Evolution of mountain plants in the region of the Qinghai-Tibetan Plateau and beyond

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SUMMARY

Biodiversity is unevenly distributed on Earth. Highly diverse biotas are particularly expected in mountain systems, because altitudinal zonation provides a number of habitat alternatives, which could lead to lower extinction rates during climatic changes. Nevertheless, the impact of environmental changes on plant diversification (especially for sub-alpine taxa) in the course of mountain orogenesis remains poorly understood. This is also true for the highest and largest plateau on Earth, the Qinghai-Tibetan Plateau (QTP) and its surrounding areas.

The predominant biome of the QTP is alpine tundra characterised by low precipitation, but especially the regions at the southern and southeastern flanks of the QTP harbour high levels of biodiversity due to more favourable climatic conditions. In total, there are four biodiversity hotspots surrounding the QTP: the Himalayas, the mountains of Central Asia, Indo-Burma, and the Hengduanshan. These hotspots are assumed to have resulted from geological and climatic changes caused by the uplift of the QTP. In this doctoral thesis, I investigated the impact of these environmental changes on plant diversification and the floristic exchange between the QTP region and biodiversity hotspots of Southeast Asia as well as other parts of the world by using the sub-alpine genera *Agapetes* and *Vaccinium* (Vaccinieae, Ericaceae) as well as *Tripterospermum* (Gentianinae, Gentianaceae) as model systems. Furthermore, I examined the role of niche evolution and conservatism in a changing environment over time, and detected possible beneficial morphological traits of plants in the surroundings of the QTP by investigating subtropical Gentianinae (*Crawfurdia, Kuepferia, Metagentiana, Sinogentiana,* and *Tripterospermum*; Gentianaceae).

For Vaccinieae, a North American origin was found from where the tribe dispersed nearly throughout the world. In this case, the QTP region acted as a sink area, because this region was colonised several times from different parts of the world and diversification followed the ancestors' arrival. The diversification of Vaccinieae started around 21.3 to 9.2 Ma in the QTP's region, which temporally coincides with the early formation of the Himalayas and the

intensification of the monsoon system, suggesting a potential causal relationship. In contrast, *Tripterospermum* originated at the southeastern fringe of the QTP at about 8.8 to 2.7 Ma, and after *in situ* diversification other regions of Asia and Southeast Asia were colonised. Therefore, the QTP acted as a source area. Such a source pattern was often found for alpine taxa, but to be able to assess a general trend for sub-alpine groups, additional genera must be investigated in future research. Nevertheless, it could be possible that the impact of the QTP's environmental changes might vary over time. During its early formation history the QTP could have acted as sink (Vaccinieae) and during more recent times as source area (*Tripterospermum*). The capsule-producing QTP genera *Kuepferia* and *Sinogentiana* (displaying a tendency towards niche conservatism) as well as *Crawfurdia* and *Metagentiana* (characterised by niche evolution) have fewer species and a smaller distribution range than *Tripterospermum* with berry-like fruits and a strong tendency towards niche evolution. Among other tested morphological traits, only the evolution of berry-like fruits has led to increased speciation rates and could therefore be viewed as potential key innovation. In the case of *Tripterospermum* it is likely that diversification was mediated by its wide niche breadth and good dispersal ability rather than environmental changes in the QTP region. This example stresses the importance to also consider the ecology of plant taxa when investigating possible correlations between environmental changes and diversification, which has only rarely been done so far for plants in the QTP region.

DECLARATION

I confirm that the work presented in this thesis is my own with the following acknowledgments.

Determination of Gentianaceae collected in a field trip to China in 2011 and re-determination of herbarium vouchers of Gentianaceae were done by Adrien Favre (chapters 2 and 3).

For the published manuscript “Two new species of the Asian genus *Tripterospermum* (Gentianaceae)” by Adrien Favre, Sabine Matuszak, and Alexandra Muellner-Riehl, published in Systematic Botany in 2013, I was involved in finding one of the two described plants (*Tripterospermum maculatum*) and in measuring as well as critically evaluating morphological traits of the two species. Furthermore, I helped to conduct comparative statistical analyses (multivariate analyses).

For the published manuscript “Two new genera of Gentianinae (Gentianaceae): *Sinogentiana* and *Kuepferia* supported by molecular phylogenetic evidence” by Adrien Favre, Sabine Matuszak, Hang Sun, Ende Liu, Yong-Ming Yuan, and Alexandra Muellner-Riehl, published in Taxon in 2014, I was involved in the lab work (PCR amplification and sequencing of the nuclear marker ITS and the plastid *trnL-trnF* region), as well as in phylogenetic analyses (chapter 5).

The use of any other material from other sources and the scientific contributions from collaborators are fully acknowledged throughout the thesis.

Sabine Matuszak Frankfurt am Main, 20th October 2015

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LIST OF ABBREVIATIONS

AIC	Akaike information criterion
a.s.l.	above sea level
AUC	Area Under the receiver operating Curve
BF	Bayes factor
BI	Bayesian inference
BIC	Bayesian information criterion
BiSSE	binary state speciation and extinction approach
BP	bootstrap percentage
DEC	dispersal-extinction-cladogenesis
DIVA	dispersal-vicariance analysis
DNA	desoxyribonucleic acid
ENM	environmental niche model
ESS	effective sample size
GAM	generalised additive model
GCM	general circulation model
GLM	generalised linear model
GTR+G	general time-reversible model with gamma distribution
GTR+G+I	general time-reversible model with gamma distribution and invariant sites
HPD	highest posterior density
ITS	internal transcribed spacer
KS	Kolmogorov-Smirnov
LDD	long-distance dispersal
Ma	million years ago
MaxEnt	maximum entropy model
MCMC	Markov chain Monte Carlo
MDI	morphological disparity index
ML	Maximum Likelihood
Myr	million years
NALB	North Atlantic Land Bridge
NTP	nucleoside triphosphate
PCA	principal component analysis

PCR	polymerase chain reaction
PP	Bayesian posterior probability
QTP	Qinghai-Tibetan Plateau
S-DIVA	statistical dispersal-vicariance analysis
SDM	species distribution modelling
SE Asia	Southeast Asia
sp.	species
spp.	species pluralis

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1. GENERAL INTRODUCTION

1.1 General aspects of biodiversity and biogeography

Biodiversity is unevenly distributed on Earth, as clearly illustrated by the geographic location of biodiversity hotspots (Myers et al., 2000; Myers et al., 2003; Myers & Mittermeier, 2003). Understanding the processes leading to the accumulation of high biodiversity levels in some regions and not in others is of growing interest for biological disciplines such as ecology and biogeography. The knowledge about the emergence of biodiversity hotspots may help to prioritise areas for conservation, and may facilitate an evaluation of the efficiency of environmental protection efforts under climate change (Favre et al., 2015). In fact, the aspect of priority in conservation is already depicted in the concept of the so-called “biodiversity hotspots”: these regions not only contain endemic species comprising at least 0.5% of all vascular plant species world-wide, but also have lost 70% or more of their primary vegetation (Myers et al., 2000; Myers, 2003; Myers & Mittermeier, 2003; Kier et al., 2005; Kier et al., 2009). To investigate the evolution of biodiversity hotspots, it is necessary to study the geographical origin and variation in time and space of species diversity, which is the main objective of the field of biogeography (Posadas et al., 2006; Lomolino et al., 2010). Over the past two decades, there has been major progress in biogeography because of methodological developments. The methods are based on the idea that taxa must have a common biogeographical history when sharing similar phylogenetic and distributional patterns (Ronquist & Sanmartin, 2011). The origin of biodiversity as well as species distribution patterns have been increasingly investigated by applying molecular phylogenetics, divergence time estimation, and ancestral area reconstructions (e.g., Antonelli & Sanmartin, 2011a; Roncal et al., 2011; Drew & Sytsma, 2012; Lexer et al., 2013; Grudinski et al., 2014b; Ornelas et al., 2014). Progress in the field of molecular genetics also enabled the use of historical specimens, which enlarged the amount of available data especially for rarely collected taxa (Wandeler et al., 2007; Roberts et al., 2011). Phylogenetic trees in combination with well determined fossils (associated with extant lineages with certainty) can provide the temporal framework, which allows testing whether the diversification of taxa might be linked to major geological or climatic events in the past, as predicted by biogeographical hypotheses (Antonelli & Sanmartin, 2011b; Pirie & Doyle, 2012; Favre et al., 2015 and references therein). However, not only the geological and climatic settings contributed to the formation of present distribution patterns of organisms, but also their efficiency to disperse.

An important aspect of biogeography is the ability of organisms to move away from their place of origin, which is defined as dispersal (Nathan et al., 2008; Gillespie et al., 2012). In plants, dispersal is mostly a passive process in which dispersal units (diaspores) are carried away from the parental population either by biotic (often insects or vertebrates) or abiotic (wind or water) vectors (Nathan, 2006). Typically, the majority of diaspores are dispersed over short distances (up to a few tens of meters; Howe & Smallwood, 1982; Nathan, 2006), but in rare cases, long-distance dispersal (LDD) might occur. LDD is of critical importance for genetic and floristic exchanges between patches of non-continuous habitats, and for the colonisation of new, empty, and distant areas (e.g. islands; Cain et al., 2000). The colonisation of the archipelagos of the Pacific Ocean is a prime example for LDD, because most of these usually volcanic islands were never directly connected to any continents (Neall & Trewick, 2008). For example, the Hawaiian flora is the result of LDD from various areas such as the Americas, Asia, Australasia, other islands of the Pacific, and even Africa, and subsequent *in situ* speciation (Baldwin & Wagner, 2010; Keeley & Funk, 2011; Cantley et al., 2014). In this case, it is assumed that LDD occurred through three primary vectors: wind, birds and ocean currents (Gillespie et al., 2012). Immigration through LDD can therefore play a major role in establishing local floristic composition. Yet, LDD alone cannot account for all disjunct distributions observed on Earth, and other processes contribute to the observed patterns of distribution of biodiversity.

Outlined in the following, another important process for continental disjunctions of plant groups is vicariance (Bartish et al., 2011). For decades, following the validation of the plate tectonic theory (Wegener, 1921) in the 1960’s, vicariance biogeography has played a dominant role in historical biogeography (Nelson & Platnick, 1980; Winterbottom, 1985; van der Spoel, 1990; van Veller, 1999; Heads, 2003), because scientists criticised that any disjunct distribution can be explained by LDD (Bartish et al., 2011). A good example for vicariance is provided by Cupressaceae. By using phylogenetic inference and divergence time estimation it could be shown that this family originated during the Triassic, before the breakup of the supercontinent Pangaea. Due to the splitting of Pangaea into Laurasia (North) and Gondwana (South), vicariance occurred between the two subfamilies of Cupressaceae: the Laurasian Cupressoideae and the Gondwanan Callitroideae. In the course of time, three further intercontinental disjunctions involving the Northern and Southern Hemisphere occurred (Mao et al., 2012). Together, LDD and vicariance might explain in most cases the base line floristic composition of a region. Yet, high levels of biodiversity cannot only be achieved by numerous parallel events of colonisation, but also by *in situ* diversification.

The underlying causes for the diversification of taxa within a region are manifold. There are genetic causes (e.g., mutation rate, genetic variability and genetic drift) and ecological causes such as environmental changes and parallel invasion of vacant niches (Purves et al., 2006; Frey & Loesch, 2010). Playing a major role in diversification, ecological factors (such as changes in the environment) can be associated in space and time to the diversification of organismic groups by biogeographical studies. Geophysical as well as climatic changes may, for example, increase diversification rates by creating vacant niches and/or strongly affect dispersal by diminishing distances between suitable habitats (Shimono et al., 2010; Couvreur et al., 2011; Wen et al., 2014). The role of environmental changes on the process of diversification is being investigated in several areas of the world, e.g. the Aegean (Jesse et al., 2011; Szarowska et al., 2014), the Isthmus of Panama (O'Dea & Jackson, 2009; Rodriguez-Reyes et al., 2014; reviewed in Bagley & Johnson, 2014), the Andes and also on the Qinghai-Tibetan Plateau (QTP). The Andes have been particularly well studied in recent years, and this region can act as a good model system for understanding the impact of geological changes on diversification. In fact, this region encompasses the most species-rich terrestrial ecosystems in the world, and has experienced drastic environmental changes in the past (Hoorn et al., 2010). The two main environmental changes in this region were the uplift of the tropical Andes in the Neogene, and the associated major modifications of the flow of the Amazon system. The orogeny of high mountain ranges had a crucial impact on the region’s climate and even influenced the atmospheric circulation of the entire Southern Hemisphere (Antonelli.et al., 2009; Hoorn et al., 2010). Not only the Andean uplift had a striking effect on the diversification of many local taxa *in situ* (Antonelli et al., 2009; Hoorn et al., 2010), but also the closure of the Panama isthmus, which allowed the immigration of pre-adapted taxa (Cody et al., 2010). Based on the distribution of hotspots of diversity on Earth, it is expected that mountain ranges host high biodiversity levels. In fact, many of them are associated with major topographical features. Whether or not the uplift of these ranges alone resulted in organismic diversification remains poorly studied. Also, the role of climate variations and their impact on immigration (and thus the increase of taxa within an area) are often ignored (Favre et al., 2015). Strikingly, other major mountain ranges are not as well studied as the Andes. An example is the highest and largest topological feature on Earth, the Qinghai-Tibetan Plateau (QTP), which is 6.4 times the size of Germany.

1.2 Geological history of the Qinghai-Tibetan Plateau

In the following, I will outline the geological and climatic history of the QTP, because within the scope of this doctoral thesis I investigated the impact of past geological and climatic events on diversification in this region. For this reason, it is necessary to rely on a sound geological and climatic scenario. Hereafter, I present a scenario based on Lippert et al. (2014) and Favre et al. (2015), with further considerations of latest geological evidence. The QTP, with an average elevation of more than 4000 m above sea level (a.s.l.) and covering a region of 2.3 million km^2 (Herzschuh et al., 2010), encompasses several large orogenic systems. Associated with a set of arc-basin systems, which formed in various periods, the QTP is surrounded by the North China, Yangtze, Tarim, and Indian cratons (Pan et al., 2012). The sequence and causes of events related to the uplift of the QTP is hotly debated, but some general scenario can nevertheless be drawn. In general, such a scenario includes 1) the proto-Tibetan Plateau resulting from an early central uplift, 2) the Indian-Asian collision, 3) the northward and southward extension, 4) the orogeny of high mountain ranges, and finally 5) the eastern extension of the plateau (summarised in Lippert et al., 2014 and Favre et al., 2015).

Until recently, it was the common scientific notion that the uplift of the QTP was the consequence of the collision of the Indian subcontinent with Eurasia (c. 55-50 million years ago, Ma, see Favre et al., 2015 and references therein). However, the evaluation of new palaeomagnetic data from the Lhasa terrane by Lippert et al. (2014) revealed a different formation history of the QTP. In latest Jurassic-earliest Cretaceous time (c. 145 Ma) the Lhasa terrane collided with the Qiangtang terrane, followed by a northward subduction of the Neotethys Ocean below the Lhasa terrane (Leier et al., 2007; Zhu et al., 2009). Probably as a result of this collision, fold and thrust fault belts developed on the Lhasa and Qiangtang terrane during the Cretaceous (Kapp et al., 2007a, b; Volkmer et al., 2007; Pullen et al., 2008), which have led to crust thickening sufficient enough to raise Tibet above sea level since c. 110 Ma (Lippert et al., 2014). Thus, it is likely that during Cretaceous-Paleogene time (well before the India-Asia collision) a proto-Tibetan Plateau developed, which began locally in central Tibet and comprised most of central Tibet by 45 Ma (Wang et al., 2008; Rohrmann et al., 2012), or even Late Cretaceous (Kapp et al., 2007a, b; Searle et al., 2011). This is also consistent with the stable oxygen isotope palaeoaltimetry record from Tibet, pointing to an Eocene proto-Plateau (reviewed in Quade et al., 2011). For example, oxygen-isotope-based studies ($^{18}O/^{16}O$) on deposits of the Lunpola basin and isotope analyses of palaeosoil carbonate in the Nima basin, both of which are located at the centre of the QTP (Fig. 1), show that this part was at an elevation of about 4000 to 4500 m for the last 35-26 million years (Rowley and Currie, 2006; DeCelles et al., 2007). The exact palaeoelevation history of the proto-Tibetan Plateau remains unknown, yet, strontium isotope analyses ($^{87}Sr/^{86}Sr$) of marine sediments indicate accelerated erosion at about 40 Ma, implying the existence of massive orographic features in the Tibetan region from this time onwards (Richter et al., 1992; Kump & Arthur, 1999). In addition, fossil pollen extracted from well-dated lacustrine sediments in the Xining basin (located in the northeastern part of the QTP; Fig. 1) suggested the existence of local high-altitude vegetation for about 38 Ma (Dupont-Nivet et al., 2008). All these studies, based on independent proxies, point to a high elevated central QTP during the last 45 Ma or even earlier, and some local high elevation in the North. Therefore, the QTP might be similar to the Altiplano of the Andes, which was uplifted above an oceanic subduction zone independent of a continent-continent collision (Lippert et al., 2014).

Even if the collision of the Indian subcontinent with Eurasia did not cause the uplift of the QTP, it appears to have led to major plateau expansion (Rohrmann et al., 2012). Some studies suggest a more recent collision (about 30 to 20 Ma or younger; Zheng et al., 2000; Aitchison et al., 2007; Wan et al., 2007), but the most accepted theory supports the collision to be around 55 to 50 Ma (Klootwijk et al., 1992; Tapponnier et al., 2001; Dupont-Nivet et al., 2010; Chen et al., 2010; Chatterjee et al., 2013; Lippert et al., 2014). This estimated age is commonly attributed to the entire Himalayan belt, though recent studies depicted an asymmetric collision/subduction. India collided with the area corresponding to the present-day eastern Himalayas at about 55-50 Ma, and the collision progressed westwards until it was completed in its westernmost edge at about 40 Ma (van Hinsbergen et al., 2012; Bouilhol et al., 2013; Chatterjee et al., 2013).

Based upon local evidence, some studies suggest several uplift phases interrupted by less active tectonic periods (Li &Fang, 1999; Tapponnier et al., 2001; Wan et al., 2007), but more global studies on palaeo-altimetry of the QTP provide strong evidence for a continuous growth (Richter et al., 1992) from south to north (Rowley & Currie, 2006; Mulch & Chamberlain, 2006). Consecutively, there was a progressive extension northwards and southwards of the QTP (Mulch & Chamberlain, 2006). A magnetostratigraphic study of Tertiary sediments of the Qaidam basin in the northern QTP (Fig. 1) revealed a main uplift of this area at about 30 Ma (Sun et al., 2005). A recent study gave evidence for the initiation of the uplift of the southern part of the Qaidam basin already at about 35 Ma (Mao et al., 2014). At the northernmost part of the QTP, the western Kunlun Mountains emerged at about 23 Ma (Jiang et al., 2013; Fig. 1), whereas the eastern range started to rise only later, by 15 Ma (Mao et al., 2014). Southern parts of the QTP could have reached an elevation similar to present-day (c. 4600m) at about 15 Ma, which was shown independently by oxygen isotope composition of pedogenic and early diagenetic carbonates, and by the physiognomy of fossil leaves from the Namling-Oiyug basin (southern Tibet, Fig. 1; Spicer et al., 2003; Currie et al., 2005). Contrastingly, a recent re-evaluation of the Namling-Oiyug basin's palaeoelevation yielded an elevation one km higher (c. 5500 m) than now (Khan et al., 2014a). This suggests a local collapse since 15 Ma due to erosion and extensional deflation (Khan et al., 2014a). A similar result was found by Saylor et al. (2009) for the peaks surrounding the Zhada basin (southwestern Tibet, Fig. 1). Followed by the early extension of the QTP, the uplift was then associated with the formation of two high mountain ranges: the Himalayas and the Tianshan (Fig. 1). I would like to comment at this point that in English language literature the term “Himalaya” is used when talking about the mountain range which separates the Indo-Gangetic Plain from the QTP excluding the so called Transhimalaya (extending in a west-east direction parallel to the main Himalayan range at the southern edge of the QTP). However, the term “Himalayas” is used when the Transhimalayan region is included.

The Himalayas are located at the southern flank of the QTP, spanning about 2500 km from the Indus River to the Brahmaputra River, reaching a width up to 250 km (Gansser, 1964; Le Fort, 1975). The higher Himalayas originated rather recently, between 15-10 Ma to present (Amano & Taira, 1992; Tapponnier et al., 2001; Wang et al., 2008), but few local peaks could have reached more than 5000 m a.s.l. already in the Early Miocene (Gebelin et al., 2013). The Hengduanshan (located at the southeastern fringe of the QTP; Fig. 1) is also relatively young. Palaeobotanical and palaeoclimatic data reveal that the Hengduanshan has arisen after the Miocene (about 5.3 Ma onwards), reaching its peak elevation before the Late Pliocene (around 2.6 Ma; Sun et al., 2011). In contrast, the Tianshan is an old mountain system. Its orogenesis was possibly re-activated by the India-Asia collision (Hendrix et al., 1992; Bullen et al., 2003; Charreau et al., 2009b; van Hinsbergen et al., 2011; Tang et al., 2012). The first orogenic phase of the Tianshan occurred during the late Palaeozoic (about 250 Ma), and was followed by a period of tectonic quiescence during which massive erosion took place until the early Cenozoic. The extent of erosion is reflected by the large accumulation of eroded material in the Tarim and Junggar basins in central Asia (Fig. 1; Hendrix et al., 1992; Charreau et al., 2009a).

Altogether, the uplift of the QTP as well as the orogenesis of the Himalayas, the Hengduanshan and the Tianshan had most probably drastic effects on air circulation in Asia.

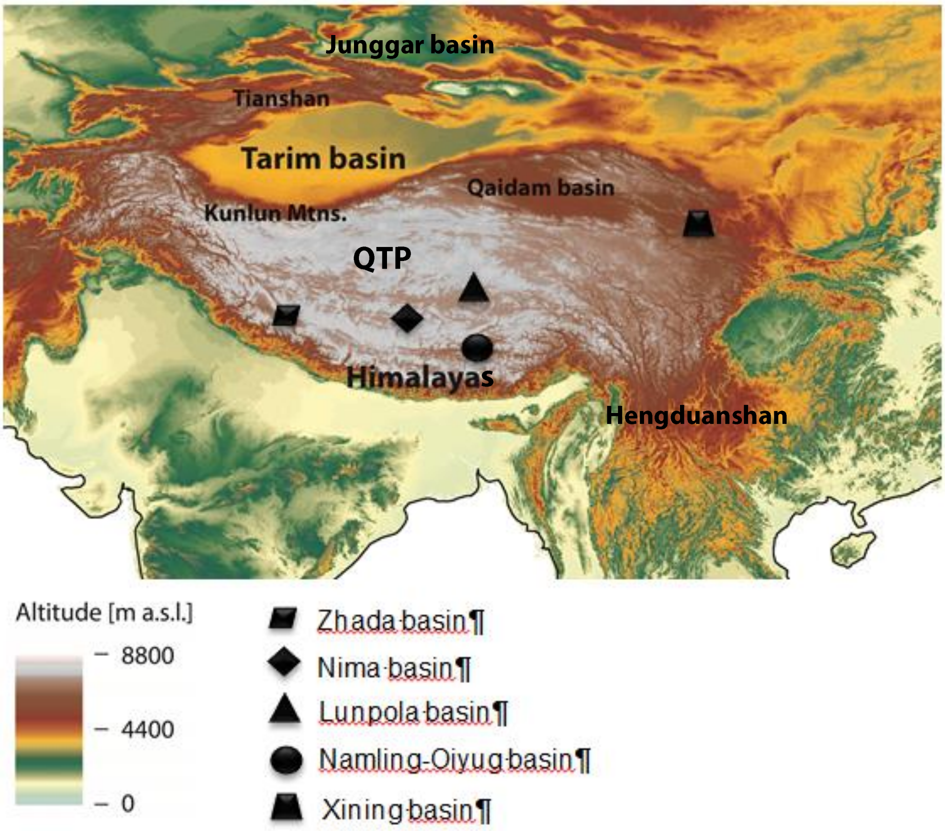

Figure 1. Topographic map of the Qinghai-Tibetan Plateau (QTP) and its surroundings. The map was generated in R (R Core Team, 2013) by using WorldClim data (Hijmans et al., 2005).

1.3 Climatic history of the Qinghai-Tibetan region and its influence on palaeovegetation

Both geological and climatic changes possibly affected organismic diversification in the QTP region. A sound scenario for the succession of climatic changes in that area is therefore also necessary. In the following, I present an overview of climatic changes based upon Favre et al. (2015) with further consideration to latest findings of palaeoclimatology.

Generally, the Earth’s climate is significantly affected by the presence of mountains and plateaus (Kutzbach et al., 1993). Therefore, it is broadly accepted that the uplift of the QTP and the Himalayas had an impact on regional (and worldwide) climate: for example, it played a role in the establishment of the Asian monsoon system and in the aridification of the Asian inland (Manabe & Terpstra, 1974; Wu & Zhang, 1998; Guo et al., 2002; Abe et al., 2013). Strong land-ocean thermal contrasts and also the height and thermal effects of the QTP lead to the Asian monsoon system (Harris, 2006; Chatterjee et al., 2013), although some evidence exists that aerial land-ocean currents were already present before the existence of the QTP in the Eocene (Licht et al., 2014; Shukla et al., 2014). The monsoon is a seasonal wind of which direction is determined by the alternating location of high air pressure zones: either on land during winter (resulting in a dry climate south of the Himalayas), or on sea during summer (causing heavy rainfalls at the southern flank of the Himalayas; Mooley & Parthasarathy, 1984; Chou et al., 2001; Sun & Wang, 2005; Harris, 2006). The regions located north of the Himalayas were progressively in the “rain shadow” of this mountain range, which contributed to the aridification of central Asia and of the QTP (Wan et al., 2007; Wu et al., 2008). Other important factors contributing to Asian inland aridification were global cooling (Miao et al., 2011, 2012, 2013) and the retreat of the Paratethys Sea (Zhang et al., 2007a; Bosboom et al., 2014). By analysing loess deposits in China the onset of the monsoon system could be dated at about 22 to 8 Ma with a progressive intensification since then. Loess deposits require: (1) a sizeable source area that is arid enough to create aeolian particles (arid central Asia and QTP) and, (2) an atmospheric circulation sufficiently powerful to carry the particles (the Asian monsoon system). Therefore palaeomagnetic measurements did not only reveal the age of the loess deposits, but also the age of the monsoon (Guo et al., 2002; Zhisheng et al., 2001). Via recording the magnetism of red-clay it could be also shown that an intensification of central Asia’s aridification started between 12 and 10 Ma (Li et al., 2014a).

It is well-established that the Asian monsoon was enhanced by the Tibetan and Himalayan topography, but recent studies indicate a possible overestimation of the impact of the QTP and found other aspects which might be equally important for the monsoon. For example, the analysis of fossil flora (Quan et al., 2012; Shukla et al., 2014) and the investigation of oxygen isotopes as well as climatic simulations (Licht et al., 2014) point to a pronounced monsoonal regime (distinct wet/dry seasonality) already in the Eocene, at the time of collision. Shukla et al. (2014) could also determine a variation of monsoon strength at that time, which cannot be explained by tectonic drivers. Moreover, based on general circulation models (GCM) it could be shown that the thermodynamic structure of the Asian monsoon (including winds and precipitation) stayed nearly the same when the topographic feature of the QTP was removed in the analysis, except for a narrow Himalaya and adjacent mountain ranges (Boos & Kuang, 2010; Abe et al., 2013). It is likely that the direct correlation of the QTP uplift with local climatic changes is too simple and that a complex combination of different variables (including the QTP uplift) have led to their occurrence, as for example the retreat of the Tarim sea (Bosboom et al., 2014), the varying content of CO_2 in the world's atmosphere (Licht et al., 2014), and global climate shifts like the Eocene-Oligocene transition (Dupont-Nivet et al., 2007) or general global cooling (Jiang & Ding, 2008). In any case, if the causes of the monsoon winds are manifold, strong precipitation gradients are most certainly due to the orographic barrier which is the Himalayan range.

1.4 Modification of Tibetan biotas over time

The geological and climatic changes gradually modified plant communities (see Table 1). Before the collision, Asia was mainly subtropical to tropical (Sun & Wang, 2005; Wang et al., 2013). Using palaeobotanical and lithological data all over China, a study revealed a subtropical humid vegetation zone in northern China, a subtropical to tropical arid to semiarid vegetation zone in central China, and a tropical humid vegetation zone of southern China during the Eocene (Sun & Wang, 2005). Furthermore, at this time a shallow epicontinental sea extended across Eurasia with the Tarim basin forming the easternmost margin of it (Bosboom et al., 2014). From the Eocene onwards the tropical to subtropical forests disappeared in the region of the QTP and the Himalayas, and grasslands, hosting an alpine flora, extended significantly (Sun & Wang, 2005). Nowadays, the southeastern part of China is humid to semi-humid (with forests), while the northwestern part is arid to semi-arid (steppe and desert). Details about typical vegetation per time slice are given in Table 1. These modifications were presumably attended by extinction, speciation, and diversification (reviewed in Wen et al., 2014). The impact of geological and climatic changes on these processes remains poorly understood, and the investigation of a possible correlation between environmental changes and species' diversification requires state of the art methods, which allow to relate phylogenies to space and time.

Table 1. Progressive shift of plant communities due to the uplift of the QTP and the Himalayas, as well as the retreat of the Tarim sea (after Sun & Wang, 2005).

		South China	Central China	North China
Paleogene without Paleocene (56-23 Ma)	**Eocene (56-33.9 Ma)**	humid tropical	subtropical to arid tropical to semiarid tropical	humid subtropical
	Oligocene (33.9-23 Ma)	tropical and humid subtropical	arid subtropical and semiarid subtropical	temperate and humid

		South of QTP	QTP	North of QTP
Neogene (23 Ma to present)	**Miocene (23-5.3 Ma)**	humid forest zone influenced by the Asian monsoon	temperate deciduous forest zone	arid
	Pliocene (5.3-2.6 Ma)	humid forest zone influenced by the Asian monsoon	subalpine forest zone	arid
	Today	humid forest zone influenced by the Asian monsoon	alpine flora, arid	arid

1.5 Studying the impact of environmental changes on diversification and biodiversity patterns

In order to investigate a possible correlation of geological and/or climatic changes with organismic diversification, a full set of complementary analyses for the reconstruction of the spatio-temporal framework of diversification is required (Favre et al., 2015). Setting the temporal framework is commonly done by molecular clock analyses, for example using Bayesian approaches such as implemented in BEAST (Drummond & Rambaut, 2007; Drummond et al., 2012). When dating a phylogenetic tree, one should pay attention to the following major steps (Sauquet, 2013):

(1) Assembling the data set. It is important to gather a reasonable density of sampled taxa, because sample size can have a strong influence on evolutionary relationships (Ives et al., 2007; Garamszegi & Moller, 2010) and consequently on age estimates (Heath et al., 2008; Milne, 2009).

(2) Choosing temporal constraints. Fossil calibration is the most common approach, because fossils are best to define absolute ages (Forest, 2009; Ho & Duchene, 2014). If the fossil record is not sufficient, secondary calibration can be used. To do so, it is necessary to obtain a broader sampling of fossil-rich lineages related to the taxon of interest. This way, the age of the crown group of the fossil-poor group can be estimated. Afterwards this estimated crown group age can be set as a secondary temporal constraint to the more densely sampled, but fossil-poor dataset (Graur & Martin, 2004; Renner, 2005; Forest, 2009). However, secondary calibration should be interpreted with care, because errors from one analysis might be transmitted to the subsequent one (Graur & Martin, 2004; Renner, 2005).

(3) Choosing a molecular clock method and a model for rate variation. For molecular clock dating, the ideal case would be that a global and constant rate of substitution exists for the entire phylogenetic tree. However, it is known that the substitution rate can vary within and between lineages and can fluctuate over time (Rutschmann, 2006). For this reason a large set of methods has been developed, which can be classified as follows: methods that use a molecular clock and a global substitution rate, methods that correct for rate heterogeneity, and methods integrating rate heterogeneity (Rutschmann, 2006; Ho & Duchene, 2014). Each of these steps needs particular care. Generally, divergence time estimation forms the basis for profound biogeographical analyses.

Important components of the spatial framework are the reconstruction of the geographic origin of the taxon and its dispersal routes and directions through time. Both can be deduced from biogeographical analyses. Lately, new approaches have been developed to reconstruct ancestral areas, e.g. the statistical dispersal-vicariance analysis (S-DIVA; Yu et al., 2010) or the dispersal-extinction-cladogenesis (DEC; Ree et al., 2005; Ree & Smith, 2008). Using a three-dimensional step matrix based on a simple biogeographic model, DIVA allows to reconstruct the ancestral distributions in a given phylogeny without any prior assumptions about area relationships (Ronquist, 1997). This approach is attended by inevitable uncertainties in phylogenetic reconstruction and ancestral area optimisation (Nylander et al., 2008; Yu et al., 2010). For example, phylogenetic uncertainties rendered it impossible to recover the origin of *Cyrtandra* J.R. Forst & G. Forst (Gesneriaceae) (Clark et al., 2008). Such limitations might be overcome by further developments of this method, as it was done in S-DIVA (Yu et al., 2010) or Bayes-DIVA (Nylander et al., 2008). In comparison, DEC, which is implemented by LAGRANGE (Ree & Smith, 2008), uses a likelihood framework to deduce the evolution of geographical ranges and incorporates divergence times and allows constraining connections between areas at specific time slices (Forest, 2009). Separately or in concert, these approaches have often yielded reliable results (e.g., Smith & Klicka, 2010; Nauheimer et al., 2012; Grudinski et al., 2014b). For example, by using DEC and S-DIVA, Zhang et al. (2014) inferred the origin of *Myricaria* Desv. (Tamaricaceae) to be located near the QTP and the Himalayas, where orogenic processes might have triggered its diversification *in situ*. From there, *Myricaria* dispersed along the mountain ranges throughout Eurasia. However, many studies investigating the role of the uplift of the QTP on plant diversification, either lack molecular dating or/and biogeographical analyses (e.g., Wang et al., 2005; Wang et al., 2009; Yunfa et al., 2010; Favre et al., 2010), rendering the association between environmental changes and diversification rather speculative. Nevertheless to date a growing number of studies involves both, the reconstruction of a temporal as well as a spatial framework (e.g., Mao et al., 2010; Zhang & Fritsch, 2010; Sun et al., 2012; Gao et al., 2013; Lei et al., 2014). Following the temporal and spatial background provided by a biogeographical analysis (which putatively allows to associate environmental changes and diversification), it is also possible to investigate the influence of climate on diversification more in detail by characterising the evolution of climatic niches in a certain plant clade.

Hutchinson (1957) defined a niche as the composition of biotic and abiotic factors in which a species is able to endure and keep a steady population size. Hutchinson (1957) also differentiated the fundamental from the realised niche. The fundamental niche comprises only the abiotic factors in which a species can exist, whereas the realised niche describes the ability of a species to exist while interacting with other species (Hutchinson, 1957; Wiens & Graham, 2005). Among other definitions of a niche (e.g., Grinnell, 1917; Elton, 1927) the one described above is the most common. In the last twenty years numerous opportunities arose for investigating the evolution of climatic tolerances and understanding how historical climatic changes contributed to shaping extant patterns of biodiversity (e.g., Graham et al., 2006; Schnitzler et al., 2012). For example, combining georeferenced occurrence data from natural history collections with high-resolution climate data (such as from WorldClim, Hijmans et al., 2005), Species Distribution Modelling (SDM) allows to quantify the climatic niche of a species as well as to predict its potential geographical distribution (Warren et al., 2008; Elith & Leathwick, 2009). There exists a wide range of methods to conduct SDM analyses. Some methods include regression-based techniques, such as generalized linear models (GLMs) or generalised additive models (GAMs), others include machine learning techniques, such as the maximum entropy model (MaxEnt; Elith et al., 2006; Graham et al., 2008). Machine learning algorithms are designed to identify patterns in large datasets and to generalise them (Mitchell, 1999). Therefore, the latter approach is more widely and more successfully used to understand patterns of biodiversity (e.g., Svenning et al., 2010; Schmidt-Lebuhn et al., 2015) and to predict distributions of species under past (e.g., Svenning et al., 2008; Nogues-Bravo, 2009) or future (e.g., Engler et al., 2011; Bystriakova et al., 2014) environmental circumstances (reviewed in Miller, 2010). Furthermore, it is possible to study (climatic) niche evolution throughout the evolutionary history of a clade as lineages diverge (Evans et al., 2009; Wiens et al., 2010; Koecke et al., 2013), for example by using relative disparity plots (Harmon et al., 2003).

Increasingly important are also measures of diversification rates (extinction rate subtracted from speciation rate; Ricklefs, 2007) to determine morphological or ecophysiological traits putatively relevant for diversification (e.g., Ackerly, 2009; Mahler et al., 2010). For example, Silvestro et al. (2014) found the Crassulacean acid metabolism and the water-impounding tank habit to correlate with increased net diversification in Bromelioideae. Altogether, these methods allow a detailed evaluation of the impact of climatic changes on diversification. Despite the suitability of the QTP and its surrounding areas to investigate the relationship between past climatic changes and diversification rate or niche shifts (see 1.3), approaches like mentioned above to analyse an organism's niche have only rarely been conducted against the background of geological and climatic changes in the QTP region.

1.6 *In situ* mechanisms of diversification and factors favouring species richness in the QTP region

The drastic geophysical and climatic changes mentioned above likely caused the development of numerous different habitats in the QTP region (ranging from tropical and seasonal rainforests at the southeastern flank of the QTP to Tibet-Qinghai cold and highland vegetation; Sun & Wang, 2005). Furthermore, extensive uplift might have promoted the fragmentation of these habitats (Xu et al., 2010). Together, the occupation of vacant niches and allopatric speciation could have led to a burst of speciation (reviewed in Wen et al., 2014). Today, the QTP and the neighbouring regions of the Himalayas and southeast China harbour a remarkable species richness and an abundance of endemics. The Plateau itself contains over 4300 plant species of 1174 genera in 189 families, 25% of its species being endemic (Chen et al., 2005a). Another very significant area for plant diversity are the Hengduan Mountains (north-west Yunnan, western Sichuan, south-east Tibet). This area alone harbours about 12000 plant species, 29% of which are endemic (Myers et al., 2000; Lopez-Pujol et al., 2011). As a comparison, Europe only has about 11500 plant species (Lopez-Pujol et al., 2011) for an area being 12.7 times bigger. In total, there are four biodiversity hotspots neighbouring the QTP (Fig. 2; homepage of Conservation International, *www.conservation.org*, 2012; homepage of Critical Ecosystem Partnership Fund CEPF, *www.cepf.net,* 2014; Sloan et al., 2014):

1. Mountains of southwest China (southeastern part of the QTP, central and southeastern China and northern Myanmar): 12000 vascular plant species, of which 3480 are endemic.
2. The Himalayas (from central Nepal to Myanmar, comprising southeast Tibet, Sikkim, North Bengal, Bhutan and northeast India): about 10000 vascular plant species, of which 3160 are endemic.
3. Indo-Burma (eastern India, southernmost China, Myanmar (excluding the northern tip), Thailand (excluding the southern tip), Cambodia, Laos and Vietnam): altogether, 13500 vascular plant species, of which 7000 are endemic.
4. Mountains of central Asia (Pamir and Tianshan range): about 5500 vascular plant species, of which 1500 are endemic.

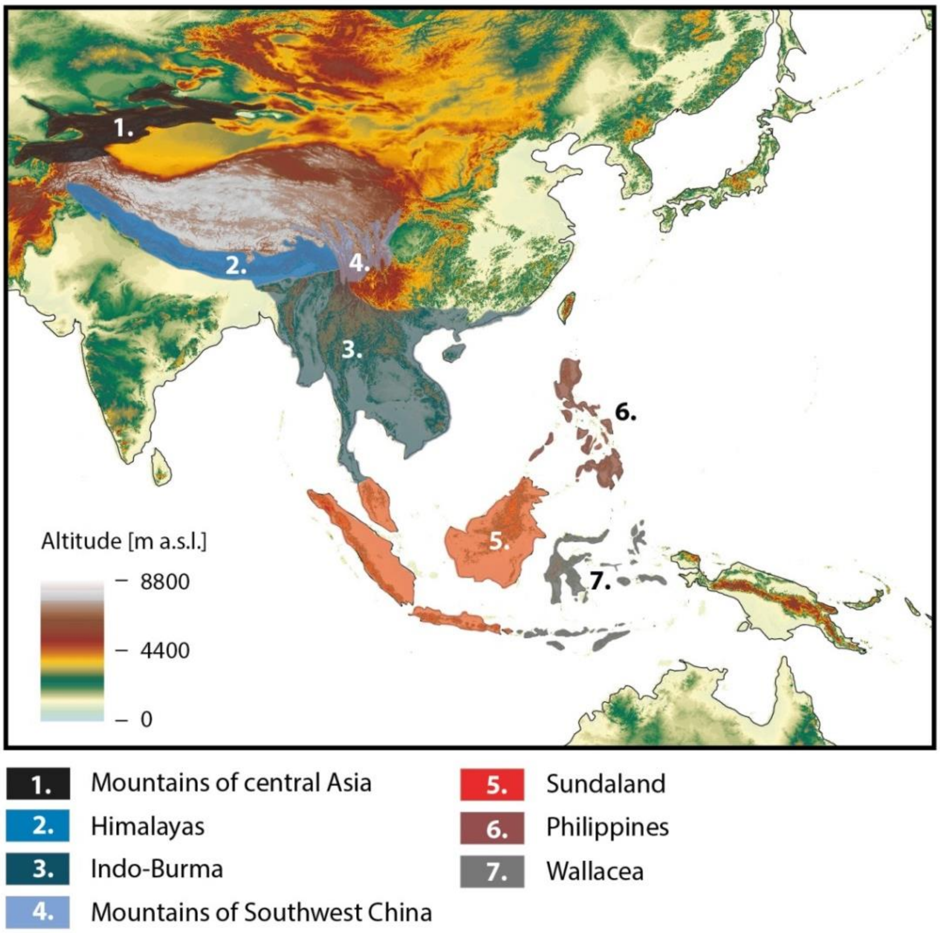

Figure 2. Biodiversity hotspots neighbouring the QTP, and of Southeast Asia (after Sloan et al., 2014). The map was generated in R (R Core Team, 2013) by using WorldClim data (Hijmans et al., 2005).

Many authors suggested that altitudinal zonation plays an important role in forming plant communities and that the uplift of the QTP could have triggered speciation by providing various vacant niches (Wang et al., 2005; Shimono et al., 2010; reviewed in Wen et al., 2014). Vacant niches are defined by Lawton (1984) as "evolutionarily novel suites of environmental conditions (biotic or abiotic) for which no species in a region are well adapted". It is well known that if many niches are vacant, adaptive radiation might occur as a response to these ecological opportunities (Lekevicius, 2009 and citations therein), which might play a crucial role in the establishment of high biodiversity levels (Benton, 1996). Predominant drivers for evolution and diversification of organisms could either be abiotic factors (e.g., climatic and tectonic events) and/or biotic factors (e.g., inter- or intraspecific competition and predation; Antonelli & Sanmartin, 2011b). Experimental laboratory work has shown a correlation between ecological opportunity and adaptive radiation. Confronted with an array of ecological opportunities (in form of spatial structure), the aerobic bacterium *Pseudomonas fluorescens* diversified morphologically, and when no ecological variation was applied, no divergence occurred (Rainey & Travisano, 1998). Of course, reaching a similar conclusion is less straightforward in nature, because more variables co-occur, and because animals and plants have a much longer generation time. Nevertheless, up-to-date methods allow to generate a temporal and spatial framework for phylogenetic reconstructions, which enables the investigation of a potential correlation between environmental changes and diversification processes (Favre et al., 2015). Such approaches have already contributed to the revelation of some relevant factors for diversification in the QTP region (reviewed in Wen et al., 2014). It is important to note that Wen et al. (2014) reviewed evidence from phylogenetic and biogeographical studies in plants against the background of the outdated geological history of the QTP, in which the existence of a proto-Tibetan Plateau was not taken into account. It was assumed that geological changes occurred after the Indian-Asian collision at about 55-50 Ma in several major uplift periods, but the uplift of central Tibet started already at about 110 Ma and was rather a continuous process (Lippert et al., 2014). Of course, this fact must be considered when interpreting the results. However, the collision of India with Eurasia might have caused a major expansion of the plateau, which resulted also in drastic environmental changes during the past 50 million years (Rohrmann et al., 2012; Lippert et al., 2014). To date, this period of time was in the focus of researchers investigating the QTP, thus, most of the reviewed findings of Wen et al. (2014) are still valid.

In their review, Wen et al. (2014) categorised six not mutually exclusive mechanisms that are of importance for diversification on the QTP. First, high elevation habitats (also known as sky islands) can be isolated from each other by low elevation habitats, which might favour allopatric divergence (He & Jiang, 2014) via genetic drift or differential selective pressure leading to local adaptation and ultimately to speciation (Hoskin et al., 2005). A striking example of diversification by allopatric speciation was found in the three genera *Soroseris* Stebbins, *Stebbinsia* Lipsch., and *Syncalathium* Lipsch. (all Asteraceae), which are all restricted to high screes of the QTP. In fact, diversification is associated in time with the fragmentation of scree habitats during the uplift (Zhang et al., 2011). The second mechanism is due to climatic changes such as glacial and interglacial climatic oscillations in the Quaternary. In this case, allopatric divergence occurs intermittently, supported by climate variations. By investigating the phylogeography of *Spiraea alpina* Pall. (Rosaceae), Khan et al. (2014b) showed that this species withstood glaciations in three different glacial refugia, resulting in strong intraspecific divergence. Other processes, such as hybridisation and introgression (third mechanism) might lead to the development of new lineages (Abbott, 1992; Arnold, 2006; Kim et al., 2008; Ren et al., 2012), as suggested for the outstanding radiation of *Rhododendron* L. (Zha et al., 2010), *Meconopsis* Vig. (Yang et al., 2012) or *Rheum* L. (Wan et al., 2014). The fourth mechanism depicts the role of morphological innovations in response to selective pressure. Key innovations, such as the cushion habitus (Wang et al., 2004) or the transparent “glasshouse” morphology (Ohba, 1988; Sun et al., 2012), might provide a selective advantage for a taxon and lead to rapid radiation. Under similar selective pressure likewise key innovations might evolve independently in several lineages, illustrating the importance of natural selection in shaping biodiversity (Liu et al., 2013; Wen et al., 2014). Often neglected when investigating diversification on and around the QTP, biotic interactions (fifth mechanism) such as pollinator-mediated reproductive isolation (reviewed in Sun et al., 2014), appear to have been crucial for the diversification of *Pedicularis* L. in the

Hengduan Mountains (Eaton et al., 2012). Finally, polyploidy (the increase in genome copy number, the sixth mechanism) is known for driving speciation because it might instantly isolate lineages of different ploidy levels by the establishment of reproductive barriers (Wood et al., 2009). Studies investigating the role of polyploidisation are extremely rare for QTP taxa, and only a few examples of polyploidy-driven diversification exist, such as in *Oxytropis* DC. species (Fabaceae). In this genus, polyploidy might have partially influenced sympatric speciation (Liu et al., 2011). The interplay of all these six mechanisms forms the basis for a spectacular richness of botanical diversity in the QTP and adjacent regions, but biological interchanges with other parts of the world might also contribute to the remarkable species richness. The role of biological interchange still remains poorly studied in the region of the QTP.

1.7 Biological relationships between the QTP and other parts of the world

To date, not much is known about biogeographical connections between the QTP and nearby biodiversity hotspots of Southeast (SE) Asia as well as the Southern Hemisphere in general. Because similar floristic elements occur throughout Asia, there is evidence for dispersal of mountain plants to (or from) SE Asia from the Himalayan region, East Asia, and Australasia (van Steenis, 1964). This is also true for lowland plants (Morley, 1998 and references therein). Nevertheless, molecular and biogeographical studies to investigate these relationships are scarce. One example is an investigation of *Nannoglottis* Maxim. (Asteraceae), which is endemic to the QTP. A phylogenetic analysis and divergence time estimation showed that *Nannoglottis* was the first diverging lineage of the Astereae, a tribe with a Southern Hemisphere origin. To explain this unusual biogeographic link, long-distance dispersal was proposed using SE Asia as a stepping stone (Liu et al., 2002). In contrast, more is known about the role of the QTP as a sink or source area for plant taxa in the Northern Hemisphere. The flora of the QTP, the Himalayas and adjacent regions show clear affinities to the flora of the temperate regions of the New and Old World, the Middle East, and also Indochina (Fig. 3; Gupta, 1964, 1982; Rau, 1974; Hajra & Rao, 1990; Zhu & Roos, 2004; Xu et al., 2014). Biogeographical analyses so far have revealed three different migration and dispersal patterns associated with the QTP (Jia et al., 2012).

The first scenario depicts a QTP origin for many plant genera (with *in situ* diversification) and subsequent colonisation of the Northern Hemisphere (e.g., Zhang et al., 2007b, 2009; Xu et al., 2010; Zhang et al., 2014). Such a pattern was observed in *Lagotis* J.Gaertn. (Plantaginaceae), which originated and diversified in the QTP during the Miocene, then dispersed several times independently to the highlands of Central Asia and further on to the Arctic. In this case, the “Central Asian Highland Corridor” might have acted as a major route for floristic exchange between the QTP and the Arctic (Li et al., 2014b). Furthermore, Donoghue & Smith (2004) found evidence that numerous temperate forest taxa originated and diversified in eastern Asia, and then dispersed to Europe and/or North America especially during the past 30 million years, suggesting Beringia (a former land bridge connecting eastern Asia with western North America) as the primary migration route between the Old and the New World, whereas the North Atlantic land bridge (between western Europe and eastern North America) was of less importance. In the second scenario, the origin of taxa was located outside the QTP and their diversification occurred only after their ancestors’ arrival in the QTP area (e.g., Liu et al., 2002; Yue et al., 2009). For example, molecular and biogeographical analyses of Mandragoreae (Solanaceae) showed two independent dispersal events from New World lineages to the Mediterranean-Turanian region and the QTP respectively. After arrival in Eurasia, the Mandragoreae diversified (Tu et al., 2010). Finally, the third scenario suggests that the QTP is a “refugium” (among others scattered throughout the Northern Hemisphere) for Tertiary relict floras (Milne & Abbott, 2002). This scenario is based upon the observation that some plant genera descended from ancient continuous Arcto-Tertiary or Madrean-Tethyan geofloras, which today display a disjunct distribution in the Northern Hemisphere, most likely because their distribution area declined due to global climatic changes (Sun, 2002a, b; Wen & Ickert-Bond, 2009; Mao et al., 2010). Knowledge about the existence of these continuous geofloras is based upon reconstructions of Tertiary fossil floras (66 to 2.6 Ma; Axelrod, 1958). *Pistacia* L. (Anacardiaceae), for example, shows a disjunct distribution throughout the Northern Hemisphere. Xie et al. (2014) found that the disjunction between America and Mediterranean Europe corresponds to Axelrod’s Madrean-Tethyan hypothesis (Axelrod, 1958), which implies vicariance between those regions in the early Tertiary, while the disjunction between the European and Asian floras can be explained by the Turgai Strait, which separated these two regions from the Paleocene to the early Oligocene (Legendre & Hartenberger, 1992).

All three biogeographic patterns together reveal that the QTP could not only have acted as a source area for the biodiversity of the Northern Hemisphere, but also as a sink for taxa from other regions (compare Fig. 3 for a summary of the biogeographic relationships between the QTP and other parts of the Northern Hemisphere). Nevertheless, further investigations using up-to-date biogeographical analyses and adequate study groups are highly needed to understand the impact of the QTP for worldwide biodiversity, especially with respect to the Southern Hemisphere.

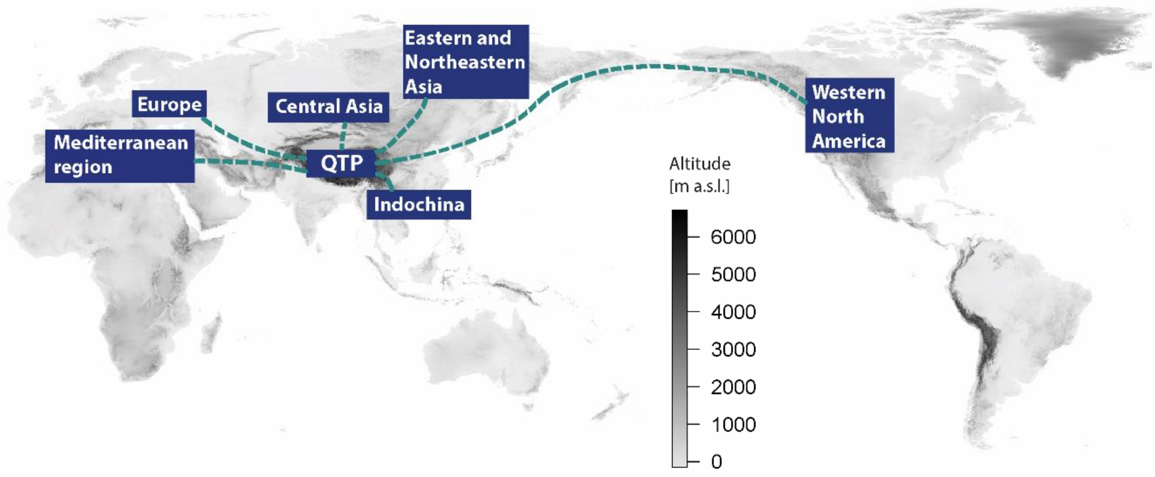

Figure 3. Regions for which strong affinities to the flora of the QTP and adjacent regions and/or dispersal have been observed (reviewed in Wen et al., 2014). Note that dispersal routes between the QTP and the Southern Hemisphere are still unknown. The map was generated in R (R Core Team, 2013) by using WorldClim data (Hijmans et al., 2005).

1.8 Study groups

When investigating the effect of geological and climatic changes on organismic diversification in the region of the QTP, the selection of appropriate taxa is of crucial importance. Favre et al. (2015) suggested some rationale to be considered when choosing taxa to address diversification on the QTP. A sound geological and climatic framework is needed (as described above), which can be compared with evolutionary patterns and processes. Non-endemic taxa should be preferred over exclusively endemic taxa because they allow a direct comparison between QTP and non-QTP lineages. Ideal system taxa have a distribution extending beyond the QTP. For these, it is necessary to gather samples from all over their distribution range. In addition, knowledge about the age of the studied model groups as well as the availability of fossils will help to infer the evolutionary history of lineages. Unfortunately, a sufficient fossil record is available only in a very few cases, therefore, secondary calibration for divergence time estimation might be considered. Furthermore, the ecological preference of a taxon should be taken into account. The uplift and associated climate changes might have had drastically different impact on organisms of contrasting ecology. Thus, general conclusions regarding the role of the uplift of the QTP for diversification will be achieved only by comparing unrelated taxa with contrasting ecology.

In this doctoral thesis, I aimed at addressing as many of these criteria as possible for choosing appropriate study taxa. Many plant genera that have been studied in the QTP region to date are alpine, therefore, I here investigate non-alpine clades in order to complement the pool of studies available. For this purpose, *Agapetes* D. Don ex G. Don and *Vaccinium* L. (Ericaceae) as well as *Tripterospermum* Blume (Gentianaceae) were chosen. They predominantly occur below tree line.

1.8.1 *Agapetes* and *Vaccinium* (Ericaceae)

Agapetes D. Don ex G. Don and *Vaccinium* L. (Fig. 4), two closely related genera, form a good model system because they have a high diversity in the QTP and the Himalayan region, and are distributed beyond the QTP. Moreover, their preferred habitats are usually located well below the alpine thermal belt. Studying their diversification in relation to the uplift of the QTP might therefore uncover different patterns than those already found for the better-studied alpine plants (e.g., Liu et al., 2014 and citations therein).

Subtropical *Agapetes* (c. 80 species) is confined to Asia including Sikkim, Bhutan, SE Tibet, Assam, Myanmar and West Yunnan. Only a few species occur in Thailand and in Peninsular Malaysia (Watthana, 2001; Stevens et al., 2004; Ruizheng & Stevens, 2005b). In contrast, *Vaccinium* (c. 450 species, Lens et al., 2004), the only predominantly temperate genus among Vaccinieae (Kron et al., 2002), is sub-cosmopolitan and occurs mainly throughout the Northern Hemisphere and in tropical mountain ranges. It is absent from Australia, New Zealand and most of Africa (only a few species in South Africa and Madagascar; Powell & Kron, 2002). The berries of many *Vaccinium* species (incl. those that are cultivated) have a high sugar content, which is characteristic for fruit dispersal via birds and mammals (Stiles, 1980). It is likely that these dispersal vectors are also important for *Agapetes* (Vaccinieae, Luteyn, 2002b; Luteyn & Pedraza-Penalosa, 2012).

Agapetes and *Vaccinium* are morphologically very similar. They are distinguished by the size and shape of their corolla (*Agapetes*: corolla 0.5 to 6 cm, tubular or cylindric, rarely urceolate; *Vaccinium*: corolla up to 1 cm, urceolate or campanulate, rarely tubular; Stevens, 1985), their inflorescence (*Agapetes*: fewer than 15 flowers; *Vaccinium* of Southeast Asia: more than 10 flowers; Stevens, 1985) and their habit (*Agapetes*: usually epiphytic; *Vaccinium*: usually terrestrial, Mingyuan et al., 2005). Because these traits are sometimes shared between these genera (and other genera in tribe Vaccinieae), the delineation of most Vaccinieae genera is poorly understood (Stevens, 1985; Kron et al., 2002). Well-resolved molecular phylogenies of Vaccinieae are lacking: based upon a small sampling size, most genera (including *Agapetes* and *Vaccinium*) appear to be polyphyletic (Kron et al., 2002; Powell & Kron, 2003). For example, Kron et al. (2002) and Stevens (2004) showed that *Agapetes* species from the SE Asian mainland and species from Australasian and Pacific islands (originally placed in *Agapetes*) did not form a monophylum. As a result, the taxonomy of Oceanic "*Agapetes"* species was modified to raise a section of *Vaccinium* (*V*. sect. *Pachyantha*) and a subgenus of *Agapetes* (*A.* subg. *Paphia*) to the generic level, namely *Dimorphanthera* (Drude) J.J.Sm and *Paphia* Seem. respectively (Stevens, 1974; Stevens, 2004; Venter & Munzinger, 2007). Morphological similarities and some phylogenetic indications (see phylogenies in Kron et al. 2002) suggest a close relationship between *Paphia* and *Dimorphanthera* (Venter & Munzinger, 2007).

For Vaccinieae, with a focus on *Agapetes* and *Vaccinium*, this doctoral thesis aims to shed more light on the complex relationships within the tribe by including more species than in former studies (e.g., Kron et al., 2002; Kron & Luteyn, 2005). Furthermore, divergence time estimation in combination with up-to-date biogeographical analyses help to refine the results of Kron & Luteyn (2005), because setting a temporal framework allows more accurate conclusions about the development of the present-day distribution pattern of Vaccinieae. The sub-cosmopolitan distribution of the tribe (particularly *Vaccinium*) enables the investigation of floristic relationships of the QTP with other parts of the world. The study on this tribe is supplemented by earlier work on Ericaceae, such as a biogeographical analysis (DIVA, without dating of the phylogenetic tree; Kron & Luteyn, 2005) and divergence time estimation of the family (Schwery et al., 2014).

Figure 4. Pictures of A. *Agapetes brachypoda* Airy Shaw, B. *Agapetes serpens* Wight (Sleumer), C. *Vaccinium delavayi* Franch., and D. *Vaccinium bracteatum* Thunb. Pictures taken by Adrien Favre and Sabine Matuszak.

1.8.2 *Tripterospermum* (Gentianaceae) and its relatives

Most biogeographical studies on plants in the QTP's region focused either on endemics, or on members of the alpine tundra (reviewed in Wen et al., 2014; Favre et al., 2015). In addition, studies which found an Asian mainland origin for plant genera which dispersed to SE Asia later on, have focused on lowland groups so far (e.g., Nauheimer et al., 2012; Thomas et al., 2012). Thus, the role of the QTP as a source or sink area for montane taxa remains largely unknown. Because of its widely extending Asian distribution (from Japan and China, incl. the surroundings of the QTP, to Southeast Asia), and its ecology (affinity with mountain flora), *Tripterospermum* Blume represents a good model system to investigate floristic exchange between biodiversity hotspots associated with the QTP and those of SE Asia.

Tripterospermum is part of the most speciose tribe of Gentianaceae, Gentianeae (20 genera with ca. 1000 spp., Struwe et al., 2002), which is divided in two subtribes, Swertiinae and Gentianinae, both being well-defined by congruent morphological and genetic data (Struwe et al., 2002; Yuan et al., 2003; Favre et al., 2010). The phylogenetic relationships within Gentianinae were uncovered by a previous study (Appendix 1; Favre et al., 2014). *Tripterospermum* was sister to the new genus *Sinogentiana* Favre & Yuan, both of which were related to *Metagentiana* T.N. Ho & S.W. Liu and *Crawfurdia* Wallich. The new genus *Kuepferia* Adr.Favre constituted the earliest diverged genus among *Crawfurdia*, *Metagentiana, Sinogentiana,* and *Tripterospermum*. *Gentiana* L. was sister to these five genera (Favre et al., 2014; Fig. 5). In addition, species coverage within *Tripterospermum* was near-complete, including several newly described species from another previous study (Appendix 2; Favre et al., 2013).

Apart from *Gentiana* (sub-cosmopolitan temperate genus, Struwe et al., 2002) and *Kuepferia* (Asian, alpine), all other genera of Gentianinae occur in sub-alpine habitats of subtropical mountains of Asia. Particularly, *Crawfurdia* and *Tripterospermum*, two easily distinguishable genera of twining vines (Ho & Pringle, 1995a, b; Favre et al. 2010), grow in montane forests and bamboo thickets (Ho & Pringle, 1995a, b; Ho et al., 2002; Favre et al., 2014). In contrast, *Metagentiana* and *Sinogentiana* mostly occur in more open habitats in the montane to sub-alpine zones of subtropical mountains in the biodiversity hotspots of South-Central China and Indo-Burma (Ho & Pringle, 1995a; Myers et al., 2000; Ho et al., 2002; Favre et al., 2014). *Tripterospermum* has the broadest distribution, occurring additionally in subtropical to tropical mountain regions of Sundaland and Wallacea (Murata, 1989). *Crawfurdia*, *Metagentiana*, *Sinogentiana* and *Kuepferia* produce capsules with winged seeds (Murata, 1989; Ho & Pringle, 1995a; Ho et al., 2002; Favre et al., 2014), indicating an adaptation to wind dispersal (Davitashvili & Karrer, 2010). *Tripterospermum* is the only genus of Gentianinae producing not only capsules (only a few species), but also berries (the vast majority of species). For the berry-producing species, dispersal vectors are unknown.

For *Tripterospermum*, this doctoral thesis aims to uncover its origin and dispersal routes among the adjacent areas of the QTP and SE Asia. Another aim is to investigate the impact of geological and climatic changes in the QTP's region on the diversification of Gentianinae (except *Gentiana*). To do so, we study the climatic divergence between *Crawfurdia, Kuepferia, Metagentiana, Sinogentiana* and *Tripterospermum*, and analyse their niche evolution through time.

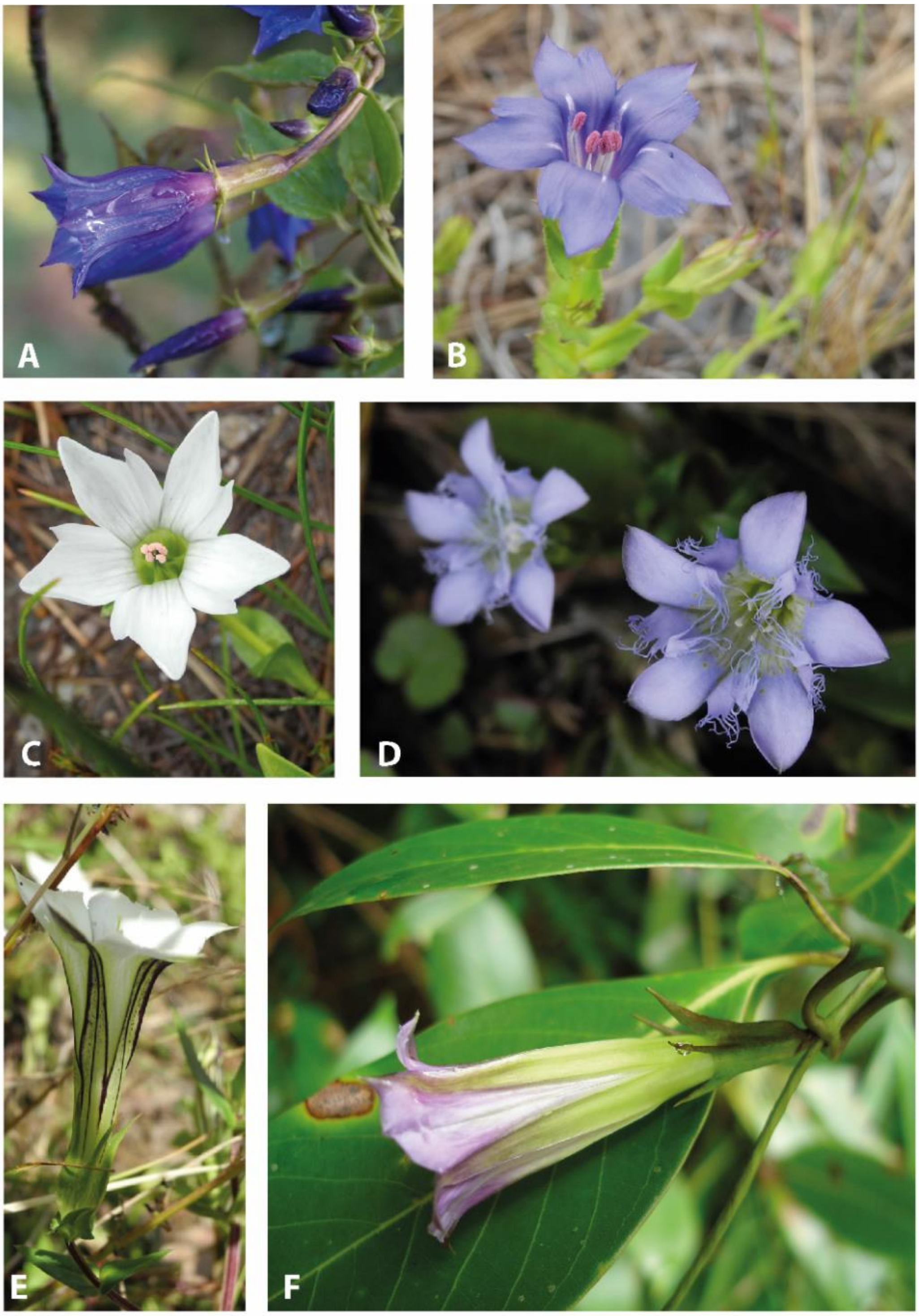

Figure 5. Pictures of A. *Crawfurdia speciosa* Wall., B. *Metagentiana serra* (Franch.) T.N. Ho, S.W. Liu & Shi L. Chen, C. *Kuepferia otophoroides* (Harry Sm.) Adr.Favre, D. *Gentiana panthaica* Burkill, E. *Sinogentiana striata* (Maxim.) Adr.Favre & Y.M. Yuan, and F. *Tripterospermum cordatum* (Marquand) Harry Sm., taken by Adrien Favre.

1.9 Thesis outline: questions and hypotheses

This doctoral thesis aims to answer the following questions:

(1) Was the QTP region a source and/or a sink area for species diversity of non-alpine plants? This question will be addressed by inferring the origin and dispersal history of the sub-alpine plant genera *Agapetes* and *Vaccinium* (Ericaceae; chapter 2) as well as *Tripterospermum* (Gentianaceae; chapter 3), using state-of-the-art biogeographical methods. If the origin of the above taxa was located in the QTP and *in situ* diversification was followed by the colonisation of other parts of the world, then the QTP would have acted as a source area. This result would be in agreement with other studies (e.g., Donoghue & Smith, 2004; Li et al., 2014b). If, in contrast, pre-adapted lineages colonised the QTP multiple times from other areas, then the QTP would have acted as a sink area. This latter result would be in contrast with the usual patterns found for alpine plants (reviewed in Wen et al., 2014). *In situ* diversification in the QTP region could either have played a minor role for toal species diversity or could have most contributed to species diversity if the number of newly evolved species exceeded the number of incoming lineages. In addition, a deviation from the patterns usually found for alpine plants, in which the QTP region acted as a source area, would show the importance of studying taxa with different ecological preferences to understand the impact of the QTP on biodiversity in its entirety.

(2) Did the diversification of *Agapetes, Vaccinium* and *Tripterospermum* temporally and spatially coincide with the uplift of the QTP and associated climate changes? To answer this question, molecular phylogenies will be reconstructed in combination with divergence time estimation and biogeographical analyses. The dated phylogenetic reconstructions will be compared with the geological and climatic scenario described above (see 1.2 and 1.3; chapter 2 and 3) to determine possible correlations. If the diversification of these taxa occurred in the QTP at the same time as major geological or climatic changes, then this would suggest there could be a potential causal relationship between environmental changes and diversification. Nevertheless, if there was temporal coincidence, then still other factors not investigated could be the causal triggers. A possible correlation would, for example, support the assumption that ecological variation and the formation of vacant niches triggered diversification in the form of adaptive radiation (Rainey & Travisano, 1998; reviewed in Lekevicius, 2009). Such a result would be in line with many other studies (reviewed in Wen et al., 2014). If in contrast, immigration (and not *in situ* diversification) dominated, or if the timing of diversification was too old (110 Ma and more) or too young (few million years), then the uplift alone could not explain diversification. In this case, other triggers might have contributed to diversification, for example, climate oscillations during the quaternary, or biological interactions (compare 1.6). Such a result would contrast with most studies performed to date with regard to diversification and uplift of the QTP (reviewed in Wen et al., 2014), and would underline the need of complementary analyses to investigate the role of the uplift on diversification.

(3) Did climatic niche evolution or conservatism play a more important role for diversification of the different genera of Asian Gentianinae with respect to the QTP's uplift and associated climatic changes? What was the impact of some newly evolved morphological traits within the tribe on diversification? To answer these questions a wide spectrum of methods will be used (chapter 4). Species Distribution Modelling as well as analysing raw data of different climatic variables and altitude will allow to define the climatic niche of *Crawfurdia, Kuepferia, Metagentiana, Sinogentiana* and *Tripterospermum*, and to predict the genera's potential geographic distribution (chapter 4). If little niche overlap in the climatic niche tolerances was detected, then this would strengthen the hypothesis that the slight fluctuations in temperature over the year in subtropical to tropical mountain systems select for narrow climatic tolerances of organisms. This might be because of nearly constant climatic gradients along the slopes, which provide great opportunities for climate-based geographical isolation, divergence and speciation (Janzen, 1967; Ghalambor et al., 2006). This result would be in line with many other studies (e.g., Kozak & Wiens, 2007; Bernal & Lynch, 2008). If a large niche overlap among the Gentianinae was found, this would show that species richness along elevational gradients may be also dependent on other factors such as the evolutionary history of species assemblages (Lomolino et al., 2010), because different rates of speciation might occur at different altitudes (Weir, 2006; Fjeldska et al., 2012). Furthermore, stochastic character mapping will allow the reconstruction of the evolution of habitat preferences of Asian Gentianinae. In parallel, the investigation of disparity through time plots of the five different genera will provide insights into their climatic niche evolution with respect to climatic (caused by geophysical) changes in the QTP's region. It will be possible to detect whether niche conservatism, evolution or an interplay of both played a role for Gentianinae. If environmental changes (due to the uplift of the QTP) triggered diversification (as proposed by many studies focusing on the past 50 Ma; reviewed in Wen et al., 2014), niche evolution over time would be expected, because rapid species diversification might be associated with accelerated climatic niche evolution among species (Kozak & Wiens, 2010). In contrast, if climatic niches were predominantly conserved in a changing environment, then this would increase the risk of local extinction of some populations given that they would not be able to track their optimal climatic range (Wiens et al., 2010; Hoorn et al., 2013). In case of niche conservatism, which describes the tendency of species to maintain already existing niche traits (Wiens et al., 2010), environmental changes would trigger species diversification mainly by separating populations from each other allowing allopatric speciation (Wiens, 2004). Although the investigation of the climatic niche allows a profound understanding of relationships between organisms and their environment (also over time), this approach has not been applied against the background of the QTP's uplift so far. Finally, we will investigate the influence of key innovations (morphological traits) on the diversification of Asian Gentianinae by estimating speciation and extinction rates using the binary state speciation and extinction approach (BiSSE). Newly evolved morphological features could enable plants to inhabit new habitats by adaptation or they could act as key innovations by offering the ability to rapidly diversify after environmental changes occurred (Simpson, 1953; Liem, 1973; Hodges & Arnold, 1995). For example, *Halenia* Borkh. (belonging to Swertiinae, which is the sister tribe of Gentianinae; Gentianaceae) comprises species with nectar spurs and some without. Von Hagen & Kadereit (2003) found an increased diversification rate only for species with spurs after their arrival in Central and

South America (coming from East Asia). Therefore, nectar spurs function as key innovation, possibly due to the presence of appropriate pollinators in the newly occupied region. Finally, this approach might contribute to the detection of beneficial traits of plants in the QTP's environment.

My thesis addresses the above mentioned questions as follows:

In chapter 2 "Transcontinental dispersals increased in the Southern Hemisphere after Miocene cooling: molecular dating and biogeography of *Agapetes*, *Vaccinium* and their relatives (Vaccinieae, Ericaceae)", the phylogenetic relationships within Vaccinieae were investigated, including c. 67% of the total extant species diversity. As part of the ingroup we used 107 out of c. 450 extant *Vaccinium* species and 12 out of c. 80 *Agapetes* species, for which our sampling provided a good representation of the overall distribution range of these genera. In total 214 sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA, containing the ITS1 spacer, the 5.8S rRNA gene, and the ITS2 spacer, were analysed using Maximum Likelihood and Bayesian Inference. With the aid of fossil data, divergence times were estimated in a Bayesian framework. Finally, potential ancestral areas and dispersal routes were identified by using two biogeographical approaches (S-DIVA and DEC). This chapter was submitted to the Journal of Systematics and Evolution on September 22nd, 2015.

In chapter 3 "Dispersal routes between biodiversity hotspots in Asia: the case of the mountain genus *Tripterospermum* (Gentianinae, Gentianaceae) and its close relatives", the historical biogeography of *Tripterospermum* and closely related genera was inferred by using Maximum Likelihood- and Bayesian-based phylogenetic reconstructions obtained from nuclear ITS and plastid *atpB-rbcL* and *trnL-trnF* data (altogether 216 accessions), molecular dating via fossil constraints, and two approaches of ancestral area reconstructions (S-DIVA and DEC). An important strength of this study is the near complete sampling, including 82% of all described species of subtropical Gentianinae. This chapter was accepted by the Journal of Biogeography on July 13th, 2015 (currently in press).

In chapter 4 “Key innovations and climatic niche divergence as drivers of diversification in subtropical Gentianinae (Gentianaceae) in the region of the Qinghai-Tibetan Plateau”, the climatic niches of five closely related genera of Gentianinae were quantified and described by conducting Species Distribution Modelling using the Maximum Entropy Modelling approach, and by performing different statistical tests (Niche Overlap-, Kolmogorov-Smirnov-, and T-Test). The species occurrence data cover ~47% of all extant *Crawfurdia*, ~42% of *Kuepferia*, 75% of *Metagentiana*, 100% of *Sinogentiana*, and ~77% of *Tripterospermum* species. Moreover, stochastic character mapping was used to reconstruct the probable evolution of habitat preferences of Gentianinae. To understand the role of niche evolution and conservatism for each genus, we determined the temporal distribution of niche disparity within versus among subclades of Asian Gentianinae using relative disparity plots. Finally, we tested the effect of five putative key innovations on the diversification of the five genera using the binary state speciation and extinction approach. This chapter was submitted to the American Journal of Botany on July 30th, 2015.

Appendix 1 comprises the publication “Two new species of the Asian genus *Tripterospermum* (Gentianaceae)”, which was published in Systematic Botany in the year 2013. This paper describes and illustrates two new species of *Tripterospermum* from China and Indonesia: *T. maculatum* Adr.Favre, Matuszak & Muellner and *T. tanatorajanense* Adr.Favre, Matuszak & Muellner. In the scope of this work, I was involved in finding *T. maculatum* in a field trip to China in 2011, and helped to measure, critically evaluate and conduct comparative statistical analyses (multivariate analyses) of flower, fruit and vegetative characters of the new species with their morphologically closest relatives.

Appendix 2 includes the publication “Two new genera of Gentianinae (Gentianaceae): *Sinogentiana* and *Kuepferia* supported by molecular phylogenetic evidence”, which was published in Taxon in the year 2014. Here, I was involved in generating nuclear (ITS) and plastid (*trnL-trnF* and *atpB-rbcL*) sequences of Gentianinae, as well as in the phylogenetic analyses. In this study, *Gentiana* section *Otophora* was elevated to generic rank under the name *Kuepferia*, whereas two species of *Metagentiana* were excluded from that genus and described under the new genus *Sinogentiana*. The taxonomic and phylogenetic treatment provided the base for further work on Gentianinae, conducted in the course of this doctoral thesis.

2. TRANSCONTINENTAL DISPERSALS INCREASED IN THE SOUTHERN HEMISPHERE AFTER MIOCENE COOLING: MOLECULAR DATING AND BIOGEOGRAPHY OF *AGAPETES, VACCINIUM* AND THEIR RELATIVES (VACCINIEAE, ERICACEAE)

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This chapter was submitted to the Journal of Systematics and Evolution on September 22nd, 2015.

2.1 Authors' 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. and A.N.M.-R. contributed to writing. S.M. and A.N.M.-R. recruited the financial support for this study (see also Appendix 3).

2.2 Confirmation: submitted to the Journal of Systematics and Evolution

22-Sep-2015

Dear Dr. Muellner-Riehl:

Your manuscript entitled "Transcontinental dispersals increased in the Southern Hemisphere after Miocene cooling: molecular dating and biogeography of Agapetes, Vaccinium and their relatives (Vaccinieae, Ericaceae)" by Matuszak, Sabine; Favre, Adrien; Sun, Hang; Muellner-Riehl, Alexandra, has been successfully submitted online and is presently being given full consideration for publication in Journal of Systematics and Evolution.

Co-authors: Please contact the Editorial Office as soon as possible if you disagree with being listed as a co-author for this manuscript.

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2.3 ABSTRACT

Biological interchanges between biodiversity hotspots throughout the world are still poorly understood. We used Vaccinieae (Ericaceae), a cosmopolitan plant group, to identify intercontinental dispersal events in the light of historical geographic and climatic settings. First, our sampling strategy focused on *Agapetes* and *Vaccinium* to be able to resolve yet unknown phylogenetic relationships. Second, we reconstructed the overall phylogeny of Vaccinieae by maximum likelihood and Bayesian analyses of the ITS region, including the largest data set on *Agapetes* and *Vaccinium* available so far. Third, we conducted molecular dating for this group, and used the phylogenetic as well as the temporal framework to conduct biogeographical analyses (DEC, S-DIVA). We found a North American origin for Vaccinieae. Possibly, the Miocene climate cooling played an important role for dispersal events within this group. Before the Miocene cooling, dispersal events mostly occurred in the Northern Hemisphere, but after the onset of the Miocene cooling (from ca. 15 million years ago, Ma, onwards) the number of dispersal events of Vaccinieae increased worldwide, especially at lower latitudes. We therefore conclude that the Miocene climate cooling might have triggered and facilitated the dispersal of Laurasian genera to and within the Southern Hemisphere.

2.4 INTRODUCTION

Biodiversity is unevenly distributed on Earth (Myers et al., 2000; Myers, 2003). Especially mountain systems harbour a higher biodiversity than expected based on area (Linder, 2008; Hoorn et al., 2013). Yet, dispersal routes and biological interchanges between these mountainous hotspots of biodiversity are not fully understood. Biogeographic studies can contribute to our understanding on floristic exchanges, but so far biogeographical studies have mostly focused on endemics occurring in one hotspot or species occurring in a few biodiversity hotspots only (e.g., Meudt & Simpson, 2006; Nauheimer et al., 2012; Grudinski et al., 2014a), rather than investigating cosmopolitan plant groups (e.g., Richardson et al., 2004; Buerki et al., 2011; Emadzade et al., 2011). In this study, we investigate the biogeography of a cosmopolitan taxon, the Vaccinieae Rchb. (Vaccinioideae, Ericaceae), with a focus on two species rich genera, *Vaccinium* L. and *Agapetes* D.Don ex G.Don, which also occur in subtropical to tropical mountain systems.

In total, tribe Vaccinieae comprise about 1250 species, classified into 34 genera (Kron et al., 2002, see Table 2). Despite recent work on the taxonomy of Vaccinieae, some uncertainties remain concerning the classification of its genera. For example, according to the results of Kron et al. (2002) and Stevens (2004), *Agapetes* species from the Southeast (SE) Asian mainland and those occurring from Papua New Guinea to the Fiji and New Caledonia (Oceanic *Agapetes*) do not form a monophyletic group. These Oceanic *Agapetes* species were raised to the rank of genus (*Paphia* Seem.), formerly a subgenus of *Agapetes* (Stevens, 2004; Venter & Munzinger, 2007). Unfortunately, no more than three *Agapetes* species from mainland Asia were included in these studies. In addition, the *Vaccinium* section *Pachyantha* Sleum. was classified as the new genus *Dimorphanthera* (Drude) J.J.Sm. (as *Dimorphanthera* section *Pachyantha* (Sleum.) Stevens) based upon anatomical characters (Stevens, 1974). *Paphia* and *Dimorphanthera* share morphological similarities and some phylogenetic evidence (see phylogenies in Kron et al., 2002) underline a close relationship between these two genera (Venter & Munzinger, 2007; distribution in Fig. 6). These and other relationships within Vaccinieae still have to be clarified. Finally, the distinction between *Agapetes* and *Vaccinium* might be problematic. Traditionally, these genera are distinguished from each other by the size and shape of the corolla (*Agapetes*: corolla 0.5 to 6 cm long, tubular or cylindric, rarely urceolate; *Vaccinium*: corolla up to 1 cm long, urceolate or campanulate, rarely tubular), their inflorescences (*Agapetes*: fewer than 15 flowers; *Vaccinium* of SE Asia: more than 10 flowers; Stevens, 1985), and their habit (*Agapetes*: usually epiphytic; *Vaccinium*: usually terrestrial; Mingyuan et al., 2005). It is however clear that these traits are not fully diagnostic since they are shared between at least some species of both genera (see Fig. 6).

Table 2. The 34 genera of Vaccinieae (Ericaceae).

Genus	Species number estimation	Reference
Agapetes D.Don ex G.Don	80	Ruizheng & Stevens, 2005a
Anthopteropsis A.C.Sm.	5	Wilbur & Luteyn, 1978
Anthopterus Hook.	12	Luteyn, 1996
Cavendishia Lindl.	100	Luteyn, 1983
Ceratostema Juss.	33	Luteyn, 2002a
Costera J.J.Sm.	10	Sleumer, 1967
Demosthenesia A.C.Sm.	9	Huamantupa, 2009
Didonica Luteyn & Wilbur	4	Luteyn, 1991
Dimorphanthera (Drude) J.J.Sm.	87	Stevens, 2004
Diogenesia Sleumer	13	Stevens et al., 2004
Disterigma (Klotzsch) Nied.	35	Stevens et al., 2004
Gaylussacia Kunth	50	Stevens et al., 2004
Gonocalyx Planch. & Linden	11	Stevens et al., 2004
Lateropora A.C.Sm.	3	Stevens et al., 2004
Macleania Hook.	50	Stevens et al., 2004
Mycerinus A.C.Sm.	3	Stevens et al., 2004
Notopora Hook.f.	5	Stevens et al., 2004
Oreanthes Benth.	7	Stevens et al., 2004
Orthaea Klotzsch	34	Stevens et al., 2004
Paphia Seem.	23	Stevens, 2004
Pellegrinia Sleumer	6	Stevens et al., 2004
Plutarchia A.C.Sm.	11	Stevens et al., 2004
Polyclita A.C.Sm.	1	Stevens et al., 2004
Psammisia Klotzsch	70	Stevens et al., 2004
Rusbya Britton	1	Stevens et al., 2004
Satyria Klotzsch	25	Luteyn, 2002b
Semiramisia Klotzsch	4	Luteyn, 1984
Siphonandra Klotzsch	3	Stevens et al., 2004
Sphyrospermum Poepp. & Endl.	22	Stevens et al., 2004
Symphysia (Vahl) Stearn	15	Vander Kloet et al., 2004
Themistoclesia Klotzsch	22	Stevens et al., 2004
Thibaudia Ruiz & Pav. ex J.St.-Hil.	60	Stevens et al., 2004
Utleya Wilbur & Luteyn	1	Stevens et al., 2004
Vaccinium L.	450	Lens et al., 2004

Both *Vaccinium* and *Agapetes* are relatively species-rich genera that are sometimes used for agricultural or medicinal purposes. *Vaccinium* encompasses about 450 species (Lens et al., 2004), some of which are cultivated for their fruits (Strik & Yarborough, 2005; Banados, 2006). The genus occurs throughout the Northern Hemisphere in temperate and subtropical to tropical mountain regions, however it is absent from Australia, New Zealand and most of Africa (a few species exist only in South Africa and Madagascar; Powell & Kron, 2002; Fig. 6). The fact that *Vaccinium* occurs in the temperate zone and in the tropics only along mountain ranges (Ruizheng & Stevens, 2005b) seems to indicate a general preference for cooler habitats. Due to its berries with high sugar content, *Vaccinium* is dispersed by birds and mammals (Stiles, 1980), which might also be the case for *Agapetes* (Vaccinieae, Luteyn, 2002b; Luteyn & Pedraza-Penalosa, 2012). In contrast, the latter genus comprises only about 80 species, and is restricted to Asia, including Sikkim, Bhutan, SE Tibet to Assam, Myanmar and China (W Yunnan). Up to now only one species has been recorded from Thailand and one from Peninsular Malaysia (Watthana, 2001; Stevens et al., 2004; Ruizheng & Stevens, 2005a; see Fig. 6). Some *Agapetes* species have medicinal properties and are used as natural remedy in Thailand and by some tribes in Arunachal Pradesh (Panda & Srivastava, 2010; Alongkornsopit et al., 2011). *Agapetes megacarpa* W.W. Smith has been reported to harbour anticancer properties (Alongkornsopit et al., 2011).

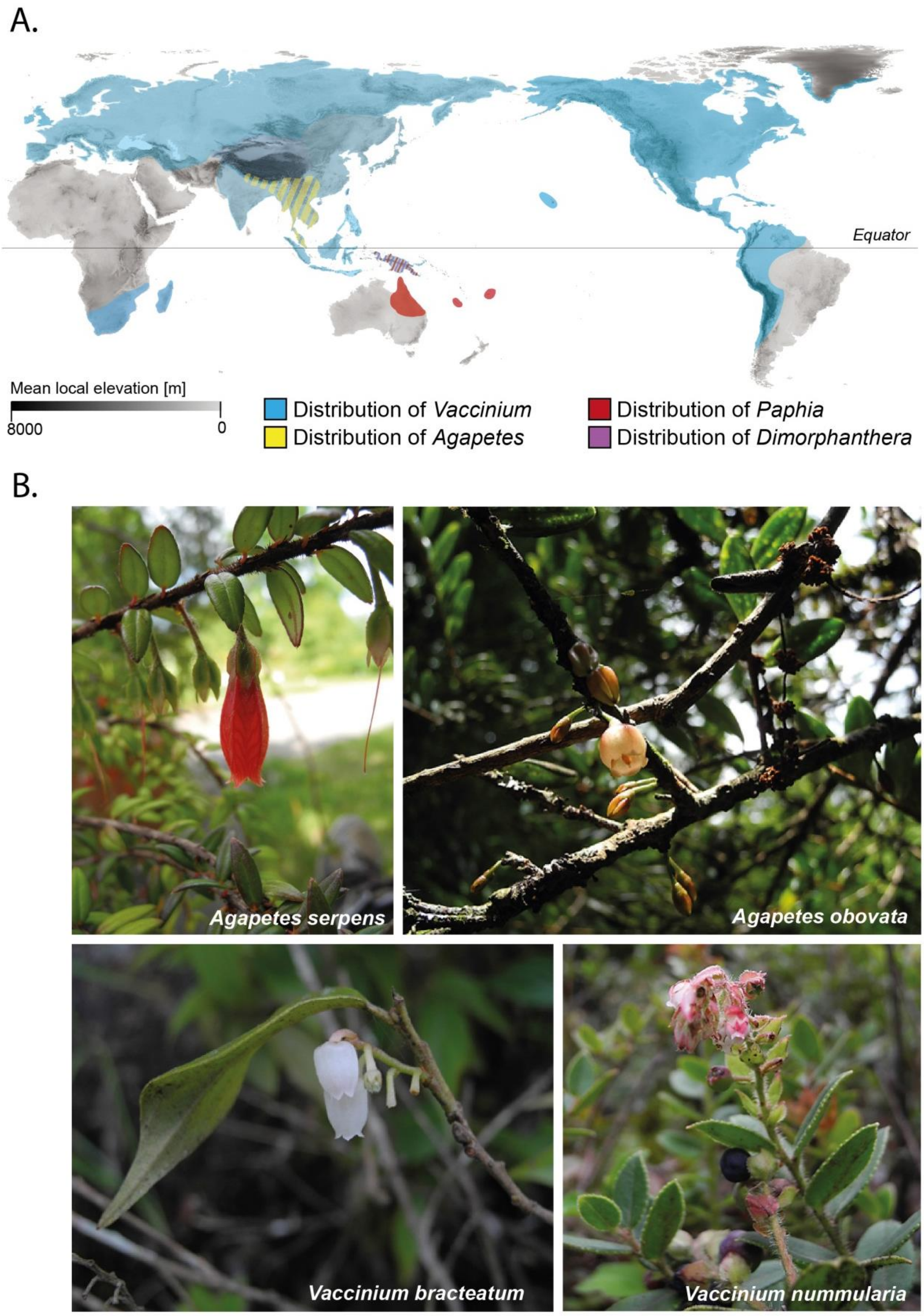


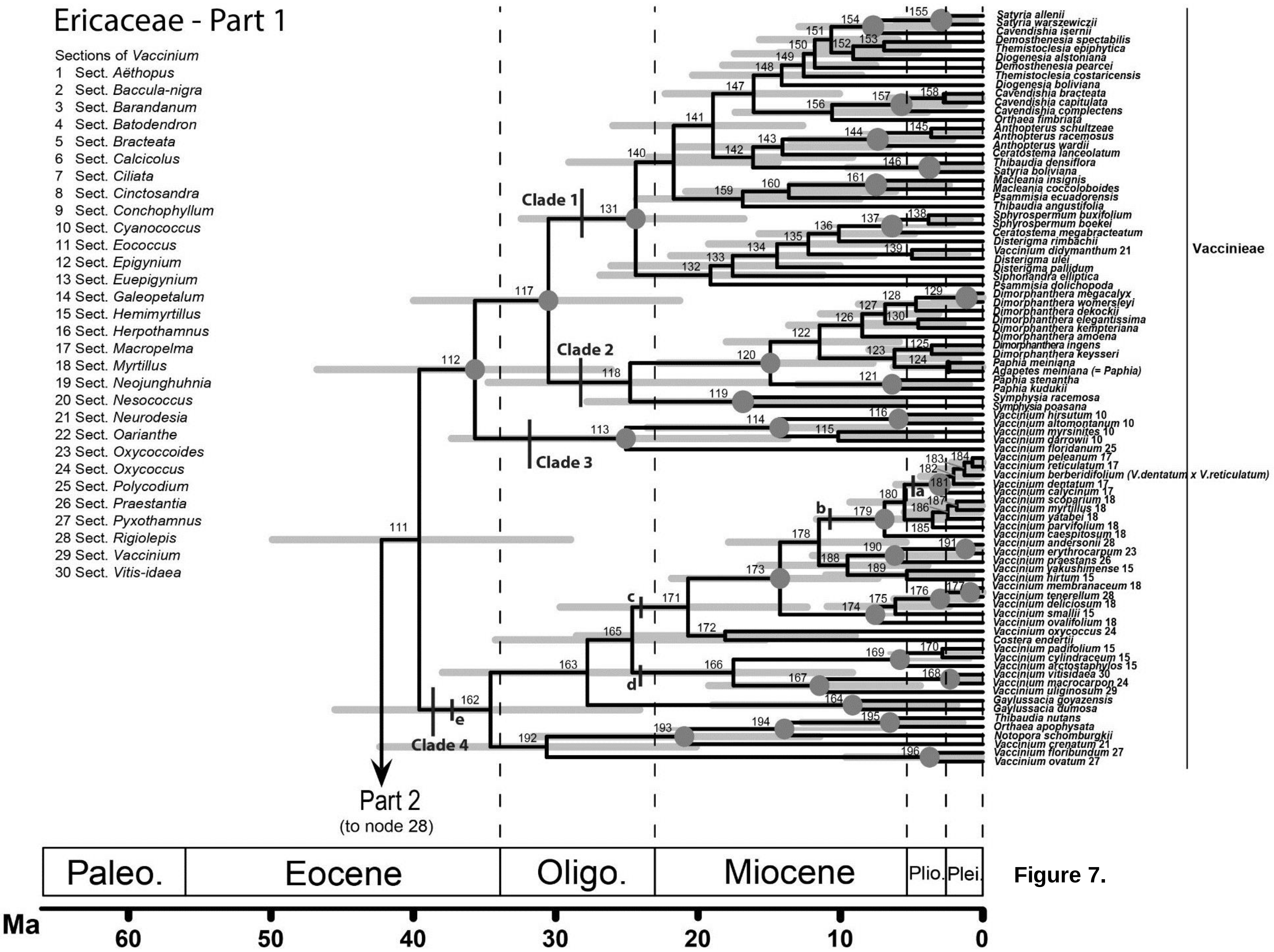
Figure 6. A. Distribution of the genera *Agapetes, Dimorphanthera, Paphia*, and *Vaccinium* (Vaccinieae, Ericaceae). B. Corollas of *Agapetes* are usually tubular (*A. serpens*), but rarely campanulate (*A. obovata*; campanulate corollas are typical for *Vaccinium*). Species of *Vaccinium* are usually terrestrial (*V. bracteatum*), but rarely epiphytic (*V. nummularia*; an epiphytic habit is typical for *Agapetes*). Pictures were taken by Adrien Favre and Sabine Matuszak. The map was created in R (R Core Team, 2013) using Worldclim data (Hijmans et al., 2005).

In this study, we reconstructed a phylogenetic framework for the biogeographical investigation of Vaccinieae by conducting maximum likelihood and Bayesian analyses of nuclear internal transcribed spacers (ITS) data. Using information on fossil Ericaceae we conducted the first molecular dating analysis for Vaccinieae, providing a temporal framework for a better understanding of dispersal events within this group, and allowing more detailed biogeographical reconstructions. The combination of divergence time estimation, information on the extant distribution of the species and knowledge about the tectonic Earth history allowed us to specifically address the following questions: (1) Will our larger data set provide better resolution of the currently still poorly known phylogenetic relationships within Vaccinieae? (2) Where and when did Vaccinieae evolve? Does the area of origin coincide with regions of high extant Vaccinieae species diversity, e.g. the Andes or the Himalayas? (3) Along which dispersal routes did Vaccinieae disperse throughout the world, and which climatic and geological settings likely contributed to their geographic expansion? Answering these questions can improve our understanding of the importance of biological interchanges between biodiversity hotspots of the Earth.

2.5 MATERIAL AND METHODS

2.5.1 Taxon sampling

This study covers c. 65% of the total generic diversity of Vaccinieae (22 out of 34 genera). As ingroup, we included a total of 170 species of Vaccinieae (c. 14% of the total species diversity) , with 105 of c. 450 extant *Vaccinium* and 12 of c. 80 *Agapetes* species, as well as 11 species of putatively synonymous genera such as *Dimorphanthera* and *Paphia*, and most of their closely related genera (*Anthopterus* Hook., *Cavendishia* Lindl., *Ceratostema* Juss., *Costera* J.J. Sm., *Demosthenesia* A.C. Sm., *Diogenesia* Sleumer, *Disterigma* (Klotzsch) Nied., *Gaylussacia* Kunth, *Macleania* Hook., *Notopora* Hook.f., *Orthaea* Klotzsch, *Psammisia* Klotzsch, *Satyria* Klotzsch, *Siphonandra* Klotzsch, *Sphyrospermum* Poepp. & Endl., *Symphysia* (Vahl) Stearn, *Themistoclesia* Klotzsch, *Thibaudia* Ruiz & Pav. ex J.St.-Hil.). Although we cover only c. 24% of all currently described *Vaccinium* species, our sampling includes a very good representation of the overall distribution range of the genus, which is likely to lead to reliable reconstructions of the biogeographic history of this group. As outgroups, we included five of the eight subfamilies of Ericaceae with two species of Cassiopeae P.F. Stevens, three of Cosmelieae Crayn & Quinn, three of Enkiantheae P.F. Stevens, two of Epacrideae Dumort., three of Ericeae DC. ex Duby, six of Gaultherieae Nied., nine of Lyonieae Kron & Judd, five of Rhodoreae DC. ex Duby, four of Richeeae Crayn & Quinn, and six of Styphelieae Bartl. (see list of species and species' authorities in Appendix 4, see Fig. 7). Leaf material for DNA extraction was retrieved from several herbaria (FR, KUN, L, WU) and botanical gardens of the universities at Frankfurt/Main, Leipzig, and Zurich, or collected in the field in China (by Sabine Matuszak and Adrien Favre in 2011). Additional sequences were retrieved from Genbank (*http://www.ncbi.nlm.nih.gov*).


Ericaceae - Part 1
Sections of *Vaccinium*
1 Sect. *Aëthopus*
2 Sect. *Baccula-nigra*
3 Sect. *Barandanum*
4 Sect. *Batodendron*
5 Sect. *Bracteata*
6 Sect. *Calcicolus*
7 Sect. *Ciliata*
8 Sect. *Cinctosandra*
9 Sect. *Conchophyllum*
10 Sect. *Cyanococcus*
11 Sect. *Eococcus*
12 Sect. *Epigynium*
13 Sect. *Euepigynium*
14 Sect. *Galeopetalum*
15 Sect. *Hemimyrtillus*
16 Sect. *Herpothamnus*
17 Sect. *Macropelma*
18 Sect. *Myrtillus*
19 Sect. *Neojunghuhnia*
20 Sect. *Nesococcus*
21 Sect. *Neurodesia*
22 Sect. *Oarianthe*
23 Sect. *Oxycoccoides*
24 Sect. *Oxycoccus*
25 Sect. *Polycodium*
26 Sect. *Praestantia*
27 Sect. *Pyxothamnus*
28 Sect. *Rigiolepis*
29 Sect. *Vaccinium*
30 Sect. *Vitis-idaea*
Clade 1
Clade 2
Clade 3
Clade 4
a
b
c
d
e
155
154
151
153
152
150
149
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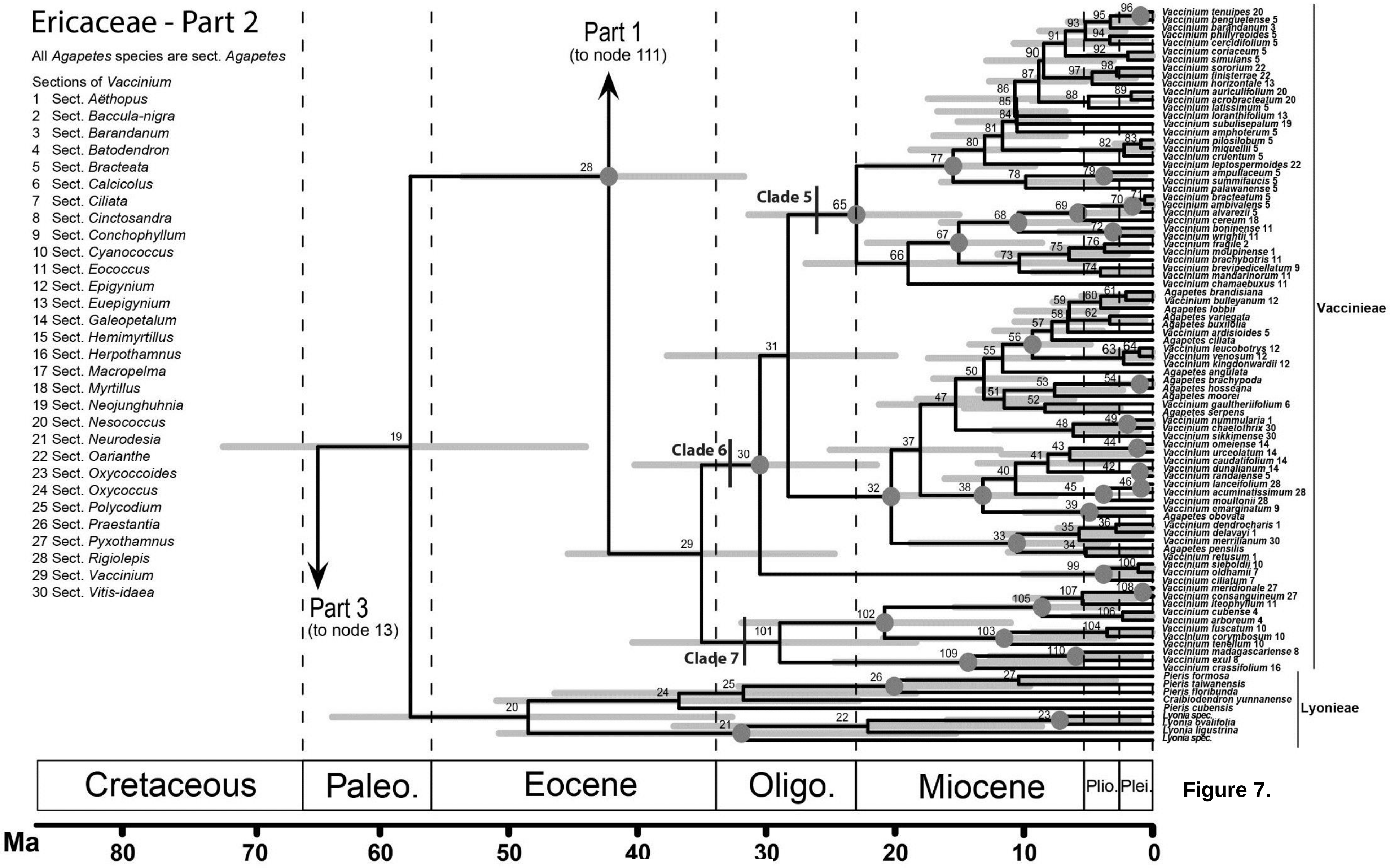
Satyria allenii
Satyria warszewiczii
Cavendishia isernii
Demosthenesia spectabilis
Themistoclesia epiphytica
Diogenesia alstoniana
Demosthenesia pearcei
Themistoclesia costaricensis
Diogenesia boliviana
Cavendishia bracteata
Cavendishia capitulata
Cavendishia complectens
Orthaea fimbriata
Anthopterus schultzeae
Anthopterus racemosus
Anthopterus wardii
Ceratostema lanceolatum
Thibaudia densiflora
Satyria boliviana
Macleania insignis
Macleania coccoloboides
Psammisia ecuadorensis
Thibaudia angustifolia
Sphyrospermum buxifolium
Sphyrospermum boekei
Ceratostema megabracteatum
Disterigma rimbachii
Vaccinium didymanthum 21
Disterigma ulei
Disterigma pallidum
Siphonandra elliptica
Psammisia dolichopoda
Dimorphanthera megacalyx
Dimorphanthera womersleyi
Dimorphanthera dekockii
Dimorphanthera elegantissima
Dimorphanthera kempteriana
Dimorphanthera amoena
Dimorphanthera ingens
Dimorphanthera keysseri
Paphia meiniana
Agapetes meiniana (= Paphia)
Paphia stenantha
Paphia kudukii
Symphysia racemosa
Symphysia poasana
Vaccinium hirsutum 10
Vaccinium altomontanum 10
Vaccinium myrsinites 10
Vaccinium darrowii 10
Vaccinium floridanum 25
Vaccinium peleanum 17
Vaccinium reticulatum 17
Vaccinium berberidifolium (V.dentatum x V.reticulatum)
Vaccinium dentatum 17
Vaccinium calycinum 17
Vaccinium scoparium 18
Vaccinium myrtillus 18
Vaccinium yatabei 18
Vaccinium parvifolium 18
Vaccinium caespitosum 18
Vaccinium andersonii 28
Vaccinium erythrocarpum 23
Vaccinium praestans 26
Vaccinium yakushimense 15
Vaccinium hirtum 15
Vaccinium membranaceum 18
Vaccinium tenerellum 28
Vaccinium deliciosum 18
Vaccinium smallii 15
Vaccinium ovalifolium 18
Vaccinium oxycoccus 24
Costera endertii
Vaccinium padifolium 15
Vaccinium cylindraceum 15
Vaccinium arctostaphylos 15
Vaccinium vitisidaea 30
Vaccinium macrocarpon 24
Vaccinium uliginosum 29
Gaylussacia goyazensis
Gaylussacia dumosa
Thibaudia nutans
Orthaea apophysata
Notopora schomburgkii
Vaccinium crenatum 21
Vaccinium floribundum 27
Vaccinium ovatum 27
Vaccinieae
Part 2
(to node 28)
Paleo.
Eocene
Oligo.
Miocene
Plio.
Plei.
Ma
60
50
40
30
20
10
0

Figure 7.


Ericaceae - Part 2
All Agapetes species are sect. Agapetes
Sections of Vaccinium
1 Sect. Aëthopus
2 Sect. Baccula-nigra
3 Sect. Barandanum
4 Sect. Batodendron
5 Sect. Bracteata
6 Sect. Calcicolus
7 Sect. Ciliata
8 Sect. Cinctosandra
9 Sect. Conchophyllum
10 Sect. Cyanococcus
11 Sect. Eococcus
12 Sect. Epigynium
13 Sect. Euepigynium
14 Sect. Galeopetalum
15 Sect. Hemimyrtillus
16 Sect. Herpothamnus
17 Sect. Macropelma
18 Sect. Myrtillus
19 Sect. Neojunghuhnia
20 Sect. Nesococcus
21 Sect. Neurodesia
22 Sect. Oarianthe
23 Sect. Oxycoccoides
24 Sect. Oxycoccus
25 Sect. Polycodium
26 Sect. Praestantia
27 Sect. Pyxothamnus
28 Sect. Rigiolepis
29 Sect. Vaccinium
30 Sect. Vitis-idaea
Part 1
(to node 111)
Part 3
(to node 13)
Clade 5
Clade 6
Clade 7
Vaccinium tenuipes 20
Vaccinium benguetense 5
Vaccinium barandanum 3
Vaccinium phillyreoides 5
Vaccinium cercidifolium 5
Vaccinium coriaceum 5
Vaccinium simulans 5
Vaccinium sororium 22
Vaccinium finisterrae 22
Vaccinium horizontale 13
Vaccinium auriculifolium 20
Vaccinium acrobracteatum 20
Vaccinium latissimum 5
Vaccinium loranthifolium 13
Vaccinium subulisepalum 19
Vaccinium amphoterum 5
Vaccinium pilosilobum 5
Vaccinium miquellii 5
Vaccinium cruentum 5
Vaccinium leptospermoides 22
Vaccinium ampullaceum 5
Vaccinium summifaucis 5
Vaccinium palawanense 5
Vaccinium bracteatum 5
Vaccinium ambivalens 5
Vaccinium alvarezii 5
Vaccinium cereum 18
Vaccinium boninense 11
Vaccinium wrightii 11
Vaccinium fragile 2
Vaccinium moupinense 1
Vaccinium brachybotris 11
Vaccinium brevipedicellatum 9
Vaccinium mandarinorum 11
Vaccinium chamaebuxus 11
Agapetes brandisiana
Vaccinium bulleyanum 12
Agapetes lobbii
Agapetes variegata
Agapetes buxifolia
Vaccinium ardisioides 5
Agapetes ciliata
Vaccinium leucobotrys 12
Vaccinium venosum 12
Vaccinium kingdonwardii 12
Agapetes angulata
Agapetes brachypoda
Agapetes hosseana
Agapetes moorei
Vaccinium gaultheriifolium 6
Agapetes serpens
Vaccinium nummularia 1
Vaccinium chaetothrix 30
Vaccinium sikkimense 30
Vaccinium omeiense 14
Vaccinium urceolatum 14
Vaccinium caudatifolium 14
Vaccinium dunalianum 14
Vaccinium randaiense 5
Vaccinium lanceifolium 28
Vaccinium acuminatissimum 28
Vaccinium moultonii 28
Vaccinium emarginatum 9
Agapetes obovata
Vaccinium dendrocharis 1
Vaccinium delavayi 1
Vaccinium merrilianum 30
Agapetes pensilis
Vaccinium retusum 1
Vaccinium sieboldii 10
Vaccinium oldhamii 7
Vaccinium ciliatum 7
Vaccinium meridionale 27
Vaccinium consanguineum 27
Vaccinium iteophyllum 11
Vaccinium cubense 4
Vaccinium arboreum 4
Vaccinium fuscatum 10
Vaccinium corymbosum 10
Vaccinium tenellum 10
Vaccinium madagascariense 8
Vaccinium exul 8
Vaccinium crassifolium 16
Pieris formosa
Pieris taiwanensis
Pieris floribunda
Craibiodendron yunnanense
Pieris cubensis
Lyonia spec.
Lyonia ovalifolia
Lyonia ligustrina
Lyonia spec.
Vaccinieae
Lyonieae
Cretaceous
Paleo.
Eocene
Oligo.
Miocene
Plio.
Plei.
Ma
80
70
60
50
40
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0

Figure 7.

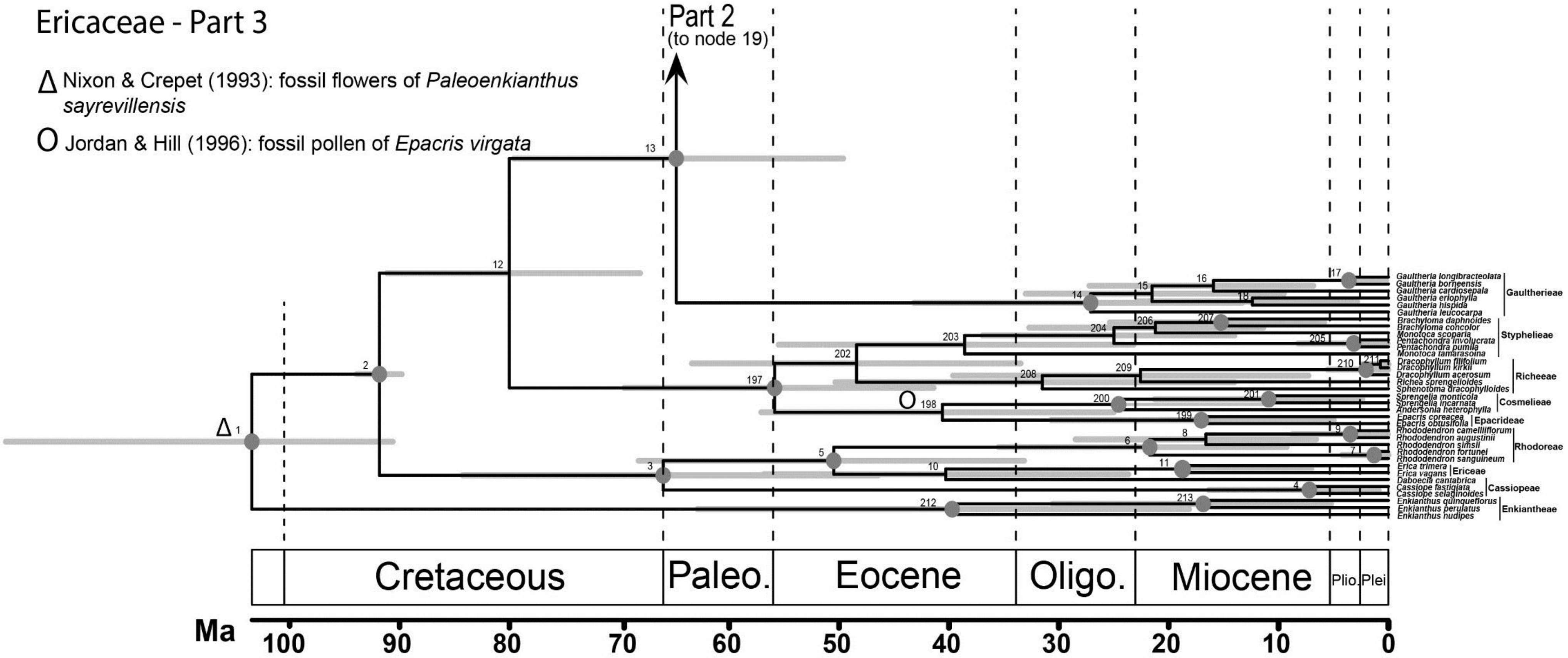

Figure 7. Chronogram for Vaccinieae (Ericaceae) based on ITS data modelled under a relaxed clock. Two fossils were used for setting temporal constraints: Δ fossil flowers of *Paleoenkianthus sayrevillensis* and ○ fossil pollen of *Epacris virgata*. Node ages represent mean ages and bars show the 95% highest posterior densisty intervals. Nodes marked with a grey dot indicate a posterior probability (PP) ≥ 0.95. The different sections of *Vaccinium* are marked by 1 (section *Aëthopus*) to 30 (section *Vitis-idaea*) after the species name. For each node, divergence times and posterior probability values are listed in Appendix 5.

2.5.2 DNA extraction, amplification, and sequencing

The DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used according to the manufacturer’s protocol to extract genomic DNA from leaf material dried with silica gel or from herbarium material, with only one minor modification: leaf samples were incubated in the lysis buffer and RNase for two hours instead of one. To amplify the ITS region, the primer pair 17SE_m, 5’-CGGTGAAGTGTTCGGATCG, and 26SE_m, 5’-CGCTCGCCGTTACTAGGG (Grudinski et al., 2014a; modified after Sun et al., 1994) was used. The reaction mix of 25 µL contained 21.9 µL Thermo Scientific 1.1X ReddyMix^{TM} PCR Master Mix (1.5 mM $MgCl_2$; ABgeneHouse, Epsom, Surrey, UK), 0.5 µL bovine serum albumin (BSA; 10 mg/mL; New England BioLabs GmbH, Frankfurt/Main, Germany), 1 µL dimethyl sulfoxide (DMSO; Carl ROTH GmbH, Essen, Germany), 1 µL of genomic DNA and 0.3 µL of each primer (10 µM). PCR reactions were performed in a Thermo Scientific Arktik Thermal Cycler (Thermo Fisher Scientific Oy, Vantaa, Finland), with initial denaturation of 2 min at 95°C, followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 53°C and extension for 1 min at 72°C, followed by a final extension step at 72°C for 10 min. When the above mentioned amplification procedure failed, ITS1 and ITS2 regions were amplified separately. The ITS1 region was amplified using the primer pair 17SE_m and ITS_middle_R1, 5'-CAACTTGCGTTCAAAGACTCG (Matuszak et al., in press), and the ITS2 region using the primer pair ITS_middle_F1, 5'-GATACTTGGTGTGAATTGCAGAATC (Matuszak et al., in press), and 26SE_m. To conduct PCR reactions for ITS1 and ITS2, the same PCR protocol as described above was used. PCR products were cleaned with a NukleoSpin® Gel and PCR clean up kit (Macherey-Nagel GmbH & Co. KG, Dueren, Germany) following the manufacturer’s instructions, and sequencing reactions were run on an ABI 3130xl Genetic Analyzer using Big Dye terminator v3.1 chemistry (Applied Biosystems, Inc., Warrington, Cheshire, UK).

In addition to ITS, we tried to amplify several plastid marker regions (*atpB-rbcL*, *matK*, *rpL16*, *rpS16*, *trnL-trnF*), with only very little success for herbarium material despite using varying PCR conditions. We used different combinations of temperatures, concentration of Mg^{2+} ions, amounts of other reagents (e.g., BSA, DMSO, template DNA), and also performed PCR reactions using a highly sensitive Taq Polymerase. In addition, we performed different Touchdown PCR and re-PCR approaches. To exclude the impact of possible mercury contamination we washed the herbarium leaf materials in a 1% SDS solution before DNA extraction, but this did not result in higher PCR success either. In contrast, freshly collected material could be amplified relatively straightforwardly, but unfortunately represented a too low proportion of samples to be reasonably included here. We are aware that the analysis of only ITS may be viewed as a weakness of our study. Future follow-up studies should therefore ideally apply ancient DNA techniques or place greater reliance on more newly collected material.

2.5.3 Sequence handling and phylogenetic analysis

PCRs did not result in more than one product, which was a hint against paralogous sequences (Feliner & Rossello, 2007). All sequences were blasted to the NCBI (National Center for Biotechnology Information; *http://www.ncbi.nlm.nih.gov/*) database to exclude the presence of laboratory contamination. We also found no evidence of polymorphism in direct sequences, indicating the absence of hybrids/polyploids in our data. Assembly and alignment of sequences were done in Geneious v5.5.6 (Drummond et al., 2011) using the multiple alignment software ClustalW (Thompson et al., 1994). The alignment required only minor manual corrections. New sequences have been deposited in GenBank under the accession numbers KM209366-KM209476 (*http://www.ncbi.nlm.nih.gov/*).

We performed Maximum Likelihood (ML) and Bayesian Inference (BI) analysis. The best fitting substitution model, based upon the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) using jModeltest v2.1.2 (Guindon & Gascuel, 2003; Posada, 2008), was found to be GTR+G. ML analyses were performed using the graphical front-end raxmlGUI v1.3 (Silvestro & Michalak, 2012) for RAxML v7.4.2 (Stamatakis, 2006). Statistical support was estimated via bootstrap analysis with 1000 replicates (Felsenstein, 1985).

Using MrBayes v3.2.1 (Huelsenbeck & Ronquist, 2001), BI analysis was performed for a total of four runs, with four Markov chains each (one cold chain and three heated ones), started simultaneously from independent random trees. Every 3000th generation was sampled for a maximum of 30 million generations. Convergence was indicated by the average standard deviation of split frequencies which was 0.0054 (< 0.01). Convergence of the parameters of the four individual runs was also determined using Tracer v1.5 (Rambaut & Drummond, 2007) and by inspecting the cumulative posterior probabilities of clades using the *Are We There Yet?* (AWTY, Wilgenbusch et al., 2004; Nylander et al., 2008) online programme. Based on the convergence diagnostics, we discarded the first 10% of the sampled trees as burn-in, and combined the four runs using MrBayes v3.2.1. The majority rule consensus tree was computed by using the remaining trees. The clade support on this tree shows the Bayesian posterior probabilities (PP).

2.5.4 Divergence time estimates

We calculated divergence times using Bayesian statistics in BEAST v1.7.5 (Rambaut & Drummond, 2007; Drummond et al., 2012), with the model of substitution set to GTR+G. We determined the best fitting speciation prior for the branching rates (Yule or Birth-Death) by estimating the marginal likelihood for each model by calculating the harmonic mean of the sampled likelihoods from an MCMC chain with 1000 bootstrap replicates (Newton & Raftery, 1994; Suchard et al., 2001) using BEAST and Tracer v1.5. The difference of the logarithmic marginal likelihoods of the two models yields the logarithmic Bayes Factor, which has to be interpreted as described by Kass & Raftery (1995).

The Yule and the Birth-Death model were compared under the conditions of an uncorrelated relaxed molecular clock and GTR+G. The logarithmic Bayes Factor was 2.399, which is positive evidence against the Birth-Death prior. For this reason, we used the Yule tree prior as tree model, which assumes a constant rate of birth per lineage (Steel & McKenzie, 2001). A likelihood ratio test conducted in MEGA6 (Tamura et al., 2013) under the Tamura-Nei model (Tamura & Nei, 1993) rejected the null hypothesis of an equal evolutionary rate throughout the tree. Therefore, the analysis was performed using an uncorrelated relaxed molecular clock model (Drummond et al., 2006) with rate variation log-normally distributed on each branch of the tree. It was run for 50 million generations, sampling every 5000th generation. We started the analysis independently four times from different random starting points, and we detected the effective sample size (ESS) for all parameters of each run and estimated the burn-in using Tracer v1.5 (Rambaut & Drummond, 2007). Three of the four independent log-files were combined using LogCombiner v1.7.5 (implemented in the BEAST package), with a burn-in of 5, 10 and 15 million generations respectively, sampling every 12000th generation. One run was excluded, because the ESS did not reach the reliable value of 200. A maximum clade credibility topology was determined with TreeAnnotator v1.7.5 (implemented in the BEAST package) and visualized using FigTree v1.4.0 (Rambaut, 2010).

There are only a few reliable fossils in Vaccinieae. There is no fossil for *Agapetes*, and for *Vaccinium*, known fossils are either very young (12000 years of age; Lawrence et al., 1967) or identification is uncertain (cf. *Vaccinium* sp.; Grimsson & Denk, 2007). For the purpose of our analysis, we avoided to use fossils for which taxonomic attribution was doubtful. Instead, we decided to use reliable fossils of other related lineages in the Ericaceae. We set temporal constraints using two fossils. (1) We used a megafossil of *Paleoenkianthus sayrevillensis* Nixon and Crepet (Enkiantheae) from the Late Cretaceous (Nixon & Crepet, 1993). This fossil flower was reported from the Turonian with an estimated age of about 90 Ma based on relative dating via stratigraphy. To take some uncertainties of this method into account, we decided to constrain the stem age of Enkiantheae with a normal prior composed of a mean of 91.85 Ma and a standard deviation of 1.05 with which we covered the whole Turonian epoch (89.8 – 93.9 Ma). The normal prior has most of the probability density around the mean, but allows a declining probability to be a bit older or younger. (2) We used microfossils (pollen) of *Epacris virgata* Hook.f. (Epacrideae) from the Middle Eocene (Jordan & Hill, 1996). The stem age of Epacrideae was also constrained with a uniform prior, but giving a hard minimum boundary at 41.3 Ma (beginning of Middle Eocene) and an extreme upper boundary (200 Ma). Epacrideae might be older than their oldest fossils. We refrained from using secondary calibration as upper boundary, for example ages estimated by Schwery et al. (2015), because of the risk of error propagation into our analysis (Graur & Martin, 2004; Renner, 2005). Due to the fact that we constrained the root with a normal prior around 90 Ma, we included already a soft-maximum-age for Epacrideae. By using an extreme upper boundary of 200 Ma we ensured that Enkiantheae and Epacrideae experience no restriction in the analysis for their maximum age (except for the indirect soft-maximum age from the root constraint). Therefore, such an extreme upper boundary would have no or only a negligible effect on the analysis.

2.5.5 Biogeographic analysis

We investigated the dispersal routes of *Agapetes* and *Vaccinium* with the dispersal-extinction-cladogenesis (DEC) model, using Lagrange (Ree et al., 2005; Ree & Smith, 2008) implemented in RASP 2.1 beta (Yu et al., 2012), and the dispersal-vicariance analysis (DIVA; Ronquist, 1997), using the statistical dispersal-vicariance programme (S-DIVA), also implemented in RASP 2.1 beta (Yu et al., 2012). By applying a model of possible dispersal routes which is based on geological history, DEC allows to make inferences about ancestral ranges in a likelihood framework (Ree et al., 2005; Clayton et al., 2009; Lamm & Redelings, 2009). In contrast, S-DIVA reconstructs ancestral distributions of clades within a parsimony-based framework favouring vicariance events. Prior assumptions about area relationships are not considered by this programme (Ronquist, 1997; Yu et al., 2010). We used these two independent biogeographical analyses to check the congruence of results, an approach which has proven useful in previous biogeographic studies (e.g., Clayton et al., 2009; Nauheimer et al., 2012; Birch & Keeley, 2013).

The BEAST MCMC trees as well as the maximum clade credibility tree from the Bayesian analysis (obtained in the course of the divergence time estimation approach) were used as input for S-DIVA, while the BEAST maximum clade credibility tree was employed for Lagrange. Detailed results of both analyses are given in Appendix 5. Using the ape package (Paradis et al., 2004) in R (R Core Team, 2013), we excluded the tips of Cassiopeae, Cosmelieae, Enkiantheae, Epacrideae, Ericeae, Gaultherieae, Rhodoreae, Richeeae, and Styphelieae from the chronogram for the purpose of our subsequent biogeographical analyses, because species coverage in these groups was too low to reflect the entire distribution of these groups and therefore could have introduced bias in our analysis. However, these outgroups were of particular importance to obtain reliable divergence time estimations, which was the reason we included them at this previous step. Another possibility to handle unequal sampling would be to keep only one representative of each outgroup genus and to attribute it to the entire distribution range of the genus (compare e.g. Grudinski et al., 2014b). In this case DEC and S-DIVA will treat this representative as one single species with a very broad distribution. The influence of this kind of information on the analysis has not been tested yet, which is why we decided to remove these outgroups with a low species coverage completely. Based on the size of our data set the programme RASP 2.1 beta (Yu et al., 2012) was only able to cope with a maximum of nine areas for DEC and S-DIVA analyses. Because *Vaccinium* has a cosmopolitan distribution, we had to define large-scale areas. For instance, we combined Sundaland with Indochina and Japan, because Japan was connected with the Asian mainland until the Miocene (Maruyama et al., 1997), and climatic oscillations with associated sea level variations during the Pleistocene resulted in the formation of land bridges between Sundaland and the Asian mainland (Voris, 2000; Chiang & Schaal, 2006). In contrast, the islands of Wallacea and the Philippines have an isolated position, and for this reason we combined these two neighbouring remote areas (Hall, 2009; van Welzen et al., 2011). Each species was assigned to one or more of the following nine areas according to its extant distribution (Fig. 8-9): (A) Qinghai-Tibetan Plateau (QTP) and its surroundings; (B) Indochina, Sundaland (Borneo, Sumatra, Java, Malay Peninsula), China (except for QTP region = A), and Japan; (C) Wallacea

(Sulawesi, the Moluccas, the Banda Arc, and the Lesser Sunda Islands), and the Philippines; (D) Sahul Shelf (New Guinea and Australia), and New Zealand; (E) Southern Polynesia and Hawaii; (F) North America; (G) Mexico, Central and South America, the Caribbean; (H) Europe and temperate/arctic Asia; and (I) Southern Africa and Madagascar. According to Chacon & Renner (2014), we tested the sensitivity of our data by using different Lagrange models: (1) no constraints, (2) only range, (3) only dispersal, and (4) range and dispersal constraints together. Details of the setting are given in Appendix 6. It is also possible to assign varying dispersal constraints to different time slices. This approach showed only minor effects on the analyses of Chacon & Renner (2014), who investigated even an older taxon than we do. Therefore, we did not test this.

Figure 8. Chronogram for Vaccinieae (Ericaceae) based on ITS data, analysed using a relaxed molecular clock approach. Node ages represent mean ages and bars show the 95% highest posterior density intervals. Node numbers in bold indicate a posterior probability (PP) of ≥ 0.95. Coloured dots at the tips depict the extant distribution of taxa. The branch colours illustrate the most likely area inferred by DEC analysis, in case the estimated probability is < 50% the branch is coloured black (see Fig. 4 for area and colour definition). Divergence times, PP values and the results inferred by DEC and S-DIVA analyses for each node are listed in Appendix 5.

Biogeographic analysis of Vaccinieae - Part 1

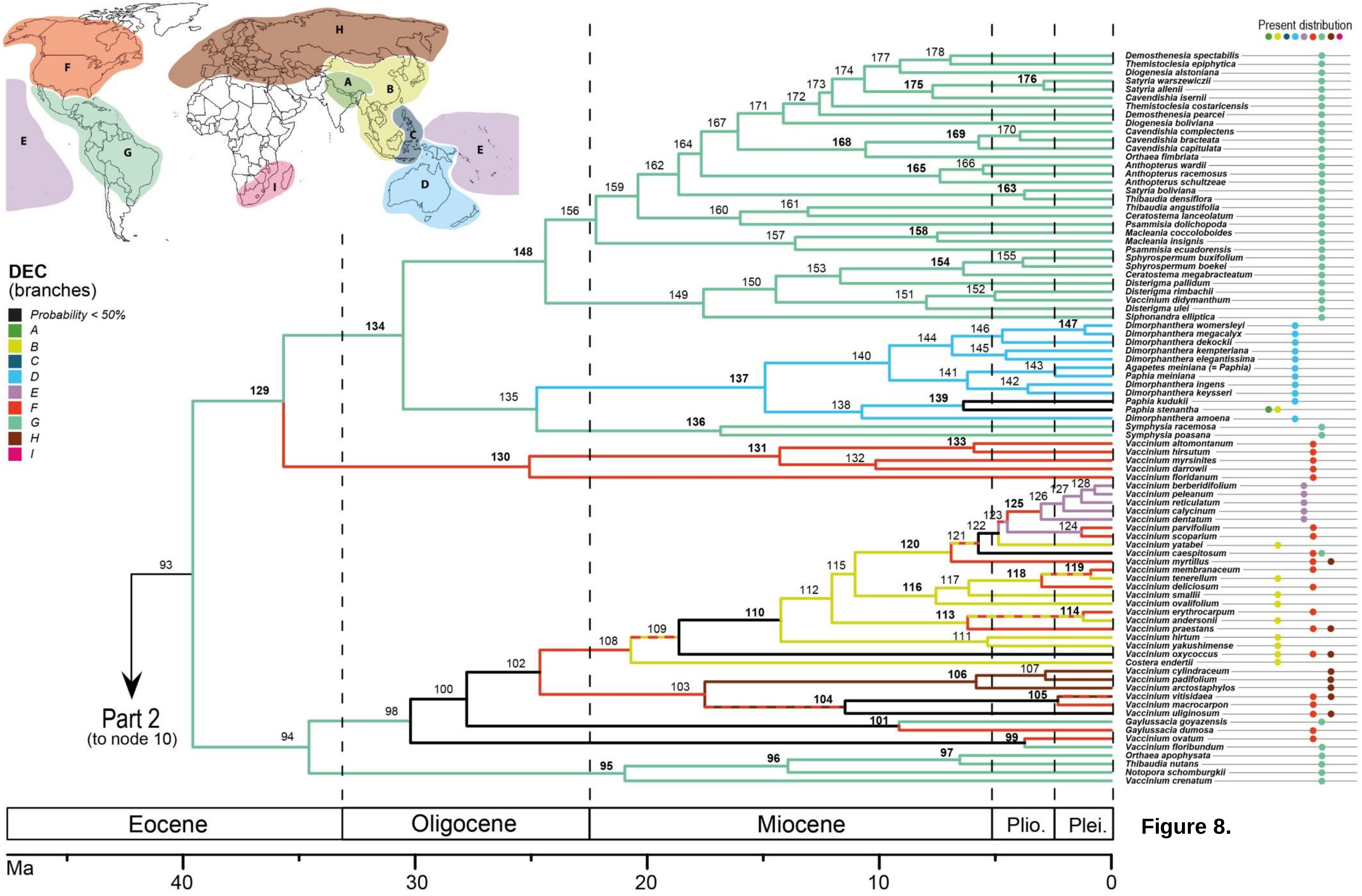

Figure 8.

Biogeographic analyses of Vaccinieae - Part 2

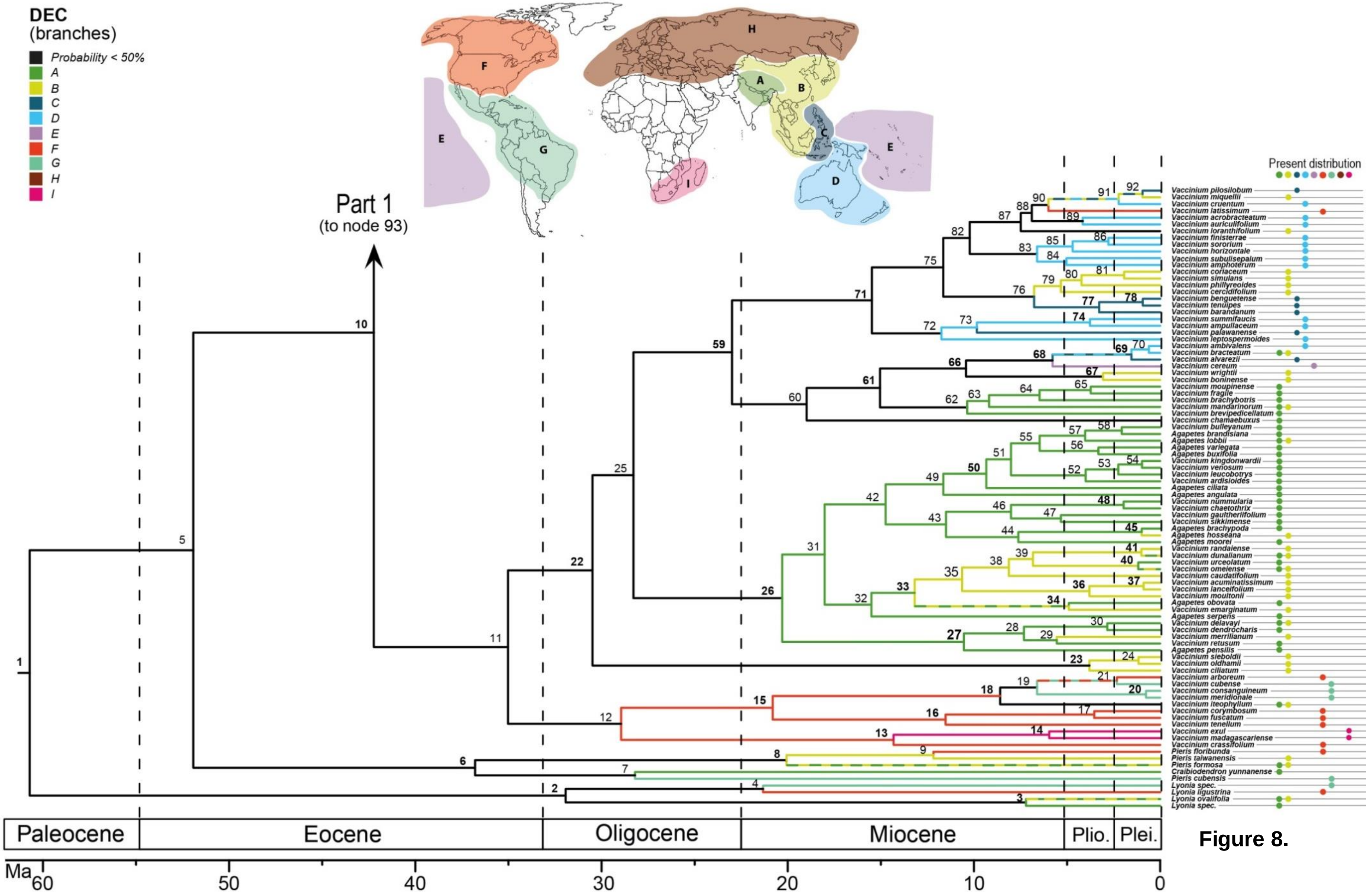

Figure 8.

2.6 RESULTS

2.6.1 Sequence data

This study includes a total of 214 sequences of ITS (110 of which were newly obtained). The aligned data matrix was 741 characters long with 446 (~60%) variable and 338 (~46%) parsimony-informative positions. We compared the most likely tree obtained from the ML analysis to the Bayesian majority rule consensus tree, and their support values. We did not detect any strongly supported topological incongruences (> 85% BP or > 0.9 PP), therefore we only present the maximum clade credibility tree of the Bayesian analysis (Fig. 7; details in Appendix 5). Throughout this paper, 0.9-0.94 PP is considered as moderate and 0.95-1 PP as strong support. Although our study only includes ITS, we are confident that our phylogeny represents a reliable base to study divergence time estimates and to conduct biogeographical reconstructions, because our results are in agreement with the topology of the taxonomically more poorly sampled phylogenies obtained by Kron et al. (2002) using the markers ITS and *matK*, by Powell & Kron (2002) using ITS, *matK*, and *ndhF*, as well as by Powell & Kron (2003) using ITS, *matK*, *ndhF*, and *rps4*.

2.6.2 Phylogenetic relationships and divergence time estimates

The ITS phylogeny (Fig. 7) reveals that not only *Agapetes* and *Vaccinium*, but also the sections within *Vaccinium* are polyphyletic. Despite a noticeable general congruence between taxonomy and phylogeny, for nearly all sections some species occur scattered throughout the tree. We divided the Vaccinieae in seven clades according to their geographical distribution. Additionally, we divided large clades (e.g., 4 and 6) into subclades that were alphabetically numbered (e.g., Clade 4a-e).

Clades 1, 2 and 4e contain only a few species of *Vaccinium* or *Agapetes* (Fig. 7). All species in Clade 1 (composed of the genera *Anthopterus, Cavendishia, Ceratostema, Demosthenesia, Diogenesia, Disterigma, Macleania, Orthaea, Psammisia, Satyria, Siphonandra, Sphyrospermum, Themistoclesia* and *Thibaudia*) have a Central and/or South American distribution.

Within *Disterigma*, one single *Vaccinium* species is nested (Fig. 7, node 93, PP 0.88), namely *V. didymanthum* Dunal (for which determination was confirmed). Clade 2 includes the genera *Dimorphanthera, Paphia* and *Symphysia*. *Dimorphanthera* and *Paphia* form a strongly supported clade of Sahul Shelf distribution, except for *Paphia stenantha* Schltr. which occurs in mainland Asia. Two *Symphysia* species from Central America form a moderately supported sister clade to *Dimorphanthera* and *Paphia*. Clade 4e consists of the genera from the American continent (*Gaylussacia*, *Notopora*, *Orthaea*, *Thibaudia*), and *Vaccinium crenatum* (D.Don ex Dunal) Sleumer. This *Vaccinium* species (of which determination was also confirmed) is closely related to *Notopora*, *Orthaea*, and *Thibaudia* with strong support.

Clade 6 is strongly supported, including all species of *Agapetes* and some species of *Vaccinium* from the southern fringe of the QTP, Asian mainland, and Japan. The phylogenetic relationships within this clade are only partially resolved, but it is certain that *Agapetes* is polyphyletic, being nested within *Vaccinium*.

Clades 3, 4a, 4b, 4c, 4d, 5, and 7 comprise mainly species of *Vaccinium* with the exception of Clade 4c which includes also *Costera endertii* J.J.Sm. Clade 3 shows a localized geographic distribution (only North American species), while clade 4, 5 and 7 are characterised by a broader distribution over several continents. In these clades, Hawaiian *Vaccinium* species (sect. *Macropelma*) group together with strong support (clade 4a), and Japanese species occur in four well supported clades (clade 4b: *V. yatabei* Makino; clade 4c: *V. hirtum* Thunb., *V. ovalifolium* Sm. in Rees, *V. oxycoccus* L., *V. smallii* A. Gray, *V. yakushimense* Makino; clade 5: *V. ciliatum* Thunb., *V. oldhamii* Miq., *V. randaiense* Hayata, *V. sieboldii* Miq.; clade 6: *V. boninense* Nakai, *V. bracteatum* Thunb., *V. wrightii* A. Gray). The species from Southern Africa are closely related to the North American species *Vaccinium crassifolium* Andrews, also with strong support (Clade 7).

The divergence time estimation (Fig. 7; for details see Appendix 5) revealed that the stem age of *Agapetes* is 20.30 [13.40-28.26] Myr (Fig. 7, node 32). The stem age of *Vaccinium*/Vaccinieae is 57.63 [44.02-72.18] Myr (Fig. 7, node 19). The crown age of Vaccinieae is 42.23 [31.72-53.60] Myr (Fig. 7, node 28).

2.6.3 Ancestral area reconstructions

The result of the likelihood-based DEC analysis is shown in Fig. 8 (see details for DEC and S-DIVA in Appendix 5). The findings of the different Lagrange model approaches were consistent. Identical percentages were obtained when using no constraints and only range constraints as well as using only dispersal and dispersal combined with range constraints (independent of the chosen maximal number of areas). For some nodes, the Lagrange model including no or only range constraints yielded slightly different percentages compared to the use of only dispersal or range and dispersal constraints, but keeping the highest probability. For example, for node 90 (Fig. 8) the model using no or only dispersal constraints depicted a probability of 53% for B_C_D|F and 37% for B_D|F, while only range constraints displayed 60% for B_C_D|F and 34% for B_D|F and range and dispersal constraints combined yielded 79% for B_C_D|F and 14% for B_D|F. For this reason, we will present only the non-constrained results allowing the highest maximal number of areas (nine).

DEC and S-DIVA analyses showed congruence for ~80% of the nodes, and both analyses inferred an origin of Vaccinieae in North America (area F; Fig. 8, node 10; DEC 8%, S-DIVA 81%). The results differ only for a few nodes between S-DIVA and DEC (nodes 1, 5, 6, 8, 11, 22, 25, 32, 33, 59, 60, 61, 66, 68, 69, 70, 71, 82, 87, 88, 93, 94, 98, 104, 105, 109, 110, 112, 113, 115, 116, 117, 118, 120, 121, and 122), potentially because of a low probability (< 50%) that S-DIVA and/or DEC scored for these nodes. Also, for the deeper nodes of the phylogeny, DEC provided only low probabilities (< 50%), while S-DIVA led mostly to higher probabilities (> 50%). DEC displayed about 29 dispersal events, while S-DIVA showed 31, 11 of which are congruent between the two analyses. In the following, we will generally refer to Vaccinieae when outlining the dispersal routes obtained by DEC and S-DIVA, because *Agapetes* and *Vaccinium* are polyphyletic.

Area F (North America; see Fig. 9 for area definition and dispersal routes) is likely to constitute the area of origin of Vaccinieae, from where this group dispersed between 5.55 to 49.89 million years ago (Ma) to four other areas, namely area B (Indochina, Sundaland, China (except for QTP region), and Japan), area G (Mexico, Central and South America, the Caribbean), area H (Europe and temperate/arctic Asia), and area I (Southern Africa and Madagascar). In total, area B was colonised ten times by Vaccinieae, namely four times from area F (Fig. 8, nodes 11, 18, 108, 113) between 1.33-45.40 Ma, four times from area A (QTP and its surroundings; Fig. 8, nodes 29, 33, 41, 45) between 19.64 Ma to present, and possibly twice from area D (Sahul Shelf and New Zealand; Fig. 8, nodes 18, 139) between 15.37 Ma to present. Area G was colonised three times directly from area F (Fig. 8, nodes 93, 18, 101) between 49.89 Ma to present. The Vaccinieae reached area H (Europe and temperate/arctic Asia) three times from area F (Fig. 8, nodes 103, 113, 120) between 27.41 Ma to present, and once either from area F or B (Fig. 8, node 109) between 28.54 Ma to present. From area F, the Vaccinieae dispersed also once to area I (Southern Africa and Madagascar; Fig. 8, node 13) at about 5.55-24.70 Ma. Area A was colonised in total five times, twice from area B (Fig. 8, nodes 25, 40) between 37.70 Ma to present, once from area F (Fig. 8, node 18) as well as twice from area D (Fig. 8, nodes 18, 70) between 15.37 Ma to present. In area D the Vaccinieae appeared earliest at about 34.74 Ma, coming from area G. In total, area D was colonised three times, once from area B at about 9.13-22.22 Ma (Fig. 8, node 71), once from area G (Fig. 8, node 135), and once either from area A or B at about 1.53-10.34 Ma (Fig. 8, node 68). The earliest occurrence of Vaccinieae in area E (Southern Polynesia and Hawaii) might be around 10.34 Ma, from area A or B. Altogether, the Vaccinieae arrived in area E twice, once from area A or B (Fig. 8, node 68), and once either from area B or F (Fig. 8, node 122) at about 1.32-8.97 Ma. Finally, the Vaccinieae reached area C (Wallacea and the Philippines) in total four times, once from area D (Fig. 8, node 73), once either from area B or D (Fig. 8, node 87), once from area B (Fig. 8, node 76), and once either from area A or B (Fig. 8, node 68) between 1.53-16.40 Ma. Area F (the potential area of origin) was also re-colonised five times, twice from area G (Fig. 8, nodes 100, 129), once from area B or D (Fig. 8, node 87), and twice from area B (Fig. 8, nodes 118, 120) between 0.43-46.76 Ma.

Figure 9.

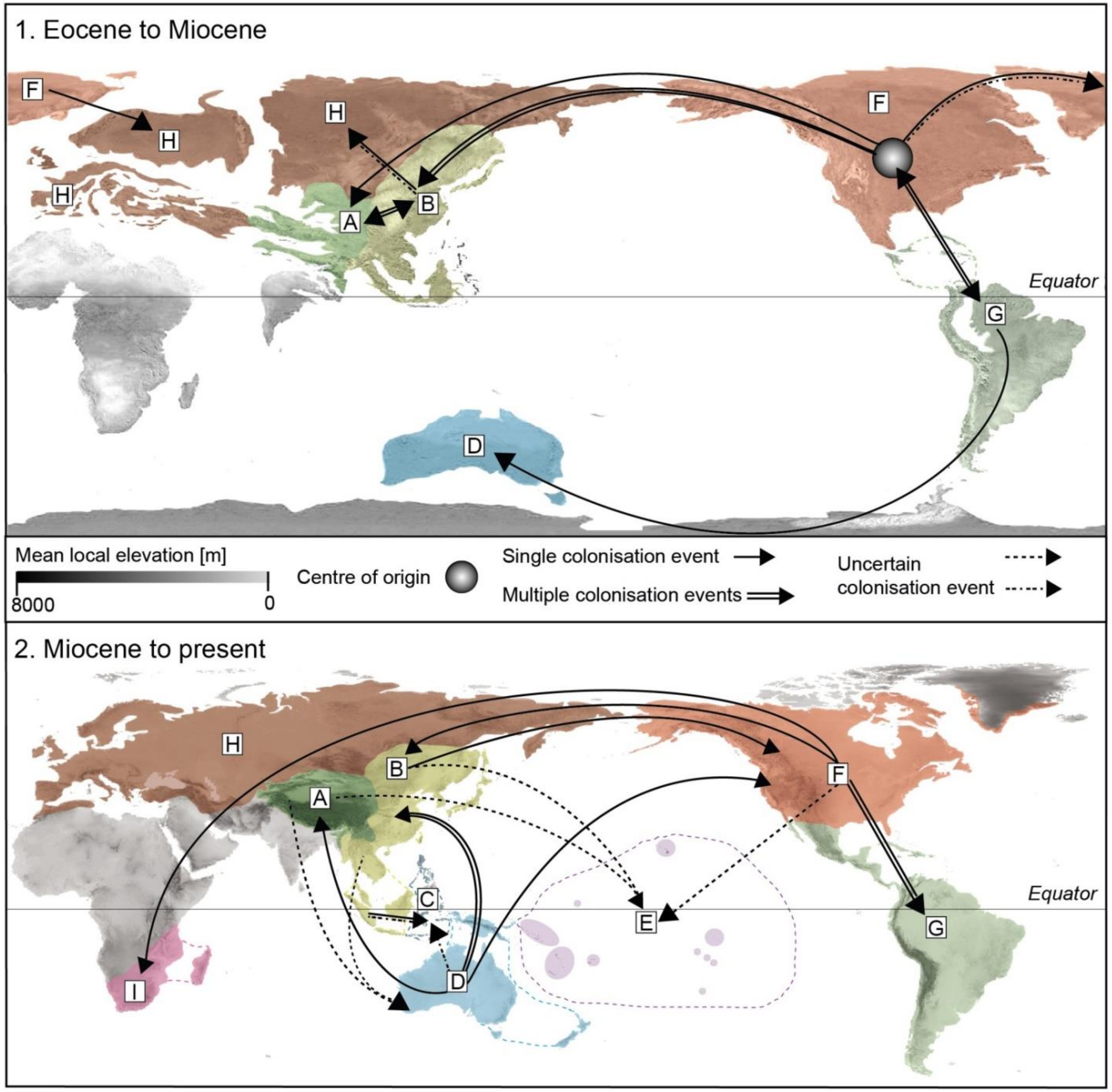
1. Eocene to Miocene
F
H
H
H
H
B
A
F
G
D
Equator
Mean local elevation [m]
8000
0
Centre of origin
Single colonisation event
Multiple colonisation events
Uncertain colonisation event
2. Miocene to present
H
B
A
F
C
E
G
D
I
Equator

Figure 9. Outline of dispersal events based on DEC and S-DIVA analyses for Vaccinieae. Area definition for ancestral area reconstructions with DEC and S-DIVA: (A) Qinghai-Tibetan Plateau (QTP) and its surroundings (green); (B) Indochina, Sundaland (Borneo, Sumatra, Java, Malay Peninsula), China (with QTP region excluded), and Japan (yellow); (C) Wallacea (Sulawesi, the Moluccas, the Banda Arc, and the Lesser Sunda Islands), and the Philippines (dark blue); (D) Sahul shelf (New Guinea and Australia), and New Zealand (light blue); (E) Southern Polynesia and Hawaii (purple); (F) North America (orange); (G) Mexico, Central and South America, the Caribbean (grey); (H) Europe and temperate/arctic Asia (brown); and (I) Southern Africa and Madagascar (pink).

2.7 DISCUSSION

This study presents the first molecular dating approach for Vaccinieae, contributing to our understanding of the evolutionary history within the large Ericaceae family. Our phylogenetic results largely agree with those of Kron & Luteyn (2005), however, our study comprises a much more extended data set for *Agapetes* and *Vaccinium*, provides a temporal framework for dispersal events within Vaccinieae, and therefore allows more detailed biogeographical reconstructions. Despite the incorporation of a larger data set (comprising all sections of *Vaccinium* and covering its entire distribution range), the phylogenetic relationships within *Vaccinium* and Vaccinieae remain only partially resolved. Our study: (1) suggests an integration of *Agapetes* in *Vaccinium*, (2) provides evidence of a North American origin for Vaccinieae in the Eocene, and (3) suggests that the Miocene cooling was an important driver for worldwide dispersal events of Vaccinieae.

2.7.1 Polyphyly of *Agapetes* and *Vaccinium*

Agapetes and *Vaccinium* are both clearly polyphyletic, with *Agapetes* nested within *Vaccinium* species from the region of the QTP, Sundaland and Indochina, in a strongly supported clade (Fig. 7, clade 6, node 32, PP 1.00). The distinction between *Agapetes* and *Vaccinium* is not straightforward, because morphological traits are shared among some species of both genera. For example, tubular and campanulate corollas can occur in both genera, although tubular corollas are more typical of *Agapetes* and campanulate corollas are usually found in *Vaccinium*. Also, both terrestrial and epiphytic plants are found in each genus, although a larger proportion of *Agapetes* is epiphytic and more *Vaccinium* is terrestrial (Mingyuan et al., 2005). Our dataset represents the morphological and ecological variability within both genera. We therefore propose to sink *Agapetes* (all species having a mainland Asian distribution) in *Vaccinium*, as already suggested by previous studies based on morphological and phylogenetic analyses including fewer *Agapetes* species (Stevens, 1972; 1985; 1997; 2004).

In the study of Kron et al. (2002) and Stevens (2004), the former species of *Agapetes* from Papua New Guinea to Fiji and New Caledonia (Oceanic *Agapetes*, now genus *Paphia*) were separated from the species of mainland Asia. *Paphia* used to be a section of *Agapetes*, but due to morphological and molecular data it was elevated to generic rank (Stevens, 2004). Clade 2 (Fig. 7) in our study forms a moderately supported group (node 118, PP 0.92) with species of *Dimorphanthera* and *Paphia* from the Sahul Shelf as well as *Symphysia* species from Central America. The re-establishment of *Paphia* might be only the beginning of a nomenclatural revision, because in the study of Kron et al. (2002), Stevens (2004), and ours, *Paphia* is not monophyletic, with *Paphia meiniana* (F. Muell.) Schltr. being nested in *Dimorphanthera*. One possibility would be to unite *Dimorphanthera* and *Paphia* under the earliest name *Paphia*. Another possibility would be to transfer only *Paphia meiniana* to *Dimorphanthera* to make the remainder of *Paphia* monophyletic. Since the sampling currently is still poor (3 species out of 21), future studies need to be extended to arrive at an accurate final taxonomic decision (Stevens, 2004).

2.7.2 General polyphyly of *Vaccinium* and its sections

The polyphyly of *Vaccinium*, with species scattered throughout the Vaccinieae, also results in the paraphyly of several other genera. For example, the Andean clade (clade 1, Fig. 7, distributions shown in Fig. 8) contains *V. didymanthum*, a species that was not included in the study by Kron et al. (2002). In our study, *V. didymanthum* occurs within *Disterigma* with low support (Fig. 7, node 134, PP 0.65), but it lacks the diagnostic feature of this genus (a pair of bracteoles at the pedicel's apex enveloping the calyx; Pedraza-Penalosa, 2009).

Vaccinium didymanthum was originally part of section *Disterigmopsis* by Sleumer (1936) along with *Disterigma*. This section was abandoned (see current sections of *Vaccinium* in Vander Kloet & Dickinson, 2009), but our results show that it should be resurrected or *V. didymanthum* should be included in a re-defined *Disterigma* genus based upon morphological characteristics of the former section *Disterigmopsis*. This is not an isolated case: in fact, a number of minor Vaccinieae genera should be (or have already been) re-defined to include some closely related *Vaccinium* species. Another example, shown in Vander Kloet et al. (2004) and confirmed by our study, is *Vaccinium poasanum* Donn.Sm. This species, formerly part of *Vaccinium* section *Oreades*, grouped together with *Symphysia racemosa* (Vahl) Stearn in a well-supported clade (Fig. 7, node 119). As a result, the entire section *Oreades* was transferred to *Symphysia* (Vander Kloet et al., 2004).

Furthermore, most sections of *Vaccinium* appear to be polyphyletic (Fig. 7), with the exception of section *Macropelma* being monophyletic but nested within the paraphyletic section *Myrtillus* (see also Kron et al., 2002). Some polyphyletic sections are distributed in a few well-supported clades (e.g. section *Cyanococcus*, Fig. 7, node 114, 103, 99) or are scattered widely throughout the tree (e.g. section *Bracteata*, Fig. 7). In this study, we covered ~24% of all *Vaccinium* species, representing the largest data set on *Vaccinium* so far, but a more extended sampling in combination with morphological investigations is necessary to arrive to a solid delineation of sections within *Vaccinium* in future studies. This work might result in the description of several genera as it occurred in the past for example for section *Oreades* (now *Symphysia*, Vander Kloet et al., 2004), section *Pachyantha* (now *Dimorphanthera*, Stevens, 1974), or section *Pseudodisterigma* (now c.f. *Disterigma*, Wilbur & Luteyn, 1978).

2.7.3 Origin and early dispersal of Vaccinieae

Both DEC and S-DIVA analyses revealed a North American origin for Vaccinieae, which is in agreement with the results of Kron & Luteyn (2005). Using Fitch parsimony character optimisation and DIVA analyses, Kron & Luteyn (2005) postulated a North American origin for Vaccinioideae. However, this study did not include molecular dating, which set limits to biogeographic interpretations. In our study, we found that the crown age of Vaccinieae dates back to the Eocene (Fig. 8, node 10, 42.23 [31.72-53.60] Ma). Despite a contrasting calibration scheme and species sampling, our estimated age for Vaccinieae is very similar to that of Schwery et al. (2015), who calculated 45.6 [37.2-54.4] Ma for the crown of Vaccinieae. Therefore, we are confident that our study provides a realistic temporal framework for our biogeographic inferences. From North America, Vaccinieae dispersed almost simultaneously to Asia and South America between the Early Eocene and the Oligocene/Miocene boundary (19.0-50.0 Ma; Fig. 8, nodes 11, 93, 101, 108).

Within this time frame, the Bering land bridge, connecting Alaska with Siberia, emerged during the Early Eocene, and the Late Eocene (Tiffney, 1985). Because an Early Eocene land bridge would partially predate the origin of Vaccinieae, it seems more likely that the crossing occurred during the latter epoch. Moreover, the very warm climate of the Early Eocene (Pross et al., 2012) could have prevented Vaccinieae to colonise the lowland of the Bering land bridge (if assuming that their past climatic niche might have been similar to the present climatic preferences of the genus). In fact, nearly all extant species of this tribe show a preference for cooler habitats (Ruizheng & Stevens, 2005b). Furthermore, during the Late Eocene, global temperature dropped significantly (Zachos et al., 2001; Liu et al., 2009a), potentially allowing cold adapted lineages to expand their distribution to lower altitudes/latitudes. In the same epoch, the southern part of the QTP had already reached an elevation of more than 4000 m (reviewed in Mulch & Chamberlain, 2006, Lippert et al., 2014, and Favre et al., 2015), potentially providing vacant niches for taxa which could cope with cooler conditions, allowing Vaccinieae to establish and diversify. About 60 species of *Vaccinium* are found nowadays in the surroundings of the QTP (Ruizheng & Stevens, 2005b). Starting during the Late Oligocene, Vaccinieae extended their distribution to Europe, either via the Bering land bridge and temperate/arctic Asia or directly from North America potentially across the North Atlantic Land Bridge (NALB). It was assumed that American-Eurasian lineages with divergence times younger than the Eocene could not have benefitted from the NALB (Tiffney, 2000; Tiffney & Manchester, 2001), thus favouring the route via the Bering land bridge (Milne, 2006).

However, Denk et al. (2010) provided and summarised evidence for an existing NALB with a temperate flora until the latest Miocene, which would be in agreement with our findings.

Vaccinieae reached South America potentially benefiting from the Central American land bridge (Late Eocene until Early Miocene), which was not fully closed at that time, but only a very narrow and shallow isthmian strait separated southern Central America from South America (Farris et al., 2011; Montes et al., 2012). It is known that plant taxa were able to disperse between these two continents for about the past 50 Ma, whereas most of the animals crossed the Isthmus only from 10 Ma onwards (Cody et al., 2010). Because reaching South America involves crossing the equator and because Vaccinieae generally display temperate preferences, long-distance dispersal across mountain regions as “stepping stones” might be postulated here. This could also be shown for other Northern American lineages, such as Malphigiaceae, Fabaceae and Annonaceae, which reached the Neotropics via the mountain chains of Central America and the young Andes (Antonelli et al., 2009). From South America, Vaccinieae dispersed further to the Sahul Shelf between the Late Eocene to Middle Miocene (15.0-35.0 Ma). About at the same time, the Sahul Shelf was connected to South America via Antarctica until c. 35 Ma (Hall, 2002; Knapp et al., 2005). Long-distance dispersal from South America to the Sahul Shelf (especially Australia) has been reported several times (e.g., Knapp et al., 2005; Muschner et al., 2012; Birch & Keeley, 2013). Dispersal from South America to the Sahul Shelf might not have occurred as a single long-distance dispersal event, but progressively via stepping stone dispersal along the coast of Antarctica (Renner et al., 2000). This dispersal route was also potentially observed in other cold-adapted taxa, for example in Gentianaceae (von Hagen & Kadereit, 2001). The early dispersal of predominantly temperate Vaccinieae seems to have occurred mostly via circumpolar land bridges, potentially because of a globally warm climate before the Miocene Cooling. Antarctica experienced near-tropical conditions during the Early Eocene epoch (Pross et al., 2012) followed by a 17-Myr-long cooling trend (Zachos et al., 2001), which might have improved the chances for *Vaccinium* to disperse to the Sahul Shelf via Antarctica.

2.7.4 Miocene to Quaternary dispersal of Vaccinieae

Since the Miocene, dispersal events continued to occur between North America and Asia as well as between North and South America. During the Late Miocene and Pliocene (3.1-12.8 Ma), the Central American archipelago probably enabled dispersal via island-hopping towards South America (Pennington & Dick, 2004; Lomolino et al., 2010). The Central American species of Vaccinieae might have descended from the northern Andean species recently (Kron & Luteyn, 2005). Floristic exchanges occurred between North America and mainland Asia (via the Bering land bridge) repeatedly even until the Late Miocene (Denk et al., 2010; Bacon et al., 2012). This can also be seen in the Vaccinieae by re-colonisation of North America from Sundaland/Eastern Asia between the Middle Miocene to Pliocene (2.4-13.5 Ma). Another re-colonisation of North America occurred simultaneously from the Sahul Shelf. Dispersal events between the Sahul Shelf and North America are rarely reported in comparison to dispersals from the Sahul Shelf to South America (Muschner et al., 2012). Nevertheless, a floristic relationship between North America and the Sahul Shelf might exist, because out of 259 North American plant genera with pantropical distribution, twenty-two comprise Australasian species (Qian, 1999). Alternatively, if we had included more species in the biogeographical analyses (DEC and S-DIVA), this dispersal route might not have been estimated as direct one, but rather have included transit via East Asia or South America. A similar scenario might be observed concerning the postulated straight colonisation of South Africa from North America between the Late Oligocene to Late Miocene (5.6-24.7 Ma). The South African Cape Flora comprises clades usually shared with Australasia, Europe and South America (Galley & Linder, 2006), whereas a North American connection is rather unusual, although Miller et al. (2011) found a similar dispersal event between the Americas and Africa for *Lycium* (Solanaceae) between the Late Miocene and Pleistocene.

From the Miocene onwards, dispersal drastically intensified between Southeast Asia and Polynesia (Fig. 9). Accelerated floristic exchanges between these tropical regions might have been encouraged by Miocene cooling, the increase of the occurrence of montane habitats in this region, and the emergence of new islands (decreasing the distance between suitable habitats). From the Middle Miocene onwards, a worldwide cooling of the climate started (Zachos et al., 2001; Shevenell et al., 2004; Passchier et al., 2013). At that point, Sundaland has been an almost permanent landmass since beginning of the Mesozoic with subsequent addition of continental fragments rifting from Australia (Hall, 2009). The New Guinean-Australian plate collided with Southeast Asia about 25 Ma leading to the formation of Wallacea, of which most islands emerged only about 5 Ma (Hall, 2009; Hall et al., 2011; van Welzen et al., 2011). This timing roughly coincides with our estimated arrival time of Vaccinieae in Wallacea: this region was colonised from the Sahul Shelf and from Sundaland (possibly also from the QTP and its surroundings via Sundaland) between Middle Miocene to present (0-16.4 Ma). Similar dispersal routes were reported during the same timeframe in other studies: from Sundaland to the Sahul Shelf (e.g., Nauheimer et al., 2012; Richardson et al., 2014; Grudinski et al., 2014b; Sirichamorn et al., 2014), and from Sundaland or Sahul Shelf to Wallacea (e.g., Muellner et al., 2008; Thomas et al., 2012; Bacon et al., 2013). More distant islands, such as the Hawaiian archipelago and Southern Polynesia were colonised twice between Late Miocene to Pleistocene (1.3-11.2 Ma), namely once from the surroundings of the QTP region or Sundaland/Northern Indochina and once either from North America or Sundaland/Northern Indochina. Plant seeds are known to have reached the archipelago from all sides of the Pacific and from regions which are even further away: America, Asia, Southeast Asia, Australia, New Zealand and Africa (Geiger et al., 2007; Keeley & Funk, 2011). The Hawaiian Islands started to emerge with the Kure atoll around 29 Ma and subsequent development of the other islands (Kim et al., 1998; Givnish et al., 2009), which corresponds to our estimated arrival time of Vaccinieae. The isolation of these islands implies long-distance dispersal, suggesting either transportation via wind or animal vectors. By producing berries with a high sugar content, Vaccinieae presumably were transported by birds (Luteyn, 2002b; Luteyn & Pedraza-Penalosa, 2012), which most likely greatly contributed to this tribe’s cosmopolitan distribution. The ability to produce berries, dispersed by animal vectors, was shown to have been an important driving factor supporting range expansion across insular regions in *Tripterospermum* Blume (Matuszak et al., in press).

2.7.5 Conclusions

Our study revealed a North American origin for Vaccinieae in the Eocene (42.23 [31.72-53.60] Ma), from where they dispersed nearly throughout the entire world possibly via avian dispersal vectors. Until the Mid-Miocene climate cooling dispersal of Vaccinieae occurred mainly in the circumpolar regions. After the Mid-Miocene climate cooling an increased number of dispersal events was observed globally, but particularly in tropical regions, indicating that climate might have been a driving factor for dispersal of genera with Laurasian origin in the Southern Hemisphere. Furthermore, it is likely that tropical mountain systems played an important role as “stepping stones” for Vaccinieae to disperse from the Northern Hemisphere to the Southern. Finally, our study highlights the complexity of the taxonomy of Vaccinieae. Some base-line taxonomic work needs to be performed in the light of phylogenetic results. To do so, and given the difficulties to obtain proper plastid sequences from herbarium material in Ericaceae, new collections should be encouraged.

2.8 ACKNOWLEDGEMENTS

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3. DISPERSAL ROUTES BETWEEN BIODIVERSITY HOTSPOTS IN ASIA: THE CASE OF THE MOUNTAIN GENUS *TRIPTEROSPERMUM* (GENTIANINAE, GENTIANACEAE) AND ITS CLOSE RELATIVES

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3.1 Authors' 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 (see also Appendix 7).

3.2 Confirmation: accepted by the Journal of Biogeography

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<u>Manuscript Title:</u> Dispersal routes between biodiversity hotspots in Asia: the case of the mountain genus Tripterospermum (Gentianinae, Gentianaceae) and its close relatives

Dear Mr. Adrien Favre;

This is confirmation that the final files you provided for JBI-14-0344.R2 meet our quality standards. The files will shortly be forwarded to the publisher for production and publication.

Sincerely,
Iris Poessé
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Journal of Biogeography

3.3 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 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 Himalayas and Hengduanshan), Taiwan, Wallacea.

Methods The evolutionary history of *Tripterospermum* was studied by using 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 *Crawfurdia*) as ingroup, and *Kuepferia*, *Gentiana* and other Gentianaceae genera as outgroups.

Results Subtropical Gentianinae originated at the southeastern fringe of the QTP (the southern Himalayas 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 Himalayas and the Hengduanshan have acted as a source area for the colonisation 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 colonised 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 hypothesise that avian transportation of berry-like fruits might have facilitated their dispersal.

3.4 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^2 (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 characterised 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 Himalayas, 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 Himalayas and the Tianshan 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 orogenesis of the Himalayas 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., 2014b). This highlights the need to investigate 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, *Crawfurdia* 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*, *Crawfurdia* 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 landmass of Sundaland promote the distribution of *Tripterospermum* during times of the Pleistocene? (3) Are there differences in dispersal patterns between lowland plants (previous studies) and mountain taxa such as *Tripterospermum* (this study) in Southeast Asia?

3.5 MATERIAL AND METHODS

3.5.1 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 *Crawfurdia*, 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 Gentianaceae species (Swertiinae, Helieae, and Potalieae; see Appendix 8). 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, 1995a, b, c, d) 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 dataset 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 8.

3.5.2 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 9). 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 9, together with information about the effect of missing data on the BEAST analysis.

3.5.3 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., 2014b). 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 (Appendix 9). 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: (A) North America, Europe and arctic/temperate Asia, (B) the southeastern fringe of the QTP (comprising the Hengduan Mountains, Yunnan (excluding Xishuangbanna), Sichuan, south and east Tibet, northern Myanmar, Nepal, Bhutan, Arunachal

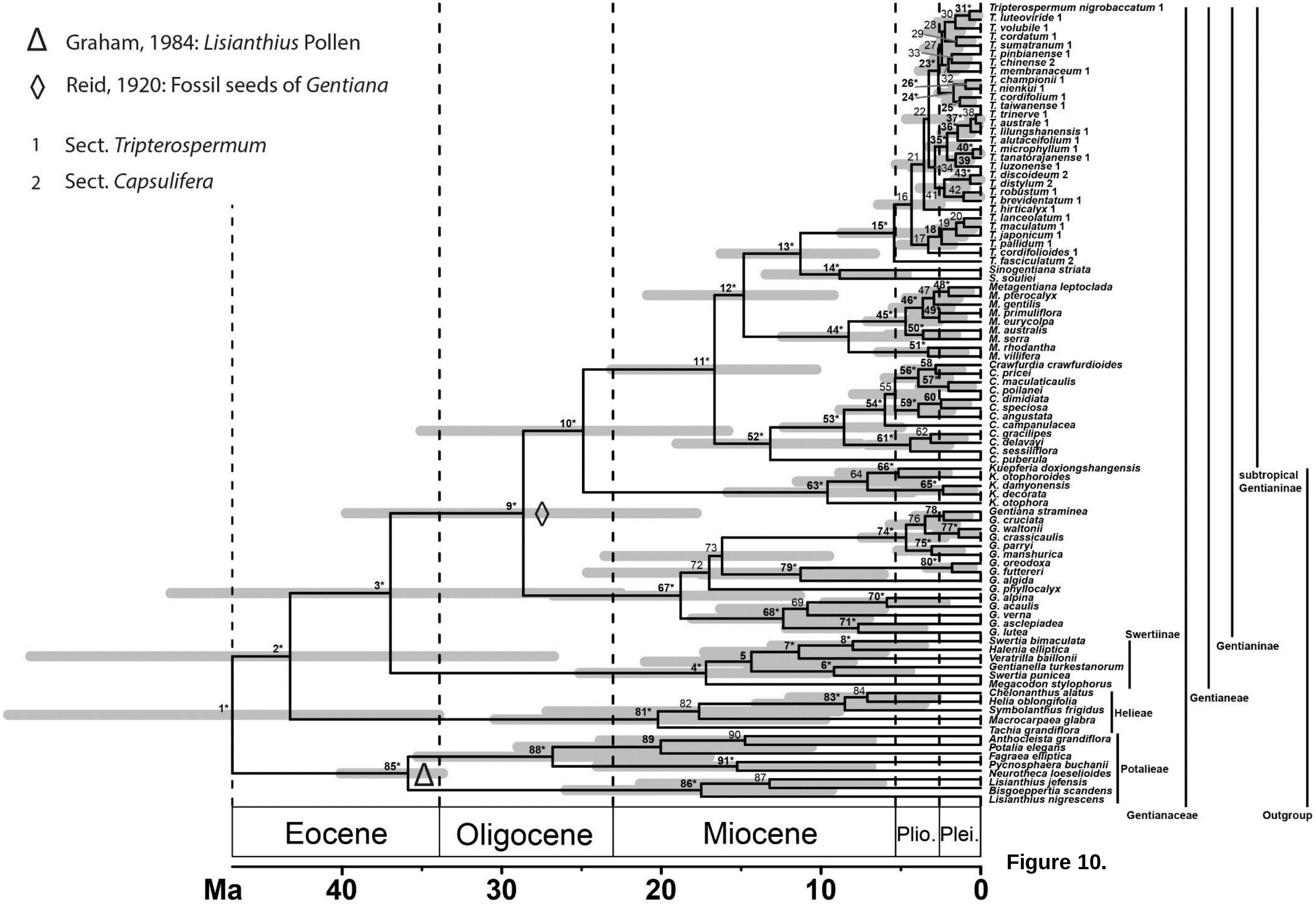
Pradesh and Sikkim), (C) the rest of China, (D) Indochina (comprising Thailand, Laos, Vietnam, Cambodia, China (Xishuangbanna), southwestern Myanmar), (E) Sundaland (Borneo, Sumatra, Java, Malay Peninsula), (F) Wallacea [Sulawesi, the Moluccas, the Banda Arc, and the Lesser Sunda islands], (G) the Philippines, (H) Japan, and (I) Taiwan (a more detailed explanation concerning our area delineation is provided in Appendix 9).

3.6 RESULTS

3.6.1 Phylogenetic relationships and divergence time estimates

Results on sequence data are summarised in Appendix 10. 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 datasets. 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. 10; see Appendix 9). 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. 10), with *Crawfurdia*, *Gentiana*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum* each forming strongly supported monophyletic groups. The phylogenetic relationships among the species of *Tripterospermum* were fully resolved (Fig. 10). 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. 11). Divergence time estimation (Fig. 10; Appendix 9) 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 *Crawfurdia* 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).


Δ Graham, 1984: *Lisianthius* Pollen
◊ Reid, 1920: Fossil seeds of *Gentiana*
1 Sect. *Tripterospermum*
2 Sect. *Capsulifera*
Tripterospermum nigrobaccatum 1
T. luteoviride 1
T. volubile 1
T. cordatum 1
T. sumatranum 1
T. pinbianense 1
T. chinense 2
T. membranaceum 1
T. championii 1
T. nienkui 1
T. cordifolium 1
T. taiwanense 1
T. trinerve 1
T. australe 1
T. lilungshanensis 1
T. alutaceifolium 1
T. microphyllum 1
T. tanatorajanense 1
T. luzonense 1
T. discoideum 2
T. distylum 2
T. robustum 1
T. brevidentatum 1
T. hirticalyx 1
T. lanceolatum 1
T. maculatum 1
T. japonicum 1
T. pallidum 1
T. cordifolioides 1
T. fasciculatum 2
Sinogentiana striata
S. souliei
Metagentiana leptoclada
M. pterocalyx
M. gentilis
M. primuliflora
M. eurycolpa
M. australis
M. serra
M. rhodantha
M. villifera
Crawfurdia crawfurdioides
C. pricei
C. maculaticaulis
C. poilanei
C. dimidiata
C. speciosa
C. angustata
C. campanulacea
C. gracilipes
C. delavayi
C. sessiliflora
C. puberula
Kuepferia doxiongshangensis
K. otophoroides
K. damyonensis
K. decorata
K. otophora
Gentiana straminea
G. cruciata
G. waltonii
G. crassicaulis
G. parryi
G. manshurica
G. oreodoxa
G. futtereri
G. algida
G. phyllocalyx
G. alpina
G. acaulis
G. verna
G. asclepiadea
G. lutea
Swertia bimaculata
Halenia elliptica
Veratrilla baillonii
Gentianella turkestanorum
Swertia punicea
Megacodon stylophorus
Chelonanthus alatus
Helia oblongifolia
Symbolanthus frigidus
Macrocarpaea glabra
Tachia grandiflora
Anthocleista grandiflora
Potalia elegans
Fagraea elliptica
Pycnosphaera buchanii
Neurotheca loeselioides
Lisianthius jefensis
Bisgoeppertia scandens
Lisianthius nigrescens
subtropical Gentianinae
Swertiinae
Gentianinae
Gentianeae
Helieae
Potalieae
Gentianaceae
Outgroup
1*
2*
3*
4*
5
6*
7*
8*
9*
10*
11*
12*
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85*
86*
87
88*
89
90
91*
Eocene
Oligocene
Miocene
Plio.
Plei.
Ma
40
30
20
10
0

Figure 10.

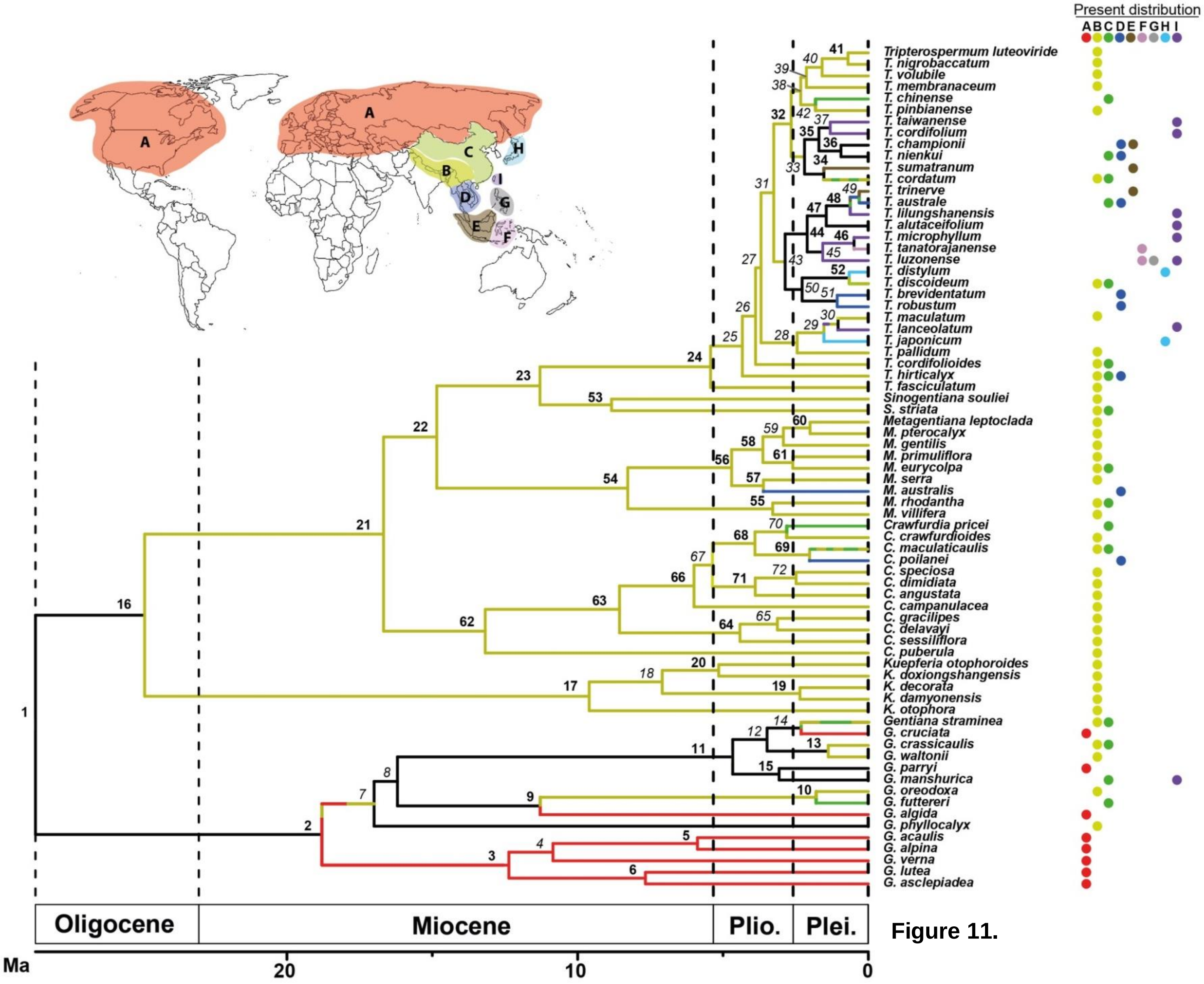
Figure 10. Chronogram for subtropical Gentianinae and Gentianaceae outgroups based on ITS, *atpB-rbcL* and *trnL-trnF* data modelled under a relaxed clock. Two fossils were used for setting temporal constraints: ∆ *Lisianthius* pollen and ◊ fossil seeds of *Gentiana*. Node ages represent mean ages and bars show the 95% highest posterior density intervals. Nodes in bold and with (*) indicate a posterior probability (PP) ≥ 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 9. Ma = million years ago.

3.6.2 Ancestral area reconstructions

The results of the DEC analysis are shown in Figs. 11 and 12 (see details for DEC and S-DIVA in Appendix 9). 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. 12) 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 ca. 360 species). For genera for which we had higher taxonomic coverage (*Tripterospermum*, *Metagentiana*, *Sinogentiana*, *Kuepferia* and *Crawfurdia*), 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* colonised area C six times (rest of China; Fig. 12; nodes 25, 26, 34, 42, 48, 52) and area D three times (Indochina; Fig. 12; nodes 25, 35, 51) between 6.4 Ma and present, area H twice (Japan; Fig. 12, nodes 29, 52) between 2.7 Ma and present, and area I three times (Taiwan; Fig. 12, nodes 29, 35, 44) between 3.3 Ma and present. From area D (Indochina), area C (rest of China; Fig. 12; node 36) as well as area E (Sundaland) were colonised 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. 12, node 48) between 1.3 Ma and present as well as twice to area E (Sundaland; Fig. 12, nodes 45, 48), twice to area F (Wallacea; Fig. 12, nodes 45, 46), and once to area G (the Phillipines; Fig. 12, 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 11. 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 and bars show the 95% highest posterior density intervals. Node numbers in bold indicate a posterior probability (PP) of ≥ 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.10 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 9. Ma = million years ago.


Present distribution
A B C D E F G H I
A
A
B
C
D
E
F
G
H
I
Tripterospermum luteoviride
T. nigrobaccatum
T. volubile
T. membranaceum
T. chinense
T. pinbianense
T. taiwanense
T. cordifolium
T. championii
T. nienkui
T. sumatranum
T. cordatum
T. trinerve
T. australe
T. lilungshanensis
T. alutaceifolium
T. microphyllum
T. tanatorajanense
T. luzonense
T. distylum
T. discoideum
T. brevidentatum
T. robustum
T. maculatum
T. lanceolatum
T. japonicum
T. pallidum
T. cordifolioides
T. hirticalyx
T. fasciculatum
Sinogentiana souliei
S. striata
Metagentiana leptoclada
M. pterocalyx
M. gentilis
M. primuliflora
M. eurycolpa
M. serra
M. australis
M. rhodantha
M. villifera
Crawfurdia pricei
C. crawfurdioides
C. maculaticaulis
C. poilanei
C. speciosa
C. dimidiata
C. angustata
C. campanulacea
C. gracilipes
C. delavayi
C. sessiliflora
C. puberula
Kuepferia otophoroides
K. doxiongshangensis
K. decorata
K. damyonensis
K. otophora
Gentiana straminea
G. cruciata
G. crassicaulis
G. waltonii
G. parryi
G. manshurica
G. oreodoxa
G. futtereri
G. algida
G. phyllocalyx
G. acaulis
G. alpina
G. verna
G. lutea
G. asclepiadea
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
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52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
Oligocene
Miocene
Plio.
Plei.
Ma
20
10
0

Figure 11.

3.7 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.

3.7.1 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 reliable fossils in Gentianaceae. Our divergence time estimates for Gentianeae, Gentianinae, and *Tripterospermum* do agree with those of other studies (see Appendix 10; von Hagen & Kadereit, 2002; Chen et al., 2005b; 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 Appendix10).

Figure 12.

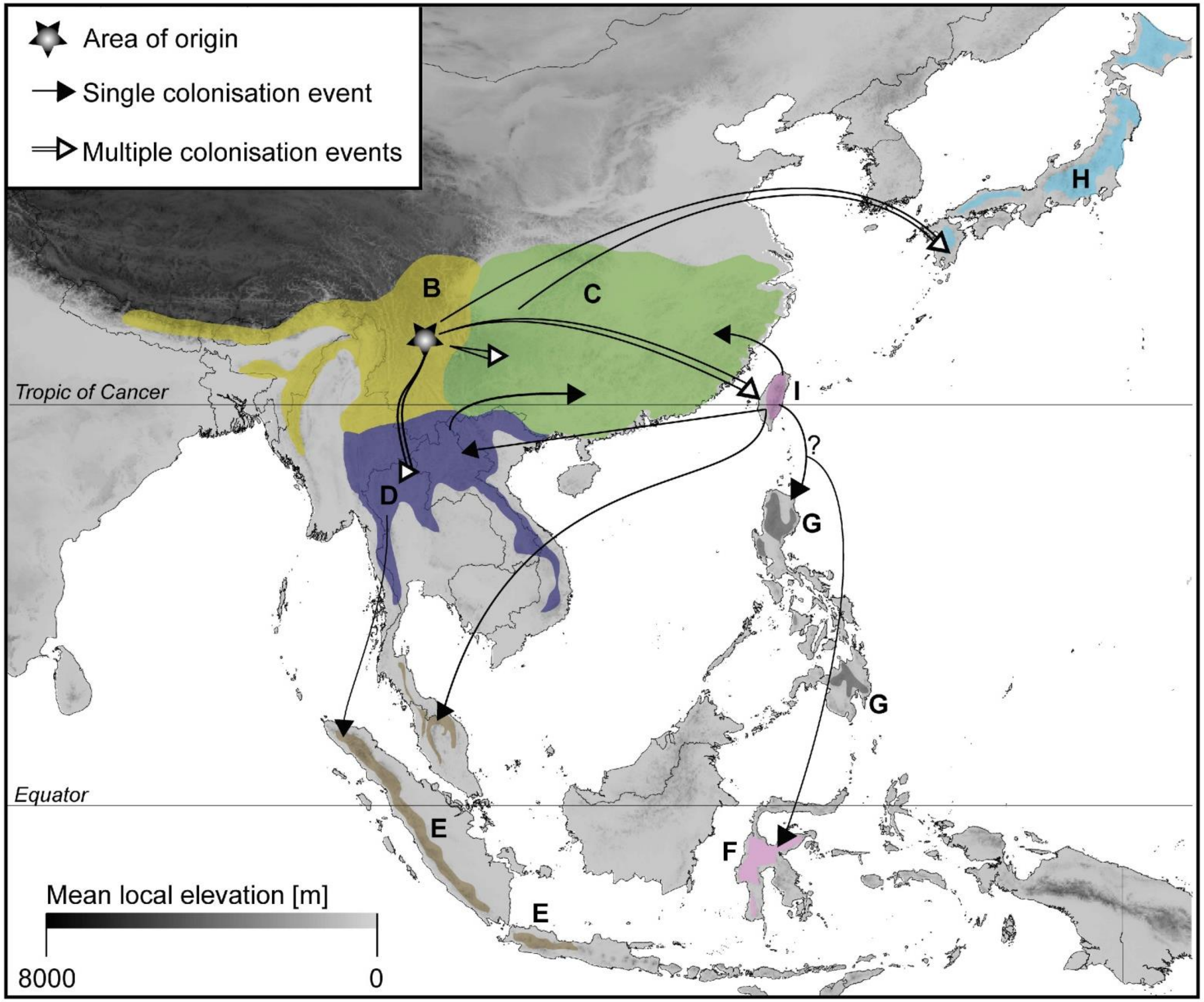
Area of origin
Single colonisation event
Multiple colonisation events
Tropic of Cancer
Equator
Mean local elevation [m]
8000
0
A
B
C
D
E
E
F
G
G
H
I
?

Figure 12. 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), southwestern 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.

3.7.2 Origin of subtropical Gentianinae

Both S-DIVA and DEC analyses suggested an origin of subtropical Gentianinae (*Tripterospermum*, *Metagentiana*, *Sinogentiana*, and *Crawfurdia*) at the southeastern fringe of the QTP around the Oligocene/Miocene boundary (Fig. 12, node 16). At this time, the southern part of the QTP had already reached its present-day altitude (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 characterised by relatively low hydric stress (Ho et al., 2002; Favre et al., 2013; Favre et al., 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. 12, node 23) and seems to have radiated much later during the Pliocene to Early Pleistocene (Fig. 12, nodes 32, 43). At about the same time (boundary of Miocene/Pliocene to Pleistocene), *Tripterospermum* colonised 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).

3.7.3 Colonisation 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 colonisation 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* colonised Taiwan three times independently (Fig. 12, 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. 12, 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).

3.7.4 Colonisation of Indochina, Sundaland, the Philippines and Wallacea

From the fringe of the QTP, *Tripterospermum* colonised Indochina three times independently (Fig. 12; 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 colonisation 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 colonisation, 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 landmass 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 colonisation events between currently adjacent landmasses (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 Southeast Asia. During the Pleistocene, *Tripterospermum* dispersed from Taiwan to Sundaland, the Philippines, and Wallacea (Fig. 12; nodes 45, 46, 48). It is likely that Wallacea was colonised 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. 12, node 45). Many studies on animals and lowland plants have confirmed repeated colonisation of Wallacea from the Sunda Shelf region (e.g., Brown et al., 2009; Nauheimer et al., 2012;

Grudinski et al., 2014b), but only a few have shown a northern colonisation 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 colonisation 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. 10, node 15; Fig. 11, 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.

3.8 CONCLUSIONS

Our study shows that in the case of *Tripterospermum* and other subtropical closely related genera (*Crawfurdia*, *Metagentiana*, and *Sinogentiana*), the southern Himalayas and the Hengduanshan (bordering the QTP) acted as source area for montane species diversity in Southeast Asia (summarised in Fig. 12). *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 *Tripterospermum* 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

Tripterospermum 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, *Tripterospermum* colonised East China, Japan, Taiwan, and Indochina multiple times. From Taiwan *Tripterospermum* 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.

3.9 ACKNOWLEDGEMENTS

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4. KEY INNOVATIONS AND CLIMATIC NICHE DIVERGENCE AS DRIVERS OF DIVERSIFICATION IN SUBTROPICAL GENTIANINAE (GENTIANACEAE) IN THE REGION OF THE QINGHAI-TIBETAN PLATEAU

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4.1 Authors' contributions

A.F., S.M. and A.N.M.-R. conceived the ideas. S.M. collected and analysed the data with instructions from J.S. S.M., A.F., J.S. 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 J.S. contributed to writing. A.F. and A.N.M.-R. recruited the financial support for this study (see also Appendix 11).

4.2 Confirmation: submitted to the American Journal of Botany

Dear Dr. Adrien Favre,

your submission entitled "Key innovations and climatic niche divergence as drivers of diversification in subtropical Gentianinae (Gentianaceae) in the region of the Qinghai-Tibetan Plateau" has been received by the American Journal of Botany.

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Yours truly,
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4.3 ABSTRACT

Premise of the study Geological and climatic changes associated with the uplift of the Qinghai-Tibetan Plateau (QTP) have been suggested as drivers for local biological diversification. To test this hypothesis, we investigated niche evolution of *Tripterospermum* (Gentianaceae) and related Asian genera through time.

Methods We conducted Species Distribution Modelling using the Maximum Entropy Modelling approach. Furthermore, we performed stochastic character mapping as well as disparity through time plots, and examined putative key innovations using the binary state speciation and extinction approach (BiSSE).

Key results *Kuepferia* and *Sinogentiana* prefer the coolest and driest habitat, having rather conserved niches over time. Despite a tendency for niche evolution, *Crawfurdia* and *Metagentiana* are probably restricted to a narrow distribution range because of poor dispersal ability. Contrastingly, *Tripterospermum* features the broadest niche width and occurs under the warmest and wettest conditions. A higher degree of niche evolution and a more efficient dispersal mechanism probably allowed this genus to diversify more and occupy a broader distribution range.

Conclusions The QTP genera producing dry capsules, whether displaying niche conservatism (*Kuepferia* and *Sinogentiana*) or a tendency for niche evolution (*Crawfurdia* and *Metagentiana*) are less species-rich and have a more restricted distribution than *Tripterospermum* (stronger niche evolution and berry-like fruits). The evolution of berry-like fruits corresponds with increased speciation rates and could therefore be viewed as a key innovation. In contrast to the majority of studies on plants occurring around the QTP, we find that speciation was probably mediated by niche breadth and dispersal ability rather than geophysical changes.

4.4 INTRODUCTION

Biological diversity is distributed unequally across the world with marked centres of terrestrial species richness in the tropics and mountainous regions (Myers et al., 2000; Mittermeier et al., 2004). Among the most important mechanisms determining these distribution patterns are the ability of organisms to adapt to changing environmental conditions in combination with the capability to disperse into new habitats (Soberon & Peterson, 2005; Fritz et al., 2013; but see Crisp et al., 2009 for the aspect of biome stasis). In particular, the evolution of specific functional traits (key innovations) has been suggested to promote the evolutionary success of taxa (Heard & Hauser, 1995; Hodges & Arnold, 1995; Hunter, 1998; Drummond et al., 2012), and thus to play a significant role in shaping the pattern of the Earth's biodiversity. In plants, the search for such key innovations typically focused on a single (usually morphological) factor related to growth form, floral morphology, and fruit type/dispersal mechanism (see Vamosi and Vamosi, 2010 and references therein). In the case of dispersal, several intrinsic (such as morphological and physiological novelties) and extrinsic (involving environmental changes) factors are likely to act together (Moore & Donoghue, 2007). Changes in dispersal modes might drive dispersal and geographic range expansion, while the colonization of novel habitats may require ecological pre-adaptations or the ability to evolve ecological tolerances. It is possible that certain traits (also a combination thereof) might trigger diversification only within a particular geographical context (De Queiroz, 2002; von Hagen & Kadereit, 2003). Thus, to understand the potential causes of high diversity, morphological and ecological correlates of diversification need to be considered.

The ecological niche has long been a central concept in ecology (Grinnell, 1917; Elton, 1927; Hutchinson, 1957; Soberon & Peterson, 2005), and can be defined as a combination of biotic and abiotic factors which allow a species to maintain a viable population size (Hutchinson, 1957). In general, one has to distinguish between the fundamental niche, comprising only abiotic factors, and the realised niche, which further includes biotic interactions with other species (Hutchinson, 1957; Wiens & Graham, 2005). Acting together, species dispersal and local niche adaptation may lead to distinct phylogeographic patterns. The following four scenarios are possible for a diversifying clade. Niche conservatism (the tendency of species to retain ancestral ecological tolerances) and a low dispersal ability will result in spatial co-occurrence and occupation of a similar niche. Niche conservatism with high dispersal ability will also lead to the occupation of a similar niche, but with larger range sizes (and likely low range overlap). In contrast, niche evolution and a poor dispersal ability should lead to spatial proximity of closely related species, but divergent ecological tolerances (and different habitats). Finally, niche evolution combined with high dispersal ability will lead to a pattern of varying range sizes, range overlap and habitat preferences within a clade. Thus, studying the evolution of the ecological niche and identifying associated key innovations is an important component of understanding macroevolutionary processes underlying present-day distribution patterns.

Whether niche conservatism or evolution is predominant in plant lineages might depend on environmental variability. For example, Janzen (1967) hypothesized that low temperature seasonality of subtropical to tropical mountains selected for organisms with narrow climatic tolerances because constant climatic barriers were present along the slopes. Simultaneously, these barriers would provide opportunities for climate-driven geographical isolation, divergence and ultimately speciation (Janzen, 1967; Ghalambor et al., 2006; Kozak & Wiens, 2007). Therefore, mountain taxa are highly suitable model investigating organisms for the evolution of climatic niches over time, because they allow to test whether climatic and geological changes in the course of orogenesis have promoted niche evolution or conservatism. An ideal, yet poorly studied, mountain system is the one surrounding the QTP and encompassing four biodiversity hotspots: the mountains of central Asia, the Himalayas, the Hengduanshan and Indo-Burma (Fig. 13; see Myers et al., 2000; Mittermeier et al., 2004). These hotspots are assumed to have resulted from environmental changes in the region which is today the QTP. Although the uplift of the Lhasa-plano (precursor of the QTP) occured very early (c. 110 million years ago, Ma; Lippert et al, 2014), the geology and climate of the region was much altered by the collision of India with Eurasia (55-50 Ma), the subsequent continuous uplift of the QTP, and the orogeny of higher mountain ranges such as the Himalayas and the Tianshan (between 20-10 Ma to present; Mulch & Chamberlain, 2006; Favre et al., 2015). In parallel, the uplift of the QTP and the orogeny of the Himalayas contributed to the onset of the Asian monsoon system (about 22 Ma) and its intensification, as well as to the aridification of central Asia (Lu & Guo, 2014). It has often been assumed that these geological and climatic changes promoted *in situ* plant diversification (reviewed in Wen et al., 2014), yet niche evolution (and associated key innovations) has not yet been studied in this context.

Here, we investigate the divergence of climatic niches and dispersal modes of five closely related genera in the vicinity of the Qinghai-Tibetan Plateau (QTP), hereafter referred to as “subtropical Gentianinae” (Gentianaceae), representing the entire sister lineage to *Gentiana* L. (Favre et al., 2014): *Crawfurdia* Wallich (19 spp.)*, Kuepferia* Adr.Favre (12 spp.), *Metagentiana* T. N. Ho & S. W. Liu

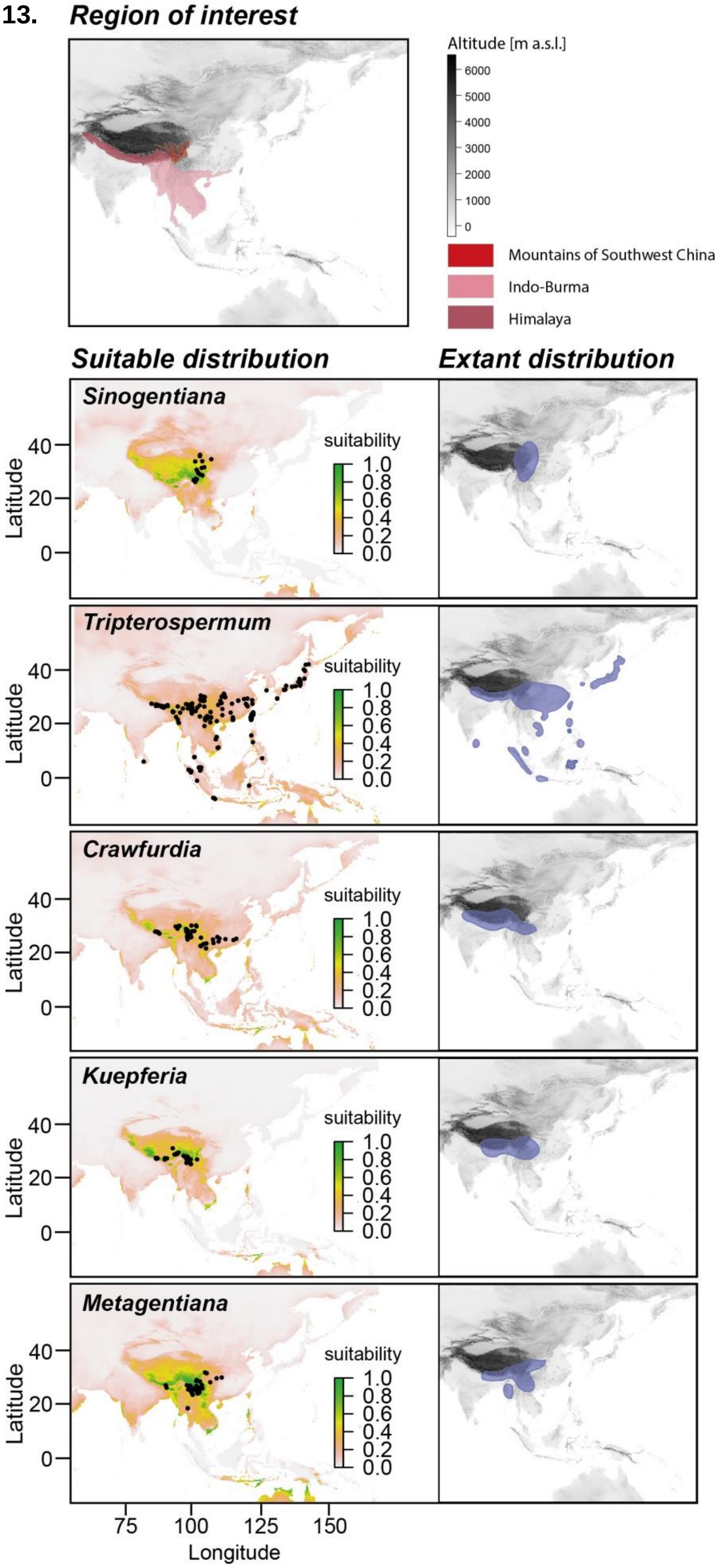
(12 spp.), *Sinogentiana* Favre & Yuan (2 spp.) and *Tripterospermum* Blume (35 spp.). All subtropical Gentianinae genera co-occur in three biodiversity hotspots surrounding the QTP (the Himalayas, the Hengduanshan and Indo-Burma; Fig. 13), whereas *Tripterospermum*, *Crawfurdia* and *Metagentiana* also occur in sympatry in southeast China and in northern Indochina (Vietnam, Thailand and Myanmar). The distribution of *Tripterospermum* extends even further to the islands of the China seas (i.e. Japan, Taiwan, Philippines) and southern Southeast Asia (Malaysia, Indonesia). These five genera are an ideal model system to investigate ecological niche divergence, because their phylogenetic relationships as well as their age and biogeographic history are well known. All genera are monophyletic (Yuan & Kuepfer, 1995; Chen et al., 2005b; Favre et al., 2010; Favre et al., 2014), originated in the vicinity of the QTP and are relatively young (Matuszak et al., in press; crown age of *Tripterospermum* 2.7 to 8.8 Ma; of *Sinogentiana* 4.6 to 13.5 Ma; of *Metagentiana* 4.6 to 12.5 Ma; of *Kuepferia* 4.3 to 15.9 Ma; of *Crawfurdia* 7.5 to 19.1 Ma). In addition, they inhabit a wide range of montane/alpine habitats: the twining vines *Crawfurdia* and *Tripterospermum* can be found in habitats such as montane forest margins and clearings, bamboo thickets and vegetation dominated by shrubs (Ho & Pringle, 1995a, b), the latter habitat being sometimes shared with *Metagentiana* and *Sinogentiana*. Species of *Kuepferia, Sinogentiana* and *Metagentiana* usually occur in more open habitats, including montane to alpine meadows or stony slopes, and only *Kuepferia* is consistently found above the tree line. Their dispersal mechanisms vary: seeds are enclosed in dry capsules and presumably dispersed by gravity alone (*Kuepferia;* Ho & Pringle, 1995c), or by wind (narrowly winged seeds in *Crawfurdia, Metagentiana, Sinogentiana,* and a few *Tripterospermum* species; Murata, 1989; Ho & Pringle, 1995a, b, c; Ho et al., 2002), or are contained in fleshy fruits (the vast majority of *Tripterospermum* species; Murata, 1989) for which dispersal vectors are unknown.

In this study, we quantify the climatic divergences among subtropical Gentianinae, evaluate their temporal dynamics, and assess patterns of trait evolution among and within this group. Specifically, we ask: 1) what are the genera’s specific climatic preferences, and 2) do they occupy the maximum extent of their potential distribution depicted by climatic and elevation data?

We used Species Distribution Modelling (SDM) to define species' climatic niches and predict their potential geographic distribution (Warren et al., 2008; Elith & Leathwick, 2009). Furthermore, we evaluated the degree of their niche overlap. If this knowledge is combined with information on the evolutionary relationships among the five genera, it is also possible to study the evolutionary dynamics. Therefore, we also ask: 3) How did niche evolution or conservatism affect the evolution and dispersal history of each genus, 4) what was the effect of geological and climatic changes in the region of the QTP on diversification of different genera in Gentianinae, and 5) what was the effect of putative key innovations on the diversification of the group?

Figure 13. Maximum Entropy Modelling of geographic distributions of *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum,* and maps of their extant distribution. Black dots represent occurrence data used in this study (see also Appendix 12 for details).

Figure 13.


Region of interest
Altitude [m a.s.l.]
6000
5000
4000
3000
2000
1000
0
Mountains of Southwest China
Indo-Burma
Himalaya
Suitable distribution
Extant distribution
Sinogentiana
Tripterospermum
Crawfurdia
Kuepferia
Metagentiana
suitability
1.0
0.8
0.6
0.4
0.2
0.0
Latitude
40
20
0
75
100
125
150
Longitude

4.5 MATERIAL AND METHODS

4.5.1 Species occurrence data

Our distribution data cover ~47% of all extant *Crawfurdia*, ~42% of *Kuepferia*, 75% of *Metagentiana*, 100% of *Sinogentiana*, and ~77% of *Tripterospermum* species. Our data set was primarily based on herbarium vouchers from numerous herbaria (see Appendix 12) *availabl*e from the Global Biodiversity Information Facility (GBIF; *www.gbif.org/) or from Tropicos (http://www.tropicos.org/). Further data were retrieved directly from online data bases of various herbaria (FR, IBK, K, KUN, PE, RBGE, TAIE) and* the literature (Murata, 1989; Zheng & Yao, 1998; Ho et al., 2002; Chen et al., 2006; Chen et al., 2008a; Favre et al., 2013; see Appendix 12 for voucher information). Finally, data were obtained during field campaigns in Asia by Adrien Favre between 2002 and 2010, and by Adrien Favre and Sabine Matuszak in 2011. Our distribution data do not include narrow endemics and/or species that are only rarely available with reliable occurrence points in databases. Altogether, our data set comprises 432 georeferenced localities, with a spatial accuracy of 5 km and with an average of ~8 data points per species. Many studies underlined the importance of having a large sample size (>30 records) for species distribution modelling (e.g., Stockwell & Peterson, 2002; Wisz et al., 2008), but Hernandez et al. (2006) showed that MaxEnt was able to produce reliable results with only 5 to 10 occurrence records.

4.5.2 Overlap statistics and niche modelling

For all species occurrence points, we obtained information about the altitude as well as climatic data from 19 variables of the WorldClim data set (see Table 3; Hijmans et al., 2005). To identify differences of abiotic environmental tolerances between *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum*, a T-test and the Kolmogorov-Smirnov test (KS test) were conducted in R (R Core Team, 2013).

We performed Maximum Entropy Modelling of geographic distributions of *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum* using MaxEnt v3.3.3k (Phillips et al., 2006; Phillips & Dudik, 2008). We used 19 climatic variables and altitude of the WorldClim data set (Hijmans et al., 2005; see Table 4). Following an initial analyses of all 20 variables, we reduced their number to eight (see Table 4) according to their relevance for the MaxEnt models (identification of important variables via the Area Under the receiver operating Curve (AUC) and Jackknife test) and a low coefficient of correlation ($r < 0.7$). The MaxEnt analyses were performed using 10 replicates.

Environmental niche models (ENMs) identify the ecological preferences of species (Warren et al., 2010) forming the basis for evaluation of niche similarity/overlap. Using the predicted habitat suitability generated by MaxEnt, we calculated niche overlap using Schoener's D (Schoener, 1968) and the I statistic (Warren et al., 2008) as implemented in ENMTools (Warren et al., 2010) via pairwise comparison of species. I and D values of the niche overlap range from 0 (species have distinct ENMs) to 1 (species have identical ENMs; Warren et al., 2010). We calculated niche overlap values both among *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum,* and among species within these genera.

Table 3. A Kolmogorov-Smirnov Test (KS-Test; to determine if two data sets differed significantly) and a T-Test (to determine if the mean of the data sets differed significantly) were performed using the original data from the WorldClim data set (Hijmans et al., 2005) obtained for all species occurrence points of *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum* for altitude and 19 bioclimatic variables. Only compared data pairs that did not differ significantly from each other in both tests or which did not differ in one of the tests are listed here (the complete list of results is presented in Appendix 14). For variable description see Table 4.

Variable	Do not differ significantly in KS- and T-Test	Differ only in one test significantly
Altitude	*Kuepferia - Sinogentiana*	\
Bio1	\	\
Bio2	\	*Crawfurdia - Metagentiana*
Bio3	*Crawfurdia - Sinogentiana*	*Crawfurdia - Kuepferia* *Crawfurdia - Metagentiana* *Crawfurdia - Tripterospermum* *Kuepferia - Metagentiana* *Kuepferia - Tripterospermum* *Metagentiana - Tripterospermum* *Sinogentiana - Tripterospermum*
Bio4	*Crawfurdia - Kuepferia*	*Crawfurdia - Metagentiana* *Kuepferia - Metagentiana*
Bio5	*Kuepferia - Sinogentiana*	\
Bio6	\	*Crawfurdia - Metagentiana*
Bio7	*Crawfurdia - Metagentiana*	*Kuepferia - Metagentiana*
Bio8	\	*Metagentiana - Tripterospermum*
Bio9	*Crawfurdia - Metagentiana*	\
Bio10	\	\
Bio11	\	\
Bio12	\	\
Bio13	\	*Kuepferia - Metagentiana* *Kuepferia - Sinogentiana*
Bio14	\	\
Bio15	*Kuepferia - Metagentiana* *Kuepferia - Sinogentiana* *Metagentiana - Sinogentiana*	*Crawfurdia - Kuepferia*
Bio16	\	*Kuepferia - Sinogentiana*
Bio17	*Kuepferia - Metagentiana*	\
Bio18	\	*Kuepferia - Metagentiana* *Kuepferia - Sinogentiana*
Bio19	*Kuepferia - Metagentiana*	\

Table 4. Bioclimatic variables and altitude variable from WorldClim (Hijmans et al., 2005). Variables marked by an asterisk (*) were used for the climatic niche models of *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum*.

Bioclimatic variables	
Altitude*	/
BIO1	Mean annual temperature
BIO2*	Mean diurnal range (mean of monthly (max temp – min temp))
BIO3	Isothermality (BIO2/BIO7)
BIO4	Temperature seasonality (standard deviation)
BIO5	Max temperature of warmest month
BIO6	Min temperature of coldest month
BIO7*	Temperature annual range (BIO5-BIO6)
BIO8*	Mean temperature of wettest quarter
BIO9*	Mean temperature of driest quarter
BIO10	Mean temperature of warmest quarter
BIO11	Mean temperature of coldest quarter
BIO12	Annual precipitation
BIO13	Precipitation of wettest month
BIO14	Precipitation of driest month
BIO15*	Precipitation seasonality (coefficient of variation)
BIO16	Precipitation of wettest quarter
BIO17	Precipitation of driest quarter
BIO18*	Precipitation of warmest quarter
BIO19*	Precipitation of coldest quarter

4.5.3 Reconstruction of habitat evolution

Stochastic character mapping (Huelsenbeck et al., 2003) was conducted to reconstruct the evolution of habitat preferences of Gentianinae. We used the *make.simmap* function implemented in the R package *phytools* (Revell et al., 2012) to project the habitats onto the maximum credibility tree from a BEAST analysis (obtained from Matuszak et al., in press). *phytools* uses a Maximum Likelihood (ML) approach to determine parameter values for the model of character evolution. Subsequently, character histories are reconstructed on trees by generating samples from a posterior distribution conditional on the ML-fitted model. We assigned five states of habitat preferences to be mapped onto the phylogenetic tree of Gentianinae: (1) open (grasslands), (2) rather open (bamboo grasslands), (3) semi-open (bushes), (4) shaded (forest margin), and (5) strongly shaded habitats (forest understorey).

4.5.4 Niche disparification and trait-correlated diversification

We quantified the temporal distribution of niche disparity within versus among subclades of subtropical Gentianinae using relative disparity plots as implemented in the R package *geiger* (Harmon et al., 2003, Harmon et al., 2008). For each subclade, which is defined by the n-1 internal nodes in the phylogeny (with n being the number of extant taxa), disparity is estimated as the mean of squared pairwise differences between extant taxa and standardised relative to the disparity of the entire clade. Because relative disparity is computed for each internal node, it declines from the stem to the tips over time. Values close to 1 point to subclades containing a substantial proportion of the total variation, whereas values near 0 indicate that subclades cover relatively little of the variation present (Harmon et al., 2003; Evans et al., 2009). In addition, the observed disparity is compared to that expected under an unconstrained Brownian motion model of evolution (using 100 simulations). Positive values of the disparity index (MDI; Harmon et al., 2003; Kozak et al., 2005) imply niche evolution within subclades, whereas negative values indicate niche evolution among subclades and conservatism within subclades.

We examined the effect of five putative key innovations on the diversification of subtropical Gentianinae using the binary state speciation and extinction approach (BiSSE; Maddison et al., 2007) as implemented in BayesRate (Silvestro et al., 2011). This specifically takes into account phylogenetic uncertainty and considers the uncertainty of the model selection by Bayesian model averaging (Silvestro et al., 2014). For a total of 58 ingroup species (out of 77), representing all five genera of subtropical Gentianinae, five traits were tested as possible key innovations (see Appendix 13): fruit coded as capsule (32 species: representing ρ_{capsule} = 61% of all species with capsules) or berry (26 species: ρ_{berry} = 88%), stamens coded as straight (17 species: ρ_{straight} = 55%) or recurved (41 species: ρ_{recurved} = 84%), internodes coded as normal (14 species: ρ_{normal} = 58%) or elongated (44 species: $\rho_{\text{elongated}}$ = 79%), habitus coded as erect (16 species: ρ_{erect} = 62%) or twining (42 species: ρ_{twining} = 78%), and corolla lobation coded as shallow (53 species: ρ_{shallow} = 78%) or deep (5 species: ρ_{deep} = 42%). For each trait, eight models were tested where the model parameters (speciation rate, λ, extinction rate, μ, and the transtion rate, q) were either linked or unlinked (see Appendix 13) using the BEAST maximum clade credibility tree (obtained from Matuszak et al., in press) by running 100000 MCMC iterations setting the first 10000 generations as burn-in, and the sampling frequency to 500. Model-fit was compared using Bayes Factors and the best-ranking models for each trait (with Bayes Factor < 2; see Appendix 13) were used in a second analysis for parameter estimation. We ran the MCMC on a random sample of 100 trees from the posterior distribution of the BEAST analysis with 200000 iterations on each tree to incorporate phylogenetic uncertainty in the parameter estimates.

4.6 RESULTS

4.6.1 Ecological differentiation and evolution of habitat preferences

We calculated niche overlap values within and between *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum* using ENMTools (Warren et al., 2008). Habitat requirements did vary partially within *Crawfurdia*, *Kuepferia*, *Metagentiana* and *Sinogentiana*, which was indicated by moderate niche overlap among species within each genus (Table 5; D: 0.48-0.71; I: 0.63-0.80). In contrast, variation within *Tripterospermum* was higher showing a lower degree of niche overlap (Table 5; D: 0.36; I: 0.57). Generally, niche overlap between genera was moderate (Table 5), with *Crawfurdia*, *Kuepferia*, *Metagentiana* and *Sinogentiana* being most similar to each other (D: 0.47-0.62; I: 0.64-0.74), while *Tripterospermum* differed from the other genera more strongly (D: 0.29-0.40; I: 0.52-0.60). The highest overlap was observed between *Crawfurdia* and *Kuepferia* (Table 5; D: 0.60; I: 0.74) as well as between *Kuepferia* and *Metagentiana* (Table 5; D: 0.62; I: 0.74), while the lowest niche overlap was detected between *Sinogentiana* and *Tripterospermum* (D: 0.29; I: ~0.52).

Based on stochastic character mapping, it is likely that the ancestor of Gentianinae evolved in an open, light-exposed habitat (Fig. 14). Counting the recent definite habitat switches, we inferred six independent transitions towards open habitats (three within *Crawfurdia*, two within *Metagentiana*, and one within *Tripterospermum*), six to semi-open habitats (three within *Crawfurdia*, two within

Metagentiana, and one within *Tripterospermum*), ten to moderately shaded habitats (two within *Crawfurdia*, one within *Metagentiana*, and seven within *Tripterospermum*), and nine shifts to strongly shaded habitats (one within *Crawfurdia*, one within *Metagentiana*, and seven within *Tripterospermum*; Fig. 14).

Table 5. Niche overlap values within and between *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum*. Using the predicted habitat suitability generated by MaxEnt, ENMTools calculate the niche overlap using Schoener's D (Schoener, 1968) and the I statistic (Warren et al., 2008) via pairwise comparison of species. Schoener's D and I values range from 0 (no overlap) to 1 (complete overlap).

Niche overlap within	**D**	**I**
Crawfurdia	0.5539	0.7041
Kuepferia	0.7117	0.7992
Metagentiana	0.6091	0.7338
Sinogentiana	0.4748	0.6321
Tripterospermum	0.3585	0.5729
between	**D**	**I**
Crawfurdia - Kuepferia	0.6033	0.7357
Crawfurdia - Metagentiana	0.5424	0.6922
Crawfurdia - Sinogentiana	0.4719	0.6387
Crawfurdia - Tripterospermum	0.3949	0.5973
Kuepferia - Metagentiana	0.622	0.7438
Kuepferia - Sinogentiana	0.614	0.7285
Kuepferia - Tripterospermum	0.3512	0.5661
Metagentiana - Sinogentiana	0.5193	0.6771
Metagentiana - Tripterospermum	0.3651	0.5739
Sinogentiana - Tripterospermum	0.2887	0.516

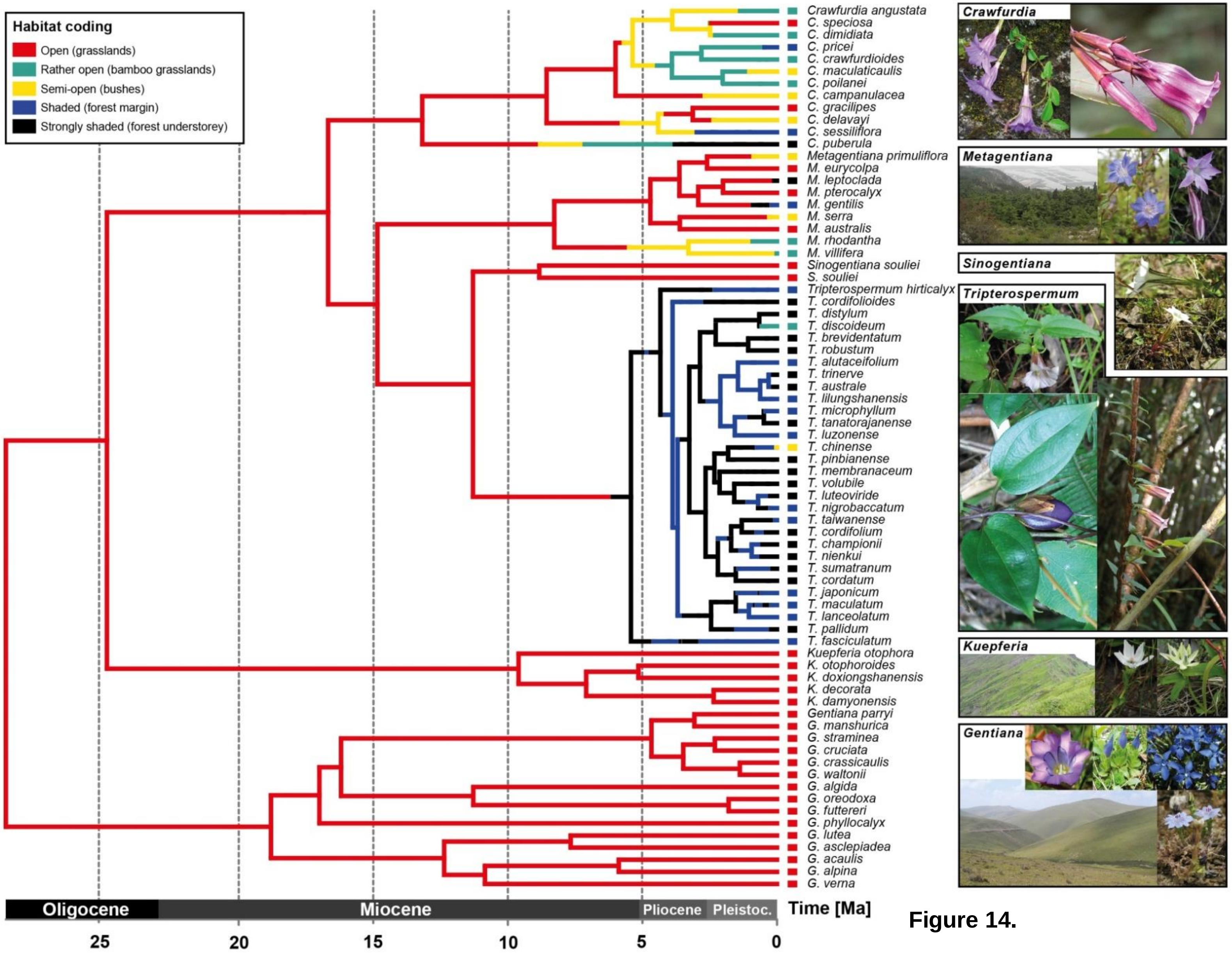

Habitat coding
🟥 Open (grasslands)
🟩 Rather open (bamboo grasslands)
🟨 Semi-open (bushes)
🟦 Shaded (forest margin)
⬛ Strongly shaded (forest understorey)
Crawfurdia angustata
C. speciosa
C. dimidiata
C. pricei
C. crawfurdioides
C. maculaticaulis
C. poilanei
C. campanulacea
C. gracilipes
C. delavayi
C. sessiliflora
C. puberula
Metagentiana primuliflora
M. eurycolpa
M. leptoclada
M. pterocalyx
M. gentilis
M. serra
M. australis
M. rhodantha
M. villifera
Sinogentiana souliei
S. souliei
Tripterospermum hirticalyx
T. cordifolioides
T. distylum
T. discoideum
T. brevidentatum
T. robustum
T. alutaceifolium
T. trinerve
T. australe
T. lilungshanensis
T. microphyllum
T. tanatorajanense
T. luzonense
T. chinense
T. pinbianense
T. membranaceum
T. volubile
T. luteoviride
T. nigrobaccatum
T. taiwanense
T. cordifolium
T. championii
T. nienkui
T. sumatranum
T. cordatum
T. japonicum
T. maculatum
T. lanceolatum
T. pallidum
T. fasciculatum
Kuepferia otophora
K. otophoroides
K. doxiongshanensis
K. decorata
K. damyonensis
Gentiana parryi
G. manshurica
G. straminea
G. cruciata
G. crassicaulis
G. waltonii
G. algida
G. oreodoxa
G. futtereri
G. phyllocalyx
G. lutea
G. asclepiadea
G. acaulis
G. alpina
G. verna
Crawfurdia
Metagentiana
Sinogentiana
Tripterospermum
Kuepferia
Gentiana
Oligocene
Miocene
Pliocene
Pleistoc.
Time [Ma]
25
20
15
10
5
0

Figure 14.

Figure 14. Chronogram of Gentianinae based on ITS, *atpB-rbcL* and *trnL-trnF* data modelled under a relaxed clock in BEAST (details about fossil contraints and other priors to be found in Matuszak et al., in press). Stochastic character map assigning five traits: (1) open (grasslands), (2) rather open (bamboo grasslands), (3) semi-open (bushes), (4) shaded (forest margin), and (5) strongly shaded habitats (forest understorey). All pictures by Adrien Favre.

4.6.2 Differentiating climatic factors

A KS Test as well as a T-Test were conducted for altitude and 19 bioclimatic variables for all species occurrence points of the five genera. Almost 90% of the pairwise comparisons between the genera showed significant differences. Of all climatic parameters, only isothermality (Bio 3; mean 0.4 to 0.45) did not differ much among the genera (Table 3; details in Appendix 14), as all genera occurr in areas where the diurnal temperature range is nearly half of the annual temperature range (as it can often be found in mountains). Significant differences between genera were found for annual mean temperature (Bio 1), mean temperature of warmest quarter (Bio 10), mean temperature of coldest quarter (Bio 11), annual precipitation (Bio 12) and precipitation of driest month (Bio 14), indicating that these five variables were most relevant to describe the differences of climatic tolerances (niches) among subtropical Gentianinae (Table 3; details in Appendix 14). Figure 3 shows density plots and the 95% HPD intervals of the raw data for the five genera and the variables mentioned above. The data show an overlap of requirements with regard to temperature and precipitation, but every genus has its own optimum range. Bio1, Bio10 and Bio11 illustrate all together that *Sinogentiana* and *Kuepferia* prefer cooler conditions compared to the other genera, with temperatures ranging from c. -10 °C (during the coldest quarter of the year) to c. 18 °C (during the warmest quarter of the year). *Crawfurdia* and *Metagentiana* prefer temperatures ranging from c. -4 °C (during the coldest quarter of the year) to c. 25 °C (during the warmest quarter of the year), while *Tripterospermum* generally occurs where temperatures are the warmest (up to c. 28 °C). Bio12 and Bio14 show the same tendency for precipitation. *Sinogentiana* and *Kuepferia* prefer relatively dry conditions (c. 360 to 1400 mm rainfall per year), while *Crawfurdia* and *Metagentiana* occur at c. 650 to 2000 mm of precipitation. Compared to these four genera, *Tripterospermum* prefers relatively wet conditions with c. 730 to 3500 mm rainfall per year.

4.6.3 Climatic niche modelling

MaxEnt models based on occurrence data were statistically strongly supported ($AUC_{Crawfurdia}$: 0.969 ± 0.017; $AUC_{Kuepferia}$: 0.990 ± 0.007; $AUC_{Metagentiana}$: 0.974 ± 0.020; $AUC_{Sinogentiana}$: 0.975 ± 0.019; $AUC_{Tripterospermum}$: 0.966 ± 0.033). For *Crawfurdia* and *Tripterospermum,* bioclimatic niche models generally predicted wide areas of suitable habitats between Kamchatka and Japan, and between the QTP and Southeast Asia (comprising Indochina, the Philippines, East Malesia, Indonesia, New Guinea, and East Timor) and Northern Australia (Fig. 13). Differences between these two genera and *Kuepferia*, *Metagentiana* and *Sinogentiana* were strongest in southeast China and the Southeast Asian region: nearly the entire region was modeled as unsuitable with the exception of the northern Philippines, Java, and parts of New Guinea. Out of the eight uncorrelated variables, which were used for the MaxEnt models, altitude as well as temperature annual range (Bio 7), precipitation of warmest quarter (Bio 18) and precipitation of coldest quarter (Bio 19) contributed the most to the potential distribution of the genera (Fig. 15).

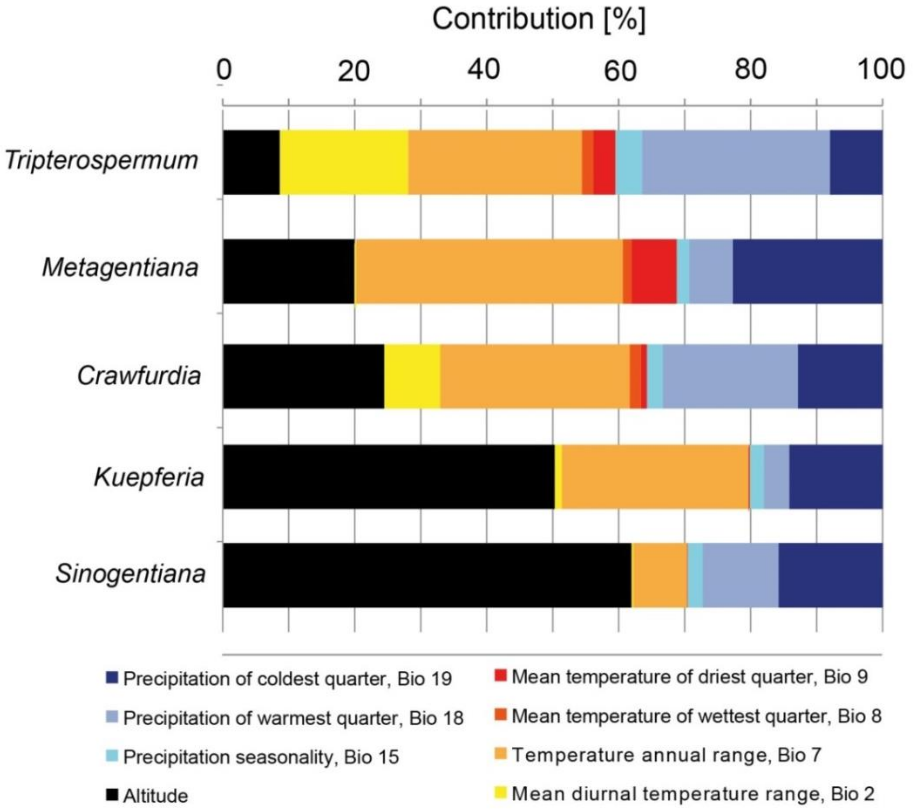


Figure 15. Contributions of altitude and bioclimatic variables to SDM predictions for *Crawfurdia, Kuepferia, Metagentiana, Sinogentiana* and *Tripterospermum*.

4.6.4 Disparity

Disparity through time plots were computed for the five climatic variables for which significant differences among all genera were found (Bio 1, annual mean temperature; Bio 10, mean temperature of warmest quarter; Bio 11, mean temperature of coldest quarter; Bio 12, annual precipitation; Bio 14, precipitation of driest month), and for the four variables which were most important for the MaxEnt models (altitude; Bio 7, temperature annual range; Bio 18, precipitation of warmest quarter; Bio 19, precipitation of coldest quarter). During the early evolutionary history of Gentianinae (Fig. 16), clade disparity does not significantly diverge from the null hypothesis of character evolution by unconstrained Brownian motion (until c. 6-4 Ma) in all tested variables. From this time onwards, a sharp rise in the average subclade disparity was found, indicating substantial divergence of climatic niches among subclades. In Bio 7, Bio 12, Bio 14 and Bio 19 disparity values of 2-3 were reached, which means that several subclades contain a substantial proportion of the total variation. Thus, adaptations to novel conditions for these four variables evolved in young subclades. The MDI values were mostly positive (pointing to niche evolution within subclades), except for Bio 10 and Bio 12 (indicating niche conservatism within subclades). A comparison among the genera of subtropical Gentianinae revealed that the overall patterns (Fig. 16) are driven predominantly by *Crawfurdia* and *Tripterospermum* (Appendix 15). *Metagentiana* shows a markedly different pattern (Appendix 15), with a peak at about 5 Ma followed by low terminal disparity values. This peak can be attributed to *M. australis* (Craib) T.N.Ho & S.W.Liu. Furthermore, only *Kuepferia* indicated niche conservatism throughout time (Appendix 15). Either disparity remained lower than the null hypothesis of Brownian motion throughout time (Bio 1, Bio 11) or disparity matched the Brownian motion model (result not shown).

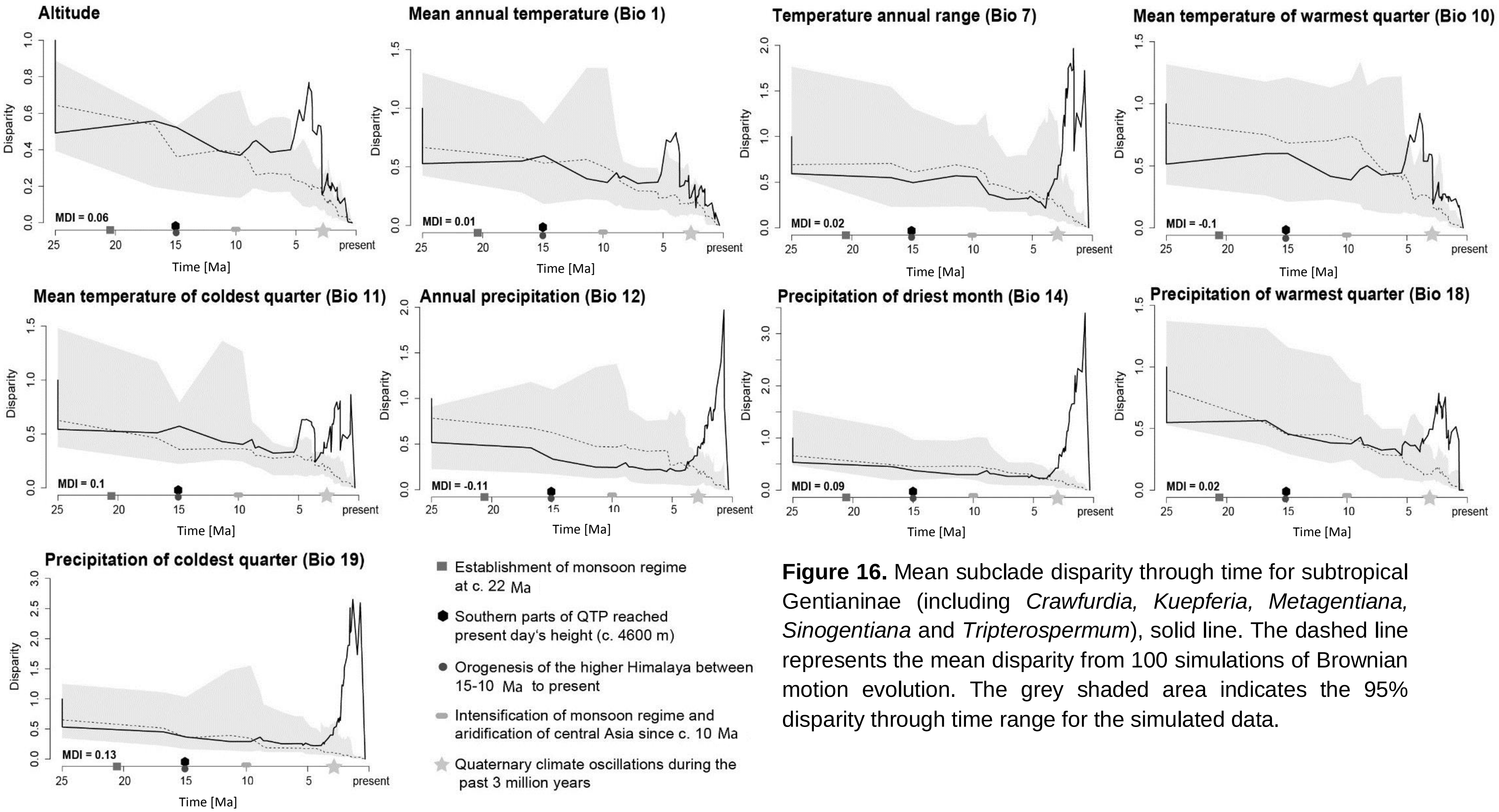


Figure 16. Mean subclade disparity through time for subtropical Gentianinae (including *Crawfurdia, Kuepferia, Metagentiana, Sinogentiana* and *Tripterospermum*), solid line. The dashed line represents the mean disparity from 100 simulations of Brownian motion evolution. The grey shaded area indicates the 95% disparity through time range for the simulated data.

4.6.5 Putative key innovations

The BiSSE analyses showed that the different states of the stamens, internodes, and corolla lobation have equal speciation, extinction and transition rates (see Appendix 13; Table 6). It is notable that while some models with unequal rates were also found to fit the data well for these traits, the simpler evolutionary model (within BF < 2) was preferred. Only the fruit types and habitus showed positive support for a model with unequal rates: different speciation rates in case of the fruits, and different extinction rates in case of the habitus. Our results showed a twofold increase in the speciation rate for lineages with fleshy fruits (λ1) compared to those with capsules (λ0), while the extinction rates stayed the same (Table 6). For the habitus we found slightly higher extinction rates for twining lineages (μ1) whereas the speciation rates for erect and twining lineages were identical (Table 6).

Table 6. Trait-correlated diversification. Posterior estimates of speciation (λ), extinction (μ), and transition (q) rates obtained by the binary state speciation and extinction approach (BiSSE). Corolla lobation: 0 = shallow, 1 = deep; fruit: 0 = capsule, 1 = berry; habitus: 0 = erect, 1 = twining; internodes: 0 = normal, 1 = elongated; stamens: 0 = straight, 1 = recurved.

		Mean posterior rates					
	Model	**λ0**	**λ1**	**μ0**	**μ1**	**q01**	**q10**
Corolla Lobation	λ0=λ1, μ0=μ1, q01=q10	0.318	0.318	0.078	0.078	0.024	0.024
Fruit	λ0≠λ1, μ0=μ1, q01=q10	0.236	0.46	0.048	0.048	0.032	0.032
Habitus	λ0=λ1, μ0≠μ1, q01=q10	0.332	0.332	0.091	0.113	0.032	0.032
Internodes	λ0=λ1, μ0=μ1, q01=q10	0.319	0.319	0.08	0.08	0.044	0.044
Stamens	λ0=λ1, μ0=μ1, q01=q10	0.312	0.312	0.074	0.074	0.009	0.009

4.7 DISCUSSION

Our analyses of ecological niches and their evolution in subtropical Gentianinae revealed a predominance of niche evolution. Only *Kuepferia* and *Sinogentiana* have rather conserved niches, preferring alpine habitat, which seems also to be the ancestral environment of the subtribe and potentially the entire tribe. In contrast, *Crawfurdia, Metagentiana* and *Tripterospermum* were able to adapt to warmer and more humid conditions, with *Tripterospermum* featuring the broadest niche width by far. All genera producing capsules (*Crawfurdia, Kuepferia, Metagentiana* and *Sinogentiana*) have a more restricted distribution and are less species-rich than *Tripterospermum*, which displays the strongest tendency for niche evolution among all subtropical Gentianinae and exclusively developed berry-like fruits. Therefore, berry-like fruits could be seen as key innovation as its development led to an increase of speciation rate by about two times. Disparification processes started c. 8-6 Ma within subtropical Gentianinae. At this point, major geological and climatic changes (such as the establishment of the monsoon regime or the development of the higher Himalayas) had already occurred and/or were ongoing. Thus, it is likely that geophysical changes were only moderately relevant (if relevant at all) for the evolution of these clades. Importantly, these geophysical changes are unlikely to have affected diversification rates, which have remained constant through time within subtropical Gentianinae, except in *Tripterospermum* where the rate changes are more likely to be associated with the development of berry-like fruits. Therefore, we suggest that a better dispersal ability together with a higher tendency for niche evolution were the triggers for the diversification in this group.

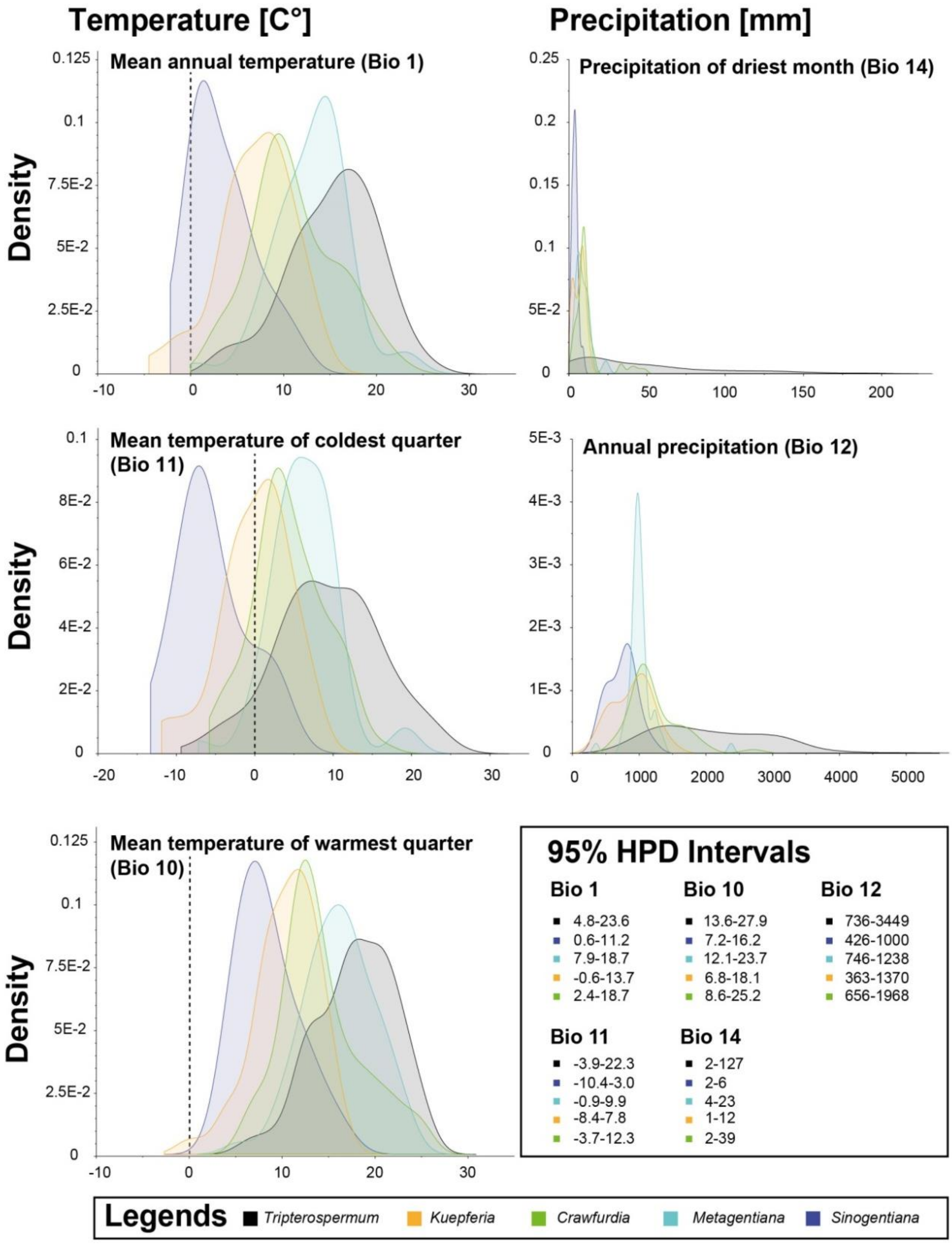
4.7.1 Ecological differentiation and phylogenetic relationships

The establishment and maintenance of reproductive isolation is essential for speciation of organisms in close proximity (Rieseberg & Willis, 2007) and could have occurred via habitat differentiation along particular niche axes between *Sinogentiana* and *Tripterospermum*. Between these two genera the lowest niche overlap was found (Table 5), even if *Tripterospermum* diverged rather recently from *Sinogentiana* (6.6 to 16.3 Ma; Fig. 14). Although the distribution of *Tripterospermum* is much broader than that of *Sinogentiana*, a recent study has shown that the former probably originated within the current distribution range of the latter (the southeastern and eastern fringe of the QTP (Matuszak et al., in press). Speciation of plants in immediate vicinity to each other (driven by ecological specialization) has also been found in Melastomataceae (Schulman et al., 2004), Arecaceae (Savolainen et al., 2006), Asteraceae (Timonin et al., 2014) and in other plants (Papadopulos et al., 2011, but see Igea et al., 2015) as well as in animals (e.g., Via, 2001; Berluenga et al., 2006).

Low niche overlap between *Tripterospermum* and other related genera likely reflects a habitat shift from open habitats, such as alpine meadows, grassy or stony slopes and small bamboo or shrub thickets, towards dense forest understorey and forest margins (Fig. 14; Ho & Pringle, 1995a, b, c). The strongly shaded habitat of *Tripterospermum* is potentially only shared with some populations of *Crawfurdia* (for example *C. pricei* (Marquand) Harry Sm.). It is striking that *Crawfurdia* and *Tripterospermum* are the only genera within Gentianinae having a climbing and twining habit. It is generally assumed that climbing plants use their supporting structures to grow fast, improving their adjustment toward irradiance (at best reaching the top of the canopy; Gartner, 1991; Schnitzer & Bongers, 2002; Salzer et al., 2006). Nevertheless, *Tripterospermum* and also some populations of *Crawfurdia* are clearly plants of the understorey, which is in agreement with the finding of Valladares et al. (2011): these authors stated that many climbers are coping with the shady conditions, which might be also true for *Tripterospermum* and *Crawfurdia*.

4.7.2 Realised distribution, dispersal potential and future variation of distribution under climate change

Based on temperature and precipitation, the ecological amplitude of *Tripterospermum* appears to be much broader than that of *Crawfurdia, Kuepferia, Metagentiana* and *Sinogentiana* (Fig. 17). In addition, *Tripterospermum* is likely to have a higher dispersal potential by being the only genus in Gentianinae producing berries (probably dispersed via zoochory; Murata, 1989; Matuszak et al., in press). Taken together, the higher dispersal potential in combination with a broader ecological amplitude, which increases the chances of survival in new environments, might explain why *Tripterospermum* succeeded to rapidly occupy its predicted range beyond the region of the QTP, whereas other genera failed to colonize distant climatically suitable areas despite their relatively older age (Fig. 14). Potentially more effective, dispersal via berry-fruits may have promoted geographic reproductive isolation followed by allopatric speciation and thus have contributed to the diversification of the genus.



95% HPD Intervals

Bio 1	Bio 10	Bio 12	Bio 11	Bio 14
4.8-23.6	13.6-27.9	736-3449	-3.9-22.3	2-127
0.6-11.2	7.2-16.2	426-1000	-10.4-3.0	2-6
7.9-18.7	12.1-23.7	746-1238	-0.9-9.9	4-23
-0.6-13.7	6.8-18.1	363-1370	-8.4-7.8	1-12
2.4-18.7	8.6-25.2	656-1968	-3.7-12.3	2-39

Figure 17. Density plots and the 95% HPD interval of the raw data from the WorldClim data set visualizing the niche width of *Crawfurdia, Kuepferia, Metagentiana, Sinogentiana* and *Tripterospermum* for mean annual temperature (Bio 1), mean temperature of warmest quarter (Bio 10), mean temperature of coldest quarter (Bio 11), annual precipitation (Bio 12) and precipitation of driest month (Bio 14).

According to the MaxEnt models, there are many climatically suitable areas outside of the current distribution range of each genus (e.g., Lesser Sunda Islands and Northern Australia), and we cannot exclude that *Crawfurdia, Kuepferia, Metagentiana* and *Sinogentiana* are still in expansion. However, these regions are separated from the current distribution range of these genera by vast inhospitable areas (Fig. 13), and a range expansion would require long-distance dispersal. Under current conditions, especially *Kuepferia, Metagentiana* and *Sinogentiana* might not be able to extend their distribution beyond the mountainous regions of the Himalayas and the QTP, because they need light exposed, forest-free habitats, whereas wooded areas are commonly found south and east of the QTP while localities north of the QTP might have temperatures of less than -20 °C during the coldest month, which potentially represents a climatic limit for these genera.

In comparison to other genera, the realised distribution of *Tripterospermum* appears to be the closest to its potential distribution, with two notable exceptions: the genus was never recorded from areas east of Sulawesi and from Borneo. The case of Borneo is particularly intriguing since this island most likely hosts suitable habitats for *Tripterospermum* (for example in mountain ranges of Sabah, Malaysia), the genus being otherwise present in all major neighbouring islands. SDMs are established as an efficient tool in ecology and conservation as well as land management (Elith et al., 2006; Williams et al., 2009), but this method could be also useful to potentially discover new species or populations of poorly sampled plant genera, which would improve our knowledge about biodiversity in general. In fact, the use of SDMs to discover new populations of rare plant species could be successfully shown in a case study by Williams et al. (2009). In the case of *Tripterospermum*, it might be promising to search for new species or unknown populations in the mountains of Northern Borneo (Fig. 13).

In this study we focused only on the climatic niche of subtropical Gentianinae using temperature and precipitation data, however, with regard to a niche's multidimensionality predictions for the future should be handled with care. During the past century (1880 to 2012), global temperatures increased about 0.85 °C, and are likely to exceed +1.5 °C by the end of the 21st century

(IPCC 2013). According to Liu et al. (2009b), the temperature in the region of the QTP might increase by 2.4 °C or more in the next 30 to 50 years, while only little change for precipitation (increase below 5%) is projected. Among Gentianinae, *Tripterospermum* will presumably cope best with the increase of temperature due to its broad niche and flexibility. *Crawfurdia* and *Metagentiana* are also able to adapt to new conditions. In contrast, *Kuepferia* and *Sinogentiana* might face a larger challenge by having a more conserved niche. Increase of temperature might, for example, modify forest/meadows relative cover in and around the QTP, potentially leading to the invasion by trees and shrubs of lower populations of light-preferring genera, such as *Kuepferia* and *Sinogentiana*. This is for example known for the actual tree-line moving at higher altitude. Nevertheless, mountain systems offer habitat alternatives within a short distance because of altitudinal zonation, which should lower extinction rates in mountains in face of climate change (Hoorn et al., 2013). Therefore, it is possible that the upper limit of occurrence for all mentioned genera will be shifted towards higher elevations. This could already be recently observed for other organisms in other mountain systems (Gehrig-Fasel et al., 2007; Chen et al., 2011), but reliable predictions of the influence of climate change on the flora need also further comprehension of non-climatic constraints (e.g. biotic interactions), which should be investigated more extensively in the future (Brown & Vellend, 2014).

In this study, we determined the realised climatic niche of *Crawfurdia*, *Kuepferia*, *Metagentiana, Sinogentiana* and *Tripterospermum* respectively, which does not have to depict the true fundamental niche, because biotic interactions may prevent establishment in further suitable habitats (Arujo & Peterson, 2012). Biotic factors are often ignored, which has been controversially discussed (reviewed in Arujo & Peterson, 2012). To overcome the problem of circularity it is possible to estimate physiological tolerances of species to climate either from mechanistic approaches (based on fitness derived from interaction between functional traits and environmental conditions) or experiments (Elith et al., 2010, Kearney et al., 2010, Arujo & Peterson, 2012), but the ecology of the investigated genera in this study is so far not known in detail. We therefore stress the importance of gathering ecological data for these genera to improve modelling work, for example via stress tolerance experiments.

4.7.3 Niche evolution through time and space

The ancestor of subtropical Gentianinae (excluding *Gentiana*) probably originated at the south-eastern fringe of the QTP at about 23 Ma (Matuszak et al., in press). At this time, the center of the QTP had already reached its present-day's height of about 4000 to 4500 m, but northern and southern parts were still uplifting (Mulch & Chamberlain, 2006). Niche disparity appears to have been initially slow in subtropical Gentianinae (because no initial divergence among the different genera occurred; Fig. 16), owing to climatic conservatism until c. 6 Ma. This finding is in agreement with that of stochastic character mapping, which depicted the initial occupation of new habitats at about 8 to 6 Ma (Fig. 14). Therefore, major climatic and geologic events such as the establishment of the monsoon regime at about 22 Ma (Guo et al., 2002), the uplift of southern parts of the QTP to present-day's altitude as well as the development of the higher Himalayas starting at about 15 Ma (Currie et al., 2005; Wang et al., 2008) seem to have had only very little or no impact on the climatic niche evolution of subtropical Gentianinae (Fig. 16).

Tripterospermum displays both a high degree of niche evolution and good dispersal ability. This combination leads to indistinct patterns among species of *Tripterospermum.* For example, dispersal events occurred in almost all clades (even several times to the same region independently), and within each clade a large range of climatic tolerances can be found (Fig. 16). Furthermore, preferences for rather open to strongly shaded habitats are present in *Tripterospermum* throughout its phylogeny (Fig. 14). In contrast to the other genera of Gentianinae, especially the variable Bio 7 (temperature annual range) showed a high disparity throughout *Tripterospermum*'s existence (Appendix 15). The ability to withstand variations in temperature probably enabled *Tripterospermum* to colonize temperate regions with stronger seasonality, such as northern Japan. Its remarkable flexibility allowed the genus to diversify and disperse greatly during only 3 million years following the development of berry-like fruits.

Crawfurdia and *Metagentiana* show also a tendency for niche evolution (although not as strong as *Tripterospermum*), but their ability to disperse is relatively poor. For *Crawfurdia*, an abrupt rise of disparity could be observed for all tested variables at about 8 to 6 Ma, which corresponds to the time of diversification in the phylogenetic tree (Fig. 14). Similar to *Tripterospermum*, subclades of *Crawfurdia* comprise a wide range of climatic tolerances (Fig. 16), which is also depicted by the random distribution of habitat preferences (open to strongly shaded) throughout the genus (Fig. 14). Despite *Crawfurdia's* flexibility, the distribution range is quite narrow. In contrast, the disparity through time plots (Appendix 15) of *Metagentiana* show one peak of disparity at about 4 Ma, which involves the node containing *M. australis* (Craib) T.N.Ho & S.W.Liu and *M. serra* (Franch.) T.N.Ho, S.W.Liu & Shi L.Chen (Fig. 14). Generally, species of *Metagentiana* have relatively concordant climatic tolerances, but *M. australis* strongly differs in terms of temperature from the other species by preferring warmer conditions. It inhabits, on average, altitudes of about 800 m, whereas the other species occur about 1000 to 2000 m higher. Nevertheless, requirements for precipitation are the same. Most species of both genera were not able to disperse beyond their area of origin (the region of the south and south-eastern fringe of the QTP), probably due to a less successful dispersal mechanism. Only *C. pricei* (Marquand) Harry Sm. and *C. maculaticaulis* C.Y.Wu ex C.J.Wu reached eastern China (probably along the Dalou and Wuling Mountains as well as the southern Nanling Mountains; Wang, 1992), whereas *C. poilanei* Hul and *M. australis* colonized northern Indochina (probably along extensions of the Hengduanshan such as the Ailaoshan). Climatically, these areas are not much different from each other, therefore no special climatic adaptations could be determined.

Only *Kuepferia* and *Sinogentiana* are restricted to the QTP region and always inhabited open habitats, *Kuepferia* being the oldest lineage among the investigated genera (Fig. 14). Both genera are an example for niche conservatism paired with a low dispersal potential, displayed by little variation for temperature as well as precipitation preferences and a narrow distribution range (Figs. 13, 15).

In summary, dispersal events occurred only in genera for which niche evolution was recovered. In case of poor dispersal potential (capsules), species managed to disperse only along mountain ranges bordering the QTP to neighbouring regions in the south and/or in the east. This also serves as explanation for the observed larger variations of temperature than of precipitation. Temperature varies strongly with altitude, which should therefore be an important factor for divergence and disparification in mountain systems. In contrast, the south and southeastern fringe of the QTP as well as its southern and eastern extensions belong to a similar precipitation zone (information from WorldClim), so divergence and disparification processes are simply not affected by this factor. This is in contrast to the situation in *Tripterospermum*, which changed its dispersal mechanism from anemochory (only short distances via capsules) to zoochory (possibly longdistances by birds) and reached more distant areas with different precipiatation regime.

4.7.4 Berry-like fruits as key innovation

Among all tested traits, only the evolution of berry-like fruits in *Tripterospermum* corresponds to an increase of speciation rate (Table 6), which is also reflected by a relatively high species number of this genus compared to the others. Other traits investigated did not seem to result in an increase of speciation rates despite their probable ecological and taxonomic importance (Murata, 1989; Chen et al., 2005b; Favre et al., 2014). For example, the evolution of elongated internodes (*Crawfurdia*, *Sinogentiana* and *Tripterospermum*) or twining habit (*Crawfurdia* and *Tripterospermum*) might have allowed an invasion of new habitats with taller vegetation (better light capture), while the climbing habit might have enabled plants to escape from disadvantageous conditions (such as light and biotic stress) in the understorey of forests (Schnitzer & Bongers, 2002; habitat-mediated speciation). Furthermore, the evolution of recurved stamens (*Metagentiana, Sinogentiana* and *Tripterospermum*) and shallow lobation of the corolla (*Crawfurdia, Metagentiana, Sinogentiana* and *Tripterospermum*) were expected to influence speciation by modifying the pollination success via switches in pollinators’ guild, pollinators’ choice, or simply the quantity of pollen transfer (Kudo, 2003; floral structure-mediated speciation, Gomez et al., 2008;

pollinator-mediated speciation). In *Pedicularis*, the shape of the galea affects the position of pollen deposition and stigma contact on the pollinators' body, leading to barriers of reproduction (floral isolation), and eventually to diversification (Eaton et al., 2012 and references therein). Nevertheless, it appears that only the transformation of fruit type fulfills the criteria for a key innovation, being a newly evolved beneficial trait which has led to increased speciation rates in *Tripterospermum* (Hodges & Arnold, 1995).

In conclusion, we argue that the evolution of berry-like fruits as key innovation promoted long-distance dispersal (and subsequent allopatric speciation), resulting in the diversification of *Tripterospermum*. A better ability to disperse in contrast to other subtropical Gentianinae genera (with which *Tripterospermum* shares the biogeographical origin, i.e. the vicinity of the QTP, and age, i.e. 15.8-35.1 Ma; Matuszak et al., in press) was further attended by its broader climatic niche, allowing a higher probability to persist in new areas. This study showed that for subtropical Gentianinae rather presence of habitat alternatives (or ecotones) promoted divergence (and also diversification if paired with a better dispersal ability) than geophysical and/or climatic changes in the QTP's region.

4.8 ACKNOWLEDGEMENTS

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5. GENERAL CONCLUSIONS

This study was designed to investigate the impact of environmental changes, such as geological and climatic changes that occurred during the uplift of the QTP, on plant diversification. It is generally assumed that major geophysical and/or climatic changes provided vacant niches and hence resulted in an increase of diversification rates (potentially leading to high species richness within a region), but the QTP and its surroundings still remain poorly studied (reviewed in Favre et al., 2015). Using Vaccinieae (focusing on *Agapetes* and *Vaccinium*; Ericaceae) and subtropical Gentianinae (focusing mainly on *Tripterospermum*; Gentianaceae) as model systems, this doctoral thesis aimed to identify the role of the QTP as a sink or source area for montane plant taxa, to reveal floristic relationships and dispersal routes between the QTP and other parts of the world, to examine the relevance of niche evolution and conservatism in a changing environment over time, and to determine possible beneficial morphological traits of plants in the QTP's region. A profound understanding of the influence of historical factors, which have shaped current patterns of biological diversity on Earth, might also help to predict the impact of present-day factors on biodiversity under climate change. This study sought to answer the following specific questions:

(1) Was the QTP region a source and/or a sink area for species diversity of non-alpine plants?
(2) Did the diversification of *Agapetes, Vaccinium* and *Tripterospermum* temporally and spatially coincide with the uplift of the QTP and associated climate changes?
(3) Did climatic niche evolution or conservatism play a more important role for diversification of the different genera of Asian Gentianinae with respect to the QTP's uplift and associated climatic changes? What was the impact of some newly evolved morphological traits within the tribe on diversification?

The main empirical findings of this study were summarised within each of the chapters 2-4. In the following, I will consolidate the empirical findings to answer this PhD study's three general research questions.

(1) Was the QTP region a source and/or a sink area for species diversity of non-alpine plants? This question was addressed by reconstructing the spatio-temporal history of the sub-alpine genera *Agapetes* and *Vaccinium* (Vaccinieae, Ericaceae; chapter 2) as well as *Tripterospermum* (Gentianinae, Gentianaceae; chapter 3). For Vaccinieae, a North American origin was found. From there, the tribe dispersed nearly throughout the entire world. Hence, the surroundings of the QTP acted rather as a sink area, because pre-adapted lineages from different parts of the world colonised this region multiple times and diversified after their ancestors' arrival. Such a sink pattern was, for example, also observed in Asparagaceae (Meng et al., 2008) or Solanaceae (Tu et al., 2010). Nevertheless, this result differed from the usual patterns found for alpine plants, in which the QTP acted as a source area (reviewed in Wen et al., 2014). The majority of alpine plants originated in the QTP region, from where they reached other parts of the world and often diversified extensively during the Quaternary when global temperatures decreased (Zhang et al., 2009; reviewed in Wen et al., 2014). In contrast to Vaccinieae, *Tripterospermum* matched the usual pattern, because the southern Himalaya and the Hengduanshan (bordering the QTP) were found to be part of its area of origin and *in situ* diversification was followed by the colonisation of East China, Japan, Taiwan, and Southeast Asia during the Pliocene to Pleistocene. To date, biogeographical reconstructions for plants occurring in the QTP mainly focused on alpine taxa, revealing a clear tendency that the QTP acted as a source area for worldwide alpine biodiversity (reviewed in Wen et al., 2014). The importance of the QTP for taxa with different ecological preferences is still unknown. By involving sub-alpine taxa, our study was a first step into this direction. The results showed that the surroundings of the QTP were a prominent area for diversification for Vaccinieae and *Tripterospermum* (as well as other subtropical Gentianinae), acting either as a sink (in case of Vaccinieae) or as a source area (for *Tripterospermum*). It could also be possible that the impact of the QTP on diversification varied over time. The QTP might have first acted as a sink area during its earlier stage of development (in case of Vaccinieae) and more as a source area during more recent epochs (in the case of *Tripterospermum*; see the "out-of-Tibet" hypothesis, Wang et al., 2015). Nevertheless, to assess the role of the QTP for the biodiversity of sub-alpine taxa in its entirety, more groups of this altitude zone need to be investigated.

(2) Did the diversification of *Agapetes, Vaccinium* and *Tripterospermum* temporally and spatially coincide with the uplift of the QTP and associated climate changes? The reconstructed spatio-temporal history of *Agapetes* and *Vaccinium* (Vaccinieae, Ericaceae; chapter 2) as well as *Tripterospermum* (Gentianinae, Gentianaceae; chapter 3) was used to answer this question. Vaccinieae appeared earliest between 37.7 to 20.0 Ma in the region of the QTP. At this time, the centre of the QTP as well as some local high elevation areas in the North exhibited already elevations similar to present day, but the northward and southward extension was still in progress. Diversification of Vaccinieae started between 21.3 to 9.2 Ma in this region, which temporally corresponds with the early formation of the higher Himalayas, and the intensification of the monsoon regime. This would suggest a potential causal relationship between environmental changes and diversification. It is striking that Vaccinieae are most diverse in the montane tropics of South America (especially the Andes) and Asia (especially the southeastern fringe of the QTP; Kron & Luteyn, 2005), two areas which both underwent major geological and climatic changes in the past. This fact supports the assumption that lineages diversify most rapidly when numerous vacant niches are present leading to subsequent sympatric speciation (Mayr, 1942; Simpson, 1944; Kawata, 2002). Especially the island-like arrangement of ecological opportunities resulting from mountain uplift might trigger diversification, which could be also shown for *Lupinus* L. (Fabaceae; Hughes & Atchison, 2015). In contrast, *Tripterospermum* turned out to be much younger than Vaccinieae, with a crown age estimated to be 8.8 to 2.7 million years. During that time, the uplift of the QTP was almost at its present stage, but the higher Himalayas were still forming, the Hengduanshan started to rise, and the monsoon regime was intensifying. Therefore, it is possible that these environmental changes have had some influence on *Tripterospermum*'s diversification, but only a minor one if at all, because we could show that the development of berry-like fruits, which probably facilitated long-distance dispersal, was of much greater importance for its diversification (see question 3). Therefore, our results showed that the impact of past geological and climatic changes might differ between the taxa considered, and could potentially, as mentioned above, vary over time.

(3) Did climatic niche evolution or conservatism play a more important role for diversification of the different genera of Asian Gentianinae with respect to the QTP's uplift and associated climatic changes? What was the impact of some newly evolved morphological traits within the tribe on diversification? These questions were answered by investigating the climatic divergences among subtropical Gentianinae (*Crawfurdia, Kuepferia, Metagentiana, Sinogentiana,* and *Tripterospermum*; Gentianaceae) as well as their temporal dynamics, and identifying patterns of trait evolution (chapter 4). Subtropical Gentianinae probably originated at the southeastern fringe of the QTP in an alpine environment. Our analyses revealed that niche evolution played an important role for their diversification. In case of *Kuepferia* and *Sinogentiana* a tendency towards niche conservatism was detected, therefore, these two genera had the fewest number of species among subtropical Gentianinae and occurred exclusively in the likely ancestral living environment of the subtribe, namely alpine habitats. Only *Crawfurdia, Metagentiana,* and *Tripterospermum* adapted to warmer and more humid conditions, with *Tripterospermum* displaying the broadest niche width among all investigated genera. In comparison with the berry-producing genus *Tripterospermum*, all other genera producing capsules (*Crawfurdia, Kuepferia, Metagentiana,* and *Sinogentiana*) occupy a smaller distribution range and have less species. Among five possible important traits for diversification (corolla lobation, fruit type, habitus, length of internodes, and shape of stamens), only the development of berry-like fruits, which might have facilitated long-distance dispersal and thus allopatric speciation, acted as key innovation by increasing the speciation rate. Due to the fact that disparification within the subtropical Gentianinae was observed at about 8 to 6 Ma, a time in which major geological and climatic changes had already occurred in the QTP region and the orogenesis of the Hengduanshan had not started yet, a significant impact of environmental changes on the diversification of this subtribe seems unlikely. For this reason, we concluded that the better dispersal ability of *Tripterospermum* paired with the tendency for niche evolution triggered its diversification.

The prevailing scientific view is that environmental changes might trigger diversification of organisms by either providing vacant niches allowing sympatric speciation (Kawata et al., 2002; reviewed in Lekevicius, 2009) or by separating populations from each other facilitating allopatric speciation (Wiens et al., 2004; Hoskin et al., 2005). A higher level of biodiversity than expected based on area is especially found in mountain systems (Linder, 2008; Sedano & Burns, 2010; Hoorn et al., 2013), such as, for example, the Andes, the Himalayas or the Tianshan (Myers et al., 2000; Myers, 2003; Myers & Mittermeier, 2003; Kier et al., 2005; Kier et al., 2009). Nevertheless, the reasons for which some regions are more species-rich than others are still not fully understood. This doctoral thesis illustrated the complexity of this topic by showing how many different methodological approaches were needed to draw sound conclusions about diversification. To date, most studies reconstructed the spatio-temporal framework of diversification, and if a coincidence in time of diversification and environmental changes could be detected, a causal relationship was assumed (reviewed in Wen et al., 2014). So far, the evolution of the ecological niche of organisms and the effect of possible beneficial traits on their evolutionary history were not investigated against the background of environmental changes in the QTP region. However, this doctoral thesis indicated the importance to consider such aspects by the example of *Tripterospermum* (chapter 4). The sole reconstruction of the spatial and temporal framework of *Tripterospermum*'s diversification would have led to the assumption that the intensification of the monsoon system as well as quaternary climate oscillations could have promoted its diversification, but additional analyses of *Tripterospermum*'s ecological niche and its evolution revealed a better dispersal ability (due to the development of berry-like fruits) and a tendency for niche evolution to be the most important triggers. This example illustrated very well that conclusions might be dependent on the scientific angle of view. In fact, the field of biogeography is closely related to ecology and phylogenetic systematics, but exchanges between these fields have been limited so far. Historical biogeography focused primarily on the following processes to explain patterns of biodiversity: vicariance, dispersal, and extinction (Wiens & Donoghue, 2004). However, these three processes are strongly dependent on ecological factors, such as dispersal ability and habitat fidelity (Wiens & Donoghue, 2004).

The case of *Tripterospermum* stressed the need to promote an integrative biogeography. Hence, for future research, it is highly recommended to take the ecology of organisms into account when investigating possible correlations between environmental changes and diversification.

According to the current state of science, the QTP acted mainly as a source area for alpine worldwide biodiversity (reviewed in Wen et al., 2014). Nevertheless, the investigation of the role of the QTP for non-alpine flora has just begun. This doctoral thesis showed that in the case of the sub-alpine tribe Vaccinieae the QTP acted as a sink area and thus differed from the studies conducted almost exclusively on alpine plants so far (reviewed in Wen et al., 2014). Due to the insufficient number of studies dealing with non-alpine plants from the surroundings of the QTP, it is not possible to make a general prediction at this point. However, our results suggest a varying role of the QTP for sub-alpine flora over time. Therefore, future research should focus more on genera from other altitude zones to better understand the impact of the QTP on patterns of biodiversity.

In conclusion, this doctoral thesis has offered new insights into the possible role of the QTP for sub-alpine plants and has stressed the importance of ecological aspects to understand the diversification of genera, which has not been sufficiently considered so far with respect to research in the QTP region.

6. ZUSAMMENFASSUNG

Ziel dieser Doktorarbeit war es, den Einfluss von Umweltveränderungen auf die Pflanzendiversifikation zu untersuchen. Die Region des Qinghai-Tibetischen Plateaus (QTP) hat seit der frühen Kreidezeit viele geophysikalische und klimatische Veränderungen erfahren, weshalb sie für solche Untersuchungen besonders gut geeignet ist. Es wird allgemein angenommen, dass starke geophysikalische und/oder klimatische Veränderungen zur Bildung neuer ökologischer Habitate führen, die wiederum eine adaptive Radiation von Organismen fördern und somit die Diversifikationsrate erhöhen. Dies könnte der Grund dafür sein, dass die Umgebung des QTPs durch hohen Artenreichtum gekennzeichnet ist, jedoch sind mögliche Zusammenhänge zwischen Umweltveränderungen und Biodiversität noch nicht ausreichend erforscht. Bisher wurde vor allem der Einfluss des QTPs auf alpine Pflanzen untersucht, wobei gezeigt werden konnte, dass viele alpine Pflanzengruppen ihren Ursprung in dieser Region haben, dort diversifizierten und anschließend andere Gebirge der Welt besiedelten. Die Rolle des QTPs für sub-alpine Pflanzen ist aber nach wie vor unbekannt. In dieser Doktorarbeit dienten daher zwei überwiegend sub-alpine Pflanzengruppen, nämlich Vaccinieae (mit Fokus auf *Agapetes* sowie *Vaccinium*, Ericaceae) und die subtropischen Gentianinae (mit Fokus auf *Tripterospermum*, Gentianaceae), als Modellsysteme, um den Einfluss des QTPs auf die sub-alpine Pflanzenwelt zu ergründen, mögliche Ausbreitungswege zwischen dem QTP und anderen Orten der Welt zu bestimmen, die Relevanz von Nischenevolution und/oder -konservatismus für die Diversifikation in einer sich verändernden Umgebung zu erfassen und mögliche morphologische Merkmale zu finden, die in der Region des QTP für Pflanzen von Vorteil sein könnten. Im Rahmen dieser Doktorarbeit sollten folgende spezifische Fragen beantwortet werden:

(1) Fungierte das QTP eher als Quelle oder als Senke für die weltweite Artenvielfalt nicht-alpiner Pflanzen?

(2) Stimmt die Diversifikation von *Agapetes, Vaccinium* und *Tripterospermum* zeitlich und räumlich mit der Hebung des QTPs und mit den damit verbundenen klimatischen Veränderungen überein?

(3) Spielte im Zuge der Hebung des QTPs und damit verbundenen klimatischen Veränderungen Nischenevolution oder -konservatismus für die Diversifikation der asiatischen Gentianinae eine größere Rolle? Welchen Einfluss hatten manche neu evolvierten morphologischen Merkmale innerhalb der asiatischen Gentianinae auf die Diversifikation?

6.1 Pflanzliche Diversifikation, Biogeografie und Nischenevolution

Biodiversität ist auf der Erde ungleich verteilt. Zu verstehen, wie und warum es zu dem heutigen Biodiversitätsmuster der Erde gekommen ist, gehört zu den wichtigsten Zielen der Biogeografie und Evolutionsbiologie. Ein fundiertes Wissen über die Entstehung von Hotspots der biologischen Vielfalt kann dabei helfen, geeignete Naturschutzgebiete zu bestimmen, um die Biodiversität in Zeiten des Klimawandels effizient zu schützen und zu bewahren. In den letzten zwei Jahrzehnten hat die Biogeografie durch die Entwicklung neuer Methoden zur anzestralen Arealrekonstruktion große Fortschritte gemacht. So konnte z.B. mit Hilfe von DEC (*dispersal-extinction-cladogenesis*) und/oder S-DIVA (*statistical dispersal vicariance analysis*) die Bedeutung von Gebirgsbildungen oder temporären Landbrücken für die Diversifikation und Ausbreitung von Pflanzenarten ermittelt werden. Die Ausbreitung von Individuen in neue Gebiete erfolgt bei Kormophyten meist passiv über Diasporen, wobei entweder biotische (vor allem Vertebraten) oder abiotische (Wind oder Wasser) Vektoren für die Ausbreitung verantwortlich sind. Zwar ist die Ausbreitung über große Distanzen hinweg (*long-distance dispersal*; LDD) seltener als jene über kurze, dennoch ist sie von entscheidender Bedeutung für die Überwindung geografischer Barrieren, um neue Orte zu besiedeln. Die Gründe, die zur Diversifikation von Pflanzentaxa innerhalb einer bestimmten Region führen, sind vielfältig. Zum einen gibt es genetische Ursachen (z.B. Mutationen, genetischer Drift), zum anderen gibt es ökologische, wie z.B. Umweltveränderungen oder die Invasion unbesetzter Nischen. Die Nische einer Art ist dabei als Gesamtheit aller biotischen und abiotischen Umweltfaktoren zu verstehen, die einer Art ihr Überleben ermöglichen. Die Tendenz zur Nischenevolution, also die Anpassung einer Art an andere oder neue Umweltbedingungen, kann auch die Diversifikation fördern. Der Einfluss von Umweltveränderungen auf die

Pflanzendiversifikation wird gerade in vielen Gegenden dieser Erde untersucht, jedoch erscheint das Qinghai-Tibetische Plateau aufgrund seiner starken geologischen und klimatischen Veränderungen besonders gut geeignet dafür.

6.2 Geologische sowie klimatische Veränderungen in der QTP-Region und ihre möglichen Auswirkungen auf die Biodiversität

Bis vor kurzem wurde davon ausgegangen, dass die Hebung des QTPs eine Folge der Plattenkollision von Indien mit Eurasien (ca. 55-50 Millionen Jahre vor heute) sei. Lippert et al. (2014) konnten jedoch durch die Auswertung palaeomagnetischer Daten des Lhasa-Terrains zeigen, dass bereits vor der Kollision ein tibetisches Protoplateau existierte. In der frühen Kreidezeit, nämlich vor ca. 145 Millionen Jahren, kollidierte das Lhasa- mit dem Qiangtang-Terrain, wodurch es zur Subduktion der Neotethys unter das Lhasa-Terrain kam. Möglicherweise führte diese frühe Kollision zu Verdickungen der Erdkruste, die ausreichend gewesen sein könnten, um vor ca. 110 Millionen Jahren Zentraltibet über den Meeresspiegel zu heben. Viele Studien weisen darauf hin, dass Zentraltibet sowie einige nördliche Gegenden des QTPs bereits im Eozän (vor ca. 45 Millionen Jahren) eine ähnliche Höhe wie heute (ca. 4000-5000 m) erreicht haben könnten. Die Kollision der indischen mit der eurasischen Kontinentalplatte veranlasste dann die nördliche, südliche und östliche Expansion des QTPs bis hin zu seinen heutigen Ausmaßen. Große an das QTP angrenzende Gebirgsketten entstanden erst relativ spät: der südlich angrenzende Himalaja entstand innerhalb von 15-10 Millionen Jahren bis heute und der südöstlich gelegene Hengduanshan türmte sich in den letzten 5,3 Millionen Jahren auf. Solche gewaltigen topografischen Veränderungen hatten vermutlich auch einen Einfluss auf das lokale sowie globale Klima.

Die Gebiete nördlich des Himalajas gelangten mit ansteigender Höhe desselben immer weiter in den Regenschatten, was gemeinsam mit der globalen Abkühlung des Klimas und dem Rückgang der Paratethys zur Wüstenbildung in Zentralasien geführt hat. Außerdem deuten Loessablagerungen in China den Beginn des asiatischen Monsuns vor ca. 22 Millionen Jahren an, der sich seitdem schrittweise intensiviert haben soll. Aktuelle Studien lassen jedoch einen älteren Monsun vermuten, für dessen

Entstehung nicht nur die Hebung des QTPs verantwortlich ist, sondern auch der Rückgang der Paratethys, die Abkühlung des weltweiten Klimas sowie der sich verändernde CO_2-Gehalt in der Atmosphäre.

Diese drastischen Umweltveränderungen in der Region des QTPs könnten, aufgrund der Entstehung neuer Habitate, adaptive Radiationen von Pflanzen begünstigt und somit die Artbildungsrate erhöht haben. Dafür spricht, dass das QTP heutzutage von vier besonders artenreichen Gegenden, sogenannten Hotspots der biologischen Vielfalt, umgeben ist: die Gebirge Südwestchinas, der Himalaja, Indo-Burma und die Gebirge Zentralasiens. Um mögliche Korrelationen zwischen Umweltveränderungen und Diversifikation ermitteln zu können, sind daher phylogenetische Rekonstruktionen nötig. Diese bekommen durch Datierungen mit Fossilien und biogeografischen Analysen einen zeitlich-räumlichen Rahmen, der mit der oben beschriebenen geologischen sowie klimatischen Geschichte des QTPs verglichen werden kann. Um die Bedeutung des QTPs für die allgemeine Biodiversität erfassen zu können, ist es wichtig, geeignete Pflanzengruppen zu untersuchen, die eine weite Verbreitung aufweisen.

6.3 *Agapetes* und *Vaccinium* (Vaccinieae, Ericaceae)

Agapetes und *Vaccinium* sind sehr artenreich in der QTP-Region vertreten, sie sind aber auch über die Grenzen des QTPs hinweg verbreitet, weshalb sich diese beiden nahe verwandten Gattungen gut als Modellsysteme eignen, um eine mögliche Korrelation zwischen Umweltveränderungen und Diversifikation zu untersuchen sowie mögliche Verbreitungswege zwischen den Hotspots der biologischen Vielfalt der QTP-Region und anderen Orten dieser Erde zu erfassen. Außerdem sind diese Gattungen sub-alpin und könnten daher die Erkenntnisse, die man bislang von alpinen Pflanzen der Region gewinnen konnte, ergänzen.

Agapetes ist subtropisch und besitzt ca. 80 Arten. Die Gattung ist in Sikkim, Bhutan, im südöstlichen Tibet, Assam, Myanmar und im westlichen Yunnan verbreitet. Nur wenige Arten kommen auch in Thailand und auf der malaysischen Halbinsel vor. *Vaccinium* hingegen ist mit 450 Arten wesentlich artenreicher als *Agapetes* und ist über die ganze Nordhemisphäre sowie in (sub-)tropischen Gebirgen verbreitet. *Vaccinium* kommt jedoch nicht in Australien, Neuseeland und in weiten Teilen Afrikas vor (mit Ausnahme einiger Arten in Südafrika und auf Madagaskar). Der hohe Zuckergehalt der Beeren von *Vaccinium* deutet auf eine Ausbreitung durch Vögel und Säugetiere hin, was wahrscheinlich auch auf *Agapetes* zutrifft.

Morphologisch sind sich *Agapetes* und *Vaccinium* sehr ähnlich. Unterschieden werden sie durch die Größe und Form ihrer Corolla (*Agapetes*: 0,5 bis 6 cm groß, röhrig oder zylindrisch, selten urnenförmig; *Vaccinium*: bis zu einem cm groß, urnen- oder glockenförmig, selten röhrig), durch ihre Infloreszenz (*Agapetes:* weniger als 15 Blüten; *Vaccinium* in Südostasien: mehr als 10 Blüten) und durch ihren Habitus (*Agapetes:* meist epiphytisch; *Vaccinium:* meist terrestrisch). Allerdings teilen die beiden Gattungen auch einige dieser morphologischen Merkmale, was eine klare Abgrenzung erschwert. In phylogenetischen Analysen von Kron et al. (2002) und Kron & Powell (2003) konnte gezeigt werden, dass *Agapetes* und *Vaccinium* sowie andere Gattungen der Vaccinieae polyphyletisch sind, jedoch wurden in den vorangegangenen Studien nur wenige Arten pro Gattung miteinbezogen. Die verwandtschaftlichen Verhältnisse innerhalb der Tribus sind demnach noch weitestgehend unbekannt. In dieser Doktorarbeit wurden die Diversifikation und die Verbreitungsgeschichte von *Agapetes* und *Vaccinium* untersucht. Zu diesem Zweck wurde der Datensatz erweitert.

6.4 *Tripterospermum* und seine nahen Verwandten (Gentianinae, Gentianaceae)

Tripterospermum ist aufgrund seiner weiten Verbreitung in Asien und Südostasien sowie seiner eher sub-alpinen Ökologie gut als Modellsystem geeignet, um die Auswirkungen der Umweltveränderungen in der QTP-Region auf die Diversifikation zu untersuchen und Ausbreitungswege zwischen dem QTP und den Hotspots der biologischen Vielfalt in Südostasien zu bestimmen. Die Gattung bildet zusammen mit *Crawfurdia, Gentiana, Kuepferia, Metagentiana* und *Sinogentiana* die Gentianinae. Unter den Gentianinae kommt nur *Gentiana* in temperaten Regionen vor, während *Crawfurdia, Kuepferia,* *Metagentiana, Sinogentiana* und *Tripterospermum* auch als die subtropischen Gentianinae bekannt sind, weil sie in sub-alpinen Habitaten subtropischer Gebirge Asiens vorkommen. Die phylogenetischen Verwandtschaftsverhältnisse innerhalb der Tribus konnten in Favre et al. (2014) gut aufgelöst werden. *Tripterospermum* ist die Schwesterlinie zu *Sinogentiana*, die beide wiederum mit *Metagentiana* und *Crawfurdia* verwandt sind. *Kuepferia* bildet die älteste Linie der subtropischen Gentianinae und *Gentiana* ist Schwester zu allen restlichen fünf Gattungen.

Crawfurdia und *Tripterospermum* sind unter den Gentianinae die einzigen Kletterpflanzen, während die anderen Gattungen eine aufrechte Wuchsform haben. Sie wachsen in Bergwäldern und Bambusdickichten in Gebirgen Süd- und Zentralchinas sowie Indo-Burmas. *Metagentiana* und *Sinogentiana* bevorzugen hingegen die offeneren Habitate der sub-alpinen Zonen dieser Regionen. *Tripterospermum* besitzt mit Abstand die weiteste Verbreitung unter den subtropischen Gentianinae, da die Gattung nicht nur in den oben genannten Gebieten vorkommt, sondern auch in Gebirgen Japans, Sundalands und Wallaceas. *Crawfurdia, Metagentiana, Sinogentiana* und *Kuepferia* produzieren Kapselfrüchte, die meist geflügelte Samen enthalten, was auf eine Ausbreitung durch Wind hindeutet. Nur *Tripterospermum* besitzt neben kapselproduzierenden Arten auch Arten mit Beerenfrüchten. Die genauen Ausbreitungsvektoren der Beeren sind unbekannt, jedoch werden Vögel, Fledermäuse und/oder landlebende Säugetiere vermutet. In dieser Doktorarbeit wurden der geografische Ursprung der subtropischen Gentianinae (mit besonderem Fokus auf *Tripterospermum*) sowie Verbreitungswege zwischen der QTP-Region und Südostasien ermittelt. Außerdem wurde der Einfluss historischer Umweltveränderungen in der Umgebung des QTPs auf die Diversifikation untersucht, wobei auch die Evolution der klimatischen Nischen der verschiedenen Gattungen berücksichtigt wurde.

6.5 Ergebnisse dieser Doktorarbeit

Die drei spezifischen Fragen dieser Doktorarbeit wurden wie folgt beantwortet:

(1) Fungierte das QTP eher als Quelle oder als Senke für die weltweite Artenvielfalt nicht-alpiner Pflanzen? Diese Frage wurde beantwortet, indem die räumlich-zeitliche Geschichte der sub-alpinen Gattungen *Agapetes* und *Vaccinium* (Vaccinieae, Ericaceae) sowie *Tripterospermum* (Gentianinae; Gentianaceae) rekonstruiert wurde. Vaccinieae sind vermutlich in Nordamerika entstanden, von wo aus sie sich nahezu über die ganze Welt ausbreiteten. Im Falle der Vaccinieae erreichten präadaptierte Linien aus verschiedenen Gegenden der Erde die QTP-Region mehrere Male unabhängig voneinander und diversifizierten anschließend vor Ort. Somit fungierte das QTP für die Vaccinieae eher als Senke für Arten von außerhalb. Obwohl solch ein Muster auch für Asparagaceae und Solanaceae gefunden wurde, ist es doch eher die Ausnahme. Für alpine Pflanzen diente das QTP nämlich hauptsächlich als Quelle für die Artenvielfalt zahlreicher anderer Gebirgsregionen. Im Gegensatz zu den Vaccinieae wurde solch ein Muster, indem das QTP als Quelle fungierte, auch für *Tripterospermum* ermittelt. Die Region des südlichen Himalajas und der Hengduanshan, der südöstlich an das QTP grenzt, stellen den geografischen Ursprung von *Tripterospermum* dar. Dort diversifizierte die Gattung und breitete sich nach China, Japan, Taiwan und Südostasien aus. Bisher lag der wissenschaftliche Fokus von Studien über die Auswirkung von Umweltveränderungen des QTPs auf die Biodiversität hauptsächlich auf alpinen Pflanzentaxa. Diese Arbeit liefert daher erste Einblicke in die Auswirkungen auf nicht-alpine Pflanzengruppen. Die Ergebnisse zeigen, dass die Umgebung des QTPs sowohl für Vaccinieae als auch für *Tripterospermum* (und seine nahen Verwandten) ein wichtiger Ort war, um zu diversifizieren. Nichtsdestotrotz wirkte die QTP-Region für Vaccinieae eher als Senke und für *Tripterospermum* als Quelle für die Artenvielfalt anderer Hotspots der biologischen Vielfalt der Erde. Ein genereller Trend, ob das QTP für sub-alpine Pflanzen eher als Quelle oder Senke fungierte, kann jedoch erst ermittelt werden, wenn weitere sub-alpine Taxa untersucht wurden. Es ist auch denkbar, dass sich die Rolle der QTP-Region für die Biodiversität im Laufe der Zeit verändert hat. Möglicherweise fungierte das QTP in seiner früheren Formationsphase eher als Senke für die

Artenvielfalt von außerhalb (z.B. Vaccinieae) und später eher als Quelle (z.B. *Tripterospermum*). Der Einfluss wäre somit epochenabhängig.

(2) Stimmt die Diversifikation von *Agapetes, Vaccinium* und *Tripterospermum* zeitlich und räumlich mit der Hebung des QTPs und mit den damit verbundenen klimatischen Veränderungen überein? Zur Beantwortung dieser Frage wurde die Rekonstruktion der räumlich-zeitlichen Geschichte von *Agapetes* und *Vaccinium* (Vaccinieae, Ericaceae) sowie *Tripterospermum* (Gentianinae, Gentianaceae) genutzt. Vaccinieae erreichten die QTP-Region frühestens vor 37,7 bis 20,0 Millionen Jahren. Zu dieser Zeit besaßen Zentraltibet sowie einige nördliche Gegenden bereits eine Höhe, die vergleichbar mit der heutigen Höhe von ca. 4000 bis 5000 m war. Die nördliche und südliche Expansion des QTPs war aber noch in vollem Gange. Die Diversifikation der Vaccinieae erfolgte vor etwa 21,3 bis 9,2 Millionen Jahren in der QTP-Region, was zeitlich mit der Hebung des Himalajas und der Intensivierung des Monsuns übereinstimmt. Dies würde einen möglichen kausalen Zusammenhang zwischen Diversifikation und Umweltveränderungen andeuten. Es ist auffällig, dass die Vaccinieae vor allem in der Anden- und der QTP-Region besonders artenreich sind, da diese beiden Gegenden gravierende Umweltveränderungen im Zuge der Gebirgshebung gemeinsam haben. Dieser Umstand spricht für die These, dass eine Vielzahl unbesetzter Nischen die Artbildung fördern könnte. Für *Tripterospermum* konnte hingegen ein Kronenalter von 8,8 bis 2,7 Millionen Jahren ermittelt werden, womit *Tripterospermum* viel jünger als die Vaccinieae ist. In dieser Zeit hatte das QTP nahezu heutige Ausmaße erreicht, aber der Himalaja formierte sich noch und die Hebung des Hengduanshan begann erst. Daher ist es möglich, dass diese Umweltveränderungen einen Einfluss auf die Diversifikation der Gattung hatten, jedoch kann dieser als sehr gering eingestuft werden, da wir in zusätzlichen Analysen zeigen konnten, dass für die Diversifikation von *Tripterospermum* die Entwicklung von Beerenfrüchten eine wesentliche Rolle gespielt hat (siehe Frage 3). Unsere Ergebnisse zeigen, dass die Bedeutung vergangener geologischer und klimatischer Veränderungen für verschiedene Pflanzengruppen unterschiedlich sein kann, was aber möglicherweise ebenfalls epochenbedingt ist.

(3) Spielte im Zuge der Hebung des QTPs und damit verbundenen klimatischen Veränderungen eher Nischenevolution und/oder -konservatismus für die Diversifikation der asiatischen Gentianinae eine größere Rolle? Welchen Einfluss hatten manche neu evolvierten morphologischen Merkmale innerhalb der asiatischen Gentianinae auf die Diversifikation? Diese Fragen wurden beantwortet, indem die klimatische Nischendivergenz der subtropischen Gentianinae (*Crawfurdia, Kuepferia, Metagentiana, Sinogentiana* und *Tripterospermum*; Gentianaceae) sowie ihre Nischenevolution über die Zeit hinweg untersucht und die Auswirkung verschiedener morphologischer Merkmale auf die Artbildungsrate bestimmt wurde. Die subtropischen Gentianinae entstanden wahrscheinlich im alpinen Habitat des südöstlichen Randbereiches des QTPs. Unsere Analysen zeigten, dass Nischenevolution eine dominante Rolle für sie spielte, mit Ausnahme von *Kuepferia* und *Sinogentiana*. Diese beiden Gattungen wiesen eine starke Präferenz für die anzestralen alpinen Habitate auf. *Crawfurdia, Metagentiana* und *Tripterospermum* hingegen passten sich an wärmere und feuchtere Bedingungen an, wobei *Tripterospermum* die größte Nischenbreite besaß. Es ist auffällig, dass *Tripterospermum* mit seinen Beerenfrüchten ein viel weiteres Verbreitungsgebiet besitzt als die kapselproduzierenden anderen Gattungen der subtropischen Gentianinae. Unter fünf getesteten morphologischen Merkmalen (die Faltung der Corolla, der Fruchttyp, der Habitus, die Länge der Internodien, und die Gestalt der Stamina), die einen Einfluss auf die Diversifikation gehabt haben könnten, erfüllte nur die Entwicklung von Beerenfrüchten die Kriterien einer Schlüsselinnovation (*key innovation*). Eine Schlüsselinnovation ist eine morphologische Neuerung, die die Artbildungsrate erhöht und adaptive Radiation fördern kann. Im Fall von *Tripterospermum* haben die Beerenfrüchte wahrscheinlich eine Ausbreitung durch Vögel über große Distanzen hinweg ermöglicht, wodurch eine verstärkte allopatrische Artbildung einsetzte. Die Analysen zeigten außerdem, dass eine Disparifikation, also eine Aufspaltung der klimatischen Nische, innerhalb der subtropischen Gentianinae erst vor ca. 8 bis 6 Millionen Jahren eintrat. Zu dieser Zeit waren jedoch die größten geologischen und klimatischen Veränderungen in der QTP-Region schon erfolgt und die Hebung des Hengduanshan hatte noch nicht begonnen. Aus diesem Grund erscheint ein bedeutender Einfluss der

Umweltveränderungen auf die Diversifikation der subtropischen Gentianinae eher unwahrscheinlich. Entscheidend für die Diversifikation von *Tripterospermum* waren hingegen die allgemeine Tendenz der Gattung zur Nischenevolution und die Ausbildung von Beerenfrüchten. Ohne die Untersuchung der klimatischen Nische sowie morphologischer Neuerungen von *Tripterospermum* hätte man wahrscheinlich die Klimaschwankungen im Quartär für die Diversifikation verantwortlich gemacht. Diese Studie unterstreicht daher die Wichtigkeit einer integrativen Biogeografie, die ökologisches Wissen miteinbezieht.

Diese Doktorarbeit hat neue Einblicke in die mögliche Rolle des QTPs für sub-alpine Pflanzengruppen gewährt und hat darüber hinaus verdeutlicht, dass die Berücksichtigung ökologischer Aspekte bei Studien mit evolutionsbiologischem Hintergrund wichtig ist. Zukünftig sollten vermehrt auch nicht-alpine Pflanzengruppen der QTP-Region untersucht werden, um den Einfluss des QTPs für die weltweite Biodiversität in Gänze zu erfassen. Außerdem sollten Studien vor dem Hintergrund des QTPs auch die ökologischen Nischen ihrer Organismen miteinbeziehen, um Fehlinterpretationen vorzubeugen.

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8. APPENDICES

Appendix 1. Favre, A., Matuszak, S. and A.N. Muellner-Riehl. 2013. Two New Species of the Asian Genus *Tripterospermum* (Gentianaceae). *Systematic Botany* 38: 224-234.

Abstract

This paper presents descriptions and illustrations for two new species of Gentianaceae, *Tripterospermum maculatum* from Sichuan Province (China) and *T. tanatorajanense* from Sulawesi (Indonesia). Based on literature search, *Tripterospermum maculatum* is morphologically close to *T. pingbianense* and *T. lanceolatum*, and *T. tanatorajanense* resembles *T. luzonense* and *T. alutaceifolium*. To test if the new species differ from their morphologically most similar species, we measured various traits on herbarium specimens and performed a principal component analysis (PCA). This analysis showed that the new species differ from similar species in gross morphology for several diagnostic traits. *Tripterospermum maculatum* differs from *T. pingbianense* and *T. lanceolatum* by having calyx lobes longer than calyx tubes, a whitish-yellow corolla maculated with purple dots, and a gynophore shorter than the ovary. *Tripterospermum tanatorajanense* is distinct from *T. luzonense* by having a narrowly winged calyx. It differs from *T. alutaceifolium* and *T. luzonense* by having a shorter ovary and a slightly longer gynophore. A key including all species of *Tripterospermum* is provided.

INTRODUCTION

The subtribes Gentianinae and Swertiinae together form the tribe Gentianeae, the most speciose tribe of the Gentianaceae family (Struwe et al., 2002). Several studies have confirmed that Gentianeae and both of its subtribes are monophyletic (Yuan & Kuepfer, 1995; Chassot et al., 2001; Yuan et al., 2003; Favre et al., 2010). The Gentianinae comprise four genera: temperate subcosmopolitan *Gentiana* L., and three Asian subtropical genera, *Tripterospermum* Blume, *Crawfurdia* Wall., and *Metagentiana* Ho & Liu.

Tripterospermum and *Crawfurdia*, two genera of twining vines, were described almost simultaneously (Blume, 1826; Wallich, 1826). Botanists often referred species to one or the other genus based on their climbing habit, regardless of other morphological traits. As a result, many herbarium specimens were wrongly attributed to *Tripterospermum* (or *Crawfurdia*), which maintained a long-lasting confusion between these two genera. Several authors even considered them a single genus (Clarke, 1885; Ridley, 1923; Ubolcholaket 1987) or included them in *Gentiana* (Marquand, 1931, 1937). Phylogenetic studies have allowed a better understanding of the relationships within the subtribe Gentianinae (Yuan & Kuepfer, 1995; Yuan et al., 1996; Favre et al., 2010). Some studies raised doubts concerning the monophyly of *Tripterospermum* and *Crawfurdia* (Chen et al., 2005b,c), but it was recently confirmed that they constitute two genetically distinct entities (Favre et al., 2010), easily distinguishable from each other by morphological synapomorphies: *Tripterospermum* has apically decurved stamens, a disc-like nectary gland at the base of the gynophore, and most species (27 out of 32) produce berry-like fruits (Ho & Pringle, 1995d; Struwe et al., 2002; Favre et al., 2010). All these traits differ in *Crawfurdia*, which has straight stamens, small and separate nectary glands at the base of the gynophore, and capsular fruits. To date, 32 species of *Tripterospermum* have been described (Murata, 1989; Hul, 2002; Chen et al., 2006; Hsu & Chung, 2012). *Tripterospermum* is divided into two sections: species with capsular fruits belong to the sect. *Capsulifera* J. Murata, whereas species producing berries are placed in sect. *Tripterospermum* (Murata, 1989). Restricted to Asia, this genus reaches its northernmost distribution on the island of Hokkaido (Japan) and extends south to the island of Java (Indonesia). With the exception of some populations on the island of Sulawesi, *Tripterospermum* is restricted to the west of Wallace´s line. The largest number of *Tripterospermum* species is found between the Himalayan range, mainland China (southeastern provinces such as Yunnan, Sichuan, and Guangxi) and the island of Taiwan. This genus is also frequently present in areas of higher elevation in southeastern Asia (Philippines, Thailand, Vietnam, Malaysia, and Indonesia) and Sri Lanka (see Fig. 18).

Here, we describe two new species of *Tripterospermum* (*T. maculatum* and *T. tanatorajanense*) and show that these new species differ from their morphologically most similar relatives by measuring various traits on herbarium specimens. The traits chosen to perform a principal component analysis (PCA) have previously been shown to be the most useful in differentiating among species of *Tripterospermum* and of related genera (*Gentiana*, *Metagentiana* and *Crawfurdia*; Murata, 1989; Hul, 2002; Chen et al., 2006).

MATERIAL AND METHODS

Taxon sampling and distribution map

Taxon sampling was performed in 2002 in Taiwan by A. Favre with the contribution of Chi-Hsiung Chen (Department of Botany, National Museum of Natural Science in Taichung), in 2008 in Sulawesi by A. Favre and in 2011 by A. Favre and S. Matuszak in Sichuan. Voucher specimens resulting from these fieldwork campaigns are deposited in three herbaria: Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN), Herbarium Senckenbergianum (FR), Senckenberg Research Institute, Frankfurt am Main, Germany, and Herbarium Universitatis Lipsiensis (LZ), Institute of Biology, University of Leipzig, Germany. See Supporting Information 1 for the detailed localities of these vouchers.

To visualise the geographical occurrence of the two new species along the entire distribution of *Tripterospermum*, a map is shown (Fig.1). This map was downloaded from WORLDCLIM (Hijmans et al., 2005) and political borders were retrieved from Esri Data and Maps (2002).

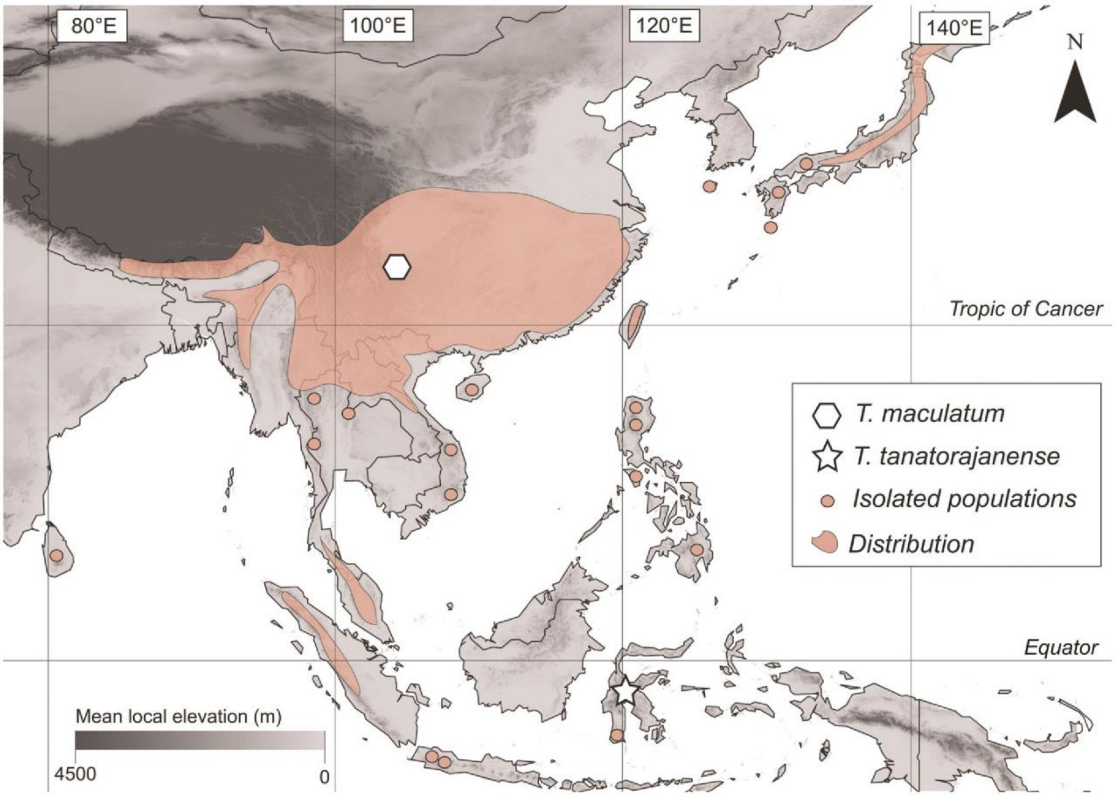


Figure 18. Distribution of Tripterospermum, and localisation of the new species *T. maculatum* and *T. tanatorajanense*. The grayscale on the continents indicates the local mean elevation (lightest gray is 0 m and the darkest 4500m and above).

Identifying morphologically similar species

This work is based on the *Flora of China* (Ho & Pringle, 1995d), the synopsis of *Tripterospermum* (Murata, 1989), and some new species descriptions (Hul, 2002; Chen et al., 2006; Hsu & Chung, 2012). To identify the species morphologically most similar to *T. maculatum* and *T. tanatorajanense*, we relied on traits and ratios previously used to differentiate *Tripterospermum* species in keys and species descriptions (Murata, 1989; Hul, 2002; Chen et al., 2006; Hsu & Chung, 2012).

Trait measurements and analysis

The specimens observed for this study are listed in Appendix I. The following traits were measured to the closest millimeter: corolla length, calyx tube length, calyx lobe length, pedicel length, leaf blade length, leaf blade width, petiole length, gynophore length, ovary length, style length. For each specimen, means of the above traits were calculated (for example, mean corolla length of the five flowers of an individual). These means were then used to calculate the following ratios: the calyx lobe/calyx tube ratio, the calyx tube/corolla ratio, the leaf length/leaf width ratio, and the petiole/leaf length ratio. Particular care was taken to investigate traits on fully developed, healthy leaves as well as on fully opened, not yet wilted flowers. To assess graphically if the new species differed from their presumably closest relatives and to understand which traits were the most relevant with regard to their identification, we performed a principal component analysis (PCA) using the *ade4* package (Chessel et al., 2004) in R.2.10.0 (R Development CoreTeam, 2009).

RESULTS

Identifying morphologically similar species

The following trait combination found in the new species *T. maculatum* is also found in *T. lanceolatum* (Hayata) H. Hara ex Satake and *T. pingbianense* Wu & Wu: fruit with marcescent corolla (type of corolla persisting until fruit maturation, losing original pigmentation and becoming dry and papery in texture), red berries ellipsoid to spindle-shaped with the fruit stipe longer than the calyx tube, and narrowly winged or wingless seeds. The second new species, *Tripterospermum tanatorajanense*, shares its globose or subglobose berries with nine other species (*T. alutaceifolium* (Liu & Kuo) Murata, *T. championii* Gardner, *T. hualienense* Hsu & Chung, *T. japonicum* (Siebold & Zuccarini) Maximowicz, *T. lilungshanensis* Chen & Wang, *T. luzonense* (Vidal) Murata, *T. robustum* Harry Smith ex Hul, *T. sumatranum* Murata, *T. trinerve* Blume). However, in *T. tanatorajanense* the fruit does not have a marcescent corolla (*T. japonicum*, *T. sumatranum*, *T. trinerve*), or winged seeds (*T. trinerve*, *T. sumatranum* and *T. championii*). Moreover, the shape of its calyx lobes clearly differs from *T. lilungshanensis* and *T. robustum* by being neither arcuate nor spreading, but erect and appressed to the corolla. Finally, *T. tanatorajanense* is not a trailing plant, does not have linear leaves, and its calyx is not wingless (*T. hualienense*). Therefore, *T. tanatorajanense* is morphologically most similar to *T. alutaceifolium* and *T. luzonense*.

Diagnostic traits

Principal component analyses included several ratios between the sizes of several organs (gynophore, ovary, calyx, calyx lobes, corolla, length and width of leaves, petiole) and showed clear morphological differentiation between the new species (*Tripterospermum maculatum* and *T. tanatorajanense*) and the species respectively most similar in gross morphology (Fig. 19; Table 7).

Tripterospermum maculatum clearly differed from *T. pingbianense* and *T. lanceolatum* by the calyx lobes being longer than the calyx tube (calyx lobes/calyx tube ratio > 1 for *T. maculatum*, and < 1 for other species) and a much smaller ratio between gynophore and ovary length (about 0.6 for *T. maculatum*, and more than 1.00 for *T. pingbianense* and *T. lanceolatum*). Additionally, *Tripterospermum maculatum* differed from *T. lanceolatum* by having fruits only partly exserted from the corolla (the fruits are almost completely exserted from the corolla in the latter species, see illustration in Murata 1989), a trait well visible in Figs. 20-21. Another morphological trait specific to *T. maculatum* was the colour of the corolla, white-yellow with purple dots.

Tripterospermum tanatorajanense differed from *T. alutaceifolium* and *T. luzonense* by having generally smaller leaves: in *T. tanatorajanense* the length of the leaves never exceeded 2 cm, whereas leaves were frequently larger than 3 or even 4 cm in the other two species (Table 7). This new species also had a shorter ovary of about 5 mm at anthesis (it was between 6 and 11 mm in the other species), and a slightly longer gynophore (Table 7; Fig. 20; Fig. 22). Therefore, the gynophore/ovary ratio was about twice bigger for *T. tanatorajanense* than for *T. alutaceifolium* and *T. luzonense*. The length of the corolla differed between *T. tanatorajanense* (always < 30 mm) and *T. alutaceifolium* (always > 38 mm). However, this trait was nearly similar in *T. tanatorajanense* and *T. luzonense* (31 to 35 mm).

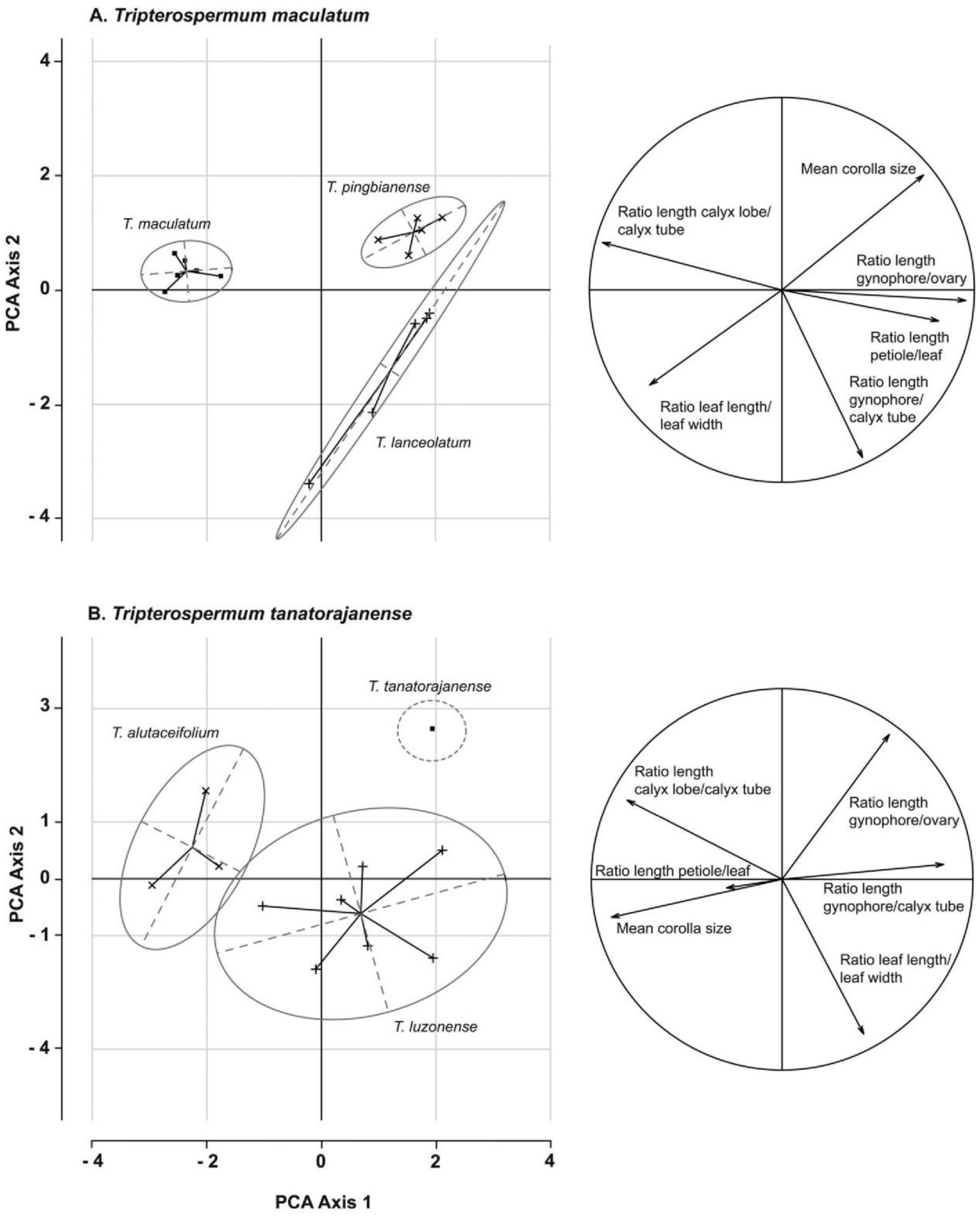


Figure 19. Principal component analyses on morphological traits comparing the two new species *T. maculatum* (A) and *T. tanatorajanense* (B) and their morphologically most similar species.

Table 7. Mean trait values and standard error for *Tripterospermum maculatum*, *T. tanatorajanense* and morphologically similar species. The number of organ observations is n. Minimum and maximum measured values per species per traits are indicated below each mean.

Traits/Species	T. maculatum	T. lanceolatum	T. pingbianense	T. tanatorajanense	T. luzonense	T. alutaceifolium
Corolla (mm)	28.5 +/– 1.36 (n = 21) min = 25, max = 30	31.6 +/– 4.22 (n = 26) min = 23, max = 37	39.7 +/– 2.25 (n = 26) min = 36, max = 42	27.6 +/– 0.89 (n = 5) min = 27, max = 29	33.6 +/– 1.67 (n = 48) min = 31, max = 37	40.6 +/– 3.35 (n = 17) min = 39, max = 45
Calyx lobes (mm)	9.2 +/– 1.14 (n = 21) min = 7, max = 11	5.8 +/– 1.59 (n = 53) min = 3, max = 12	8.9 +/– 1.31 (n = 56) min = 6, max = 13	7.1 +/– 0.79 (n = 12) min = 6, max = 8	5.6 +/– 1.49 (n = 81) min = 2, max = 9	10.6 +/– 1.34 (n = 28) min = 8, max = 12
Calyx tube (mm)	7.9 +/– 0.75 (n = 26) min = 7, max = 9	8.0 +/– 1.77 (n = 27) min = 6, max = 11	11.0 +/– 0.93 (n = 27) min = 10, max = 14	9.3 +/– 0.46 (n = 8) min = 9, max = 10	7.4 +/– 1.69 (n = 46) min = 5, max = 10	10.0 +/– 1.09 (n = 16) min = 9, max = 12
Gynophore (mm)	6.3 +/– 1.03 (n = 4) min = 4, max = 8	9.3 +/– 0.53 (n = 9) min = 7, max = 11	9.71 +/– 0.29 (n = 7) min = 9, max = 11	3.50 +/– 0.84 (n = 6) min = 3, max = 5	2.98 +/– 0.60 (n = 21) min = 2, max = 4	2.17 +/– 0.43 (n = 9) min = 2, max = 3
Ovary (mm)	7.8 +/– 1.11 (n = 4) min = 5, max = 10	8.1 +/– 0.61 (n = 9) min = 4, max = 10	9.57 +/– 0.43 (n = 7) min = 8, max = 11	5.5 +/– 0.55 (n = 6) min = 5, max = 6	8.6 +/– 1.54 (n = 21) min = 6, max = 11	6.28 +/– 1.15 (n = 9) min = 6, max = 8
Leaflength (cm)	5.99 +/– 1.09 (n = 24) min = 4.1, max = 7.9	4.68 +/– 1.28 (n = 30) min = 2.3, max = 7.1	5.07 +/– 1.03 (n = 23) min = 3.0, max = 6.6	1.78 +/– 0.45 (n = 8) min = 1.1, max = 2.0	3.20 +/– 1.24 (n = 36) min = 1.3, max = 4.7	3.05 +/– 1.18 (n = 11) min = 2, max = 5.3
Leaf width (cm)	1.42 +/– 0.31 (n = 24) min = 0.9, max = 2.0	1.44 +/– 0.56 (n = 30) min = 0.5, max = 2.5	1.91 +/– 0.48 (n = 23) min = 1.2, max = 2.8	0.99 +/– 0.21 (n = 8) min = 0.7, max = 1.3	1.24 +/– 0.56 (n = 36) min = 0.4, max = 2.4	1.51 +/– 0.47 (n = 11) min = 1.1, max = 2.6
Petiole (cm)	0.72 +/– 0.35 (n = 24) min = 0.5, max = 1.5	0.89 +/– 0.33 (n =30) min = 0.4, max = 1.5	0.87 +/– 0.25 (n = 23) min = 0.3, max = 1.4	0.61 +/– 0.14 (n = 8) min = 0.4, max = 0.8	0.93 +/– 0.40 (n = 36) min = 1.0, max = 2.0	1.11 +/– 0.49 (n = 11) min = 0.5, max = 2.0

DISCUSSION

The new species *T. maculatum* from Sichuan is morphologically similar to *T. lanceolatum* and *T. pingbianense*, two species from Taiwan and Yunnan respectively. Whether or not *T. pingbianense* and *T. lanceolatum* are two distinct species was discussed in a previous study: Murata (1989) included *T. pingbianense* sensu Wu (1984) in a broader delineation of *T. lanceolatum*. However, the most recent phylogenetic reconstruction suggested that *T. pingbianense* and *T. lanceolatum* were not very closely related (Favre et al., 2010). Leaf traits and leaf trait ratios were not allowing the distinction between these three species. However, we found a number of other traits that supported the description of *T. maculatum* as a distinct species (Table 8): it differed from both *T. pingbianense* and *T. lanceolatum* by having calyx lobes longer than calyx tubes, a much smaller ratio between gynophore and ovary length, and a whitish-yellow corolla maculated with purple dots. *Tripterospermum maculatum* also differed from *T. lanceolatum* by having fruits only partly exserted from the corolla, and from *T. pingbianense* by having much smaller flowers and a calyx tube which was angled or inconspicuously keeled.

The new species *T. tanatorajanense* shares morphological similarities with *T. alutaceifolium* and *T. luzonense* (globose or subglobose berries, white corolla, deciduous (not marcescent) corolla in fruit). This is consistent with Murata (1989), who suggested that *Tripterospermum* populations from Sulawesi should be included in *T. luzonense* (Taiwan, Philippines) because of a strong morphological resemblance between plants from these different islands. Nevertheless, Murata (1989) reported that specimens from Sulawesi were generally of smaller size (leaves and flowers) than those from Taiwan, a trend supported by our analysis. In addition to these observations, we were able to show that there are additional substantial morphological differences in the Sulawesi populations supporting the recognition of a new species (Table 9). *Tripterospermum tanatorajanense* differs from *T. luzonense* by having a narrowly winged calyx (*T. luzonense* has a wingless calyx) and by a gynophore/ovary ratio about two times bigger than in *T. alutaceifolium* and *T. luzonense*. Our description of *T. tanatorajanense* is based upon a complete specimen (with several flowers and fruits) from a population of about 50 plants. The morphological resemblance of *T. tanatorajanense* to the Taiwanese species (*T. luzonense* and *T. alutaceifolium*) could indicate close phylogenetic relatedness and the occurrence of past dispersal events between Taiwan and Sulawesi.

Investigating the biogeography of *Tripterospermum* in future studies will contribute to the growing (yet insufficient) understanding of floristic exchanges between southeastern China, the Himalayas, Sundaland and Wallacea. Two limitations still exist to perform a solid biogeographic analysis: first, the taxonomic sampling needs to be increased in comparison to previous studies (Favre et al., 2010), and second, more DNA markers need to be analyzed to obtain better resolved phylogenetic reconstructions.

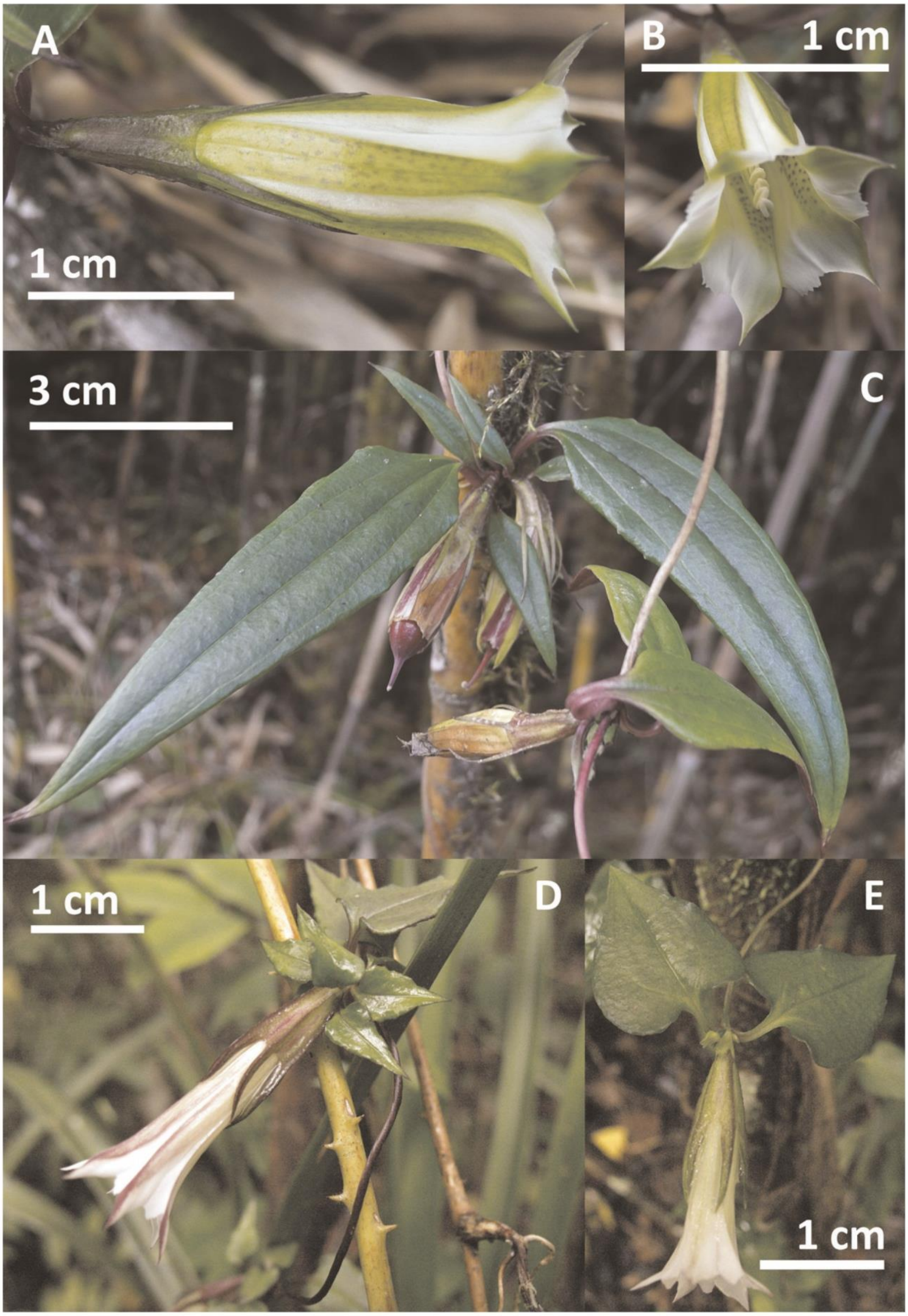


Figure 20. A-C, *Tripterospermum maculatum*. A, outside view of the corolla (note the dots) and the calyx; B, corolla lobes, plicae and corolla throat; C, narrowly lanceolate leaves, red berries and marcescent corolla. D-E, *Tripterospermum tanatorajanense*: corolla, calyx tube and calyx lobes decurrent into wings on the calyx tube, cordate leaves.

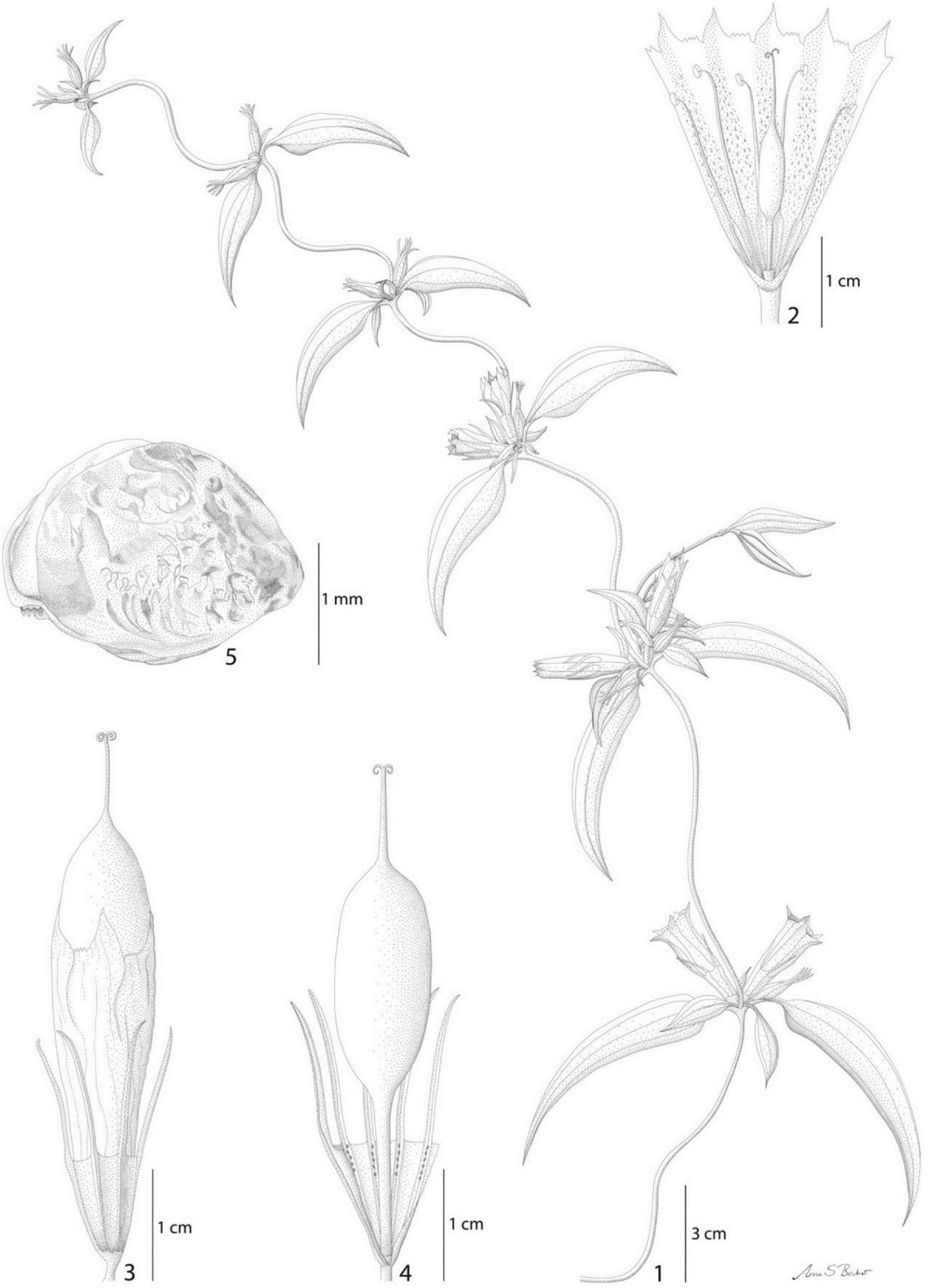


Figure 21. *Tripterospermum maculatum* Favre, Matuszak and Muellner-Riehl. 1. Habitus; 2. Dissected corolla with stamens and pistil; 3. Berry with marcescent corolla; 4. Berry with dissected calyx; 5. Seed. Drawn from Favre 321A and Favre 321B.

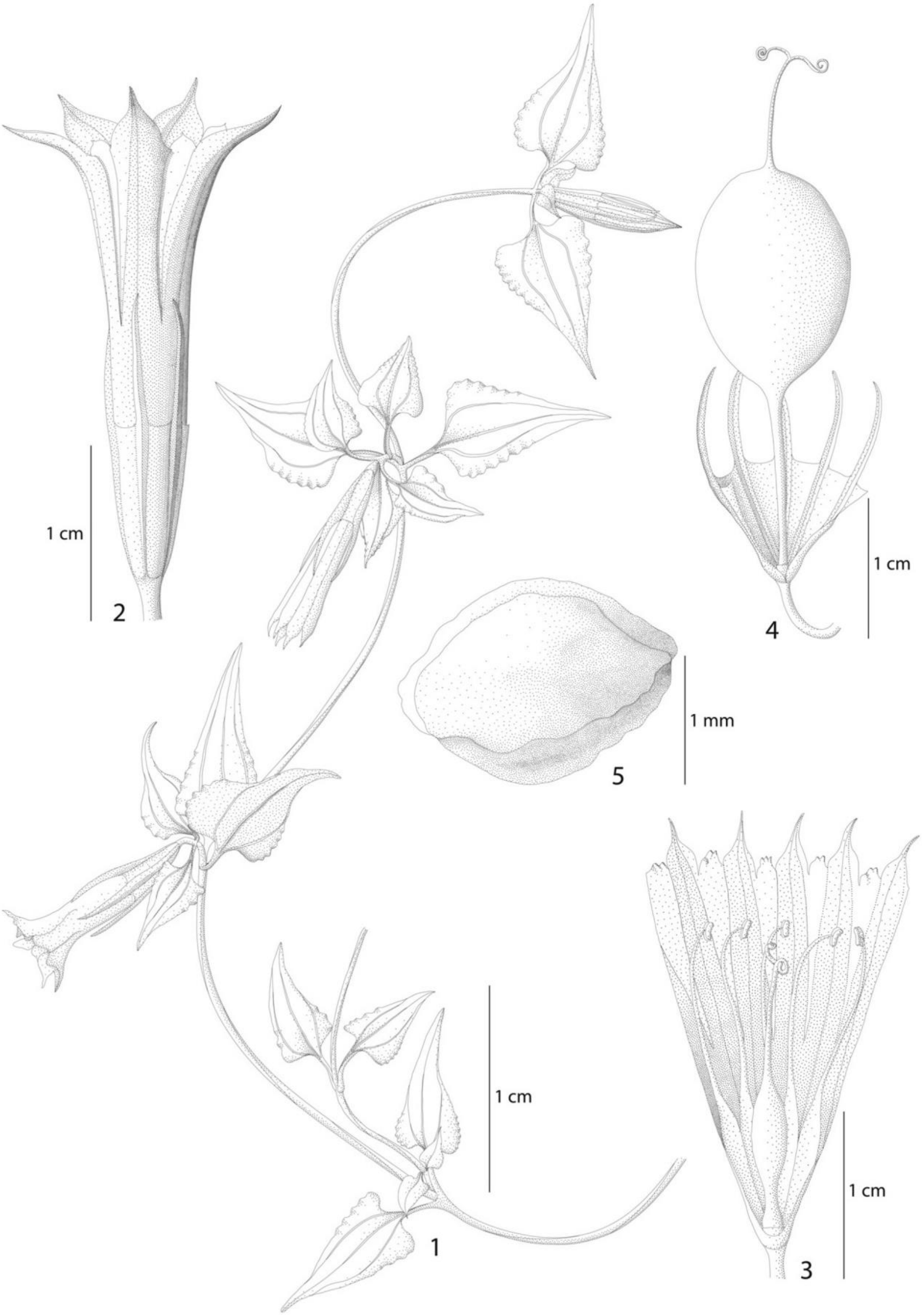
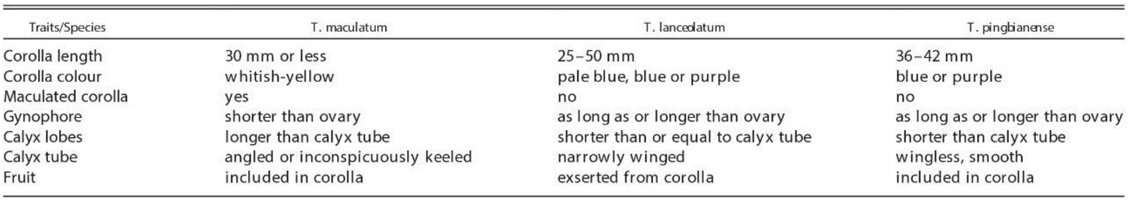


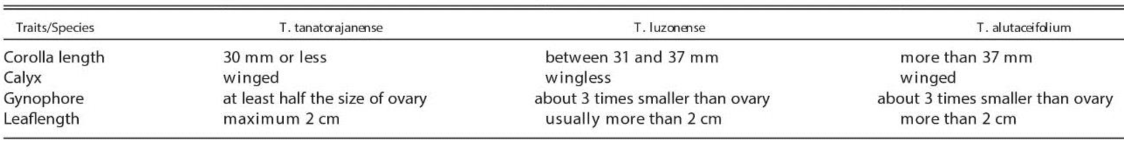
Figure 22. *Tripterospermum tanatorajanense* Favre, Matuszak and Muellner-Riehl. 1. Habitus; 2. Corolla and calyx; 3. Dissected corolla with stamens and pistil; 4. Berry with dissected calyx (the corolla is not marcescent); 5. Seed. Drawn from Favre 400.

Table 8. Diagnostic features of *Tripterospermum maculatum* with regards to its morphologically most similar species.



Traits/Species	T. maculatum	T. lanceolatum	T. pingbianense
Corolla length	30 mm or less	25–50 mm	36–42 mm
Corolla colour	whitish-yellow	pale blue, blue or purple	blue or purple
Maculated corolla	yes	no	no
Gynophore	shorter than ovary	as long as or longer than ovary	as long as or longer than ovary
Calyx lobes	longer than calyx tube	shorter than or equal to calyx tube	shorter than calyx tube
Calyx tube	angled or inconspicuously keeled	narrowly winged	wingless, smooth
Fruit	included in corolla	exserted from corolla	included in corolla

Table 9. Diagnostic features of *Tripterospermum tanatorajanense* with regards to its morphologically most similar species.



Traits/Species	T. tanatorajanense	T. luzonense	T. alutaceifolium
Corolla length	30 mm or less	between 31 and 37 mm	more than 37 mm
Calyx	winged	wingless	winged
Gynophore	at least half the size of ovary	about 3 times smaller than ovary	about 3 times smaller than ovary
Leaflength	maximum 2 cm	usually more than 2 cm	more than 2 cm

KEY TO THE SPECIES OF *TRIPTEROSPERMUM*

The following key is based on Murata (1989) and *Flora of China* (Ho & Pringle 1995d). It includes all species attributed to *Tripterospermum*. To determine *Tripterospermum* species, information on fruit type (capsular or berry), fruit shape (globose or spindle-shaped), fruit colour, and seed morphology (winged or wingless) is necessary. This information is missing for *T. filicaule* (Hemsley) Smith, a narrow endemic species from Shennongjia (Hubei, China). Thus, this species cannot be accurately placed in the following key, but is nevertheless referred to under couplet 1.

1. Fruits berries (section *Tripterospermum*, see also *T. filicaule*) 2
    2. Marcescent corolla at fruit maturation; stipe of fruit usually longer than calyx tube (except in *T. microcarpum*).. 3
        3. Fruit body spindle-shaped to ellipsoid.. 4
            4. Calyx tube longer than 6 mm; berries red to purple 5
                5. Corolla mauve, pale blue, blue or purple 6
                    6. Calyx tube ridged or narrowly winged 7
                        7. Fruit exserted from corolla................................*T. lanceolatum*
                        7. Fruit almost completely included in corolla......*T. microcarpum*
                    6. Calyx tube wingless...*T. pingbianense*

5. Corolla white or yellowish green 8

8. Seeds distinctly winged; corolla without dots............... *T. volubile*

8. Seeds wingless or narrowly ridged; corolla with purple dots ....*T. maculatum*

4. Calyx tube shorter than 6 mm; berries black............*T. nigrobaccatum*

3. Fruit body subglobose to globose 9

9. Peduncle longer than corolla; calyx lobes linear to broadly linear........ .... *T. trinerve*

9. Peduncle distinctly shorter than corolla (usually less than 1 cm); calyx lobes lanceolate to subulate 10

10. Leaf blade oblong; calyx lobes distinctly spreading .... *T. sumatranum*

10. Leaf blade lanceolate to triangular ovate; calyx lobes not distinctly spreading 11

11. Corolla light purple, pale blue or blue; seeds ridged or slightly winged; fruit almost entirely exserted from corolla; berries red . ...*T. japonicum*

11. Corolla white; seeds distinctly winged; fruit only partially exserted from corolla; berries purple.................... *T. championii*

2. Corolla deciduous before fruit maturation; stipe of the fruit shorter than calyx tube (slightly longer in *T. lilungshanensis* 12

12. Stem long trailing, neither spirally twisted nor twining (sometimes twining in the upper part in *T. hualienensis*) 13

13. Leaf blade cordate 14

14. Calyx lobes oblanceolate and recurved; stipe of fruits as long as calyx tube; style distinctly exserted from corolla.......*T. cordifolium*

14. Calyx lobes linear-subulate to lanceolate, not recurved; fruit very shortly stipitate; style included in corolla....... *T. cordifolioides*

13. Leaf blade not cordate 15

15. Leaf blade deltoid-lanceolate to lanceolate; ovary 5-6 mm long; berries 8-10 × 5-7; seeds 1.5 mm or longer .... *T. microphyllum*

15. Leaf blade linear to linear-lanceolate; ovary 7-9 mm long; Berries 10-16 × 8-12 mm; seeds up to 1.3 mm long...*T. hualienense*

12. Stem spirally twisted and twining for most if its length 16

16. Ovary completely included or only slightly exserted from calyx tube at anthesis 17
  17. Corolla purple, pink or pale blue 18
    18. Calyx tube cylindrical, with ciliate ridges .... *T. hirticalyx*
    18. Calyx tube campanulate, smooth 19
      19. Calyx lobes shorter than calyx tube (max. 4 mm) ....*T. brevidentatum*
      19. Calyx lobes as long as or much longer than calyx tube20
        20. Fruit body globose to subglobose .... *T. robustum*
        20. Fruit body ellipsoid to narrowly ellipsoid 21
          21. Peduncle with scalelike or subulate bracts; calyx lobes arcuate spreading ....*T. australe*
          21. Peduncle with leafy bracts; calyx lobes ascending or recurved only near tip .... *T. cordatum*
  17. Corolla white, at most tinged with purple outside 22
    22. Seeds narrowly winged ....*T. pallidum*
    22. Seeds wingless 23
      23. Base of calyx lobes distinctly narrowed 24
        24. Corolla more than 35 mm long; leaf blade 4-11 cm long; stipe of the fruit shorter than calyx tube......... .... *T. alutaceifolium*
        24. Corolla 25 to 35 mm long; leaf blade 2-5 cm long; stipe of the fruit longer than calyx tube ....*T. lilungshanensis*
      23. Base of calyx lobes not distinctly narrowed 25
        25. Leaf blade up to 2 cm long ....*T. tanatorajanense*
        25. Leaf blade 5 cm long or more .... *T. robustum*
16. Upper half of ovary or entire ovary exserted from calyx tube at anthesis 26
  26. Fruit body ellipsoid or spindle-shaped 27
    27. Corolla white, greenish white or yellowish inside, sometimes tinged with purple outside 28
      28. Fruit body 25-40 mm long; seeds narrowly winged; berries bright red..*T. luteoviride*

        - 28. Fruit body less than 25 mm long; seeds wingless, berries dark purple.. *T. taiwanense*
      - 27. Corolla purplish, mauve, blue purple or dark purple............ 29
        - 29. Leaves herbaceous; corolla dark purple or blue purple, narrowly campanulate, with triangular lobes......*T. nienkui*
        - 29. Leaves membranaceous, particularly when dried; corolla mauve, infundibuliform, with broadly triangular lobes....... .... *T. membranaceum*
    - 26. Fruit body globose or subglobose ....*T. luzonense*

1. Fruits capsules (section *Capsulifera*, see also *T. filicaule*) 30
  - 30. Stipe of the ovary nearly as long as or longer than calyx tube at anthesis and distinctly longer than calyx tube in fruit 31
    - 31. Corolla campanulate, less than 35 mm long; calyx tube less than 9 mm long; stem leaves petiolate, petiole more than ¼ of the blade length..*T. distylum*
    - 31. Corolla narrowly campanulate, usually more than 35 mm long; calyx tube more than 10 mm long; stem leaves sessile or subsessile...... 32
      - 32. Calyx lobes 2-4 mm, tube wingless or narrowly winged .... *T. discoideum*
      - 32. Calyx lobes 6-9 mm, tube with five-keeled ....*T. chinense*
  - 30. Stipe of the ovary much shorter than calyx tube at anthesis and in fruit. 33
    - 33. Ovary about twice as long as calyx tube..........................*T. coeruleum*
    - 33. Ovary shorter than or nearly as long as calyx tube...... *T. fasciculatum*

TAXONOMIC TREATMENT

Tripterospermum maculatum

> Favre, Matuszak and Muellner-Riehl sp. nov.— TYPE: CHINA. Sichuan: eastern flank of Luojishan, Puge, south of Xichang, 27°35'18.00'' N, 102°23'22.22'' E, 3179 m, 28 September 2011, (fl., fr.), *Favre 321 A* (holotype: KUN!; isotypes: KUN! FR! LZ!)

Ut videtur *Tripterospermo pingbianense* C. Y. Wu & C. J. Wu et *Tripterospermo lanceolato* (Hayata) H. Hara ex Satake affinis, sed a primo breviore corolla angusteque alato calyce et a secundo magna parte fructu in corolla includitur differt. *Tripterospermum maculatum* alba luteave corolla cum purpureis maculis et calycis lobis longioribus quam calycis tubo ab utroque differt.

Perennial herb, stems spirally twisted, twining, slender, terete, up to 2 m long, commonly with few secondary branches. Leaves opposite, leaf blade lanceolate to narrowly lanceolate, 4-7 × 1-2 cm, base rounded, margin entire or crenulate and slightly revolute, apex acuminate, sometimes caudate; veins 3. Inflorescences axillary, as 1-flowered or few-flowered cymes; bracts 1-3 pairs, leaf-like, to 1 cm. Pedicel 0.3-0.7 cm. Flowers 5-merous. Calyx campanulate; tube 7-10 mm, angled or inconspicuously keeled; lobes linear 8-12 mm, apex acuminate. Corolla campanulate, 2.5-3.1 cm, white or yellowish with green-yellow stripes with purple dots inside the throat also visible on the outside and in immature flowers; lobes triangular, 2-5 mm, apex acuminate to cuspidate; plicae horizontally truncate, 1.5-3 mm, margin denticulate. Stamens inserted at basal part of corolla tube, unequal, apically recurved; filaments linear-subulate to linear, 1.3-1.7 cm; anthers ellipsoid, ca. 1 mm. Nectaries conspicuously developed, forming a collar-like disc surrounding the base of the ovary, ca. 1mm. Ovary 1-locular, 10-13 mm; ovules many; gynophore 4-7 mm; style 7-10 mm. Berries ellipsoid to spindle-shaped, red, 1.5-2 cm, partially exserted from marcescent corolla. Seeds dark brown to black, triquetrous, wingless.

***Etymology*—**The epithet “*maculatum*” refers to the numerous purple dots which are present on the corolla, a rather uncommon trait in this genus.

Phenology*—Tripterospermum maculatum* was found with both flowers and mature fruits by the end of September.

Distribution and Habitat—*Tripterospermum maculatum* is known only from the type locality, but is likely to occur in other populations in the Luojishan range. It is especially abundant among bamboo thickets, but also grows at the edge of mixed deciduous forests, forest clearings and among bushes of secondary vegetation between 2800 and 3400 m, a typical habitat for most species of *Tripterospermum* in China. Bamboo stems or trunks of young trees are commonly the support for the long, twining stem of this species. *Tripterospermum maculatum* may be restricted to a single mountain range, and the information available is not sufficient for a proper assessment of its conservation status. Therefore, the species has to be classified as Data Deficient according to IUCN Red List categories. The recent establishment of the Luojishan National Park might contribute to the protection of this species.

Tripterospermum tanatorajanense

> Favre, Matuszak and Muellner-Riehl sp. nov.— TYPE: INDONESIA. Sulawesi: South Sulawesi Province, pass between Pulu-Pulu and Baruppu, on the flanks of Gunung Mandoangin and Gunung Neosing, 2°47'06.35'' S, 119°46'03.30'' E, 2407 m, 15 February 2008, (fl., fr.), Favre 400 (type:LZ!)

Ut videtur *Tripterospermo luzonense* (Vidal) J. Murata et *Tripterospermo alutaceifolio* (Liu & Kuo) J. Murata affinis, sed a primo anguste alato calyce et a secundo minore corolla differt. *Tripterospermum tanatorajanense* breviore ovario et foliis minoribus ab utroque differt.

Perennial herb, stems spirally twisted, twining, slender, terete, up to 1.5 m long, commonly with few secondary branches. Leaves opposite, leaf blade cordate, 0.5-25 × 0.5-15 mm, base rounded, margin entire or crenulate, apex acuminate; veins 3. Inflorescences axillary, as 1-flowered or few-flowered cymes; bracts 1-3 pairs, leaf-like. Flowers usually sessile or pedicel to 3 mm long. Flowers 5-merous. Calyx campanulate; tube 8-10 mm, conspicuously keeled or winged, wings decurrent from lobes; lobes narrowly triangular, 7-10 mm, apex acuminate. Corolla campanulate, 2.5-3 cm, white or at most tinged with purple; lobes triangular, 2-4 mm, apex acuminate; plicae semiorbicular, 0.5-1.5 mm, margin irregularly denticulate or sometimes entire. Stamens inserted at basal part of corolla tube, unequal, apically recurved; filaments linear-subulate to linear, 1.3-1.7 cm; anthers ellipsoid, ca. 1 mm. Nectaries conspicuously developed, forming a collar-like disc surrounding the base of the ovary, ca. 1 mm. Ovary 1-locular, short, 4-6 mm; ovules many; gynophore 2- 4.5 mm; style 7-10 mm. Berries globose to subglobose, red, 1-1.5 cm, visible after early decay of the corolla. Seeds dark brown to black, triquetrous, wingless.

Etymology—The epithet "*tanatorajanense*" refers to the region where this species occurs: Tana Toraja, the land around Rantepao in Sulawesi, Indonesia.

Phenology—*Tripterospermum tanatorajanense* was found with both flowers and fruits by mid-February.

Distribution and Habitat—*Tripterospermum tanatorajanense* is known only from the type locality but is likely to occur in other populations in the provinces of Central and South Sulawesi. For example, Murata (1989) observed specimens of *Tripterospermum* from the regions of Masamba and Erekan (South Sulawesi), deposited in Bogor Herbarium. This species is abundant at the type locality, in mossy forests between 2000 and 2500m. It develops in the forest understory and uses small tree trunks or ferns as climbing support. *Tripterospermum tanatorajanense* also occurs at the edge of forests and in secondary vegetation along roads or trails. The typical habitat of *Tripterospermum* species in southeast Asia (*T. sumatranum*, *T. trinerve*, *T. championii, T. tanatorajanense*) is the mossy forest (also called cloud or fog forest), a forest type occurring at higher elevations (1800 m and higher) in tropical regions. This forest type is characterised by a frequent cloud cover providing a high humidity favorable to the development of mosses. With the Tana Toraja region being rather densely populated, this habitat is likely to be affected by logging and agricultural use. Because the distribution of this species is not well understood and potential threats by human activities exist, the species might be classified as Data Deficient or Vulnerable according to IUCN Red List categories.

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SUPPORTING INFORMATION 1

Vouchers specimens that were measured for the principal component analysis:

Tripterospermum alutaceifolium (Liu and Kuo) J. Murata. TAIWAN. Taipei: Yangmingshan, Summit of Mt. Chihsingshan (1000m), N 25°10'5.04", E 121°33'21.23", 22 Sep. 2002, *Favre A. 130*. (KUN). Taipei: Yangmingshan, Summit of Mt. Chihsingshan (1000m), N 25°10'5.04", E 121°33'21.23", 22 Sep. 2002, *Favre A. 131*. (LZ). Taipei: Yangmingshan, Summit of Mt. Chihsingshan (1000m), N 25°10'5.04", E 121°33'21.23", 22 Sep. 2002, *Favre 132*. (TNM). ***Tripterospermum lanceolatum*** (Hayata) H. Hara ex Satake. TAIWAN. Nantou: between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 8 Sep. 2002, *Favre A. 102* (FR). Nantou: between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 8 Sep. 2002, *Favre A. 103* (TNM). Nantou: Hohanshan, road to Tayuling (2500m), roadside, N 24° 06'085, E121°11'329, 10 Sep. 2002, *Favre A. 104* (LZ). Nantou:

Hohanshan, road to Tayuling (2500m), roadside, N 24° 06'085, E121°11'329, 10 Sep. 2002, *Favre A. 105* (LZ). Nantou: Hohanshan, between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 15 Sep. 2002, *Favre A. 106* (KUN). ***Tripterospermum luzonense*** (Vidal) J. Murata. TAIWAN. Nantou: between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 9 Sep. 2002, *Favre A. 094* (TNM). Nantou: between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 9 Sep. 2002, *Favre A. 095* (FR). Nantou: between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 8 Sep. 2002, *Favre A. 096* (LZ). Nantou: between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 9 Sep. 2002, *Favre A. 097* (TNM). Nantou: between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 9 Sep. 2002, *Favre A. 098* (FR). Nantou: between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 7 Sep. 2002, *Favre A. 099* (KUN). Nantou: between Tsuifeng and Meifeng (2250m), roadside, N 24° 6'11.52", E 121°11'37.69", 9 Sep. 2002, *Favre A. 100* (LZ). Chiayi: Alishan (2200m), roadside, N 23° 29'940, E 120° 47'676, 15 Sep. 2002, *Favre A. 101* (FR). ***Tripterospermum maculatum*** Favre, Matuszak and Muellner-Riehl. CHINA. Sichuan: Luojishan south of Xichang (3179 m), 27°35'18.00'' N, 102°23'22.22'' E, 28 Sep. 2011, (fl., fr.), *Favre 321 A* (KUN!). Sichuan: Luojishan south of Xichang (3179 m), 27°35'18.00'' N, 102°23'22.22'' E, 28 Sep. 2011, (fl., fr.), *Favre 321 B* (KUN!). Sichuan: Luojishan south of Xichang (3179 m), 27°35'18.00'' N, 102°23'22.22'' E, 28 Sep. 2011, (fl., fr.), *Favre 321 C* (FR!). Sichuan: Luojishan south of Xichang (3179 m), 27°35'18.00'' N, 102°23'22.22'' E, 28 Sep. 2011, (fl., fr.), *Favre 321 D* (FR!). Sichuan: Luojishan south of Xichang (3179 m), 27°35'18.00'' N, 102°23'22.22'' E, 28 Sep. 2011, (fl., fr.), *Favre 321 E* (LZ!). Sichuan: Luojishan south of Xichang (3179 m), 27°35'18.00'' N, 102°23'22.22'' E, 28 Sep. 2011, (fl., fr.), *Favre 321 F* (LZ!). ***Tripterospermum pingbianense*** C. Y. Wu and C. J. Wu. CHINA. Yunnan: HongHe prefecture, Pingbian, Daweishan (2054m), N 22°54'45.89'', E 103°41'52.41'', 6 Sep. 2002, *Favre A. 108* (FR). Yunnan: HongHe prefecture, Pingbian, Daweishan (2054m), N 22°54'45.89'', E 103°41'52.41'', 6 Sep. 2002, *Favre A. 109* (FR). Yunnan: HongHe prefecture, Pingbian, Daweishan (2054m), N 22°54'45.89'', E 103°41'52.41'', 6 Sep. 2002, *Favre A. 110* (KUN). Yunnan:

HongHe prefecture, Pingbian, Daweishan (2054m), N 22°54'45.89'', E 103°41'52.41'', 6 Sep. 2002, *Favre A. 111* (FR). HongHe prefecture, Pingbian, Daweishan (2054m), N 22°54'45.89'', E 103°41'52.41'', 6 Sep. 2002, *Favre A. 112* (LZ). HongHe prefecture, Pingbian, Daweishan (2054m), N 22°54'45.89'', E 103°41'52.41'', 6 Sep. 2002, *Favre A. 113* (LZ). ***Tripterospermum tanatorajanense*** Favre, Matuszak and Muellner-Riehl. INDONESIA. Sulawesi: pass between Pulu-Pulu and Baruppu, on the flanks of Gunung Mandoangin and Gunung Neosing (2407m), 2°47'06.35'' S, 119°46'03.30'' E, 15 Feb. 2008, (fl., fr.), *Favre 400* (LZ!).

Appendix 2. Favre, A., Matuszak, S. and A.N. Muellner-Riehl. 2014. Two new genera of Gentianinae (Gentianaceae): *Sinogentiana* and *Kuepferia* supported by molecular phylogenetic evidence. *Taxon* 63: 342-354.

Abstract

Subtribe Gentianinae, encompassing *Gentiana*, *Tripterospermum*, *Metagentiana* and *Crawfurdia*, represents one of the most species-rich clades of Gentianaceae. Only a few taxonomic uncertainties have remained at generic level in the subtribe Gentianinae: the inclusion of *Gentiana* section *Otophora* in *Gentiana*, and the polyphyletic nature of *Metagentiana*. In both cases, data were lacking in earlier studies to resolve ambiguities and provide the baseline for a solid taxonomic treatment of these lineages. For the present study, we increased the number of species sequenced for *Gentiana* section *Otophora* and *Metagentiana*, using a combination of nuclear (ITS) and plastid (*trnL-F* and *atpB-rbcL*) markers. We reconstructed phylogenetic relationships in Gentianinae conducting Maximum Likelihood and Bayesian analyses. Our results show that *Gentiana* section *Otophora* is monophyletic and more closely related to *Metagentiana* than to *Gentiana*. We suggest excluding *Gentiana* section *Otophora* from *Gentiana* and elevating this group to the rank of genus described here under the name *Kuepferia* Favre. *Metagentiana* is monophyletic when excluding two species, *M. striata* and *M. souliei*, here described as the new genus *Sinogentiana* Favre & Y.-M. Yuan.

INTRODUCTION

Tribe Gentianeae, with its two subtribes Gentianinae and Swertiinae, exhibits the highest species diversity of the Gentianaceae family (Struwe et al., 2002). Gentianinae and Swertiinae are easily distinguishable from each other by well-defined synapomorphies: all species of Gentianinae (*Gentiana* L., *Tripterospermum* Blume, *Metagentiana* T.N. Ho & S.W. Liu, and *Crawfurdia* Wallich) display glands at the base of the ovary, and plicae or folds between the corolla lobes (with the exception of *Gentiana sceptrum* Griseb. and *G. lutea* L.), whereas species of Swertiinae (e.g., *Swertia* L., *Gentianella* Moench, *Comastoma* (Wettstein) Toyokuni, *Lomatogonium* Braun, *Halenia* Borkh.) have epipetalous glands and no plicae. Several phylogenetic studies have confirmed the monophyly of both subtribes (Yuan & Kuepfer, 1995; Struwe et al., 2002; Favre et al., 2010). Whereas the generic delineation within Swertiinae is rather complicated, with *Swertia* being paraphyletic (Chassot et al., 2001), the generic composition of Gentianinae is relatively simple. The subtribe includes four genera: temperate sub-cosmopolitan *Gentiana* and subtropical *Tripterospermum*, *Crawfurdia*, and *Metagentiana*. *Gentiana*, equivalent to subgenus *Eugentiana* of Kusnezow, encompasses approximately 360 to 400 species classified into 15 sections (Ho & Liu, 1990; Ho & Pringle, 1995d; Ho & Liu, 2001; Struwe et al., 2002) and is present in all temperate regions of the world as well as in tropical mountain systems. Since at least 2000 years, some species of *Gentiana* have been used in traditional medicine in Asia (Zhao et al., 2010; Zhou et al., 2010) and in Europe. Contrastingly, *Tripterospermum* (31 species), *Crawfurdia* (16 species) and *Metagentiana* (14 species) are restricted to Asia and are less often used in traditional medicine (but see Li et al., 2006). *Crawfurdia* and *Tripterospermum*, two lianescent genera of which taxonomic treatment was repeatedly discussed and modified in the past (Clarke, 1885; Ridley, 1923; Marquand, 1931, 1937; Ubolcholaket, 1987), formed strongly supported clades in a recent phylogenetic study (Favre et al., 2010). The fourth and most recently described genus of Gentianinae, *Metagentiana* (Ho et al., 2002), includes all the species of the former *Gentiana* section *Stenogyne*, which was excluded from *Gentiana* in an earlier study (Yuan et al., 1996).

Despite several phylogenetic studies in the past 20 years, two uncertainties have remained concerning generic delimitation within subtribe Gentianinae. First, species of *Gentiana* section *Otophora* Kusn. appear to be more closely related to *Metagentiana*, *Crawfurdia* and *Tripterospermum*, than to the remaining sections of *Gentiana* (Favre et al., 2010). Only two species of *Gentiana* section *Otophora* and only one DNA marker per species had been included in the latter study. Based on this limited dataset, the phylogenetic analyses by Favre et al. (2010) were unable to confirm the monophyly of this section or provide strongly supported evidence for its phylogenetic position within Gentianinae. Nevertheless, species of *Gentiana* section *Otophora* exhibit floral features that differ from *Gentiana*, for example plicae reduced to a small tooth or auricle on side of the corolla lobe (other Gentianinae species display well-developed plicae, see Fig. 23). Second, *Metagentiana* was found to be polyphyletic, with species forming three well supported clades (Chen et al., 2005b; Favre et al., 2010), without obvious morphological evidence to support them. This pattern might either result from the repeated divergence of *Metagentiana* lineages that have retained ancestral morphological characters or might be due to convergent evolution. Alternatively, specimens might have been misidentified (determination admittedly difficult) in these studies.

In the present study, we aim at resolving phylogenetic relationships among genera of Gentianinae by using an extended sampling of the taxa for which phylogenetic uncertainties remain, and by using an additional plastid marker, the *trnL-trnF* intergenic spacer. Specifically, we will 1) test the monophyly of *Gentiana* section *Otophora* and elucidate its phylogenetic relationships with other taxa in the Gentianinae, and 2) test the level of polyphyly of *Metagentiana* by using new sequences based on specimens that have been carefully determined. This will enable us to arrive at an improved taxonomic treatment for Gentianinae.

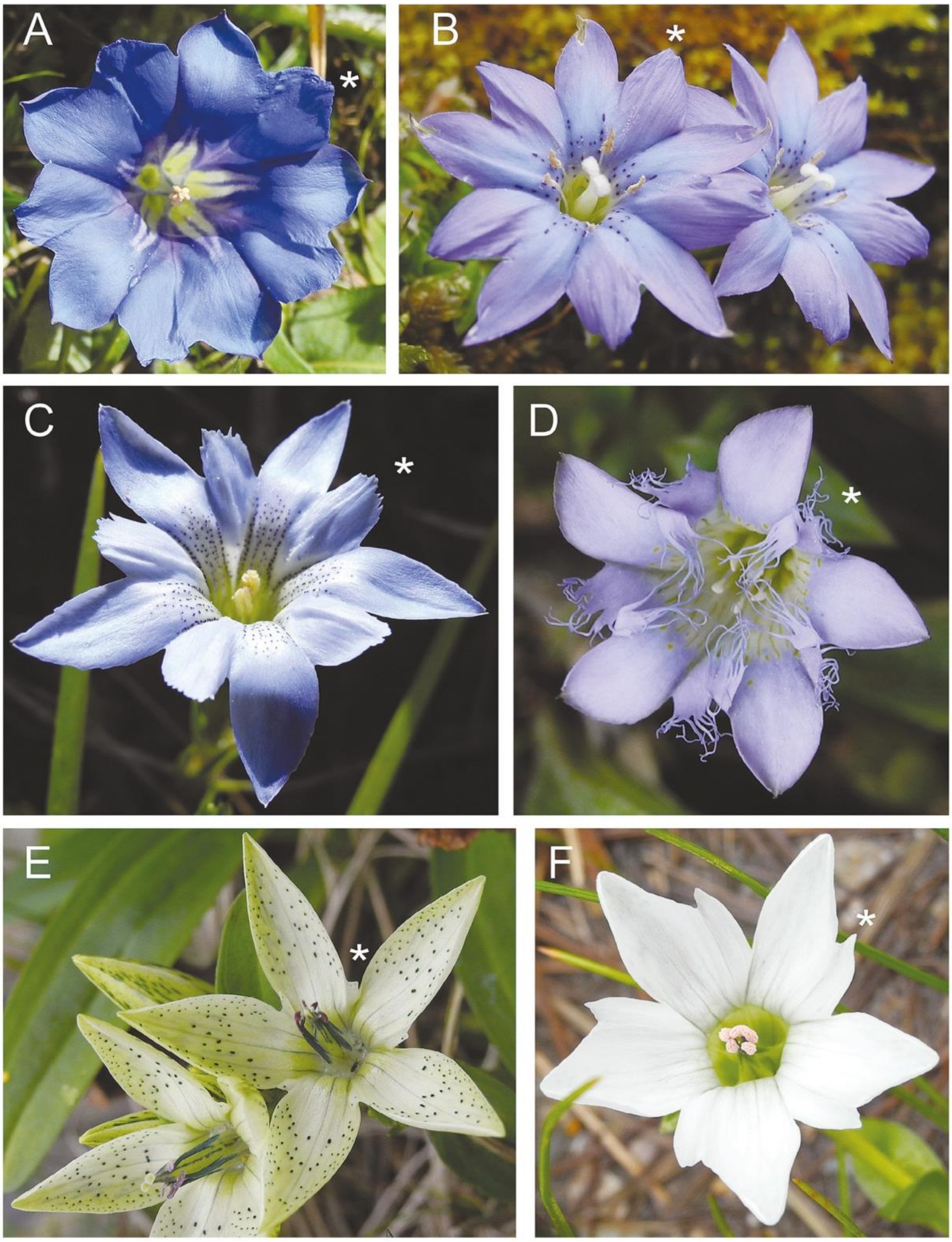


Figure 23. Morphology of plicae (indicated by an *) in *Gentiana* **(A–D),** and reduced plicae in *Kuepferia* **(E–F). A,** *Gentiana sino-ornata* Balf.f., petal-like plicae with entire margin; **B,** *Gentiana piasezkii* Maxim., petal-like plicae with slightly two-cleft margin; **C,** *Gentiana haynaldii* Kanitz, erose or denticulate plicae margin; **D,** *Gentiana panthaica* Burkill, fringed plicae margin; **E,** *Kuepferia otophora* (Franch.) Adr.Favre and **F,** *Kuepferia otophoroides* (Harry Sm.) Adr.Favre, plicae auricle-like or reduced to a tooth. — Photographs by A. Favre.

MATERIALS AND METHODS

Sampling design and material collection

This study includes a total of 62 species of Gentianinae (ingroup) as well as nine species of Swertiinae (outgroup). To improve our understanding of phylogenetic relationships between *Metagentiana*, *Gentiana* section *Otophora* and the remaining clades of Gentianinae, our sampling was designed to cover each genus of Gentianinae. Particular care was taken to increase the sampling for *Metagentiana* and *Gentiana* section *Otophora*. For these two genera, herbarium vouchers were carefully re-determined using the *Flora of China* (Ho & Pringle, 1995d), and leaf material was retrieved from unambiguously identified specimens. Furthermore, at least one species of each of the remaining 13 sections of *Gentiana* (Ho & Liu, 1990) was included. Plant specimens were collected during several field campaigns in China, Nepal, Austria and Switzerland between 2002 and 2012 by Adrien Favre, Sabine Matuszak and other collectors (see Supporting Information 2). Leaf material was dried in silicagel, and specimens resulting from our field collections were deposited at the Herbarium of Kunming (KUN) and/or Leipzig (LZ). To complete our dataset, we additionally retrieved material from herbarium vouchers deposited at Kunming (KUN), Leiden (L), Vienna (WU) and Leipzig (LZ) and sequences from Genbank: vouchers information and accession numbers are given in Supporting Information 2.

DNA extraction, amplification and sequencing

Extraction of total DNA was conducted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol with a minor modification: the samples were incubated in the lysis buffer and RNase for two hours instead of one.

In addition to *atpB-rbcL* spacer sequences that were available from GenBank (*http://www.ncbi.nlm.nih.gov*), some sequences of nuclear ITS region and the plastid *trnL-F* spacer were obtained. These markers have been successfully used in the past to reconstruct phylogenetic relationships within Gentianaceae (Chassot et al., 2001; Mansion and Struwe, 2004; Mansion & Zeltner, 2004;

Prieto et al., 2012). The ITS region was amplified using the newly created primer pair 17SE_m, 5’-CGGTGAAGTGTTCGGATCG, and 26SE_m, 5’-CGCTCGCCGTTACTAGGG (Grudinski et al., 2014a; modified after Sun et al., 1994). The reaction mix of 25 µL contained 21.9 µL Thermo Scientific 1.1X ReddyMix^{TM} PCR Master Mix (1.5 mM $MgCl_2$; ABgeneHouse, Epsom, Surrey, UK), 0.5 µL bovine serum albumin (BSA; 10 mg/mL; New England BioLabs GmbH, Frankfurt/Main, Germany), 1 µL dimethyl sulfoxide (DMSO; Carl ROTH GmbH, Essen, Germany), 1 µL of genomic DNA and 0.3 µL of each primer (10 µM). Polymerase chain reaction (PCR) was performed using a Thermo Scientific Arktik Thermal Cycler (Thermo Fisher Scientific Oy, Vantaa, Finland) with initial denaturation of 2 min at 95°C, followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 53°C and extension for 1 min at 72°C, followed by a final extension step at 72°C for 10 min. For material that did not yield amplification product of sufficient quality, ITS1 and ITS 2 regions were amplified separately. The ITS1 region was amplified using the primer pair 17SE_m, 5’-CGGTGAAGTGTTCGGATCG (Grudinski et al., 2014a; modified after Sun et al., 1994), and ITS_R1, 5'-CAACTTGCGTTCAAAGACTCG (Grudinski, unpublished), and the ITS2 region using the primer pair ITS_F1, 5'-GATACTTGGTGTGAATTGCAGAATC (Grudinski, unpublished), and 26SE_m, 5’-CGCTCGCCGTTACTAGGG (Grudinski et al., 2014a; modified after Sun et al., 1994). For ITS1 and ITS2 PCR reactions, the same set of chemicals and the same programme were used as described above.

For *trnL-F*, the primer pairs trnLF_c, 5'-CGAAATCGGTAGACGCTACG, and trnLF_d, 5'-GGGGATAGAGGGACTTGAAC as well as trnLF_e, 5'-GGTTCAAGTCCCTCTATCCC, and trnLF_f, 5'-ATTTGAACTGGTGACACGAG (Taberlet et al., 1991) were used. The reaction mix of 25 µL volume contained 21.9 µL of Thermo Scientific 1.1x ReddyMix PCR Master Mix (2.5 mM $MgCl_2$, ABgeneHouse, Epsom, Surrey, UK), 1 µL bovine serum albumin (BSA; 10 mg/mL; New England BioLabs GmbH, Frankfurt/Main, Germany), 0.5 µL dimethyl sulfoxide (DMSO; Carl ROTH GmbH, Essen, Germany), 1 µL of genomic DNA and 0.3 µL of each primer (10 µM). PCR was performed using a Thermo Scientific Arktik Thermal Cycler (Thermo Fisher Scientific Oy, Vantaa, Finland), with template denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 1 min, primer annealing at 56°C for 1 min, primer extension at 72°C for 1 min and a final extension step at 72°C for 5 min. PCR products were cleaned using a NukleoSpin® Gel and PCR clean up kit (Macherey-Nagel GmbH & Co. KG, Dueren, Germany) according to the manufacturer's protocol. Sequencing reactions for ITS and *trnL-F* were run on an ABI 3130xl Genetic Analyzer using Big Dye terminator v3.1 chemistry (Applied Biosystems, Inc., Warrington, Cheshire, UK).

Data handling and phylogenetic analyses

Complementary sequences were assembled and aligned in Geneious v.5.6.3. (Biomatter Ltd., *http://www.geneious.com*) using the default settings. For *trnL-F*, five regions in the aligned matrix, accounting for a total of 111 basepairs (bp) and characterised by mono- to polynucleotide repeats, could not be aligned unambiguously. To test the effect of these alignment positions on phylogenetic reconstruction, the following analyses were performed twice, either including or excluding these five regions.

Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were performed on both the individual and combined datasets (ITS, *atpB-rbcL* and *trnL-F*). For the analysis of the combined dataset, two partitions were used with ITS on the one hand, and *atpB-rbcL* and *trnL-F* combined on the other hand. It was recently shown that partitioning by genome source (nuclear vs. plastid) might be more advantageous than partitioning by gene because it decreases the risk of overparametrization (Miller et al., 2009). Because a complete set of target markers is scarce in Genbank for Swertiinae, and because multiple sources have shown that Swertiinae and Gentianinae are monophyletic (Yuan & Kuepfer, 1995; Struwe et al., 2002; Favre et al., 2010), topological constraint was applied to the outgroup (species of the subtribe Swertiinae) in both ML and BI analyses. This artefact did not change the topology of Gentianinae.

ML analyses were run with RAxML v.7.4.2 (Stamatakis, 2006), using the graphical interface raxmlGUI v.1.3 (Silvestro & Michalak, 2012). Model of sequence evolution was set to GTR + I + G as suggested by jModeltest 2.1.4 (Posada, 2008). The number of bootstraps was determined during the analysis with the autoMRE "bootstopping" criterion (Pattengale et al., 2010), and a rapid hill climbing ML search was conducted (Stamatakis et al., 2007). BI analyses were conducted using MrBayes v.3.2.1 (Ronquist et al., 2012). Three parallel Metropolis Coupling Markov Chain Monte Carlo ($(MC)^3$) runs with three chains each (one cold, two hot chains with default temperature t= 0.2) were started from independent random trees (Geyer, 1991; Altekar et al., 2004). A total of ten million generations were computed, with trees sampled every 1000th generation. We used Tracer v.1.5 (Rambaut & Drummond, 2007) to confirm the convergence of the nine independent analyses. Trees that preceded stabilization of the likelihood value were excluded as burn-in (25%).

RESULTS

Sequences and alignment

A total of 84 new sequences were generated (31 for ITS and 53 for *trnL-F*, see list in Supporting Information 2). Additional sequences were retrieved from Genbank (*http://www.ncbi.nlm.nih.gov*). The combined DNA data matrix contained 2845 characters. The ITS sequences ranged from 606 to 638 bp, the *trnL-F* sequences from 755 to 1095, and *atpB-rbcL* sequences from 650 to 816 bp in length.

Phylogenetic analyses

We relied on visual inspection of the individual ML bootstrap consensus trees as well as the individual Bayesian majority rule consensus trees to determine combinability of the nuclear and plastid data sets. Given the absence of topological conflict (defined as nodes with < 85% bootstrap support, BS, or < 90 posterior probabilities, PP), we combined the chloroplast and nuclear data partitions. Throughout this paper, 75 – 84% BS and 90-94 PP is considered moderate, and 85 – 100% BS and 95-100 PP strong support. BI and ML analyses resulted in largely similar topologies and node support, no matter whether the five ambiguous regions (mono- to polynucleotide repeats) were included or not. Due to the absence of strongly supported topological conflict, we only present the tree obtained from the BI analysis of the combined dataset, additionally plotting the corresponding BS values obtained from the ML analysis (Fig. 24). During the Bayesian analysis run of the combined data set, Effective Sample Size (ESS) and Potential Scale Reduction Factor (PSRF) values were well within acceptable values showing the robustness of the analyses: ESS were well above 200 and PSRF varied between 0.999 and 1.001. After exclusion of the burn-in, the remaining trees were used to calculate posterior probabilities via the construction of a majority rule consensus tree. The latter was plotted using FigTree v.1.3.1 (Rambaut & Drummond, 2010).

Phylogenetic relationships

Phylogenetic relationships at the generic level within this subtribe Gentianinae are well resolved (Fig. 24). *Tripterospermum* and *Crawfurdia* form two strongly supported clades (PP= 1.0, BS= 99, for both genera). *Gentiana* forms a strongly supported clade (PP= 1.0, BS= 99), except for the five species of *Gentiana* section *Otophora* which are found in a separate clade (PP= 1.0, BS= 99, labelled *Kuepferia* in Fig. 24). The clade containing these species is more closely related to *Metagentiana*, *Tripterospermum* and *Crawfurdia* than to *Gentiana*. *Metagentiana* is polyphyletic. Most species of *Metagentiana* form a strongly supported cluster (PP= 1.0, BS= 100), except for *M. souliei* and *M. striata*. The latter two species form a clade which is sister to *Tripterospermum* (PP= 1.0, BS= 100, labelled *Sinogentiana* in Fig. 24).

Within each of the above mentioned six clades, phylogenetic relationships are only partially resolved. The polytomy in *Gentiana* and several very poorly supported nodes at the backbone of the genus render it difficult to assess the phylogenetic relationships between the different sections. Nevertheless, the European species of *Gentiana* cluster together (PP= 1.0, BS= 93), with a clear delimitation of three sections, including *G.* sect. *Ciminalis* (*G. clusii* and *G. acaulis*; PP= 1.0, BS= 97), *G.* sect. *Calathianae* (*G. brachyphylla*, *G. bavarica*, *G. nivalis*; PP= 1.0, BS= 99), and *G.* sect. *Gentiana* (*G. lutea*, *G. asclepiadea*, *G. purpurea*; PP= 1.0, BS= 93). A predominantly Asian section also appears to be monophyletic: *G.* sect. *Chondrophyllae* (*G. epichysantha*, *G. panthaica*; PP= 0.96, BS= 95).

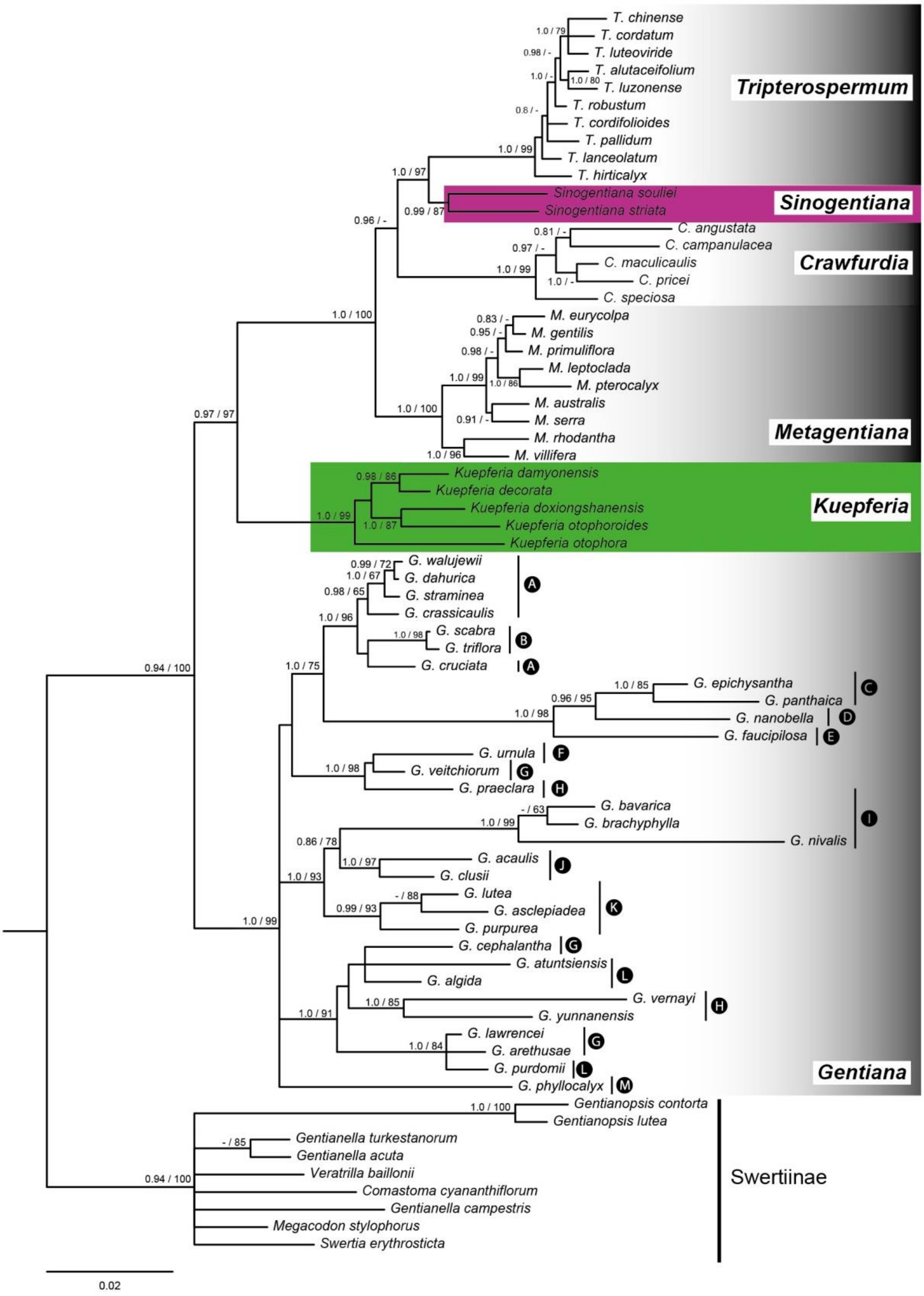


Figure 24. Bayesian majority-rule consensus tree based on the combined dataset of ITS, *atpBrbcL* and *trnLF* sequences with average branch length. Bayesian posterior probabilities (PP) and ML bootstrap support values (BS) are shown on branches, separated by a slash. Values for nodes with less than 80 PP or 60 BS are not shown. The sectional placement of *Gentiana* species is indicated by letters in black circles as follows: A, *Gentiana* sect. *Cruciata* Gaudin; B, *Gentiana* sect. *Pneumonanthe* (Gled.) Gaudin; C, *Gentiana* sect. *Chondrophyllae*; D, *Gentiana* sect. *Dolichocarpa* T.N.Ho; E, *Gentiana* sect. *Fimbricornata* T.N.Ho; F, *Gentiana* sect. *Isomeria* Kusn.; G, *Gentiana* sect. *Kudoa* (Masam.) Satake & Toyok. ex Toyok.; H, *Gentiana* sect. *Microsperma* T.N.Ho; I, *Gentiana* sect. *Calathianae*; J, *Gentiana* sect. *Ciminalis*; K, *Gentiana* sect. *Gentiana*; L, *Gentiana* sect. *Frigida* Kusn.; M, *Gentiana* sect. *Phyllocalyx* T.N.Ho.

DISCUSSION

Generic delimitation and phylogenetic relationships

The generic composition of Gentianinae has experienced several re-arrangements during the past two decades. Using three gene regions, our study presents substantial improvements compared to previous phylogenetic reconstructions of Gentianinae. Based on our results, we recognise six different genera within this subtribe. The monophyly of *Crawfurdia* and *Tripterospermum* was shown recently, but *Gentiana* and *Metagentiana* appeared to be unnatural entities (Favre et al., 2010). We here suggest *Metagentiana* to be maintained, but to exclude two species now referred to the new genus *Sinogentiana*. Additionally, we show that *Gentiana* section *Otophora* should be excluded from *Gentiana* and placed in a new genus, *Kuepferia*. The phylogenetic reconstructions show strong support for the monophyly of each of the six genera and resolve the phylogenetic relationships among them. *Gentiana* is sister to the clade formed by all other genera. In the latter clade, *Kuepferia* appears to have diverged first. *Sinogentiana* is sister to *Tripterospermum*. In the ML analysis, the relative position of *Crawfurdia* to *Metagentiana* is unresolved, with either one of these genera being more closely related to *Sinogentiana* and *Tripterospermum*. In the BI analysis, the relationship is resolved, with *Crawfurdia* being more closely related to *Sinogentiana* and *Tripterospermum* than *Metagentiana* is. By including all main lineages of Gentianinae and all sections of *Gentiana*, our study provides the base to arrive at a valid taxonomic delimitation of the genera of Gentianinae which is unlikely to change with increased species sampling.

The new genus *Kuepferia*

Our results support the exclusion of *Gentiana* section *Otophora* from the genus *Gentiana* and the description of the new genus *Kuepferia*. The sectional epithet “*Otophora*” could not be established as generic name because it already exists for a genus of Sapindaceae, *Otophora* Blume. Our analyses include five out of described 12 species of *Gentiana* section *Otophora*, and clearly show the monophyly of this taxon. *Gentiana* section *Otophora* has more phylogenetic affinity to the clade of *Metagentiana*, *Crawfurdia* and *Tripterospermum* than to other sections of *Gentiana*. Based on morphological evidence, *G.* section *Otophora* had previously been divided into two distinct series, Ser. *Otophorae* C. Marquand and Ser. *Decoratae* C. Marquand. We included species of both series in our dataset. It is therefore very unlikely that the addition of further species would result in a different topology. Moreover, synapomorphies support *Kuepferia* as a distinct genus in Gentianinae (summarised in Table 10): the corolla of all *Kuepferia* species has plicae reduced to a small tooth or auricle, a unique trait in Gentianinae of which other genera consistently display large petal-like plicae. In addition, the corolla of *Kuepferia* species is usually lobed to the middle or below (with the corolla lobes being about as long as the tube), another diagnostic trait for *Kuepferia*, possibly found in only one other species, *G. prainii*. These floral traits were considered as highly derived in an earlier morphological study (Ho & Liu, 1990). We here argue that the shape of the plicae and the lobation of the corolla in *Kuepferia* represent an intermediate stage in comparison to other Gentianaceae (plicae absent and corolla usually deeply lobed), and other genera of Gentianinae (plicae well developed and corolla lobed to less than half of its length).

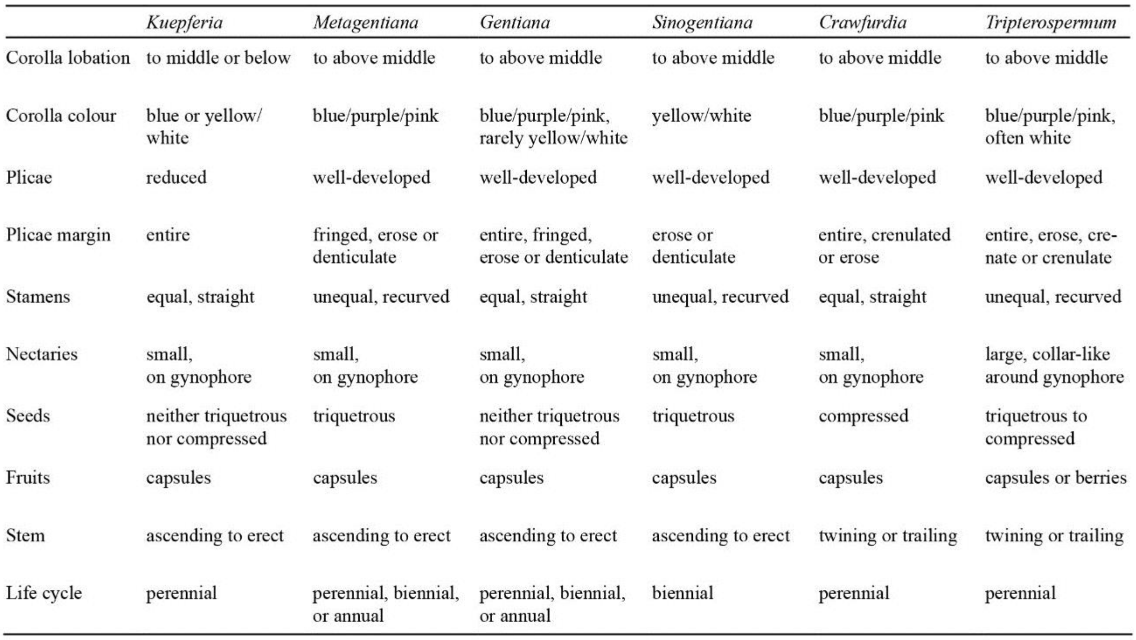
The new genus *Sinogentiana*

The description of the genus *Metagentiana* was based upon the exclusion of *Gentiana* section *Stenogyne* from the genus *Gentiana* (Ho et al., 2002). Because *Metagentiana* displays a combination of traits either resembling to *Gentiana* or *Tripterospermum*, it has previously been suggested that this genus could have resulted from hybridisation and reticulate evolution between *Gentiana* and the *Tripterospermum*/*Crawfurdia* lineage (Ho et al., 2002; Chen et al., 2005b). Past hybridisation events sometimes result in conflicts between the topologies reconstructed with nuclear and plastid markers. In the absence of such conflicts in our study, the hypothesis of a hibrid origin of *Metagentiana* could neither be confirmed nor refuted. *Metagentiana* more recently has been revealed to be polyphyletic (Chen et al., 2005b; Favre et al., 2010). We here show that species of *Metagentiana* cluster in two clades. The first clade encompasses most of the species originally attributed to *Metagentiana*, whereas the second clade includes two species, *M. striata* and *M. souliei*, described here as the new genus *Sinogentiana*. Our findings contrast with an earlier study showing that *M. eurycolpa* and *M. leptoclada* formed together a third well-supported clade of *Metagentiana* (Favre et al., 2010). There is however no obvious morphological evidence that would support these two species as distinct taxonomic entity. For the present study, we took a particular care to determine all *Metagentiana* vouchers we extracted DNA from. We did not use previously available sequences of *M. eurycolpa* and *M. leptoclada* derived from vouchers we could not check. We therefore confirm that *M. eurycolpa* and *M. leptoclada* are part of *Metagentiana*.

The phylogenetic support for *Sinogentiana* is slightly lower than that of other genera of Gentianinae, particularly in the ML analysis. In comparison to species of *Tripterospermum* (their closest relative), *S. striata* and *S. souliei* display a higher number of autapomorphies (Fig.24). This is in congruence with findings on the phylogeography of *S. striata* (under the name *M. striata*) where the level of differentiation among populations was found to be very high, particularly in their putative glacial refugia (Chen et al., 2008b). This might be due to either ancient origin of *S. striata* and *S. souliei*, or a rapid genetic differentiation potentially driven by repeated cycles of range expansion and fragmentation during the climate fluctuations of the Quaternary, as suggested by Chen et al. (2008). Only a few morphological synapomorphies support the description of *Sinogentiana* (summarised in Table 10). The main differences to *Metagentiana* lie in the colour of the corolla, the relatively long corolla tube in comparison to the corolla lobes (the tube is at least 4 times longer than the lobes, a trait shared only with *M. villifera*), and the average length of the internodes. Flowers of *Metagentiana* are always pink, purple or blue, whereas the corolla of *Sinogentiana* is consistently whitish or yellow (Fig. 25). The pigmentation of the corolla is a rather consistent trait in Gentianinae: anthocyanin pigmentation largely dominates the entire subtribe, with only few exceptions of whitish or yellow flowers, predominantly found in *Tripterospermum* (the closest relative to *Sinogentiana*), a few species of *Kuepferia*, and a few isolated cases in *Gentiana* (for example *G. lutea*, *G. mirandae* Paray and *G salpinx* Griseb.). Moreover, internodes of *Sinogentiana* species are generally longer than those of other

Metagentiana species, with the exception of *M. pterocalyx* (Franch.) T.N. Ho & S.W. Liu. In the Bayesian tree topology of Favre and colleagues (2010), the latter species appeared closely related to *Sinogentiana*, whereas it is clearly clustering with core *Metagentiana* in the present study. It is possible that older sequences of *Metagentiana pterocalyx* were obtained from a misidentified voucher of either species of *Sinogentiana. Sinogentiana* and *M. pterocalyx* share a similar habit (elongated internodes) and sometimes their unusual corolla colour. In fact, Marquand identified a yellow-flowered *Gentiana pterocalyx* (var. *flavo-viridis*). Whether or not yellow-flowered *M. pterocalyx* exists or if this variety is a synonym for either *Sinogentiana* species remains unclear. In this study, we took particular care to generate new sequences for *M. pterocalyx* based on an unambiguously re-determined voucher deposited in KUN. We are therefore confident that *M. pterocalyx* belongs to the core *Metagentiana*.

Table 10. List for diagnostic traits for all six genera of subtribe Gentianinae (Gentianaceae).



	Kuepferia	*Metagentiana*	*Gentiana*	*Sinogentiana*	*Crawfurdia*	*Tripterospermum*
Corolla lobation	to middle or below	to above middle	to above middle	to above middle	to above middle	to above middle
Corolla colour	blue or yellow/ white	blue/purple/pink	blue/purple/pink, rarely yellow/white	yellow/white	blue/purple/pink	blue/purple/pink, often white
Plicae	reduced	well-developed	well-developed	well-developed	well-developed	well-developed
Plicae margin	entire	fringed, erose or denticulate	entire, fringed, erose or denticulate	erose or denticulate	entire, crenulated or erose	entire, erose, crenate or crenulate
Stamens	equal, straight	unequal, recurved	equal, straight	unequal, recurved	equal, straight	unequal, recurved
Nectaries	small, on gynophore	small, on gynophore	small, on gynophore	small, on gynophore	small, on gynophore	large, collar-like around gynophore
Seeds	neither triquetrous nor compressed	triquetrous	neither triquetrous nor compressed	triquetrous	compressed	triquetrous to compressed
Fruits	capsules	capsules	capsules	capsules	capsules	capsules or berries
Stem	ascending to erect	ascending to erect	ascending to erect	ascending to erect	twining or trailing	twining or trailing
Life cycle	perennial	perennial, biennial, or annual	perennial, biennial, or annual	biennial	perennial	perennial

TAXONOMIC TREATMENT

As clearly shown by our molecular phylogenetic reconstruction, the description of two new genera of Gentianinae is necessary to maintain the monophyly of *Gentiana* and *Metagentiana*. First, *Gentiana* section *Otophora* is excluded from *Gentiana* and described as the new genus *Kuepferia* Favre. Second, two species of *Metagentiana* are excluded from the latter to form the new taxon *Sinogentiana* Favre and Y.-M. Yuan. The determination key to the species of *Kuepferia* corresponds to the key for *Gentiana* section *Otophora,* and the key to the species of *Metagentiana* and *Sinogentiana* corresponds to the key of *Gentiana* section *Stenogyne* in the *Flora of China* (Ho & Pringle, 1995d). The following determination key to the genera of subtribe Gentianinae is based upon information from the *Flora of China* (Ho & Pringle, 1995d), the description of *Metagentiana* by Ho et al. (2002) and the *Synopsis of Tripterospermum* (Murata, 1989).

Key to the genera of subtribe Gentianinae

1. Stems terete, twining or trailing...**2**

1. Stems ascending to erect, neither twining nor trailing...............................**3**

2. Nectaries conspicuously developed, forming a collar-like disc around gynophore; stamens asymmetrical, unequal in length, apically recurved; fruit a capsule or berry ....***Tripterospermum***

2. Glands small, on gynophore; stamens equal in length, straight; fruits a capsule ....***Crawfurdia***

3. Plicae reduced to a small tooth or auricle (usually less than 1 mm); corolla usually lobed to middle or below (corolla lobes about as long as tube)..***Kuepferia***

3. Plicae usually wider and/or longer than 1 mm; corolla usually lobed to above middle (corolla lobes shorter than tube)..**4**

4. Stamens equal or unequal in length, usually straight; flowers ebracteate (except for species of sect. *Pneumonanthe* (Gled.) Gaudin); style linear to cylindrical, shorter than ovary; seeds ellipsoid, ovoid to globose, not triquetrous..***Gentiana***

4. Stamens unequal in length and apically recurved; flowers bracteates; style filiform, as long as to longer than ovary; seeds triquetrous with three winged edges (rarely wingless)..**5**

5. Flowers yellow to pale-yellow; stem leaves 1-3 cm; plicae never fringed ....***Sinogentiana***

5. Flowers blue, purple or pink; stem leaves 0.3-1.5 cm, up to 3 cm in *M. rhodantha* but then plicae fringed...***Metagentiana***

The new genus *Kuepferia* Favre

Kuepferia A. Favre, **gen. nov.** ≡ *Gentiana* sect. *Otophora* Kusnezow, Trudy S.-Peterburgsk. Obshch. Estestvoisp., Otd. Bot. 24(2): 102. 1894. ≡ *Gentiana* subg. *Otophora* (Kusnezow) Halda, Acta Mus. Richnov., Sect. Nat. 3(1): 15. 1995. —Type: *Gentiana otophora* Franchet ex F. B. Forbes & Hemsley.

Diagnosis. – This new genus differs from all other genera of Gentianinae by its very small or auriculate plicae and by its corolla tube which is shorter than the lobes. It differs from *Tripterospermum*, *Sinogentiana* and *Metagentiana* by having straight stamens (recurved in these other three genera). It differs from other sections of *Gentiana* by its stout cylindrical or fusiform, persistent taproot.

Description. – Perennials. Taproot slightly fleshy, stout, cylindrical or fusiform. Stem branching monopodially, prostrate to ascending, simple, in some species with dense brown membranous remnants of old petioles. Basal leaves either very small to absent, sometimes in few rosettes occasionally crowded in a cushion. Stem leaves opposite, with 2 to many pairs usually widely spaced, sometimes more crowded; veins 1 to 3. Flowers terminal and solitary, rarely in cymes. Pedicel absent or up to 3 cm long. Calyx cupular; tube up to to 6 mm long; lobes usually unequal. Corolla lobed to the middle or below, the tube shorter than, or equalling, the lobes (except in *G. doxiongshanensis* of which corolla is lobed to above middle); plicae very small, auriculate, attached on the right side of each lobe. Stamens straight, inserted at basal part or middle of the corolla tube. Capsule ellipsoid, 0.8-1.5 cm long, sessile or subsessile (gynophore rarely exceeding 4 mm). Seeds usually minutely reticulate, rarely covered with honeycomb-like, hexagonal, simple, shallow pits.

Etymology. ─ The new genus *Kuepferia* is named in honour of Prof. Philippe Kuepfer of the University of Neuchatel (Switzerland), for his significant contribution to our knowledge on the taxonomy and evolution of the family Gentianaceae.

Distribution. ─ Species of this genus are distributed in the south-eastern part of the Tibetan Plateau and in the Himalayas, including China (S and E Tibet, NW Yunnan, SW Sichuan), India (Arunachal Pradesh, Sikkim), N Myanmar, Bhutan and Nepal (Fig. 26).

Habitat. ─ *Kuepferia* species grow in alpine meadows, grassy and stony slopes of high altitudes usually between (2800) 3200 m and 4600 m, and sometimes along alpine streams (*K. decorata* and *K. otophora*).

IUCN conservation assessment. ─ Because populations of *Kuepferia* species are only rarely reported (and collected), it is difficult to assess the number of existing populations and the number of individuals they contain. Nevertheless, it is possible to estimate roughly their distribution range and the frequency of occurrence of their habitat (provided by sources such as *Flora of China* and herbarium specimens). Five *Kuepferia* species (*K. damyonensis*, *K. infelix*, *K. otophora*, *K. otophoroides* and *K. sichitoensis*) are distributed across large areas over several countries and/or Chinese provinces, occupy fairly common habitats and tolerate a rather wide elevation gradient. These species might therefore be considered as Least Concern (LC) according to the *IUCN Red List Categories and Criteria* (IUCN 2001, 2012). Three other species (*K. decorata*, *K. doxiongshangensis*, *K. leucantha*) occupy similar or smaller distribution ranges but their respective habitats are likely to be localized and fragmented (for example *K. decorata* occurs only along streams). They should be considered as Near Threatened (NT). *Kuepferia caryophyllea*, *K. chateri*, *K. hicksii* and *K. mas*onii are likely to have an area of occupancy smaller than 20´000 km^2 and occur in high altitude habitats (always above 4000 m) that are likely to be highly fragmented and particularly vulnerable to disturbance. These species should be considered as Vulnerable (VU B1ab(iii)). Despite the establishment of several nature reserves particularly in China and Nepal, the respective habitats of all *Kuepferia* species might be compromised by touristic development at higher altitudes, by drainage for water use and potentially by overgrazing of alpine meadows. The status of the different species of *Kuepferia* should therefore be evaluated regularly.

1. ***Kuepferia caryophyllea*** (Harry Smith) Favre, **comb. nov.** ≡ *Gentiana caryophyllea* Harry Smith in Anz. Akad. Wiss. Wien, Math.-Naturwiss. Kl. 63: 101. 1926 — Type: China, Yunnan, Prope fines Tibeto-birmanicas inter fluvios Lu-djiang (Salween) et Djoudiang, 4200m, 15 July 1916, *Handel-Mazzetti 7876* (holotype: NMNH, image!).

2. ***Kuepferia chateri*** (T. N. Ho) Favre, **comb. nov.** ≡ *Gentiana chateri* T. N. Ho in Bull. Nat. Hist. Mus. London, Bot. 23(2): 55–56. 1993 — Type: Nepal, Kasuwa khola, 4000 m, 23 August 1975, *Beer 25363* (holotype: BM, image!).

3. ***Kuepferia damyonensis*** (C. Marquand) Favre, **comb. nov.** ≡ *Gentiana damyonensis* C. Marquand in Bull. Misc. Inform. Kew 1928(2): 51. 1928 — Type: China, Sichuan, alpine pastures at Damyon, 4800-5200 m, 5 September 1922, *Kingdon-Ward 5377* (holotype: E, image!).

4. ***Kuepferia decorata*** (Diels) Favre, **comb. nov.** ≡ *Gentiana decorata* Diels in Notes Roy. Bot. Gard. Edinburgh. 5(25): 220–221. 1912 — Lecotype (T.N. Ho, Worldwide Monogr. *Gentiana* 160. 2001): China, Yunnan, Lijiang Range eastern flank, 13-14,000 ft., September 1906, *Forrest 3021* (E; isolectotype: BM, image!).

= *Gentiana tsarongensis* Balf. f. & G. Forrest in Bull. Misc. Inform. Kew 1928(2): 62. 1928 — Type: China: Xizang: Tsarong, Doker-la, August 1918, *Forrest 16876* (holotype: HT; Syntype: K).

5. ***Kuepferia doxiongshanensis*** (T. N. Ho) Favre, **comb. nov.** ≡ *Gentiana doxiongshangensis* T. N. Ho in Bull. Bot. Res., Harbin. 4(1): 73–75, pl. 1. 1984 — Type: China, Xizang, Medog-Mailing, Doxiongshan, in clivis, 4200 m, 7 August 1973, *NWBI Xizang Exped. 1892* (holotype: HWBI).

6. ***Kuepferia hicksii*** (Harry Smith) Favre, **comb. nov.** ≡ *Gentiana hicksii* Harry Sm. ex T.N. Ho & S.W. Liu in Bull. Nat. Hist. Mus. London, Bot. 23(2): 56. 1993 ≡ *Gentiana damyonensis* subsp. *hicksii* (Harry Sm. ex T.N. Ho & S.W. Liu) Halda in Acta Mus. Richnov. Sect. Nat. 3: 15. 1995 — Type:

Bhutan, Pang la, on open wet slopes, 4000 m, 21 September 1949, *Ludlow, Sherriff & Hicks 21456* (holotype: BM, image!; isotype: UPS).

7. *Kuepferia infelix* (C. B. Clarke) Favre, **comb. nov.** ≡ *Gentiana infelix* C. B. Clarke in Fl. Brit. India 4(10): 111. 1883 ≡ *Varasia infelix* (C.B.Clarke) Soják in Čas. Nár. Muz. Praze, Rada Přír. 148(3-4): 201. 1980 — Lectotype T.N. Ho, Worldwide Monogr. *Gentiana* 163. 2001): India, Sikkim, Yeumtong and Kankola, 14-15,000 ft., *J.D. Hooker s.n.* (K, isolectotypes; GH, P).

= *Gentiana microtophora* C. Marquand in Bull. Misc. Inform. Kew 1937(3): 181–182. 1937 — Type: China, Xizang, frontier with Myanmar, alpine meadows, 4100-4500 m, *Kingdon Ward 9921* (holotype: BM).

= *Gentiana minuta* N. E. Br in Bull. Misc. Inform. Kew 1912(7): 341. 1912 — Type: India: Himalaya, at Tunkra Pass, *J.D. Hooker & T. Thomson s.n.* (holotype: K).

8. *Kuepferia leucantha* (Harry Sm. ex T. N. Ho & S. W. Liu) Favre, **comb. nov.** ≡ *Gentiana leucantha* Harry Sm. ex T. N. Ho & S. W. Liu in Bull. Nat. Hist. Mus. London, Bot. 23(2): 55. 1993 ≡ *Gentiana decorata* subsp. *leucantha* (Harry Sm. ex T.N. Ho & S.W. Liu) Halda in Acta Mus. Richnov. Sect. Nat. 3: 16. 1995 — Type: China, S. E. Tibet, Kucha La near Paka, 14000-15000 ft, 25 July 1938, *Ludlow, Sherriff & Taylor 5934* (holotype: BM, image!).

9. *Kuepferia masonii* (T. N. Ho) Favre, **comb. nov.** ≡ *Gentiana masonii* T. N. Ho in Bull. Nat. Hist. Mus. London, Bot. 23(2): 56, f. 1. 1993 — Type: Upper Burma, N'Maikha-Salwin divide, 26°50' N 98°48' E, on alpine meadows, 4270 m, September 1925, *Forrest 27222* (holotype: BM, image!; isotype: K).

10. *Kuepferia otophora* (Franch.) Favre, **comb. nov.** ≡ *Gentiana otophora* Franch. in J. Linn. Soc., Bot. 26(174): 130–131. 1890 — Type: China, Yunnan, grassland on slopes or in valleys, 2800-4200 m, (holotype: K).

= *Gentiana otophora* var. *ovatisepala* C. Marquand in Bull. Misc. Inform. Kew 1937(3): 182. 1937 — Type: Myanmar, near frontier with Xizang, *Kingdon Ward 9927* (holotype: BM).

11. ***Kuepferia otophoroides*** (Harry Sm.) Favre, **comb. nov.** ≡ *Gentiana otophoroides* Harry Sm. in Anz. Akad. Wiss. Wien, Math.-Naturwiss. Kl. 63: 101. 1926 — Type: China: Yunnan: Chamutong, meadows on rocky slopes, 4050 m, 15-17 August 1916, *Handel-Mazzetti 9894* (holotype: E, image!; isotypes: K, P, UPS, US).

12. ***Kuepferia sichitoensis*** (C. Marquand) Favre, **comb. nov.** ≡ *Gentiana sichitoensis* C. Marquand in Bull. Misc. Inform. Kew. 1928(2): 56–57. 1928 ≡ *Gentiana otophora* subsp. *sichitoensis* (C. Marquand) Halda in Acta Musei Richnov. Sect. Nat. 3: 15. 1995 — Type: China: Xizang: Tsarong, open alpine meadows on the salween-Kiu chiang divide, NW of Sichito, October 1922, *Forrest 22795* (holotype: E, image!; syntype: K).

Figure 25. Morphology and corolla colour in *Metagentiana* **(A–B)** and *Sinogentiana* **(C–D). A,** *Metagentiana gentilis* (Franch.) T.N.Ho & S.W.Liu; **B,** *Metagentiana rhodantha* (Franch.) T.N.Ho & S.W.Liu; **C,** *Sinogentiana striata* (Maxim.) Adr.Favre & Y.M.Yuan; **D,** *Sinogentiana souliei* (Franch.) Adr.Favre & Y.M.Yuan. — Photographs by A. Favre.

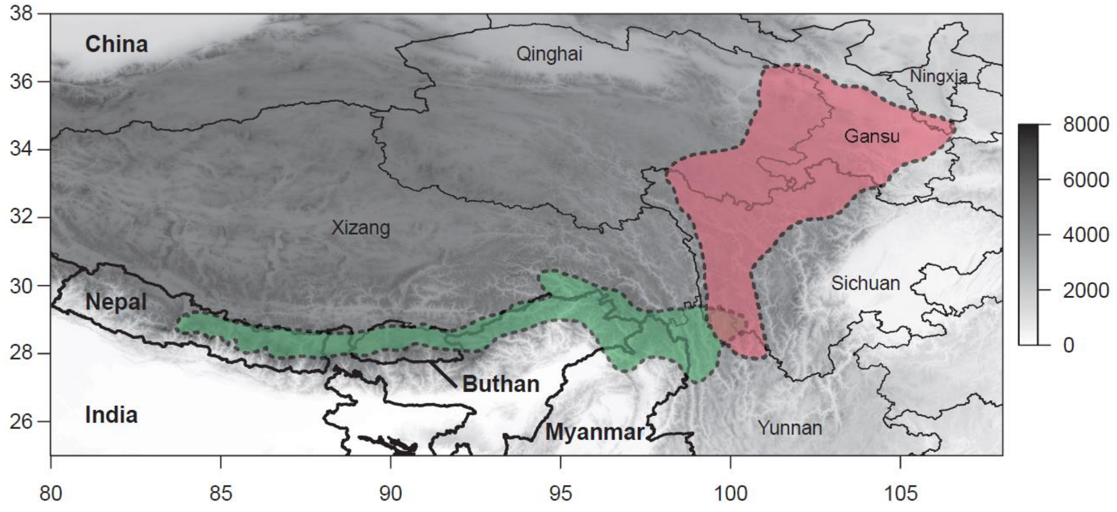


Figure 26. Approximate distribution of *Kuepferia* (green) and *Sinogentiana* (pink) in the Himalayan and the Hengduanshan regions. The grayscale indicates the local mean elevation. This map was downloaded from WORLDCLIM (Hijmans & al., 2005) and political borders were retrieved from Esri Data and Maps (2002).

Sinogentiana

Sinogentiana Favre & Y.-M. Yuan, **gen. nov**. —Type: *Gentiana souliei* Franchet, Bull. Soc. Bot. France 43: 491. 1896. China, W. Sichuan, Kangding, Tongolo and Tizou (Dzeura), September 1891, *Soulie 194* (holotype: P, image!; isotype: K).

Diagnosis. – This new genus differs from *Tripterospermum* and *Crawfurdia* by its ascending to erect flowering stems (flowering stems are trailing or climbing in *Tripterospermum* and *Crawfurdia*). In contrast to *Gentiana*, *Kuepferia* and *Crawfurdia*, the new genus *Sinogentiana* has recurved stamens. Finally, *Sinogentiana* is easily distinguished from *Metagentiana* by the yellow colour of its corolla, its plicae never fringed and its corolla tube 4 to 8 times longer that the corolla lobes.

Description. – Biennials. Stems ascending to erect. Basal leaves withered at anthesis. Stem leaves 1-3 cm x 5-12 mm, widely spaced, shorter than internodes, abaxially weakly to densely pubescent on midvein; base rounded; margin scabrous to ciliolate; apex acuminate to acute; veins 1-3. Petioles of stem leaves 0.5-1 mm, entirely connate. Flowers terminal, solitary, sessile. Calyx keeled or winged; tube campanulate or tubular, 1-1.3 cm; lobes linear-lanceolate, 8-11 mm, margin ciliolate, apex acuminate, midvein outside strongly keeled and decurrent into wings of calyx tube. Corolla pale yellow to yellow, tubular to funnelform, 3-6 cm; lobes ovate, 5-7 mm, margin entire, apex acuminate; plicae asymmetrical, very oblique, horizontally truncate; margin denticulate or erose. Stamens unequal; filaments 0.5-1.8 cm; anthers narrowly ellipsoid, 2.5-3 mm. Style filiform, longer to slightly shorter than the ovary. Capsules ellipsoid, 2-3.5 cm, wingless. Seeds brown, ellipsoid, 2-3.5 mm, triquetrous with three winged edges, rarely wingless or areolate.

Etymology. ― The generic name refers on the one hand to the exclusive Chinese distribution of this genus, and on the other hand to its phylogenetic position within the Gentianinae.

Distribution. ― Both species are endemic to China, *S. striata* occurs in the provinces of Gansu, Ningxia, Qinghai, and Sichuan, and *S. souliei* occurs in Sichuan and Yunnan (Fig. 26).

Habitat. ― Grassland and scrub slopes, *Abies* forests, between (2200) 3200 and 3900 m.

IUCN conservation assessment. ― Both *Sinogentiana striata* and *S. souliei* are widespread species that occupy a fairly common habitat within their relatively vast distribution range (see a non-exhaustive list of populations of S. striata in Chen et al. 2008). The area of occupancy of each species exceeds the threshold of 20´000 km2 for threatened categories and therefore can be considered as Least Concern (LC) according to the *IUCN Red List Categories and Criteria* (IUCN 2001, 2012). However, because these species largely depend on grasslands, which are increasingly threatened by overgrazing in this region of China, their status should be re-assessed regularly.

1. *Sinogentiana souliei* (Franch.) Favre & Y.-M. Yuan, **comb. nov.** ≡ *Metagentiana souliei* (Franch.) T. N. Ho, S. W. Liu & S. L. Chen in Bot. Bull. Acad. Sin. 43: 83–89. 2002 ≡ *Gentiana souliei* Franch., Bull. Soc. Bot. France 43(8): 491. 1896 — TYPE: China, W. Sichuan, Kangding, Tongolo and Tizou (Dzeura), September 1891, *Soulie 194* (holotype: P, image !; isotype: K).

= *Gentiana pterocalyx* Franch. var*. flavo-viridis* C. Marquand, Bull. Misc. Inform. Kew 1928: 54. 1928 ≡ *Gentiana souliei* Franch. var. *flavo-viridis*

(C. Marquand) C. Marquand, Bull. Misc. Inform. Kew 1937: 154. 1937— TYPE: China, Yunnan, *Forrest 6531, Schneider 2340, 3002, 3135* (syntype: E, image!).

2. ***Sinogentiana striata*** (Maxim.) Favre & Y.-M. Yuan, **comb. nov.** ≡ *Metagentiana striata* (Maxim.) T. N. Ho, S. W. Liu & S. L. Chen in Bot. Bull. Acad. Sin. 43: 89. 2002 ≡ *Gentiana striata* Maxim., in Bull. Acad. Imp. Sci. Saint-Petersbourg 27(4): 501–502. 1881 — TYPE: China, W Gansu, in alpine meadows, in 1872, *Przewalski s. n.* (holotype: LE).

= *Gentiana tricholoba* Franch., Bull. Soc. Bot. France 43(8): 490–491. 1896 — TYPE: China, W Sichuan, Kanding (Ta Tsien Lu), *Soulie 134* (holotype: P).

= *Gentiana schlechteriana* H. Limpr., Repert. Spec. Nov. Regni Veg. Beih. 12: 467. 1922 — TYPE: China, W Sichuan, Kanding (Ta Tsien Lu), 3900 m, *Limpricht 1768* (holotype: WRSL; isotype: UPS).

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SUPPORTING INFORMATION 2

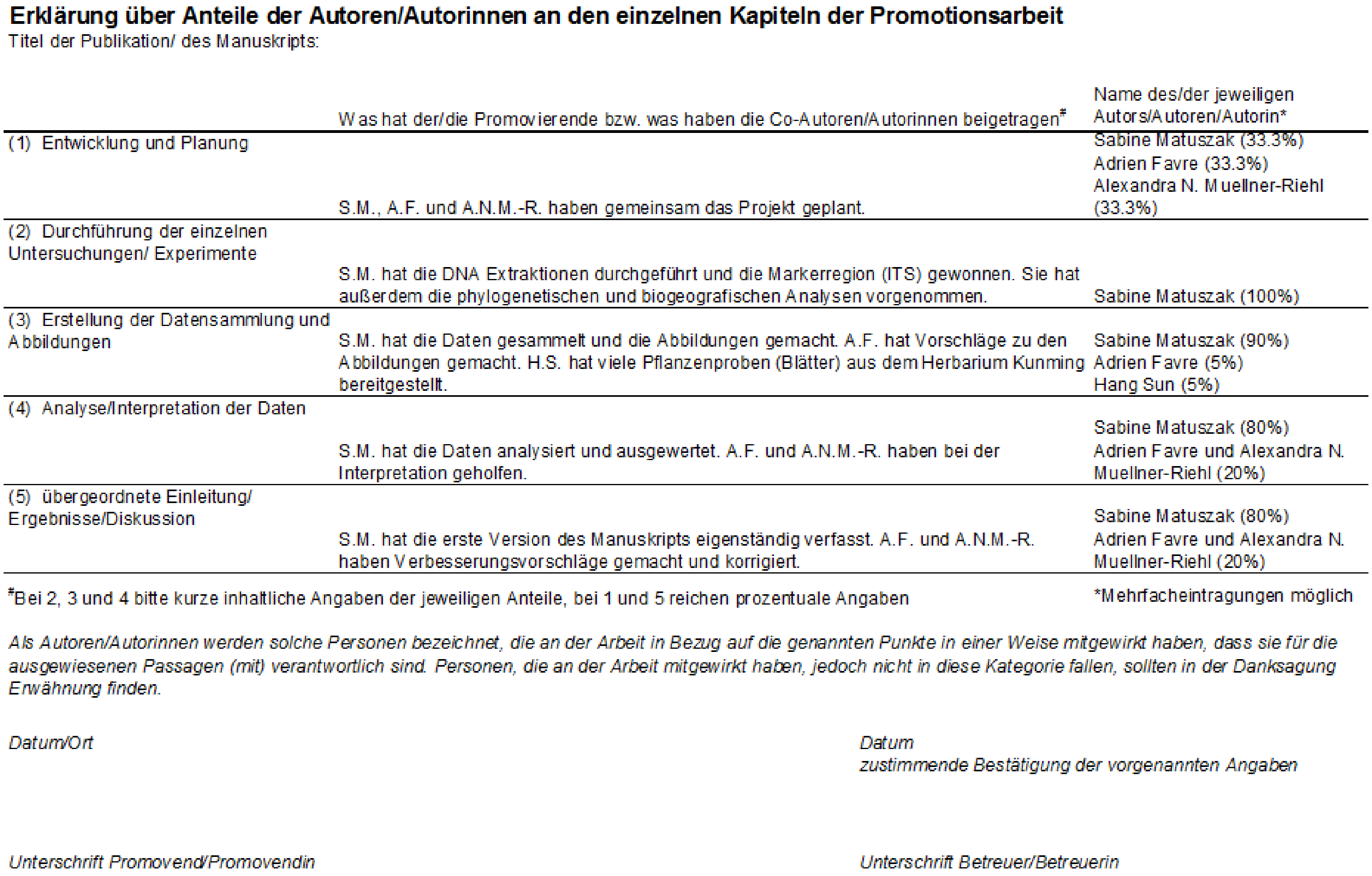
Samples of Gentianeae used in this study: Taxon, voucher, Genbank accession numbers for ITS (sometimes ITS1, ITS2), *atpB-rbcL*, and *trnL-F*. Sequences newly obtained for the present study are indicated with a (*) and missing sequences are indicated with a (–).

Comastoma cyananthiflorum (Franch.) Holub, –, GU250970, –; ***Crawfurdia angustata*** C. B. Clarke in J. D. Hooker, Dulongjiang, Yunnan, China, *Favre 229* (KUN), GU251013, GU250971, KF563978*; ***Crawfurdia campanulacea*** Wallich & Griffith ex C. B. Clarke in J. D. Hooker, Dulongjiang, Yunnan, China, *Favre 214* (KUN), GU251014, GU250972, KF563979*; ***Crawfurdia maculicaulis*** C. J. Wu, Malipo, Yunnan, China, *Favre 215* (LZ), GU251017, GU250975, KF563980*; ***Crawfurdia pricei*** (C. Marquand) Harry Smith, Dayaoshan, Guangxi, China, *Favre 245* (LZ), GU251019, GU250977, KF563981*; ***Crawfurdia speciosa*** Wallich, Darjeeling, West Bengal, India, *Favre 207* (KUN), GU251021, GU250979, KF564004*; ***Gentiana acaulis*** L., Grand-St-Bernhard, Valais, Switzerland, *Favre 1003* (LZ), KF563947*, –, KF563982*; ***Gentiana algida*** Pall., GU251024, DQ398622, AB219622; ***Gentiana arethusae*** Burkill, Baimaxuashan, Yunnan, China, *Favre & Matuszak 177a* (KUN), KF563955*, –, KF563993*; ***Gentiana asclepiadea*** L., AJ580549, GU250980, AJ580515; ***Gentiana atuntsiensis*** W. W. Smith, Dali, Yunnan, China, *Favre & Matuszak 060a* (KUN), –, –, KF563991*; ***Gentiana bavarica*** L., Sanetsch, Valais, Switzerland, *Favre 1002* (LZ), KF563948*, –, KF563983*; ***Gentiana brachyphylla*** Vill., Sanetsch, Valais, Switzerland, *Favre 1000a* (LZ), KF563949*, –, KF563984*; ***Gentiana cephalantha*** Franch. ex Hemsl., *Favre 325* (KUN), KF563950*, –, KF563985*; ***Gentiana clusii*** Perr. & Songeon, Fionnay, Valais, Switzerland, *Favre CH001* (LZ), AJ223107, –, KF563986*; ***Gentiana crassicaulis*** Duthie ex Burkill, Lijiang, Yunnan, China, *Favre & Matuszak 116a* (KUN), DQ398636, DQ398603, KF564005*; Gentiana cruciata L., DQ398634, –, DQ398712; ***Gentiana dahurica*** Fisch., Xiahe, Gansu, China, *Favre 319* (KUN), KF563971*, DQ398598, KF564009*; ***Gentiana epichysantha*** Hand.-Mazz., Bashuitai, Yunnan, China, *Favre & Matuszak 131a* (KUN), KF563953*, –, KF563989*; ***Gentiana faucipilosa*** Harry Sm., Bingzhongluo, Yunnan, China, *Favre & Matuszak, 37a* (KUN), KF563954*,–, KF563990*; ***Gentiana lawrencei*** Brukill, Langmusi, Sichuan, China, *Favre 315* (KUN), AF346006, –, KF563992*; ***Gentiana lutea*** L., –, EU370939, –; ***Gentiana nanobella*** C.Marquand, Baimaxuashan, Yunnan, China, Favre & Matuszak 179a (KUN), KF563956*, –, –; ***Gentiana nivalis*** L., Ferpècle, Valais, Switzerland, *Jochum 1201* (LZ), KF563958*, –, JF748589; ***Gentiana***

panthaica Prain & Burkill, Dali, Yunnan, Switzerland, *Favre & Matuszak 061a* (KUN), KF563960*, –, KF563995*; ***Gentiana phyllocalyx*** C.B.Clarke, Bingzhongluo, Yunnan, China, *Favre & Matuszak 33a* (KUN), KF563961*, –, KF563996*; ***Gentiana praeclara*** C. Marquand, Lugu Hu, Yunnan, China (KUN), KF563962*, –, KF563997*; ***Gentiana purdomii*** C. Marquand, Songpan, Sichuan, China, *Favre 311* (KUN), KF563963*, –, KF563998*; ***Gentiana purpurea*** L., Val di Blenio, Ticino, Switzerland, *Triponez 1001* (no specimen), KF563964*, –, KF563999*; ***Gentiana scabra*** Bunge, Gangwon-do, North Korea, *Sun BY 2001-102* (WU), KF563965*, –, GQ864088; ***Gentiana straminea*** Maxim., DQ398630, DQ398595, –; ***Gentiana triflora*** Pall., GQ864019, –, GQ864092; ***Gentiana urnula*** Harry Sm., Boshan, Tibet, China, *Qing Zangbudian 751961* (KUN), Z48071, Z48090, GU250983, KF564000*; ***Gentiana veitchiorum*** Hemsl., Langmusi, Sichuan, China, *Favre 314* (KUN), KF563966*, –, KF564001*; ***Gentiana vernayi*** C. Marquand, Ridang, Tibet, China, *Qingzang Team 151454* (KUN), AY858670, –, KF564002*; ***Gentiana walujewii*** Regel & Schmalh., –, DQ398611, –; ***Gentiana yunnanensis*** Franch., Daxueshan, Yunnan, China, *Favre & Matuszak 183a* (KUN), KF564003*, –, KF564003*; ***Gentianella acuta*** (Michx.) Hiitonen, –, EU370942, –; ***Gentianella campestris*** (L.) Börner, AJ580557, –, AJ580523; ***Gentianella turkestanorum*** (Gand.) Holub, –, EU370941, –; ***Gentianopsis contorta*** (Royle) Ma, Shangrila, Yunnan, China, *Favre & Matuszak 170* (KUN), KF563968*, –, AJ315237; ***Gentianopsis lutea*** (Burkill) Ma, Kunming, Yunnan, China, Favre 331 (KUN), KF563969*, , –, KF564006*; ***Kuepferia damyonensis*** (Marquand) Favre, Ridong, Xiang, Tibet, China, *Expedition Team to Qinghai-Tibet Plateau 10232* (KUN), KF563951*, –, KF563987*; ***Kuepferia decorata*** (Diels) Favre, Medog-Millin, Tibet, China, *Yuan Yong-Ming 2003-38* (KUN), KF563970*, GU250981, KF564008*; ***Kuepferia doxiongshanensis*** (Ho) Favre, Medog-Millin, Tibet, China, *Yuan Yong-Ming, 2003-33* (KUN), KF563952*, –, KF563988*; ***Kuepferia otophora*** (Franchet in Forbes & Hemsley) Favre, Dali, Yunnan, China, *Favre & Matuszak 056a* (KUN), KF563957*, –, –; ***Kuepferia otophoroides*** (Harry Smith) Favre, Bingzhongluo, Yunnan, China, *Favre & Matuszak 034a* (KUN), KF563959*, –, KF563994*; ***Megacodon stylophorus*** (C.B. Clarke) Harry Sm., Bingzhongluo, Yunnan, China, *Favre & Matuszak 35* (KUN), AY858679, –, KF564007*; ***Metagentiana australis*** (Craib) T. N. Ho & S. W. Liu, Mt. Doi Chiang Dao, Chiang Mai, Thailand, *Tagawa & al. 4151* (L), GU251029, GU250986, KF564010*; ***Metagentiana eurycolpa*** (C. Marquand) T. N. Ho & S. W. Liu, Gunapo in Wuding, Yunnan, China (KUN), KF563972*, –, KF564011*; ***Metagentiana gentilis*** (Franch.) T. N. Ho & S. W. Liu, Kunming, Yunnan, China (KUN), GU251030, , GU250987, KF564012*; ***Metagentiana leptoclada*** (Balf & Forrest) T. N. Ho & S. W. Liu, Lijiang, Yunnan, China, Li & al. 0758

(KUN), KF563973*, –, KF564013*; ***Metagentiana primuliflora*** (Franch.) T. N. Ho & S. W. Liu, GU251031, GU250988, –; ***Metagentiana pterocalyx*** (Franch.) T. N. Ho & S. W. Liu, Lijiang, Yunnan, China, *Liu & Yang 421* (KUN), KF563974*, –, KF564014*; ***Metagentiana rhodantha*** (Franch.) T. N. Ho & S. W. Liu, Lijiang, Kunming, China, *Favre 301* (KUN), GU251032, GU250989, KF564015*; ***Metagentiana serra*** (Franch.) T. N. Ho, S. W. Liu & Shi L. Chen, Lijiang, Yunnan, China, *Favre & Matuszak 125a* (KUN), GU251033, GU250990, KF564016*; ***Metagentiana villifera*** (H. W. Li ex T. N. Ho) T. N. Ho & S. W. Liu, Niutouzhai, Sichuan, China, *Expedition Team to Economic Plants of Sichuan 0018* (KUN), KF563975*, –, KF564019*; ***Sinogentiana souliei*** (Franchet) Favre & Y.-M. Yuan, Yading, Sichuan, China, *Favre & Matuszak 202a* (KUN), GU251034 ; GU250991, KF564017*; ***Sinogentiana striata*** (Maximowicz) Favre & Y.-M. Yuan, Songpan, Sichuan, China, *Favre 305* (KUN), GU251035, GU250992, KF564018*; ***Swertia erythrosticta*** Maxim., Huanglong, Sichuan, China, *Favre 309* (KUN), KF563976*, –, KF564020*; ***Tripterospermum alutaceifolium*** (Liu & Kuo) J. Murata, Chihchengshan, Taiwan, *Favre 130k1* (KUN), GU251037, GU250994, KF564021*; ***Tripterospermum chinense*** (Migo) Harry Sm., Luofushan, Guangdong, China, *Favre 137* (KUN), GU251040, GU250997, KF564022*; ***Tripterospermum cordatum*** (C. Marquand) Harry Sm., Emeishan, Sichuan, China, *Favre 084* (KUN), GU251055, GU251012, KF564023*; ***Tripterospermum cordifolioides*** J. Murata, Wolong, Sichuan, China, *Favre 123* (LZ), GU251041, GU250998, KF564024*; ***Tripterospermum hirticalyx*** C. Y. Wu & C. J. Wu, GU251043, GU251000, –; ***Tripterospermum lanceolatum*** (Hayata) H. Hara ex Satake**,** Meifeng, Taiwan, *Favre 106* (KUN), GU251044, GU251001, KF564025*; ***Tripterospermum luteoviride*** (C.B.Clarke) J.Murata, Gangtok, Sikkim, India, Favre 015 (LZ), GU251045, GU251002, KF564026*; ***Tripterospermum luzonense*** (Vidal) J. Murata, Tsuifeng, Taiwan, Favre 100 (KUN), GU251046, GU251003, KF564027*; ***Tripterospermum pallidum*** Harry Sm., Emeishan, Sichuan, China, Favre 089b (KUN), GU251050, GU251007, KF564028*; ***Tripterospermum robustum*** Harry Sm. ex Hul, Bana hill station, Danang, Viet Nam, Favre 25 (LZ), GU251052, GU251009, KF564029*; ***Veratrilla baillonii*** Franch., Dali, Yunnan, China, Favre & Matuszak 057a (KUN), KF563977*, –, KF564030*.

Appendix 3. Erklärung zu Kapitel 2 „Transcontinental dispersals increased in the Southern Hemisphere after Miocene cooling: molecular dating and biogeography of Agapetes, Vaccinium and their relatives (Vaccinieae, Ericaceae)".



Erklärung über Anteile der Autoren/Autorinnen an den einzelnen Kapiteln der Promotionsarbeit

Titel der Publikation/ des Manuskripts:

	Was hat der/die Promovierende bzw. was haben die Co-Autoren/Autorinnen beigetragen#	Name des/der jeweiligen Autors/Autoren/Autorin*
(1) Entwicklung und Planung	S.M., A.F. und A.N.M.-R. haben gemeinsam das Projekt geplant.	Sabine Matuszak (33.3%) Adrien Favre (33.3%) Alexandra N. Muellner-Riehl (33.3%)
(2) Durchführung der einzelnen Untersuchungen/ Experimente	S.M. hat die DNA Extraktionen durchgeführt und die Markerregion (ITS) gewonnen. Sie hat außerdem die phylogenetischen und biogeografischen Analysen vorgenommen.	Sabine Matuszak (100%)
(3) Erstellung der Datensammlung und Abbildungen	S.M. hat die Daten gesammelt und die Abbildungen gemacht. A.F. hat Vorschläge zu den Abbildungen gemacht. H.S. hat viele Pflanzenproben (Blätter) aus dem Herbarium Kunming bereitgestellt.	Sabine Matuszak (90%) Adrien Favre (5%) Hang Sun (5%)
(4) Analyse/Interpretation der Daten	S.M. hat die Daten analysiert und ausgewertet. A.F. und A.N.M.-R. haben bei der Interpretation geholfen.	Sabine Matuszak (80%) Adrien Favre und Alexandra N. Muellner-Riehl (20%)
(5) übergeordnete Einleitung/ Ergebnisse/Diskussion	S.M. hat die erste Version des Manuskripts eigenständig verfasst. A.F. und A.N.M.-R. haben Verbesserungsvorschläge gemacht und korrigiert.	Sabine Matuszak (80%) Adrien Favre und Alexandra N. Muellner-Riehl (20%)

#Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben

*Mehrfacheintragungen möglich

Als Autoren/Autorinnen werden solche Personen bezeichnet, die an der Arbeit in Bezug auf die genannten Punkte in einer Weise mitgewirkt haben, dass sie für die ausgewiesenen Passagen (mit) verantwortlich sind. Personen, die an der Arbeit mitgewirkt haben, jedoch nicht in diese Kategorie fallen, sollten in der Danksagung Erwähnung finden.

Datum/Ort

Datum
zustimmende Bestätigung der vorgenannten Angaben

Unterschrift Promovend/Promovendin

Unterschrift Betreuer/Betreuerin

Appendix 4. List of species of Ericaceae included in this study, with their classification, authorities and Genbank accession numbers for ITS sequences. Sequences newly obtained are indicated by an *.

Name	ITS
Agapetes angulata (Griff.) Hook.f.	KM209366*
Agapetes brachypoda Airy Shaw	KM209367*
Agapetes brandisiana W.E.Evans	KM209378*
Agapetes buxifolia Nutt. ex Hook.f.	AF382650
Agapetes ciliata S.H.Huang	KM209368*
Agapetes hosseana Diels	KM209369*
Agapetes lobbii C.B. Clarke	KM209371*
Agapetes moorei Hemsl.	KM209373*
Agapetes obovata (Wight) Hook.f.	KM209374*
Agapetes pensilis Airy Shaw	KM209375*
Agapetes serpens (Wight) Sleumer	KM209376*
Agapetes variegata D.Don ex G.Don	KM209377*
Andersonia heterophylla Sond.	KM209379*
Anthopterus racemosus Hook.	AF382655
Anthopterus schultzeae (Sleumer) Luteyn	KM209380*
Anthopterus wardii Ball	AF382656
Brachyloma concolor Benth.	KM209381*
Brachyloma daphnoides (Sm.) Benth.	KM209382*
Cassiope fastigiata D.Don	JF976084
Cassiope selaginoides Hook.f. & Thomson	KM209383*
Cavendishia bracteata (Ruiz & Pav. ex J.St.-Hil.) Hoerold	KM209384*
Cavendishia capitulate Donn.Sm.	KM209385*
Cavendishia complectens Hemsl.	KM209386*
Cavendishia isernii (Sleumer) Luteyn	KM209387*
Ceratostema lanceolatum Benth.	AF382660
Ceratostema megabracteatum Luteyn	AF382661
Costera endertii J.J. Sm.	AF382662
Craibiodendron yunnanense W.W. Sm.	EU547685
Daboecia cantabrica (Huds.) K.Koch f. *albiflora* (D.C.McClint.) E.C.Nelson	JX262456
Demosthenesia pearcei (Britton) A.C.Sm.	AF382663
Demosthenesia spectabilis (Rusby) A.C.Sm.	AF382665
Dimorphanthera amoena Sleumer	AF382666
Dimorphanthera dekockii J.J.Sm.	AF382667
Dimorphanthera elegantissima K.Schum.	KM209388*
Dimorphanthera ingens (Sleumer) P.F.Stevens	KM209446*
Dimorphanthera kempteriana Schltr.	AF382668
Dimorphanthera keysseri (Schltr. ex Diels) P.F.Stevens	AF382669
Dimorphanthera megacalyx Sleumer	AF382670
Dimorphanthera womersleyi Sleumer	AF382671
Diogenesia alstoniana Sleumer	AF382672
Diogenesia boliviana (Britton) Sleumer	AF382673
Disterigma pallidum A.C.Sm.	AF382674
Disterigma rimbachii (A.C.Sm.) Luteyn	FJ001695
Disterigma ulei Sleumer	FJ001701
Dracophyllum acerosum Berggr.	AY649407
Dracophyllum filifolium Hook.f.	KM209389*

Name	ITS
Dracophyllum kirkii Berggr.	AY649410
Enkianthus nudipes (Honda) Ueno	AB726228
Enkianthus perulatus C.K.Schneid.	JF976287
Enkianthus quinqueflorus Lour.	JF976292
Epacris coriacea A.Cunn. ex DC.	KM209390*
Epacris obtusifolia Sm.	KM209391*
Erica trimera (Engl.) Beentje	HQ859308
Erica vagans L.	HQ859320
Gaultheria borneensis Stapf	KM209392*
Gaultheria cardiosepala Hand.-Mazz.	KM209393*
Gaultheria eriophylla (Pers.) Mart. ex Sleumer	JF801589
Gaultheria hispida R.Br.	FJ665707
Gaultheria leucocarpa Blume	KM209394*
Gaultheria longibracteolata R.C.Fang	KM209395*
Gaylussacia dumosa (Andrews) A.Gray	AF382677
Gaylussacia goyazensis Sleumer	KM209396*
Lyonia ligustrina (L.) DC.	HM182083
Lyonia ovalifolia (Wall.) Drude	KM209397*
Lyonia spec. (was determined as *Vaccinium duclouxii* Hand.-Mazz.)	KM209441*
Lyonia spec. (was determined as *Vaccinium leucanthum* Schltdl.)	KM209450*
Macleania coccoloboides A.C.Sm.	AF382680
Macleania insignis M.Martens & Galeotti	AF382681
Monotoca scoparia (Sm.) R.Br.	KM209398*
Monotoca tamariscina F.Muell.	KM209399*
Notopora schomburgkii Hook.f.	AF382683
Orthaea apophysata (Griseb.) A.C.Sm.	AF382685
Orthaea fimbriata Luteyn	AF382686
Paphia meiniana (F.Muell.) Schltr.	AF382652
Paphia meiniana (F.Muell.) Schltr.	KM209372*
Paphia stenantha Schltr.	AF382654
Paphia kudukii (Veldkamp)P.F.Stevens	KM209370*
Pentachondra involucrata R.Br.	KM209400*
Pentachondra pumila R.Br.	KM209401*
Pieris cubensis Small	EU547688
Pieris floribunda Benth. & Hook.f.	EU547694
Pieris formosa D.Don	EU547689
Pieris taiwanensis Hayata	AF432431
Psammisia dolichopoda A.C.Sm.	AF382690
Psammisia ecuadorensis Hoerold	AF382691
Rhododendron augustinii Hemsl.	KM209402*
Rhododendron camelliiflorum Hook.f.	KM209403*
Rhododendron fortunei Lindl.	KM209404*
Rhododendron sanguineum Franch. & Cowan	KM209405*
Rhododendron simsii Planch.	KM209406*
Richea sprengelioides (R.Br.) F.Muell.	KM209407*
Satyria allenii A.C.Sm.	AF382692
Satyria boliviana Luteyn	AF382693
Satyria warszewiczii Klotzsch	AF382698
Siphonandra elliptica Klotzsch	AF382700
Sphenotoma dracophylloides Sond.	KM209408*
Sphyrospermum boekei Luteyn	KM209409*

Name	ITS
Sphyrospermum buxifolium Poepp. & Endl.	AF382702
Sprengelia incarnata Sm.	KM209410*
Sprengelia monticola (DC.) Druce	KM209411*
Symphysia poasana (Donn.Sm.) Kloet	AF382736
Symphysia racemosa (Vahl) Stearn	AF382705
Themistoclesia costaricensis Luteyn & Wilbur	AF382706
Themistoclesia epiphytica A.C.Sm.	AF382707
Thibaudia angustifolia Hook.	KM209412*
Thibaudia densiflora (Herzog) A.C.Sm.	AF382708
Thibaudia nutans Klotzsch & Mansf.	KM209413*
Vaccinium acrobracteatum K.Schum.	AY274562
Vaccinium acuminatissimum Miq.	KM209425*
Vaccinium alto-montanum Ashe	AF273710
Vaccinium alvarezii Merr.	KM209414*
Vaccinium ambivalens Sleumer	KM209415*
Vaccinium amphoterum Sleumer	KM209416*
Vaccinium ampullaceum Sleumer	KM209417*
Vaccinium andersonii Sleumer	KM209418*
Vaccinium arboreum Marshall	KM209419*
Vaccinium arctostaphylos L.	KM209420*
Vaccinium ardisioides Hook.f. ex C.B.Clarke	KM209421*
Vaccinium auriculifolium Sleumer	KM209422*
Vaccinium barandanum S.Vidal	AY274563
Vaccinium benguetense S.Vidal	KM209423*
Vaccinium berberidifolium Skottsb. (= *V.dentatum x V.reticulatum*)	KM209424*
Vaccinium boninense Nakai	AB623184
Vaccinium brachybotris Hand.-Mazz.	KM209426*
Vaccinium bracteatum Thunb.	KM209427*
Vaccinium brevipedicellatum C.Y.Wu	KM209428*
Vaccinium bulleyanum (Diels) Sleumer	KM209429*
Vaccinium caespitosum Michaux	KM209430*
Vaccinium calycinum Sm.	AF419776
Vaccinium caudatifolium Hayata	AF382715
Vaccinium cercidifolium J.J.Sm.	AF382716
Vaccinium cereum G.Forst.	KM209431*
Vaccinium chaetothrix Sleumer	KM209432*
Vaccinium chamaebuxus C.Y.Wu	KM209433*
Vaccinium ciliatum Thunb.	AB623188
Vaccinium consanguineum Klotzsch	AF382717
Vaccinium coriaceum Hook.f.	KM209434*
Vaccinium corymbosum L.	AF419778
Vaccinium crassifolium Andrews	AF382718
Vaccinium crenatum (D.Don exDunal) Sleumer	KM209435*
Vaccinium cruentum Sleumer	KM209436*
Vaccinium cubense Griseb.	KM209437*
Vaccinium cylindraceum Sm.	AF382720
Vaccinium darrowii Camp	AY274573
Vaccinium delavayi Franch.	KM209438*
Vaccinium deliciosum Piper	AF419790, AF419791
Vaccinium dendrocharis Hand.-Mazz.	KM209439*

Name	ITS
Vaccinium dentatum Sm.	AF382721
Vaccinium didymanthum Dunal	KM209440*
Vaccinium dunalianum Wight	AF432427
Vaccinium emarginatum Hayata	AB623181
Vaccinium erythrocarpum Michx.	AF419781
Vaccinium exul Bolus	KM209443*
Vaccinium finisterrae Schltr.	AF382723
Vaccinium floribundum Kunth	KM209444*
Vaccinium floridanum (Nutt.) Schwein. ex Nutt. & Sleumer	KM209445*
Vaccinium fragile Franch.	AF382725
Vaccinium fuscatum Aiton	AF084322
Vaccinium gaultheriifolium (Griff.) Hook.f. ex C.B.Clarke	AF382726
Vaccinium hirsutum Buckley	AF419780
Vaccinium hirtum Thunb.	AF382727
Vaccinium horizontale Sleumer	AF382728
Vaccinium iteophyllum Hance	KM209447*
Vaccinium kingdon-wardii Sleumer	KM209448*
Vaccinium lanceifolium (Ridl.) Sleumer	AF382738
Vaccinium latissimum J.J.Sm.	KM209449*
Vaccinium leptospermoides J.J.Sm.	AF382729
Vaccinium leucobotrys (Nutt.) G.Nicholson	KM209451*
Vaccinium loranthifolium Ridl.	KM209452*
Vaccinium macrocarpon Aiton	FJ010588
Vaccinium madagascariense (Thou. ex Poir.) Sleumer	KM209442*
Vaccinium mandarinorum Diels	KM209453*
Vaccinium membranaceum Douglas ex Hook.	KM209454*
Vaccinium meridionale Sw.	KM209455*
Vaccinium merrilianum Hayata	AF432451
Vaccinium miquellii Boerl.	KM209456*
Vaccinium moultonii Merr.	AF382722
Vaccinium moupinense Franch.	KM209457*
Vaccinium myrsinites Lam.	AY274572
Vaccinium myrtillus L.	KM209458*
Vaccinium nummularia Hook.f. & Thomson ex C.B.Clarke	KM209459*
Vaccinium oldhamii Miq.	AF419783
Vaccinium omeiense Fang	KM209460*
Vaccinium ovalifolium Sm.	AF419784
Vaccinium ovatum Pursh	AY274568
Vaccinium oxycoccus L.	KM209461*
Vaccinium padifolium Sm.	AF382734
Vaccinium palawanense Merr.	KM209462*
Vaccinium parvifolium Sm.	KM209463*
Vaccinium peleanum Skottsb.	KM209464*
Vaccinium phillyreoides Sleumer	KM209465*
Vaccinium pilosilobum J.J.Sm.	KM209466*
Vaccinium praestans Lamb.	AF419785
Vaccinium randaiense Hayata	AF432452
Vaccinium reticulatum Sm.	KM209467*
Vaccinium retusum Hook.f. ex C.B.Clarke	AF419786
Vaccinium scoparium Leiberg ex Coville	AF419787
Vaccinium sieboldii Miq.	AB623191

Name	ITS
Vaccinium sikkimense C.B.Clarke	KM209468*
Vaccinium simulans Sleumer	KM209469*
Vaccinium smallii A.Gray	AF382739
Vaccinium sororium J.J.Sm.	KM209470*
Vaccinium subulisepalum J.J.Sm.	KM209471*
Vaccinium summifaucis Sleumer	AF382740
Vaccinium tenellum Aiton	AF382741
Vaccinium tenerellum Sleumer	KM209472*
Vaccinium tenuipes Merr.	KM209473*
Vaccinium uliginosum L.	GU361897
Vaccinium urceolatum Hemsl.	KM209474*
Vaccinium venosum Wight	KM209475*
Vaccinium vitis-idaea L.	KM209476*
Vaccinium wrightii A.Gray	AB623192
Vaccinium yakushimense Makino	AB623183
Vaccinium yatabei Makino	AF419789

Appendix 5. Clade support (posterior probabilities, PP, and bootstrap percentages, BP), divergence time estimates of Ericaceae taxa, and ancestral area reconstruction using dispersal-extinction-cladogenesis (DEC) and statistical dispersal-vicariance (S-DIVA) models. Node numbers refer to Fig. 7 or Fig. 8. For reconstructions by DEC, a vertical line (e.g. H|G) splits into upper branches (left) and lower branches (right). Nine areas were defined as follows: (A) Qinghai-Tibetan Plateau (QTP) and its surroundings (green); (B) Indochina, Sundaland (Borneo, Sumatra, Java, Malay Peninsula), China (except for QTP region = A), and Japan (yellow); (C) Wallacea (Sulawesi, the Moluccas, the Banda Arc, and the Lesser Sunda Islands), and the Philippines (dark blue); (D) Sahul shelf (New Guinea and Australia), and New Zealand (light blue); (E) Southern Polynesia and Hawaii (purple); (F) North America (orange); (G) Mexico, Central and South America, the Caribbean (grey); (H) Europe and temperate/arctic Asia (brown); and (I) Southern Africa and Madagascar (pink).

Node No.	**BEAST**			**Node No.**	**BEAST**		**DEC**		**S-DIVA**	
(Fig. 7)	**PP**	**BP**	**Age in Ma [95% HPD interval]**	**(Fig. 8)**	**PP**	**Age in Ma**	**Ancestral Split**	**Relative probability [%]**	**Area**	**Relative probability [%]**
1 fossil calibration	1.00	100	103.44 [90.62-125.83]	**1**	1.00	60.71 [44.02-72.18]	many possibilities	all 1	ABFG AFG	70 21
2	1.00	100	91.83 [89.80-93.90]	**2**	1.00	31.93 [15.26-50.75]	F_G|A G|A F|A F_G|B G|A_B F|A_B	31 10 10 9 5 4	AFG AG AF FG ABFG ABG	30 25 19 10 9 8
3	1.00	91	66.00 [46.43-84.27]	**3**	1.00	7.20 [1.09-15.97]	A_B|A A|A	51 46	A AB	78 22

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
4	1.00	100	7.20 [0.89-16.33]	**4**	0.63	21.33 [8.59-37.18]	G|F	73	FG	100
5	0.98	80	50.49 [33.16-68.24]	**5**	0.69	51.92 [32.66-63.70]	G|G A|A F|F B|B A_B_F_G|A A_B_D_F_G|A F_G|G	6 4 4 2 2 2 2	ABF BFG ABFG BF B	23 23 23 23 7
6	1.00	95	21.73 [9.27-35.48]	**6**	0.95	36.79 [22.80-50.96]	A|A A|A_G B|A_G A_B|A B|G B|A	32 14 10 8 6 5	ABG BG ABFG AB BFG AFG FG ABF AF AG	24 18 12 12 9 8 6 6 4 2
7	1.00	100	1.30 [0.00-4.20]	**7**	0.46	28.20 [18.36-46.44]	A|G A|A	63 17	AG	100
8	0.45	49	16.61 [6.58-28.48]	**8**	1.00	20.07 [9.53-32.15]	B|A_B B|B B_F|B B_F|A	50 17 14 4	BF B ABF AF	64 26 5 5
9	1.00	96	3.47 [0.04-8.73]	**9**	0.26	12.18 [2.86-19.05]	F|B	85	BF	100

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
10	0.83	62	40.30 [23.68-56.87]	**10**	1.00	42.23 [31.72-53.60]	F|F	8	F	81
							F_G |F	6		
							G|A_B_C_D_E_F	4		
							G|A_B_C_D_F	4		
							G|A_B_F	4		
							G|A_B_D_F	4		
							F|B_F	4		
							G|A_B_D_E_F	3		
							F|A_F	3		
							G|G	3		
							F|A_B_F	2		
							G|A_B_C_F	2		
							G|B_F	2		
							G|A_B_C_E_F	2		

Node No.	**BEAST**			**Node No.**	**BEAST**		**DEC**		**S-DIVA**	
(Fig. 7)	**PP**	**BP**	**Age in Ma [95% HPD interval]**	**(Fig. 8)**	**PP**	**Age in Ma**	**Ancestral Split**	**Relative probability [%]**	**Area**	**Relative probability [%]**
11	1.00	100	18.74 [6.88-31.13]	**11**	0.90	35.03 [24.70-45.40]	B|F A_B|F A_B_C_D_E|F A_B_C_D|F A_B_D|F A_B_D_E|F B|B_F B|B A_B_C_E|F A_B_C|F F|F A|F A_B_E|F B|B_F_G B|F_I	9 8 8 5 5 5 4 3 3 3 3 2 2 2 2	AF BF ABF	53 24 20
12	0.87	\	80.01 [68.11-91.13]	**12**	0.90	28.95 [18.37-40.38]	F|F	67	F	93
13	1.00	\	64.18 [49.62-79.59]	**13**	1.00	14.32 [5.55-24.70]	I|F	90	FI	100
14	1.00	95	27.10 [13.31-43.18]	**14**	0.99	5.96 [0.94-12.63]	I|I	98	I	100
15	0.45	57	21.52 [9.51-33.02]	**15**	0.99	20.81 [11.01-31.92]	F|F	62	F FG	48 46
16	0.34	50	15.93 [6.83-27.21]	**16**	0.98	11.52 [6.11-18.30]	F|F	100	F	100
17	1.00	99	3.60 [0.07-9.08]	**17**	0.85	3.54 [0.04-9.32]	F|F	100	F	100

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
18	0.47	\	12.39 [2.84-22.90]	**18**	1.00	8.59 [2.72-15.37]	F_G|B F_G|A F|A_B G|A_B F|B	37 33 8 6 5	BFG AFG ABFG FG AF BF ABF	17 17 17 16 11 11 11
19	0.55	\	57.63 [44.02-72.18]	**19**	0.09	6.61 [1.33-10.68]	F_G|G	89	FG G	50 50
20	0.23	\	48.48 [32.66-63.70]	**20**	1.00	0.77 [0.00-2.45]	G|G	100	G	100
21	1.00	67	31.93 [15.26-50.75]	**21**	0.90	2.33 [0.04-6.18]	F|G	99	FG	100
22	0.34	45	22.10 [8.59-37.18]	**22**	0.98	30.49 [21.43-40.22]	B|B A_B|B A_B_C_D_E|B A_B_C_D|B A_B_D|B A_B_D_E|B A|B	22 14 9 5 5 5 3	AB	88
23	1.00	95	7.20 [1.09-15.97]	**23**	1.00	3.80 [0.77-7.74]	B|B	100	B	100
24	0.95	\	36.79 [22.80-50.96]	**24**	0.42	1.16 [0.00-3.50]	B|B	100	B	100

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
25	0.38	\	31.78 [18.36-46.44]	**25**	0.58	28.29 [19.98-37.70]	A_B|A B|B A_B_C_D_E|A A_B_D|A A_B_C_D|A A_B_D_E|A A|A A_B_C|A A_B_C_E|A A_C_D_E|A	17 13 9 6 5 5 4 3 2 2	A	81
26	1.00	\	20.07 [9.53-32.15]	**26**	1.00	20.30 [13.40-28.26]	A|A	79	A	95
27	0.73	45	10.41 [2.86-19.05]	**27**	1.00	10.54 [3.46-18.80]	A|A A_B|A	78 21	A AB	80 20
28	1.00	30	42.23 [31.72-53.60]	**28**	0.19	7.31 [3.46-18.80]	A|A A|A_B	58 31	A AB	81 19
29	0.90	\	35.03 [24.70-45.40]	**29**	0.11	5.55 [0.81-11.58]	B|A	92	AB	100
30	0.98	\	30.49 [21.43-40.22]	**30**	0.43	2.84 [0.02-7.35]	A|A A_B|A	71 29	A	99
31	0.58	\	28.29 [19.98-37.70]	**31**	0.79	18.02 [11.82-25.04]	A|A A|A_B	87 13	A AB	58 42
32	1.00	73	20.30 [13.40-28.26]	**32**	0.28	15.51 [9.19-21.25]	A|A A_B|A	74 23	AB A	56 44
33	1.00	52	10.54 [3.46-18.80]	**33**	0.97	13.19 [7.53-19.64]	B|A_B	84	B AB	65 35
34	0.32	72	5.17 [0.55-11.21]	**34**	1.00	4.91 [0.74-9.94]	A|B	97	AB	100

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
35	0.26	\	5.70 [0.81-11.58]	**35**	0.56	10.64 [5.57-16.14]	B|B	89	B AB	72 28
36	0.43	71	2.84 [0.02-7.35]	**36**	1.00	3.8 [0.77-7.74]	B|B	100	B	100
37	0.79	\	18.02 [11.82-25.04]	**37**	0.98	0.89 [0.00-2.68]	B|B	100	B	100
38	0.97	47	13.19 [7.53-19.64]	**38**	0.45	8.13 [3.26-13.57]	B|B A_B|B	86 14	B AB	87 14
39	1.00	84	4.91 [0.74-9.94]	**39**	0.25	6.84 [1.72-11.72]	B|B	77	AB B	50 50
40	0.56	47	10.64 [5.57-16.14]	**40**	1.00	1.18 [0.00-3.88]	A|A_B	95	A AB	53 47
41	0.45	36	8.13 [3.26-13.57]	**41**	1.00	1.01 [0.00-3.21]	B|A_B B|B	55 45	B	95
42	1.00	100	1.01 [0.00-3.21]	**42**	0.41	14.74 [9.19-21.25]	A|A	100	A	100
43	0.32	\	6.43 [1.72-11.72]	**43**	0.14	11.50 [6.11-18.30]	A|A	99	A	100
44	1.00	99	1.18 [0.00-3.88]	**44**	0.92	7.62 [2.31-13.52]	A|A	90	A	100
45	1.00	100	3.80 [0.77-7.74]	**45**	1.00	0.99 [0.00-3.15]	A|B	99	AB	100
46	0.99	89	0.89 [0.00-2.68]	**46**	0.21	8.01 [1.06-12.32]	A|A	100	A	100
47	0.63	23	15.29 [9.19-21.25]	**47**	0.3	5.33 [0.79-11.03]	A|A	100	A	100
48	0.21	\	6.16 [1.06-12.32]	**48**	0.98	1.97 [0.00-6.07]	A|A	100	A	100
49	0.98	60	1.97 [0.00-6.07]	**49**	0.42	11.65 [6.91-17.43]	A|A	100	A	100
50	0.01	\	13.10 [8.50-17.01]	**50**	1.00	9.34 [3.87-12.27]	A|A	100	A	100
51	0.01	\	11.52 [6.11-18.30]	**51**	0.17	8.01 [1.06-12.32]	A|A	100	A	100
52	0.06	\	8.35 [2.48-14.63]	**52**	0.32	4.00 [0.45-7.74]	A|A	100	A	100
53	0.92	82	7.62 [2.31-13.52]	**53**	0.67	2.25 [0.03-6.19]	A|A	100	A	100
54	1.00	99	0.99 [0.00-3.15]	**54**	0.28	0.98 [0.00-3.88]	A|A	100	A	100

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
55	0.42	37	11.65 [6.91-17.43]	**55**	0.14	6.47 [2.79-10.56]	A|A	99	A	100
56	1.00	50	9.33 [4.82-14.20]	**56**	0.63	3.32 [0.59-6.81]	A|A	100	A	100
57	0.17	27	7.81 [3.87-12.27]	**57**	0.12	4.03 [0.45-7.74]	A|A	90	A	100
58	0.2	\	6.58 [2.96-10.71]	**58**	0.28	2.07 [0.00-5.51]	A|A	100	A	100
59	0.14	25	6.47 [2.79-10.56]	**59**	1.00	22.99 [15.02-31.36]	B|A_B B|B D|A_B_C_D_E D|A_B_D D|A_B_D_E D|A_B_C_D D|A_D C|A_B_C_D_E B_C_D_F|A D|A_B_C_E B_D_F|A B_D|A B|A_B_C_D_E	11 9 7 5 4 3 3 2 2 2 2 2 2	AD ABD BD AB ABCDE	76 7 4 3 2

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
60	0.12	\	4.03 [0.45-7.74]	**60**	0.94	18.99 [11.36-26.93]	A_B|A A_B_C_D_E |A A|A A_B_D_E|A A_B_D|A A_B_C_D|A A_B_C_E|A A_B_C|A A_B_E|A	19 15 11 7 7 6 5 4 4	A AB	84 11
61	0.28	\	2.07 [0.00-5.51]	**61**	0.98	15.05 [8.59-22.16]	B_C_D_E|A B_D_E|A B|A B_C_E|A B|A_B B_C_D|A B_D|A B_E|A B_C|A	22 9 7 7 7 7 6 6 5	A AB	55 41
62	0.63	68	3.32 [0.59-6.81]	**62**	0.82	10.36 [4.86-16.41]	A|A	90	A	99
63	0.67	\	2.25 [0.03-6.19]	**63**	0.01	9.19 [4.53-14.33]	A|A	88	A	100
64	0.31	\	1.01 [0.00-3.38]	**64**	0.66	6.48 [1.95-12.04]	A|A	100	A	100
65	1.00	29	22.99 [15.02-31.36]	**65**	0.71	3.73 [0.47-7.74]	A|A	100	A	100

Node No.	**BEAST**			**Node No.**	**BEAST**		**DEC**		**S-DIVA**	
(Fig. 7)	**PP**	**BP**	**Age in Ma [95% HPD interval]**	**(Fig. 8)**	**PP**	**Age in Ma**	**Ancestral Split**	**Relative probability [%]**	**Area**	**Relative probability [%]**
66	0.94	29	18.99 [11.36-26.93]	**66**	0.96	10.43 [4.86-16.41]	C_D_E|B D_E|B C_E|B C_D|B E|B	36 11 10 6 6	B ABCE ABE ABCDE ABDE ABC AB ABCD ABD	12 11 11 11 11 11 11 11 11
67	0.98	32	15.05 [8.59-22.16]	**67**	1.00	3.07 [0.03-7.25]	B|B	100	B	100
68	0.96	40	10.43 [4.86-16.41]	**68**	1.00	5.78 [1.53-10.34]	C_D|E D|E C|E	53 8 8	ABDE ABCE ABE ABCDE ACDE ADE ACE AE BCDE BE BCE BDE	8 8 8 8 8 8 8 8 8 8 8 8

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
69	1.00	78	5.78 [1.53-10.34]	**69**	1.00	1.55 [0.02-3.89]	D|C C_D|C	61 10	ABCD ACD BCD ABD ABC AD AC BC BD	15 15 14 10 9 9 9 9 9
70	1.00	66	1.55 [0.02-3.89]	**70**	0.35	0.61 [0.00-1.93]	D|D D|C C_D	62 12 12	ABD AD BD	34 34 32
71	0.32	63	0.61 [0.00-1.93]	**71**	0.99	15.48 [9.13-22.22]	B_D|D D|D B_D_F|D B_C_D|D B_C_D_F|D	31 24 6 6 5	D	88
72	1.00	87	3.07 [0.03-7.25]	**72**	0.15	11.75 [6.78-16.99]	D|D C_D|D	67 33	D CD	84 16
73	0.82	\	10.36 [4.56-16.68]	**73**	0.35	9.85 [3.64-16.40]	D|C D|D	81 16	CD	100
74	0.94	\	4.06 [0.24-9.29]	**74**	1.00	3.77 [0.47-8.33]	D|D	100	D	100

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
75	0.66	\	6.48 [1.95-12.04]	**75**	0.25	11.65 [6.78-16.99]	B_D|B B_D_F|B B_C_D_F|B B_C_D_F|C C_D|C	41 11 6 6 6	BD BDF CD BCD DF	53 13 12 12 8
76	0.71	\	3.73 [0.47-7.74]	**76**	0.31	6.77 [2.83-10.76]	B|C	96	BC	100
77	0.99	48	15.48 [9.13-22.22]	**77**	0.95	3.28 [0.57-6.56]	C|C	99	C	100
78	0.35	41	9.85 [3.64-16.40]	**78**	1.00	0.94 [0.00-2.75]	C|C	100	C	100
79	1.00	96	3.77 [0.47-8.33]	**79**	0.11	5.33 [0.79-11.03]	B|B	100	B	100
80	0.12	\	13.07 [7.28-18.78]	**80**	0.20	4.23 [0.24-9.29]	B|B	100	B	100
81	0.25	\	11.65 [6.78-16.99]	**81**	0.15	1.94 [0.00-5.02]	B|B	100	B	100
82	0.90	60	2.23 [0.04-5.55]	**82**	0.05	10.23 [3.51-17.80]	B_D|D B_C_D_F|D B_D_F|D B_C_D|D D|D C_D|D	40 16 15 12 6 6	D DF BDF	81 15 4
83	0.35	60	0.92 [0.00-2.93]	**83**	0.05	6.61 [1.06-12.32]	D|D	100	D	100
84	0.01	\	10.53 [6.54-15.13]	**84**	0.04	5.04 [1.17-9.47]	D|D	100	D	100
85	0.01	\	10.57 [6.80-16.69]	**85**	0.53	4.70 [1.40-8.73]	D|D	100	D	100
86	0.00	\	10.70 [6.35-17.44]	**86**	0.37	2.78 [0.27-6.04]	D|D	100	D	100
87	0.00	\	8.85 [5.15-12.69]	**87**	0.01	7.48 [2.36-13.52]	B_C_D_F|B B_D_F|B B_D|B B_C_D|B	33 25 24 11	BD	100

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
88	0.03	\	4.97 [1.17-9.47]	**88**	0.01	6.89 [3.11-11.40]	B_C_D_F|D B_D_F|D B_D|D B_C_D|D	38 28 17 8	DF	100
89	0.23	\	1.67 [0.00-5.03]	**89**	0.06	4.16 [0.24-9.29]	D|D	100	D	100
90	0.01	\	8.46 [3.05-12.90]	**90**	0.05	6.00 [0.94-12.63]	B_C_D|F B_D|F	53 37	BDF BCDF CDF DF BCF BF	23 23 20 20 8 6
91	0.31	34	6.77 [2.83-10.76]	**91**	0.90	2.23 [0.04-5.55]	B_C|D	87	BCD CD BD BC	42 27 24 7
92	0.15	37	1.94 [0.00-5.02]	**92**	0.32	0.94 [0.00-2.75]	C|B	99	BC	100
93	0.06	\	5.23 [2.12-8.77]	**93**	0.31	39.58 [28.96-49.89]	G|G F_G|G F|F	52 21 13	F G	76 23
94	0.13	\	3.31 [0.39-6.80]	**94**	0.79	34.58 [24.10-45.50]	G|G F_G|G	69 19	FG G	76 14
95	0.95	63	3.28 [0.57-6.56]	**95**	1.00	20.97 [11.45-31.59]	G|G	99	G	100
96	1.00	85	0.94 [0.00-2.75]	**96**	1.00	13.94 [6.62-22.70]	G|G	100	G	100
97	0.53	53	4.70 [1.32-8.97]	**97**	0.99	6.53 [1.42-12.71]	G|G	100	G	100

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
98	0.38	17	2.78 [0.27-6.04]	**98**	0.18	30.20 [20.13-42.34]	G|G F|F_G F|F F_G|F F_G|G	32 30 8 8 8	F	80
99	1.00	72	3.80 [0.14-10.10]	**99**	1.00	3.73 [0.47-7.74]	F|G	98	FG	100
100	0.38	41	1.10 [0.00-3.50]	**100**	0.37	27.78 [17.12-37.95]	F|F F|F_G H|G	38 29 10	F	82
101	0.90	\	28.95 [18.37-40.38]	**101**	1.00	9.15 [1.84-18.99]	G|F	93	FG	100
102	0.99	26	20.81 [11.01-31.92]	**102**	0.48	24.63 [15.25-34.22]	F|F H|H B|H B|F B_F|F	50 9 6 6 6	F BF BFH BH	69 18 10 3
103	0.98	74	11.52 [3.00-20.92]	**103**	0.9	17.52 [9.15-27.41]	H|F_H H|H	61 29	FH	97
104	0.85	70	3.54 [0.04-9.32]	**104**	0.98	11.47 [4.40-19.25]	F|F_H H|H F_H|H F_H|F H|F_H F|F	30 20 15 15 11 7	F	97
105	1.00	86	8.59 [2.72-15.37]	**105**	1.00	2.30 [0.03-5.85]	F_H|F F|F	75 24	F	98
106	0.90	61	2.33 [0.04-6.18]	**106**	1.00	5.83 [0.87-12.07]	H|H	100	H	100

Node No.	**BEAST**			**Node No.**	**BEAST**		**DEC**		**S-DIVA**	
(Fig. 7)	**PP**	**BP**	**Age in Ma [95% HPD interval]**	**(Fig. 8)**	**PP**	**Age in Ma**	**Ancestral Split**	**Relative probability [%]**	**Area**	**Relative probability [%]**
107	0.64	58	5.44 [1.33-10.68]	**107**	0.87	2.84 [0.19-6.58]	H|H	100	H	100
108	1.00	97	0.77 [0.00-2.45]	**108**	0.32	20.70 [12.35-29.68]	B_F|B B|B B_H|B	53 21 15	BF B	66 30
109	1.00	90	14.32 [5.55-24.70]	**109**	0.13	18.63 [8.97-28.54]	B|B_F B|B B|B_H B_F|B B_F|F B|B_F_H	25 20 14 12 12 7	F B	80 20
110	0.99	90	5.96 [0.94-12.63]	**110**	0.97	14.24 [7.37-21.86]	B|B B_F|B	62 30	BF	88
111	0.31	\	39.58 [28.96-49.89]	**111**	0.70	5.33 [0.79-11.03]	B|B	100	B	100
112	0.97	72	35.67 [26.10-46.76]	**112**	0.09	12.04 [6.78-16.99]	B|B B_F|F F|B_F B|B_F	50 23 7 6	F	96
113	0.99	27	25.07 [13.70-37.31]	**113**	0.95	6.19 [1.33-11.96]	B_F|F F|F	66 26	F	91
114	1.00	81	14.29 [6.19-23.58]	**114**	1.00	1.22 [0.00-3.88]	F|B	99	BF	100
115	0.65	50	10.16 [3.51-17.80]	**115**	0.10	11.04 [4.40-19.25]	B|B F|B_F F|F B_F|B	50 14 13 12	F	100

Node No.	**BEAST**			**Node No.**	**BEAST**		**DEC**		**S-DIVA**	
(Fig. 7)	**PP**	**BP**	**Age in Ma [95% HPD interval]**	**(Fig. 8)**	**PP**	**Age in Ma**	**Ancestral Split**	**Relative probability [%]**	**Area**	**Relative probability [%]**
116	0.96	83	5.92 [0.78-12.55]	**116**	0.99	7.56 [2.95-12.96]	B|B B|B_F	51 17	F BF FH BFH	38 26 24 13
117	1.00	72	30.52 [21.29-39.98]	**117**	0.29	6.14 [2.35-10.92]	B|B B_F|B	69 24	BFH BF FH	35 35 31
118	0.92	47	24.76 [14.96-34.74]	**118**	1.00	3.00 [0.43-6.24]	B_F|F	95	F BF	75 25
119	0.96	62	16.83 [5.45-27.79]	**119**	0.98	0.89 [0.00-2.65]	F|B	100	BF	100
120	1.00	97	14.92 [7.71-22.76]	**120**	0.95	6.90 [2.68-11.68]	B_F|F F|F B_E_F|F	50 22 20	F EF	67 29
121	1.00	80	6.37 [0.94-13.01]	**121**	0.07	5.73 [1.29-11.21]	B_E_F|F B_F|F F|F	38 37 19	EF F BEF	43 40 13
122	0.83	51	11.46 [5.87-18.01]	**122**	0.05	4.87 [1.32-8.97]	E_F|B F|B	72 22	EF BEF	58 33
123	0.49	\	6.20 [1.69-11.48]	**123**	0.04	4.48 [1.39-8.21]	E|F	84	EF	100
124	0.86	54	2.42 [0.02-6.28]	**124**	0.32	1.28 [0.00-3.88]	F|F	100	F	100
125	0.4	\	3.60 [0.21-7.99]	**125**	0.99	3.03 [0.72-6.10]	E|E	86	E	100
126	0.25	31	8.47 [3.86-13.63]	**126**	0.17	2.05 [0.32-4.37]	E|E	86	E	100
127	0.65	56	6.86 [3.11-11.40]	**127**	0.17	1.29 [0.03-3.13]	E|E	100	E	100
128	0.45	\	4.70 [1.40-8.73]	**128**	0.22	0.72 [3.11-11.40]	E|E	100	E	100

Node No.	BEAST			Node No.	BEAST		DEC		S-DIVA	
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]	(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
129	0.99	96	1.14 [0.00-3.49]	**129**	0.97	35.67 [26.10-46.76]	G|F G|G	67 13	FG	99
130	0.55	62	4.54 [1.39-8.21]	**130**	0.99	25.07 [13.70-37.31]	F|F	92	F	100
131	1.00	97	24.38 [16.79-32.41]	**131**	1.00	14.29 [6.19-23.58]	F|F	100	F	100
132	0.53	21	19.13 [11.22-26.88]	**132**	0.65	10.16 [3.51-17.80]	F|F	100	F	100
133	0.64	\	17.58 [10.03-26.11]	**133**	0.96	5.92 [0.78-12.55]	F|F	100	F	100
134	0.65	\	14.46 [7.23-21.91]	**134**	1.00	30.52 [21.29-39.98]	G|G G|D_G	74 18	G	99
135	0.36	\	12.25 [6.34-19.25]	**135**	0.92	24.76 [14.96-34.74]	D|G G|G	72 11	DG	100
136	0.01	\	10.11 [4.79-15.71]	**136**	0.96	16.84 [8.63-24.32]	G|G	99	G	100
137	1.00	98	6.37 [2.00-11.24]	**137**	1.00	14.92 [7.71-22.76]	D|D	78	D	100
138	0.93	77	3.81 [0.74-7.43]	**138**	0.09	10.75 [5.96-15.68]	D|D B_D|D A_D|D	67 12 12	D	100
139	0.34	34	4.98 [0.93-10.13]	**139**	1.00	6.37 [2.00-11.24]	D|B D|A D|A_B	31 31 31	ABD BD AD	33 33 33
140	0.4	\	21.71 [14.40-29.06]	**140**	0.56	9.56 [3.87-15.68]	D|D	100	D	100
141	0.23	\	18.96 [12.66-25.97]	**141**	0.49	6.20 [1.69-11.48]	D|D	100	D	100
142	0.12	\	16.14 [9.15-23.47]	**142**	0.40	3.60 [0.07-9.08]	D|D	100	D	100
143	0.26	\	14.07 [6.58-21.51]	**143**	0.86	2.42 [0.02-6.28]	D|D	100	D	100
144	1.00	93	7.39 [2.12-13.53]	**144**	0.64	6.86 [3.11-11.40]	D|D	100	D	100
145	0.66	\	3.63 [0.08-8.51]	**145**	0.55	4.54 [1.39-8.21]	D|D	100	D	100
146	0.99	81	3.75 [0.14-9.51]	**146**	0.45	4.70 [1.32-8.97]	D|D	100	D	100

Node No.	**BEAST**			**Node No.**	**BEAST**		**DEC**		**S-DIVA**	
(Fig. 7)	**PP**	**BP**	**Age in Ma [95% HPD interval]**	**(Fig. 8)**	**PP**	**Age in Ma**	**Ancestral Split**	**Relative probability [%]**	**Area**	**Relative probability [%]**
147	0.65	21	16.09 [10.08-22.30]	**147**	0.99	1.14 [0.00-3.49]	D|D	100	D	100
148	0.77	18	14.13 [8.50-20.37]	**148**	1.00	24.38 [16.79-32.41]	G|G	100	G	100
149	0.62	21	12.58 [7.53-18.26]	**149**	0.64	17.58 [10.03-26.11]	G|G	100	G	100
150	0.17	21	11.81 [7.11-17.44]	**150**	0.65	14.46 [7.23-21.91]	G|G	100	G	100
151	0.84	20	10.64 [5.96-15.68]	**151**	0.88	7.97 [3.26-13.57]	G|G	100	G	100
152	0.39	\	9.11 [4.53-14.33]	**152**	0.51	5.02 [0.74-9.94]	G|G	100	G	100
153	0.89	55	6.94 [2.43-11.72]	**153**	0.52	11.68 [5.34-17.85]	G|G	100	G	100
154	0.97	56	7.70 [3.25-12.90]	**154**	1.00	6.37 [0.94-13.01]	G|G	100	G	100
155	1.00	99	2.91 [0.53-6.10]	**155**	0.93	3.81 [0.74-7.43]	G|G	100	G	100
156	0.95	42	10.59 [3.91-17.39]	**156**	0.35	22.21 [8.59-37.18]	G|G	100	G	100
157	0.99	70	5.72 [1.29-11.21]	**157**	0.88	13.62 [6.14-20.85]	G|G	100	G	100
158	0.59	\	2.75 [0.07-6.70]	**158**	0.99	7.50 [2.36-13.52]	G|G	100	G	100
159	0.21	\	16.87 [8.63-24.32]	**159**	0.05	20.40 [12.35-29.68]	G|G	100	G	100
160	0.88	51	13.62 [6.14-20.85]	**160**	0.08	15.99 [6.83-27.21]	G|G	100	G	100
161	0.99	73	7.50 [2.36-13.52]	**161**	0.15	13.08 [7.28-18.78]	G|G	100	G	100
162	0.79	\	34.58 [24.10-45.50]	**162**	0.39	18.65 [6.88-31.13]	G|G	100	G	100
163	0.37	\	27.78 [17.12-37.95]	**163**	0.99	3.75 [0.14-9.51]	G|G	100	G	100
164	1.00	93	9.15 [1.84-18.99]	**164**	0.11	17.67 [9.15-27.41]	G|G	100	G	100
165	0.48	\	24.63 [15.25-34.22]	**165**	1.00	7.38 [2.12-13.53]	G|G	100	G	100
166	0.90	41	17.52 [9.15-27.41]	**166**	0.20	5.53 [2.15-9.33]	G|G	100	G	100
167	0.98	62	11.47 [4.40-19.25]	**167**	0.65	16.09 [10.08-22.30]	G|G	100	G	100
168	1.00	85	2.30 [0.03-5.85]	**168**	0.95	10.59 [3.91-17.39]	G|G	100	G	100
169	1.00	94	5.83 [0.87-12.07]	**169**	0.98	5.72 [1.29-11.21]	G|G	100	G	100

Node No.	BEAST		
(Fig. 7)	PP	BP	Age in Ma [95% HPD interval]
170	0.87	63	2.84 [0.19-6.58]
171	0.32	\	20.70 [12.35-29.68]
172	0.23	\	18.09 [8.97-28.54]
173	0.97	37	14.24 [7.37-21.86]
174	0.99	57	7.56 [2.95-12.96]
175	0.29	31	6.14 [2.35-10.92]
176	1.00	81	3.00 [0.43-6.24]
177	0.98	\	0.89 [0.00-2.65]
178	0.2	37	11.51 [5.34-17.85]
179	0.95	51	6.90 [2.68-11.68]
180	0.12	\	5.50 [2.15-9.33]
181	0.99	60	3.03 [0.72-6.10]
182	0.18	\	2.05 [0.32-4.37]
183	0.17	\	1.29 [0.03-3.13]
184	0.22	\	0.73 [0.00-2.17]
185	0.16	\	3.52 [0.57-7.11]
186	0.11	\	2.46 [0.18-5.51]
187	0.12	\	1.83 [0.05-4.57]
188	0.18	\	9.50 [3.87-15.68]
189	0.7	42	5.33 [0.79-11.03]
190	0.95	45	6.19 [1.33-11.96]
191	1.00	83	1.22 [0.00-3.88]
192	0.19	\	30.63 [20.13-42.34]

Node No.	BEAST		DEC		S-DIVA	
(Fig. 8)	PP	Age in Ma	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
170	0.16	3.94 [0.24-9.29]	G|G	100	G	100
171	0.77	14.13 [8.50-20.37]	G|G	100	G	100
172	0.62	12.58 [7.53-18.26]	G|G	100	G	100
173	0.21	12.02 [2.84-22.90]	G|G	100	G	100
174	0.84	10.64 [5.57-16.14]	G|G	100	G	100
175	0.97	7.70 [3.25-12.90]	G|G	100	G	100
176	1.00	2.91 [0.53-6.10]	G|G	100	G	100
177	0.39	9.11 [4.53-14.33]	G|G	100	G	100
178	0.89	6.94 [2.43-11.72]	G|G	100	G	100

Node No. **(Fig. 7)**	**BEAST**		
	PP	**BP**	**Age in Ma [95% HPD interval]**
193	1.00	74	20.97 [11.45-31.59]
194	1.00	84	13.94 [6.62-22.70]
195	0.99	77	6.53 [1.42-12.71]
196	1.00	97	3.73 [0.11-9.64]
197	1.00	96	55.87 [41.40-69.53]
198 fossil calibration	0.91	50	40.59 [25.00-57.08]
199	1.00	93	17.03 [4.98-30.70]
200	1.00	76	24.56 [11.03-39.53]
201	1.00	95	10.90 [2.37-21.27]
202	0.76	38	48.41 [33.47-63.39]
203	0.59	61	38.58 [23.19-55.49]
204	0.92	98	24.98 [14.01-36.93]
205	1.00	100	3.16 [0.08-8.20]
206	0.23	\	21.21 [11.39-32.70]
207	0.95	95	15.24 [5.85-25.36]
208	0.47	\	31.51 [14.03-50.34]
209	0.09	\	22.57 [7.28-39.62]
210	1.00	100	2.04 [0.02-5.53]
211	0.34	33	0.73 [0.00-2.34]
212	1.00	100	39.71 [18.15-62.87]
213	1.00	100	16.86 [5.22-30.56]

Appendix 6. Different Lagrange models were tested: (1) no, (2) only range, (3) only dispersal, and (4) range and dispersal constraints together. The different matrices used for these approaches are shown here. To define the area-dispersal matrix (dispersal constraints) we followed Buerki et al. (2011) using three categories: a low value (0.01) was given to non-neighbouring areas, a medium value (0.5) was given to areas which have been connected in former times or for which a floral relationship was proven, and a high value (1.0) was given to directly neighbouring areas. See Appendix 5 for area delineation.

(1) No constraints

Range matrix

	B	**C**	**D**	**E**	**F**	**G**	**H**	**I**
A	X	X	X	X	X	X	X	X
B		X	X	X	X	X	X	X
C			X	X	X	X	X	X
D				X	X	X	X	X
E					X	X	X	X
F						X	X	X
G							X	X
H								X

Dispersal matrix

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
A	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1
I	1	1	1	1	1	1	1	1	1

(2) Only range constraints

Range matrix

	B	**C**	**D**	**E**	**F**	**G**	**H**	**I**
A	X				X		X	
B					X		X	
C								
D					X	X		
E								
F							X	
G							X	
H								

Dispersal matrix

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
A	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1
I	1	1	1	1	1	1	1	1	1

(3) Only dispersal constraints

Range matrix

	B	**C**	**D**	**E**	**F**	**G**	**H**	**I**
A	X	X	X	X	X	X	X	X
B		X	X	X	X	X	X	X
C			X	X	X	X	X	X
D				X	X	X	X	X
E					X	X	X	X
F						X	X	X
G							X	X
H								X

Dispersal matrix

	A	B	C	D	E	F	G	H	I
A	1	1	0.01	0.01	0.01	0.01	0.01	1	0.01
B	1	1	0.5	0.01	0.01	0.5	0.01	1	0.01
C	0.01	0.5	1	0.5	0.01	0.01	0.01	0.01	0.01
D	0.01	0.01	0.5	1	0.01	0.01	0.5	0.01	0.01
E	0.01	0.01	0.01	0.01	1	0.01	0.01	0.01	0.01
F	0.01	0.5	0.01	0.01	0.01	1	1	0.5	0.01
G	0.01	0.01	0.01	0.5	0.01	1	1	0.01	0.01
H	1	1	0.01	0.01	0.01	0.5	0.01	1	0.01
I	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1

(4) Range and dispersal constraints

Range matrix

	B	C	D	E	F	G	H	I
A	X				X		X	
B					X		X	
C								
D					X	X		
E								
F							X	
G							X	
H								

Dispersal matrix

	A	B	C	D	E	F	G	H	I
A	1	1	0.01	0.01	0.01	0.01	0.01	1	0.01
B	1	1	0.5	0.01	0.01	0.5	0.01	1	0.01
C	0.01	0.5	1	0.5	0.01	0.01	0.01	0.01	0.01
D	0.01	0.01	0.5	1	0.01	0.01	0.5	0.01	0.01
E	0.01	0.01	0.01	0.01	1	0.01	0.01	0.01	0.01
F	0.01	0.5	0.01	0.01	0.01	1	1	0.5	0.01
G	0.01	0.01	0.01	0.5	0.01	1	1	0.01	0.01
H	1	1	0.01	0.01	0.01	0.5	0.01	1	0.01
I	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1

Appendix 7. Erklärung zu Kapitel 3 „Dispersal routes between biodiversity hotspots in Asia: the case of the mountain genus *Tripterospermum* (Gentianinae, Gentianaceae) and its close relatives".

Erklärung über Anteile der Autoren/Autorinnen an den einzelnen Kapiteln der Promotionsarbeit

Titel der Publikation/ des Manuskripts:

	Was hat der/die Promovierende bzw. was haben die Co-Autoren/Autorinnen beigetragen[#]	Name des/der jeweiligen Autors/Autoren/Autorin*
(1) Entwicklung und Planung	S.M., A.F. und A.N.M.-R. haben gemeinsam das Projekt geplant.	Sabine Matuszak (33.3%) Adrien Favre (33.3%) Alexandra N. Muellner-Riehl (33.3%)
(2) Durchführung der einzelnen Untersuchungen/ Experimente	S.M. hat die DNA Extraktionen durchgeführt und die Markerregionen (ITS, *atpB-rbcL* , *trnL-trnF*) gewonnen. Sie hat außerdem die phylogenetischen und biogeografischen Analysen vorgenommen.	Sabine Matuszak (100%)
(3) Erstellung der Datensammlung und Abbildungen	S.M. und A.F. haben die Daten gesammelt. Die Abbildungen wurden von S.M. gemacht, wobei A.F. Verbesserungsvorschläge zu den Abbildungen geliefert hat. H.S. hat viele Pflanzenproben (Blätter) aus dem Herbarium Kunming bereitgestellt.	Sabine Matuszak (80%) Adrien Favre (15%) Hang Sun (5%)
(4) Analyse/Interpretation der Daten	S.M. hat die Daten analysiert und ausgewertet. A.F. und A.N.M.-R. haben bei der Interpretation geholfen.	Sabine Matuszak (80%) Adrien Favre und Alexandra N. Muellner-Riehl (20%)
(5) übergeordnete Einleitung/ Ergebnisse/Diskussion	S.M. hat die erste Version des Manuskripts eigenständig verfasst. A.F. und A.N.M.-R. haben Verbesserungsvorschläge gemacht und korrigiert. H.S. hat kleinere Korrekturen am fertigen Manuskript gemacht.	Sabine Matuszak (80%) Adrien Favre und Alexandra N. Muellner-Riehl (18%) Hang Sun (2%)

[#] Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben

*Mehrfacheintragungen möglich

Als Autoren/Autorinnen werden solche Personen bezeichnet, die an der Arbeit in Bezug auf die genannten Punkte in einer Weise mitgewirkt haben, dass sie für die ausgewiesenen Passagen (mit) verantwortlich sind. Personen, die an der Arbeit mitgewirkt haben, jedoch nicht in diese Kategorie fallen, sollten in der Danksagung Erwähnung finden.

Datum/Ort

Datum
zustimmende Bestätigung der vorgenannten Angaben

Unterschrift Promovend/Promovendin

Unterschrift Betreuer/Betreuerin

Appendix 8. List of species of Gentianaceae included in this study (Appendix 8.1). Information on DNA extraction, PCR amplification, and sequencing (Appendix 8.2). Information on sequence handling, and phylogenetic analysis (Appendix 8.3).

Appendix 8.1 List of species of Gentianaceae (with authorities) included in this study, and GenBank accession numbers for all sequences. Sequences newly obtained are indicated by an *, missing sequences are indicated by a /. Outgroups are indicated by a ~ before species names.

Name	ITS	*trnL-trnF*	*atpB-rbcL*
Helieae			
~*Chelonanthus alatus* (Aubl.) Pulle	EU709790	AF102396	/
~*Helia oblongifolia* Mart.	EU709794	/	/
~*Macrocarpaea glabra* (L.f.) Gilg	EU528098	EU528060	EU528171
~*Symbolanthus frigidus* (Sw.) Struwe & K.R. Gould	EU709802	/	/
~*Tachia grandiflora* Maguire & Weaver	DQ401418	/	/
Gentianeae/Gentianinae			
Crawfurdia angustata C.B. Clarke	GU251013	KF563978	GU250971
C. campanulacea Wall. & Griff. ex C.B. Clarke	GU251014	KF563979	GU250972
C. crawfurdioides (Marquand) C.J. Wu	KJ570863*	KJ570877*	/
C. delavayi Franch.	GU251015	AY563391	GU250973
C. dimidiata (Marquand) H. Sm.	KJ570864*	KJ570878*	/
C. gracilipes H. Sm.	GU251016	/	GU250974
C. maculaticaulis C.Y. Wu ex C.J. Wu	GU251017	KF563980	GU250975
C. poilanei Hul	GU251018	KJ570879*	GU250976
C. pricei (Marquand) H. Sm.	GU251019	KF563981	GU250977
C. puberula C.B. Clarke	KJ570865*	/	/
C. sessiliflora (Marquand) H. Sm.	KJ570866*	KJ570880*	GU250978

Name	ITS	*trnL-trnF*	*atpB-rbcL*
C. speciosa C.B. Clarke	GU251021	KF564004	GU250979
~*Gentiana acaulis* L.	AJ223108, AJ223109	JF748586	/
~*G. algida* Pall.	GU251024	DQ398729	DQ398622
~*G. alpina* Vill.	AJ223128, AJ223126	JF748587	/
~*G. asclepiadea* L.	GU251025	AJ580515	GU250980
~*G. crassicaulis* Duthie ex Burkill	DQ398638	DQ398711	DQ398603
~*G. cruciata* L.	DQ398635	DQ398713	DQ398600
~*G. futtereri* Diels & Gilg	DQ398658	DQ398730	DQ398623
~*G. lutea* L.	DQ358878	EU370935	EU370939
~*G. manshurica* Kitag.	GQ864018	GQ864091	/
~*G. oreodoxa* H. Sm.	DQ398657	DQ398728	DQ398621
~*G. parryi* Engelm.	X85370, Z48096	DQ398731	DQ398624
~*G. phyllocalyx* C.B. Clarke	KF563961	KF563996	/
~*G. straminea* Maxim.	DQ398631	DQ398705	DQ398596
~*G. verna* L.	GU251028	JF748712	GU250984
~*G. waltonii* Burkill	DQ398628	DQ398708	DQ398593
~*Kuepferia damyonensis* (Marquand) Favre	KF563951	KF563987	/
~*K. decorata* (Diels) Favre	KF563970	KF564008	GU250981
~*K. doxiongshangensis* (T.N. Ho) Favre	KF563952	KF563988	/
~*K. otophora* (Franchet in Forbes & Hemsley) Favre	KF563957	/	/
~*K. otophoroides* (H. Smith) Favre	KF563959	KF563994	/
Metagentiana australis (Craib) T.N. Ho & S.W. Liu	GU251029	KF564010	GU250986
M. eurycolpa (C. Marquand) T.N. Ho & S.W. Liu	KF563972	KF564011	/
M. gentilis (Franch.) T.N. Ho & S.W. Liu	AY562177	KF564012	GU250987
M. leptoclada (Balf.f. & Forrest) T.N. Ho & S.W. Liu	KF563973	KF564013	/
M. primuliflora (Franch.) T.N. Ho & S.W. Liu	AY562178	AY563385	GU250988
M. pterocalyx (Franch.) T.N. Ho & S.W. Liu	KF563974	KF564014	/
M. rhodantha (Franch.) T.N. Ho & S.W. Liu	AY562174	KF564015	GU250989
M. serra (Franch.) T.N. Ho, S.W. Liu & S.L. Chen	AY562175	KF564016	GU250990
M. villifera (H.W. Li ex T.N. Ho) T.N. Ho & S.W. Liu	KF563975	KF564019	/
*Sinogentiana souliei (*Franch.) Favre & Y.-M. Yuan	AY562170	KF564017	GU250991
S. striata (Maximowicz) Favre & Y.-M. Yuan	AY562173	KF564018	GU250992

Name	ITS	*trnL-trnF*	*atpB-rbcL*
Tripterospermum alutaceifolium (Liu & Kuo) J. Murata	GU251037	KJ570881*	GU250994
T. australe J. Murata	GU251038	/	GU250995
T. brevidentatum Hul	KJ570867*	/	/
T. championii Gardner	GU251039	/	GU250996
T. chinense (Migo) H. Smith	GU251040	KF564022	GU250997
T. cordatum (C. Marquand) H. Smith	GU251055	KF564023	GU251012
T. cordifolioides J. Murata	GU251041	KF564024	GU250998
T. cordifolium (Yamam.) Satake	GU251042	KJ570882*	GU250999
T. discoideum (C. Marquand) H. Smith	KJ570868*	/	/
T. distylum J. Murata & Yahara	KJ570869*	/	/
T. fasciculatum (Wall.) Chater	KJ570870*	/	/
T. hirticalyx C.Y. Wu & C.J. Wu	GU251043	KJ570883*	GU251000
T. japonicum Maxim.	KJ570871*	KJ570884*	/
T. lanceolatum (Hayata) H. Hara ex Satake	GU251044	KF564025	GU251001
T. lilungshanensis C.H. Chen & J.C. Wang	KJ570872*	KJ570885*	/
T. luteoviride (C.B. Clarke) J. Murata	GU251045	KF564026	GU251002
T. luzonense (Vidal) J. Murata	GU251046	KF564027	GU251003
T. maculatum Favre, Matuszak & Muellner-Riehl	KJ570873*	KJ570886*	KJ570859*
T. membranaceum (C. Marquand) H. Smith	KJ570874*	KJ570887*	KJ570860*
T. microphyllum Hul	GU251047	/	GU251004
T. nienkui (C. Marquand) C.J. Wu	GU251048	KJ570888*	GU251005
T. nigrobaccatum H. Hara	GU251049	KJ570889*	GU251006
T. pallidum H. Smith	GU251050	KF564028	GU251007
T. pinbianense C.Y. Wu & C.J. Wu	GU251051	KJ570890*	GU251008
T. robustum H. Smith ex Hul	GU251052	KF564029	GU251009
T. sumatranum J. Murata	KJ570875*	KJ570891*	KJ570861*
T. taiwanense (Masam.) Satake	GU251053	KJ570892*	GU251010
T. tanatorajanense Favre, Matuszak & Muellner-Riehl	KJ570876*	KJ570893*	KJ570862*
T. trinerve Blume	GU251054	KJ570894*	GU251011
T. volubile (D. Don) H. Hara	AY858667	/	/
Gentianeae/Swertiinae			

Name	ITS	*trnL-trnF*	*atpB-rbcL*
~*Gentianella turkestanorum* (Gand.) Holub	AJ294689, AJ294629	EU370936	EU370941
~*Halenia elliptica* D. Don	JX569822	KJ570895*	/
~*Megacodon stylophorus* (C.B. Clarke) H. Smith	AJ294636, AJ294696	KJ570896*	/
~*Swertia bimaculata* (Siebold & Zucc.) Hook.f. & Thomson ex C.B. Clarke	JF978820	KJ570897*	/
~*S. punicea* Hemsl.	AJ410348, AJ318569	KJ570898*	/
~*Veratrilla baillonii* Franch.	KF563977	KF564030	/
Potalieae			
~*Anthocleista grandiflora* Gilg	AJ489864	AY251777	/
~*Bisgoeppertia scandens* (Spreng.) Urb.	FJ232556	/	/
~*Fagraea elliptica* Roxb.	FJ232579	JX217753	/
~*Lisianthius jefensis* A. Robyns & T.S. Elias	EU709782	/	/
~*L. nigrescens* Schltdl. & Cham.	FJ232564	/	/
~*Neurotheca loeselioides* (Spruce ex Progel) Baill.	FJ232570	/	/
~*Pycnosphaera buchananii* (Baker) N.E. Br.	KC535863	/	/

Appendix 8.2 DNA extraction, amplification, and sequencing.

Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions with one minor modification: leaf samples were incubated in lysis buffer and RNase for two hours instead of one. We amplified two plastid regions (*atpB-rbcL; trnL-trnF*) and one nuclear region (ITS), all of which had been proven to be informative in previous phylogenetic studies of Gentianaceae (Chassot et al., 2001; Favre et al., 2010; Favre et al., 2014). All PCR reactions were conducted in a Thermo Scientific Arktik Thermal Cycler (Thermo Fisher Scientific Oy, Vantaa, Finland). We amplified the *atpB-rbcL* spacer with the primer pair S2R and RBCL1 (Hoot et al., 1995), the *trnL-trnF* region with the primers trnLF c, d, e and f

(Taberlet et al., 1991), and ITS with 17SE_m and 26SE_m (Grudinski et al., 2014a; modified after Sun *et al.*, 1994). PCR procedures were standard, as described in Favre et al. (2014) for *trnL-trnF*, and as in Muellner et al. (2005) for ITS (but the primer annealing temperature was 53°C for ITS). In case amplification failed, ITS1 and ITS2 were amplified separately using the following newly created internal primers: 17SE_m (Grudinski et al., 2014a) and ITS_middle_R1, 5'-CAACTTGCGTTCAAAGACTCG (designed by M. Grudinski in 2013, unpublished), and the ITS2 region using the primer pair ITS_middle_F1, 5'-GATACTTGGTGTGAATTGCAGAATC (designed by M. Grudinski in 2013, unpublished), and 26SE_m (Grudinski et al., 2014a). For ITS1 and ITS2 the same PCR protocol was used as mentioned above.

For *atpB-rbcL*, the Phusion® High-Fidelity DNA Polymerase (New England BioLabs GmbH, Frankfurt/Main, Germany) was used. The reaction mix of 25 µL included 0.3 µL Phusion Taq, 5 µL 5x Phusion HF Buffer, 11.7 µL distilled water, 0.6 µL BSA (10 mg/mL; New England BioLabs GmbH, Frankfurt/Main, Germany), 0.8 µL DMSO (Carl ROTH GmbH, Essen, Germany), 2 µL dNTPs (2.5 mM each; Thermo Fisher Scientific Oy, Vantaa, Finland), 2 µL of genomic DNA (3-6 µg/mL), and 1.3 µL of each primer (10 µM). The PCR reaction was conducted with template denaturation at 98°C for 30 s, followed by 35 cycles of denaturation at 98°C for 10 s, primer annealing at 52°C for 30 s, primer extension at 72°C for 30 s, and a final extension step at 72°C for 10 min. All PCR products were cleaned with a NukleoSpin® Gel and PCR clean up kit (Macherey-Nagel GmbH & Co. KG, Dueren, Germany) according to the manufacturer’s protocol, and sequencing reactions were run on an ABI 3130xl Genetic Analyzer using Big Dye terminator 3.1 chemistry (Applied Biosystems, Inc., Warrington, Cheshire, UK).

Appendix 8.3 Sequence handling and phylogenetic analysis.

Sequences were assembled and aligned using Geneious 5.4.2 (Drummond et al., 2011) with the multiple alignment software CLUSTALW (Thompson et al., 1994). The alignment required only minor manual corrections. For the analysis, ambiguously aligned regions of mono- and polynucleotide repeats were excluded. New sequences have been deposited in GenBank under the accession numbers KJ570859–KJ570898 (*http://www.ncbi.nlm.nih.gov/*).

Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were conducted individually for each gene region (*atpB-rbcL*, *trnL-trnF* and ITS) as well as in combination because no strongly supported (> 85% bootstrap percentage, BP; > 0.9 Bayesian posterior probability, PP) topological conflicts were detected between separately analysed data sets. ML analyses were performed using the graphical front-end raxmlGUI 1.3 (Silvestro & Michalak, 2012) for RAXML 7.4.2 (Stamatakis, 2006). The best model of sequence evolution, based upon the AIC and BIC criterion using jModeltest 2.1.2 (Guindon & Gascuel, 2003; Posada, 2008), was GTR+G+I for *trnL-trnF*, ITS and the combined dataset, and GTR+G for *atpB-rbcL*. The combined dataset was analysed using only one substitution model, because choosing different models was not possible in raxmlGUI 1.3. Statistical support was estimated via bootstrap analysis with 1000 replicates (Felsenstein, 1985).

BI analyses were performed using MrBayes 3.2.1 (Huelsenbeck & Ronquist, 2001) with the appropriate substitution model for each partition. Four runs with four Markov chains each (one cold chain and three heated ones) were started simultaneously from independent random trees. Every 3000th generation was sampled for a maximum of 30 million generations. The average standard deviation of split frequencies was 0.002961 (< 0.01), indicating convergence. Additionally, convergence of the parameters of the four individual runs was assessed by Tracer 1.5 (Rambaut & Drummond, 2007) and by examining the cumulative posterior probabilities of clades using the *Are We There Yet?* (AWTY; Wilgenbusch et al., 2004; Nylander et al., 2008) online programme. Based on the outcome we discarded the first 10% of the sampled trees as burn-in and combined the four runs using MrBayes 3.2.1. Using the remaining trees, a majority rule consensus tree was computed to calculate Bayesian posterior probabilities.

Appendix 9. Fossils and priors for divergence time estimation (Appendix 9.1). Information about area delineation for biogeographical analyses, and range as well as area-dispersal matrices used for different Lagrange models (Appendix 9.2). Clade support, divergence time estimates of Gentianaceae taxa, and ancestral area reconstruction using dispersal-extinction-cladogenesis (DEC) and statistical dispersal-vicariance (S-DIVA) models (Appendix 9.3).

Appendix 9.1 Fossils and priors for divergence time estimation.

To determine the best fitting speciation prior for the branching rates (Yule or Birth-Death), we used Beast 1.7.5 and Tracer 1.5 to estimate the marginal likelihood for each model by calculating the harmonic mean of the sampled likelihoods from an MCMC chain with 1000 bootstrap replicates (Newton & Raftery, 1994; Suchard et al., 2001). The difference of the logarithmic marginal likelihoods of the two models yields the logarithmic Bayes Factor, which was interpreted as described in Kass & Raftery (1995). The Yule and the Birth-Death model were compared under the conditions of an uncorrelated relaxed molecular clock and GTR+G+I. The logarithmic Bayes Factor was 4.255, which is positive evidence against the Birth-Death prior. Therefore, we used the Yule prior as tree model. A likelihood ratio test conducted in MEGA6 (Tamura et al., 2013) rejected the null hypothesis of an equal evolutionary rate throughout the tree. Therefore, the analysis was performed using an uncorrelated relaxed molecular clock model (Drummond et al., 2006) with rate variation log-normally distributed on each branch in the tree.

Reliable fossil records for Gentianaceae are scarce. In our analysis, we refrained from considering fossils for which attribution to Gentianaceae is uncertain (e.g., *Pistillipollenites macgregorii* from the lower Eocene; Crepet & Daghlian, 1981; Stockey & Manchester, 1988; Struwe & Albert, 2002). We refrained from applying secondary calibration because errors of the first analysis may be propagated to the second (Graur & Martin, 2004; Renner, 2005). Instead, we used the following fossils for setting temporal constraints: (1) pollen of *Lisianthius* (Potalieeae) from the Eocene (Graham, 1984), and (2) seeds of *Gentiana* from the Pliocene (Reid, 1920). Graham (1984) assigned the age of the *Lisianthius* pollen confidently to the Late Eocene, but Middle Eocene sediments might also be included. To account for this possibility, we decided to constrain the stem age of *Lisianthius* (corresponding to the crown age of Potalieeae) with a log-normal prior composed of an offset of 33.6 Myr (giving a hard minimum bound at the end of the Eocene), a mean of 0.7, and a standard deviation of 1.0 (median: 36.61, 2.5%: 33.88, 97.5%: 47.9; covering the time span of the Late Eocene as well as the Middle Eocene, and allowing a declining probability to be older). The only certain fossil of *Gentiana* is 2.6 Myr old, which is clearly underestimating the genus' true age (Favre et al., 2010; Merckx et al., 2013). As to our knowledge there is no other evidence we could deduce a constraint from, the most conservative approach was to invoke a uniform prior on *Gentiana's* stem, with a hard minimum bound at 2.6 Myr and an extreme upper bound, which would have no or only negligible influence on the analysis. We decided to define this upper boundary as 200 Myr, providing practically no limitation (we also performed an additional analysis using 150 Myr, which is closer to the presumed age of the oldest angiosperms, but this did not change the results; data not shown).

Additionally, we tested the effect of missing data on the estimation of branch lengths of the Beast analysis. Our data are indeed slightly unbalanced with 91, 72 and 53 sequences for ITS, *trnL-trnF*, and *atpB-rbcL* respectively. According to Lemmon et al. (2009), missing data could have an influence on the analysis by reducing the estimated ages. To test the effect, we randomly removed 30% of our sequence data, keeping the number of taxa for a better comparison of the nodes, and repeated the analysis as described above. Despite the missing data we obtained very similar estimated ages. We are therefore confident that missing sequences did not affect our estimated ages, similarly to Wiens & Morrill (2011), who detected only a minor influence (if any) of missing data on their results.

Appendix 9.2 Information about area delineation for biogeographical analyses, and range as well as area-dispersal matrices used for different Lagrange models. Likelihood ratio test for different Lagrange models.

We defined the areas as follows: (A) North America, Europe and arctic/temperate Asia, (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), (C) the rest of China, (D) Indochina [Thailand, Laos, Vietnam, Cambodia, China (Xishuangbanna), southwestern Myanmar], (E) Sundaland [Borneo, Sumatra, Java, Malay Peninsula], (F) Wallacea [Sulawesi, the Moluccas, the Banda Arc, and the Lesser Sunda islands], (G) the Philippines, (H) Japan, and (I) Taiwan. Our area delineation primarily follows climatic regions (tropical for areas DEFG, subtropical for areas BCI and temperate for areas AH). This coarse delineation is further refined based upon permanent or intermittent geographic barriers such as water bodies. Generally, our delineation follows geological regions, except for area B (southeastern fringe of the QTP), into which we included regions directly bordering the QTP. As suggested by Chacon & Renner (2014), we tested different approaches for Lagrange by varying constraints as follows: (1) no constraints, (2) range constraints, (3) dispersal constraints, and (4) range as well as dispersal constraints. To define the range constraints, we used an adjacency matrix, which is based on the assumption that organisms disperse more likely to adjacent areas than to non-adjacent areas (Chacon & Renner, 2014). For this reason, we allowed the combination of ABCDEHI, because these areas are or have been connected in former times, but we did not allow the combination of G and F with any other area, because these areas have been rather isolated (Hall, 2009). To define the dispersal constraints, we used an area-dispersal matrix, which specifies the probability to disperse from one area to the other (Chacon & Renner, 2014). Following Buerki *et al.* (2011), we used three categories: a low value (0.01) was given to non-neighbouring areas (G and F), a medium value (0.5) was given to areas which have been connected in former times or for which a strong floristic relationship has been proven (e.g., DE, IC, HC, HI), and a high value (1.0) was given to directly neighbouring areas (ABCD).

(1) No constraints

Range matrix

	B	C	D	E	F	G	H	I
A	X	X	X	X	X	X	X	X
B		X	X	X	X	X	X	X
C			X	X	X	X	X	X
D				X	X	X	X	X
E					X	X	X	X
F						X	X	X
G							X	X
H								X

Dispersal matrix

	A	B	C	D	E	F	G	H	I
A	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1
I	1	1	1	1	1	1	1	1	1

(2) Only range constraints

Range matrix

	B	C	D	E	F	G	H	I
A	X	X					X	
B		X	X					
C			X				X	X
D				X				
E								
F								
G								
H								

Dispersal matrix

	A	B	C	D	E	F	G	H	I
A	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1
I	1	1	1	1	1	1	1	1	1

(3) Only dispersal constraints

Range matrix

	B	C	D	E	F	G	H	I
A	X	X	X	X	X	X	X	X
B		X	X	X	X	X	X	X
C			X	X	X	X	X	X
D				X	X	X	X	X
E					X	X	X	X
F						X	X	X
G							X	X
H								X

Dispersal matrix

	A	B	C	D	E	F	G	H	I
A	1	1	1	0.01	0.01	0.01	0.01	0.5	0.01
B	1	1	1	1	0.01	0.01	0.01	0.01	0.01
C	1	1	1	1	0.01	0.01	0.01	0.5	0.5
D	0.01	0.01	0.01	1	0.5	0.01	0.01	0.01	0.01
E	0.01	1	1	0.5	1	0.01	0.01	0.01	0.01
F	0.01	0.01	0.01	0.01	0.01	1	0.01	0.01	0.01
G	0.01	0.01	0.01	0.01	0.5	0.01	1	0.01	0.01
H	0.5	0.01	0.5	0.01	0.01	0.01	0.01	1	0.5
I	0.01	0.01	0.5	0.01	0.01	0.01	0.01	0.5	1

(4) Range and dispersal constraints

Range matrix

	B	C	D	E	F	G	H	I
A	X	X					X	
B		X	X					
C			X				X	X
D				X				
E								
F								
G								
H								

Dispersal matrix

	A	B	C	D	E	F	G	H	I
A	1	1	1	0.01	0.01	0.01	0.01	0.5	0.01
B	1	1	1	1	0.01	0.01	0.01	0.01	0.01
C	1	1	1	1	0.01	0.01	0.01	0.5	0.5
D	0.01	0.01	0.01	1	0.5	0.01	0.01	0.01	0.01
E	0.01	1	1	0.5	1	0.01	0.01	0.01	0.01
F	0.01	0.01	0.01	0.01	0.01	1	0.01	0.01	0.01
G	0.01	0.01	0.01	0.01	0.5	0.01	1	0.01	0.01
H	0.5	0.01	0.5	0.01	0.01	0.01	0.01	1	0.5
I	0.01	0.01	0.5	0.01	0.01	0.01	0.01	0.5	1

Additionally, we tested which of these four models described above fitted our data best, by conducting a Likelihood ratio test in R (R Core Team, 2013), using the BioGeoBears package (Matzke, 2013). The likelihood ratio is between 0 and 1. A value closer to 0 means that the more complex model is preferred, while a value closer to 1 means that there is no change of the likelihood.

DEC	No Constraints	Range Constraints	Dispersal Constraints	Range and Dispersal Constraints
Log-likelihood	209.579	209.579	207.693	207.693
number of parameters	0	36	36	72

	No Constraints	Range Constraints	Dispersal Constraints
No Constraints	/	/	/
Range Constraints	1	/	/
Dispersal Constraints	1	1	/
Range and Dispersal Constraints	1	1	1

The pairwise comparison of the four different models showed no change of the likelihood. Each model was therefore found to fit our data as good as the other models.

Appendix 9.3 Clade support (posterior probabilities, PP, and bootstrap percentages, BP), divergence time estimates of Gentianaceae taxa, and ancestral area reconstruction using dispersal-extinction-cladogenesis (DEC) and statistical dispersal-vicariance (S-DIVA) models. Node numbers refer to Fig. 10 and Fig. 11, respectively. For reconstructions by DEC, a vertical line (e.g. B|G) splits into upper branches (left) and lower branches (right). Eight areas were defined as follows: (A) North America, Europe and arctic Asia, (B) southeastern fringe of the QTP [Yunnan (excluding Xishuangbanna), Sichuan, South and East Tibet, Northern Myanmar, Nepal, Bhutan, North India], (C) Rest of China, (D) Indochina [Thailand, Laos, Vietnam, Cambodia, China (Xishuangbanna), southwestern Myanmar], (E) Sundaland [Borneo, Sumatra, Java, Malay Peninsula], (F) Wallacea [Sulawesi, the Moluccas, the Banda Arc, and the Lesser Sunda islands], (G) Philippines, (H) Japan, and (I) Taiwan.

Node No.	**Maximum Likelihood**	**BEAST**		**Node No.**	**BEAST**		**DEC**		**S-DIVA**	
(Fig. 10)	**BP**	**PP**	**Age in Ma [95% HPD interval]**	**(Fig. 11)**	**PP**	**Age in Ma [95% HPD interval]**	**Ancestral Split**	**Relative probability [%]**	**Area**	**Relative probability [%]**
1	100	1	46.88 [33.91-60.91]	1	1	28.65 [17.79-39.76]	B|A_B	41	ABCI	30
							B|B	31	AB	26
							B|A	10	B	23
							B|A_B_C	7	ABC	21
2	100	1	43.26 [26.73-59.53]	2	1	18.80 [11.32-26.80]	A_B|A	50	ABCI	25
							B|A	22	ABC	19
							A_B_C |A	13	AB	18
									A	15
									ACI	12
									AC	11

Node No.	Maximum Likelihood	BEAST		Node No.	BEAST		DEC		S-DIVA	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]	(Fig. 11)	PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
3	100	1	36.97 [22.53-50.75]	3	1	12.37 [6.99-18.10]	A|A	100	A	100
4	100	1	17.21 [0.95-25.15]	4	0.68	10.85 [6.04-16.33]	A|A	100	A	100
5	71	0.93	14.37 [8.01-21.04]	5	1	5.88 [2.20-9.82]	A|A	100	A	100
6	92	1	9.19 [4.41-14.66]	6	1	7.67 [3.59-12.22]	A|A	100	A	100
7	79	0.99	11.39 [6.01-17.33]	7	0.45	17.00 [10.06-24.66]	A_B|B	40	AB	21
							B|B	22	ABCI	21
							A|B	12	BCI	21
							A_B_C |B	11	B	16
							A_C |B	6	BC	16
									ACI	2
									ABC	2
									CI	2
8	81	0.99	8.01 [3.55-13.10]	8	0.47	16.20 [9.50-23.57]	A|A_B	20	A	27
							B|B	19	B	18
							A|A	11	C	17
							B|A_B	10	BCI	10
							A_B|A	5	ABCI	10
							A|A_B_C	4	CI	10
							A_B|B	3	ACI	10
							C|A_B_C	3		

Node No.	Maximum Likelihood	BEAST		Node No.	BEAST		DEC		S-DIVA	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]	(Fig. 11)	PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
9	100	1	28.65 [17.79-39.76]	9	1	11.28 [0.61-17.52]	B|A	52	ABC	35
							B_C|A	32	AB	35
							C|A	16	AC	29
10	99	1	24.89 [15.80-35.09]	10	1	1.80 [0.50-3.41]	B|C	100	BC	100
11	100	1	16.68 [10.29-23.14]	11	1	4.67 [2.24-7.46]	A_B|A	16	A	16
							B|A_C_I	12	ABCI	15
							A_B_C|A	12	BCI	15
							A_B_C|C	7	AB	12
							C|A_C_I	5	BC	10
							C|A_C	5	C	9
							A|A	5	ABI	7
							B|A_C	5	ABC	7
							B|A_I	5	BI	7
							B_C|C	4		
							A|A_C_I	4		
							A|A_C	3		
							A|A_I	3		

Node No. (Fig. 10)	Maximum Likelihood	BEAST		Node No. (Fig. 11)	BEAST		DEC		S-DIVA	
	BP	PP	Age in Ma [95% HPD interval]		PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
12	61	0.95	14.85 [9.20-20.90]	12	0.75	3.48 [1.54-5.71]	A_B|B	28	B	36
							A_B_C|B	23	AB	25
							B|B	19	ABC	18
							C|C	7	C	11
							B_C|B	5	BC	11
							A_C|C	5		
							A_B_C|C	4		
13	94	1	11.30 [6.62-16.29]	13	1	1.38 [0.25-2.77]	B|B	64	B	72
							B_C|B	36	BC	29
14	92	1	8.83 [4.62-13.48]	14	0.93	2.31 [0.75-4.12]	B_C|A	55	ABC	36
							B|A	34	AB	36
							C|A	11	AC	28
15	99	1	5.43 [2.66-8.76]	15	1	3.07 [1.25-5.23]	A|C_I	42	ACI	43
							A|C	35	AC	29
							A|I	23	AI	29
16	\	0.57	4.33 [2.50-0.64]	16	1	24.89 [15.80-35.09]	B|B	100	B	100
17	\	0.26	0.03 [1.72-5.06]	17	1	9.60 [4.27-15.86]	B|B	100	B	100
18	\	0.91	2.45 [1.06-4.06]	18	0.89	7.09 [3.32-11.52]	B|B	100	B	100
19	\	0.75	1.53 [0.48-2.73]	19	1	0.02 [0.46-0.48]	B|B	100	B	100
20	\	0.31	1.04 [0.23-2.04]	20	1	5.15 [2.06-8.87]	B|B	100	B	100
21	\	0.18	3.56 [2.07-5.34]	21	1	16.68 [10.29-23.14]	B|B	100	B	100

Node No.	Maximum Likelihood	BEAST		Node No.	BEAST		DEC		S-DIVA	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]	(Fig. 11)	PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
22	\	0.88	3.26 [1.91-4.69]	22	0.95	14.85 [9.20-20.90]	B|B	99	B	100
23	\	0.96	2.65 [1.57-3.89]	23	1	11.30 [6.62-16.29]	B|B	98	B	100
24	55	0.99	1.70 [0.82-2.66]	24	1	5.43 [2.66-8.76]	B|B	91	B	100
25	58	0.94	1.31 [0.54-2.18]	25	0.57	4.33 [2.50-6.40]	B|B	81	B	100
26	66	0.98	0.01 [0.02-1.82]	26	0.33	3.88 [2.35-5.73]	B|B	91	B	99
27	\	0.14	2.43 [1.38-3.55]	27	0.17	3.69 [2.27-5.39]	B|B	90	B	100
28	\	0.08	2.24 [1.24-0.03]	28	0.91	2.45 [1.06-4.06]	B|B	68	B	79
							B_H|B	15	BHI	8
							B_I |B	10	BI	7
									BH	6
29	56	0.87	1.53 [0.58-2.53]	29	0.75	1.53 [0.48-2.73]	B_I|H	70	BHI	46
							B|H	30	BH	29
									BI	25
30	53	0.88	1.59 [0.58-2.72]	30	0.31	1.04 [0.23-2.04]	B|I	100	BI	100
31	90	1	0.70 [0.16-1.35]	31	0.88	3.26 [1.91-4.69]	B|B	56	B	55
							B_C|C	12	BD	16
							B|B_D	8	BI	15
									BDI	9

Node No.	Maximum Likelihood	BEAST		Node No.	BEAST		DEC		S-DIVA	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]	(Fig. 11)	PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
32	\	0.16	2.03 [0.97-3.05]	32	0.96	2.65 [1.57-3.89]	B|B	50	B	78
							B_C |C	19	BC	6
							B|B_E	8	BI	5
									BDI	4
									BD	3
									BCDI	1
									BCI	1
									BEI	1
									BCD	1
33	\	0.49	1.82 [0.80-2.94]	33	0.17	2.20 [1.23-3.35]	E|B_E	36	BDI	8
							C|C	15	BCEI	8
							C|B_C	12	BEI	8
							E|B_C_E	6	BCDEI	8
							C|B_C_E	6	BEDI	8
									BCDI	8
									BED	8
									BCD	8
									BD	8
									BCI	8
									BI	8
									BCDE	8

Node No.	Maximum Likelihood	BEAST		Node No.	BEAST		DEC		S-DIVA	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]	(Fig. 11)	PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
34	\	0.37	2.86 [1.68-0.43]	34	0.87	1.53 [0.58-2.53]	E|B_C E|B E|C	50 34 16	BCE BE	50 50
35	50	0.97	2.10 [1.01-3.25]	35	0.99	1.70 [0.82-2.66]	I|E I|C I|D_E I|C_D I|D	28 24 19 18 11	DI CDEI CEI DEI EI CDI CI	89 3 2 2 2 1 1
36	61	1	1.45 [0.59-2.46]	36	0.98	0.09 [0.02-1.82]	D_E|D D|C_D D|D	46 41 9	D CDE CE ED CD	90 4 3 3 1
37	59	1	0.63 [0.01-1.26]	37	0.94	1.31 [0.54-2.18]	I|I	100	I	100
38	77	0.52	0.31 [0.01-0.74]	38	0.09	2.33 [1.32-3.45]	B|B B|B_C	66 34	B BC	75 25
39	55	0.9	1.57 [0.07-2.60]	39	0.11	0.45 [0.21-1.11]	B|B	100	B	100
40	84	1	0.49 [0.00-1.19]	40	0.88	1.59 [0.58-2.72]	B|B	100	B	100
41	\	0.38	2.28 [0.92-3.84]	41	1	0.70 [0.16-1.35]	B|B	100	B	100

Node No.	Maximum Likelihood	BEAST		Node No.	BEAST		DEC		S-DIVA	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]	(Fig. 11)	PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
42	\	0.63	1.07 [0.02-2.64]	42	0.49	1.82 [0.80-2.94]	C|B	100	BC	100
43	89	1	0.66 [0.01-1.65]	43	0.37	1.68 [0.43-2.86]	D|D	3	BDI	12
							I|B_D_H	2	BCDI	12
							I|B_C_D	2	BDHI	12
							I|B_H	2	BCDHI	12
							I|B_C	2	BI	8
							D|C_D	1	BCHI	8
							C|B_C_H	1	BCI	8
							C_I|C	1	BHI	8
							I|B_C_D_H	1	BCDH	5
							D|B_C_D_H	1	BDH	5
							I|B_C_H	1	BCD	5
							D_I|D	1	BD	5
							E|B	1	DI	1
44	99	1	8.28 [4.56-12.46]	44	0.97	2.10 [1.01-3.25]	D_I|I	33	I	100
							I|I	22		
							C_I|I	18		
							C_D_I |I	6		
							D_E_I |I	5		
45	99	1	4.71 [2.61-7.12]	45	0.9	1.57 [0.07-2.60]	I|I	63	I	99
46	65	1	3.63 [1.88-5.52]	46	1	0.49 [0.00-1.19]	I|F	100	FI	100

Node No.	Maximum Likelihood	BEAST		Node No.	BEAST		DEC		S-DIVA	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]	(Fig. 11)	PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
47	\	0.84	2.92 [1.38-4.70]	47	1	1.45 [0.59-2.46]	D_I|I	23	I	100
							I|I	20		
							C_I|I	13		
							C_D_I|I	12		
							D_E_I|I	11		
							C_D_E_I|I	10		
							C_E_I|I	7		
							E_I|I	3		
48	75	1	2.01 [0.07-0.35]	48	1	0.63 [0.01-1.26]	C_D_E |I	52	CEI	18
							D_E |I	16	DEI	18
							C_E|I	11	CDEI	18
							C_D |I	10	EI	15
									CI	10
									DI	10
									CDI	10
49	69	1	2.60 [1.12-4.31]	49	0.52	0.31 [0.01-0.74]	E|C_D	81	CDE	33
							E|D	11	DE	33
									CE	33

Node No.	Maximum Likelihood	BEAST		Node No.	BEAST		DEC		S-DIVA	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]	(Fig. 11)	PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
50	58	0.97	3.61 [1.53-5.85]	50	0.38	2.28 [0.92-3.84]	B|D B_C|D B_H |D B_C_H |D C|D	32 23 17 15 8	BDH BCDH BD BCD	25 25 25 25
51	94	1	3.29 [1.01-6.43]	51	0.63	1.07 [0.02-2.64]	D|D	100	D	100
52	94	0.99	13.18 [7.54-19.05]	52	1	0.66 [0.01-1.65]	H|B_C H|B	81 15	BCH BH CH	46 46 8
53	97	1	8.56 [4.97-12.35]	53	1	8.83 [4.62-13.48]	B|B	96	B	100
54	90	1	6.00 [4.97-12.35]	54	1	8.28 [4.56-12.46]	B|B	90	B	100
55	\	0.62	5.36 [3.04-8.00]	55	1	3.29 [1.01-6.43]	B|B B_C |B	81 19	B	100
56	68	1	3.90 [0.20-6.02]	56	1	4.71 [2.61-7.12]	B|B B|B_D	62 37	B	98
57	60	0.99	2.02 [0.54-3.84]	57	0.97	3.61 [1.53-5.85]	B|D	100	BD	100
58	50	0.94	2.81 [1.21-4.74]	58	1	3.63 [1.88-5.52]	B|B	99	B	100
59	65	1	3.89 [1.83-6.07]	59	0.84	2.92 [1.38-4.70]	B|B	100	B	100
60	68	0.92	2.49 [0.84-4.34]	60	1	2.01 [0.07-0.35]	B|B	100	B	100
61	93	1	4.42 [1.96-7.10]	61	1	2.60 [1.12-4.31]	B|B	93	B	100
62	\	0.83	3.13 [1.06-5.39]	62	0.99	13.18 [7.54-19.05]	B|B	100	B	100

Node No.	Maximum Likelihood	BEAST	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]
63	100	1	9.60 [4.27-15.86]
64	71	0.89	7.09 [3.32-11.52]
65	94	1	0.47 [0.46-0.48]
66	93	1	5.15 [2.06-8.87]
67	100	1	18.80 [11.32-26.80]
68	100	1	12.37 [6.99-18.10]
69	81	0.68	10.85 [6.04-16.33]
70	100	1	5.88 [2.20-9.82]
71	100	1	7.67 [3.59-12.22]
72	\	0.45	17.00 [10.06-24.66]
73	\	0.47	16.20 [9.50-23.57]
74	97	1	4.67 [2.24-7.46]
75	89	1	3.07 [1.25-5.23]
76	\	0.75	3.48 [1.54-5.71]
77	91	1	1.38 [0.25-2.77]
78	53	0.93	2.31 [0.75-4.12]
79	96	1	11.28 [0.61-17.52]

Node No.	BEAST		DEC		S-DIVA	
(Fig. 11)	PP	Age in Ma [95% HPD interval]	Ancestral Split	Relative probability [%]	Area	Relative probability [%]
63	1	8.56 [4.97-12.35]	B|B	99	B	100
64	1	4.42 [1.96-7.10]	B|B	100	B	100
65	0.83	3.13 [1.06-5.39]	B|B	100	B	100
66	1	6.00 [3.32-8.85]	B|B	98	B	100
67	0.62	5.36 [3.04-8.00]	B|B	95	B	100
68	1	3.90 [0.20-6.02]	B|B B_C |B B_C|C	56 13 13	B	94
69	0.99	2.02 [0.54-3.84]	B_C |D B|D C|D	51 42 7	BCD BD	50 50
70	0.94	2.81 [1.21-4.74]	C|B	100	BC	100
71	1	3.89 [1.83-6.07]	B|B	100	B	100
72	0.92	2.49 [0.84-4.34]	B|B	100	B	100

Node No.	Maximum Likelihood	BEAST	
(Fig. 10)	BP	PP	Age in Ma [95% HPD interval]
80	100	1	1.80 [0.50-3.41]
81	100	1	20.22 [9.72-30.45]
82	55	0.45	17.64 [0.89-27.19]
83	100	1	8.51 [3.53-14.08]
84	\	0.39	7.09 [2.73-11.97]
85	100	0.95	35.86 [33.68-40.11]
86	100	1	17.51 [9.31-2.60]
87	58	0.67	13.23 [6.17-21.34]
88	50	0.98	26.81 [16.98-35.30]
89	54	0.93	20.04 [10.58-29.02]
90	69	0.89	14.76 [6.82-23.87]
91	84	1	15.26 [6.85-24.05]

Appendix 10. Sequence data results (Appendix 10.1). Comparison of divergence time estimates for Gentianaceae obtained in different studies (Appendix 10.2).

Appendix 10.1 Sequence data results.

The combined data matrix included a total of 216 sequences, 40 of which had been newly generated for this study. Fifty-three *atpB-rbcL* sequences resulted in a data matrix of 957 characters, from which we excluded 32 because of a poly-T run. Of the remaining 925 positions, 193 (~21%) sites were variable and 89 (~10%) were potentially parsimony-informative. The *trnL-trnF* matrix consisted of 72 sequences, which resulted in a data matrix of 1146 characters. We excluded 148 ambiguous sites (one polynucleotide repeat, one TA dinucleotide repeat, and one TAA trinucleotide repeat). The remaining 998 characters contained 269 (~27%) variable and 155 (~16%) parsimony-informative sites. Finally, we used 91 sequences of the nuclear marker ITS. The aligned ITS data matrix consisted of 656 characters, with 390 (~60%) variable and 287 (~44%) potentially parsimony-informative sites. The total length of the combined (*atpB-rbcL*, *trnL-trnF*, and ITS) molecular matrix was 2579 characters, of which 852 (~33%) sites were variable and 531 (~21%) were potentially parsimony-informative.

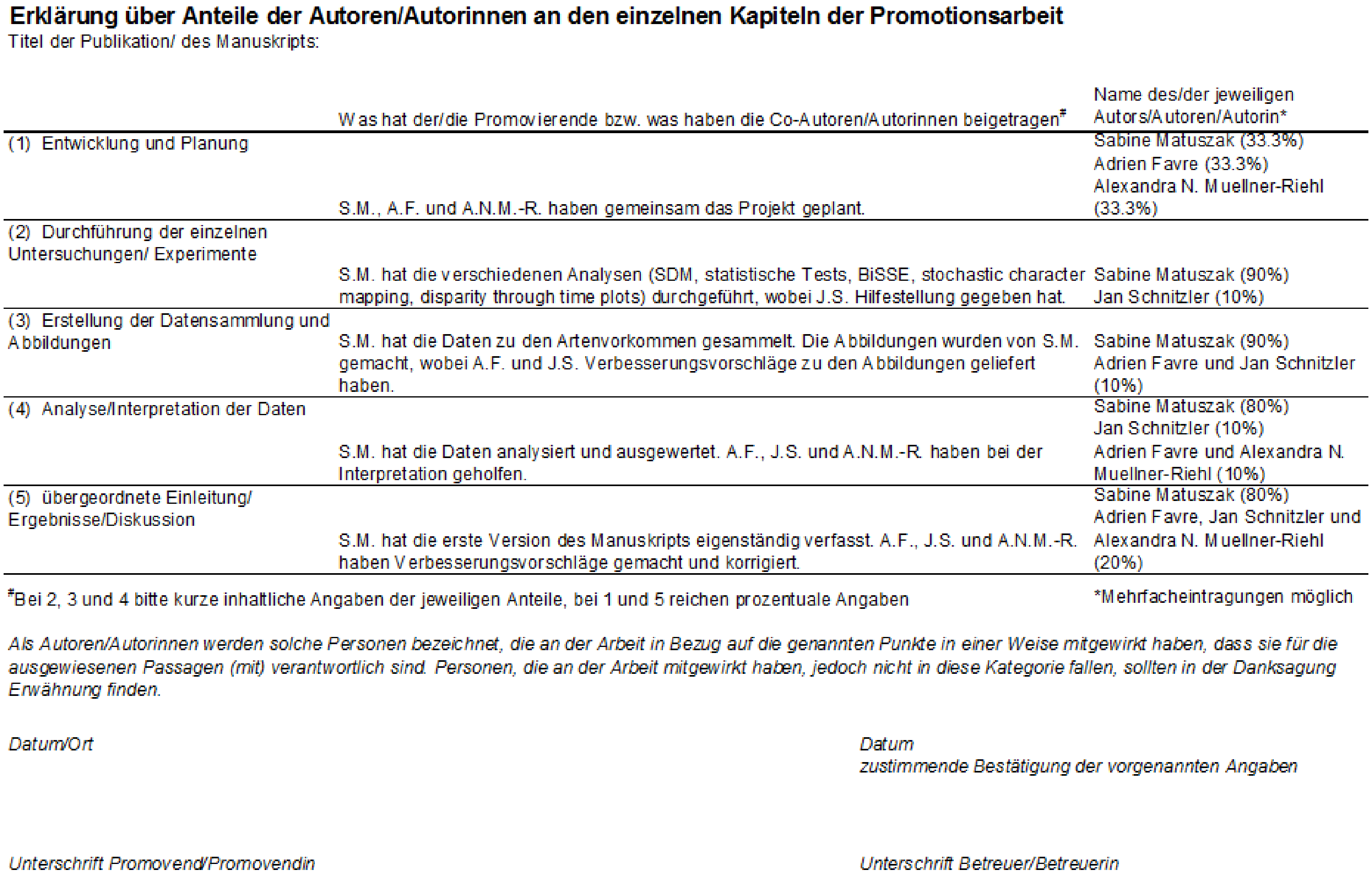
Appendix 10.2 Comparison of divergence time estimates for Gentianaceae obtained in different studies.

In addition to differences in sampling design, the calibration approach adopted in this study might explain the slightly different divergence time estimates and larger error bars we obtained in comparison to other studies (we refrained from using fossils of uncertain age and identification, and from secondary calibration, see Appendix 9). Our stem age inferred for Gentianeae

(26.7-59.5 Myr; Fig. 10, node 2) overlaps with that estimated by Merckx et al. (2013) who obtained a stem age of 25.0-37.9 Myr. The crown age of Gentianinae was estimated to be 17.79-39.76 Myr (Fig. 10, node 9), whereas the estimate of Chen et al. (2005) is younger (11.4-21.4 Myr), though also largely overlapping. For *Tripterospermum*, our results suggested a stem age of 6.6-16.3 Myr (Fig. 10, node 13) and a crown age of 3.5-9.5 Myr (Fig. 10, node 15). This result is very similar to that of Favre et al. (2010; Scenario 3).

		This study		**Other studies**	**Reference**
Potalieeae	**crown**	Fig.10, node 85	33.68-40.11	34.7-38.5	Merckx et al., 2013
	stem	Fig.10, node 1	33.91-60.91	35.6-44.9	Merckx et al., 2013
Helieae	**crown**	Fig.10, node 81	9.72-30.45	13.1-24.8	Merckx et al., 2013
	stem	Fig.10, node 2	26.73-59.53	25.0-37.9	Merckx et al., 2013
Gentianeae	**crown**	Fig.10, node 3	22.53-50.75	20.6-32.8	Merckx et al., 2013
	stem	Fig.10, node 2	26.73-59.53	25.0-37.9	Merckx et al., 2013
Swertiinae	**crown**	Fig.10, node 4	0.95-25.15	15	von Hagen & Kadereit, 2002
	stem	Fig.10, node 3	22.53-50.75	\	\
Gentianinae	**crown**	Fig.10, node 9	17.79-39.76	11.4-21.4	Chen et al., 2005
	stem	Fig.10, node 3	22.53-50.75	15-39.1	Favre et al., 2010
Tripterospermum	**crown**	Fig.10, node 15	2.66-8.76	3.5-9.5	Favre et al., 2010
	stem	Fig.10, node 13	6.62-16.29	6.2-16.7	Favre et al., 2010

Appendix 11. Erklärung zu Kapitel 4 „Key innovations and climatic niche divergence as drivers of diversification in subtropical Gentianinae (Gentianaceae) in the region of the Qinghai-Tibetan Plateau".



Erklärung über Anteile der Autoren/Autorinnen an den einzelnen Kapiteln der Promotionsarbeit

Titel der Publikation/ des Manuskripts:

	Was hat der/die Promovierende bzw. was haben die Co-Autoren/Autorinnen beigetragen#	Name des/der jeweiligen Autors/Autoren/Autorin*
(1) Entwicklung und Planung	S.M., A.F. und A.N.M.-R. haben gemeinsam das Projekt geplant.	Sabine Matuszak (33.3%) Adrien Favre (33.3%) Alexandra N. Muellner-Riehl (33.3%)
(2) Durchführung der einzelnen Untersuchungen/ Experimente	S.M. hat die verschiedenen Analysen (SDM, statistische Tests, BiSSE, stochastic character mapping, disparity through time plots) durchgeführt, wobei J.S. Hilfestellung gegeben hat.	Sabine Matuszak (90%) Jan Schnitzler (10%)
(3) Erstellung der Datensammlung und Abbildungen	S.M. hat die Daten zu den Artenvorkommen gesammelt. Die Abbildungen wurden von S.M. gemacht, wobei A.F. und J.S. Verbesserungsvorschläge zu den Abbildungen geliefert haben.	Sabine Matuszak (90%) Adrien Favre und Jan Schnitzler (10%)
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#Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben

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Datum/Ort

Datum
zustimmende Bestätigung der vorgenannten Angaben

Unterschrift Promovend/Promovendin

Unterschrift Betreuer/Betreuerin

Appendix 12. Specimen information and GPS coordinates obtained for *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum*.

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
***Crawfurdia* Wallich**						
C. angustata	98.901225	26.911697	H. T. Tsai	54498	HUH	00113178
C. angustata	98.62	27.996111	Gaoligong Shan Biotic Survey Expedition - Autumn 2004	22601	E	E00320935
C. angustata	98.45	27.68	Li Heng	9575	MO	1403370
C. angustata	98.34829712	27.71150017	Dulong Jiang Investigation Team	1460	CAS	1004534
C. angustata	98.342619	27.88215	A. Favre	229	FR	FR-0105083
C. angustata	98.31619263	28.01413918	Gaoligong Shan Biodiversity Survey	21675	CAS	1060110
C. angustata	98.32627869	28.07691956	Gaoligong Shan Biodiversity Survey	21532	CAS	1060032
C. angustata	98.570875	28.0495	C. W. Wang	67544	HUH	00113175
C. angustata	98.32595062	28.08807945	Gaoligong Shan Biodiversity Survey	21404	CAS	1060178
C. angustata	98.61641693	27.63214302	Gaoligong Shan Biodiversity Survey	22339	CAS	1061458
C. angustata	98.734967	27.745378	K. M. Feng	8669	PE	\
C. angustata	98.342622	27.882144	A. Favre	229	KUN	\
C. campanulacea	98.715278	25.635	Gaoligong Shan Expedition 1998	11278	E	E00258935
C. campanulacea	98.778333	25.306389	H. Li, B. Bartholomew & Z. L. Dao	11645	HUH	00129831

Species	**Longitude**	**Latitude**	**Collector**	**Collector-No.**	**Herbarium**	**Catalogue-No.**
C. campanulacea	98.66055298	25.67389297	H. Li, B. Bartholomew & Z. L. Dao	11033	CAS	1012924
C. campanulacea	98.9	26.9667	H. T. Tsai	59004	HUH	00113158
C. campanulacea	98.661944	26.000556	H. Li, B. Bartholomew & Z. L. Dao	10228	HUH	00129832
C. campanulacea	98.733333	27.183333	Gaoligong Shan Expedition 1996	7910	E	E00095296
C. campanulacea	98.348767	27.899367	A. Favre	214	FR	\
C. campanulacea	98.59814453	27.78616333	Gaoligong Shan Biodiversity Survey	22571	CAS	1061370
C. campanulacea	98.32083893	28.00386047	Gaoligong Shan Biodiversity Survey	21710	CAS	1060156
C. campanulacea	98.325833	28.088056	Gaoligong Shan Biotic Survey Expedition - Autumn 2004	21404	E	E00264599
C. crawfurdioides	98.481111	27.968611	Gaoligong Shan Biotic Survey Expedition - Summer 2006	31715	E	E00640174
C. crawfurdioides	98.468333	27.780556	Gaoligong Shan Biotic Survey Expedition - Autumn 2002	16813	E	E00629870
C. crawfurdioides	98.456944	27.691667	Gaoligong Shan Expedition 1997	9595	E	E00095908
C. crawfurdioides	98.61	28.03	C. W. Wang	67482	HUH	00113166
C. crawfurdioides	98.25	28.67	Forrest	18794	K	K000195236
C. crawfurdioides	98.49	28.08	J. F. Rock	22162	HUH	00113896
C. crawfurdioides	98.25	28.666667	G. Forrest	18974	E	E00001639

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
C. crawfurdioides	100.064744	25.633475	G. Forrest	11564	E	E00001636
C. crawfurdioides	95.325403	29.770936	Luo Jian et al.	LuoJian-XZ-0931	PE	\
C. crawfurdioides	95.717339	29.8942	Tibet-Expedition	73-1452	PE	\
C. crawfurdioides	100.082944	25.667525	Ph. Chassot & Yong-Ming	c99-SIN-10	NEU	\
C. delavayi	100.108097	25.674103	A. Favre	217	FR	FR-0105090
C. delavayi	98.45	27.683333	Gaoligong Shan Expedition 1997	9575	E	E00095883
C. delavayi	98.4675	27.981111	Gaoligong Shan Biotic Survey Expedition - Summer 2006	31345	E	E00640173
C. delavayi	98.659444	27.906111	Gaoligong Shan Biotic Survey Expedition - Autumn 2002	15551	E	E00629867
C. delavayi	98.456944	27.691667	Gaoligong Shan Expedition 1997	9588	E	E00095896
C. delavayi	98.81	28.17	T. T. Yü	23151	HUH	00113168
C. delavayi	99.845672	26.536147	Ching	24713	PE	\
C. delavayi	100.106367	25.672619	Ph. Chassot and Yong-Ming	c99-SIN-07	NEU	\
C. dimidiata	99.017875	25.753694	G. Forrest	25225	K	\
C. dimidiata	103.778864	25.454228	T. T. Yü	20221	HUH	00113170
C. gracilipes	98.758333	26.441667	Gaoligong Shan Biotic Survey Expedition - Summer 2005	25956	E	E00320940
C. gracilipes	98.456944	27.691667	Gaoligong Shan Expedition 1996	7755	E	E00095299
C. gracilipes	98.455833	27.755	Gaoligong Shan	32206	E	E00640172

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
			Biotic Survey Expedition - Summer 2006			
C. gracilipes	98.59	28.04	C. W. Wang	67293	HUH	00113171
C. gracilipes	98.455489	27.772392	A. Favre	231k4	KUN	\
C. maculaticaulis	98.7678	24.8308	H. Li, Z. L. Dao & L. W. Yin	s.n.	HUH	43997
C. maculaticaulis	104.733494	23.1447	Luo Yi-bo	514	PE	\
C. maculaticaulis	105.794881	23.333494	Gao Peng Zinn	55983	IBK	\
C. maculaticaulis	104.576822	22.966422	Favre	215	LZ	\
C. nyingchiensis	94.352069	29.625869	W. L. Zheng	628	XZE	\
C. nyingchiensis	95.785092	29.839683	Tsangpo River Expedition	1189	XZE	\
C. poilanei	103.77835	22.3072	A. Favre & Rebeaud	200	FR	FR-0105087
C. poilanei	103.7775	22.364872	E. Poilane	17014	MNHN	P00348848
C. pricei	116.908275	25.077061	W. R. Price	1169	K	K000195237
C. pricei	108.236011	24.793878	Dianqiangui Team	70250	IBK	\
C. pricei	115.884117	24.604097	Deng Liang	5880	WUK	\
C. pricei	109.885158	25.735297	Luqing Hua, Wie Yuzong	20233	IBK	\
C. pricei	108.435067	23.478917	Yuan Yong-Ming	c93-SIN-04	NEU	\
C. pricei	110.316872	24.027436	A. Favre	245	LZ	\
C. semialata	100.206361	29.975236	F. Kingdon-Ward	4984	E	E00001642
C. semialata	101.297272	27.942828	F. Kingdon-Ward	4880	E	E00001641
C. sessiliflora	112.882289	24.929428	Liangbao Han	83846	IBK	\
C. sessiliflora	110.642397	25.121283	Chen Chiu-chou	53777	IBK	\
C. sessiliflora	102.130042	29.668883	Yuan Yong-Ming	c93-SIN-01	NEU	\
C. sessiliflora	100.448331	27.850139	Liu Ende et al.	1209074	LZ	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
C. speciosa	87.4	27.583333	Edinburgh Makalu Expedition 1991	873	E	E00114959
C. speciosa	87.3	27.733333	Edinburgh Makalu Expedition 1991	712	E	E00114958
C. speciosa	88.2842	26.99495	A. Favre	208	FR	FR-0105086
C. speciosa	103.6	22.42	Unknown	\	MO	100174068
C. speciosa	95.236981	29.409442	F. Kingdon-Ward	6234	E	E00001646
C. speciosa	87.897294	27.475878	C. Totty	46	E	19911958
C. speciosa	105.644056	21.465989	P. A. Pételot	4584	MNHN	P00348849
C. speciosa	86.713556	27.734222	C. S. Chang et al.	NE020501	PE	\
C. speciosa	95.055336	28.835706	Ph. Chassot	99-NEP-01	NEU	\
C. speciosa	88.277444	27.007611	A. Favre	207	KUN	\
C. thibetica	98.458889	27.806944	Gaoligong Shan Biotic Survey Expedition - Autumn 2002	16900	E	E00629863
C. thibetica	101.944736	30.004572	J. A. Soulie	851	UPS	V-089231
***Kuepferia* Adr.Favre**						
K. chateri	87.203925	27.675878	L. W. Beer, C. R. Lancaster and D. Morris	9545	BM	\
K. chateri	87.361392	27.941769	L. W. Beer	25363	BM	\
K. damyonensis	98.831089	28.055103	T. T. Yü	22754	HUH	00113355
K. damyonensis	98.483611	27.985	Gaoligong Shan Biotic Survey Expedition - Summer 2006	31498	E	E00625474
K. damyonensis	98.518147	28.472147	C. W. Wang	65651	HUH	00113354
K. damyonensis	98.968711	28.284075	F. Kingdon-Ward	5377	E	E00001656

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
K. damyonensis	94.995286	29.481708	Chen Weilie	15157	PE	\
K. damyonensis	102.417175	27.393794	Guan Zhongtian	8039	PE	\
K. decorata	94.816411	29.577975	S. Bowes Lyon	11202	E	E00075615
K. decorata	98.38005	28.449556	C. W. Wang	65982	HUH	00113357
K. decorata	100.1	25.67	J. F. Rock	6345	HUH	00113359
K. decorata	98.470556	27.983889	Gaoligong Shan Biotic Survey Expedition - Summer 2006	31582	E	E00625477
K. decorata	100.1	27.39	K. M. Feng	2208	HUH	00113356
K. decorata	94.94745	29.487733	Yuan Yong-Ming	2003-38	KUN	\
K. decorata	98.9	28.0667	C. W. Wang	68955	HUH	00113361
K. decorata	94.144333	29.256186	Qinghai-Tibet-Sammelexpedition	7513636	QTPMB	\
K. decorata	95.486764	29.934497	Junsheng, Yuan	651193	PE	\
K. decorata	97.407047	28.692903	Zhang Jing Wie	1742	PE	\
K. doxiongshangensis	94.4098	29.2551	F. Ludlow, G. Sherriff & G. Taylor	s.n.	UPS	V-136833
K. doxiongshangensis	94.94745	29.487733	Yuan Yong-Ming	2003-33	KUN	\
K. hicksii	91.229614	27.787147	Ludlow, Shernff and Hicks	21456	BM, UPS	\
K. hicksii	87.20935	27.6812	Edinburgh Makalu Expedition 1991	360	E	E00114963
K. infelix	90.355683	27.340439	G. & S. Miehe	00-315-17	E	E00190609
K. infelix	87.078772	27.984261	H. Birks, B. & H. H. John	s.n.	E	E00289347
K. infelix	87.745664	27.806436	J. Stainton	1030	MNHN	P00513799
K. leucantha	90.457758	27.860183	F.Ludlow,G.Sherriff,& J.H.Hicks	16886	PE	\
K. leucantha	94.000658	29.25215	F. Ludlow, G.	5934	UPS	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
			Sherriff, G. Taylor			
K. masonii	98.478736	26.084136	Forrest	24944	K	\
K. masonii	98.586806	26.409883	Forrest	27222	BM, K	\
K. otophora	98.572694	28.023642	C. W. Wang	67108	HUH	00113581
K. otophora	98.880275	28.024031	T. T. Yü	22438	HUH	00113579
K. otophora	98.45	27.683333	Gaoligong Shan Expedition 1997	9620	E	E00095271
K. otophora	98.474722	27.975	Gaoligong Shan Biotic Survey Expedition - Summer 2006	31401	E	E00640184
K. otophora	98.76	27.97	T. T. Yü	22604	HUH	00113580
K. otophora	98.972544	26.774397	H. T. Tsai	58202	HUH	00113571
K. otophora	100.1	25.67	J. F. Rock	6356	HUH	00113567
K. otophora	99.222303	27.171011	K.M.Feng	8684	PE	\
K. otophora	100.098847	25.660586	Favre and Matuszak	56	FR, KUN, LZ	\
K. otophoroides	98.48	28.02	J. F. Rock	21977	HUH	00113564
K. otophoroides	98.464722	27.991389	Gaoligong Shan Biotic Survey Expedition - Summer 2006	31606	E	E00625472
K. otophoroides	98.444722	27.773056	Gaoligong Shan Biotic Survey Expedition - Autumn 2002	17022	E	E00619879
K. otophoroides	98.45	27.683333	Gaoligong Shan Expedition 1997	9456	E	E00095270
K. otophoroides	93.36625	31.531742	Handel-Mazzetti	9894	K	\
K. otophoroides	98.494906	27.95065	Favre and Matuszak	34	FR, KUN, LZ	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
K. otophoroides	94.94745	29.487733	Yuan Yong-Ming	Cn2k66	LZ	\
***Metagentiana* T.N. Ho & S.W. Liu**						
M. alata	90.670247	27.988514	F. Ludlow, G. Sherriff, J. H. Hicks	19759	UPS	V-136804
M. alata	91.017336	27.091881	Sherriff, Hicks	17212	MNHN	P00517470
M. alata	102.598881	25.001075	Delavay	s.n.	P	\
M. australis	98.873753	19.394308	A. F. G. Kerr	2865	K	\
M. australis	98.888719	19.44605	E. Hennipman	3269	MNHN	P00350296
M. eurycolpa	99.089919	26.396142	Maire	7404	K, UC, US	\
M. eurycolpa	103.305903	26.395378	Maire	2910	K, UC	\
M. eurycolpa	104.336711	26.842247	Y. Tsiang	9101	PE	\
M. eurycolpa	103.240831	26.108858	S. B. Lan	193	PE	\
M. expansa	104.175669	28.105372	Semon Ten	282	B, UPS	\
M. expansa	100.646267	25.866072	Ducloux	4875	P	\
M. expansa	100.742003	24.427119	M. K. Li	2154	PE	\
M. expansa	99.804353	27.741481	Ten	282	B	\
M. gentilis	103.0333	25.3667	F. Ducloux	6833	HUH	00113410
M. gentilis	102.633133	24.950531	A. Favre	302	FR	FR-0105085
M. gentilis	102.486347	24.991825	Delavay	s.n.	P	\
M. gentilis	102.365836	25.537136	Y.P.Chang	386	PE	\
M. gentilis	102.629589	24.962175	K.M.Feng	10438	PE	\
M. gentilis	102.633125	24.950528	A. Favre	302	KUN	\
M. gentilis	102.633136	24.950331	A. Favre	332	KUN	\
M. leptoclada	100.334489	25.223131	T. T. Yü	18277	HUH	00113470
M. leptoclada	99.494633	27.79385	Forrest	7409	E, K	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
M. leptoclada	99.365425	27.342375	Forrest	11444	BM, E, K	\
M. leptoclada	101.65045	26.641144	T. T. Yu	1700	PE	\
M. primuliflora	102.071633	29.599508	F. Ducloux	3702	MNHN	P00430602
M. primuliflora	103.0333	25.3667	F. Ducloux	7323	HUH	00113648
M. primuliflora	102.25	26.68333	H. R. E. Handel-Mazzetti	5092	HUH	00113645
M. primuliflora	100.042725	26.378439	Delavay	9	E, GH, K, P, UPS	\
M. primuliflora	103.267428	25.330017	Delavay	s.n.	K	\
M. pterocalyx	99.746267	26.643008	Delavay	1553	E, GH, IBSC, K, P	\
M. pterocalyx	102.19135	26.794083	T.T.Yu	1731	PE	\
M. pterocalyx	102.991753	26.987431	H.T.Tsai	52007	PE	\
M. pterocalyx	100.101094	26.328267	Ching	24180	PE	\
M. rhodantha	100.190378	26.821797	Adrien Favre	301	KUN	\
M. rhodantha	98.592742	28.026536	C. W. Wang	67555	HUH	00113700
M. rhodantha	100.05	27.18	K. M. Feng	3172	HUH	00113691
M. rhodantha	99.2667	27.2167	H. T. Tsai	63118	HUH	00113699
M. rhodantha	102.2	29.9333	W. P. Fang	3721	HUH	00113682
M. rhodantha	103.463428	29.375125	H. C. Chow	9081	HUH	00113680
M. rhodantha	105.955825	32.490956	F. T. Wang	22583	HUH	00113685
M. rhodantha	107.26667	29.116667	W. P. Fang	5677	HUH	00113683
M. rhodantha	102.7407	25.139	K. M. Feng	183	HUH	00113689
M. rhodantha	102.07	27.73	H. R. E. Handel-Mazzetti	5613	HUH	00113684
M. rhodantha	111.549225	30.877519	Maries	s.n.	K	\
M. rhodantha	109.644214	30.596492	Ho-Chang Chow	1696	PE	\
M. rhodantha	105.327706	32.762886	T. P. Wang	8021	PE	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
M. rhodantha	102.400733	27.583903	A. Favre	322	LZ	\
M. serra	99.797022	27.846333	R. C. Ching	30655	HUH	00113740
M. serra	100.234164	27.130808	J. Murata, N. Kanayama, Y. Murakami, Z. S. Ren & S. W. Yu	538	HUH	00113747
M. serra	100.14	26.86	R. C. Ching	21065	HUH	00113739
M. serra	102.953692	26.909539	F. Ducloux	5768	HUH	00113746
M. serra	99.734544	26.176819	J. M. Delavay	1238	K	\
M. serra	102.318192	25.875503	Y. P. Chang	453	PE	\
M. serra	100.251203	27.100919	Favre & Matuszak	125a	FR	\
***Sinogentiana* Favre & Yuan**						
S. souliei	100.823661	28.157614	T. T. Yü	14536	HUH	00113787
S. souliei	99.6	27.8333	C. Schneider	3002	HUH	00113784
S. souliei	100.01	27.65	K. M. Feng	2316	HUH	00113780
S. souliei	100.214028	27.139083	J. F. Rock	10815	HUH	00113782
S. souliei	101.297619	30.529925	Harry Smith	11837	PE	\
S. souliei	101.992672	29.986628	Soulie	194	K	\
S. souliei	100.37775	28.38885	Adrien Favre	202a	KUN	\
S. striata	100.744444	31.725	D. E. Boufford, M. J. Donoghue & R. H. Ree	27833	HUH	00196602
S. striata	103.601317	32.6356	A. Favre	305	FR	FR-0105136
S. striata	102.93	29.65	E. H. Wilson	4144	HUH	00113825
S. striata	101.918333	36.807778	S. L. Chen	4001	HNWP	\
S. striata	100.236667	34.807778	S. L. Chen	3012	HNWP	\
S. striata	102.013611	37.2325	S. L. Chen	4024	HNWP	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
S. striata	102.022778	37.2175	S. L. Chen	4029	HNWP	\
S. striata	102.8325	34.903889	S. L. Chen	4047	HNWP	\
S. striata	106.202778	35.667222	S. L. Chen	4053	HNWP	\
S. striata	102.6025	32.405	S. L. Chen	3060	HNWP	\
S. striata	101.279722	30.831944	S. L. Chen	381	HNWP	\
***Tripterospermum* Blume**						
T. alutaceifolium	121.555897	25.168067	Favre	131k1	KUN	\
T. alutaceifolium	121.534533	25.187528	Murata & Yang	17846	TI	\
T. alutaceifolium	121.606886	25.176556	Tian-Chuan Hsu	1674	HCT	\
T. alutaceifolium	121.618847	24.967058	Chuan-Chieh Chen	982	HCT	\
T. alutaceifolium	121.483397	24.893489	Ho-Ming Chang	4535	HCT	\
T. alutaceifolium	120.95528	24.24139	Shao-Ting Chiu	s.n.	TAIF	293290
T. alutaceifolium	121.55	25.16667	Kuo-Shih Hsu	s.n.	TAIF	190143
T. alutaceifolium	121.66056	24.89	Ho-Ming Chang	s.n.	TAIF	200861
T. alutaceifolium	121.43333	24.73333	Sheng-You Lu	s.n.	TAIF	182818
T. alutaceifolium	121.65	25	Jenn-Che Wang	s.n.	TAIF	128404
T. australe	110.116669	24.155567	A. Favre	093a1	LZ	\
T. australe	108.357469	11.991431	E. Poilane	23436	MNHN	\
T. australe	103.437222	20.078056	Khampheng Homsombath & Mark F. Newman	1416	E	\
T. australe	113.197208	25.092847	Chun	42413	K	\
T. australe	108.045506	21.908803	Tsang	24442	A, NY	\
T. australe	105.703261	25.293714	Tsiang	4637	E	\
T. australe	103.829253	22.288914	Cavalerie	29413	P	\
T. brevilobum	105.8515	23.382211	D. Fang et al.	3-1340.1	GXMI	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
T. brevilobum	105.633483	23.295361	D. Fang et al.	3-1340.2	GXMI	\
T. brevilobum	105.908808	23.1914	D. Fang et al.	3-1340.3	GXMI	\
T. championii	101.361344	4.650572	A. Favre	007	FR	\
T. championii	99.729033	8.494522	A. Favre	010	LZ	\
T. championii	101.400783	4.471669	A. Favre	006	LZ	\
T. championii	101.382717	4.519381	A. Favre	001	FR	\
T. championii	101.782283	3.439983	S. N. Phoon	FRI60476	SIN	FRI 60476
T. championii	80.787944	6.810936	C. P. Gardner	137	K	K000195223
T. championii	108.398714	11.897903	Tixier	05	P	\
T. championii	102.236439	4.630853	Hulttum	20680	BM	\
T. championii	99.7107	8.532803	Kerr	15528	K	\
T. chinense	114.016056	23.283661	A. Favre	134	FR	\
T. chinense	116.020761	29.608575	Ce-Ming Tan	s.n.	TAIF	237656
T. chinense	121.31655	28.354675	Hangzhou Botanical Garden Herbarium	\	TAIF	317044
T. chinense	119.175903	27.894636	X. F. Jin	808B, 808C	HTC	\
T. chinense	118.881944	28.354978	F. G. Zhang, M. H. Wu & Z. Y. Li	4406	HTC	\
T. chinense	119.505	27.453	Chengqi Ao	10001	WZU	\
T. chinense	109.836756	28.630306	Unknown	\	SIMM	TC-052004
T. coeruleum	103.33	29.52	W. P. Fang	2919	HUH	00114575
T. coeruleum	101.652617	27.802544	Handel-Mazzeti	5556	A, E, W	\
T. coeruleum	103.395022	27.321233	T. T. Yu	4111	PE	\
T. cordatum	102.067053	31.481381	D. E. Boufford & B. Bartholomew	24703	HUH	00114577
T. cordatum	113.692236	28.415231	Ziyunshan Exp.	\	TAIF	TAIF197779
T. cordatum	103.277325	29.509511	E. Zala	171	MNHN	P00353453

Species	**Longitude**	**Latitude**	**Collector**	**Collector-No.**	**Herbarium**	**Catalogue-No.**
T. cordatum	103.380483	29.549222	A. Favre	087	FR	\
T. cordatum	102.832367	29.500333	H. Smith	13520	UPS	V-082129
T. cordifolioides	103.560003	30.904119	Li	47139	PE	\
T. cordifolioides	103.178664	31.033769	A. Favre	120	LZ	\
T. cordifolioides	98.56138889	27.77416667	Gaoligong Shan Biodiversity Survey	16515	CAS	\
T. cordifolioides	102.0277778	29.58638889	D. E. Boufford, M. J. Donoghue & R. H. Ree	27331	HUH	\
T. cordifolioides	109.358786	29.49465	Li	7434	PE	\
T. cordifolioides	108.583778	27.971028	Tsiang	7890	E, K, NY	\
T. cordifolioides	101.883258	30.004889	Pratt	431	BM, K	\
T. cordifolioides	98.524997	28.01315	Favre and Matuszak	036	FR, LZ	\
T. cordifolium	120.938822	23.266689	A. Favre	118	LZ	
T. cordifolium	121.3983333	24.67	Unknown	\	TAI	180784
T. cordifolium	120.91667	23.25	Pi-Fong Lu	s.n.	TAIF	220639
T. cordifolium	120.823333	22.9725	Yoshimatsu Yamamoto, Kunihiko Mori	456	TAI	091071
T. cordifolium	120.937778	23.249167	S. Sasaki	s.n.	TAI	091801
T. cordifolium	121.443333	24.361667	S. Sasaki	s.n.	TAI	091070
T. cordifolium	120.913056	23.278889	K. C. Yang, S. T. Chiu, C. C. Chou	s.n.	TAI	224644
T. cordifolium	120.670556	22.854167	Tsai-Wen Hsu	10197	TAIE	017640
T. cordifolium	120.679167	23.509722	Ming-Yea Tseng	2432	TAIE	007688
T. cordifolium	121	23.05	Tsai-Wen Hsu	9313	TAIE	013289
T. discoideum	115.990325	29.577511	Steward	2732	E, K	\
T. discoideum	110.504222	31.487067	Sino-amer. Exped.	566	A	\

Species	**Longitude**	**Latitude**	**Collector**	**Collector-No.**	**Herbarium**	**Catalogue-No.**
T. discoideum	118.174067	30.133314	Wu	51	TUS	\
T. discoideum	116.008319	29.545892	K. Yao	8563	MNHN	P00353458
T. discoideum	109.890725	31.045875	A. Henry	7091	K	K000195212
T. discoideum	121.205708	29.740047	W. R. Carles	134	K	\
T. discoideum	113.953142	22.388761	Merrill	10348	A, NY	\
T. discoideum	110.664447	32.138861	Silvestri	1828	K	\
T. discoideum	111.189906	30.831103	Henry	4463	K, NY	\
T. distylum	130.559686	30.320281	Murata,Yahara, & Im	15831	A, KYO, TI	\
T. distylum	130.502192	30.345428	Murata,Yahara, & Im	15831	A, KYO, TI	\
T. distylum	130.571658	30.304569	Murata,Yahara, & Im	15831	A, KYO, TI	\
T. fasciculatum	91.762978	25.446989	Kingdon-Ward	16019	BM	\
T. fasciculatum	91.902911	25.307406	Biswas	4059	A	\
T. fasciculatum	91.731383	25.283144	Clarke	15169	K	\
T. fasciculatum	93.921953	21.234578	Kingdon-Ward	22753	BM, E	\
T. fasciculatum	93.801972	21.386339	Gale	9181	E	\
T. fasciculatum	91.633333	25.583333	J. D. Hooker, T. Thomson	s.n.	MNHN	P00353463
T. fasciculatum	92.874125	23.489472	Wenger	366	K	\
T. fasciculatum	93.512217	22.490953	Venning	140	K	\
T. filicaule	111.096814	30.817258	A. Henry	Catalogue-No. P00353468	MNHN	P00353468
T. filicaule	110.808394	31.847583	A. Henry	6842	A, BM, K, P	\
T. filicaule	110.499981	31.500017	1980 Sino- Amer. Exped.	39	A, NY	\
T. hirticalyx	103.697317	22.912947	A. Favre	s.n.	FR	\
T. hirticalyx	103.769667	22.352392	A. Favre	s.n.	LZ; FR	\

Species	**Longitude**	**Latitude**	**Collector**	**Collector-No.**	**Herbarium**	**Catalogue-No.**
T. hirticalyx	101.754433	23.67085	A. Favre	047	FR	\
T. hirticalyx	101.754433	23.67085	H. T. Tsai	56521	A	\
T. hirticalyx	98.9	26.9667	Gaoligong Shan Biotic Survey Expedition - Summer 2006	34207	E	\
T. hirticalyx	98.603056	27.765278	Gaoligong Shan Biotic Survey Expedition - Summer 2006	32503	E	\
T. hirticalyx	98.298611	27.693611	A. Favre	068	FR	\
T. hirticalyx	104.569178	22.954092	A. Favre	78	NEU	\
T. hirticalyx	105.909047	23.331719	A. Favre and Y. Song	59	NEU	\
T. hirticalyx	104.006975	23.276314	A. Favre	63	NEU	\
T. hirticalyx	104.781183	23.371917	A. Favre and F. Rebeaud	42	NEU	\
T. hirticalyx	103.773153	22.304772	A. Favre	055	KUN	\
T. japonicum	133.203	33.5351	\	\	KPM	KPM-NA0158043
T. japonicum	133.849	35.0869	\	\	KURA	124799
T. japonicum	136.1	34.75	\	\	HYO	C1225350
T. japonicum	137.433	34.9168	\	\	KPM	KPM-NA0057050
T. japonicum	139.213	36.9058	\	\	KPM	KPM-NA0080327
T. japonicum	138.477778	36.488889	P. Brownless, T. Clark, R. Jamieson, V. Marrocco, B. McNamara, S. Tsukie	123	E	E00399212
T. japonicum	138.809019	37.705381	\	\	KPM	KPM-NA0194035
T. japonicum	126.542942	33.392733	Nakai	6415	TI	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
T. japonicum	139.189	35.9076	\	\	KPM	KPM-NA0028071
T. japonicum	139.053	35.1241	\	\	KPM	KPM-NA0190452
T. lanceolatum	121.190242	24.102006	Favre	103	TNM	\
T. lanceolatum	121.274722	24.123611	Favre	104	LZ	\
T. lanceolatum	121.082844	24.337486	Ohashi & Nemoto	20899	TUS	\
T. lanceolatum	121.4169	24.386928	Taiteishi et al.	18715	TUS	\
T. lanceolatum	120.752578	22.621472	Taiteishi et al.	19406	TI, TUS	\
T. lanceolatum	120.972147	24.239428	Su-Wen Chung	3528	HCT	
T. lanceolatum	120.901389	23.47861	S.Sasaki	s.n.	TAI	TAI091087
T. lanceolatum	120.88333	23.48333	Sheng-You Lu	s.n.	TAIF	TAIF182886
T. lanceolatum	121.295	24.698056	Ya-Yi Huang	1265	TAI	252626
T. lanceolatum	121.25222	23.95917	Chia-Chun Hsu	s.n.	TAIF	148970
T. lanceolatum	119.426269	30.339561	Law	898	K	\
T. lanceolatum	105.697692	25.315219	Tsiang	4637	K	\
T. luteoviride	88.277444	27.007611	Favre	012	LZ	\
T. luteoviride	88.618025	27.335936	Favre	015	LZ	\
T. luteoviride	98.7894	24.8111	H. Li, B. Bartholomew, Z. L. Dao	10768	CAS	\
T. luteoviride	98.767778	24.830833	H. Li, B. Bartholomew, Z. L. Dao	10678	CAS	\
T. luteoviride	98.660556	25.673889	H. Li, B. Bartholomew, Z. L. Dao	11041	CAS	\
T. luteoviride	88.2842	26.99495	A. Favre	014	FR	\
T. luteoviride	88.623128	27.371172	Hara	s.n.	TI	\
T. luteoviride	88.100033	26.996286	Kanai et al.	721178	TI	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
T. luteoviride	85.384236	27.569967	Kanai	673402	TI	\
T. luteoviride	87.936519	27.097031	Hara et al.	6302574	TI	\
T. luteoviride	83.755694	28.410028	A. Favre	1267a	LZ	\
T. luzonense	121.193803	24.1032	A. Favre	098	FR	\
T. luzonense	120.971111	23.744444	A. Favre	101	FR	\
T. luzonense	120.735844	22.615133	Pi-Fong Lu	17117	HCT	\
T. luzonense	120.75	22.4167	Ta-Yi Liu	s.n.	HAST	HAST25160
T. luzonense	120.7447	22.6114	Chih-Hsiung Chen	s.n.	HAST	HAST45214
T. luzonense	121.55	24.8333333	Pi-Fong Lu	s.n.	TAIF	TAIF224349
T. luzonense	120.9	16.6	Jacobs	7105	K	\
T. luzonense	121.480314	14.067822	Merrill	7511	BM	\
T. luzonense	124.924711	8.078875	Ramos and Edano	s.n.	BM, K	\
T. luzonense	120.054733	-2.121878	Eyma	1326	BO	\
T. membranaceum	100.136125	25.661039	Favre and Matuszak	104	FR, LZ	\
T. membranaceum	98.8	26.383333	G. Forrest	25060	E	E00001650
T. membranaceum	98.85	26.9	Li Heng	9755	MO	1403550
T. membranaceum	98.571667	27.713333	Gaoligong Shan Expedition 1996	7688	E	E00095307
T. membranaceum	97.4908	28.072781	Kingdon-Ward	7440	K	\
T. membranaceum	96.583319	28.249992	Kingdon-Ward	8599	K	\
T. membranaceum	97.275083	26.669894	Keenan, TuhAung and ThaHla	3177	K	\
T. membranaceum	98.167453	27.086817	Kingdon-Ward	21573	A, BM	\
T. membranaceum	98.745517	25.453317	Favre and Matuszak	24	FR, LZ	\
T. microphyllum	121.047667	24.296058	Ohashi & Iketani	21092	TUS	
T. microphyllum	120.8797	22.7317	Wai-Chao Leong	s.n.	HAST	HAST95213
T. microphyllum	120.9097	23.4769	Chien-I Huang	s.n.	HAST	HAST102012

Species	**Longitude**	**Latitude**	**Collector**	**Collector-No.**	**Herbarium**	**Catalogue-No.**
T. microphyllum	121.6353	24.2408	Chien-I Huang	s.n.	HAST	HAST111467
T. microphyllum	121.3967	24.5789	Ching-I Peng	s.n.	HAST	HAST101965
T. microphyllum	121.0139	24.2703	Jenn-Che Wang	s.n.	HAST	HAST110555
T. microphyllum	121.4539	24.4761	Chia-Hua Lin	s.n.	HAST	HAST84169
T. microphyllum	121.01389	24.27028	Jenn-Che Wang, Summer collection team	s.n.	TAIF	245200
T. microphyllum	121.26667	24.55	Tien-Chuan Hsu	s.n.	TAIF	233236
T. microphyllum	121.5237	24.493961	Suzuki	s.n.	A, TAI	\
T. nienkui	114.016506	23.283939	A. Favre	030	FR	\
T. nienkui	114.122944	22.410161	A. Favre	029	KUN	\
T. nienkui	105.64665	21.463644	A. Favre	26	NEU	\
T. nienkui	108.65	12.1	L. Averyanov et al.	VH4458	MO	1409883
T. nienkui	107.637925	15.130347	L. Averyanov, N.T. Ban, A. Budantzev, L. Budantzev, N.T. Hiep, D.D. Huyen, P.K. Loc, G. Yakovlev	VH1919	MNHN	P00348810
T. nienkui	105.633025	21.477914	P. A. Eberhardt	3968	MNHN	P00348815
T. nienkui	114.118142	22.415947	K. Y. Chan	1273	MNHN	P00348807
T. nienkui	100.110514	25.690767	Chungtien, Lijiang & Dali Expedition	1415	E	19911067
T. nienkui	110.118875	26.455144	Qin-Zhong Lin	s.n.	TAIF	TAIF316517
T. nienkui	103.850006	22.334389	A. J. B. Chevalier	29453	MNHN	P00348813
T. nigrobaccatum	85.437075	27.791011	Kanai, Hara & Ohba	72731	TI	\
T. nigrobaccatum	89.745033	27.491172	Sinclair & Long	4762	E	\
T. nigrobaccatum	89.727542	27.903614	Sinclair & Long	5004	E	\
T. nigrobaccatum	87.636883	27.543947	Kanai et al.	6300522	TI	\

Species	**Longitude**	**Latitude**	**Collector**	**Collector-No.**	**Herbarium**	**Catalogue-No.**
T. nigrobaccatum	87.494472	27.273133	Ohashi et al.	772993	TI	\
T. nigrobaccatum	86.202528	27.6592	Banerjii	s.n.	A	\
T. pallidum	103.33	29.52	S. C. Sun & K. Chang	1258	HUH	00114592
T. pallidum	98.5783333	27.7575	Gaoligong Shan Biodiversity Survey	17136	CAS	1033238
T. pallidum	98.660556	25.673889	Gaoligong Shan Expedition 1998	11041	E	E00268534
T. pallidum	104.42955	23.784228	F. Ducloux leg. M. Prey	2940	MNHN	P00354060
T. pallidum	103.344053	29.556653	A. Favre	088	LZ	\
T. pingbianense	103.697317	22.912947	A. Favre	110	KUN	\
T. pingbianense	103.708122	22.961189	A. Favre	Field observation	\	\
T. pingbianense	103.695822	22.99035	H.T.Tsai	61590	PE	\
T. pingbianense	100.752025	26.949447	Chris Stapleton	1030	E	E00031004
T. pingbianense	103.697892	22.912747	A. Favre	108	FR	\
T. robustum	108.008664	16.004211	M. Poliane	7098	UPS	V-139969
T. robustum	107.994833	15.99665	A. Favre	025	FR	\
T. sumatranum	98.384869	3.175964	A. Favre	018	LZ	\
T. sumatranum	98.526917	3.236944	A. Favre	021	FR	\
T. sumatranum	97.918094	3.521006	van Steenis	8306	K	K000195224
T. sumatranum	97.495319	3.774875	Iwatsuki et al.	1092	BO, KYO	\
T. sumatranum	98.397608	2.7689	Alston	14949	BM	\
T. sumatranum	98.504881	3.241197	Ridley	s.n.	K	\
T. taiwanense	120.792181	23.496342	A. Favre	127	TNM	\
T. taiwanense	120.696808	23.506781	Unknown	199	HCT	\
T. taiwanense	121.409089	24.82435	Pi-Fong Lu	17697	HCT	\

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
T. taiwanense	121.305556	24.348611	J. C. Wang, S. F. Huang, K. C. Yang, W. S. Tang	3972	TAI	\
T. taiwanense	120.81667	22.98333	Unknown	\	TAIF	139168
T. taiwanense	120.90056	22.36139	Sheng-You Lu	\	TAIF	183048
T. taiwanense	120.7603	22.4114	Chien-I Huang	\	HAST	HAST115551
T. taiwanense	121.03333	22.9	Unknown	\	TAIF	171707
T. taiwanense	120.75111	23.08083	Kuoh-Cheng Yang, Sheng-Chieh Wu	s.n.	TAIF	147792
T. taiwanense	120.94722	24.42361	Pi-Fong Lu	s.n.	TAIF	187180
T. trinerve	106.732914	-6.715589	Van den Brinck	4123	K	\
T. trinerve	106.977706	-6.779119	A. Favre	140	LZ	\
T. trinerve	100.670106	-0.327922	Nagamasu	3363	TI	\
T. trinerve	99.666	8.5	K. Larsen	s.n.	AAU	K.Larsen45974
T. trinerve	106.732831	-6.714972	H. Zollinger	1701	MNHN	P00354068
T. trinerve	106.951514	-6.760233	H. Zollinger	s.n.	MNHN	P00354069
T. trinerve	107.400439	-7.161128	Backer	12768	BO	\
T. trinerve	97.817333	3.69625	Wilde and Wilde-Dufjes	14247	K	\
T. trinervium	130.50967	30.3138	M. Kusaka et Y. Kobayashi	s.n.	FFPRI	25946
T. trinervium	133.056	33.5708	\	\	MBK	11731
T. trinervium	133.519	33.6625	\	\	MBK	11738
T. trinervium	138.939	36.7258	\	\	GMNHJ	BS0065427
T. trinervium	138.413	36.5417	\	\	GMNHJ	BS0071822
T. trinervium	140.8771	40.6592	Hosoi	s.n.	FFPRI	FFPRI:TFA:25928
T. trinervium	140.402	41.142	M. Kusaka	s.n.	FFPRI	FFPRI:TFA:25935
T. trinervium	141.152419	42.950305	M. Kusaka	s.n.	FFPRI	FFPRI:TFA:25938

Species	**Longitude**	**Latitude**	**Collector**	**Collector-No.**	**Herbarium**	**Catalogue-No.**
T. trinervium	142.2506	43.09665	M. Kusaka	s.n.	FFPRI	FFPRI:TFA:25930
T. trinervium	140.704	37.7736	\	\	FKSE	60783
T. volubile	98.7894	24.8111	H. Li, B. Bartholomew & Z. L. Dao	41637	HUH	41637
T. volubile	86.716667	27.7075	Third Darwin Nepal Fieldwork Training Expedition	BX302	E	E00248422
T. volubile	86.705278	27.757222	Third Darwin Nepal Fieldwork Training Expedition	BY222	E	E00248413
T. volubile	88.6	27.35	Edinburgh Expedition to Northern Sikkim 1996	11	E	E00048379
T. volubile	98.460833	27.784444	Gaoligong Shan Biotic Survey Expedition - Summer 2006	33939	E	E00640169
T. volubile	85.995522	27.987011	Tibetan Chinese Traditional Medicine Expedition	1160	PE	\
T. volubile	85.45	28.183333	Edinburgh Nepal Expedition 2001	386	E	E00210277
T. volubile	98.88	28.54	Danica M. Anderson, Jan Salick, B. F. Gunn, Yin Xuezhen & Senadorji	311	MO	1934375
T. volubile	98.8	28.18	Kurt Hoffmeister, Deng Zhiwei, Anthony Amend	7	MO	3010967
T. volubile	98.6606	25.6739	H. Li, B. Bartholomew & Z. L. Dao	s.n.	HUH	41912

Species	Longitude	Latitude	Collector	Collector-No.	Herbarium	Catalogue-No.
T. volubile	93.506328	27.374694	Stonor	1	E	\
T. volubile	95.428325	26.615217	Bor	6762	K	\
T. volubile	94.388606	25.093464	Kingdon-Ward	17777	NY	\
T. volubile	83.755694	28.410028	A. Favre	1267a	LZ	\

Appendix 13. Model fit of trait-dependent diversification of five putative key innovations: fruit type (capsule or berry), stamens (straight or recurved), internodes (normal or elongated), habitus (erect or twining), and corolla lobation (shallow or deep). Eight models with different constraint settings: speciation rate λ equal or unequal, extinction rate μ equal or unequal, and transition rate q equal or unequal. To estimate the best model for each trait we used the Bayes factor (BF) test, which is defined as the ratio between the marginal likelihoods. It should be interpreted as outlined in Kass & Raftery (1995): a value of 0 to 2 indicates no evidence against M_0, but 2 to >10 provide evidence against M_0. The best model has only a slightly better fit than the following, therefore, the simpler evolutionary model (within BF < 2) was preferred for parameter estimation (marked in bold).

Model	**log Marginal Likelihood**	**BF**
Corolla lobation		
λ0=λ1, μ0=μ1, q01=q10	-156.8051	0
λ0=λ1, μ0≠μ1, q01=q10	-157.3958	1.1814
λ0=λ1, μ0≠μ1, q01≠q10	-159.8462	6.0822
λ0=λ1, μ0=μ1, q01≠q10	-160.2981	6.986
λ0≠λ1, μ0=μ1, q01=q10	-162.7125	11.8148
λ0≠λ1, μ0≠μ1, q01=q10	-163.7625	13.9148
λ0≠λ1, μ0=μ1, q01≠q10	-165.2282	16.8462
λ0≠λ1, μ0≠μ1, q01≠q10	-166.7322	19.8542
Fruits		
λ0≠λ1, μ0=μ1, q01=q10	-166.3829	0
λ0≠λ1, μ0≠μ1, q01=q10	-166.9236	1.0814
λ0=λ1, μ0≠μ1, q01=q10	-167.379	1.9922
λ0=λ1, μ0=μ1, q01=q10	-167.3912	2.0166
λ0=λ1, μ0≠μ1, q01≠q10	-167.7128	2.6598
λ0≠λ1, μ0≠μ1, q01≠q10	-168.3576	3.9494
λ0≠λ1, μ0=μ1, q01≠q10	-169.725	6.6842
λ0=λ1, μ0=μ1, q01≠q10	-171.0796	9.3934
Habitus		
λ0=λ1, μ0≠μ1, q01=q10	-160.5379	0
λ0=λ1, μ0=μ1, q01=q10	-161.9507	2.8256
λ0≠λ1, μ0=μ1, q01=q10	-163.8301	6.5844
λ0≠λ1, μ0≠μ1, q01=q10	-164.4472	7.8186
λ0=λ1, μ0≠μ1, q01≠q10	-165.3783	9.6808
λ0=λ1, μ0=μ1, q01≠q10	-166.0987	11.1216
λ0≠λ1, μ0=μ1, q01≠q10	-167.9554	14.835

Model	log Marginal Likelihood	BF
λ0≠λ1, μ0≠μ1, q01≠q10	-168.3231	15.5704
Internodes		
λ0=λ1, μ0=μ1, q01=q10	-160.2378	0
λ0=λ1, μ0≠μ1, q01=q10	-160.8294	1.1832
λ0=λ1, μ0=μ1, q01≠q10	-163.4364	6.3972
λ0=λ1, μ0≠μ1, q01≠q10	-164.8805	9.2854
λ0≠λ1, μ0=μ1, q01=q10	-165.7324	10.9892
λ0≠λ1, μ0≠μ1, q01=q10	-167.1899	13.9042
λ0≠λ1, μ0≠μ1, q01≠q10	-168.7277	16.9798
λ0≠λ1, μ0=μ1, q01≠q10	-168.7816	17.0876
Stamens		
λ0=λ1, μ0≠μ1, q01=q10	-158.8323	0
λ0=λ1, μ0=μ1, q01=q10	-159.1544	0.6442
λ0≠λ1, μ0=μ1, q01=q10	-162.7587	7.8528
λ0≠λ1, μ0≠μ1, q01=q10	-163.1543	8.644
λ0=λ1, μ0=μ1, q01≠q10	-163.3713	9.078
λ0=λ1, μ0≠μ1, q01≠q10	-163.4028	9.141
λ0≠λ1, μ0=μ1, q01≠q10	-167.7429	17.8212
λ0≠λ1, μ0≠μ1, q01≠q10	-169.2155	20.7664

Genus	Species	Fruit	Stamens	Internodes	Habitus	Corolla lobation
Crawfurdia	*angustata*	capsule	straight	elongated	twining	shallow
Crawfurdia	*speciosa*	capsule	straight	elongated	twining	shallow
Crawfurdia	*dimidiata*	capsule	straight	elongated	twining	shallow
Crawfurdia	*pricei*	capsule	straight	elongated	twining	shallow
Crawfurdia	*crawfurdioides*	capsule	straight	elongated	twining	shallow
Crawfurdia	*maculaticaulis*	capsule	straight	elongated	twining	shallow
Crawfurdia	*poilanei*	capsule	straight	elongated	twining	shallow
Crawfurdia	*campanulacea*	capsule	straight	elongated	twining	shallow
Crawfurdia	*gracilipes*	capsule	straight	elongated	twining	shallow
Crawfurdia	*delavayi*	capsule	straight	elongated	twining	shallow
Crawfurdia	*sessiliflora*	capsule	straight	elongated	twining	shallow
Crawfurdia	*puberulenta*	capsule	straight	elongated	twining	shallow
Metagentiana	*primuliflora*	capsule	recurved	normal	erect	shallow
Metagentiana	*eurycolpa*	capsule	recurved	normal	erect	shallow
Metagentiana	*leptoclada*	capsule	recurved	normal	erect	shallow
Metagentiana	*pterocalyx*	capsule	recurved	normal	erect	shallow
Metagentiana	*gentilis*	capsule	recurved	normal	erect	shallow
Metagentiana	*serra*	capsule	recurved	normal	erect	shallow
Metagentiana	*australis*	capsule	recurved	normal	erect	shallow
Metagentiana	*rhodantha*	capsule	recurved	normal	erect	shallow
Metagentiana	*villifera*	capsule	recurved	normal	erect	shallow

Genus	Species	Fruit	Stamens	Internodes	Habitus	Corolla lobation
Sinogentiana	*souliei*	capsule	recurved	elongated	erect	shallow
Sinogentiana	*striata*	capsule	recurved	elongated	erect	shallow
Tripterospermum	*hirticalyx*	berry	recurved	elongated	twining	shallow
Tripterospermum	*cordifolioides*	berry	recurved	elongated	twining	shallow
Tripterospermum	*distylum*	capsule	recurved	elongated	twining	shallow
Tripterospermum	*discoideum*	capsule	recurved	elongated	twining	shallow
Tripterospermum	*brevidentatum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*robustum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*alutaceifolium*	berry	recurved	elongated	twining	shallow
Tripterospermum	*trinerve*	berry	recurved	elongated	twining	shallow
Tripterospermum	*australe*	berry	recurved	elongated	twining	shallow
Tripterospermum	*lilungshanensis*	berry	recurved	elongated	twining	shallow
Tripterospermum	*microphyllum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*tanatorajanense*	berry	recurved	elongated	twining	shallow
Tripterospermum	*luzonense*	berry	recurved	elongated	twining	shallow
Tripterospermum	*chinense*	capsule	recurved	elongated	twining	shallow
Tripterospermum	*pinbianense*	berry	recurved	elongated	twining	shallow
Tripterospermum	*membranaceum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*volubile*	berry	recurved	elongated	twining	shallow
Tripterospermum	*luteoviride*	berry	recurved	elongated	twining	shallow
Tripterospermum	*nigrobaccatum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*taiwanense*	berry	recurved	elongated	twining	shallow
Tripterospermum	*cordifolium*	berry	recurved	elongated	twining	shallow
Tripterospermum	*championii*	berry	recurved	elongated	twining	shallow
Tripterospermum	*nienkui*	berry	recurved	elongated	twining	shallow
Tripterospermum	*sumatranum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*cordatum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*japonicum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*maculatum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*lanceolatum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*pallidum*	berry	recurved	elongated	twining	shallow
Tripterospermum	*fasciculatum*	capsule	recurved	elongated	twining	shallow
Kuepferia	*otophora*	capsule	straight	normal	erect	deep
Kuepferia	*otophoroides*	capsule	straight	normal	erect	deep
Kuepferia	*doxiongshangensis*	capsule	straight	normal	erect	deep
Kuepferia	*decorata*	capsule	straight	normal	erect	deep
Kuepferia	*damyonensis*	capsule	straight	normal	erect	deep

Appendix 14. A Kolmogorov-Smirnov Test (KS-Test; to determine if two data sets differed significantly) and a T-Test (to determine if the mean of the data sets differed significantly) were performed using the original data from the WorldClim data set (Hijmans et al., 2005) obtained for all species occurrence points of *Crawfurdia*, *Kuepferia*, *Metagentiana*, *Sinogentiana* and *Tripterospermum* for altitude and 19 bioclimatic variables. The mean, standard deviation and the 95% HPD interval of the original data are shown for each genus and variable.

Variable [unit]	**Data sets between**	**KS-Test**		**T-Test**		**Genus**	**Mean**	**St.dev.**	**95% HPD Interval**
		p-Value	**differ data?**	**p-Value**	**differ mean?**				
Altitude [m]	*Crawfurdia - Kuepferia*	0.0002908	yes	7.03E-08	yes	*Crawfurdia*	2514.96	1012.7	[649, 4203]
	Crawfurdia - Metagentiana	0.0008072	yes	0.02219	yes				
	Crawfurdia - Sinogentiana	0.0002101	yes	6.28E-05	yes	***Kuepferia***	3432.27	739.46	[2312, 4751]
	Crawfurdia - Tripterospermum	<2.2E-16	yes	5.24E-16	yes				
	Kuepferia - Metagentiana	4.46E-09	yes	2.31E-12	yes	*Metagentiana*	2213.55	735.03	[947, 3563]
	Kuepferia - Sinogentiana	**0.4858**	**no**	**0.8143**	**no**				
	Kuepferia - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	***Sinogentiana***	3468.53	735.93	[2196, 4480]
	Metagentiana - Sinogentiana	1.53E-06	yes	3.46E-07	yes				
	Metagentiana - Tripterospermum	3.05E-09	yes	1.85E-08	yes	*Tripterospermum*	1478.65	917.2	[98, 3050]
	Sinogentiana - Tripterospermum	1.27E-09	yes	1.11E-10	yes				
Bio1 (annual mean temperature) [°C]	*Crawfurdia - Kuepferia*	0.0005066	yes	6.99E-07	yes	*Crawfurdia*	11.34	4.7	[2.4, 18.7]
	Crawfurdia - Metagentiana	0.002572	yes	0.008708	yes				
	Crawfurdia - Sinogentiana	2.74E-07	yes	2.74E-09	yes	*Kuepferia*	7.07	4.15	[-0.6, 13.7]
	Crawfurdia - Tripterospermum	4.23E-10	yes	3.47E-11	yes				
	Kuepferia - Metagentiana	2.72E-08	yes	1.73E-11	yes	*Metagentiana*	12.86	3.03	[7.9, 18.7]
	Kuepferia - Sinogentiana	0.002419	yes	0.000838	yes				
	Kuepferia - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Sinogentiana*	3.23	3.54	[0.6, 11.2]

Variable [unit]		KS-Test		T-Test					
	Data sets between	**p-Value**	**differ data?**	**p-Value**	**differ mean?**	**Genus**	**Mean**	**St.dev.**	**95% HPD Interval**
	Metagentiana - Sinogentiana	3.28E-08	yes	1.53E-11	yes				
	Metagentiana - Tripterospermum	8.22E-06	yes	0.000278	yes	*Tripterospermum*	14.98	4.8	[4.8, 23.6]
	Sinogentiana - Tripterospermum	4.42E-11	yes	1.88E-12	yes				
Bio2 (mean diurnal range) [°C]	*Crawfurdia - Kuepferia*	0.0004219	yes	8.92E-07	yes	*Crawfurdia*	10.32	1.56	[7.5, 12.9]
	Crawfurdia - Metagentiana	0.005227	yes	**0.06205**	**no**				
	Crawfurdia - Sinogentiana	1.05E-06	yes	2.53E-05	yes	*Kuepferia*	11.65	1.25	[10.3, 13.9]
	Crawfurdia - Tripterospermum	2.44E-15	yes	4.85E-14	yes				
	Kuepferia - Metagentiana	0.01542	yes	0.00142	yes	*Metagentiana*	10.68	1.25	[7.4, 12]
	Kuepferia - Sinogentiana	0.02043	yes	0.01746	yes				
	Kuepferia - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Sinogentiana*	12.84	1.95	[10.4, 15.4]
	Metagentiana - Sinogentiana	2.37E-05	yes	0.000267	yes				
	Metagentiana - Tripterospermum	7.11E-15	yes	<2.2E-16	yes	*Tripterospermum*	8.8	1.61	[6.2, 11.7]
	Sinogentiana - Tripterospermum	1.53E-10	yes	2.38E-08	yes				
Bio3 (isothermality) [no unit]	*Crawfurdia - Kuepferia*	**0.341**	**no**	0.005217	yes	***Crawfurdia***	0.43	0.05	[0.31, 0.49]
	Crawfurdia - Metagentiana	2.48E-06	yes	**0.4015**	**no**				
	Crawfurdia - Sinogentiana	**0.2865**	**no**	**0.1408**	**no**	*Kuepferia*	0.45	0.02	[0.42, 0.49]
	Crawfurdia - Tripterospermum	0.001865	yes	**0.1726**	**no**				
	Kuepferia - Metagentiana	0.004034	yes	**0.3741**	**no**	*Metagentiana*	0.43	0.07	[0.26, 0.49]
	Kuepferia - Sinogentiana	0.02043	yes	0.01076	yes				
	Kuepferia - Tripterospermum	2.63E-05	yes	**0.8928**	**no**	***Sinogentiana***	0.4	0.06	[0.31, 0.48]
	Metagentiana - Sinogentiana	0.002342	yes	**0.06571**	**no**				
	Metagentiana - Tripterospermum	0.0001032	yes	**0.6073**	**no**	*Tripterospermum*	0.45	0.03	[0.22, 0.83]
	Sinogentiana - Tripterospermum	**0.1915**	**no**	0.03126	yes				

Variable [unit]		**KS-Test**		**T-Test**					
	Data sets between	**p-Value**	**differ data?**	**p-Value**	**differ mean?**	**Genus**	**Mean**	**St.dev.**	**95% HPD Interval**
Bio4 (temperature seasonality) [°C]	***Crawfurdia - Kuepferia***	**0.0394**	**no**	**0.07959**	**no**	***Crawfurdia***	49.46	6.96	[37.41, 61.18]
	Crawfurdia - Metagentiana	0.001849	yes	**0.8343**	**no**				
	Crawfurdia - Sinogentiana	1.23E-05	yes	0.000101	yes	***Kuepferia***	51.59	5.88	[42.46, 57.95]
	Crawfurdia - Tripterospermum	1.45E-08	yes	0.000973	yes				
	Kuepferia - Metagentiana	0.0001045	yes	**0.1675**	**no**	*Metagentiana*	50.02	9.86	[41.56, 74.5]
	Kuepferia - Sinogentiana	9.49E-05	yes	0.000431	yes				
	Kuepferia - Tripterospermum	5.30E-08	yes	1.45E-05	yes	*Sinogentiana*	65.62	13	[46.16, 83.32]
	Metagentiana - Sinogentiana	4.49E-05	yes	9.20E-05	yes				
	Metagentiana - Tripterospermum	1.29E-06	yes	0.01384	yes	*Tripterospermum*	44.35	22.94	[2.61, 83.6]
	Sinogentiana - Tripterospermum	5.93E-06	yes	1.84E-06	yes				
Bio5 (max. temperature of warmest month) [°C]	*Crawfurdia - Kuepferia*	0.002403	yes	8.63E-07	yes	*Crawfurdia*	21.62	4.38	[14.6, 30.4]
	Crawfurdia - Metagentiana	5.73E-05	yes	0.002902	yes				
	Crawfurdia - Sinogentiana	5.61E-05	yes	3.42E-05	yes	***Kuepferia***	18.2	2.97	[12.1, 22.1]
	Crawfurdia - Tripterospermum	4.69E-11	yes	5.75E-10	yes				
	Kuepferia - Metagentiana	6.69E-10	yes	8.30E-12	yes	*Metagentiana*	23.47	3.66	[16.9, 29.4]
	Kuepferia - Sinogentiana	**0.09463**	**no**	**0.2828**	**no**				
	Kuepferia - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	***Sinogentiana***	17.22	3.26	[13.4, 23.4]
	Metagentiana - Sinogentiana	1.23E-05	yes	3.00E-08	yes				
	Metagentiana - Tripterospermum	0.003243	yes	0.02956	yes	*Tripterospermum*	24.73	4.37	[17.3, 33]
	Sinogentiana - Tripterospermum	1.59E-07	yes	1.36E-09	yes				
Bio6 (min. temperature of coldest month) [°C]	*Crawfurdia - Kuepferia*	7.43E-05	yes	6.09E-07	yes	*Crawfurdia*	-2.27	5.57	[-11.7, 7.2]
	Crawfurdia - Metagentiana	0.003171	yes	**0.07739**	**no**				
	Crawfurdia - Sinogentiana	1.17E-07	yes	1.14E-08	yes	*Kuepferia*	-7.69	5.38	[-18.8, 0.3]
	Crawfurdia - Tripterospermum	7.52E-12	yes	6.57E-15	yes				

Variable [unit]	**Data sets between**	**KS-Test**		**T-Test**		**Genus**	**Mean**	**St.dev.**	**95% HPD Interval**
		p-Value	**differ data?**	**p-Value**	**differ mean?**				
	Kuepferia - Metagentiana	4.46E-09	yes	7.16E-10	yes	*Metagentiana*	-1.19	3.12	[-6.4, 3.7]
	Kuepferia - Sinogentiana	0.000135	yes	9.44E-05	yes				
	Kuepferia - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Sinogentiana*	-14.72	5.7	[-19.3, -2.1]
	Metagentiana - Sinogentiana	1.44E-08	yes	1.35E-09	yes				
	Metagentiana - Tripterospermum	3.33E-10	yes	1.02E-09	yes	*Tripterospermum*	3.41	7.26	[-11.7, 16.6]
	Sinogentiana - Tripterospermum	5.62E-11	yes	7.05E-12	yes				
Bio7 (temperature annual range) [°C]	*Crawfurdia - Kuepferia*	0.005055	yes	0.000425	yes	***Crawfurdia***	23.9	2.69	[17.9, 28.8]
	Crawfurdia - Metagentiana	**0.2388**	**no**	**0.07214**	**no**				
	Crawfurdia - Sinogentiana	1.12E-06	yes	2.02E-06	yes	*Kuepferia*	25.89	2.96	[22.5, 30]
	Crawfurdia - Tripterospermum	5.44E-11	yes	5.20E-08	yes				
	Kuepferia - Metagentiana	**0.1162**	**no**	0.03619	yes	***Metagentiana***	24.65	2.15	[22.4, 30.2]
	Kuepferia - Sinogentiana	0.0002681	yes	6.76E-05	yes				
	Kuepferia - Tripterospermum	8.45E-12	yes	6.41E-13	yes	*Sinogentiana*	31.94	4.83	[25, 38.4]
	Metagentiana - Sinogentiana	1.18E-13	yes	7.85E-06	yes				
	Metagentiana - Tripterospermum	1.18E-13	yes	3.68E-11	yes	*Tripterospermum*	21.32	6.45	[10.6, 32.3]
	Sinogentiana - Tripterospermum	6.27E-08	yes	1.30E-08	yes				
Bio8 (mean temperature of wettest quarter) [°C]	*Crawfurdia - Kuepferia*	0.0005066	yes	7.66E-07	yes	*Crawfurdia*	16.89	4.19	[9.5, 23.9]
	Crawfurdia - Metagentiana	0.0003591	yes	0.004075	yes				
	Crawfurdia - Sinogentiana	7.29E-06	yes	1.04E-07	yes	*Kuepferia*	13.26	3.48	[6.8, 18.1]
	Crawfurdia - Tripterospermum	1.64E-09	yes	8.96E-09	yes				
	Kuepferia - Metagentiana	7.07E-09	yes	5.03E-12	yes	*Metagentiana*	18.57	3.17	[12.1, 23.7]
	Kuepferia - Sinogentiana	0.02043	yes	0.01722	yes				
	Kuepferia - Tripterospermum	4.44E-16	yes	<2.2E-16	yes	*Sinogentiana*	10.99	3.11	[7.2, 15]
	Metagentiana - Sinogentiana	3.28E-08	yes	1.98E-10	yes				

Variable [unit]	Data sets between	KS-Test p-Value	KS-Test differ data?	T-Test p-Value	T-Test differ mean?	Genus	Mean	St.dev.	95% HPD Interval
	Metagentiana - Tripterospermum	**0.07991**	**no**	0.02285	yes	*Tripterospermum*	19.6	4.13	[13, 27.9]
	Sinogentiana - Tripterospermum	3.68E-10	yes	5.41E-11	yes				
Bio9 (mean temperature of driest quarter) [°C]	*Crawfurdia - Kuepferia*	8.49E-05	yes	1.47E-06	yes	***Crawfurdia***	5.38	4.68	[-2.6, 13.9]
	Crawfurdia - Metagentiana	**0.09568**	**no**	**0.1274**	**no**				
	Crawfurdia - Sinogentiana	1.59E-07	yes	1.89E-09	yes	*Kuepferia*	0.83	4.81	[-8.4, 7.9]
	Crawfurdia - Tripterospermum	2.76E-08	yes	5.96E-13	yes				
	Kuepferia - Metagentiana	1.73E-06	yes	2.34E-08	yes	***Metagentiana***	6.03	3.09	[-0.9, 9.9]
	Kuepferia - Sinogentiana	0.000135	yes	2.36E-05	yes				
	Kuepferia - Tripterospermum	4.44E-16	yes	<2.2E-16	yes	*Sinogentiana*	-5.58	4.54	[-10.4, 3]
	Metagentiana - Sinogentiana	1.44E-08	yes	1.17E-10	yes				
	Metagentiana - Tripterospermum	4.33E-07	yes	9.07E-07	yes	*Tripterospermum*	9.77	6.63	[-2.7, 22.8]
	Sinogentiana - Tripterospermum	7.53E-12	yes	1.26E-12	yes				
Bio10 (mean temperature of warmest quarter) [°C]	*Crawfurdia - Kuepferia*	0.0008667	yes	7.29E-07	yes	*Crawfurdia*	17.13	4.55	[8.6, 25.2]
	Crawfurdia - Metagentiana	0.0003591	yes	0.006984	yes				
	Crawfurdia - Sinogentiana	1.17E-05	yes	4.01E-07	yes	*Kuepferia*	13.27	3.44	[6.8, 18.1]
	Crawfurdia - Tripterospermum	3.11E-11	yes	1.06E-09	yes				
	Kuepferia - Metagentiana	7.07E-09	yes	3.81E-12	yes	*Metagentiana*	18.7	3.32	[12.1, 23.7]
	Kuepferia - Sinogentiana	0.02043	yes	0.03339	yes				
	Kuepferia - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Sinogentiana*	11.17	3.32	[7.2, 16.2]
	Metagentiana - Sinogentiana	1.61E-07	yes	9.74E-10	yes				
	Metagentiana - Tripterospermum	0.003125	yes	0.002793	yes	*Tripterospermum*	20.2	4.2	[13.6, 27.9]
	Sinogentiana - Tripterospermum	4.87E-10	yes	7.79E-11	yes				
Bio 11	*Crawfurdia - Kuepferia*	0.000373	yes	2.16E-06	yes	*Crawfurdia*	4.57	4.73	[-3.7, 12.3]
	Crawfurdia - Metagentiana	0.001517	yes	0.01566	yes				

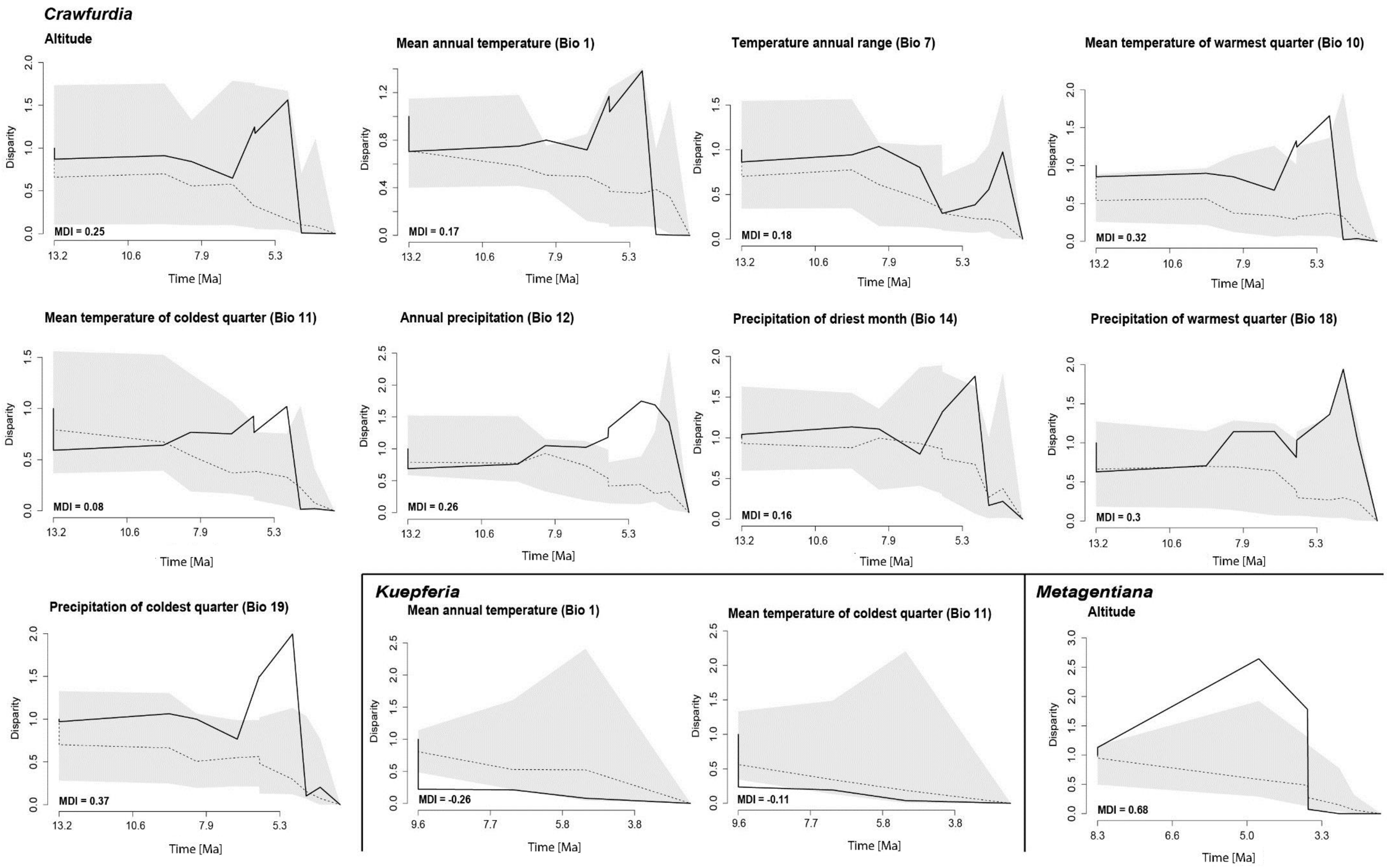
Variable [unit]		KS-Test		T-Test					
	Data sets between	**p-Value**	**differ data?**	**p-Value**	**differ mean?**	**Genus**	**Mean**	**St.dev.**	**95% HPD Interval**
Bio11 (mean temperature of coldest quarter) [°C]	*Crawfurdia - Sinogentiana*	1.59E-07	yes	8.50E-09	yes	*Kuepferia*	0.17	4.7	[-8.4, 7.8]
	Crawfurdia - Tripterospermum	1.13E-07	yes	1.91E-12	yes				
	Kuepferia - Metagentiana	1.11E-08	yes	5.92E-10	yes	*Metagentiana*	5.95	3.12	[-0.9, 9.9]
	Kuepferia - Sinogentiana	0.0002681	yes	9.62E-05	yes				
	Kuepferia - Tripterospermum	4.89E-15	yes	<2.2E-16	yes	*Sinogentiana*	-5.7	4.58	[-10.4, 3]
	Metagentiana - Sinogentiana	1.44E-08	yes	1.67E-10	yes				
	Metagentiana - Tripterospermum	1.95E-06	yes	4.21E-05	yes	*Tripterospermum*	8.93	6.74	[-3.9, 22.3]
	Sinogentiana - Tripterospermum	5.33E-11	yes	4.30E-12	yes				
Bio12 (annual precipitation) [mm]	*Crawfurdia - Kuepferia*	0.0009741	yes	5.41E-07	yes	*Crawfurdia*	1248.75	416.83	[656, 1968]
	Crawfurdia - Metagentiana	3.89E-05	yes	0.000162	yes				
	Crawfurdia - Sinogentiana	2.15E-07	yes	1.52E-09	yes	*Kuepferia*	888.63	298.83	[363, 1370]
	Crawfurdia - Tripterospermum	3.13E-14	yes	<2.2E-16	yes				
	Kuepferia - Metagentiana	0.007572	yes	0.03557	yes	*Metagentiana*	995.43	117.3	[746, 1238]
	Kuepferia - Sinogentiana	0.016	yes	0.02108	yes				
	Kuepferia - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Sinogentiana*	738.59	211.05	[426, 1000]
	Metagentiana - Sinogentiana	1.53E-06	yes	5.05E-05	yes				
	Metagentiana - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Tripterospermum*	2104.35	921.44	[736, 3449]
	Sinogentiana - Tripterospermum	7.96E-12	yes	<2.2E-16	yes				
Bio13 (precipitation of wettest month) [mm]	*Crawfurdia - Kuepferia*	0.00268	yes	2.33E-05	yes	*Crawfurdia*	265.32	104.54	[141, 422]
	Crawfurdia - Metagentiana	0.0005075	yes	0.002944	yes				
	Crawfurdia - Sinogentiana	1.23E-05	yes	2.82E-07	yes	*Kuepferia*	195.17	61.34	[92, 289]
	Crawfurdia - Tripterospermum	5.26E-11	yes	6.86E-12	yes				
	Kuepferia - Metagentiana	0.01223	yes	**0.05735**	**no**	*Metagentiana*	216.2	27.46	[167, 265]
	Kuepferia - Sinogentiana	**0.07748**	**no**	0.02603	yes				

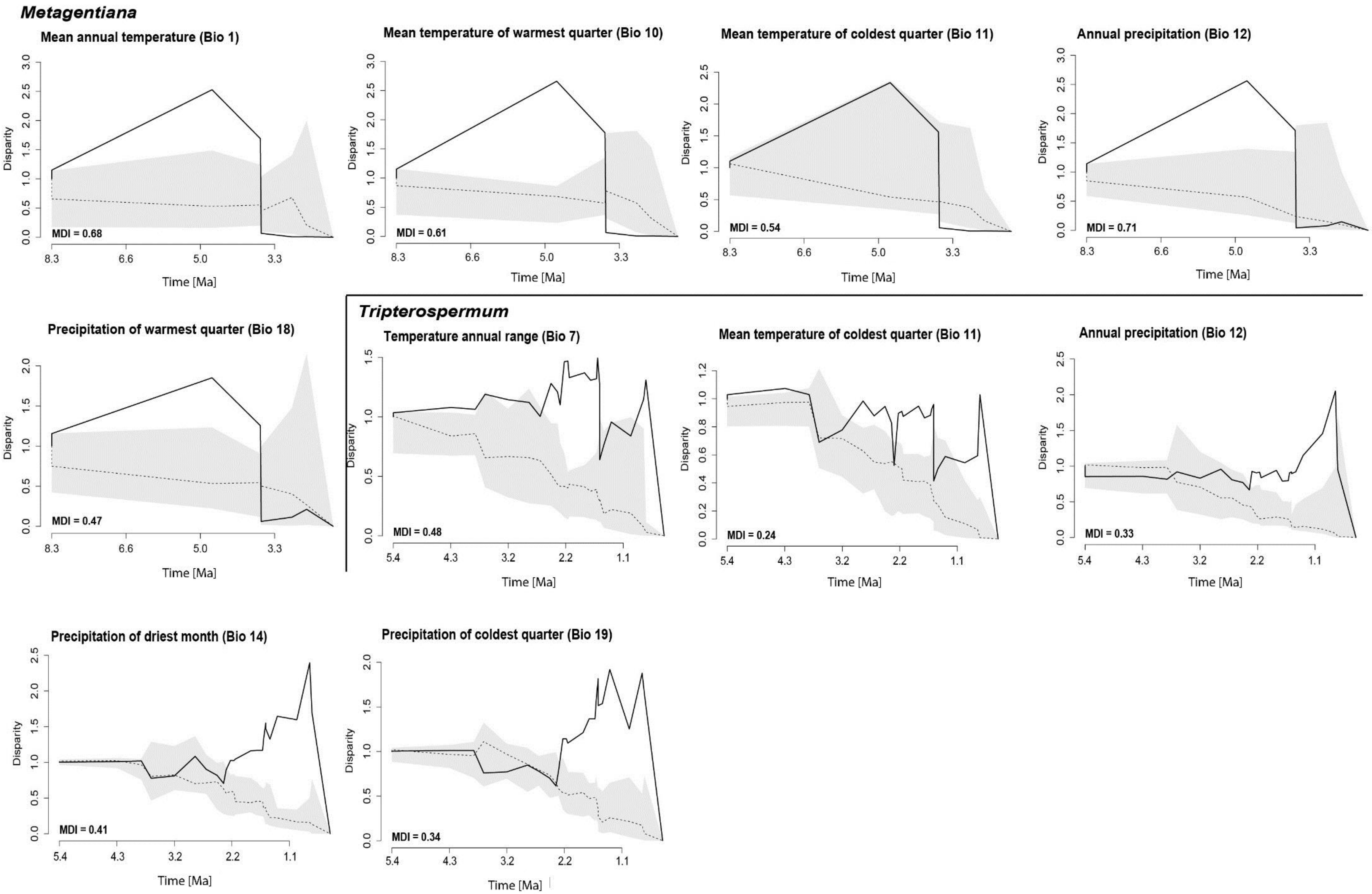
Variable [unit]		KS-Test		T-Test					
	Data sets between	p-Value	differ data?	p-Value	differ mean?	Genus	Mean	St.dev.	95% HPD Interval
	Kuepferia - Tripterospermum	1.14E-12	yes	<2.2E-16	yes	*Sinogentiana*	159.71	52.03	[91, 235]
	Metagentiana - Sinogentiana	0.0004807	yes	0.000341	yes				
	Metagentiana - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Tripterospermum*	387.87	206.68	[149, 719]
	Sinogentiana - Tripterospermum	2.78E-09	yes	<2.2E-16	yes				
Bio14 (precipitation of driest month) [mm]	*Crawfurdia - Kuepferia*	0.001928	yes	3.69E-05	yes	*Crawfurdia*	12.23	10.47	[2, 39]
	Crawfurdia - Metagentiana	0.006257	yes	0.01777	yes				
	Crawfurdia - Sinogentiana	4.95E-07	yes	1.66E-09	yes	*Kuepferia*	6.56	3.74	[1, 12]
	Crawfurdia - Tripterospermum	5.88E-14	yes	<2.2E-16	yes				
	Kuepferia - Metagentiana	0.01542	yes	0.008675	yes	*Metagentiana*	9.18	4.74	[4, 23]
	Kuepferia - Sinogentiana	0.0009789	yes	0.000263	yes				
	Kuepferia - Tripterospermum	1.67E-15	yes	<2.2E-16	yes	*Sinogentiana*	4	1.87	[2, 6]
	Metagentiana - Sinogentiana	4.49E-05	yes	1.94E-08	yes				
	Metagentiana - Tripterospermum	3.66E-15	yes	<2.2E-16	yes	*Tripterospermum*	41.96	38.6	[2, 127]
	Sinogentiana - Tripterospermum	2.32E-10	yes	<2.2E-16	yes				
Bio15 (precipitation seasonality) [no unit]	*Crawfurdia - Kuepferia*	**0.06401**	**no**	0.002682	yes	*Crawfurdia*	83.81	10.87	[56, 101]
	Crawfurdia - Metagentiana	1.17E-06	yes	0.002855	yes				
	Crawfurdia - Sinogentiana	0.0001749	yes	0.000594	yes	***Kuepferia***	89.2	9.39	[79, 107]
	Crawfurdia - Tripterospermum	1.83E-09	yes	1.82E-14	yes				
	Kuepferia - Metagentiana	**0.0671**	**no**	**0.959**	**no**	***Metagentiana***	88.78	9.82	[60, 102]
	Kuepferia - Sinogentiana	**0.07748**	**no**	**0.2355**	**no**				
	Kuepferia - Tripterospermum	3.29E-10	yes	<2.2E-16	yes	***Sinogentiana***	91.24	7.5	[82, 106]
	Metagentiana - Sinogentiana	**0.1376**	**no**	**0.2172**	**no**				
	Metagentiana - Tripterospermum	9.40E-11	yes	<2.2E-16	yes	*Tripterospermum*	69.21	24.03	[21, 104]
	Sinogentiana - Tripterospermum	7.32E-06	yes	2.52E-13	yes				

Variable [unit]	Data sets between	KS-Test		T-Test		Genus	Mean	St.dev.	95% HPD Interval
		p-Value	differ data?	p-Value	differ mean?				
Bio16 (precipitation of wettest quarter) [mm]	*Crawfurdia - Kuepferia*	0.001456	yes	1.17E-05	yes	*Crawfurdia*	702.9	260.92	[388, 1095]
	Crawfurdia - Metagentiana	2.31E-05	yes	0.002489	yes				
	Crawfurdia - Sinogentiana	1.17E-05	yes	8.88E-08	yes	*Kuepferia*	515.42	165.21	[306, 846]
	Crawfurdia - Tripterospermum	8.44E-11	yes	4.80E-13	yes				
	Kuepferia - Metagentiana	0.005211	yes	0.03788	yes	*Metagentiana*	577.02	62.13	[479, 683]
	Kuepferia - Sinogentiana	**0.09463**	**no**	0.02881	yes				
	Kuepferia - Tripterospermum	7.69E-13	yes	<2.2E-16	yes	*Sinogentiana*	428.18	129.85	[244, 601]
	Metagentiana - Sinogentiana	0.0004807	yes	0.000163	yes				
	Metagentiana - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Tripterospermum*	1029.06	532.68	[380, 1920]
	Sinogentiana - Tripterospermum	9.15E-10	yes	<2.2E-16	yes				
Bio17 (precipitation of driest quarter) [mm]	*Crawfurdia - Kuepferia*	0.001032	yes	9.38E-06	yes	*Crawfurdia*	50.25	34.32	[12, 134]
	Crawfurdia - Metagentiana	0.0002063	yes	0.000205	yes				
	Crawfurdia - Sinogentiana	2.43E-08	yes	5.87E-13	yes	***Kuepferia***	28.51	16.6	[4, 57]
	Crawfurdia - Tripterospermum	1.67E-13	yes	<2.2E-16	yes				
	Kuepferia - Metagentiana	**0.05007**	**no**	**0.19**	**no**	***Metagentiana***	33.84	15.2	[14, 74]
	Kuepferia - Sinogentiana	0.0009789	yes	2.37E-05	yes				
	Kuepferia - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Sinogentiana*	16.12	6.98	[7, 25]
	Metagentiana - Sinogentiana	8.35E-05	yes	7.83E-09	yes				
	Metagentiana - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Tripterospermum*	150.7	127.99	[11, 432]
	Sinogentiana - Tripterospermum	7.53E-12	yes	<2.2E-16	yes				
Bio18 (precipitation of warmest quarter) [mm]	*Crawfurdia - Kuepferia*	0.001456	yes	3.34E-05	yes	*Crawfurdia*	692.07	260.97	[388, 1095]
	Crawfurdia - Metagentiana	0.0001607	yes	0.00235	yes				
	Crawfurdia - Sinogentiana	1.17E-05	yes	2.15E-07	yes	*Kuepferia*	515.2	165.56	[306, 846]
	Crawfurdia - Tripterospermum	2.78E-07	yes	1.35E-08	yes				

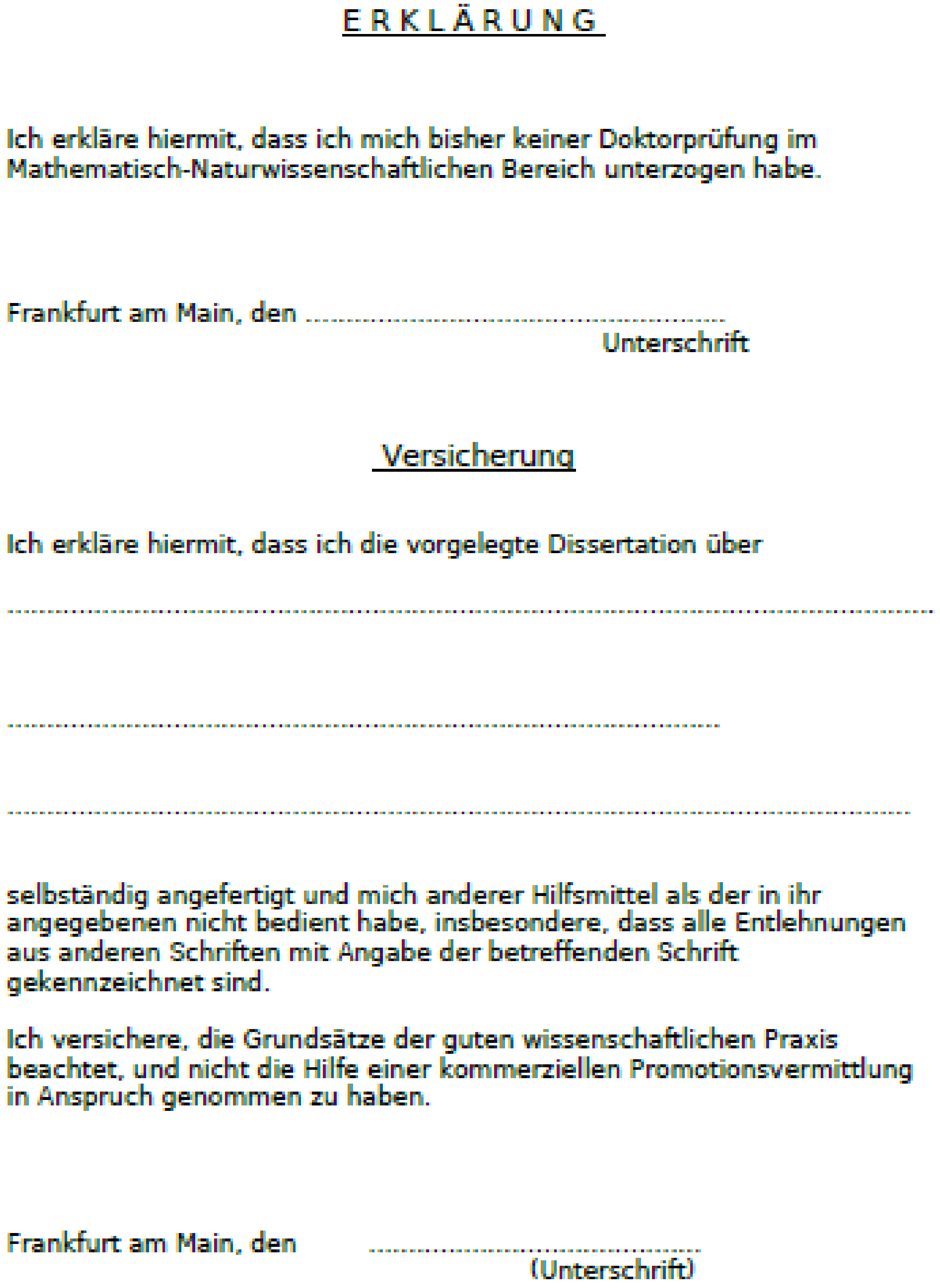
Variable [unit]	Data sets between	KS-Test		T-Test		Genus	Mean	St.dev.	95% HPD Interval
		p-Value	differ data?	p-Value	differ mean?				
Bio 18	*Kuepferia - Metagentiana*	0.01728	yes	**0.09281**	**no**	*Metagentiana*	575.06	62.32	[479, 683]
	Kuepferia - Sinogentiana	**0.09463**	**no**	0.02711	yes				
	Kuepferia - Tripterospermum	1.93E-08	yes	<2.2E-16	yes	*Sinogentiana*	426	131.51	[242, 601]
	Metagentiana - Sinogentiana	0.001407	yes	0.000432	yes				
	Metagentiana - Tripterospermum	9.99E-15	yes	<2.2E-16	yes	*Tripterospermum*	942.13	506	[378, 1920]
	Sinogentiana - Tripterospermum	5.36E-08	yes	<2.2e-16	yes				
Bio19 (precipitation of coldest quarter) [mm]	*Crawfurdia - Kuepferia*	0.003696	yes	9.39E-06	yes	*Crawfurdia*	58.95	44.54	[12, 185]
	Crawfurdia - Metagentiana	3.61E-09	yes	8.57E-06	yes				
	Crawfurdia - Sinogentiana	5.38E-09	yes	7.44E-13	yes	***Kuepferia***	31.51	18.37	[8, 66]
	Crawfurdia - Tripterospermum	1.87E-11	yes	<2.2E-16	yes				
	Kuepferia - Metagentiana	**0.08886**	**no**	**0.7635**	**no**	***Metagentiana***	34.12	15.34	[14, 74]
	Kuepferia - Sinogentiana	0.0003741	yes	1.57E-06	yes				
	Kuepferia - Tripterospermum	2.22E-16	yes	<2.2E-16	yes	*Sinogentiana*	16.12	6.98	[7, 25]
	Metagentiana - Sinogentiana	8.35E-05	yes	5.66E-09	yes				
	Metagentiana - Tripterospermum	<2.2E-16	yes	<2.2E-16	yes	*Tripterospermum*	185.89	177.96	[13, 499]
	Sinogentiana - Tripterospermum	7.61E-13	yes	<2.2E-16	yes				

Appendix 15. Relative disparity as a function of time for *Crawfurdia, Kuepferia, Metagentiana* and *Tripterospermum* (Gentianinae, Gentianaceae; solid line) in comparison with mean disparity from 100 simulations of Brownian motion evolution (dashed line). The grey area indicates the 95% disparity through time range for the simulated data. The most meaningful nine variables were tested (see Materials and Methods). If disparity matched the Brownian motion model, result not shown. The abbreviation “Ma” means “million years ago”.


Crawfurdia
Altitude
MDI = 0.25
Mean annual temperature (Bio 1)
MDI = 0.17
Temperature annual range (Bio 7)
MDI = 0.18
Mean temperature of warmest quarter (Bio 10)
MDI = 0.32
Mean temperature of coldest quarter (Bio 11)
MDI = 0.08
Annual precipitation (Bio 12)
MDI = 0.26
Precipitation of driest month (Bio 14)
MDI = 0.16
Precipitation of warmest quarter (Bio 18)
MDI = 0.3
Precipitation of coldest quarter (Bio 19)
MDI = 0.37
Kuepferia
Mean annual temperature (Bio 1)
MDI = -0.26
Mean temperature of coldest quarter (Bio 11)
MDI = -0.11
Metagentiana
Altitude
MDI = 0.68
Disparity
Time [Ma]
13.2
10.6
7.9
5.3
9.6
7.7
5.8
3.8
8.3
6.6
5.0
3.3
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.4
0.8
1.2


Metagentiana
Mean annual temperature (Bio 1)
MDI = 0.68
Mean temperature of warmest quarter (Bio 10)
MDI = 0.61
Mean temperature of coldest quarter (Bio 11)
MDI = 0.54
Annual precipitation (Bio 12)
MDI = 0.71
Precipitation of warmest quarter (Bio 18)
MDI = 0.47
Tripterospermum
Temperature annual range (Bio 7)
MDI = 0.48
Mean temperature of coldest quarter (Bio 11)
MDI = 0.24
Annual precipitation (Bio 12)
MDI = 0.33
Precipitation of driest month (Bio 14)
MDI = 0.41
Precipitation of coldest quarter (Bio 19)
MDI = 0.34
Disparity
Time [Ma]
8.3
6.6
5.0
3.3
5.4
4.3
3.2
2.2
1.1
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.2
0.4
0.6
0.8
1.2

Appendix 16. Erklärung und Versicherung.



ERKLÄRUNG

Ich erkläre hiermit, dass ich mich bisher keiner Doktorprüfung im Mathematisch-Naturwissenschaftlichen Bereich unterzogen habe.

Frankfurt am Main, den ……………………………………………………
Unterschrift

Versicherung

Ich erkläre hiermit, dass ich die vorgelegte Dissertation über

……

……………………………………………………………………………………

……

selbständig angefertigt und mich anderer Hilfsmittel als der in ihr angegebenen nicht bedient habe, insbesondere, dass alle Entlehnungen aus anderen Schriften mit Angabe der betreffenden Schrift gekennzeichnet sind.

Ich versichere, die Grundsätze der guten wissenschaftlichen Praxis beachtet, und nicht die Hilfe einer kommerziellen Promotionsvermittlung in Anspruch genommen zu haben.

Frankfurt am Main, den ……………………………………………
(Unterschrift)