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WITHDRAWN: Tracing ancient migration routes: Did Gloydius pit vipers disperse across Paleo-Tibet through temperate and subtropical corridors?

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Abstract

The Himalayan–Tibetan orogen has undergone complex paleoenvironmental transformations that shaped its biodiversity, yet the timing and sequence of surface uplift—and corresponding biogeographic scenarios for endemic Himalayan taxa—remain debated. We investigated the evolutionary history of *Gloydius* pit vipers (Serpentes: Crotalinae) from the Himalaya and Hindu Kush by integrating a time-calibrated mitochondrial phylogeny with paleoclimatic reconstructions and species distribution modeling. Our results indicate that *Gloydius* originated in East and Southeast Asia during the early Miocene (~ 18.6 Ma), with divergence of the Himalaya–Hindu Kush clade at ~ 14.5 Ma and subsequent diversification from ~ 11.7–6.9 Ma. Phylogenetic patterns suggest that ancestral lineages expanded westward across Paleo-Tibet via subtropical to warm-temperate corridors, likely through a Central Paleo-Trans-Tibet Valley between the Gangdese and Tanggula Mountains, rather than along the Himalayan arc. A secondary corridor along the Indus–Yarlung Suture Zone may have allowed access to Himalayan transverse valleys, facilitating colonization of the western and central Himalaya, while the eastern Himalaya remained unoccupied despite suitable climate. Paleoclimatic models reveal dynamic habitat shifts over the last 3 million years, highlighting the sensitivity of these low-dispersal taxa to climatic fluctuations. This study provides a plausible scenario for the paradoxical phylogenetic topology of *Gloydius* in the Himalaya and Hindu Kush, offering a biogeographic model for disjunct trans-Tibet distributions of low-dispersal taxa and a biotic perspective on the Miocene tectonic and environmental evolution of the Himalayan–Tibetan Orogen.

Introduction

The Himalayan-Tibetan orogen (HTO), renowned for its geological complexity and biodiversity hotspots [1], has undergone significant paleoenvironmental transformations throughout its uplift. Understanding these changes is critical for reconstructing the region's climatic and ecological history, and for shedding light on how these shifts have influenced the distribution and evolution of the species rich biodiversity in the Himalaya and East Asia [2–4].

The timing, magnitude, and sequence of surface uplift in the HTO remain a subject of ongoing debate. Several geoscientific studies, primarily based on stable isotope paleoaltimetry, suggest a proto-Plateau that was already at elevations of 4,000 to 5,000 m a.s.l. by the middle Oligocene (e.g., [5–8], implying the presence of a large-scale alpine biome by that time. These findings align with earlier tectonic models that propose a high-elevation Paleogene Tibet [9–13]. However, fossil evidence contradicts these elevation scenarios, with no signs of alpine biota before the late Miocene. Instead, tropical and warm-temperate species appear to have been widespread until the early Miocene [14–19].

While traditional methods, such as sedimentary records, isotopic analyses, and paleontological data, have significantly contributed to our understanding of the HTO's evolution, they still provide inconsistent insights. To complement these approaches, a promising yet underexplored approach lies in examining the seemingly paradoxical phylogenies of Himalayan species groups that have evolved within this dynamic landscape. Such phylogenies reveal unexpected evolutionary relationships that challenge conventional models of Himalayan species' historical biogeography and the associated paleoenvironmental scenarios. Specifically, these phylogenies often show an intriguing pattern: taxa from the far western Himalaya represent ancient lineages or are more closely related to those from the eastern margin of the Tibetan Plateau, thousands of kilometers away, rather than to geographically proximate taxa in the Central Himalaya. This surprising finding—observed in groups such as Himalayan ground beetles [20] and amphibians [21, 22]—contradicts the widely accepted view that species distribution follows a stepwise migration pattern from east to west (or vice versa), a framework often used to explain the colonization and evolution of Himalayan biodiversity. These unexpected phylogenetic relationships indicate that the region's evolutionary history is much more complex than previously thought, offering new insights into the processes shaping the biodiversity of the Himalaya and surrounding areas. The HTO, therefore, presents a unique opportunity to explore how species have responded to changing paleoenvironmental conditions, providing a clearer understanding of the Paleogene Tibetan landscape and its topography.

Recently, pit vipers (Crotalinae) of the genus *Gloydius* have emerged as promising models for biogeographic research in the Himalaya and Hindu Kush—two of Asia's least explored mountain systems. A comprehensive taxonomic revision of the *G. himalayanus* complex uncovered several deeply divergent, geographically structured lineages with restricted ranges, highlighting their potential for reconstructing historical patterns of isolation and dispersal [23] (Fig. 1). By integrating dated phylogenetic data with paleoclimatic reconstructions, this study provides new insights into the origin and historical biogeography of these taxa and the paleoenvironmental evolution of the HTO.

Material and Methods

We used a recently published mitochondrial sequence data set of *Gloydius* species from the Himalaya and Hindu Kush which comprised sequences of 12S, 16S, cytochrome b (cyt*b*) and NADH dehydrogenase 4 (nd4) gene regions [23]. Additional sequence data of all currently recognized species of the genus, as well as data of appropriate outgroups corresponding to the target loci were retrieved from GenBank (accession numbers in Supplementary Table S2), aligned with MEGA11 [24] and concatenated. The final alignment contained 179 taxa (2,693 bp: 12s 408 bp; 16s 482 bp; cyt*b* 1,125 bp; nd4 678 bp). Based on this locus partitioned dataset, a maximum likelihood (ML) phylogeny was inferred with IQ-TREE2 v2.1.2 [25] using the partition scheme and substation models as selected by ModelFinder [26] and PartitionFinder [27] implemented in IQ-TREE 2, and 100,000 bootstrap replicates.

Divergence time estimation

For estimating divergence dates we used BEAST2 v.2.7 [28] and the concatenated mtDNA dataset. We imposed the following 11 secondary time constraints from the fossil calibrated Viperinae phylogeny of Šmíd and Tolley [29]. All constraints were implemented through normally distributed priors: i) MRCA (Most Recent Common Ancestor) of Azemiopinae and Crotalinae: 31.6 Ma, range 26.9–36.3; ii) MRCA of *Protobothrops*, *Gloydius, Agkistrodon, Crotalus*, and *Sistrurus*: 24.7 Ma, range 21.0–28.4; iii) MRCA of *Agkistrodon*, *Crotalus*, and *Sistrurus*: 20.2 Ma, range 16.9–23.5; iv) MRCA of *Crotalus* and *Sistrurus*: 13.3 Ma, range 10.8–15.9; v) MRCA of *Macrovipera* and *Montivipera*: 12.6 range 11.7–13.5; vi) MRCA of *Daboia mauritanica* and *D. russellii* split: 16.11 Ma range 13.6–18.7; vii) separation of *Vipera ammodytes* from all other *Vipera* in the tree: 13.4 Ma, range 11.5–15.3; viii) MRCA of *Vipera aspis* and *V. latastei* split: 7.1 Ma, range 5.6–8.7; ix) MRCA of *Vipera berus, V. seoanei*, *V. berus walser, V. kaznakovi, V. graeca, V. ursinii, V. eriwanensis, V. lotievi*, and *V. renardi*: 6.0 Ma, range 5.1–6.9; x) MRCA of *Vipera berus* and *V. seoanei*: 4.7 Ma, range 3.8–5.7; xi) MRCA of *Vipera ursinii* and *V. graeca*: 2.6 Ma, range 2.0–3.2.

We performed three independent BEAST2 runs, defining a chain length of 10 million for each run, a thinning interval of 1000, a lognormal relaxed clock model, a birth-death tree prior, and a random starting tree. Substitution models were inferred using bModelTest [30]. Runs were then combined with BEAST2 LogCombiner v.2.6.7. Convergence and stationary levels were verified with Tracer by a standard deviation of split frequencies < 0.01 as well as an effective sample size value > 200 of the traces. The final tree was obtained with TreeAnnotator v.2.6.2 and visualized with FigTree v.1.4 [31].

Species distribution modelling

To assess the potential geographic distribution of the *Gloydius* species across the Himalayan arc and adjacent Hindu Kush (*G. hazarensis*, *G. himalayanus*, *G. hindukushensis*, and *G. nepalensis*), we computed species distribution models (SDMs). The models were computed based on the species' currently realized climatic niches and subsequently projected onto paleoclimatic scenarios. Our species distribution modeling approach relies on a static characterization of the species' realized niches derived from present-day taxon occurrence data. A clear distinction between the records of *G. himalayanus* and *G. chambensis* could not be made due to the potential overlap in their geographic distributions and their significant morphological similarity [23]. Therefore, it could not be excluded that some records identified as *G. himalayanus* might correspond to *G. chambensis* and vice versa.

Species locality records associated with geographical coordinates were obtained from our previous study [23]–except those that were provided as "provisional"–and are listed in Supplementary Figure S1. As environmental layers, we used a set of bioclimatic variables for the present time (1979–2013) from the CHELSEA database v.2.1 [32] at a spatial resolution of 2.5 arc minutes. Collinearity between variables may result in model overfitting. To account for this potential error, we selected only one predictor among pairs with a Spearman's pairwise rank correlation of higher than |0.75| focusing on predictors reflecting thermal energy and water availability as expected to be relevant for an ectothermic organism (i.e., annual mean temperature = bio1, annual precipitation = bio12, precipitation of the driest month = bio14, precipitation seasonality = bio15, precipitation of the warmest quarter = bio18, and precipitation of the coldest quarter = bio19). Corresponding paleoclimatic variables for the mid-Pliocene warm period (mPWP v1.0, 3.205 Ma) [33], and mid-Pliocene cooling period (Marine Isotope Stage M2 v1.0, 3.3 Ma) [34] were downloaded with a spatial resolution of 2.5 arc min from the paleoclimate data base [35].

Currently realized niches of each species were compared using density kernel estimates, as computed with the sm.density.compare function of the sm [36] package for R.

For SDM calculation and model fitting, we used MaxEnt ver. 3.4.4 [37–39] using a model optimization procedure based on AICc as described in [40] using the relevant functions of the ENMeval package for R [41]. For further details of the analysis pipeline, we refer to the Supporting Information of [42]. We tested all possible combinations of regularization parameters (0.5 to 5 in steps of 0.1) and feature classes each 100 times using a data split of 80% of the records used for training and 20% for model testing using the area under the receiver operating characteristic curve (AUC). For the final models, we selected those settings with an average AUC > 0.7 and the lowest AICc and used the same data split strategy. Additionally, to AUC, we computed the average True Skills Statistic (TSS) across all replicates to assess model performance [43]. As lower threshold, we selected the average 10% training omission. Potential areas with non-analogous climatic conditions exceeding the training range of the models were identified using multivariate environmental similarity surfaces [44].

Results

Phylogenetic relationships and divergence times in Gloydius

We provide a dated phylogeny for the genus *Gloydius*, comprising all currently recognized species including those of the Himalaya and Hindu Kush (HHK) clade. The topologies of both BI and ML phylogenetic trees are largely consistent, with full concordance observed for the HHK lineages and their sister clades (Fig. 2, Supplementary Fig. S1). Most divergences are strongly supported. Higher-level nodes show a sister relationship between *Gloydius* and the East Asian pit vipers *Ovophis okinavensis* and *Trimeresurus gracilis*, as well as a closer relationship between the genera *Gloydius*,

Ovophis, and *Trimeresurus* and the New World pit vipers. The placement of the Southeast Asian *O. monticola* and the enigmatic central-eastern Himalayan genus *Himalayophis* was only weakly supported.

Within *Gloydius*, two main clades are recovered: a clade comprising warm- to cold-temperate taxa from regions north and east of the Tibetan Plateau, spanning Central Asia, northern, eastern, and central China, the Caucasus, Korea, and Kazakhstan (Asian clade), and a high montane clade from the HHK clade. The latter includes two monophyletic subclades. The first is a warm-temperate subclade from the Hazara range—part of the Hazara-Kashmir Syntaxis at the northwestern margin of the Himalayan orogen—and the Hindu Kush, represented by *G. hazarensis* and *G. hindukushensis*. The second comprises temperate to cold-temperate Himalayan taxa: *G. chambensis* from the westernmost Lesser Himalaya near the Sutlej River, which is sister to a temperate to cold temperate lineage from the western and central parts of the Greater Himalaya formed by *G. himalayalanus* and *G. nepalensis*.

Of note, several sequence data and the associated *Gloydius* taxa were inconsistently reported, either in GenBank or in publications, e.g. the *G. rubromaculatus* holotype IOZ002317 was assigned to *G. angusticeps* [45], *G. rubromaculatus* voucher SAFS2019001 (GenBank accession numbers MW690593 and NC_064056) may represent *G. qinlingensis* (Fig. 2), *G. intermedius* voucher GP188 (GenBank accession numbers JQ687469, JQ687488, JQ687507, KP403594) probably represents *G. cognatus* (Fig. 2), *G*. sp. isolate CD2 and GR1 (accession numbers OK184551, OK210582, OK239647, OK239652) has been described as *G. swild* (holotype IVPPOV2725) but sequences of it were later assigned to the recently described *G. variegatus* (voucher CD2; accession numbers OK184550, OK210581, OK239647, OK239652) [46].

The stem age of *Gloydius* is estimated at *ca*. 18.6 Ma (95% HPDi: 15.6–21.7 Ma), with the two main clades diverging approximately 14.5 Ma (95% HPDi: 11.9–17.4 Ma) (Fig. 2). Diversification within the HHK clade occurred during the Mid- to Late Miocene (11.7 Ma, 95% HPDi: 8.9–14.8 Ma) and continued within its subclades until the early Pleistocene. The Central/East Asian clade began to diversify somewhat later, around the upper Miocene (9.8 Ma, 95% HPDi: 7.8–12.1 Ma).

The split between the warm-temperate subclade from the Hazara and Hindu Kush and the Himalayan subclade occurred around 11.7 Ma (95% HPDi: 8.9–14.8 Ma), followed by a simultaneous separation at approximately 9.3 Ma (95% HPDi: 6.3–12.7 Ma and 6.4–12.1 Ma) within these two subclades. This led to the formation of the five species mentioned above (Hindu Kush region: *G. hindukushensis*; Hazara region: *G. hazarensis*); Chamba valley of the Western Lesser Himalaya: *G. chambensis*; Western Greater Himalaya: *G. himalayanus*; Central Greater Himalayas: *G. nepalensis*). The crown age of the Greater Himalayan subclade formed by *G. himalayanus* and *G. nepalensis* is estimated at *ca*. 6.9 Ma (95% HPDi: 4.6–9.4 Ma).

Niche quantification and species distribution models

Comparing the realized niches of all Taxa revealed pronounced overlaps in niche space, which is strongest in the annual mean temperature and annual precipitation and more divergent in precipitation related variables (Fig. 3).

Summary statistics of the SDMs are provided in Table 1. Overall, the SDMs performed well in terms of AUC and TSS scores. Across all taxa, the annual mean temperature (bio1) made the highest contribution, followed by precipitation of the warmest quarter (bio18) in *G. harzarensis* and *G. himalayanus*, and precipitation of the coldest quarter (bio19) in *G. nepalensis*.

Table 1
Final model settings and summary statistics of species distribution models for *Gloydius* species under study.

Species	***G. hazarensis***	***G. hindukushensis***	***G. himalayanus***	***G. nepalensis***
Feature classes	LQ	LQ	LQ	LH
Regularization	1	1.2	0.6	0.6
TSS	0.71	0.64	0.65	0.78
AUCtest	0.85	0.75	0.85	0.90
AUCtrain	0.89	0.81	0.88	0.85
Variable	Percent contribution			
bio1	58.5	83.1	70.4	47.5
bio12	1.8	0.4	2.2	6.1
bio14	0.6	8.9	3.9	2.8
bio15	2.3	2.2	3.4	7.7
bio18	34.0	4.2	13.9	7.0
bio19	2.8	1.3	6.2	28.8

The species distribution models for four *Gloydius* taxa under different climatic scenarios reveal predicted shifts in suitable habitat across the last 3 million years, highlighting a strong potential sensitivity to paleoclimatic fluctuations (Fig. 34-L). Under present climatic conditions, *G. hindukushensis* is predicted to have a highly restricted suitable range, limited to higher-elevation valleys in the eastern Hindu Kush (Fig. 4A), which is consistent with the species' known distribution range [23] (Fig. 1).

The species *G. hazarensis* is predicted to occupy mainly the southwestern margin of the Himalaya, broadly consistent with its known distribution in northern Pakistan (Fig. 4D). Although the models also indicate additional suitable areas extending into the central and eastern Himalaya and the southeastern edge of the Tibetan Plateau, the species has not been recorded from these regions [23]. Its current distribution appears geographically constrained by the Upper Indus Valley to the west, the Karakoram range to the north, and the Indus Plains to the south (Fig. 1). The eastern range limit remains uncertain, particularly in the Kashmir Valley, where potential overlap with *G. chambensis* complicates range delineation and warrants further sampling and molecular analysis.

In contrast, *G. himalayanus* is predicted by the model to have a broader distribution along the Himalayan arc, extending from the westernmost ranges through the central Himalaya and into the southeastern margin of the Tibetan Plateau (Fig. 4G). In reality, however, the species appears to be restricted to the Northwestern Himalaya of India, with no confirmed records outside this region [23] (Fig. 1).

The model predicts the distribution of *G. nepalensis* to be primarily centered in the eastern Himalaya, with suitable habitats extending eastward to the southeastern margin of the Tibetan Plateau and the mountains of Indochina (Fig. 4J). In contrast, the confirmed range of this taxon is limited to the central Himalaya of western and central Nepal, with verified records from the Karnali and Gandaki zones. To date, there is no credible evidence of its occurrence east of the Annapurna region [23] (Fig. 1).

During the Mid-Pliocene Warm Period (~ 3.205 Ma), representing a globally warmer and wetter interglacial interval, the models predict substantial expansions in suitable habitat for all four taxa (Fig. 4B, E, H, K); especially *G. hazarensis*, *G. himalayanus*, and *G. hindukushensis* show particularly extensive expansions into the northern and eastern periphery of the today's Tibetan Plateau, including areas that are climatically unsuitable under current conditions. *G. nepalensis* is predicted to occupy a broader range extending from the eastern Himalaya across the Hengduan Mountains and adjoining montane zones. Interestingly, despite the predicted broad-range expansions, habitat suitability along the main Himalayan arc becomes more restricted according to the model. Notably, there are no known occurrences of the four species—or their close relatives—within these proposed expansion zones. Instead, their distributions are confined to the Himalaya and Hindu Kush regions.

By contrast, under the cooler and likely drier conditions of Marine Isotope Stage M2 (~ 3.3 Ma), all four taxa are predicted to have experienced a substantial reduction in suitable habitat (Fig. 4C, F, I, L). *G. hazarensis* and *G. hindukushensis* are modeled to persist only in small, isolated refugial areas near their present-day range. *G. himalayanus* retains a fragmented distribution across the central Himalaya, whereas *G. nepalensis* is predicted to have been restricted to a few discrete areas within the eastern and central Himalaya. The present-day range of the latter species is centered in the western Greater Himalaya of central Nepal.

Discussion

In this study, we present the first time-calibrated phylogeny of *Gloydius* pit vipers from the Himalaya and Hindu Kush based on mitochondrial sequence data, complemented by paleoclimatic modelling to explore the evolutionary history of the group within the framework of different concepts of Himalayan-Tibetan orogeny.

The higher-level relationships recovered in our topology are broadly consistent with previous studies, which have suggested a sister-group relationship between *Gloydius* and the East Asian pit vipers *Ovophis okinavensis* and *Trimeresurus gracilis* [47–51]. These studies, however, disagree regarding the exact placement of the genera *Protobothrops*, *Trimeresurus* sensu lato, and *Ovophis* within the subfamily Crotalinae. Most of these studies, including the present work, support a sister relationship of the New World pit vipers and the clade containing *Gloydius*. A more recent multilocus phylogenetic study based on nearly 100 molecular markers provided strong support for a sister relationship between *Ovophis* and *Gloydius*, which together form the sister clade to the green pit vipers (*Trimeresurus* sensu lato) and *Protobothrops* [52]. It should be noted, however, that this multilocus dataset did not include *O. okinavensis* or *T. gracilis*. A sister relationship of *Gloydius* and *Ovophis* (and a different placement of *O. okinavensis* and *T. gracilis*) had been suggested previously, albeit with weak support [53]. Despite differences among individual studies, a clear pattern emerges regarding the early biogeography of the group: the closest extant relatives of *Gloydius* are species distributed across South, East, and Southeast Asia. Consistently, recent ancestral range estimations suggest that *Gloydius* most likely originated in Southeast Asia or the Indo-Malayan region and that the family Viperidae as a whole has an Asian origin [48].

According to our results, *Gloydius* evolved in the early Miocene (~ 18.6 Ma), with major lineage divergences between the HHK and Asian clade occurring during the Mid- to Late Miocene (17.4–11.9 Ma). The HHK clade began diversifying around 11.7 Ma, with subsequent splits among the five focal species dated to between ~ 11.7 and 6.9 Ma. These divergence ages align closely with those observed in other taxa from the Himalaya and its western adjoining mountains, ranging from herpetofauna—such as spiny frogs of the genera *Chrysopaa* and *Nanorana* [54], lazy toads of the genus *Scutiger* [21], and Himalayan lineages of the gekkonid genus *Cyrtodactylus* [55]—to entirely unrelated organisms, including ground beetles [20, 56], indicating comparable temporal frameworks and potentially shared biogeographic histories across multiple taxa.

These temporal patterns, consistent across multiple Himalayan taxa, raise intriguing questions about the influence of past orogenic processes and climatic fluctuations on lineage distributions. Our paleoclimatic projections over the past 3 million years illustrate the potentially highly dynamic ranges of the four *Gloydius* taxa in response to climate oscillations, highlighting their pronounced sensitivity to paleoclimate changes. This may suggest that ancestral lineages likely tracked suitable environmental niches over time, although their dispersal was constrained by the region's complex terrain within the tectonically active and topographically dynamic landscape of the HTO.

Our results offer a potential temporal and spatial basis for interpreting diversification patterns in relation to geological and climatic processes in the region. In the following, we discuss alternative hypotheses for the phylogeography of *Gloydius* and evaluate their plausibility considering current scientific perspectives on the timing and mechanisms underlying the formation of the HTO.

Was the Tibetan Plateau already alpine during the Early to Mid-Miocene, forcing dispersal either east- or westward along the Himalaya?

Decades of geoscientific research across the HTO have produced a wide range of hypotheses regarding its topographic evolution, differing substantially in the timing, magnitude, and sequence of surface uplift across the orogen (reviewed in [57]). Stable isotopic paleoaltimetry studies frequently suggest that large parts of the Tibetan Plateau attained elevations close to modern values as early as the Paleogene (e.g., [5, 6, 8, 58], supporting models of an early high-elevated plateau [9–13, 57, 59, 60] and the evolution of alpine biota as early as the Oligocene [61, 62]. If an alpine plateau did exist during this period, the ancestral lineages of today's temperate *Gloydius* in the Himalaya and Hindu Kush would likely have been forced to avoid these high-altitude environments, entering the Himalaya via alternative dispersal corridors. A long-standing hypothesis proposes that such dispersal occurred in an east–west or west–east direction parallel to the Himalaya [63, 64].

However, the phylogenetic topology within *Gloydius* reveals patterns that are unusual compared to other reptile species groups of the HTO and difficult to reconcile with either proposed geological scenarios. The closest extant relatives of the HHK clade occur in East Asia, east of the modern Tibetan Plateau, whereas the earliest-diverging lineages of the genus are found in the far western Himalaya and adjacent Hindu Kush. At the time of the earliest divergence within the HHK clade (about 11.7 Ma), temperate conditions must have prevailed along the western edge of Paleo-Tibet, providing suitable habitats for ancestral *Gloydius* lineages. This inference is broadly consistent with geological and paleontological evidence of a significantly uplifted Miocene Tibetan Plateau [57, 65], irrespective of whether this plateau had already attained alpine elevations by that time.

From a paleogeographic and paleoecological perspective, the timing of divergence between the HHK and East Asian *Gloydius* clade (~ 14.5 Ma; 11.9–17.4 Ma) and the first cladogenetic event within the HHK clade (~ 11.7 Ma; 8.9–14.8 Ma) are particularly noteworthy. During this interval, the ancestor of the HHK clade must have colonized the western margin of Paleo-Tibet. If the plateau had already attained alpine elevations by that time, dispersal of ancestral *Gloydius* may have been possible only along the southern slope of the HTO. However, *Gloydius* occurs today exclusively in the western and, with a single terminal(!) clade, in the central Himalaya but is entirely absent from the eastern Himalaya. For the latter region to have once been inhabited, ancestral populations would have had to be extirpated from the entire central and eastern Himalayan arc—an unlikely scenario given the presence of multiple *Gloydius* species along the eastern margin of the Tibetan Plateau and the complete absence of the genus from the eastern Himalaya. This interpretation is consistent with phylogenetic niche models, which predict potentially suitable habitats for *G.*

hazarensis, *G. himalayanus*, and *G. nepalensis* in the eastern Himalaya that appear to remain unoccupied. Collectively, these findings suggest that the striking absence of *Gloydius* from the eastern Himalaya and the lack of early-diverging lineages in the central Himalaya cannot be explained by a dispersal route confined to the Himalayan arc.

A dispersal route parallel to the Himalayan arc would have necessitated that ancestral *Gloydius* traversed the north–south oriented mountain ranges and deeply incised transverse valleys that have characterized the region since its initial uplift. Rivers and mountains, however, are well established as effective barriers to snake dispersal, including pit vipers [23, 66, 67]. Although crossing some deeply incised transverse valleys may have been possible—facilitated by the geomorphodynamics of the Himalaya, such as landslides and valley infilling [68, 69]—continuous east–west dispersal along the entire Himalayan arc would have constituted an enormous, and likely insurmountable, barrier for *Gloydius*. Moreover, such a scenario should have left phylogenetic signatures, at minimum in the form of old, relic lineages in parts of the eastern and central Himalaya, which are entirely absent. Thus, accounting for the ancestral origins of the Himalayan and Hindu Kush species—and the routes by which they arrived—requires consideration of alternative scenarios for long-distance dispersal in *Gloydius*.

Warm-temperate corridors across Paleo-Tibet as potential dispersal pathways into the rising Himalaya?

The estimated crown age of *Gloydius* (~ 14.5 Ma) in our dated phylogeny marks the earliest possible existence of a shared ancestral range for the present-day HHK clade and its Asian sister group. Phylogenetic relationships and current distributions strongly suggest that their common ancestor inhabited tropical to warm-temperate environments in East and Southeast Asia, consistent with earlier reconstructions [48]. This raises two central questions: (i) if east–west migration parallel to the Himalaya is implausible, by which routes did ancestral *Gloydius* colonize the western Himalaya and Hindu Kush following divergence from their Asian relatives east of the modern Tibetan Plateau and (ii) what ecological and evolutionary processes occurred during the interval between this divergence and the first split within the HHK clade?

Answering these questions requires integration of recent paleontological evidence with reconstructions of Tibet's paleoenvironmental history. Contrary to models proposing a highly elevated Paleogene Tibetan Plateau, the Cenozoic fossil record of the HTO (compiled in [56, 65, 70] indicates that large parts of Tibet remained at comparatively low elevations and supported subtropical to temperate biomes at least until the Early Miocene (reviewed in [71]; [19]). Importantly, no fossil evidence currently substantiates cold-temperate or alpine habitats in central or southern Paleo-Tibet until the end of the Paleogene. This absence casts doubt on early high-elevation scenarios and, by extension, undermines models that constrain dispersal exclusively to the Himalayan arc.

We therefore propose that, beginning in the early to mid-Miocene, the emergence of warm-temperate habitats across Paleo-Tibet created ecologically suitable corridors that enabled westward dispersal of ancestral *Gloydius*. These corridors may have facilitated their separation from tropical–subtropical congeners, ultimately giving rise to the distinct HHK clade. A comparable paleogeographic scenario has previously been proposed for the evolutionary history of Himalayan amphibians of the genera *Chrysopaa*, *Nanorana*, and *Scutiger* [21, 54]; see [56] for details). Fossil evidence supports this interpretation: the Namling flora documents temperate forests in the central Gangdese Shan during the Mid-Miocene (~ 15 Ma; [14]), while subtropical to warm-temperate fossil floras have been found in the Lunpola and Qiabulin basins in central-southern Tibet, and dated to the Oligocene-Miocene boundary/late early Miocene (e.g., [15, 17, 72]. Moreover, geochemical data from the Qaidam Basin [73] indicate that uplift to present elevations (~ 3000 m) occurred at earliest in the Mid-Miocene, reinforcing the view that east–west warm-temperate "niche corridors" were available to Asian biota [56, 57]. Recent geoscientific work even emphasizes a warm-humid climate in central Tibet persisting until the Mid-Miocene, with peak humidity around 15–14 Ma [74].

Nevertheless, knowledge of past environments and elevations across different tectonic domains of Tibet remains fragmentary and controversial (compiled in [65]. For example, reconstructions for central Tibet diverge widely: some infer elevations exceeding 4000–5000 m already in the late Eocene/early Oligocene [5, 75], while others suggest < 2300 m in the latest Oligocene [17, 76], ~ 3000 m in the early Miocene [15, 77], or < 2500m and > 4500 m during the middle Miocene [74, 78]. Such discrepancies stem from methodological differences as well as from uncertainties in paleoelevational and paleogeographic reconstructions. Competing models—such as PALEOMAP [79], GPlates [80, 81], or Getech's Globe/Neftex reconstructions—adopt contrasting assumptions on the timing and style of uplift (and the resulting topography), ranging from an "early high plateau" to more gradual, mosaic patterns of elevation. Consequently, no single geoscientific framework can yet be regarded as definitive. This underlines the value of dated phylogenetic patterns, such as those in *Gloydius*, which provide an independent biological archive that can contribute to disentangle the complex paleoenvironmental history of the HTO.

Assuming an east-west trans-Tibetan movement of subtropical to warm-temperate ancestral *Gloydius* lineages during the Miocene, their presence across Paleo-Tibet may have been facilitated by an east–west-oriented low-elevation zone in central Paleo-Tibet characterized by a subtropical to temperate climate. The most recent geoscientific model, supported by fossil evidence, indicate the existence of such suitable environments between the east–west-trending Gangdese and Tanggula Mountains, the latter forming the present-day Qiangtang Terrane [57]. We refer to this corridor hereafter as the Central Paleo-Trans-Tibet Valley (Fig. 5). It should be noted that nearly two decades ago, subtropical primary Tertiary ranges along the Transhimalaya had already been proposed for ground beetles endemic to the Himalaya [82]—a model later considered most plausible for explaining the historical biogeography of Himalayan-endemic amphibians [83, 84].

The Gangdese Mountains likely represented a southern barrier for species inhabiting this valley—provided these mountains had already attained considerable elevation at that time. It is plausible that ancestral *Gloydius* lineages occupied this valley extensively during the Middle Miocene and reached as far as the western slopes of the Gangdese Mountains. During the Late Miocene, the Central Paleo-Trans-Tibet Valley was further elevated and, based on the paleontological record, characterized by high montane forests adapted to colder temperatures ("alpine forest" sensu [65]. This environmental change may have displaced *Gloydius* populations toward the opposite margins of the HTO, leading to the evolution of the western clade (the HHK clade) now separated from its eastern relatives.

Regardless of the exact elevation of the surrounding terrain, temperate habitats must have persisted widespread along western slopes of Paleo-Tibet and the rising Himalaya—such as along the catchment areas of the paleo-Indus River—allowing these snakes to survive and expand along drainage systems that formed during the further uplift in this part of the HTO. The Indus River, originating in western Tibet and the Karakoram, predates the main phase of Himalayan uplift and is among the oldest documented rivers in the HTO [85, 86]. These pre-existing and newly formed drainage systems likely facilitated the dispersal of ancestral *Gloydius* along the intramontane Indus–Yarlung Suture Zone, which extended from northwest to southeast between the Gangdese Mountains and the rising Himalaya. This corridor would have enabled southward and eastward range expansion of *Gloydius*, while its western continuation probably corresponded to the present-day Indus River valley. By following this moderately elevated longitudinal valley of the Indus–Yarlung Suture Zone, *Gloydius* may have accessed epigenetic Himalayan transverse valleys, such as the Sutlej, Karnali, and Thakkohla, subsequently colonizing the rising Himalaya.

The absence of *Gloydius* in the eastern Himalaya, together with the genus' current distributional limit in the Himalaya of central Nepal, may indicate that the highest-elevation section of this ancient corridor acted as a barrier to ancestral *Gloydius*, although this remains speculative. Interestingly, other Tertiary West-Tibetan faunal elements of the Himalaya (i.e., faunal groups primarily distributed in the western Himalaya with a Tertiary origin in West Tibet) also reach their eastern range limits in the Nepal Himalaya (e.g., ground beetles of the genera *Calathus*, *Carabus*, and *Pristosia* [56, 87, 88])—a pattern not readily explained by climatic factors. Consistently, our species distribution models reveal climatically suitable habitats throughout the eastern Himalaya, yet *Gloydius* remain entirely absent, with no records from this region.

Divergence among the ancestral lineages then occurred after their arrival in the rising Himalaya, driven by continuous uplift and the resulting increase in topographic barriers, leading to allopatric separation. According to our dated phylogenetic tree, this process took place from the mid- to late Miocene (~ 11.7–6.9 Ma), a timeframe that aligns with most geoscientific models of Himalayan uplift [12, 89, 90]. The ongoing uplift of the Tibetan Plateau ultimately resulted in the loss of ancestral *Gloydius*' primary ranges across Paleo-Tibet driven by the associated cooling and aridification, a pattern similarly observed in other endemic Himalayan taxa [21, 54, 56, 91].

Conclusion

This study provides a framework for the timing and spatial patterns of *Gloydius* evolution in the Himalaya and Hindu Kush, integrating a time-calibrated phylogeny with paleoclimatic and geological evidence. Our results suggest that ancestral lineages may have expanded across Paleo-Tibet during the Miocene via subtropical to warm-temperate, low-elevation corridors. The Central Paleo-Trans-Tibet Valley, situated between the Gangdese and Tanggula Mountains, may have served as the primary route facilitating westward movement from East and Southeast Asia, where the closest extant relatives of Himalayan *Gloydius* occur.

As the plateau continued to uplift during the Late Miocene, high montane environments and emerging topographic barriers may have fragmented populations. A secondary corridor along the intramontane Indus–Yarlung Suture Zone may have guided lineages into epigenetic transverse valleys, which acted as the primary entrance into the rising Himalaya. Through these valleys, *Gloydius* may have colonized the western and central Himalayan ranges, while the eastern Himalaya remained unoccupied, probably due to persistent geographic barriers, despite climatically suitable habitats. Once established within the Himalayan ranges, ancestral *Gloydius* may have undergone allopatric divergence in response to increasing topographic barriers during the ongoing Himalayan uplift.

By providing a plausible scenario for the paradoxical phylogenetic topology of *Gloydius*, this study offers new insights into the origin, historical biogeography, and diversification of these taxa. Importantly, it presents a biogeographic model explaining the disjunct trans-Tibet distributions of low-dispersal organisms, suggesting specific paleoenvironmental corridors across Paleo-Tibet that may have guided their movement and persistence, offering a biotic perspective on the Miocene tectonic and environmental evolution of the Himalayan–Tibetan Orogen.

Declarations

Animal ethics and consent to participate

Not applicable.

Consent to publish

Not applicable.

Competing interest

The authors declare no competing interests.

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Author Contribution

S.H. and J.S. conceived and designed the work; S.H., E.P.G., and D.R. compiled and analysed the data; S.H., D. R., and J.S. interpreted the data; D.J. and C.B.B. provided resources; S.H. wrote the main manuscript text and M.F. prepared figures 3 and 4. All authors edited, readand approved the final manuscript.

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Figures

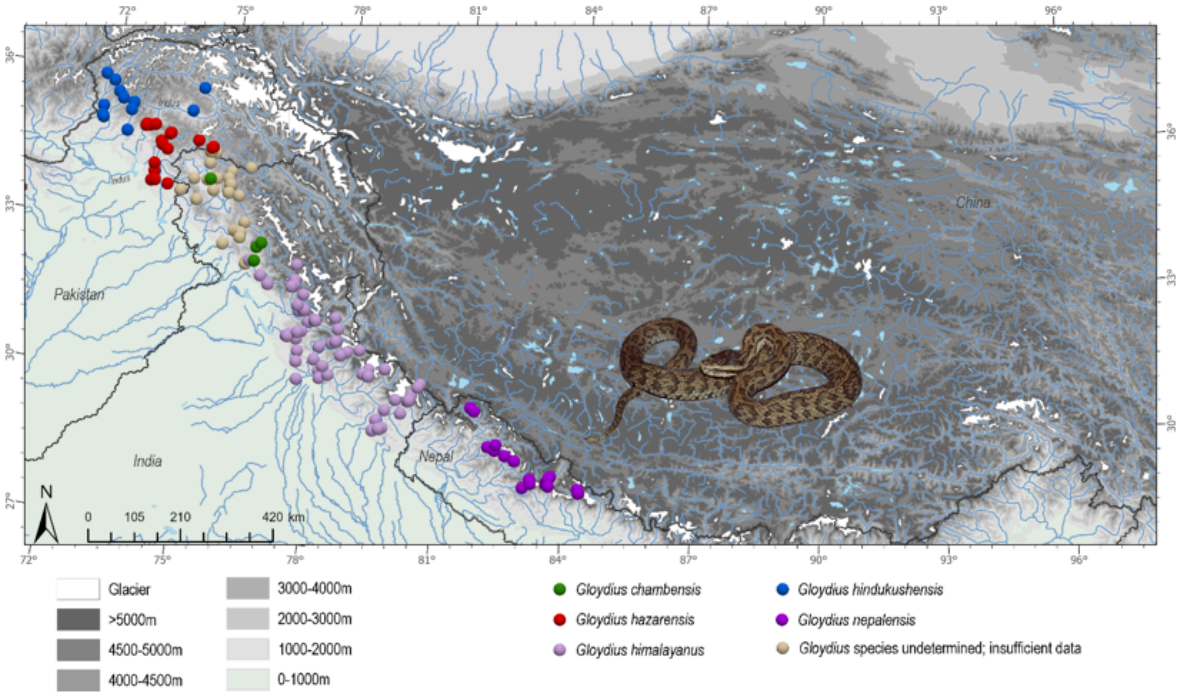

Figure 1

Geographic distribution of recognized *Gloydius* species distributed in the Himalaya and Hindu Kush. Records labeled as "undetermined" species were originally assigned to *G. himalayanus* but may include individuals of *G. chambensis* or *G. hazarensis*. Inserted photo showing *G. nepalensis*(holotype ZMB 65613); photo credit: Frank Tillack. For coordinates see Supplementary Table S1.

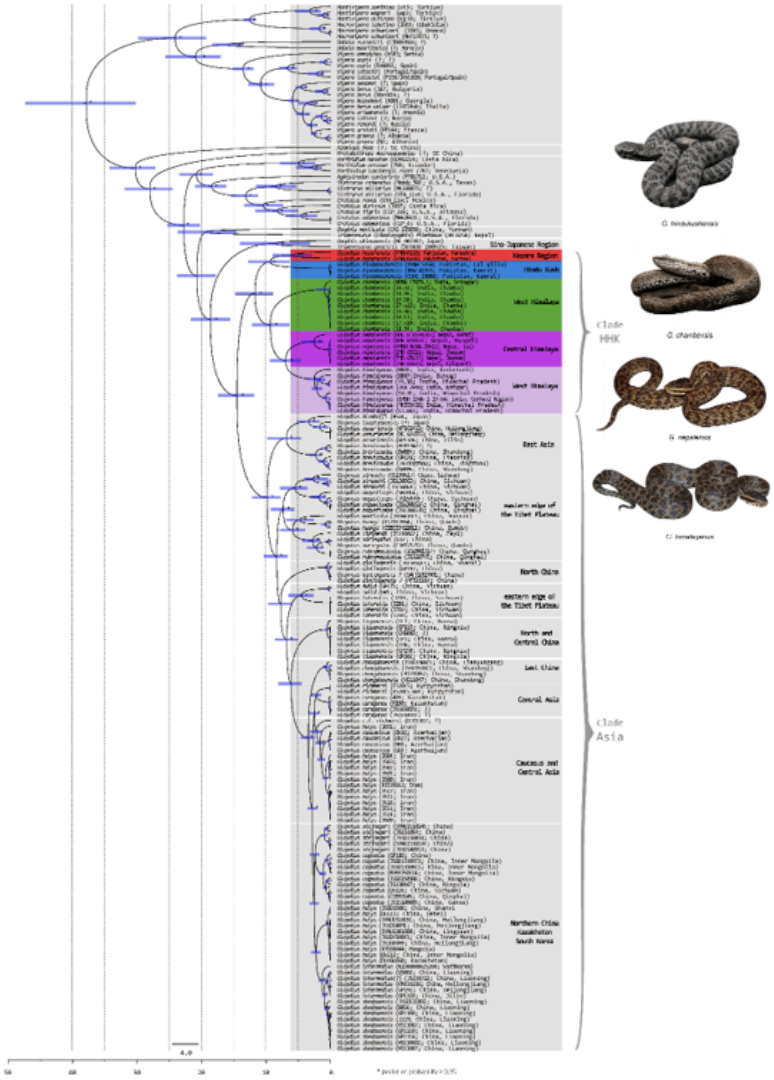

Figure 2

Time-calibrated phylogeny of *Gloydius* with selected outgroups. Divergence times were inferred in BEAST2 using a relaxed molecular clock model. Tip labels include the species name, followed (in brackets) by the associated voucher, isolate, field, or GenBank accession number, and locality information (country and, where available, more specific locality details). Horizontal bars at nodes indicate 95% highest posterior density (HPD) intervals. Nodes with posterior probability support ≥ 0.95 are marked with an asterisk. Species of the Himalaya-Hindu Kush (HHK) clade are color-coded according to the geographic map (Fig. 1). Photo credit: D. Jablonski (*G. hindukushensis*), G. Choure (*G. chambensis*), and F. Tillack (*G. nepalensis*, *G. himalayanus*). A high-resolution version is available as Supplementary Fig. S2.

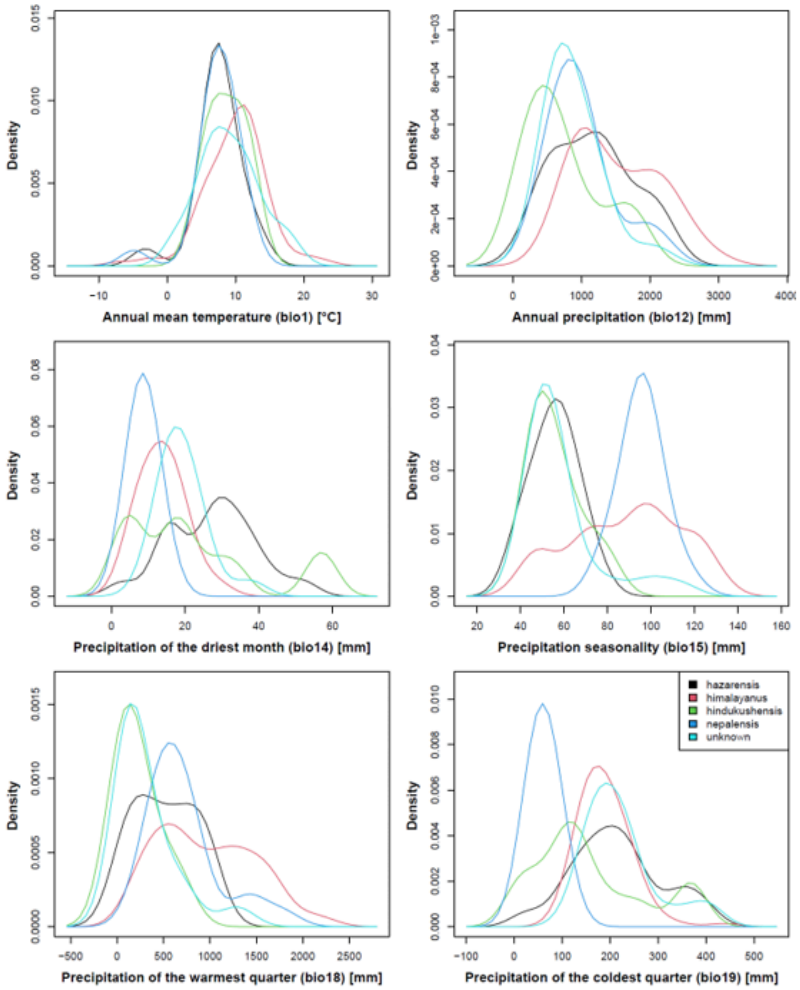

Figure 3

Comparison of climatic niches among *Gloydius* species based on density distributions of selected bioclimatic variables. Each curve represents the range of values occupied by different *Gloydius* species: *G. hazarensis*, *G. himalayanus*, *G. hindukushensis*, *G. nepalensis*, and unidentified populations, originally assigned to *G. himalayanus* but possibly including misidentified individuals of *G. chambensis* or *G. hazarensis*. Variables correspond to BIO1 = annual mean temperature, BIO12 = annual precipitation, BIO14 = precipitation of driest month, BIO15 = precipitation seasonality, BIO18 = precipitation of warmest quarter, and BIO19 = precipitation of coldest quarter.

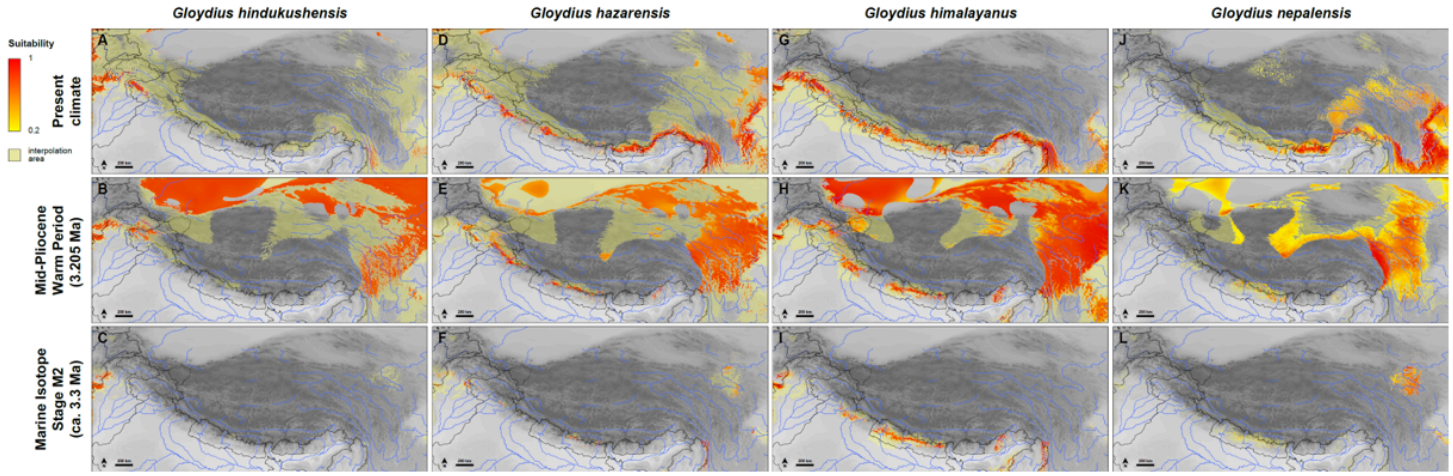

Figure 4

Species distribution models for four Himalayan *Gloydius* taxa under three climatic scenarios: present, Mid-Pliocene Warm Period (3.205 Ma), and Marine Isotope Stage M2 (ca. 3.3 Ma). (A–C) *G. hindukushensis*, (D–F) *G. hazarensis*, (G–I) *G. himalayanus*, and (J–L) *G. nepalensis*, shown in order as present, Mpwp, and M2.

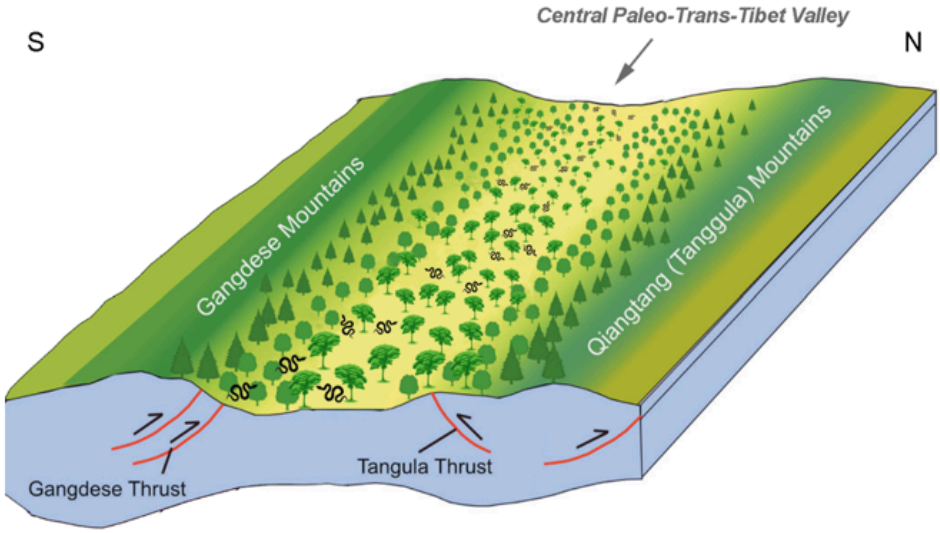

Figure 5

Schematic illustration of the lower elevated Central Paleo-Trans-Tibet valley. We assume the existence of such subtropical to warm-temperate corridor for the early to middle Miocene (figure modified from [57]).

Supplementary Files

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- SupplMat1.pdf
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