous biodiversity hotspot, where rivers also act as barriers for dispersal/gene flow [38], but most of the studies found asynchronous divergence events (e.g. [39]; but see [40]). We suggest the extremely rugged terrain and rivers made the impact of glaciations on species community more punctuated in MSWC, generating clusters of divergence times (i.e. co-divergence pulses).

## (b) Factors contribute to heterogenous diversification in the mountains of southwest China

While our results suggest abiotic factors (i.e. geographical features and climate history) as the most dominant driving force for diversification, our results do not support the complete synchronous hypothesis (figure 1e). Partial synchrony suggests heterogeneity among species in the community.

The potential impact of biotic factors cannot be totally discounted. Even though taxonomically grouped species lack synchronous divergence history (after excluding birds from the analyses; figure 3), our result revealed heterogeneity among species sharing the same geographical break (i.e. the synchronous hypothesis H2 is not supported; figure 1e). All three rivers have the signature of an older and a more recent divergence pulse (figure 4), which we suggest might arise as a function of differences in altitude such that when species’ distributions shifted to the south, the rivers were more permeable. However, the permeability of the river barrier clearly varied among species (i.e. not all species exhibit recent divergence; some older divergence events indicate some species have not crossed the barrier, whereas other species did). It is an outstanding question why some species (but not all) dispersed across the river recently. Second, clustering taxa into two groups—mammals versus amphibians/reptiles—is obviously a broad grouping for investigating how biotic factors might structure divergence events. As such, synchrony based on shared taxonomy might not be a reasonable expectation if the species within each group are characterized by many different traits that would impact their propensity to disperse (or not) across a river barrier [13,41].

Moreover, the primary exception to the role of rivers in MSWC structuring divergence was observed in the bird species, which showed less spatial concordance of phylogeographic structure and/or very little evidence of geographical structuring of genetic variation (i.e. lack of genealogical splits). This suggests that the narrow rivers in the MSWC probably do not constitute effective dispersal barriers for birds. Likewise, previous studies have shown that the phylogeography breaks of some MSWC plants do not correspond to rivers—the genetic exchange of plants could be facilitated by insects or birds flying over rivers; the high mountain ridges might have functioned as dispersal barriers instead (e.g. in *Terminalia franchetii* [42] and *Buddleia crispa* [43]). For those animals dwelling in arid-hot valleys, icy mountain ridges might have acted as barriers as well. The majority of



**Figure 5.** Differences in the permeability of river barriers as a function of the magnitude of climate-induced distributional shifts associated with the Pleistocene glacial cycles. During drastic glacial cycles, the phylogeographic divergence of montane species included dispersal across river barriers as species tracked their climate niche altitudinally, but also latitudinally where the mountains are lower (i.e. refugia, marked with red orange patches). This contrasts with mild glacial cycles, where the environmental pressures are not harsh enough to promote cross-river dispersal. Instead, species could track climatic niche additionally using elevational gradients. (Online version in colour.)

species considered here were living in mid-elevation broad-leaved forests or alpine regions, because hot-arid valleys are the least studied area in MSWC for land vertebrates. Future studies can expand the taxon sampling to explore the connection among biological traits and niche, the type of geographical barrier and divergence pulses for communities of the MSWC.

### (c) The neglected Quaternary co-diversification among species in the mountains of southwest China

Understanding the mechanism of recent diversifications is indispensable for understanding how biodiversity was generated and maintained in the MSWC. Note that divergence events in the MSWC encompass a broad time range, even

for phylogeographic divisions within species. For example, phylogeographic divergences in subnival plants across the Mekong–Salween Divide—a classic biogeographic boundary, also known as the Ward Line—varied from the late Pleistocene (0.37–0.48 Ma) [44] to the Pliocene (4.49 Ma) [45]. Mosbrugger *et al.* [11] recently reviewed the Cenozoic evolution of geobiodiversity in the general Tibeto-Himalayan region and pointed out three peaks of diversification: the first flourishing of modern families and genera during the mid-Miocene (20–15 Ma), the second during the Miocene–Pliocene boundary, and the third, the focus of our study, at the early and mid-Pleistocene (2.0 Ma–150 ka).

Many phylogenetic or phylogeographic studies in this region aim to find concordance between speciation events and geographical events, but neglect whether diversification

events co-occur with each other in the later stage. One main reason is that the MSWC is adjunct to Tibeto-Himalayan region, and the majority of the phylogeographic studies in this region focus on finding the footprint of the orogenesis process on biodiversity. The popular ‘cradle’ hypothesis posits that the accumulation of species diversity in this region is tied to the mountain uplifting process, while the role of the MSWC during the Pleistocene is more considered as a ‘museum’ preserving species diversity as it provided refuge for relict species during glacial cycles [46]. Many studies reported young divergence events (2.0 Ma–150 ka), but were ‘mired’ by this ‘uplift story’, and falsely ascribed them to orogenesis processes including the river captures. Only recently, careful reviews of geographical evidence questioned the often-cited literature on the third uplift phase of the Tibetan Plateau (15–0.5 Ma) [26], and the current geographical consensus is that the orogeny in this region (i.e. uplifting of the Hengduan Mountains and formation of the three rivers) finished long before the Pleistocene. This leaves very few studies recognizing and investigating the effect of Pleistocene climate change on generating species diversity in the MSWC [47]. Phylogeographic concordance on community level, a powerful tool for examining the role of extrinsic factors on diversity, has never been tested for the recent phylogeographic divergences in animals in the MSWC.

Studying recent divergence (i.e. phylogeographic processes) in the MSWC is also difficult, yet crucial for conservation. Many taxa in this region have taxonomic ambiguity. In fact, more than half of our studied species are cryptic, and many are still placed in species complexes. Given the MSWC is a crucial region for conservation—over 30% of plant species (approx. 12 000 species), and about 50% vertebrates (approx. 1100 species) of China live here (according to the Catalogue of Life China 2019 Annual Checklist), including many endangered species. Characterizing this cryptic and endemic biodiversity—how they are distributed spatially and how climate change might affect

them—is important for comprehensive conservation planning in the MSWC [48,49].

## 5. Conclusion

With complex terrain, mountains harbour a substantial proportion of the world’s species. This disproportionately high species richness, as well as the mechanisms of evolutionary diversification, have long intrigued biologists. With the help of comparative phylogeographic methods, our study explored the fascinating phylogeographic diversity harboured in the MSWC and identified that spatial concordance is not associated with temporal synchrony. Instead, multiple pulses of co-divergence, which coincide with the more pronounced glacial cycles suggests a complex biogeographic process akin to a Pleistocene’s species pump effect across the extremely rugged terrain of the MSWC. Future studies in the MSWC with genomic-scale data and more species sampling would enhance our understanding of the accumulation of mountain biodiversity evolution across space and time.

**Data accessibility.** The present study also sequenced the *Cytb* gene in six mammal species. The assembled sequence data for comparative phylogeography analyses are in the Source Data file and archived in Dryad Digital Repository: <https://doi.org/10.5061/dryad.mgqnk98x6> [50].

**Authors’ contributions.** T.W., H. H., X.-L.J. carried out the design of this study. L.L.K. provided analysis instruction and key discussion for the manuscript. T.W., H.H. and J.R.O. performed the analyses and writing. All authors have read and approved the content of this manuscript.

**Competing interests.** The authors declare no competing interests.

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