Molecular Phylogenetics and Evolution 157 (2021) 107062

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Diversification in Qinghai-Tibet Plateau: Orchidinae (Orchidaceae) clades exhibiting pre-adaptations play critical role

Yang-Jun Lai [a,1], Yu Han [a,1], Andre Schuiteman [b], Mark W. Chase [c,d], Song-Zhi Xu [a], Jian-Wu Li [e], Jian-Yong Wu [f], Bo-Yun Yang [g,*], Xiao-Hua Jin [a,*]

[a] *State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 10093, China*
[b] *Identification and Naming Department, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK*
[c] *Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK*
[d] *Department of Environment and Agriculture, Curtin University, Bentley, Western Australia 6102, Australia*
[e] *Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun Township, Mengla County, Yunnan 666303, China*
[f] *Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment (MEE), China*
[g] *School of Life Sciences, Nanchang University, Nanchang 330031, China*

ARTICLE INFO

Keywords:
Dispersal
Diversification
Habenaria
Herminium
Orchidoideae
Pre-adaptation
Platanthera

ABSTRACT

We explore the origins of the extraordinary plant diversity in the Qinghai-Tibetan Plateau (QTP) using Orchidinae (Orchidaceae) as a model. Our results indicate that six major clades in Orchidinae exhibited substantial variation in the temporal and spatial sequence of diversification. Our time-calibrated phylogenetic model suggests that the species-richness of Orchidinae arose through a combination of *in situ* diversification, colonisation, and local recruitment. There are multiple origins of species-richness of Orchidinae in the QTP, and pre-adaptations in clades from North Temperate and alpine regions were crucial for *in situ* diversification. The geographic analysis identified 29 dispersals from Asia, Africa and Europe into the QTP and 15 dispersals out. Most endemic species of Orchidinae evolved within the past six million years.

1. Introduction

It is well known that higher diversity than would be expected from chance alone occurs within large complex montane regions, especially the Andes, the Cape Fold Mountains, New Guinea, and the Qinghai-Tibet Plateau (Favre et al., 2015; Myers et al., 2000; Perez-Escobar et al., 2017; Schwery et al., 2015; Vollering et al., 2016). Previous studies indicated that much of this diversity evolved in recent, rapid bursts of diversification, although the origin and processes that generate and maintain diversity remain largely unresolved (Badgley et al., 2017; Hughes and Atchison, 2015; Wen et al., 2014). Studies of these high-diversity areas have led to two contrasting hypotheses to explain diversity in mountain areas: (a) the pre-adaptation hypothesis suggests that niche conservatism and pre-adaptation play an over-arching role in the origin of diversity, for example, in the extraordinary species diversity in tropical Asian Mt. Kinabalu (Merckx et al., 2015); whereas (b) the niche evolution hypothesis suggests that diversification involves substantial niche shifts, for example, establishment of *Hypericum* in the Andean Paramos (Nuerk et al., 2018) and diversification of North American Cyperaceae (Spalink et al., 2016).

The Qinghai-Tibetan Plateau s.l. (QTP, here advocated), including part of the Himalayas, Tibetan Plateau, Karakorum Mountains, Hengduan Mountains, and surrounding mountainous regions, is the highest and most extensive plateau in the world, with a mean elevation exceeding 4500 m and a surface area of 2.5 million km^2 (Zhang et al., 2002; Zhang et al., 2016). Although the dimensions and timescales of uplifts within the QTP remain unclear (Deng and Ding, 2015; Fang et al., 2017; Su et al., 2019), multiple phased uplifts have been proposed (Deng and Ding, 2015; Fang, 2017; Favre et al., 2015). The QTP is noteworthy for its geographical and ecological heterogeneity. The QTP is largely alpine meadow, but the eastern, southeastern, and southern border regions exhibit zones from tropical evergreen forest or tropical seasonal forest to alpine meadows (Ni and Herzschuh, 2011; The Comprehensive Investigation Team of Tibetan Plateau, 1988; Zhang et al., 1982). In contrast to tropical mountains, such as geographically isolated Mt. Kinabalu and the Andean Páramos, huge mountain ranges and rivers

---

* Corresponding authors.
E-mail addresses: ljw@xtbg.org.cn (J.-W. Li), yangboyun@163.com (B.-Y. Yang), xiaohuajin@ibcas.ac.cn (X.-H. Jin).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ympev.2020.107062
Received 7 December 2019; Received in revised form 22 December 2020; Accepted 24 December 2020

extend from the QTP into the different climatic zones of Asia, especially the temperate regions (Zhang et al., 1982). The QTP is famous for its extraordinary species richness. It has over 12,000 species (ca. 1500 genera) of vascular plants (Li and Li, 1993; Wu et al., 1995; Wu, 2008) and is characterized by the rapid diversification of many temperate and alpine groups (Hughes et al., 2015; Wen et al., 2014; Xing and Ree, 2017, Ding et al., 2020). Based on floristic analyses, Wu (1987) proposed that QTP species richness was derived from rapid species diversification from tropical ancestors. The alternative is that pre-adapted temperate (or alpine) clades played the predominant role in species diversification in the QTP. Here we use the subtribe Orchidinae, a group rich in QTP endemics, to test these alternative hypotheses. In addition, we want to identify some of the potential abiotic and biotic drivers of Orchidinae diversification.

Orchidinae s.l. (Orchidaceae) comprise ~1800 species in about 50 genera and is the most species-rich orchid clade in the QTP (Chen et al., 2009; Pearce and Cribb, 2002). Approximately 180 species in 12 genera in Orchidinae occur in the QTP, including ca. 120 endemic species. Two genera, *Herminium* (50-60 species) and *Hsenhsua* (1 species), are alpine specialists, the latter endemic to the QTP whereas the former has only a few species outside the QTP (Jin et al., 2014; Raskoti et al., 2017). Two thirds of *Ponerorchis* s.l. (about 60 species) are endemic in the alpine region of the QTP, whereas the remaining species of this genus are found in subtropical and alpine region outside the QTP. *Habenaria*, the largest genus of Orchidinae with approximately 800 species, is mainly pantropical, but there are about 20 species endemic in the QTP alpine region. *Platanthera* (about 100 species) is widespread in Europe, Asia, northern America, Hawaii and Azores, with approximately 40 species endemic in the QTP.

Four types of underground vegetative organs (rhizomes, globular or finger-like tubers, and tuberous roots, Fig. S1) that allow these species to survive dry and/or cold seasons (Pridgeon et al., 2001) occur in QTP Orchidinae. For example, tuberous roots (i.e., tubers distally tapering to a root) occur in *Platanthera*, rhizomes in *Galearis* and finger-like tubers in *Dactylorhiza* and *Gymnadenia* (Pridgeon et al., 2001). In contrast, another subtribe within subfamily Orchidoideae, namely subtribe Goodyerinae, is species-rich in tropical and subtropical regions just south of the QTP, but they have diversified little in the QTP. Members of this subtribe do not show such clear adaptations for cold and drought like those of Orchidinae. Because Orchidinae occur both in tropical and temperate regions, they make a good model group to understand the origins of species diversity in the QTP.

2. Materials and methods

2.1. Taxon sampling and phylogenetic analysis

In total, 368 species in 46 genera were sampled (Table S1) to reconstruct a robust phylogenetic tree for subtribe Orchidinae. We included 164 species of Orchidinae from QTP, among which 103 species are endemic to the QTP; the remaining 61 species occur in the QTP plus adjacent regions in Eurasia. In order to reduce issues with long branches in phylogenetic and biogeographical analyses, 184 species that are not distributed in the QTP were used as placeholders, including 57 from Asia, 15 from Europe, 44 species from Africa, 8 from North America, 17 from South America, and 53 from two or more continents (Table S1). Most genera in Orchidinae with available nucleotide data in National Center for Biotechnology Information (NCBI), such as *Anacamptis*, *Himantoglossum*, *Neotinea,* and *Pseudorchis*, were included in our analyses (Table S1). Based on previous results (Jin et al. 2014, 2017), 18 species from tribes Cranichideae and Diurideae, representing the geographical distribution of these tribes, were used as outgroups. Seven markers comprising two nuclear (ribosomal internal spacer, nrITS, and xanthine dehydrogenase, *Xdh*) and five plastid markers (*matK*, *psaB*, *psbA-trnH*, *rbcL,* and *trnL-F*) were employed. In total, 1805 sequences were included (all available in NCBI; Table S1).

Sequences were aligned using MAFFT v. 7.221 (Katoh and Standley, 2013) with the auto strategy and default settings. Aligned sequences were then manually adjusted using BioEdit v. 7.25 (Hall, 1999). Ambiguously aligned characters in the *psbA-trnH* and *trnL-F* datasets (both are spacers/introns) were excluded from phylogenetic analyses. The combinability in terms of heterogeneity between nuclear (nrITS and *Xdh*) and plastid (*matK*, *psaB*, *psbA-trnH*, *rbcL* and *trnL-F*) datasets was tested using incongruence length difference (ILD) (Farris et al., 1995) implemented in PAUP v4.0b10 (Swofford, 2003). For the ILD test we used 100 replicates with random addition sequences and tree-bisection-reconnection (TBR) swapping and no more than 15 trees with a score (length) ≥ 5 were saved for each replicate. Heterogeneity between datasets was also assessed by comparing topologies and support produced in the three independent data partitions (plastid and the two nuclear markers). The thresholds for hard incongruence of cladograms were as described in Pelser et al. (2010). These tests showed that for five species, the topologies for nrITS on the one hand and plastid, *Xdh* and plastid + *Xdh* on the other were possibly incongruent. The incongruence disappeared when the nrITS data for these five conflicting taxa were eliminated from the combined matrix. After removal of the five incongruent nrITS sequences, we combined the nuclear and plastid datasets in SequenceMatrix v1.7.8 (Vaidya et al., 2011) to perform further phylogenetic analyses.

Platanthera and *Herminium* have the most QTP endemic species. Our analyses indicate that the topology of *Platanthera* was unresolved (Fig. S2), and the results of molecular dating based on it were not reliable (results not shown here). In order to infer a relatively robust tree of *Platanthera* for use in dating, molecular trees for Orchidinae were inferred in two steps following supertree methods (Gordon 1986; Bininda-Emonds, 2004; Givnish et al. 2016). Firstly, matrix I (368 species in 46 genera) was used for molecular dating and biogeographic and diversification rate analyses, even though relationships within *Platanthera* were not well resolved. We then created matrix II (*Platanthera* with five *Herminium* species as outgroup) to infer the *Platanthera* tree and used it in subsequent divergence time estimation. The time-calibrated tree for *Platanthera* was then grafted onto the Orchidinae supertree for the ancestral areas, diversification and trait-dependent analyses (following methods of Givnish et al. 2016). Phylogenetic analyses were performed for each matrix using maximum likelihood (ML) and Bayesian inference (BI). The best fitting evolutionary models for each data partition were determined using jModelTest v.2.1.10 (Darriba et al., 2012) relying on a likelihood ratio test (LRT) and the Akaike information criterion (AIC) (Table S2). ML approach was implemented in RAxML-HPC2 v8.2.10 (Stamatakis, 2014) and BI was inferred with MrBayes v3.2.3 (Ronquist et al., 2012), both executed on the CIPRES Science Gateway computing facility (Miller et al., 2010). Two separate, four Markov chain Monte Carlo (MCMC) analyses were performed, starting with a random tree, proceeding for 10 million generations and sampling every 1000th generations. Trees and PPs were created after discarding the burn-in phase converged (the first 25% of sampled trees).

2.2. Divergence time estimation

All three undoubtedly, unambiguous orchid macrofossils (*Dendrobium winikaphyllum*, *Earina fouldenensis,* and *Meliorchis caribea*) (Conran et al., 2009; Ramirez et al., 2007) are in clades distantly related to Orchidinae. To reduce the bias of calibration by using distantly related taxa, we also applied secondary calibrations (Shaul and Graur, 2002; Hipsley and Mueller, 2014; Xiang et al., 2016, 2017; Ehl et al., 2019). Using two loci (*matK* and *rbcL*), we generated a fossil-calibrated tree with 203 orchid species evenly sampled along the tree based on the previous matrix and results (Xiang et al., 2016).

Three fossil calibration points were used for the crown clades: (1) Goodyerinae (Orchidoideae and Orchidaceae; offset = 15, mean = 1.0, sigma = 1.25) (Ramirez et al., 2007); (2) *Dendrobium* (offset = 23.2, mean = 1.0, sigma = 1.25) (Conran et al., 2009); and (3)

Agrostophyllinae (offset = 23.2, mean = 1.0, sigma = 1.25) (Conran et al., 2009). Prior probabilities were also placed on the node at which Orchidaceae split from other monocots (offset = 120 Ma, sigma = 1.0) based on previous results (Givnish et al., 2015) (Fig. S2).

We conducted a likelihood ratio (Felsenstein, 1988) and χ^2 test to determine the suitable models. Considering that rate constancy along all branches of the tree was rejected ($\delta = 6234.84$, d.f. = 353, $P < 0.0001$), we chose the Bayesian relaxed clock methodology. Divergence times for Orchidaceae and subtribe Orchidinae were estimated using BEAST v2.3.0 (Bouckaert et al., 2014) with an uncorrelated lognormal relaxed clock on the CIPRES Science Gateway (Miller et al., 2010). The substitution model was the same as that used in the BI analysis. The best fitting tree speciation model was selected using Bayes factors calculated by comparing the Yule, birth death and birth–death sampling models in Tracer v1.4.1 (Rambaut et al., 2014) (Tables S3 and S4). Two independent analyses of 100 million generations were performed, each sampling every 10,000 trees. Log files of all runs were combined with LogCombiner v1.8.0 (in BEAST package) after discarding 25% of samples as burn-in for each run, and convergence of chains was obtained when the effective sample size (ESS) was greater than 200 according to Tracer v1.6 (Rambaut et al., 2014). A maximum clade credibility (MCC) tree was constructed in TreeAnnotator v1.8.0 (in the BEAST package).

2.3. *Ancestral area reconstruction*

To infer the geographic origins of QTP Orchidinae, we coded six biogeographical areas employing the distribution records of the *World Checklist of Selected Plant Families* (WCSP, 2015) and eMonocot (http://e-monocot.org/): (A) Africa, (B) Asia, (C) Europe, (D) North America (including Greenland), (E) the Americas (including the Caribbean areas and Central America, but not North America) and (F) Australasia (Australia, New Zealand, New Guinea and neighbouring islands in the Pacific Ocean). To investigate biological interchange between the QTP and adjacent regions, we coded three areas: QTP (Q), Asia (apart from QTP) (P), and outside of Asia (O).

To estimate ancestral areas in Orchidinae, we used the R package BioGeoBEARS (Matzke, 2013). Considering the recent criticism of DEC + J parameter in the estimation of likelihoods of historical biogeography (Ree and Sanmartín, 2018), we compared the three biogeographical estimation models: dispersal-extinction-cladogenesis (DEC) model (Ree et al., 2005), dispersal-vicariance analysis (DIVA) (Ronquist, 1997) and BayArea model (Landis et al., 2013). The best fit model was tested by the Akaike information criterion (Table S5).

2.4. *Diversification and trait-dependent analyses*

To determine whether Orchidinae diversity in the QTP has been derived from pre-adapted clades, vegetation types/ecoregions, elevations are used as surrogates for the climatic zone inhabited by each species. We followed the classification of major QTP ecoregions proposed previously (Ni and Herzschuh, 2011; The Comprehensive Investigation Team of Tibetan Plateau, 1988; Zhang et al., 1982). Forest or grassland areas occurring at elevations higher than 2900 m in the QTP were considered to be alpine conifer forest or grassland, forests at 2300–2900 m were considered a mix of conifer and deciduous broadleaf forests, 1100–2299 m was considered subtropical evergreen forest and lower than 1099 m wet tropical forest. For ecoregions of other parts of the world, we followed the classification of Olson et al (2001). Elevation was treated as continuous using distribution ranges. We obtained ecoregion information and elevations for every taxon sampled in our tree from herbarium records (E, K, KATH, PE, KUN), fieldwork, existing literature (such as Chen et al., 2009; Pearce and Cribb, 2002), and online data (Global Biodiversity Information Facility, gbif.org; Internet Orchid Species Photo Encyclopedia, http://www.orchidspecies.com/). To account for biotic variables as drivers of QTP orchid diversification, one potential variable, underground organ, was compiled. Detailed collection data are included in Appendix S1.

The state-dependent speciation and extinction (SSE) branching process was used to assess if the states of character are associated with different rates of speciation or extinction. Types of underground organs (Table S1) were compiled mainly based on specimens, fieldwork and the literature (Chen et al., 2009; Pearce and Cribb, 2002).

To estimate rates of speciation (λ), extinction (μ) and state transformations associated with underground organs, we used the binary state speciation and extinction model (BiSSE, (Maddison et al., 2007)); to illustrate diversification rate patterns in different regions and vegetation classes (as proxy for climate zone), we utilized the geographic state speciation and extinction model (GeoSSE, (Goldberg et al., 2011)); and to investigate relationships between elevations and diversification in the Orchidinae, we utilised the quantitative state speciation and extinction model (GeoSSE, (FitzJohn, 2010)). All analyses were implemented in the R program diversitree (v.0.9–8) (FitzJohn, 2012), and models were compared using the Akaike information criterion (AIC).

For the BiSSE analysis, we tested eight models, including the full model, in which all six parameters were estimated, and constrained models, in which we tested the effects of a given trait on specific parameters (Table S7). We accounted for random taxon sampling by utilizing the skeletal tree approach (FitzJohn et al., 2009). Sampling schemes were based on the proportion of sampled taxa (Table S1). Models were compared using a likelihood ratio test. The best model was then used to estimate parameters using the MCMC approach implemented in diversitree. We parameterized the selected model with an exponential prior probability $1/(2r)$, where r is the character-independent diversification rate as estimated from the BiSSE-ML searches. This prior probability was used in the estimation of each parameter, the chain was run for 100,000 generations and the first 25% of trees were discarded as burn-in.

GeoSSE expands upon the BiSSE binary model by incorporating a third, polymorphic state for geographic characteristics, since taxa are often present in more than one region (Goldberg et al., 2011). We estimated ML parameters for five models (Table S8). Models comparing and parameters selection were following the BiSSE analysis. We assigned each species to QTP, outside the QTP or both. We classified the states of vegetation as alpine + montane, tropical + subtropical, and temperate.

For QuaSSE analysis, we constructed seven models for relationships between elevation and speciation (λ): (a) λ is constant and independent of the index; (b) a linear relationship; (c) a sigmoid one; (d) a unimodal relationship represented by a vertically offset Gaussian function; and another three models: (e) linear, (f) sigmoid, and (g) unimodal with a directional tendency (Table S8). QuaSSE models were implemented in diversitree. We compared models using the Akaike information criterion (AIC).

To estimate the rates of speciation, extinction, and diversification independently of geography in Orchidinae and specific clades within the subtribe we used BAMM (Bayesian analyses of macroevolutionary mixtures) v.2.4 and R package ‘BAMMtools’ (Rabosky et al., 2014). The sampling fraction was specified for each genus (Table S2) to account for non-random incomplete taxon sampling in this analysis. Priors for speciation and extinction were set with the setBAMMpriors function under R package ‘BAMMtools’. Each BAMM Markov chain Monte Carlo analysis was run for 5 million generations and sampled every 500 generations. After plotting the likelihoods trace, the first 25% of these were discarded as the burn-in, and the ESS for likelihood was calculated to assess convergence. Plots of phylorate and rate over time were generated to visualize rate distribution patterns along clades and trends over time. Outgroups were excluded in all diversification analyses.

To determine whether the diversification rates associated with vegetation types/ecoregions, biogeographic regions and vegetation classes detected in BiSSE and GeoSSE could be artefacts because of Type I errors biases in SSE models (Rabosky et al., 2014), we followed Perez-Escobar et al. (2017) to simulate neutral characters by randomly reshuffling the coding onto the phylogenetic tree. We carried out 1000 iterations, estimating the AICc of the best model for each iteration to get the AICc null distribution and compare it with AIC model value (ΔAIC) of our real dataset (Fig. S8). The simulation analyses without QuaSSE due to the elevation were treated as quantitative traits.

3. Results

3.1. Phylogenetics of Orchidinae

These analyses (Fig. S2) agree with previous results demonstrating that Orchidinae sensu Chase et al.(2015) are monophyletic and subdivided into three main clades: *Satyrium*, Orchidinae s.s. and the formerly recognised Habenariinae (see (Jin et al., 2017). Orchidinae s.s. include two superclades (I and II) with *Schizochilus* and *Brachycorythis* as their successive sister clades. Habenariinae include four major superclades and shows unresolved positions for five clades. Superclade III comprises the genera *Herminium* and *Hsenhsua*, whereas IV includes *Pecteilis* and ~50 species of *Habenaria*. Superclade V includes *Peristylus* sister to superclades III plus IV with weak support.

3.2. Divergence times and biogeography of Orchidinae in the QTP

The results of divergence time estimation with three models were consistent, and we compared the 95% highest posterior density (HPD)

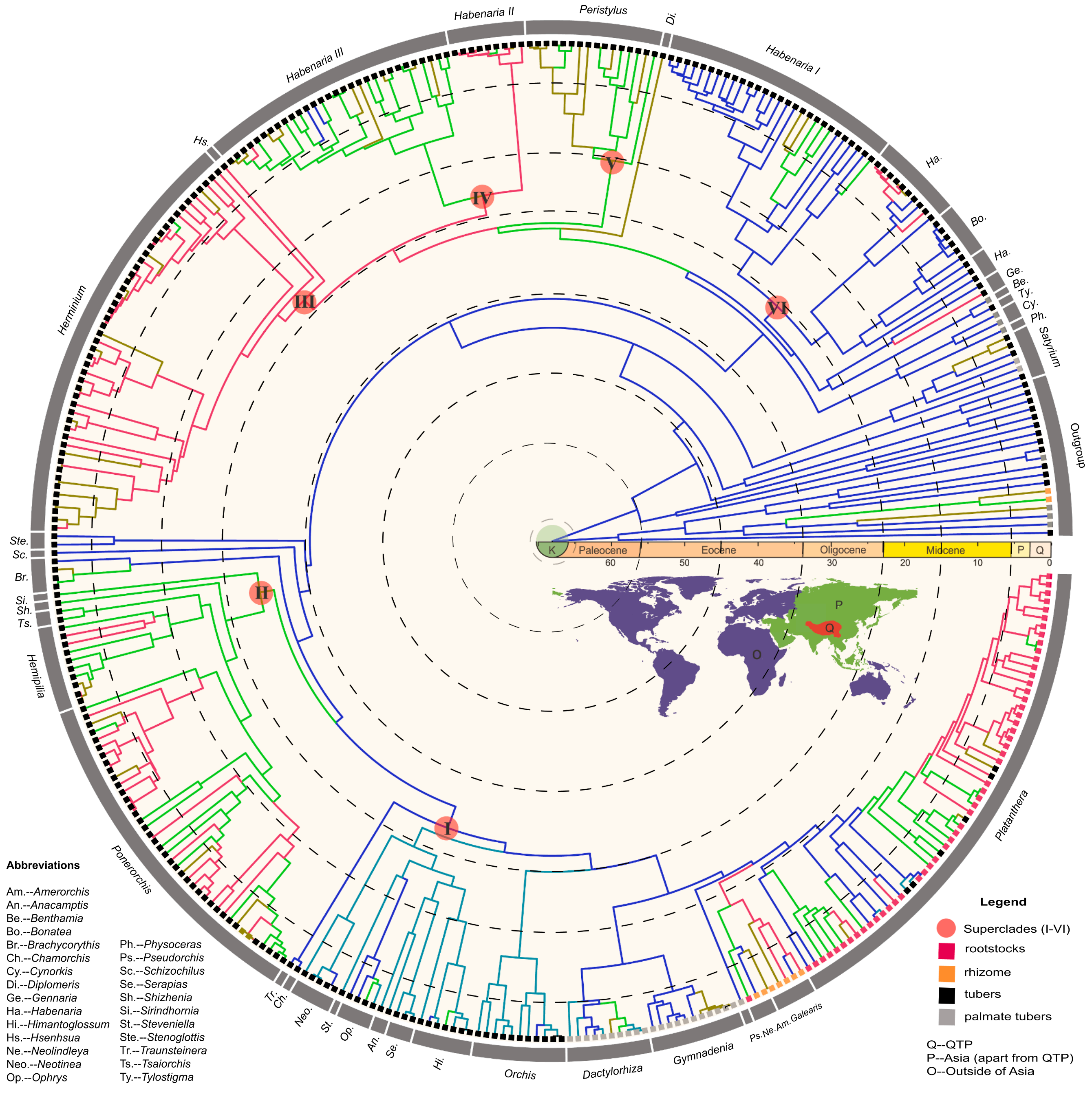

Fig. 1. Chronogram and ancestral area reconstruction of biogeographical patterns in QTP Orchidinae. Map showing three biogeographical regions in colours as defined. Terminal squares on tips indicate four types of underground vegetative organs (rhizomes, globular or palmate tubers, and tuberous roots). Dark and light grey arcs represent different clades. The six superclades are shown as orange circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

values of the Beast analysis for the clades of interest between Yule and birth–death models (Table S3). The best fitting model for biogeographical analyses was BAYAREALIKE (Table S5). The results from these analyses indicate that Orchidinae split from their sister group at 38.0 (32.4–43.3) Ma (Fig. 1; Figs. S3 and S4). Orchidinae s.l. originated in Africa around 33.9 (28.7–39.0) Ma, and 28 dispersals into the QTP and 15 dispersals from the QTP were detected (Figs. S5 and S6). Four genera, *Habenaria*, *Herminium, Platanthera,* and *Ponerorchis*, underwent *in situ* diversification in the QTP (Fig. 1).

Orchidinae s.s. dispersed from Africa into Asia and diverged into two clades, superclades I and II, at 26.69 (22.4–31.1) Ma (Fig. 1; Figs. S3–S6). QTP elements of superclade I were members of *Platanthera* and *Galearis*, which split 15.1 (11.3–17.9) Ma. *Galearis* reached the QTP once ~11.0 Ma. *Platanthera* originated in temperate regions of northeastern Asia at 14.0 (11.3–16.6) Ma and then dispersed into QTP three times during the Late Miocene–Early Pliocene. One *in situ* diversification and two dispersals from the QTP were detected. All QTP elements of superclade II originated in subtropical Asia and reached the QTP at least five times from the Mid-Miocene to the Pleistocene and experienced four *in situ* diversification events.

Superclades III (including *Herminium* and *Hsenhua*) and IV (including *Habenaria* p.p. and *Pecteilis*) diverged at 21.4 (17.2–25.8) Ma. *Herminium* and *Hsenhsua* originated in the QTP at 18.1 (14.2–22.1) Ma and are almost endemic in the QTP, with at least eight dispersals out of the QTP (Fig. 1; Figs. S3–S6). Superclade IV produced two smaller clades, one diversifying *in situ* in the QTP around 2.2 Ma, and the other in tropical and subtropical Asian areas adjacent to the QTP, but some species dispersed to the QTP only recently. *Peristylus* originated in tropical Asia around 13.4 (8.6–17.9) Ma with five dispersals to the QTP. *Habenaria* s.l. originated and diversified *in situ* in Africa 20.6 (16.6–24.7) Ma and dispersed to the QTP at least twice.

In total, 73.8% of all sampled QTP species (121 species) originated in the QTP, whereas 26.2% (43 species) dispersed to the QTP. In addition, 18 species originated in the QTP and then dispersed to surrounding areas of Asia, some reaching even Europe and Africa. Among 103 QTP endemics, 88.5% (92 species) underwent diversification *in situ*. The estimated median stem-node ages of 103 endemic species span a wide range, 0.2–20.0 million years (Fig. 1). Including endemic species of *Habenaria*, *Herminium*, *Platanthera,* and *Ponerorchis*, 85 species (83%) originated within the last six million years.

GeoSSE modelling shows that species distributed in the QTP experienced a higher speciation rate than those in other areas with an equal

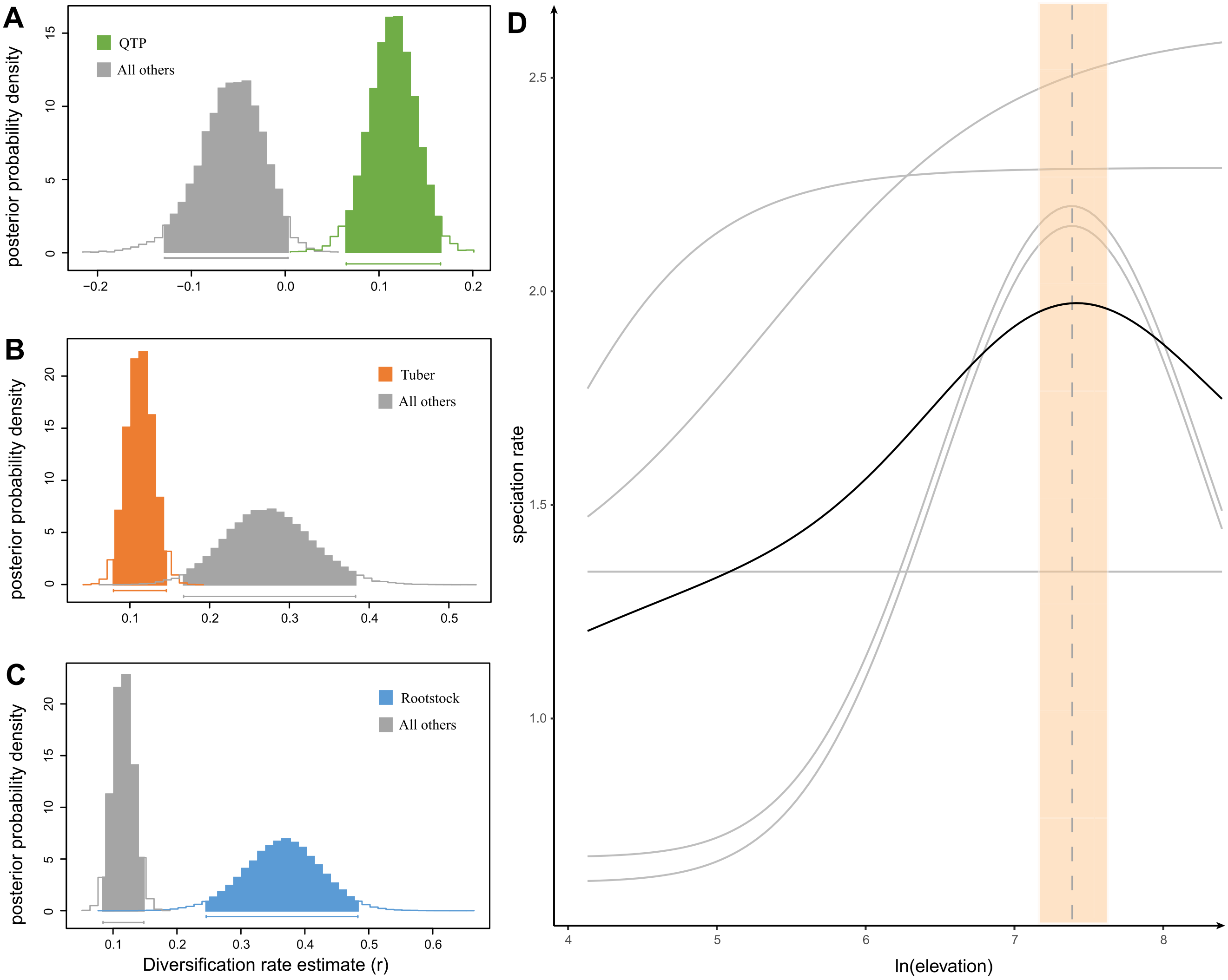

Fig. 2. Rates of speciation, extinction, and diversification dynamics associated with geography, underground vegetative organs, and elevation in Orchidinae inferred from the GeoSSE, BiSSE, and QuaSSE models. (A) Diversification rate between the QTP, adjacent areas, and outside the QTP (GeoSSE model); (B, C) diversification rate associated with underground organs (tubers & rhizomes) (BiSSE analysis); (D) relationship between elevation and speciation rates in Orchidinae as inferred from the QuaSSE models. The grey lines represent six models with varying speciation rates (Table S8). The black line represents the model-averaged prediction. Brown area represents the peak value of speciation corresponding to elevation.

extinction rate, which led to a higher net diversification rate (Fig. 2A).

3.3. Diversification and trait-dependent analysis

BiSSE analysis suggests that tuberous-rooted taxa have similar extinction rates but higher speciation rates compared to non-tuberous-rooted taxa (Fig. 2 B, C). In the QuaSSE analysis, we detected a trend for the rate of the speciation to increase with elevation, accelerating to reach a maximum when the elevation is approximately 1800 m and then decreasing with increasing elevation (Fig. 2 D). This implies that, within certain limits, high-elevation areas had a relatively higher speciation than those at lower elevations. Montane + alpine vegetation accelerated the speciation rate of Orchidinae, whereas temperate vegetation decreased the extinction rate (Fig. S7A). Our simulation indicates that our results of BiSSE and GeoSSE analyses are not artefacts caused by Type I error biases in SSE models (Fig. S8).

3.4. Diversification patterns

Our results show that each clade displays a different history of diversification (Fig. 3A, B). Most genera have low speciation and extinction rates, except for *Habenaria* and *Platanthera*. *Habenaria* (superclades IV and VI) exhibits a higher speciation rate and more significant differences between members than other clades, but its diversification rate is similar to the background, and only clade XXIV, endemic to the QTP, displays a large increase in net diversification rate (Fig. 3B, *Habenaria* II). Diversification of *Herminium* was initially high, but then decreased and was lower than the background (the average rate

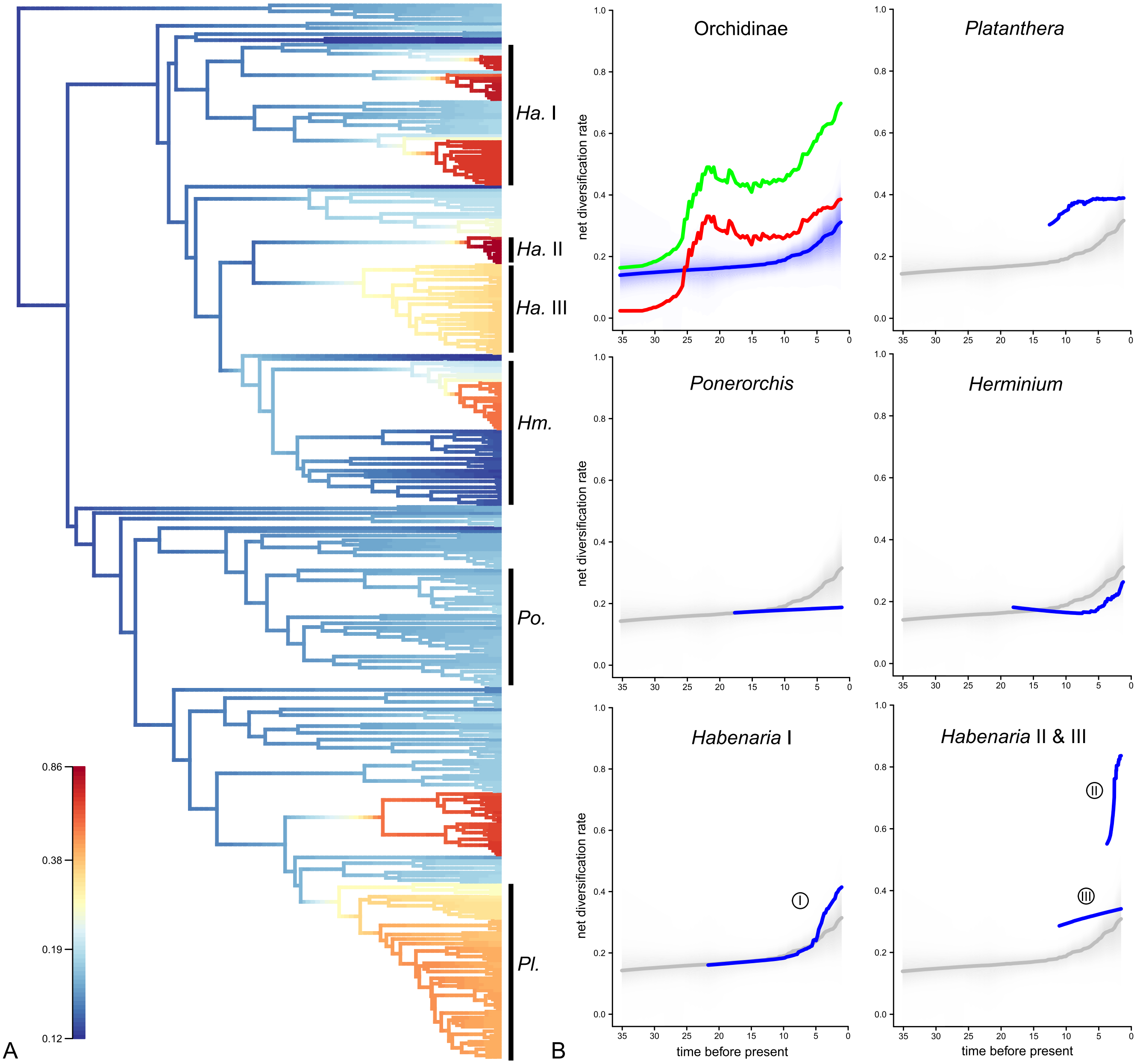

Fig. 3. Net diversification rates over time among Orchidinae clades. (A) The phylogenetic tree is shown as a time-calibrated grafted maximum clade credibility (MCC) tree without outgroups; branches coloured by reconstructed net diversification rates. (B) Rate-over-time curves depicting Orchidinae and clade-specific net diversification trajectories. The green, red, and blue represent speciation, extinction, and net diversification, respectively. The grey line denotes the mean diversification rate-over-time curve across all Orchidinae as background for focal clades. The shading intensity of the coloured line for each species reflects the relative probability of a given diversification trajectory. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of diversification of Orchidinae) for the last 10 Ma (Fig. 3B, *Herminium*). That for *Platanthera* was initially high then lower but has remained higher than that of other clades (Fig. 3B, *Platanthera*). The net rate of diversification has declined up to the present in *Platanthera*, *Herminium*, and *Ponerorchis,* whereas it is increasing in two clades with the most recent diversification, XXIV and superclade VI.

4. Discussion

4.1. Diversification of pre-adaptation clades in QTP

Our results indicate that clades coming either from temperate low-elevation regions or alpine regions had crucial roles in the origin of the QTP Orchidinae, which supports the hypothesis that temperate and alpine clades would play a predominant role in species diversification in the QTP. Diversification in some clades, such as that of *Herminium* and *Platanthera* originating in humid and cold northeastern temperate Asia and alpine regions, closely followed their dispersal into the QTP or their origin in the QTP. Only two possible tropical clades have been identified as showing *in situ* diversification in QTP. One clade, the *Habenaria glaucifolia* group, diverged from its tropical to subtropical sister group at 17.4 Ma and experienced an *in situ* QTP alpine diversification approximately 2.0 Ma (Fig. 1; Fig. S4). We suggest that such tropical groups were slow to adapt to the highly different QTP alpine habitats before their diversification could occur. On the other hand, clades with the highest species richness in the Mediterranean region with totally different habitats compared to the QTP, such as *Anacamptis*, *Ophrys*, *Orchis*, *Serapias*, etc., are absent from the QTP. Conversely, clades with highest species richness in the QTP, such as *Herminium*, *Platanthera*, or *Ponerorchis*, are absent from or diversified little in the Mediterranean region although they do occur further north in Europe. These observations suggest that clades exhibiting different pre-adaptations may have played different major roles in the Mediterranean region and the QTP.

It is worth noting that *Brachycorythis*, a genus relatively diverse in tropical Asia and even more so in tropical and southern Africa, is absent from the QTP except along its fringes below 2300 m elevation. Like most clades of *Habenaria*, this genus would seem to be pre-adapted through the possession of underground tubers, but, apparently, lack of cold resistance traits may present an additional obstacle that it has been unable to overcome. Both principally tropical *Habenaria* and *Brachycorythis* did not manage to colonize the Boreal temperate zone, despite possessing underground tubers.

Recent results of other studies have indicated that alpine floras of tropical and subtropical mountains are mixed derivatives of immigrants with temperate ancestry and descendants from nearby lowland clades (Hughes and Atchison, 2015; Merckx et al., 2015; Nuerk et al., 2018; Perez-Escobar et al., 2017; Wen et al., 2014). Dispersal of clades and descendants from local lowland ancestors made a major contribution to the biodiversity of Mount Kinabalu (Merckx et al., 2015) and the Andean orchid flora (Perez-Escobar et al., 2017). Diversification of descendants from temperate ancestors has been documented in many alpine plant clades, such as *Hypericum* in the Andes (Nuerk et al., 2018) and *Rheum* in the QTP (Sun et al., 2012). In the QTP, there is relatively little evidence for a tropical lowland origin of most of Orchidaceae found there, and tropical clades have diversified little there.

4.2. Abiotic and biotic factors are drivers of diversification in the QTP

Our results suggest that tuberous roots are among the key innovations associated with diversification of *Platanthera*, one of the youngest genera in Orchidinae. Our fieldwork indicates that horizontal tuberous roots are associated with shallow soils on rocks and growth along humid mossy barks of trees in the QTP. In addition, five pollination syndromes have been recorded in this subtribe (Jersakova et al., 2006), and future studies on pollination biology of QTP Orchidinae will undoubtedly provide more insights in biotic factors as drivers of diversification.

Key innovations associated with diversification have been identified or suggested for many clades in the alpine zone, such as the shift from an annual to perennial life history in *Lupinus* (Hughes et al., 2015) or development of caulescent rosettes in *Dendrosenecio* and *Lobelia* (Hedberg and Hedberg, 1979). Abiotic factors associated with the uplift of the QTP, such as geographic isolation, climatic oscillations, and biotic factors, such as pollination systems, hybridization and introgression, morphological innovation and polyploidy, have been suggested as drivers of diversification in the QTP (Song et al., 2015; Wen et al., 2014; Zhang et al., 2011; Zhou et al., 2013). Several key innovations have been identified as triggers of diversification in certain clades, such as “glasshouse” bracts in *Rheum* (Zhang et al., 2010), or “cushion” habit in *Androsace* (Wang et al., 2004).

4.3. The uplift of QTP s.l. and the species richness of alpine flora

Correlations between orogeny and diversification of clades in the QTP have been debated (Favre et al., 2015; Renner, 2016; Spicer, 2017; Xing and Ree, 2017), and recent results of time-calibrated phylogenetic analyses suggest that phased uplift of the QTP and the formation of the Asian monsoon climate might have been the main triggers of the spectacular rapid diversification (Liu et al., 2002, 2006; Uzma et al., 2019; Wang et al., 2009; Wen et al., 2014; Xing and Ree, 2017; Zhang et al., 2014; Zhou et al., 2013). However, few studies have combined ecological and historical factors to illustrate species diversification of the QTP (Cai et al., 2018). Our results indicate that five major clades in Orchidinae exhibit substantial variation in the temporal and spatial sequence of diversification in QTP, which suggests that the uplift of the QTP might not have had a uniform cross-lineage effect on the alpine flora.

The origin and diversification of *Herminium* in the QTP indicates that alpine meadows habitats were well established in the QTP approximately 20 million years ago, which agrees with previous studies on rapid diversification of alpine flora in the QTP, such as the *Ligularia–Cremanthodium–Parasenecio* complex (Asteraceae; Liu et al., 2006), and alpine gingers (Zingiberaceae; Zhao et al., 2016). However, most QTP endemic species originated within the past six million years, and only a few taxa are older than 15 million years, which agrees with the timing of radiation of many alpine taxa in the QTP, such as *Cyananthus* (Campanulaceae; Zhou et al., 2013), *Lilium* (Liliaceae; Gao et al., 2013), and *Saxifraga* subsection *Kabschia* (Saxifragaceae; Ebersbach et al., 2017). Some studies indicate that the recent rapid diversification of these clades might have been driven by the final uplift of QTP s.l. in Late Miocene or Pliocene and/or the Tertiary and Quaternary climatic oscillations (Chen et al., 2012; Ren et al., 2015; Zhou et al., 2013). Recent studies suggested that ‘sky islands’ (isolated mountains) could have promoted very recent diversifications during the Pliocene to Quaternary (Gizaw et al., 2016; Luo et al., 2016; Zhang et al., 2018).

Besides the pre-adaptation and niche evolution hypotheses mentioned above, which are predominantly about the processes underpinning speciation, two other hypotheses have been proposed to explain species richness in mountain areas: the cradle hypothesis, i.e., accelerated diversification compared with lowlands, and the museum hypothesis, i.e., long-term accumulation of species coupled with little extinction (Badgley et al., 2017; Hughes, 2017; Hughes and Atchison, 2015; Kier et al., 2009; Merckx et al., 2015; Schwery et al., 2015; Wen et al., 2014). The variety of diversification patterns across six major clades suggests that the QTP is a museum of clades with low speciation and low extinction rates except for three clades (Fig. 3A, B; Fig. S7). Two clades (clade XXIV and XXV) exhibit both the highest speciation and extinction rates among Orchidinae. In contrast, *Platanthera* in the QTP exhibits a high speciation rate but with much lower extinction rate than in QTP *Habenaria*. It seems that the QTP is both museum and cradle for *Platanthera*. However, we should caution that the net speciation rate of *Platanthera* obtained here may be biased by dissimilar sampling intensities throughout Orchidinae for parameters related to the net diversification rate, such as speciation, extinction and age estimates. Rapid endemic speciation and bidirectional dispersal of QTP elements indicate that the QTP is not only a cradle and museum, but also displays significant species turnover.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We sincerely thank María José Saní and Dr. Ran Wei for methodological assistance and suggestions.

Data accessibility

Vouchers and GenBank accession numbers are listed in Table S1. Final DNA sequence assembly has been uploaded in online files.

Funding

Research was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA19050201 and XDA19050103), Biodiversity Survey, Observation and Assessment Program of Ministry of Ecology and Environment of China, and the Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences (Grant No. Y4ZK111B01).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2020.107062.

References

Badgley, C., Smiley, T.M., Terry, R., Davis, E.B., DeSantis, L.R.G., Fox, D.L., Hopkins, S.S.B., Jezkova, T., Matocq, M.D., Matzke, N., McGuire, J.L., Mulch, A., Riddle, B.R., Roth, V.L., Samuels, J.X., Stroemberg, C.A.E., Yanites, B.J., 2017. Biodiversity and topographic complexity: modern and geohistorical perspectives. Trends Ecol. Evol. 32, 211–226.

Bininda-Emonds, O.R.P., 2004. The evolution of supertrees. Trends Ecol. Evol. 19, 315–322.

Bouckaert, R., Heled, J., Kuehnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10.

Cai, T., Fjeldsa, J., Wu, Y., Shao, S., Chen, Y., Quan, Q., Li, X., Song, G., Qu, Y., Qiao, G., Lei, F., 2018. What makes the Sino-Himalayan mountains the major diversity hotspots for pheasants? J. Biogeogr. 45, 640–651.

Chase, M.W., Cameron, K.M., Freudenstein, J.V., Pridgeon, A.M., Salazar, G., Van den Berg, C., Schuiteman, A., 2015. An updated classification of Orchidaceae. Bot. J. Linn. Soc. 177, 151–174.

Chen, S., Xing, Y., Su, T., Zhou, Z., Dilcher, E.D.L., Soltis, D.E., 2012. Phylogeographic analysis reveals significant spatial genetic structure of *Incarvillea sinensis* as a product of mountain building. BMC Plant Biol. 12.

Chen, X.-Q., Liu, Z.-J., Zhu, G.-H., Lang, K.-Y., Ji, Z.-H., Luo, Y.-B., Jin, X.-H., Cribb, P.J., Wood, J.J., Gale, S.W., Ormerod, P., Vermeulen, J.J., Wood, H.P., Clayton, D., Bell, A., 2009. Flora of China, vol. 25. Science Press, Beijing.

Conran, J.G., Bannister, J.M., Lee, D.E., 2009. Earliest orchid macrofossils: Early Miocene *Dendrobium* and *Earina* (Orchidaceae: Epidendroideae) from New Zealand. Am. J. Bot. 96, 466–474.

Darriba, D., Taboada, G., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 30 (9), 772–772.

Deng, T., Ding, L., 2015. Paleoaltimetry reconstructions of the Tibetan Plateau: progress and contradictions. Natl. Sci. Rev. 2, 417–437.

Ding, W.N., Ree, R.H., Spicer, R.A., Xing, Y.W., 2020. Ancient orogenic and monsoon-driven assembly of the world's richest temperate alpine flora. Science 369, 578–581.

Ebersbach, J., Muellner-Riehl, A.N., Michalak, I., Tkach, N., Hoffmann, M.H., Roeser, M., Sun, H., Favre, A., 2017. In and out of the Qinghai-Tibet Plateau: divergence time estimation and historical biogeography of the large arctic-alpine genus *Saxifraga* L. J. Biogeogr. 44, 900–910.

Ehl, S., Vences, M., Veith, M., 2019. Reconstructing evolution at the community level: A case study on Mediterranean amphibians. Mol. Phylogenet. Evol. 134, 211–225.

Fang, Q., Hong, H.L., Zhao, L.L., Furnes, H., Lu, H.Y., Han, W., Liu, Y., Jia, Z.Y., Wang, C., Yin, K., Algeo, T.J., 2017. Tectonic uplift-influenced monsoonal changes promoted hominin occupation of the Luonan Basin: Insights from a loess-paleosol sequence, eastern Qinling Mountains, central China. Quat. Sci. Rev. 169, 312–329.

Fang, X., 2017. Phased uplift of the Tibetan Plateau. Sci. Technol. Rev. 35, 42–50.

Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1995. Constructing a significance test for incongruence. Syst. Biol. 44, 570–572.

Favre, A., Paeckert, M., Pauls, S.U., Jaehnig, S.C., Uhl, D., Michalak, I., Muellner-Riehl, A.N., 2015. The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. Biol. Rev. 90, 236–253.

Felsenstein, J., 1988. Phylogenies from molecular sequences: inference and reliability. Annu. Rev. Genet. 22, 521–565.

FitzJohn, R.G., 2010. Quantitative traits and diversification. Syst. Biol. 59, 619–633.

FitzJohn, R.G., 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3, 1084–1092.

FitzJohn, R.G., Maddison, W.P., Otto, S.P., 2009. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58, 595–611.

Gao, Y.D., Harris, A.J., Zhou, S.D., He, X.J., 2013. Evolutionary events in *Lilium* (including *Nomocharis*, Liliaceae) are temporally correlated with orogenies of the Q-T plateau and the Hengduan Mountains. Mol. Phylogenet. Evol. 68, 443–460.

Givnish, T.J., Spalink, D., Ames, M., Lyon, S.P., Hunter, S.J., Zuluaga, A., Iles, W.J.D., Clements, M.A., Arroyo, M.T.K., Leebens-Mack, J., Endara, L., Kriebel, R., Neubig, K.M., Whitten, W.M., Williams, N.H., Cameron, K.M., 2015. Orchid phylogenomics and multiple drivers of their extraordinary diversification. Proc. R. Soc. B-Biol. Sci. 282, 171–180.

Givnish, T.J., Spalink, D., Ames, M., Lyon, S.P., Hunter, S.J., Zuluaga, A., Doucette, A., Caro, G.G., McDaniel, J., Clements, M.A., Arroyo, M.T.K., Endara, L., Kriebel, R., Williams, N.H., Cameron, K.M., 2016. Orchid historical biogeography, diversification, Antarctica and the paradox of orchid dispersal. J. Biogeogr. 43, 1905–1916.

Gizaw, A., Brochmann, C., Nemomissa, S., Wondimu, T., Masao, C.A., Tusiime, F.M., Abdi, A.A., Oxelman, B., Popp, M., Dimitrov, D., 2016. Colonization and diversification in the African 'sky islands': insights from fossil-calibrated molecular dating of *Lychnis* (Caryophyllaceae). New Phytol. 211, 719–734.

Goldberg, E.E., Lancaster, L.T., Ree, R.H., 2011. Phylogenetic inference of reciprocal effects between geographic range evolution and diversification. Syst. Biol. 60, 451–465.

Gordon, A.D., 1986. Consensus supertrees - the synthesis of rooted trees containing overlapping sets of labeled leaves. J. Classif. 3, 335–348.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 95–98.

Hedberg, I., Hedberg, O., 1979. Tropical-alpine life-forms of vascular plants. Oikos 33, 297–307.

Hipsley, C.A., Mueller, J., 2014. Beyond fossil calibrations: realities of molecular clock practices in evolutionary biology. Front. Genet. 5, 138. https://doi.org/10.3389/fgene.2014.00138.

Hughes, C.E., 2017. Are there many different routes to becoming a global biodiversity hotspot? Proc. Natl. Acad. Sci. U. S. A. 114, 4275–4277.

Hughes, C.E., Atchison, G.W., 2015. The ubiquity of alpine plant radiations: from the Andes to the Hengduan Mountains. New Phytol. 207, 275–282.

Hughes, C.E., Nyffeler, R., Linder, H.P., 2015. Evolutionary plant radiations: where, when, why and how? New Phytol. 207, 249–253.

Jersakova, J., Johnson, S.D., Kindlmann, P., 2006. Mechanisms and evolution of deceptive pollination in orchids. Biol. Rev. 81, 219–235.

Jin, W.T., Jin, X.H., Schuiteman, A., Li, D.Z., Xiang, X.G., Huang, W.C., Li, J.W., Huang, L.Q., 2014. Molecular systematics of subtribe Orchidinae and Asian taxa of Habenariinae (Orchideae, Orchidaceae) based on plastid *matK*, *rbcL* and nuclear ITS. Mol. Phylogenet. Evol. 77, 41–53.

Jin, W.T., Schuiteman, A., Chase, M.W., Li, J.W., Chung, S.W., Hsu, T.C., Jin, X.H., 2017. Phylogenetics of subtribe Orchidinae s.l. (Orchidaceae; Orchidoideae) based on seven markers (plastid *matK*, *psaB*, *rbcL*, *trnL-F*, *trnH-psbA*, and nuclear nrITS, *Xdh*): implications for generic delimitation. BMC Plant Biol. 17.

Katoh, K., Standley, D.M., 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Kier, G., Kreft, H., Lee, T.M., Jetz, W., Ibisch, P.L., Nowicki, C., Mutke, J., Barthlott, W., 2009. A global assessment of endemism and species richness across island and mainland regions. Proc. Natl. Acad. Sci. U. S. A. 106, 9322–9327.

Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian analysis of biogeography when the number of areas is large. Syst. Biol. 62, 789–804.

Li, X.W., Li, J., 1993. A preliminary floristic study on the seed plants from the region of Hengduan Mountain. Acta Botanica Yunnanica 15, 217–231.

Liu, J.Q., Gao, T.G., Chen, Z.D., Lu, A.M., 2002. Molecular phylogeny and biogeography of the Qinghai-Tibet Plateau endemic *Nannoglottis* (Asteraceae). Mol. Phylogenet. Evol. 23, 307–325.

Liu, J.Q., Wang, Y.J., Wang, A.L., Hideaki, O., Abbott, R.J., 2006. Radiation and diversification within the *Ligularia-Cremanthodium-Parasenecio* complex (Asteraceae) triggered by uplift of the Qinghai-Tibetan Plateau. Mol. Phylogenet. Evol. 38, 31–49.

Luo, D., Yue, J.-P., Sun, W.-G., Xu, B., Li, Z.-M., Comes, H.P., Sun, H., 2016. Evolutionary history of the subnival flora of the Himalaya-Hengduan Mountains: first insights from comparative phylogeography of four perennial herbs. J. Biogeogr. 43, 31–43.

Maddison, W.P., Midford, P.E., Otto, S.P., 2007. Estimating a binary character's effect on speciation and extinction. Syst. Biol. 56, 701–710.

Matzke, N.J., 2013. BioGeoBEARS: biogeography with Bayesian (and likelihood) evolutionary analysis in R scripts. R package, version 0.2 1, 2013.

Merckx, V.S.F.T., Hendriks, K.P., Beentjes, K.K., Mennes, C.B., Becking, L.E., Peijnenburg, K.T.C.A., Afendy, A., Arumugam, N., de Boer, H., Biun, A., Buang, M. M., Chen, P.-P., Chung, A.Y.C., Dow, R., Feijen, F.A.A., Feijen, H., Soest, C.F.-V., Geml, J., Geurts, R., Gravendeel, B., Hovenkamp, P., Imbun, P., Ipor, I., Janssens, S. B., Jocque, M., Kappes, H., Khoo, E., Koomen, P., Lens, F., Majapun, R.J., Morgado, L.N., Neupane, S., Nieser, N., Pereira, J.T., Rahman, H., Sabran, S., Sawang, A., Schwallier, R.M., Shim, P.-S., Smit, H., Sol, N., Spait, M., Stech, M., Stokvis, F., Sugau, J.B., Suleiman, M., Sumail, S., Thomas, D.C., van Tol, J., Tuh, F.Y. Y., Yahya, B.E., Nais, J., Repin, R., Lakim, M., Schilthuizen, M., 2015. Evolution of endemism on a young tropical mountain. Nature 524, 347–350.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Ni, J., Herzschuh, U., 2011. Simulating biome distribution on the tibetan plateau using a modified global vegetation model. Arct. Antarct. Alp. Res. 43, 429–441.

Nuerk, N.M., Michling, F., Linder, H.P., 2018. Are the radiations of temperate lineages in tropical alpine ecosystems pre-adapted? Glob. Ecol. Biogeogr. 27, 334–345.

Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'Amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P., Kassem, K.R., 2001. Terrestrial ecoregions of the world: A new map of life on Earth. Bioscience 51, 933–938.

Pearce, N.R., Cribb, P.J., 2002. The Orchids of Bhutan. Charlesworth Group, Huddersfield.

Pelser, P.B., Kennedy, A.H., Tepe, E.J., Shidler, J.B., Nordenstam, B., Kadereit, J.W., Watson, L.E., 2010. Patterns and causes of incongruence between plastid and nuclear Senecioneae (Asteraceae) phylogenies. Am. J. Bot. 97, 856–873.

Perez-Escobar, O.A., Chomicki, G., Condamine, F.L., Karremans, A.P., Bogarin, D., Matzke, N.J., Silvestro, D., Antonelli, A., 2017. Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. New Phytol. 215, 891–905.

Pridgeon, A.M., Cribb, P.J., Chase, M.W., Rasmussen, F.N., 2001. Genera Orchidacearum. Vol. 2. Orchidoideae (Part 1). Oxford University Press, Oxford.

Rabosky, D.L., Grundler, M., Anderson, C., Title, P., Shi, J.J., Brown, J.W., Huang, H., Larson, J.G., 2014. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. Methods Ecol. Evol. 5, 701–707.

Rambaut, A., Suchard, M., Xie, D. & Drummond, A., 2014. Tracer v1. 6 http://tree.bio.ed.ac.uk/software/tracer/ (last accessed 5 March 2018).

Ramirez, S.R., Gravendeel, B., Singer, R.B., Marshall, C.R., Pierce, N.E., 2007. Dating the origin of the Orchidaceae from a fossil orchid with its pollinator. Nature 448, 1042–1045.

Raskoti, B.B., Schuiteman, A., Jin, W.-T., Jin, X.-H., 2017. A taxonomic revision of *Herminium* L (Orchidoideae, Orchidaceae). Phytokeys 1–74.

Ree, R.H., Moore, B.R., Webb, C.O., Donoghue, M.J., 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59, 2299–2311.

Ree, R.H., Sanmartín, I., 2018. Conceptual and statistical problems with the DEC+ J model of founder-event speciation and its comparison with DEC via model selection. J. Biogeogr. 45, 741–749.

Ren, G., Conti, E., Salamin, N., 2015. Phylogeny and biogeography of *Primula* sect. *Armerina*: implications for plant evolution under climate change and the uplift of the Qinghai-Tibet Plateau. BMC Evol. Biol. 15.

Renner, S.S., 2016. Available data point to a 4-km-high Tibetan Plateau by 40Ma, but 100 molecular-clock papers have linked supposed recent uplift to young node ages. J. Biogeogr. 43, 1479–1487.

Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Schwery, O., Onstein, R.E., Bouchenak-Khelladi, Y., Xing, Y.W., Carter, R.J., Linder, H.P., 2015. As old as the mountains: the radiations of the Ericaceae. New Phytol. 207, 355–367.

Shaul, S., Graur, D., 2002. Playing chicken (*Gallus gallus*): methodological inconsistencies of molecular divergence date estimates due to secondary calibration points. Gene 300, 59–61.

Song, B., Stoecklin, J., Peng, D.L., Gao, Y.Q., Sun, H., 2015. The bracts of the alpine 'glasshouse' plant *Rheum alexandrae* (Polygonaceae) enhance reproductive fitness of its pollinating seed-consuming mutualist. Bot. J. Linn. Soc. 179, 349–359.

Spalink, D., Drew, B.T., Pace, M.C., Zaborsky, J.G., Li, P., Cameron, K.M., Givnish, T.J., Sytsma, K.J., 2016. Evolution of geographical place and niche space: Patterns of diversification in the North American sedge (Cyperaceae) flora. Mol. Phylogenet. Evol. 95, 183–195.

Spicer, R.A., 2017. Tibet, the Himalaya, Asian monsoons and biodiversity - In what ways are they related? Plant Divers. 39, 233–244.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.

Su, T., Farnsworth, A., Spicer, R.A., Huang, J., Wus, F.X., Liu, J., Li, S.F., Xing, Y.W., Huang, Y.J., Deng, W.Y.D., Tang, H., Xu, C.L., Zhao, F., Srivastava, G., Valdes, P.J., Deng, T., Zhou, Z.K., 2019. No high Tibetan Plateau until the Neogene. Sci. Adv. 5, eaav2189.

Sun, Y.H., Wang, A.L., Wan, D.S., Wang, Q., Liu, J.Q., 2012. Rapid radiation of *Rheum* (Polygonaceae) and parallel evolution of morphological traits. Mol. Phylogenet. Evol. 63, 150–158.

Swofford, D.L., 2003. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10., Sinauer, Sunderland, Massachusetts.

The Comprehensive Investigation Team of Tibetan Plateau, C.A.O.S.C., 1988. Vegetation of Xizang. Science Press, Beijing.

Uzma, Jimenez-Mejias P., Amir, R., Hayat, M.Q., Hipp, A.L., 2019. Timing and ecological priority shaped the diversification of sedges in the Himalayas. PeerJ 7, 6792.

Vaidya, G., Lohman, D.J., Meier, R., 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27, 171–180.

Vollering, J., Schuiteman, A., de Vogel, E., van Vugt, R., Raes, N., 2016. Phytogeography of New Guinean orchids: patterns of species richness and turnover. J. Biogeogr. 43, 204–214.

Wang, Y.J., Susanna, A., Von Raab-Straube, E., Milne, R., Liu, J.Q., 2009. Island-like radiation of *Saussurea* (Asteraceae: Cardueae) triggered by uplifts of the Qinghai-Tibetan Plateau. Biol. J. Linn. Soc. 97, 893–903.

Wang, Y.J., Li, X.J., Hao, G., Liu, J.Q., 2004. Molecular phylogeny and biogeography of *Androsace* (Primulaceae) and the convergent evolution of cushion morphology. Acta Phytotaxonom. Sin. 42, 481–499.

WCSP, 2015. World Checklist of Selected Plant Families. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://apps.kew.org/wcsp/ Retrieved.

Wen, J., Zhang, J.-Q., Nie, Z.-L., Zhong, Y., Sun, H., 2014. Evolutionary diversificatons of plants on the Qinghai-Tibetan Plateau. Front. Genet. 5.

Wu, C.-Y., 1987. Flora Xizangica, Vol. 5. Science Press, Beijing.

Wu, S.-G., Yang, Y.-P., Fei, Y., 1995. On the flora of the alpine region in the Qinghai-Xizang (Tibet) Plateau. Acta Botanica Yunnanica 17, 233–250.

Wu, Y.-H., 2008. The Vascular Plants and Their Eco-geographical Distribution of the Qinghai-Tibet Plateau. Science Press, Beijing.

Xiang, X.-G., Mi, X.-C., Zhou, H.-L., Li, J.-W., Chung, S.-W., Li, D.-Z., Huang, W.-C., Jin, W.-T., Li, Z.-Y., Huang, L.-Q., Jin, X.-H., 2016. Biogeographical diversification of mainland Asian *Dendrobium* (Orchidaceae) and its implications for the historical dynamics of evergreen broad-leaved forests. J. Biogeogr. 43, 1310–1323.

Xiang, K.-L., Zhao, L., Erst, A.S., Yu, S.-X., Jabbour, F., Wang, W., 2017. A molecular phylogeny of *Dichocarpum* (Ranunculaceae): Implications for eastern Asian biogeography. Mol. Phylogenet. Evol. 107, 594–604.

Xing, Y., Ree, R.H., 2017. Uplift-driven diversification in the Hengduan Mountains, a temperate biodiversity hotspot. Proc. Natl. Acad. Sci. U. S. A. 114, E3444–E3451.

Zhang, D.-C., Ye, J.-X., Sun, H., 2016. Quantitative approaches to identify floristic units and centres of species endemism in the Qinghai-Tibetan Plateau, south-western China. J. Biogeogr. 43, 2465–2476.

Zhang, D., Liu, B., Zhao, C., Lu, X., Wan, D., Ma, F., Chen, L., Liu, J., 2010. Ecological functions and differentially expressed transcripts of translucent bracts in an alpine 'glasshouse' plant *Rheum nobile* (Polygonaceae). Planta 231, 1505–1511.

Zhang, J.-Q., Meng, S.-Y., Allen, G.A., Wen, J., Rao, G.-Y., 2014. Rapid radiation and dispersal out of the Qinghai-Tibetan Plateau of an alpine plant lineage *Rhodiola* (Crassulaceae). Mol. Phylogenet. Evol. 77, 147–158.

Zhang, J.-W., Nie, Z.-L., Wen, J., Sun, H., 2011. Molecular phylogeny and biogeography of three closely related genera, *Soroseris*, *Stebbinsia*, and *Syncalathium* (Asteraceae, Cichorieae), endemic to the Tibetan Plateau, SW China. Taxon 60, 15–26.

Zhang, R.-Z., Zhen, D., Yang, Q.-Y., 1982. Physical Geography of Xizang (Tibet). Science Press, Beijing.

Zhang, Y.-Z., Zhu, R.-W., Zhong, D.-L., Zhang, J.-Q., 2018. Nunataks or massif de refuge? A phylogeographic study of *Rhodiola crenulata* (Crassulaceae) on the world's highest sky islands. BMC Evol. Biol. 18.

Zhang, Y., Li, B., Zheng, D., 2002. A discussion on the boundary and area of the Tibetan Plateau in China. Geogr. Res. 21, 1–8.

Zhao, J.-L., Xia, Y.-M., Cannon, C.H., Kress, W.J., Li, Q.-J., 2016. Evolutionary diversification of alpine ginger reflects the early uplift of the Himalayan-Tibetan Plateau and rapid extrusion of Indochina. Gondwana Res. 32, 232–241.

Zhou, Z., Hong, D., Niu, Y., Li, G., Nie, Z., Wen, J., Sun, H., 2013. Phylogenetic and biogeographic analyses of the Sino-Himalayan endemic genus *Cyananthus* (Campanulaceae) and implications for the evolution of its sexual system. Mol. Phylogenet. Evol. 68, 482–497.