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# Dispersal routes between biodiversity hotspots in Asia: the case of the mountain genus *Tripterospermum* (Gentianinae, Gentianaceae) and its close relatives

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## ABSTRACT

**Aim** We investigated the biogeography of the subtropical mountain genus *Tripterospermum* Blume (Gentianaceae), disjunctly distributed at the southern fringe of the Qinghai–Tibetan Plateau (QTP) and other mountain systems within the biodiversity hotspots of Southeast Asia. This study reveals dispersal routes among these areas.

**Location** East China, Indochina, Japan, the Philippines, southeastern fringe of the Qinghai–Tibetan Plateau (southern Himalaya and Hengduanshan), Taiwan, Wallacea.

**Methods** The evolutionary history of *Tripterospermum* was studied using the phylogenetic reconstructions (Maximum Likelihood and Bayesian Inference using ITS, *atpB-rbcL* and *trnL-trnF*), molecular dating (using BEAST with a relaxed clock model and fossil constraints), and two approaches of ancestral area reconstructions (DEC, S-DIVA). Our sampling design included 82% of the extant species of subtropical Gentianinae (*Tripterospermum*, *Metagentiana*, *Sinogentiana* and *Crawfordia*) as ingroup, and *Kuepferia*, *Gentiana* and other Gentianaceae genera as outgroups.

**Results** Subtropical Gentianinae originated at the southeastern fringe of the QTP (the southern Himalaya and Hengduanshan) between 16 and 35 million years ago (Ma). With a crown age estimated to be 2.7–8.8 million years (Myr), *Tripterospermum* originated at the southeastern fringe of the QTP, from where it dispersed to East China, Indochina, Sundaland, Taiwan, Japan and Wallacea.

**Main conclusions** For *Tripterospermum*, the southern Himalaya and the Hengduanshan have acted as a source area for the colonization of East and Southeast Asia. This study depicts dispersal routes among the biodiversity hotspots neighbouring the QTP and those located on Sundaland and Wallacea. Mountain plants seem to have colonized Wallacea using a northern route via Taiwan rather than a more southern route via Sundaland. The latter route has previously been recorded for many lowland lineages. Because *Tripterospermum* species-producing berries have dispersed more often across geographical barriers than those producing capsules, we hypothesize that avian transportation of berry-like fruits might have facilitated their dispersal.

## Keywords

biogeography, Gentianaceae, molecular dating, phylogeny, Qinghai–Tibetan Plateau, Southeast Asia, *Tripterospermum*

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## INTRODUCTION

Biodiversity is unevenly distributed on Earth, some regions and habitats exhibiting considerably higher species numbers than others. For example, highly diverse biotas are expected in mountain systems because the proximity of habitat alternatives provided by altitudinal zonation could result in lower extinction rates during climatic changes (Hoorn *et al.*, 2013). Mountain systems, particularly in tropical regions, can conceptually be seen as island systems (Sklenar *et al.*, 2014), because in both islands and mountains, the biotas are not continuous but form clearly defined subunits separated by persistent barriers to dispersal (Whittaker *et al.*, 2008). In mountain and island systems, biodiversity increases with the area, whereas floristic similarities between two mountain ranges or islands usually decrease with distance (Whittaker *et al.*, 2008; Sklenar *et al.*, 2014). Biodiversity levels on islands can also be influenced by island age and geological processes (Whittaker *et al.*, 2008), both aspects being poorly studied in mountain systems. Among other factors, geophysical and climatic changes resulting from mountain building can play a predominant role in shaping patterns of biodiversity (Hoorn *et al.*, 2013). These changes may, for example, affect dispersal routes by modifying geographical connectivity and diversification rates by providing new and therefore unoccupied niches. In the Andes, the uplift had both a promoting effect on diversification *in situ* via allopatric speciation (Antonelli *et al.*, 2009; Hoorn *et al.*, 2010) and on the immigration of cold-tolerant plants from North America (Cody *et al.*, 2010). The fact that mountain building is attended by the accumulation of species due to triggering diversification and/or promoting immigration is clearly reflected in the distribution of hotspots of biodiversity (Myers *et al.*, 2000). Yet, the origin and evolution of biotas in some mountain systems remain poorly studied, including the most prominent topological feature on Earth, the Qinghai–Tibetan Plateau (QTP) and its surroundings.

The QTP is the highest and largest plateau on Earth with a mean elevation above 4000 m covering a region of 2.3 million km<sup>2</sup> (Herzschuh *et al.*, 2010), and which provides numerous habitats (from forests to high alpine meadows). The vast proportion of the QTP interior is occupied by alpine tundra characterized by low precipitation (Sun & Wang, 2005), but several regions surrounding the QTP benefit from more favourable climatic conditions and harbour an enormous level of biological diversity. Four biodiversity hotspots, including the Himalaya, the mountains of Central Asia, the northern part of the Indo-Burmese hotspot, and the Hengduanshan (Myers *et al.*, 2000) are located there. These hotspots of biodiversity are considered to have resulted from geological and climatic changes that occurred from the Palaeocene to the present. The sequence of geological events include the collision of India with Eurasia (55–50 Ma), an uplift phase of the QTP far beyond sea level (c. 45–35 Ma), further uplift of higher mountain ranges such as the Himalaya and the Tian Shan starting between

20–10 Ma and continuing to the present (Mulch & Chamberlain, 2006; Lippert *et al.*, 2014; Favre *et al.*, 2015). The uplift of the QTP and the orogeny of the Himalaya likely contributed to the Asian monsoon system at c. 22 Ma and its progressive intensification, as well as the aridification of the Asian interior (Lu & Guo, 2014). A plethora of studies on plant evolution claim that these environmental changes have triggered speciation bursts (reviewed in Wen *et al.*, 2014). However, some of the mountainous hotspots of biodiversity bordering the QTP are in direct contact (or at least in close proximity) with further highly diverse regions, such as Sundaland and the rest of the Malay Archipelago. It may therefore be expected that some degree of floristic exchange from either side increased the biodiversity of the QTP region. The contribution of immigration to the accumulation of species in the areas surrounding the QTP has remained poorly studied (Favre *et al.*, 2015), and the role of the QTP region as a source and/or sink for mountain lineages is unknown. Tackling these questions requires several approaches, including molecular dating and biogeographical analyses, but so far these kinds of studies are scarce for the QTP and Southeast Asia (reviewed in Lohmann *et al.*, 2011; Wen *et al.*, 2014; Favre *et al.*, 2015). Moreover, biogeographical studies on plant taxa from Southeast Asia have mainly focused on lowland groups (e.g. Nauheimer *et al.*, 2012; Grudinski *et al.*, 2014). This highlights the need to study mountain taxa to investigate potential differences in their dispersal patterns. Because of its well-resolved phylogenetic relationships, its distribution at the southeastern fringe of the QTP and Southeast Asia, and its mountainous habitat (Murata, 1989), *Tripterospermum* Blume is very suitable to investigate the origin and evolution of diversity hotspots associated with the QTP as well as floristic exchanges with species-rich areas adjacent to Southeast Asia.

*Tripterospermum* belongs to Gentianinae (Gentianeae, Gentianaceae), along with five other genera: *Gentiana* L., *Metagentiana* T. N. Ho & S. W. Liu, *Crawfordia* Wallich, *Sinogentiana* Favre & Yuan and *Kuepferia* Adr. Favre (Favre *et al.*, 2014). Except for *Gentiana* (alpine, sub-cosmopolitan) and *Kuepferia* (alpine, Asian), these genera occur in subtropical montane forests of Asia (Favre *et al.*, 2010, 2014). In this study, we refer to *Tripterospermum*, *Metagentiana*, *Crawfordia* and *Sinogentiana* as ‘subtropical Gentianinae’, all together forming a monophyletic clade (Favre *et al.*, 2014). With 35 species of climbing vines, *Tripterospermum* consists of two sections: sect. *Capsulifera*, producing capsules, and sect. *Tripterospermum*, producing berries (Murata, 1989). Dispersal vectors of *Tripterospermum* are unknown. Section *Capsulifera* produces dry capsules with distinct winged seeds (Murata, 1989; Ho *et al.*, 2002), indicating anemochory. In contrast, the seeds of species of section *Tripterospermum* are strongly attached to the sticky pericarp of the berries and might become fixed to the plumage of frugivorous birds and potentially to the fur of other vectors such as frugivorous bats or also terrestrial animals. The distribution of species with capsules is restricted to Northeast India, China and Japan

(Murata, 1989), while species with berries have a broader distribution: the QTP region, China, Taiwan, Japan, Sundaland (Peninsular Malaysia, Sumatra and Java), Wallacea (Sulawesi), the Philippines and Sri Lanka (Murata, 1989). A large proportion (about 70%) of all species (whether capsular or berry-producing) are endemic to an island or a mountain range. In China, Taiwan and Japan, *Tripterospermum* occurs in mountain forests and in bamboo thickets between 1000 and 4000 m above sea level (a.s.l.), whereas southern populations (Sundaland, Wallacea, Philippines) are found above 1800 to 2500 m a.s.l. in the mossy forest.

This study on *Tripterospermum* aims at unveiling dispersal routes and directions for mountain plants among the hotspots of biodiversity neighbouring the QTP, and those located on the Sunda Shelf and Wallacea, applying both molecular and biogeographical analyses. By combining information from the fossil-constrained phylogenetic tree of *Tripterospermum*, present distributional data and knowledge about the geological history of the regions involved, we addressed the following questions: (1) Did *Tripterospermum* originate at the southeastern fringe of the QTP and disperse from there to Southeast Asia, as may be expected based on higher species numbers in the QTP region compared to other areas in Asia and Southeast Asia? (2) Did the intermittent continuous land mass of Sundaland promote the distribution of *Tripterospermum* during times of the Pleistocene? (3) Are there any differences in dispersal patterns between lowland plants (previous studies) and mountain taxa such as *Tripterospermum* (this study) in Southeast Asia?

## MATERIALS AND METHODS

### Taxon sampling and phylogenetic analysis

We sampled 82% of subtropical Gentianinae (our ingroup), including 30 of 35 extant *Tripterospermum*, 9 of 12 *Metagentiana*, 12 of 16 *Crawfordia*, and 2 of 2 *Sinogentiana* species. The outgroup included 5 *Kuepferia*, 15 *Gentiana* (covering most of the distribution range of the genus) as well as 19 other Gentianeae species (Swertiinae, Helieae, and Potalieae; see Appendix S1 in Supporting Information). Leaf samples were collected during field campaigns in Asia and dried in silica-gel. Additional material was retrieved from KUN, L, LZ, P, TNM and WU. Vouchers were re-determined using the *Flora of China* (Ho & Pringle, 1995) and other sources (Murata, 1989; new species descriptions: Chen *et al.*, 2006; Favre *et al.*, 2013). Additional sequences were retrieved from Genbank (<http://www.ncbi.nlm.nih.gov>). We amplified two plastid regions (*atpB-rbcL* spacer, *trnL-trnF* spacer and part of the *trnL* gene) and one nuclear region (ITS). Our data set contained 91, 72 and 53 sequences for ITS (14 newly generated), *trnL-trnF* (22 newly generated) and *atpB-rbcL* (four newly generated) respectively. Information on DNA extraction, PCR amplification and sequencing as well as on sequence handling and phylogenetic analysis is provided in Appendix S1 in Supporting Information.

### Divergence time estimation

Divergence time estimates were generated using Bayesian statistics in BEAST 1.7.5 (Rambaut & Drummond, 2007; Drummond *et al.*, 2012), with the Yule model and uncorrelated relaxed molecular clock model as best fitting priors (Drummond *et al.*, 2006; see Appendix S2). Analyses were run for 50 million generations, sampling every 5000th generation. Bayesian MCMC analyses were performed independently four times starting from different random starting points. The effective sample size (ESS) for all parameters of each run was detected (> 200) and the burn-in was assessed using TRACER 1.5 (Rambaut & Drummond, 2007). The four independent log-files were combined using LOGCOMBINER 1.7.5 with a burn-in according to the individual runs reaching convergence (once 12 million generations and for the three remaining runs 5 million generations), sampling every 15,000th generation. A maximum clade credibility topology was determined with TREEANNOTATOR 1.7.5 and was visualized using FIGTREE 1.4.0 (Rambaut, 2010). A detailed description of the fossils and the priors that were used to calibrate the tree is provided in Appendix S2, together with information about the effect of missing data on the BEAST analysis.

### Ancestral area reconstructions

We investigated the origin and dispersal routes (as well as their directions) of *Tripterospermum* and closely related genera with two approaches: (1) dispersal-extinction-cladogenesis (DEC), using LAGRANGE (Ree *et al.*, 2005; Ree & Smith, 2008) implemented in RASP 2.1 beta (Yu *et al.*, 2012), and (2) dispersal–vicariance analysis (DIVA; Ronquist, 1997), using the statistical dispersal–vicariance programme (S-DIVA) in RASP 2.1 beta. The DEC model infers ancestral ranges in a likelihood framework, applying a model of possible dispersal routes based on geological history (Ree *et al.*, 2005), whereas S-DIVA is parsimony-based and allows the reconstruction of ancestral distributions of a clade without any prior assumptions about area relationships, therefore favouring vicariance events (Ronquist, 1997; Yu *et al.*, 2010). The outcome of these methods is commonly compared in biogeographical studies (e.g. Grudinski *et al.*, 2014). Based on the combined data set, the BEAST MCMC trees as well as the maximum clade credibility tree derived from the Bayesian analysis were used as input for S-DIVA, while the BEAST maximum clade credibility tree was employed for LAGRANGE (see Appendix S2). Our species coverage in some outgroup clades (Helieae, Potalieae and Swertiinae) was too low to reflect their entire distribution, which might introduce bias in biogeographical analyses. Therefore, we excluded the tips representing these clades from the chronogram using the APE package (Paradis *et al.*, 2004) in R (R Core Team, 2013), leaving only the closest outgroups to subtropical Gentianinae (*Gentiana* and *Kuepferia*) of which the distribution range was sufficiently covered by our data. Each species was attributed to one or more of the following areas

according to their extant distribution: (1) North America, Europe and arctic/temperate Asia, (2) the southeastern fringe of the QTP (comprising the Hengduan Mountains, Yunnan (excluding Xishuangbanna), Sichuan, South and East Tibet, northern Myanmar, Nepal, Bhutan, Arunachal Pradesh and Sikkim), (3) the rest of China, (4) Indochina (comprising Thailand, Laos, Vietnam, Cambodia, China (Xishuangbanna), southeastern Myanmar), (5) Sundaland (Borneo, Sumatra, Java, Malay Peninsula), (6) Wallacea [Sulawesi, the Moluccas, the Banda Arc, and the Lesser Sunda islands], (7) the Philippines, (8) Japan and (9) Taiwan (a more detailed explanation concerning our area delineation is provided in Appendix S2).

## RESULTS

### Phylogenetic relationships and divergence time estimates

Results on sequence data are summarized in Appendix S3 in Supporting Information. We compared the most likely trees of the ML analysis, derived from *atpB-rbcL*, *trnL-trnF*, ITS, and the combined data matrix visually, and with the Bayesian majority rule consensus tree of the individual as well as the combined data sets. No topological incongruence (> 85% bootstrap percentage, BP, or > 0.9 posterior probability, PP) was detected. For this reason, we only present the maximum clade credibility tree of the Bayesian analysis (Fig. 1; see Appendix S2). Throughout this paper, 0.9–0.94 PP is considered as moderate and 0.95–1 PP as strong support.

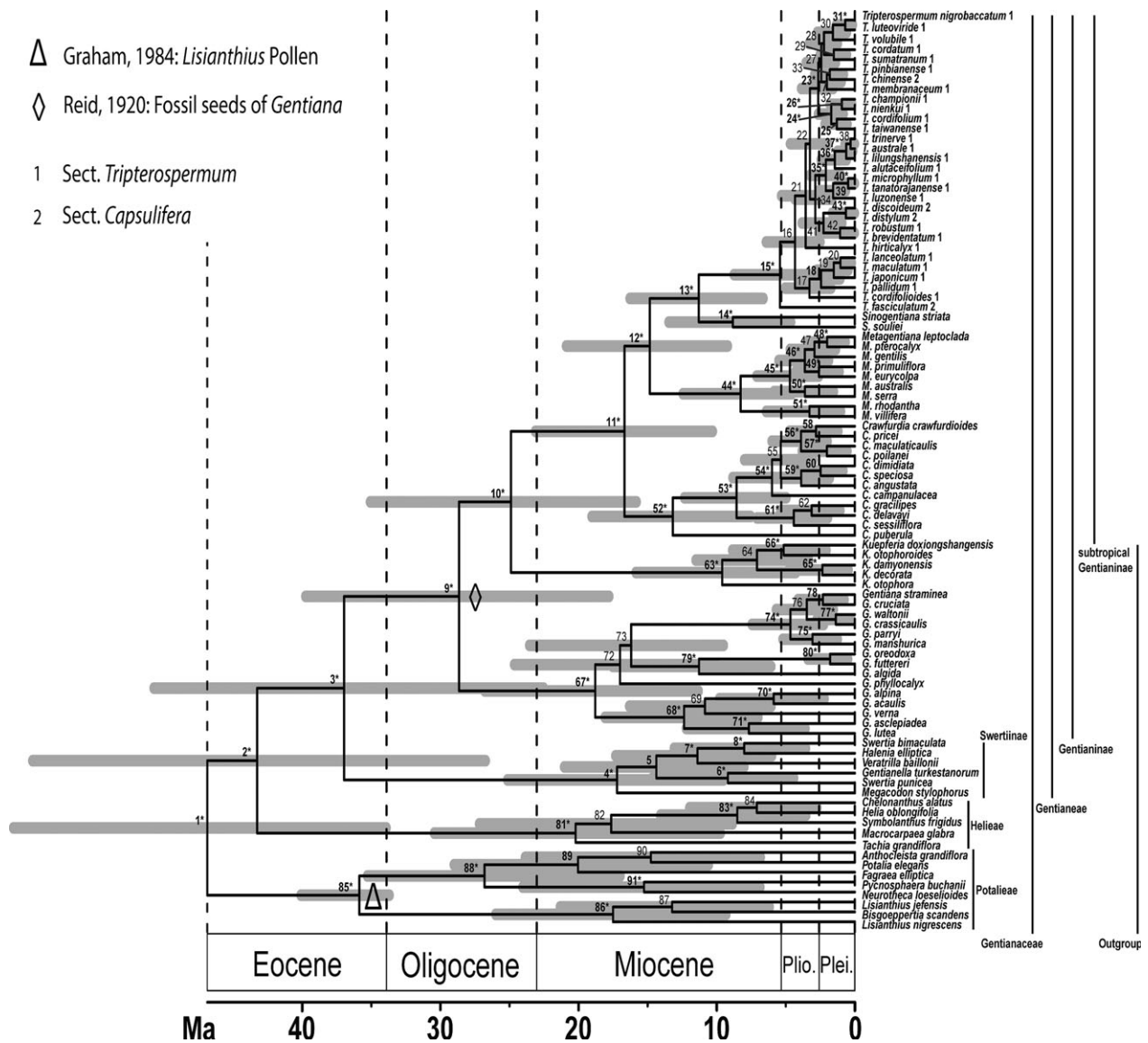
The phylogenetic relationships within Gentianinae were strongly supported (Fig. 1), with *Crawfordia*, *Gentiana*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum* each forming strongly supported monophyletic groups. The phylogenetic relationships among the species of *Tripterospermum* were fully resolved (Fig. 1). Section *Capsulifera* was polyphyletic, with species appearing in three strongly supported clades among species with berries (sect. *Tripterospermum*). The seven Taiwanese species did not form a monophyletic group, but appeared in three strongly supported clades (Fig. 2). Divergence time estimation (Fig. 1; see Appendix S2) revealed the crown age of Gentianinae (node 9; 28.7 [17.8–39.8] Myr). *Tripterospermum* was the youngest group with 5.4 [2.7–8.8] Myr (node 15), followed by *Sinogentiana* with 8.8 [4.6–13.5] Myr (node 14) and *Metagentiana* with 8.3 [4.6–12.5] Myr (node 44). *Kuepferia* and *Crawfordia* were potentially older with a crown age of 9.6 [4.3–15.9] Myr (node 63) and 13.2 [7.5–19.1] Myr (node 52) respectively. *Gentiana* was the potentially oldest group with 18.8 [11.3–26.8] Myr (node 67).

### Ancestral area reconstructions

The results of the DEC analysis are shown in Figs 2 and 3 (see details for DEC and S-DIVA in Appendix S2). We tested different approaches for LAGRANGE by using (1) no

constraints, (2) range constraints, (3) dispersal constraints and (4) range and dispersal constraints allowing different maximum numbers of areas. The outcome of these approaches was highly similar. Using no and only range constraints yielded the same percentages (except for a few nodes, but without changing the highest probability) as using only dispersal and dispersal combined with range constraints (independent of the maximum number of allowed areas). For example, for node 9 (Fig. 3) the approach using no or only range constraints displayed a probability of 52% for B|A, 32% for B\_C|A and 16% for C|A, while only dispersal or range and dispersal constraints showed 46% for B|A, 34% for B\_C|A and 20% for C|A. Therefore, we present only the results of the non-constrained approach allowing the highest maximum number of areas (nine) in this study.

The ancestral area reconstructions in both analyses led to congruent results, with the exception of nodes 1, 2, 8, 11, 12, 33, 35, 36, 43 and 44. These differences might be due to the low relative probability (< 50%) that S-DIVA and/or DEC yielded for these nodes. According to S-DIVA and DEC, the common ancestor of the Gentianinae might have had a broad distribution range (node 1; S-DIVA: areas ABCI; DEC: areas AB; Eurasia and North America), but the probabilities given for this node are low (< 50%). This is potentially due to the low coverage of *Gentiana* in this study (15 out of c. 360 species). For genera for which we had higher taxonomic coverage (*Tripterospermum*, *Metagentiana*, *Sinogentiana*, *Kuepferia* and *Crawfordia*), we found a clear origin in the southeastern fringe of the QTP (area B), supported by a relative probability of 100% for S-DIVA and DEC. Out of the 17 divergence events within *Tripterospermum*, DEC revealed 11 dispersal events, while S-DIVA recovered 9. Because S-DIVA favours vicariance, combined ancestral areas appeared more often in S-DIVA than in DEC. Both analyses suggested that starting from area B (southeastern fringe of the QTP), *Tripterospermum* colonized area C six times (rest of China; Fig. 3; nodes 25, 26, 34, 42, 48, 52) and area D three times (Indochina; Fig. 3; nodes 25, 35, 51) between 6.4 Ma and present, area H twice (Japan; Fig. 3, nodes 29, 52) between 2.7 Ma and present, and area I three times (Taiwan; Fig. 3, nodes 29, 35, 44) between 3.3 Ma and present. From area D (Indochina), area C (rest of China; Fig. 3; node 36) as well as area E (Sundaland) were colonized once between 1.8 Ma and present. Furthermore, *Tripterospermum* dispersed from area I (Taiwan) once to area C (rest of China) and D (Indochina; Fig. 3, node 48) between 1.3 Ma and present as well as twice to area E (Sundaland; Fig. 3, nodes 45, 48), twice to area F (Wallacea; Fig. 3, nodes 45, 46), and once to area G (the Philippines; Fig. 3, node 45) between 2.6 Ma and present. It is likely that *Tripterospermum* might have reached area F (Wallacea) via area G (the Philippines) from area I (Taiwan). Because these dispersal events occurred very recently, this route could not be deduced in detail from the results of our analyses.



**Figure 1** Chronogram for subtropical Gentianinae and Gentianaceae outgroups based on ITS, *atpB-rbcL* and *trnL-trnF* data modelled under a relaxed clock. Two fossils were used for setting temporal constraints:  $\Delta$  *Lisianthus* pollen and  $\diamond$  fossil seeds of *Gentiana*. Node ages represent mean ages and bars show the 95% highest posterior density intervals. Nodes in bold and with (\*) indicate a posterior probability (PP)  $\geq 0.95$ . The different sections of *Tripterospermum* are marked by 1 (section *Tripterospermum*) or 2 (section *Capsulifera*) after the species name. For each node, divergence times and posterior probability values are listed in Appendix S2. Ma = million years ago.

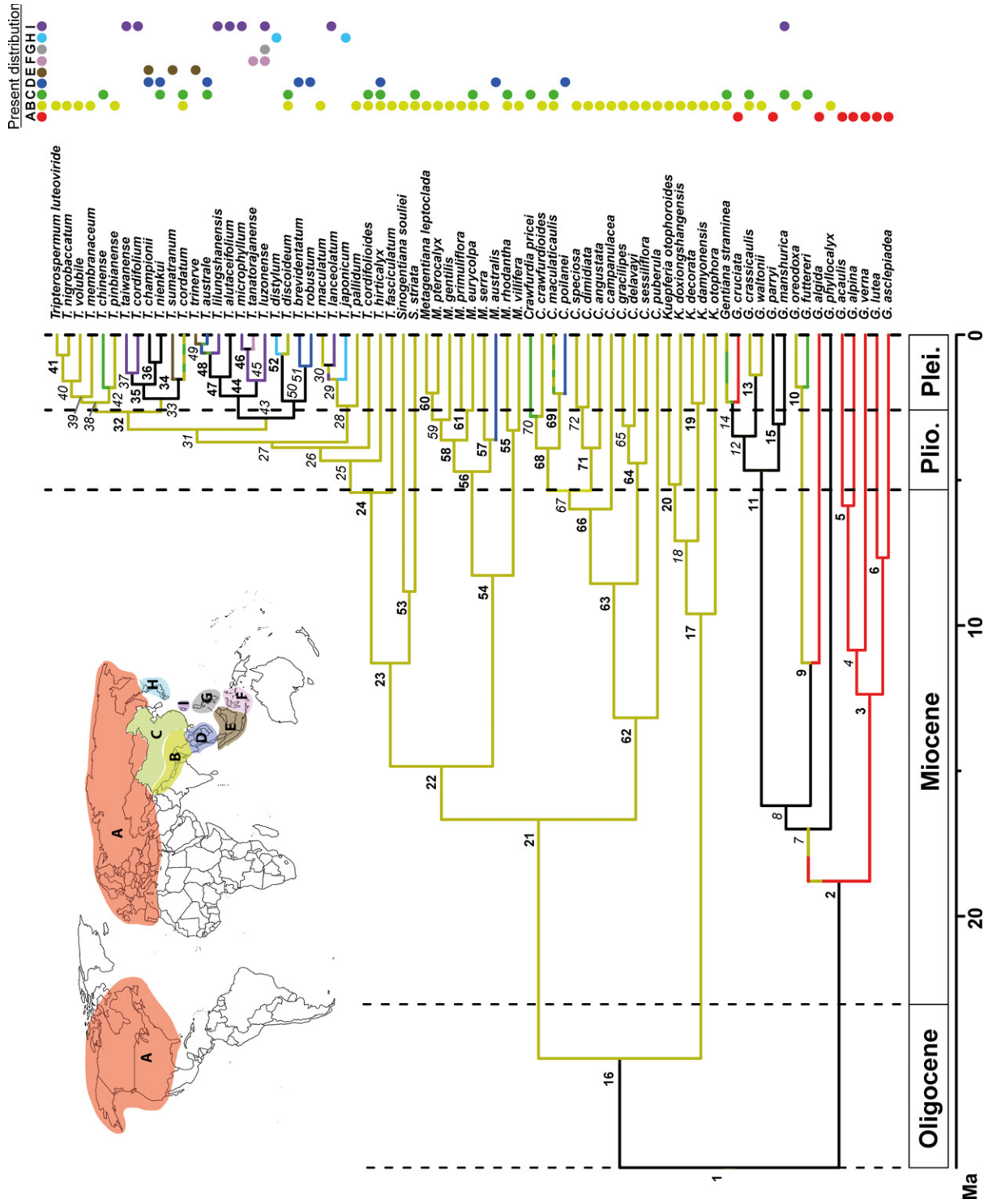
**DISCUSSION**

This study of the biogeography of *Tripterospermum* contributes to our understanding of the dispersal of elements of the mountain flora, conceptually similar to island flora systems in tropical areas, among the tropical biodiversity hotspots in SE Asia and the southeastern fringe of the QTP (Myers et al., 2000; Sklenar et al., 2014). So far, biogeographical studies on plants in the QTP region have focused either on endemics or plants of the QTP’s alpine tundra (reviewed in Wen et al., 2014; Favre et al., 2015), and studies describing the origin of plant genera in the Asian mainland with dispersal events to the Sunda Shelf have focused

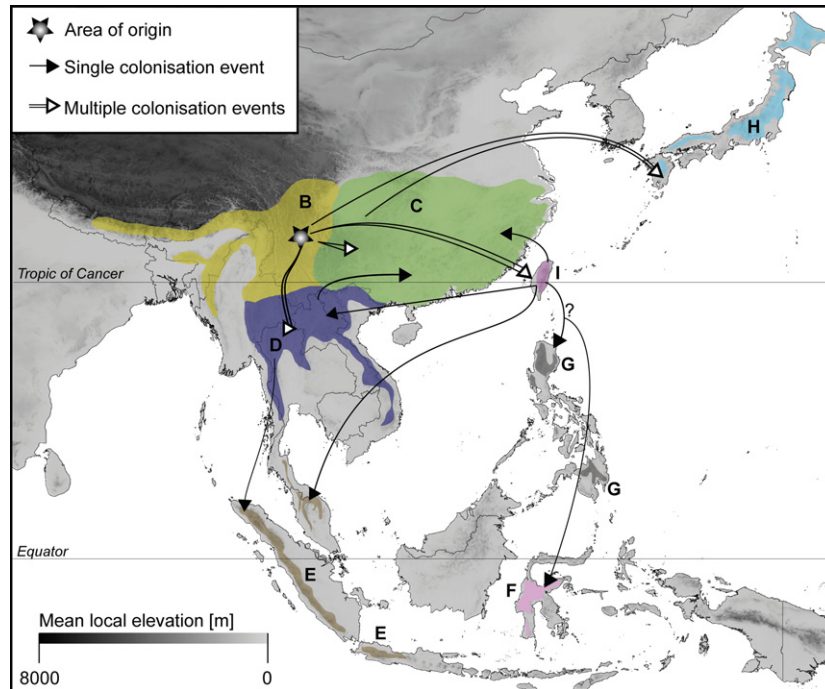
on lowland taxa (e.g. Nauheimer et al., 2012; Thomas et al., 2012). To understand the evolution of biodiversity hotspots and their relations, the investigation of species of different habitats is of crucial importance to assign possible differences and processes. Up to now, not much is known about the historical distribution of plants that are restricted to mountainous habitats in the Asian and SE Asian region.

**Divergence time estimation**

Evolutionary relationships among genera of Gentianinae were well resolved, and this constituted an ideal framework for the estimation of divergence times despite the scarcity of reli-



**Figure 2** Chronogram for *Tripterospermum* and outgroups based on ITS, *atpB-rbcL* and *trnL-trnF* data, analysed using a relaxed molecular clock approach. Node ages represent mean ages. Node numbers in bold indicate a posterior probability (PP) of  $\geq 0.95$ . Coloured dots at the tips depict the extant distribution of the taxa. The branch colours illustrate the most likely area inferred by the DEC analysis, with black indicating estimated probability  $< 50\%$  (see Fig. 1 for area and colour definition). For clarity, only the result of DEC is shown in this figure. The result of S-DIVA is to be found in Appendix S2. Ma = million years ago.



**Figure 3** Area definition for ancestral area reconstructions with DEC and S-DIVA: (A) North America, Europe and Arctic/temperate Asia (not highlighted), (B) the southeastern fringe of the QTP (comprising the Hengduan Mountains, Yunnan (excluding Xishuangbanna), Sichuan, South and East Tibet, northern Myanmar, Nepal, Bhutan, Arunachal Pradesh and Sikkim) (yellow), (C) the rest of China (green), (D) Indochina (comprising Thailand, Laos, Vietnam, Cambodia, China (Xishuangbanna), southeastern Myanmar) (dark blue), (E) Sundaland (Borneo, Sumatra, Java, Malay Peninsula) (brown), (F) Wallacea (Sulawesi, the Moluccas, the Banda Arc and the Lesser Sunda islands) (pink), (G) the Philippines (grey), (H) Japan (turquoise) and (I) Taiwan (purple). The distribution of extant *Tripterospermum* species as well as proposed dispersal events based on DEC and S-DIVA analyses are outlined.

able fossils in Gentianaceae. Our divergence time estimates for Gentianeae, Gentianinae, and *Tripterospermum* do agree with those of other studies (see Appendix S3; von Hagen & Kadereit, 2002; Chen *et al.*, 2005; Favre *et al.*, 2010; Merckx *et al.*, 2013) despite contrasting calibration schemes and species sampling, and we are confident that our study provides a realistic temporal framework for the investigation of the evolution of Gentianinae (see Appendix S3).

### Origin of subtropical Gentianinae

Both S-DIVA and DEC analyses suggested an origin of subtropical Gentianinae (*Tripterospermum*, *Metagentiana*, *Sinogentiana*, and *Crawfordia*) at the southeastern fringe of the QTP around the Oligocene/Miocene boundary (Fig. 3, node 16). At this time, the southern part of the QTP had already reached its present-day elevation (4000 m a.s.l.; Mulch & Chamberlain, 2006). This timeframe also corresponds to the establishment and the intensification of the monsoon regime (Sun & Wang, 2005) and the aridification of the Asian interior (Lu & Guo, 2014). Because all extant species of these four genera occur in tropical or subtropical mountain systems characterized by relatively low hydric stress (Ho *et al.*, 2002; Favre *et al.*, 2013, 2014), we assume that they were all derived from a common ancestor who lived in

a similar habitat at the southern or eastern fringe of the expanding QTP (southern Himalaya and Hengduanshan). Niche differences of those genera are currently under investigation (Matuszak *et al.*, submitted).

*Tripterospermum* diverged from *Sinogentiana* between the Middle to Late Miocene (Fig. 3, node 23) and seems to have radiated much later during the Pliocene to Early Pleistocene (Fig. 3, nodes 32, 43). At about the same time (boundary of Miocene/Pliocene to Pleistocene), *Tripterospermum* colonized the neighbouring area of East China six times from the QTP's surroundings. From the Southwest of China, plants could possibly disperse to East China along the following mountain systems: the northern Qinling–Daba Mountains, the centrally placed Dalou and Wuling Mountains as well as the southern Nanling Mountains (Wang, 1992). At this time, the QTP had nearly reached its present-day dimensions (Mulch & Chamberlain, 2006) and the monsoon regime was already in place, but still intensifying (Lu & Guo, 2014; Favre *et al.*, 2015). The diversification of *Tripterospermum* in the surroundings of the QTP thus coincided temporally with climatic changes (Asian monsoon and climate oscillations during the Quaternary) rather than orogenic processes. The climatic changes might have caused repeated expansion and contraction of distribution ranges, favouring allopatric speciation. This result would be in contrast to the general

assumption that plant diversification in the region of the QTP would have been triggered by geological processes (reviewed in Favre *et al.*, 2015).

### Colonization of Taiwan and Japan

Despite a number of poorly supported nodes in the phylogenetic reconstruction of *Tripterospermum*, clear patterns of dispersal were observed. For example, the colonization of Taiwan by *Tripterospermum* happened simultaneously with the orogenesis of Taiwanese mountain ranges (i.e. 6–2 Ma; Liu *et al.*, 2001; Sibuet & Hsu, 2004). *Tripterospermum* colonized Taiwan three times independently (Fig. 3, nodes 29, 35, 44) from the southeastern fringe of the QTP during the Late Pliocene to Pleistocene. Climatic oscillations and associated sea level variations during the Pleistocene, which also temporally coincided with the arrival of *Tripterospermum* in Taiwan, allowed land bridges to connect this island to the Asian mainland (Voris, 2000; Chiang & Schaal, 2006), potentially facilitating dispersal. *Tripterospermum* also reached Japan twice in the very Late Pliocene to Pleistocene (Fig. 3, nodes 29, 52), most likely by long-distance dispersal (possibly via frugivorous birds). Japan has been an archipelago since the Miocene (15–25 Ma), when the Japanese Sea separated it from East Asia (Maruyama *et al.*, 1997). Our phylogenetic reconstruction provides strong support for a close relationship between *T. maculatum* from mainland China, *T. lanceolatum* from Taiwan and *T. japonicum* from Japan, pointing towards dispersal events between these three areas. This example does not represent an isolated case because the floras of Taiwan and Japan share strong similarities with the flora of mainland China (Hsieh, 2003; Chiang & Schaal, 2006).

### Colonization of Indochina, Sundaland, the Philippines and Wallacea

From the fringe of the QTP, *Tripterospermum* colonized Indochina three times independently (Fig. 3; nodes 25, 35, 51), arriving earliest in this region at the Miocene/Pliocene boundary to Early Pleistocene. Some of the species that evolved after these colonization events occur geographically close to Southwest China (*T. hirticalyx*, *T. robustum*), whereas other species are distributed further south in Peninsular Malaysia, Sumatra and Java (Sundaland; *T. sumatranum*, *T. championii* and *T. trinerve*). At the time of colonization, the geography of SE Asia was very similar to its present configuration (about 3 Ma; Hall, 2009; van Welzen *et al.*, 2011). During the Pleistocene, climate oscillations led to fluctuations of the sea level, resulting in the intermittent continuous land mass of Sundaland (van Welzen *et al.*, 2011), but this situation is not restricted to the Pleistocene, as the reconstructions of Hall (2009) indicated a connection of mainland Asia and the Sunda Shelf throughout most of the last 25 Myr. Sea level oscillations probably facilitated dispersal of *Tripterospermum* by shortening distances for animal vectors between islands. In

our study, we could observe more frequent colonization events between currently adjacent land masses (e.g. Taiwan-mainland Asia or Sundaland-mainland Asia) especially during the time of the Pliocene/Pleistocene than between remote areas (Philippines and Wallacea). This finding is consistent with numerous other studies (de Bruyn *et al.*, 2014).

Our results revealed an important role of Taiwan for *Tripterospermum* as a stepping stone to the islands of South-east Asia. During the Pleistocene, *Tripterospermum* dispersed from Taiwan to Sundaland, the Philippines, and Wallacea (Fig. 3; nodes 45, 46, 48). It is likely that Wallacea was colonized via the Philippines, but the analyses were not able to detail this route. There is only one species of *Tripterospermum* occurring in the Philippines (*T. luzonense*), and two in Wallacea (*T. luzonense* and the recently newly described *T. tanatorajanense* from Sulawesi; Favre *et al.*, 2013). These two species are morphologically very similar, and our phylogeny strongly supports a close relationship between them (Fig. 3, node 45). Many studies on animals and lowland plants have confirmed repeated colonization of Wallacea from the Sunda Shelf region (e.g. Brown *et al.*, 2009; Nauheimer *et al.*, 2012; Grudinski *et al.*, 2014), but only a few have shown a northern colonization route from Taiwan (Wu *et al.*, 2009; Esselstyn & Oliveros, 2010). However, there is a strong floristic similarity between Taiwan and the Philippines (Li, 1953) and van Steenis hypothesized already in 1964 that Taiwan-Luzon might be a major track for mountain plants. The colonization of the Philippines from Taiwan is and was only possible by long-distance dispersal, because these areas were always isolated from each other by the Bashi Strait (Wu *et al.*, 2009). The evolution of berry-like fruits (Fig. 1, node 15; Fig. 2, node 24) might have favoured long-distance dispersal of *Tripterospermum* as illustrated by the broader distribution of berry-producing species compared to capsule-producing species. The winged seeds of the capsular species might only very rarely be transported by wind across permanent water bodies (as it is probably only the case for *T. distylum* on the island of Yakushima, Japan). Moreover, our analyses showed that those long-distance dispersals occurred very recently (about 2 Ma), which supposes only a short time for *in situ* radiation and might also explain the relatively small number of species observed in the Philippines, Wallacea and Sundaland. Therefore, *Tripterospermum* illustrates the well-established theory of island biogeography (Whittaker *et al.*, 2008): the greater the distance of islands (here: mountains on islands) to the mainland, the less floristic similarities (*Tripterospermum* as a floristic element) occur.

### CONCLUSIONS

Our study shows that in the case of *Tripterospermum* and other subtropical closely related genera (*Crawfordia*, *Metagentiana*, and *Sinogentiana*), the southern Himalaya and the Hengduanshan (bordering the QTP) acted as source area for montane species diversity in Southeast Asia (summarized in Fig. 3). *Tripterospermum* evolved in the surroundings of



the QTP around the Middle to Late Miocene. Because the extension of the QTP was almost at its present stage by the time *Tripterosperrum* diversified (Pliocene, Pleistocene), geological changes (the uplift) alone might not have triggered the accumulation of species in the region of the QTP. Rather, we argue that the combination of geographical features (mountain chains and islands) and climate oscillations favoured speciation throughout the range of this genus. Dispersal events of *Tripterosperrum* from the southeastern fringe of the QTP to other areas occurred at the same epochs. Long-distance dispersal possibly by animal vectors was probably facilitated by sea level variations (resulting in the exposure of land bridges) and by the production of berries. From the QTP's surroundings, *Tripterosperrum* colonized East China, Japan, Taiwan and Indochina multiple times. From Taiwan, *Tripterosperrum* dispersed to Sundaland, the Philippines and Wallacea. This northern dispersal route via Taiwan, which we could confirm in our study, was already proposed as a major track for mountain plants by van Steenis (1964). For lowland plants more often a southern route via Sundaland was proposed. More biogeographical studies on mountain taxa should be performed in order to confirm that Taiwan has acted as a secondary source area for the mountain flora of southern tropical hotspots of biodiversity.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Voucher information and GenBank accession numbers; protocols for laboratory work and phylogenetic analysis.

**Appendix S2** Fossils and priors for divergence time estimation; information about area delineation; additional information for Figs. 2 and 3 (node information, divergence time estimates and results of the ancestral area analyses, DEC and S-DIVA).

**Appendix S3** Sequence data results; comparison of divergence time estimates for Gentianinae/Gentianaceae with previous studies.

## BIOSKETCH

This research is part of **Sabine Matuszak's** doctoral dissertation. She studies *Tripterospermum* as part of her research and education programme at Goethe University Frankfurt and the University of Leipzig. Her research focuses on systematics and biogeography of selected taxa of Ericaceae and Gentianaceae, focusing on the region of the Qinghai–Tibetan Plateau.

Author contributions: A.F., S.M. and A.N.M.-R. conceived the ideas. S.M. collected and analysed the data. H.S. provided plant material and supported fieldwork logistics. S.M., A.F. and A.N.M.-R. were involved in interpreting the results. S.M. wrote the first draft of the manuscript; A.F., A.N.M.-R. and H.S. contributed to writing. A.F. and A.N.M.-R. recruited the financial support for this study.

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