Journal of Biogeography

WILEY

Journal of Biogeography

RESEARCH ARTICLE

Dramatic Increases in Net Diversification Rates and Key Innovations Explain Rich Diversity and High Biomass of Arethuseae (Orchidaceae) in Asian Tropical and Subtropical Mountain Ecosystems

Chongbo Ma[1,2,3] | Jian-Wu Li[4] | Himmah Rustiami[5] | Arief Hidayat[5] | Xiao-Hua Jin[1,2]

[1]State Key Laboratory of Plant Diversity and Specialty Crops, Institute of Botany, Chinese Academy of Sciences, Beijing, China | [2]China National Botanical Garden, Beijing, China | [3]Yunnan Academy of Forestry and Grassland, Kunming, China | [4]Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, China | [5]Research Center for Biosystematics and Evolution, National Research and Innovation Agency, Cibinong, Indonesia

Correspondence: Xiao-Hua Jin (xiaohuajin@ibcas.ac.cn)

Received: 12 September 2024 | **Revised:** 9 June 2025 | **Accepted:** 4 July 2025

Funding: This work was supported Chinese Academy of Sciences (International partnership program, Grant. No. 063GJHZ2024075GC; Sustainable Development Research Project, Gant No. CAS-ANSO-SDRP-2024–02; Taxonomy Funds, Grant Nos. CAS-TAX-24–010) and the National Natural Science Foundation of China (32270214 to X.-H.J.).

Keywords: Arethuseae | biogeography | diversification | key innovation | pseudobulbs

ABSTRACT

Aims: Asian tropical and subtropical mountain systems (ATSME) encompass a diverse group of complex ecosystems ranging from tropical rainforests and subtropical rainforests to alpine grassland. ATSME provide a good chance to explore the mechanisms underlying the pattern of mountain biodiversity patterns, and various hypotheses have been proposed. We aim to characterise diversification in Arethuseae and its driving factors in Asian tropical and subtropical mountain ecosystems and test the hypothesis that specific key innovations had an influence on the diversification rates.

Location: Asian tropical and subtropical mountain ecosystems.

Taxon: Arethuseae (Orchidaceae), a group of 700 species mainly endemic in Asian tropical and subtropical mountain ecosystems.

Methods: The phylogenetic relationships of Arethuseae were reconstructed based on protein-coding genes of plastid genomes of 75 species + nrITS + 8 plastid genes for 191 species of Arethuseae, representing 96% of the genera (except *Aglossorrhyncha*) and 30% of the species in the Arethuseae. Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) methods. Divergence times were analysed using BEAST v1.10.4. Diversification rates were estimated using BAMM v2.5.0. Ancestral areas of Arethuseae were reconstructed using RASP v.4.3. The D statistic was used to test the phylogenetic signal of pseudobulb traits and epiphytic habits during the evolution of the phylogenetic tree of Arethuseae. A maximum entropy algorithm was used to model habitat suitability of Arethuseae.

Results: Arethuseae originated in the Asian tropical and subtropical monsoon areas, with diversification at the Oligocene–Miocene boundary. It was not until the mid-Miocene that Arethuseae began to diversify rapidly. The genera that diverged from the mid-Miocene were all epiphytic and most had pseudobulbs. Dramatic increases in situ diversification rates of Arethuseae began to diversify rapidly from mid-Miocene.

Main Conclusions: Asian tropical and subtropical mountain ecosystems might serve as both cradles and museums for diversification in Arethuseae. The innovation of pseudobulbs and epiphytic habit may have provided evolutionary opportunities for

© 2025 John Wiley & Sons Ltd.

adaptive radiation of Arethuseae. The intensification of the Asian monsoon and expansion of montane rainforests as well as their interactions were crucial for the formation and maintenance of mountain biodiversity in Asia.

1 | Introduction

Mountain ecosystems encompass rich biodiversity (Antonelli et al. 2018; Badgley et al. 2017; Favre et al. 2016, 2015; Lai et al. 2021; Myers et al. 2000; Trethowan et al. 2023), covering approximately 25% of the land but supporting one-third of all terrestrial biodiversity (Fjeldsa et al. 2012; Rahbek, Borregaard, Colwell, et al. 2019; Spehn et al. 2011). The causes of this rich mountain biodiversity have been a focus of research in ecology and evolutionary biology (Antonelli et al. 2018; Pandit et al. 2025; Rahbek, Borregaard, Colwell, et al. 2019). Although the complexity of mountain ecosystems is strongly correlated with species richness, the processes and mechanisms underlying this association are not clear (Perrigo et al. 2020). Various hypotheses, including pre-adaptation, niche conservatism and museum or cradle concepts, have been proposed to explain the rich biodiversity in mountain ecosystems (Dagallier et al. 2020; Fjeldsa et al. 2012; Gonzalez-Orozco 2023; Hoorn et al. 2013; Lai et al. 2021; Meseguer et al. 2022; Rahbek, Borregaard, Colwell, et al. 2019). Specifically, global or regional geological activity, historical climate and habitat heterogeneity may determine species diversification and biodiversity (Perrigo et al. 2020; Rahbek, Borregaard, Antonelli, et al. 2019).

Asian tropical and subtropical mountain systems (ATSME) encompass a diverse group of complex ecosystems ranging from tropical rainforests (e.g., New Guinea) and subtropical rainforests (e.g., Hengduan Mountains) to alpine grassland (e.g., the Himalayas). Seven of 35 global biodiversity hotspots are found in Asian tropical and subtropical mountain ecosystems (Myers et al. 2000; Spicer 2017). ATSME are characterised by the Asian monsoonal climate system and huge mountain chains ranging from the Himalayas (highest mountain system) through the Hengduanshan Mountains to southeast Asian tropical mountains (Ding et al. 2017; Spicer 2017). The Asian monsoon includes the South Asian monsoon and East Asian monsoon (Tada et al. 2016; Zhang et al. 2012), which may have occurred asynchronously (Renner 2016), are among the largest and strong monsoon in the world. The uplift of the Himalayas, the Tibetan Plateau and the collision between Sahul and Sunda shaped the palaeoclimate of Asia (Boos and Kuang 2010; Ding et al. 2017; Kooyman et al. 2019; Wilson and Moss 1999). These mountain ecosystems play a crucial role in biodiversity formation and maintenance, carbon cycling and sustainable human development. They provide a good chance to explore the mechanisms underlying the patterns of mountain biodiversity and various hypotheses have been proposed. For example, Rahbek, Borregaard, Antonelli, et al. (2019) and Rahbek, Borregaard, Colwell, et al. (2019) proposed that the mountain regions of Southeast Asia are primarily occupied by species that are recently diverged.

The orchid tribe Arethuseae (Orchidaceae, Epidendroideae) contains approximately 700 species in 13–27 genera (Chase et al. 2021; Huang et al. 2022; Li et al. 2015; Pridgeon et al. 2005), 99% of them are endemic in tropical and subtropical Asian mountain ecosystems with only six species in two genera distributed in North America. Plants of Arethuseae have high ornamental, medicinal and economic value (Cribb and Butterfield 1999; Han et al. 2021; Pridgeon et al. 2005; Sympli 2021; Zhang et al. 2018). They have diverse vegetative organs for the winter and dry seasons, including terrestrial corms in all species (approximately 8 species) belonging to *Anthogonium*, *Arethusa*, *Eleorchis* and *Calopogon*; tuberous rhizomes in four species of *Bletilla*; rhizomes in about 140 species of *Aglossorrhyncha* and *Glomera*; and pseudobulbs in about 550 species of *Coelogyne*, *Otochilus* and *Pholidota* (Chase et al. 2015, 2021; Li et al. 2015; Pridgeon et al. 2005). With the exception of a few terrestrial species, plants of Arethuseae (about 90% of species) are restricted to and grow well in tropical and subtropical humid rain forest mountain ecosystems and are epiphytic on branches and trunks, occupying an interlayer ecological niche (Cribb and Butterfield 1999; Pridgeon et al. 2005; Wood 2001). With the exception of *Aglossorrhyncha* and *Glomera*, epiphytic plants of Arethuseae are characterised by large pseudobulbs, leathery leaves and a clonal habit (Cribb and Butterfield 1999; Pridgeon et al. 2005; Wood 2001). Although Arethuseae is among the smallest tribes in Epidendroideae with respect to the number of species (Chase et al. 2015), our fieldwork indicated that plants in Arethuseae might have the largest biomass among orchid tribes in Asia. Many species of *Otochilus* and *Pholidota* form dense communities larger than 100 m^2. Yet, little is known about how and when Arethuseae diversified in tropical and subtropical Asian mountain ecosystems as well as the factors that triggered diversification.

In this study, we assembled the largest data sets for Arethuseae to date, with data for 191 species in 27 genera and 71 plastomes with aims to (1) characterise diversification in Arethuseae and its driving factors in Asian tropical and subtropical mountain ecosystems, and (2) test the hypothesis that specific key innovations had influence on the diversification rates.

2 | Materials and Methods

2.1 | Taxon Sampling

The plastid genomes of 71 species in Arethuseae were obtained, representing 15 genera of the two subtribes in tribe Arethuseae, of which plastid genomes and nuclear genes (ITS) of 49 species were newly sequenced in this study (Tables S1 and S2). Fifteen genera included *Anthogonium*, *Arundina*, *Bletilla*, *Bulleyia*, *Coelogyne*, *Dendrochilum*, *Glomera*, *Ischnogyne*, *Neogyna*, *Otochilus*, *Panisea*, *Pholidota*, *Pleione*, *Thunia* and *Thuniopsis*. All materials were from specimens kept in the herbarium (PE), Institute of Botany, Chinese Academy of Sciences or from living plants in the China National Botanical Gardens (Tables S1 and S2).

To generate a more comprehensive phylogenetic framework, data for eight plastid genes (*matK*, *ycf1*, *rbcL*, *trnF*, *psaB*, *rpoC1*,

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

rpoC2 and *ndhJ*) and one nuclear gene (ITS) from 120 species of Arethuseae available in NCBI were used (Table S3). In total, 191 species (~30% of 700 species) in 27 genera (~96% of 28 genera) were sampled in Arethuseae (Table S4). Twenty-seven genera included *Anthogonium*, *Arethusa*, *Arundina*, *Bletilla*, *Bracisepalum*, *Bulleyia*, *Calopogon*, *Chelonistele*, *Coelogyne*, *Dendrochilum*, *Dickasonia*, *Dilochia*, *Eleorchis*, *Entomophobia*, *Geesinkorchis*, *Gynoglottis*, *Glomera*, *Ischnogyne*, *Mengzia*, *Nabaluia*, *Neogyna*, *Otochilus*, *Panisea*, *Pholidota*, *Pleione*, *Thunia* and *Thuniopsis*. According to previous studies (Chase et al. 2015; Zhang et al. 2023), *Epipactis veratrifolia*, *Elleanthus sodiroi*, *Sobralia bouchei* and *Sobralia callosa* were used as outgroup taxa.

2.2 | DNA Extraction, Sequencing, Assembly and Annotation

The NuClean Plant Genomic DNA Kit was used to extract total DNA from silica-dried leaves. Before the library was prepared, the genomic DNA of each sample was assessed for quality using agarose gel electrophoresis. High-quality DNA was sent to Majorbio (Beijing, China) for genomic library construction and sequencing using the Illumina HiSeq 2500 sequencing platform to generate 150 bp paired-end reads. Raw reads were filtered using NGSQCTOOLKIT ver. 2.3.3 (Patel and Jain 2012) to remove low-quality bases. Ultimately, for each individual, 5 Gb of clean data was generated for subsequent plastid genome assembly.

GetOrganelle v1.7.5 (Jin et al. 2020) was used for chloroplast genome assembly, and the results were imported to Bandage v. 0.80 (Wick et al. 2015) for the visualisation of complete circular plastomes. For data that could not be assembled into a loop using GetOrganelle v1.7.5 (Jin et al. 2020), the scaffold was used as a reference to manually extend the assembly in Geneious Prime (Kearse et al. 2012), and mapping was repeated multiple times to obtain circular plastomes. The nrITS assembly was performed initially using GetOrganelle v1.7.5 (Jin et al. 2020), and the results were then imported into Geneious Prime for filtering to assemble the complete nrITS. *Pleione bulbocodioides*, *Bulleyia yunnanensis* and *Bletilla striata* were used as references for annotation using PGA and Perl scripts, and the genes were checked manually using Geneious Prime (Kearse et al. 2012).

2.3 | Phylogenetic Inference

To evaluate whether the ITS and coding regions (CDS) of plastid genome datasets (PGD) support statistically different phylogenetic hypotheses, we conducted a partition homogeneity test on the ITS and plastid genome datasets (PGD). The incongruence length difference (ILD) test (Farris et al. 1994) resulted in a value of 0.01, which may be attributed to significant differences in the number of uninformative characters between the partitions. We further conducted topology tests for the ITS+ PGD tree and PGD tree using the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999). The results indicated a consistency between the ITS+ PGD tree and the PGD tree (Table S4).

Arethuseae phylogenetic relationships were then reconstructed using two datasets: (1) M1, including CDS of plastid genomes of 75 species (49 species were newly sequenced), and (2) M2, a matrix of the CDS of plastid genomes of 75 species + nrITS + 8 plastid sequences (*matK*, *ycf1*, *rbcL*, *trnF*, *psaB*, *rpoC1*, *rpoC2*, *ndhJ*) for 191 species of Arethuseae, representing 96% of the genera (except *Aglossorrhyncha*) and 30% of the species in the tribe. All these plastid sequences were downloaded from NCBI (Table S4). Missing CDS and sequences were treated as gaps. MAFFT was used to align CDS, sequences and nrITS. Aligned sequences were trimmed using GBLOCK 0.91b (Talavera and Castresana 2007), manually checked using BioEdit and finally concatenated into a matrix using PhyloSuite (Zhang et al. 2020). The best-fit nucleotide substitution models were evaluated using ModelFinder based on the corrected Akaike information criterion (AICc). Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) methods. For the first dataset in RAxML 8.2.10 (Stamatakis 2014), the GTR + I + G4 model and 1000 bootstrap replicates were used. IQ-Tree v.2.0-rc1 (Nguyen et al. 2015) was used for the reconstruction of the ML tree for the second dataset using an automatic selection model with 100,000 ultra-fast bootstrap replicates. The BI analysis was conducted in MrBayes 3.2.6 (Ronquist et al. 2012). GTR + I + G4 was the best-fit substitution model for the first dataset and GTR + I + G + F was selected for the other. Two independent runs were conducted, each consisting of four Markov chain Monte Carlo (MCMC) chains, with sampling every 10,000 generations for a total of 10,000,000,000 generations. Stationarity was determined using Tracer v.1.7.2 (Rambaut et al. 2018). The first 25% of the sampled trees from all runs were obtained as burn-in, and consensus trees were constructed from the remaining trees to estimate posterior probabilities (PP).

2.4 | Divergence Time Estimation

BEAST v1.10.4 (Drummond and Rambaut 2007) and the matrix M2 were used to calculate divergence times. The marginal likelihood, as estimated through path sampling, was used to evaluate competing molecular clock and coalescent model combinations. The optimal configuration integrated an Uncorrelated Relaxed Clock with a Yule Process coalescent model (Table S5) (Baele et al. 2012; Drummond et al. 2005). Six time points were selected to calibrate the phylogeny of Arethuseae based on 95% HPD intervals for node ages from the fossil-calibrated study by Ji et al. (2024): (1) Crown age of *Dendrochilum*: 5.05–17.01 Ma; (2) Crown age of *Coelogyne* + *Pleione*: 14.11–33.07 Ma; (3) Crown age of *Pleione*: 3.29–17.58 Ma; (4) Crown age of Arethusinae + Coelogyninae: 19.67–42.3 Ma; (4) Crown age of Arethusinae: 13.97–36.6 Ma; and (6) Root age: 47.8–76.5 Ma. Calibrations were applied as uniform priors to accommodate uncertainty in fossil age assignments.

The BEAUti interface was used to generate input files for BEAST v1.10.4 (Drummond and Rambaut 2007). Three independent runs were evaluated, with each MCMC chain set to run for 200,000,000 generations and sampling every 20,000 generations. LogCombiner v1.10.4 (Drummond and Rambaut 2007) was used to merge log and tree files from three independent

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

runs, discarding the top 25% of samples from each log and tree file as burn-in before merging. Convergence was then checked using Tracer v.1.7.2 (Rambaut et al. 2018).

2.5 | Diversification Rates

Bayesian Analysis of Macroevolutionary Mixtures (BAMM v2.5.0) (Rabosky et al. 2014) was used to analyse diversification rates with a maximum clade credibility tree. To account for 30% sampling of total species in Arethuseae, the sampling fraction was estimated for each clade reconstructed in our phylogeny. The evolutionary rate parameter priors were set using the BAMMtools v2.1.6 (Rabosky et al. 2014) package in R with the setBAMMpriors function. We ran 1,000,000 generations with sampling every 1000 generations. The coda package was used to assess convergence, ensuring that the effective sample size was >200 and the first 10% of samples were discarded as burn-in. The BAMM output data were analysed in R using the BAMMtools package (Rabosky et al. 2014). To visualise the change in diversification rates through time, logarithmic lineage-through-time (LTT) plots were constructed using the R package APE (Paradis et al. 2004).

2.6 | Ancestral Geographical Range Analysis

Five biogeographic regions for Arethuseae were defined based on the existing distribution, climate and geological characteristics of the tribe: North America (A), Asian tropical islands (B, including the Malay Archipelago, Indonesia and New Guinea), Asian tropical monsoon area (C, including the Central South Peninsula, the Indian Peninsula and Hainan Island in China), Asian subtropical monsoon area (D) and Asian temperate monsoon area (E, including northern Japan and the Thousand Islands).

Ancestral areas of Arethuseae were reconstructed using RASP v.4.3 (Yu et al. 2020). The differences between the distribution of the outgroup and Arethuseae species may affect the reconstruction of the ancestral region of Arethuseae; therefore, we removed the outgroup (Table S6). The Dispersal-Extinction Cladogenesis (DEC) model, Dispersal-Vicariance Analysis (DIVA) model, BayArea model and each model '+J' were fitted, where '+J' is jumping species formation. The maximum allowable distribution area was set to three based on the distribution of extant Arethuseae.

2.7 | Ancestral State Reconstruction

The presence of a pseudobulb and epiphytic habit of Arethuseae was coded based on previous studies on extant species (Table S6). To determine the appropriate trait evolution model, we independently fitted an equal rates model (ER), an all-rates-different model (ARD) and a symmetric rates model (SYM) using the fitMk function in the phytools package in R. Ancestral state reconstructions of pseudobulb and epiphytic habit were then estimated using stochastic character mapping with 500 simulations using the make.simmap function in the package phytools (Revell 2012).

2.8 | Evolutionary Rates and Phylogenetic Signal Testing

To test the phylogenetic signal of pseudobulb traits and epiphytic habits during the evolution of the phylogenetic tree of Arethuseae, the D statistic was calculated using the phylo.d function in the package caper in R (Fritz and Purvis 2010). A value of $D=1$ indicates that the observed trait has a phylogenetically random distribution (i.e., no phylogenetic signal), while $D=0$ indicates that the observed trait showed a pattern of evolution consistent with Brownian motion (i.e., a strong phylogenetic signal). $D<0$ indicates high conservation of the trait of interest and $D>1$ indicates overdispersion. We performed 1000 permutations to evaluate the significance of D.

The key innovation hypothesis suggests that increases in diversification over time are coincident with the evolution of key traits and that changes in diversification rates can influence the evolution and distribution of traits (Heard and Hauser 1995). In this study, trait-diversification rate correlations were tested using a state-dependent speciation and extinction (SSE) model. We simulated speciation rates (λ), extinction rates (μ), and transition rates (q) with and without pseudobulbs using the BiSSE model (binary-state speciation and extinction model) in the diversitree package under the R language. Based on the prevailing view of the key innovation hypothesis, we focused on the speciation rate (λ). We fitted eight BiSSE models with decreasing complexity that included constraints on speciation, extinction, and transition rates between trait states (Table S7). The best-fitting models were selected based on likelihood-ratio tests under a chi-squared distribution and Akaike's information criterion (AIC). ML was used to optimise full and constrained models. MCMC chains were run for 10,000 generations for the best-fitting models in 1000 randomly sampled phylogenetic trees of Arethuseae.

2.9 | Calculation of Maximal Number of Occurrence Events

To quantify the temporal dynamics of species diversification, we calculated the Maximal Number of Occurrence Events (MOE) based on divergence time estimates. A maximum clade credibility tree, generated using BEAST v1.10.4 (Drummond and Rambaut 2007), was used to extract node-specific divergence times. For each node, the 95% HPD intervals of node heights were obtained using the R package treeio v1.32.0 (Wang et al. 2020). We defined a time slice range from 0 to 40 million years ago (Ma) with a step size of 1 Ma. For each 1 Ma time slice, we counted the number of nodes whose 95% HPD intervals overlapped with the slice, generating raw MOE values.

To reduce stochastic fluctuations and avoid over-interpretation of short-term variations, raw MOE values were smoothed using a sliding window spanning 5 million years. The smoothed MOE at each time point was computed as the mean of raw values within the window, with edge effects excluded from subsequent analyses. Significant shifts in MOE trends were identified using the e.divisive function in the R package ecp v3.1.6 (James and Matteson 2015). The following parameters: the maximum

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

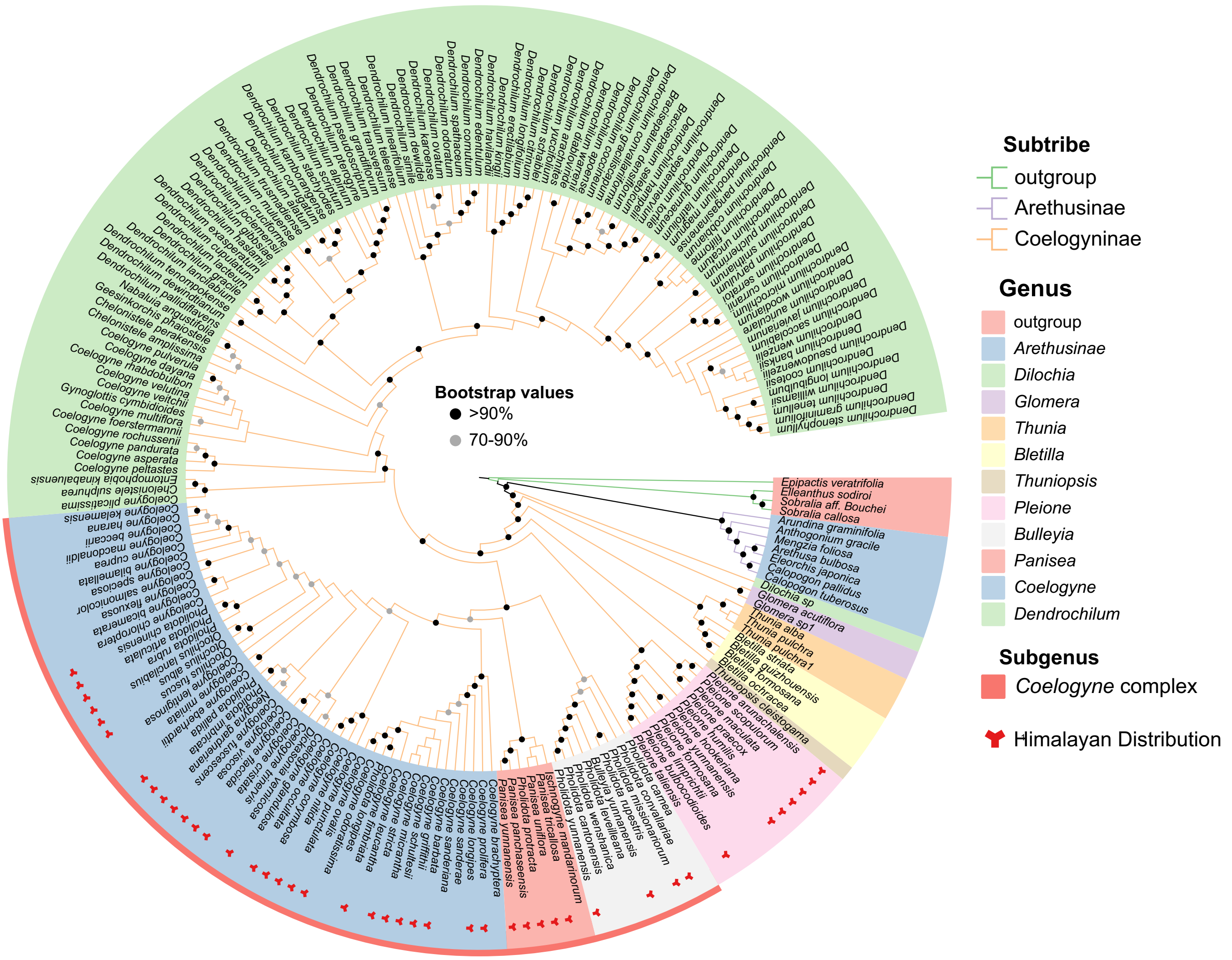

FIGURE 1 | Maximum likelihood (ML) tree reconstruction for Arethuseae based on plastid genes and ITS. Black dots above branches indicate bootstrap support (BS) values of 100%, and grey dots indicate BS > 90%.

number of random permutations = 1000, the significance level = 0.05, the minimum number of observations between change points = 5 and moment index alpha = 1.

2.10 | Ecological Niche Modelling

We obtained distribution data for 28 genera of Arethuseae from the Global Biodiversity Information Facility (GBIF http://www.gbif.org) and field records accumulated by our research team. After excluding localities with ambiguous coordinates, potential misidentifications and spatial duplicates within a 10-km radius, we retained 4451 georeferenced records (Table S8) to map the observed distribution of Arethuseae. For these records, 19 bioclimatic variables were extracted from the WorldClim2 database (Fick and Hijmans 2017) with a spatial resolution of 30-arc-second using ArcGIS v.10.6 (ESRI).

To model habitat suitability, we used the maximum entropy algorithm implemented in MaxEnt v.3.4.4 (Phillips et al. 2006, 2017). Collinear variables ($|r| \geq 0.80$) were systematically removed through pairwise correlation analysis, resulting in seven predictors for final model calibration (Table S9). Model parameters were set as follows: (1) random test percentage: 25%; (2) regularisation multiplier: 2; (3) cross-validation replicates: 10; (4) maximum background points: 10^4; (5) the output format was set to logistic; and (6) the jackknife method was set to detect the results. All other parameters retained default settings.

3 | Results

3.1 | Phylogenetic Inferences

Plastid genomes (plastomes) for 49 species of Arethuseae were newly sequenced and 26 were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). Two datasets, matrix 1 (M1, 75 species with all 76 plastid protein coding sequences (ptCDS), in total 63,376 bp) and matrix 2 (M2, 76 ptCDS + nrITS data for 195 species, in total 65,389 bp), were used for phylogenetic inference. The phylograms constructed using Bayesian inference (BI) and maximum likelihood (ML) methods based on M1 were highly consistent and strongly supported (Figure S1). The phylogenetic relationships based on M2 were consistent with the topology obtained based on M1 (Figure 1, Figure S2). Considering the

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

representativeness of species, the ML phylogram based on M2 was selected for all subsequent analyses.

3.2 | Divergence Time Estimation

The stem age of Arethuseae was 38.66 Ma (95% HPD: 49.48–31.89 Ma) (Figure S3). Arethusinae and Coelogyninae diverged during the early Oligocene at 28.75 Ma (95% HPD: 35.33–22.48 Ma). In Arethusinae, four genera, *Arundina*, *Anthogonium*, *Mengzia* and *Calopogon*, diverged successively along the backbone of Arethusinae at 26.14, 21.54, 18.68 and 12.02 Ma, respectively. In Coelogyninae, five genera without pseudobulbs, *Dilochia*, *Glomera*, *Thunia*, *Bletilla* and *Thuniopsis*, diverged successively along the backbone of Coelogyninae from the middle to late Oligocene (26.07–23.13 Ma) (Figure S3). Genera with pseudobulbs, such as *Pleione*, *Bulleyia* and *Coelogyne*, diverged from the backbone of Coelogyninae in the early-to-middle Miocene (22.01–11.96 Ma). With a few exceptions, most species of Arethuseae diverged in the middle Miocene to late Miocene during a period of global cooling (Figure S3).

3.3 | Ancestral Geographical Range

The BAYAREALIKE+J model with the smallest corrected Akaike's information criterion (AICc) value and the largest AICc_wt value was identified as the best-fit biogeographical model for our data (Tables S10 and S11). Our results indicated that Arethuseae originated in tropical and subtropical monsoon areas of Asia (Figure S4). At least two long-distance dispersal events were identified in Arethusinae: (1) the dispersal of the ancestor of *Calopogon* from the tropical and subtropical monsoon areas of Asia to North America in the early Miocene, and (2) the dispersal of the ancestor of *Eleorchis* from North America to Japan in the late Miocene (Figure S4, V1 and V2). Three main dispersal events within tropical and subtropical Asia were identified: (1) the dispersal of the common ancestor of Dilochia and Glomera from tropical and subtropical mainland Asia to tropical islands of Asia during the late Oligocene, (2) the dispersal of Dendrochilum s.l. from mainland Asia to the tropical island of Asia in the middle Miocene and (3) the dispersal of *Coelogyne* from mainland Asia to tropical islands in the late Miocene (Figure S4, V3–V5).

3.4 | Diversification and Rate Shifts

We detected nine diversification rate shifts; however, only three had a posteriori probability of >0.5 (Figure S5). The most likely rate shift node was determined using the getBestShiftConfiguration function (Figure 2A). As visualised using a phylorate plot of speciation rates, most genera had high diversification rates and diversification rates were significantly higher in the pseudobulb taxa than in non-pseudobulb taxa (Figure 2B). Rate-through-time plots suggested that the speciation rate and net diversification rate of Arethuseae accelerated significantly near the Oligocene–Miocene boundary. About 18 Ma, the speciation rate exceeded the extinction rate and reached a peak during the late Miocene (about 8 Ma). Moreover, the diversification rate remained relatively high thereafter (Figure 2C). The increases in speciation rate and net diversification rates were consistent with the location of rate shifts in the phylogeny.

3.5 | Ancestral State Reconstruction of Pseudobulbs and Epiphytism

The all-rates-different model (ARD) was selected as the best-fit model based on likelihood and AIC values (Table S12). Ancestral state reconstructions showed that epiphytic habits arose independently at least twice in Arethuseae. Pseudobulbs originated at least once in Arethuseae (Figures S6 and S7).

3.6 | Evolutionary Rates and Phylogenetic Signal

Phylogenetic signals based on D statistics were evaluated for pseudobulb traits and epiphytic habits of Arethuseae. Pseudobulb traits ($D=-1.32$) and epiphytic habits ($D=-1.35$) were highly conserved, suggesting that closely related species share this trait (Table S13). Equal speciation and transition rate models were selected as the best models based on likelihood ratio tests under a chi-squared distribution and AIC (Table S7). Our results showed that net diversification rates of taxa with pseudobulbs were higher than those of taxa without pseudobulbs, while the extinction rate shows the opposite trend (Figure S8).

3.7 | Temporal Dynamics of Arethuseae Occurrence

The smoothed MOE curve revealed a pronounced intensification of diversification rates in Arethuseae over the 0–40 Ma timeframe (Figure 3). A marked acceleration initiated near the Oligocene–Miocene boundary, with the MOE increasing linearly until reaching its first peak at ca. 5 Ma. Subsequent to this peak, the MOE trajectory entered a secondary acceleration phase, continuing into the present.

The e.divisive algorithm identified seven statistically potential change points ($p \leq 0.05$) in the MOE curve, partitioning the diversification history into three distinct phases:(1) 38.01–23.5 Ma: characterised by the initial divergence of the subtribes Arethusinae and Coelogyninae; (2) 23.5–8.5 Ma: a rapid radiation phase coinciding with the emergence of pseudobulb-bearing lineages; (3) 8.5 Ma–present: a renewed acceleration phase (Figure 3).

3.8 | Influential Climatic Factors for the Distribution of Arethuseae

We obtained 4451 valid georeferenced records of 28 genera of Arethuseae and extracted 19 bioclimatic variables for each record from the WorldClim2 database (Fick and Hijmans 2017). After removing collinear variables ($|r| \geq 0.80$), seven predictors were retained to model the climatically suitable distribution of Arethuseae (Figure S9): Mean Diurnal Range (Bio2), Max Temperature of Warmest Month (Bio5), Mean Temperature of Driest Quarter (Bio9), Annual Precipitation (Bio12), Precipitation Seasonality (Bio15), Precipitation of Driest Quarter (Bio17)

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

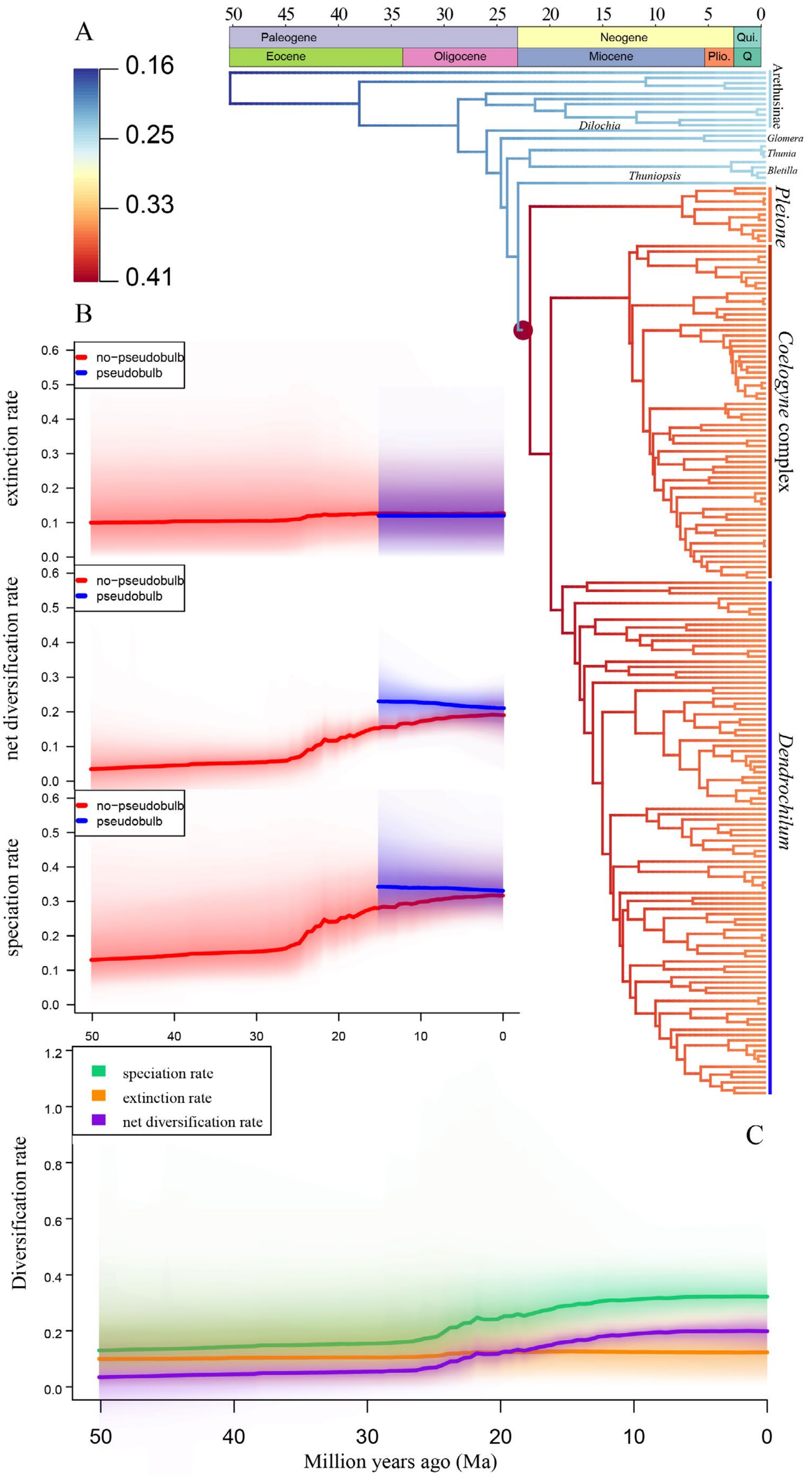

FIGURE 2 | Legend on next page.

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

FIGURE 2 | (A) Best shift configurations based on phylorate plots for Arethuseae. (B) Comparison of species formation rates, extinction rates and net species diversification rates for taxa with and without pseudobulbs. (C) Rate-through-time plots for the speciation rate (green), extinction rate (yellow) and net diversification (purple), with shaded areas indicating 95% confidence intervals.

and Precipitation of Warmest Quarter (Bio18) (Table S14). The MaxEnt model demonstrated high predictive accuracy, achieving a mean training AUC of 0.879 ± 0.001 across 10 replicate runs (Figure S10). Jackknife analysis revealed Precipitation of Warmest Quarter (Bio18) as the dominant climatic driver of Arethuseae distribution, contributing 62.6% to the model, followed by annual precipitation (Bio12; 29.3%) and max temperature of warmest month (Bio5; 2.8%) (Table S14).

4 | Discussion

4.1 | Phylogeny of Arethuseae

Recent molecular phylogenetics studies indicated that generic delimitation in Arethuseae is necessary (Chase et al. 2021; Goldman et al. 2001; Huang et al. 2022; Li et al. 2015; van den Berg et al. 2005; Wati et al. 2021). Our phylograms were consistent with previous results but provide new insights into the phylogenetic relationships and classification of Arethuseae based on more samples and plastome data. For example, we found that *Dilochia*, *Glomera*, *Thunia* + *Bletilla*, *Thuniopsis* and *Pleione* are successive sister groups along the backbone of Coelogyninae, different from the comb-like or weakly supported phylograms in previous studies (Chase et al. 2021; Li et al. 2015; Wati et al. 2021) (Figure S11).

Based on phylograms and morphological characters of Arethuseae, we propose the following taxonomic treatments for the *Coelogyne* complex: (1) the expansion of *Bulleyia* to include some *Pholidota* species (*Bulleyia* s.l. is characterised by long and pendulous inflorescences, persistent and large floral bracts); (2) the expansion of *Panisea* to include *Ischnogyne* (*Panisea* s.l. is characterised by racemes with 1–2 flowers, floral bracts small, petals similar to sepals, lip flat or slightly depressed at base but never saccate); (3) the expansion of *Coelogyne* to include *Neogyna*, *Otochilus*, *Dickasonia* and some species of *Pholidota*; and (4) the expansion of *Dendrochilum* to include *Geesinkorchis*, *Nabaluia*, *Chelonistele*, *Gynoglottis*, *Entomophobia* and some *Coelogyne* species distributed only in the tropical island region of Asia. *Coelogyne* s.l., *Panisea* s.l. and *Bulleyia* s.l. form the *Coelogyne* complex (Figure 1, Figure S2).

4.2 | Key Innovations in Arethuseae

The formation of the monsoon climate forced organisms in the East Asia and Southeast Asia region to adapt to seasonal climatic changes and to endure months of water saturation and extreme drought conditions (Spicer 2017). Some ferns adapt to seasonal changes through defoliation and dormant stages (Wang et al. 2012). The emergence of pseudobulbs in the Arethuseae facilitated a shift from a terrestrial to an epiphytic habit to cope with these conditions.

Adaptive radiations require geographic, ecological and evolutionary opportunities (Losos 2010; Simpson 1953), where evolutionary opportunity refers to the ability of a lineage to diversify to utilise available resources (Miller et al. 2023; Valen 1971; Wahlert 1965), specifically to generate new interactions with the environment. In the present study, the diversification rate differed between taxa with and without pseudobulbs, and a rate shift occurred in pseudobulb groups (Figure 2A,B). The key innovation hypothesis suggests that increased diversification is associated with the evolution of key traits and that changes in diversification rates can influence the evolution of traits (Miller et al. 2023). Based on state-dependent speciation and extinction (SSE) models (Figure S9), we found that net diversification rates were significantly higher in pseudobulb clades than in clades without pseudobulbs, while the extinction rate shows the opposite trend. These results further suggest that the evolution of pseudobulbs facilitated the habit shift from terrestrial to epiphytic in Arethuseae.

Ancestral state reconstruction revealed two independent evolutionary origins of the epiphytic habit and a single origin of the pseudobulb trait. Furthermore, phylogenetic signal tests revealed that pseudobulb traits and epiphytic habits showed highly conserved evolutionary patterns (Figures S6 and S7, Table S13). These conserved traits are closely related to plant nutrient utilisation and may be related to selection in different habitats and correlated evolution of structural and functional traits. Arethuseae did not diversify immediately after the rapid divergence of two subtribes at the Mid-Oligocene, which were mainly terrestrial in habit (except for *Glomera*) and without pseudobulb traits (Figure 3). Coelogyninae diversified rapidly until the Oligocene–Miocene boundary (23 Ma) (Figures 2C and 3). These genera shifted from a geophytic habit to a lithophytic and/or epiphytic habit and from rhizomes to pseudobulbs. These changes may be adaptations to the Asian monsoonal climate (i.e., to endure the complex habitat of several months of water saturation and extreme drought) (Spicer 2017). On the one hand, pseudobulbs can store large amounts of water, minerals and carbohydrates, enabling the growth and survival of epiphytic orchids in the dry season (He 2018). On the other hand, the evolution of pseudobulbs facilitated the habitat transition from terrestrial to epiphytic in Coelogyninae, thus allowing these species to access ecological niches in the middle or upper layers of the rainforest and utilise the available resources in these niches. Interestingly, *Glomera* includes about 155 epiphytic species (https://www.ipni.org) but did not show a high diversification rate; it is possible that the epiphytic habit enabled the genus to diversify only in humid tropical island areas due to the absence of pseudobulbs.

4.3 | The Diversification Dynamics of Arethuseae

Our ancestral region reconstructions and molecular dating analyses indicate that Arethuseae originated in Asian

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

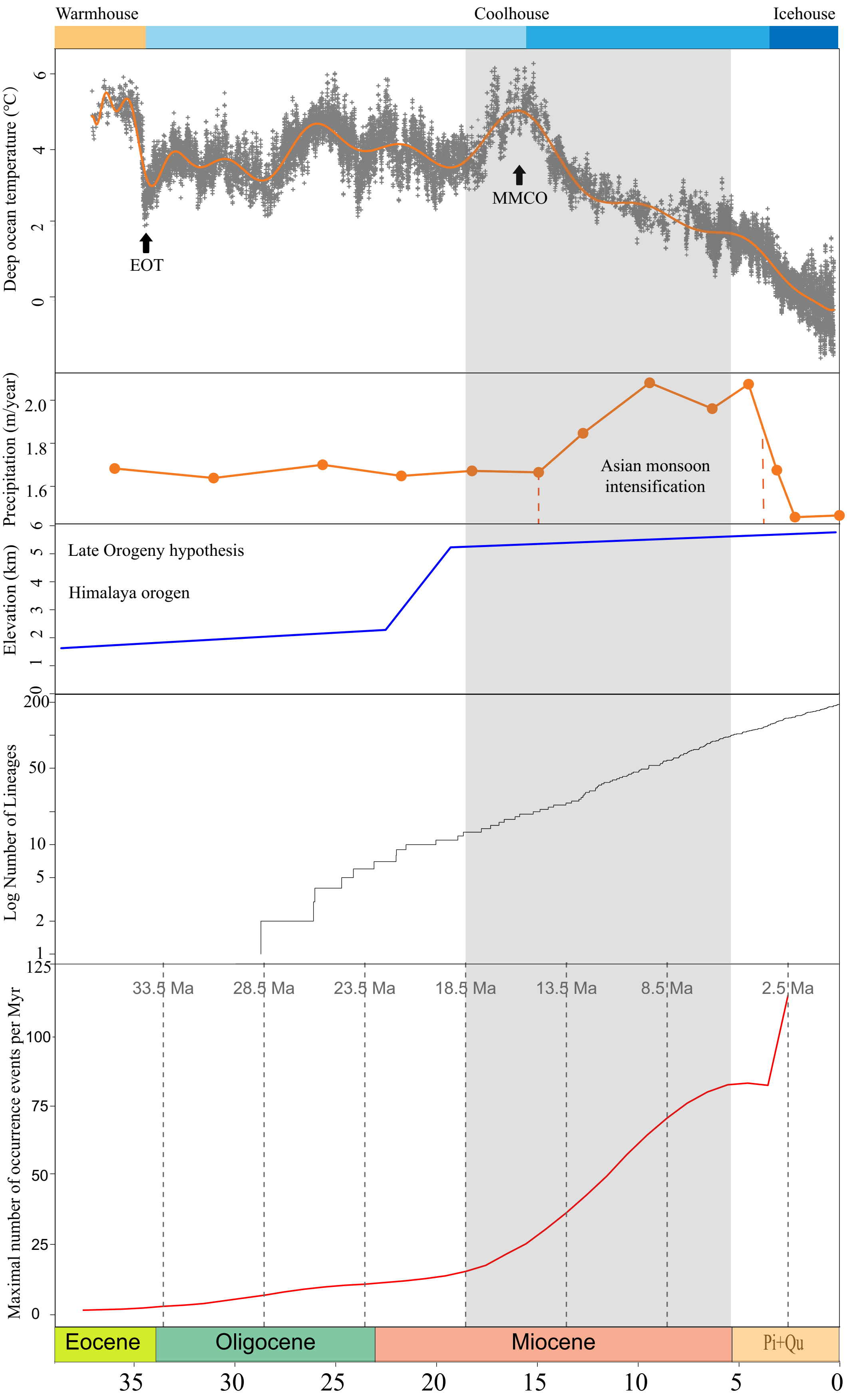

FIGURE 3 | Legend on next page.

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

FIGURE 3 | Diversification of Arethuseae in relation to orogenesis and palaeoclimate. Global climate change curves were derived from deep ocean temperatures estimated by Hansen et al. (2013). Mean annual precipitation on the Tibetan Plateau and neighbouring areas simulated by Farnsworth et al. (2019) under ideal CO_2 indicates the evolutionary trend of the Asian monsoon. The blue curve describes the 'stepwise hypothesis' of Himalayan uplift proposed by Ding et al. (2017). EOT, Eocene–Oligocene Transition; NMCO, Middle Miocene Climatic Optimum. The red curve describes the maximal number of occurrence events (MOE) per million years of Arethuseae. Seven grey dotted lines indicate estimated significant change points, respectively ($p \leq 0.05$). The black line describes the lineage-through-time (LTT).

tropical and subtropical monsoon areas (Central-South China) at 38.66 Ma (95% HPD: 49.48–31.89 Ma; Figure S3). Subsequently, at least two long-distance dispersal events were identified, facilitating the disjunct distribution of Arethuseae between North America and East Asia (Figure S4). This biogeographic pattern exhibits parallels with numerous orchid genera, such as *Bulbophyllum* (south American, Africa and Asia) (Pridgeon et al. 2014), *Calanth*e (Asia, Africa and Central America) (Pridgeon et al. 2005), *Gastrodia* (Asia, Africa, Oceania) (Huang et al. 2018), *Pogonia* (northern American and East Asia) (Pansarin et al. 2008), display current-day disjunct distribution. LDD in orchids were facilitated by the dust-like orchid seeds that predominantly adapted to wind dispersal (Jersakova and Malinova 2007). In addition, three main dispersal from mainland Asia to the tropical islands of Asia were detected and followed by in situ diversification; instead, dispersal from tropical islands of Asia to Asian mainland was not detected (Figure S4). Our results indicate that the tropical and subtropical monsoon regions of Asia are the centres of dispersal for the radiation of Arethuseae.

The maximal number of occurrence events (MOE) began increasing at 33.5 Ma, coinciding with the initial divergence of subtribes Arethusinae and Coelogyninae (Figure 3). The MOE began to increase at 23.5 Ma, which coincides with tectonic activities in the Himalayas (Figure 3). This tectonic activity strengthened the summer monsoons in East and South Asia, potentially enhancing regional precipitation. Concurrently, the pseudobulb trait emerged at this period, facilitating the diversification of Arethuseae in East and South Asia. Rate-through-time analyses further confirmed that net diversification rates exceeded extinction rates at the Oligocene–Miocene boundary (Figure 3). Ecological niche modelling identified Precipitation of Warmest Quarter (Bio18) as the primary climatic determinant of Arethuseae distribution (Table S14). Thus, we suggest that environmental drivers and key innovations synergistically promote the diversification of Arethuseae.

From 18.5 to 5 Ma, the MOE showed a linear increase and reached the first peak about 5 Ma (Figure 3). This coincided with the global Mid-Miocene Climatic Maximum (ca. 17–14 Ma) and the establishment of the East Asian 'Super Monsoon' period (ca. 12–4 Ma) (Farnsworth et al. 2019). Meanwhile, the flourishing continuously of Asian evergreen broadleaf forests during the Miocene (Li et al. 2022) likely provided ample ecological opportunities for the diversification of Coelogyninae. Additionally, the widespread uplift of Borneo during the middle Miocene (Morley 2018), the dramatic uplift of New Guinea in the late Miocene (Toussaint et al. 2014) and significant sea-level oscillations in Southeast Asia may have further facilitated the dispersal of Coelogyninae from Asia to the tropical islands.

The MOE declined transiently at 4 Ma but rapidly rebounded, indicating that Northern Hemisphere glaciation ('Icehouse State') did not influence Arethuseae diversification (Westerhold et al. 2020). This suggests that the tropical and subtropical mountainous regions of Asia serve as both cradles and museums for the diversification of Arethuseae. From 5 Ma to the preindustrial period, even as precipitation in East Asia was declining, the MOE showed a secondary acceleration, which may be attributed to the adaptive advantages brought by pseudobulbs, which buffered the impact of Arethuseae on hydroclimate change, especially during the dry season, and maintained diversity during the Neogene–Quaternary transition.

The main limitation of this study is limited taxon sampling, which included approximately 30% of species in Arethuseae. The incomplete sampling of species may affect our estimations in several aspects, such as impacting inferred ancestral states (Brightly et al. 2024; Salisbury and Kim 2001), altering the estimations of species diversification rate over time (Wu et al. 2025) and even the reconstruction of phylogeny. The diversification rates and ancestral state reconstruction should be interpreted with caution.

5 | Conclusion

Using plastid genes and nrITS data, we established a robust phylogenetic framework for the tribe Arethuseae, which reveals novel relationships within the *Coelogyne* complex and proposes revised generic delimitations. Arethuseae originated in the tropical and subtropical monsoon regions of Asia during the middle Oligocene (ca. 28 Ma) and subsequently experienced accelerated diversification during the Oligocene–Miocene (O–M) boundary and the middle Miocene. This diversification coincided with the uplift of the Himalayas, the intensification of the Asian monsoon and the emergence of pseudobulb traits. Our results indicate that environmental drivers and key innovations synergistically promoted the diversification of Arethuseae. This study emphasises how key innovations interact with environmental factors to shape tropical biodiversity and provides a model for testing evolutionary hypotheses in other plant lineages. Furthermore, the Asian tropical–subtropical mountains serve as both a cradle (generating new diversity) and a museum (preserving ancient lineages) for Arethuseae, underscoring their conservation importance.

Author Contributions

X.-H.J. conceived and designed the research. C.-B.M. performed the research. C.-B.M. and X.-H.J. analysed the data. C.-B.M. and X.-H.J. wrote the paper. J.-W.L., H.R., A.H. and X.-H.J. revised the manuscript. All authors read and approved the manuscript.

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Acknowledgements

This work was supported by Chinese Academy of Sciences (International partnership program, Grant. No. 063GJHZ2024075GC; Sustainable Development Research Project, Gant No. CAS-ANSO-SDRP-2024–02; Taxonomy Funds, Grant Nos. CAS-TAX-24–010) and the National Natural Science Foundation of China (32270214 to X.-H.J.).

Conflicts of Interest

The authors declare there are no conflicts of interest.

Data Availability Statement

All Newly Sequenced and Annotated Plastid Genomes and nrITS Generated in This Study Have Been Submitted to NCBI (https://www.ncbi.nlm.nih.gov/) Under Accession Numbers Listed in Tables S1–S3. Upon Acceptance of the Manuscript, all Newly Sequenced Plastid Genome Assemblies and the Corresponding Annotation Data Will Be Made Publicly Available. The Online Resources of Genomic Data (Tables S1–S3) Were Downloaded From NCBI (https://www.ncbi.nlm.nih.gov/).

References

Antonelli, A., W. D. Kissling, S. G. A. Flantua, et al. 2018. "Geological and Climatic Influences on Mountain Biodiversity." *Nature Geoscience* 11, no. 10: 718–725. https://doi.org/10.1038/s41561-018-0236-z.

Badgley, C., T. M. Smiley, R. Terry, et al. 2017. "Biodiversity and Topographic Complexity: Modern and Geohistorical Perspectives." *Trends in Ecology & Evolution* 32, no. 3: 211–226. https://doi.org/10.1016/j.tree.2016.12.010.

Baele, G., P. Lemey, T. Bedford, A. Rambaut, M. A. Suchard, and A. V. Alekseyenko. 2012. "Improving the Accuracy of Demographic and Molecular Clock Model Comparison While Accommodating Phylogenetic Uncertainty." *Molecular Biology and Evolution* 29, no. 9: 2157–2167. https://doi.org/10.1093/molbev/mss084.

Boos, W. R., and Z. Kuang. 2010. "Dominant Control of the South Asian Monsoon by Orographic Insulation Versus Plateau Heating." *Nature* 463, no. 7278: 218–222. https://doi.org/10.1038/nature08707.

Brightly, W. H., A. M. Bedoya, M. M. Carlson, M. G. Rottersman, and C. A. E. Stroemberg. 2024. "Correlated Evolution of Dispersal Traits and Habitat Preference in the Melicgrasses." *American Journal of Botany* 111, no. 10: e16406. https://doi.org/10.1002/ajb2.16406.

Chase, M. W., K. M. Cameron, J. V. Freudenstein, et al. 2015. "An Updated Classification of Orchidaceae." *Botanical Journal of the Linnean Society* 177, no. 2: 151–174. https://doi.org/10.1111/boj.12234.

Chase, M. W., B. Gravendeel, B. P. Sulistyo, R. K. Wati, and A. Schuiteman. 2021. "Expansion of the Orchid Genus Coelogyne (Arethuseae; Epidendroideae) to Include Bracisepalum, Bulleyia, Chelonistele, Dendrochilum, Dickasonia, Entomophobia, Geesinkorchis, Gynoglottis, Ischnogyne, Nabaluia, Neogyna, Otochilus, Panisea and Pholidota." *Phytotaxa* 510, no. 2: 94–134. https://doi.org/10.11646/phytotaxa.510.2.1.

Cribb, P., and I. Butterfield. 1999. *The Genus Pleione*. Natural History Publications.

Dagallier, L.-P. M. J., S. B. Janssens, G. Dauby, et al. 2020. "Cradles and Museums of Generic Plant Diversity Across Tropical Africa." *New Phytologist* 225, no. 5: 2196–2213. https://doi.org/10.1111/nph.16293.

Ding, L., R. A. Spicer, J. Yang, et al. 2017. "Quantifying the Rise of the Himalaya Orogen and Implications for the South Asian Monsoon." *Geology* 45, no. 3: 215–218. https://doi.org/10.1130/g38583.1.

Drummond, A. J., and A. Rambaut. 2007. "BEAST: Bayesian Evolutionary Analysis by Sampling Trees." *BMC Evolutionary Biology* 7: 214. https://doi.org/10.1186/1471-2148-7-214.

Drummond, A. J., A. Rambaut, B. Shapiro, and O. G. Pybus. 2005. "Bayesian Coalescent Inference of Past Population Dynamics From Molecular Sequences." *Molecular Biology and Evolution* 22, no. 5: 1185–1192. https://doi.org/10.1093/molbev/msi103.

Farnsworth, A., D. J. Lunt, S. A. Robinson, et al. 2019. "Past East Asian Monsoon Evolution Controlled by Paleogeography, Not CO_2." *Science Advances* 5, no. 10: eaax1697. https://doi.org/10.1126/sciadv.aax1697.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1994. "Testing Significance of Incongruence." *Cladistics: The International Journal of the Willi Hennig Society* 10, no. 3: 315–319. https://doi.org/10.1111/j.1096-0031.1994.tb00181.x.

Favre, A., I. Michalak, C.-H. Chen, et al. 2016. "Out-Of-Tibet: The Spatio-Temporal Evolution of Gentiana (Gentianaceae)." *Journal of Biogeography* 43, no. 10: 1967–1978. https://doi.org/10.1111/jbi.12840.

Favre, A., M. Paeckert, S. U. Pauls, et al. 2015. "The Role of the Uplift of the Qinghai-Tibetan Plateau for the Evolution of Tibetan Biotas." *Biological Reviews* 90, no. 1: 236–253. https://doi.org/10.1111/brv.12107.

Fick, S. E., and R. J. Hijmans. 2017. "WorldClim 2: New 1-Km Spatial Resolution Climate Surfaces for Global Land Areas." *International Journal of Climatology* 37, no. 12: 4302–4315. https://doi.org/10.1002/joc.5086.

Fjeldsa, J., R. C. K. Bowie, and C. Rahbek. 2012. "The Role of Mountain Ranges in the Diversification of Birds." *Annual Review of Ecology, Evolution, and Systematics* 43: 249–265.

Fritz, S. A., and A. Purvis. 2010. "Selectivity in Mammalian Extinction Risk and Threat Types: A New Measure of Phylogenetic Signal Strength in Binary Traits." *Conservation Biology* 24, no. 4: 1042–1051. https://doi.org/10.1111/j.1523-1739.2010.01455.x.

Goldman, D. H., J. V. Freudenstein, P. J. Kores, et al. 2001. "Phylogenetics of Arethuseae (Orchidaceae) Based on Plastid *matK* and *rbcL* Sequences." *Systematic Botany* 26, no. 3: 670–695. https://doi.org/10.1043/0363-6445-26.3.670.

Gonzalez-Orozco, C. E. 2023. "Unveiling Evolutionary Cradles and Museums of Flowering Plants in a Neotropical Biodiversity Hotspot." *Royal Society Open Science* 10, no. 10: 230917. https://doi.org/10.1098/rsos.230917.

Han, S. W., X. J. Wang, B. S. Cui, et al. 2021. "Hepatoprotective Glucosyloxybenzyl 2-Hydroxy-2-Isobutylsuccinates From *Pleione yunnanensis*." *Journal of Natural Products* 84, no. 3: 738–749. https://doi.org/10.1021/acs.jnatprod.0c01117.

Hansen, J., M. Sato, G. Russell, and P. Kharecha. 2013. "Climate Sensitivity, Sea Level, and Atmospheric Carbon Dioxide." *Philosophical Transactions of the Royal Society A* 371: 20120294. https://doi.org/10.1098/rsta.2012.0294.

He, J. 2018. "Physiological Roles of the Green Pseudobulb in Tropical Epiphytic Orchids." *Advances in Plants & Agriculture Research* 8, no. 1: 00295.

Heard, S. B., and D. L. Hauser. 1995. "Key Evolutionary Innovations and Their Ecological Mechanisms." *Historical Biology* 10, no. 2: 151–173.

Hoorn, C., V. Mosbrugger, A. Mulch, and A. Antonelli. 2013. "Biodiversity From Mountain Building." *Nature Geoscience* 6, no. 3: 154. https://doi.org/10.1038/ngeo1742.

Huang, W. C., Z. J. Liu, K. Jiang, et al. 2022. "Phylogenetic Analysis and Character Evolution of Tribe Arethuseae (Orchidaceae) Reveal a New Genus Mengzia." *Molecular Phylogenetics and Evolution* 167: 107362. https://doi.org/10.1016/j.ympev.2021.107362.

Huang, W. C., Z. W. Wang, N. Wei, et al. 2018. "Gastrodia Elatoides (Orchidaceae: Epidendroideae: Gastrodieae), a New Holomycoheterotrophic Orchid From Madagascar." *Phytotaxa* 349, no. 2: 167–172. https://doi.org/10.11646/phytotaxa.349.2.7.

James, N. A., and D. S. Matteson. 2015. "Ecp: An R Package for Nonparametric Multiple Change Point Analysis of Multivariate Data."

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Journal of Statistical Software 62, no. 7: 1–25. https://doi.org/10.18637/jss.v062.i07.

Jersakova, J., and T. Malinova. 2007. "Spatial Aspects of Seed Dispersal and Seedling Recruitment in Orchids." *New Phytologist* 176, no. 2: 237–241. https://doi.org/10.1111/j.1469-8137.2007.02223.x.

Ji, H. Y., C. Ye, Y. Q. Chen, et al. 2024. "Phylogenomics and Biogeographical Diversification of Collabieae (Orchidaceae) and Its Implication in the Reconstruction of the Dynamic History of Asian Evergreen Broadleaved Forests." *Molecular Phylogenetics and Evolution* 196: 108084. https://doi.org/10.1016/j.ympev.2024.108084.

Jin, J. J., W. B. Yu, J. B. Yang, et al. 2020. "GetOrganelle: A Fast and Versatile Toolkit for Accurate de Novo Assembly of Organelle Genomes." *Genome Biology* 21, no. 1: 241. https://doi.org/10.1186/s13059-020-02154-5.

Kearse, M., R. Moir, A. Wilson, et al. 2012. "Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data." *Bioinformatics* 28, no. 12: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

Kooyman, R. M., R. J. Morley, D. M. Crayn, et al. 2019. "Origins and Assembly of Malesian Rainforests." *Annual Review of Ecology, Evolution, and Systematics* 50: 119–143. https://doi.org/10.1146/annurev-ecolsys-110218-024737.

Lai, Y. J., Y. Han, A. Schuiteman, et al. 2021. "Diversification in Qinghai-Tibet Plateau: Orchidinae (Orchidaceae) Clades Exhibiting Pre-Adaptations Play Critical Role." *Molecular Phylogenetics and Evolution* 157: 107062. https://doi.org/10.1016/j.ympev.2020.107062.

Li, L., D. P. Ye, M. Niu, H. F. Yan, T. L. Wen, and S. J. Li. 2015. "*Thuniopsis*: A New Orchid Genus and Phylogeny of the Tribe Arethuseae (Orchidaceae)." *PLoS One* 10, no. 8: e0132777. https://doi.org/10.1371/journal.pone.0132777.

Li, X. Q., X. G. Xiang, F. Jabbour, et al. 2022. "Biotic Colonization of Subtropical East Asian Caves Through Time." *Proceedings of the National Academy of Sciences of the United States of America* 119, no. 34: e2207199119. https://doi.org/10.1073/pnas.2207199119.

Losos, J. B. 2010. "Adaptive Radiation, Ecological Opportunity, and Evolutionary Determinism." *American Naturalist* 175, no. 6: 623–639. https://doi.org/10.1086/652433.

Meseguer, A. S., A. Michel, P.-H. Fabre, et al. 2022. "Diversification Dynamics in the Neotropics Through Time, Clades, and Biogeographic Regions." *eLife* 11: e74503. https://doi.org/10.7554/eLife.74503.

Miller, A. H., J. T. Stroud, and J. B. Losos. 2023. "The Ecology and Evolution of Key Innovations." *Trends in Ecology & Evolution* 38, no. 2: 122–131. https://doi.org/10.1016/j.tree.2022.09.005.

Morley, R. J. 2018. "Assembly and Division of the South and South-East Asian Flora in Relation to Tectonics and Climate Change." *Journal of Tropical Ecology* 34: 209–234. https://doi.org/10.1017/s0266467418000202.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. "Biodiversity Hotspots for Conservation Priorities." *Nature* 403, no. 6772: 853–858. https://doi.org/10.1038/35002501.

Nguyen, L.-T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. "IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies." *Molecular Biology and Evolution* 32, no. 1: 268–274. https://doi.org/10.1093/molbev/msu300.

Pandit, M. K., K. Manish, S. Sen, et al. 2025. "Evolutionary Triggers of Plant Speciation in the Himalaya-Tibet-Hengduan Biodiversity Epicentre." *Journal of Biogeography* 52: e15152. https://doi.org/10.1111/jbi.15152.

Pansarin, E. R., A. Salatino, and M. L. F. Salatino. 2008. "Phylogeny of South American Pogonieae (Orchidaceae, Vanilloideae) Based on Sequences of Nuclear Ribosomal (ITS) and Chloroplast (psaB, rbcL, rps16, and trnL-F) DNA, With Emphasis on Cleistes and Discussion of Biogeographic Implications." *Organisms Diversity & Evolution* 8, no. 3: 171–181. https://doi.org/10.1016/j.ode.2007.09.003.

Paradis, E., J. Claude, and K. Strimmer. 2004. "APE: Analyses of Phylogenetics and Evolution in R Language." *Bioinformatics* 20, no. 2: 289–290. https://doi.org/10.1093/bioinformatics/btg412.

Patel, R. K., and M. Jain. 2012. "NGS QC Toolkit: A Toolkit for Quality Control of Next Generation Sequencing Data." *PLoS One* 7, no. 2: e30619. https://doi.org/10.1371/journal.pone.0030619.

Perrigo, A., C. Hoorn, and A. Antonelli. 2020. "Why Mountains Matter for Biodiversity." *Journal of Biogeography* 47, no. 2: 315–325. https://doi.org/10.1111/jbi.13731.

Phillips, S. J., R. P. Anderson, M. Dudík, R. E. Schapire, and M. E. Blair. 2017. "Opening the Black Box: An Open-Source Release of Maxent." *Ecography* 40, no. 7: 887–893. https://doi.org/10.1111/ecog.03049.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. "Maximum Entropy Modeling of Species Geographic Distributions." *Ecological Modelling* 190, no. 3: 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026.

Pridgeon, A. M., P. J. Cribb, M. W. Chase, and F. N. Rasmussen. 2005. *Genera Orchidacearum Volume 4: Epidendroideae (Part 1)*. Oxford University Press.

Pridgeon, A. M., P. J. Cribb, M. W. Chase, and F. N. Rasmussen. 2014. *Genera Orchidacearum Volume 6: Epidendoideae (Part Three)*. Oxford University Press.

Rabosky, D. L., M. Grundler, C. Anderson, et al. 2014. "BAMMtools: An R Package for the Analysis of Evolutionary Dynamics on Phylogenetic Trees." *Methods in Ecology and Evolution* 5, no. 7: 701–707. https://doi.org/10.1111/2041-210X.12199.

Rahbek, C., M. K. Borregaard, A. Antonelli, et al. 2019. "Building Mountain Biodiversity: Geological and Evolutionary Processes." *Science* 365, no. 6458: 1114–1119. https://doi.org/10.1126/science.aax0151.

Rahbek, C., M. K. Borregaard, R. K. Colwell, et al. 2019. "Humboldt's Enigma: What Causes Global Patterns of Mountain Biodiversity?" *Science* 365, no. 6458: 1108–1113. https://doi.org/10.1126/science.aax0149.

Rambaut, A., A. J. Drummond, D. Xie, G. Baele, and M. A. Suchard. 2018. "Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7." *Systematic Biology* 67, no. 5: 901–904.

Renner, S. S. 2016. "Available Data Point to a 4-Km-High Tibetan Plateau by 40 Ma, but 100 Molecular-Clock Papers Have Linked Supposed Recent Uplift to Young Node Ages." *Journal of Biogeography* 43, no. 8: 1479–1487. https://doi.org/10.1111/jbi.12755.

Revell, L. J. 2012. "Phytools: An R Package for Phylogenetic Comparative Biology (And Other Things)." *Methods in Ecology and Evolution* 3, no. 2: 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x.

Ronquist, F., M. Teslenko, P. van der Mark, et al. 2012. "MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space." *Systematic Biology* 61, no. 3: 539–542. https://doi.org/10.1093/sysbio/sys029.

Salisbury, B. A., and J. H. Kim. 2001. "Ancestral State Estimation and Taxon Sampling Density." *Systematic Biology* 50, no. 4: 557–564. https://doi.org/10.1080/106351501750435103.

Shimodaira, H., and M. Hasegawa. 1999. "Multiple Comparisons of Log-Likelihoods With Applications to Phylogenetic Inference." *Molecular Biology and Evolution* 16, no. 8: 1114–1116. https://doi.org/10.1093/oxfordjournals.molbev.a026201.

Simpson, G. G. 1953. *The Major Features of Evolution*. Columbia Univ. Press.

Spehn, E. M., K. Rudmann-Maurer, and C. Körner. 2011. "Mountain Biodiversity." *Plant Ecology and Diversity* 4, no. 4: 301–302. https://doi.org/10.1080/17550874.2012.698660.

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Spicer, R. A. 2017. "Tibet, the Himalaya, Asian Monsoons and Biodiversity-In What Ways Are They Related?" *Plant Diversity* 39: 233–244. https://doi.org/10.1016/j.pld.2017.09.001.

Stamatakis, A. 2014. "RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies." *Bioinformatics* 30, no. 9: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Sympli, H. D. 2021. "Estimation of Drug-Likeness Properties of GC-MS Separated Bioactive Compounds in Rare Medicinal Pleione Maculata Using Molecular Docking Technique and SwissADME in Silico Tools." *Network Modeling Analysis in Health Informatics and Bioinformatics* 10, no. 1: 14. https://doi.org/10.1007/s13721-020-00276-1.

Tada, R., H. Zheng, and P. D. Clift. 2016. "Evolution and Variability of the Asian Monsoon and Its Potential Linkage With Uplift of the Himalaya and Tibetan Plateau." *Progress in Earth and Planetary Science* 3: 4. https://doi.org/10.1186/s40645-016-0080-y.

Talavera, G., and J. Castresana. 2007. "Improvement of Phylogenies After Removing Divergent and Ambiguously Aligned Blocks From Protein Sequence Alignments." *Systematic Biology* 56, no. 4: 564–577. https://doi.org/10.1080/10635150701472164.

Toussaint, E. F. A., R. Hall, M. T. Monaghan, et al. 2014. "The Towering Orogeny of New Guinea as a Trigger for Arthropod Megadiversity." *Nature Communications* 5: 4001. https://doi.org/10.1038/ncomms5001.

Trethowan, L. A., B. E. Walker, S. P. Bachman, et al. 2023. "Plant Species Biogeographic Origin Shapes Their Current and Future Distribution on the World's Highest Island Mountain." *Journal of Ecology* 111, no. 2: 372–379. https://doi.org/10.1111/1365-2745.14022.

Valen, L. V. 1971. "Adaptive Zones and the Orders of Mammals." *Evolution* 25, no. 2: 420–428. https://doi.org/10.1111/j.1558-5646.1971.tb01898.x.

van den Berg, C., D. H. Goldman, J. V. Freudenstein, A. M. Pridgeon, K. M. Cameron, and M. W. Chase. 2005. "An Overview of the Phylogenetic Relationships Within Epidendroideae Inferred From Multiple DNA Regions and Recircumscription of Epidendreae and Arethuseae (Orchidaceae)." *American Journal of Botany* 92, no. 4: 613–624. https://doi.org/10.3732/ajb.92.4.613.

Wahlert, G. 1965. "The Role of Ecological Factors in the Origin of Higher Levels of Organization." *Systematic Biology* 14, no. 4: 288–300. https://doi.org/10.2307/sysbio/14.4.288.

Wang, L., H. Schneider, X.-C. Zhang, and Q.-P. Xiang. 2012. "The Rise of the Himalaya Enforced the Diversification of SE Asian Ferns by Altering the Monsoon Regimes." *BMC Plant Biology* 12: 210. https://doi.org/10.1186/1471-2229-12-210.

Wang, L. G., T. T. Lam, S. Xu, et al. 2020. "Treeio: An R Package for Phylogenetic Tree Input and Output With Richly Annotated and Associated Data." *Molecular Biology and Evolution* 37, no. 2: 599–603. https://doi.org/10.1093/molbev/msz240.

Wati, R. K., E. F. de Graaf, D. Bogarin, et al. 2021. "Antimicrobial Activity of Necklace Orchids Is Phylogenetically Clustered and Can Be Predicted With a Biological Response Method." *Frontiers in Pharmacology* 11: 586345. https://doi.org/10.3389/fphar.2020.586345.

Westerhold, T., N. Marwan, A. J. Drury, et al. 2020. "An Astronomically Dated Record of Earth's Climate and Its Predictability Over the Last 66 Million Years." *Science* 369, no. 6509: 1383–1387. https://doi.org/10.1126/science.aba6853.

Wick, R. R., M. B. Schultz, J. Zobel, and K. E. Holt. 2015. "Bandage: Interactive Visualization of de Novo Genome Assemblies." *Bioinformatics (Oxford, England)* 31, no. 20: 3350–3352. https://doi.org/10.1093/bioinformatics/btv383.

Wilson, M. E. J., and S. J. Moss. 1999. "Cenozoic Palaeogeographic Evolution of Sulawesi and Borneo." *Palaeogeography Palaeoclimatology Palaeoecology* 145, no. 4: 303–337. https://doi.org/10.1016/s0031-0182(98)00127-8.

Wood, J. J. 2001. *Dendrochilum of Borneo*. Natural History Publications.

Wu, G., Q. Ye, H. Liu, et al. 2025. "Shaded Habitats Drive Higher Rates of Fern Diversification." *Journal of Ecology* 113, no. 5: 1200–1208. https://doi.org/10.1111/1365-2745.70026.

Yu, Y., C. Blair, and X. He. 2020. "RASP 4: Ancestral State Reconstruction Tool for Multiple Genes and Characters." *Molecular Biology and Evolution* 32, no. 2: 604–606.

Zhang, D., F. Gao, I. Jakovlic, et al. 2020. "PhyloSuite: An Integrated and Scalable Desktop Platform for Streamlined Molecular Sequence Data Management and Evolutionary Phylogenetics Studies." *Molecular Ecology Resources* 20, no. 1: 348–355. https://doi.org/10.1111/1755-0998.13096.

Zhang, G., Y. Hu, M. Z. Huang, et al. 2023. "Comprehensive Phylogenetic Analyses of Orchidaceae Using Nuclear Genes and Evolutionary Insights Into Epiphytism." *Journal of Integrative Plant Biology* 65, no. 5: 1204–1225. https://doi.org/10.1111/jipb.13462.

Zhang, Q. Q., D. K. Ferguson, V. Mosbrugger, Y. F. Wang, and C. S. Li. 2012. "Vegetation and Climatic Changes of SW China in Response to the Uplift of Tibetan Plateau." *Palaeogeography Palaeoclimatology Palaeoecology* 363: 23–36. https://doi.org/10.1016/j.palaeo.2012.08.009.

Zhang, W., J. Qin, R. Yang, Y. Yang, and S.-B. Zhang. 2018. "Two New Natural Hybrids in the Genus Pleione (Orchidaceae) From China." *Phytotaxa* 350, no. 3: 247–258. https://doi.org/10.11646/phytotaxa.350.3.4.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

13652699, 2025, 10, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbi.70018 by Xishuangbanna Tropical Botanical Garden, Wiley Online Library on [11/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License