In and out of the Qinghai-Tibet Plateau: divergence time estimation and historical biogeography of the large arctic-alpine genus Saxifraga L.

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ABSTRACT

Aim Geologically dynamic areas often harbour remarkable levels of biodiversity. Among other factors, mountain building is assumed to be a precondition for species radiation, and yet, the potential role of immigration as a source of biodiversity prior to radiation is often neglected. Here, we studied the biogeographical history of the large genus Saxifraga to unravel the role played by the Qinghai-Tibet Plateau (QTP) for the diversification of this genus and to understand factors that have led to the establishment of high biodiversity in and around this region.

Location QTP and surrounding mountain ranges and worldwide distribution range of Saxifraga.

Methods Using a total of 420 taxa (321 ingroup taxa) comprising more than 60% of extant Saxifraga species, we studied the evolutionary history of Saxifraga by performing phylogenetic analyses (maximum likelihood and Bayesian inference on nuclear ITS and plastid trnL–trnF, matK sequences), divergence time estimation (using uncorrelated log-normal clock models and four fossil constraints in BEAST) and ancestral range estimation (using BioGeoBEARS).

Results Saxifraga originated in North America around 74 (64–83) Ma, dispersed to South America and northern Asia during its early diversification and colonized Europe and the QTP region by the Late Eocene. The QTP region was colonized several times independently, followed in some lineages by rapid radiations, temporally coinciding with recent uplifts of the Hengduan Mountains at the southeastern fringe of the QTP. Subsequently, several lineages dispersed out of Tibet.

Main conclusions Immigration, recent rapid radiation and lineage persistence were all important processes for the establishment of a rich species stock of Saxifraga in the QTP region. Because floristic exchanges between the neighbouring areas and the QTP region were bi-directional, the spatio-temporal evolution of Saxifraga contrasts with the ‘out of QTP’ pattern, which has often been assumed for northern temperate plants.

Keywords historical biogeography, in situ alpine diversification, molecular dating, phylogeny, Qinghai-Tibet Plateau, Saxifraga
(Hoorn et al., 2013; Linder et al., 2014) usually characterized by complex physiography. Among other factors, these areas offer a wide range of ecological opportunities while buffering changing environmental conditions, and they can foster diversification over relatively short geological timeframes (Linder, 2008; Lancaster & Kay, 2013). Such rapid or explosive radiations are well-known from isolated habitats, such as oceanic islands, lakes and mountain tops (Hughes et al., 2015). In fact, mountains (also called ‘sky-islands’) and mountainous regions house half of the currently defined biodiversity hotspots and have hosted a large number of species radiations, prompting increased interest in the origin of mountain biodiversity (Kohler & Maselli, 2009; Hughes & Atchison, 2015). However, little is known about the relative contribution of immigration and in situ diversification for the evolution of mountainous biodiversity hotspots of the world, including those adjacent to the Qinghai-Tibet Plateau (QTP) (Antonelli, 2015).

The QTP, located in Central Asia and East Asia, is the Earth’s largest continental highland (Mulch & Chamberlain, 2006). It stands out as an ideal system to study the evolution of mountain biodiversity because the mountain ranges flanking it to the south (Himalaya) and the southeast (Hengduan Mountains) constitute major biodiversity hotspots (Myers et al., 2000) with large proportions of alpine elements (Wen et al., 2014; Xu et al., 2014). Several spectacular in situ radiations have been reported, and as a result, the Hengduan Mountains sensu lato have been referred to as ‘largest evolutionary front of the North Temperate Zone’ (López-Pujol et al., 2011; Hughes & Atchison, 2015). Many of these radiations were attributed to the uplift of the QTP (Wen et al., 2014). However, previous studies assessing a temporal correlation between uplift and biological diversification have relied on varying geological scenarios which were often too young (Renner, 2016). In fact, a synthesis of various types of data clearly points out that the average altitudes of QTP were likely above 4 km by 40 Ma (Renner, 2016). In addition, biotic interchange has been largely disregarded in previous studies, and thus, the overall role of the QTP for the biogeography and diversification of plants in the Northern Hemisphere is still poorly understood (but see Wen et al., 2014). In this study, we investigate the relative roles of biotic interchange as well as in situ radiation along with lineage persistence in the establishment of the circum-Tibetan biodiversity hotspots with respect to a widespread arctic-alpine taxon, Saxifraga L. (Saxifragaceae), relying on latest geological scenarios.

Comprising c. 450–500 species in at least 13 sections (Pan et al., 2001; Tkach et al., 2015), Saxifraga is the largest genus of Saxifragaceae. Its distribution is concentrated in the Northern Hemisphere, with only a few occurrences in the Andes. Besides the mountain ranges flanking the QTP, considerable levels of diversity are found in mountains of southern Europe, the Caucasus, and in the Arctic, demonstrating a clear affinity of the genus to treeless habitats (Soltis et al., 1996). With over 200 species (more than half of which are oftentimes referred to as endemics), Saxifraga is a prominent element of the high altitude habitats throughout the QTP region (Pan et al., 2001; Xu et al., 2014), mainly represented by the two species-rich sections Ciliatae and Porphyrion.

While several divergence time estimates and biogeographical scenarios exist for Saxifragales, Saxifragaceae and subgroups within Saxifraga, many of these previous studies have used datasets with limited taxonomic sampling. For instance, very recent rapid radiation(s) within the last 2 Myr have been suggested for section Ciliatae subset. Hirculoideae based on a dataset of 73 Saxifragaceae taxa (c. 14% Saxifraga sampling, Gao et al., 2015). This would make this group one of the youngest among those that have experienced explosive radiations in the Hengduan Mountains (Hughes & Atchison, 2015) and suggest tremendously fast diversification rates (Gao et al., 2015). Deng et al. (2015) recently studied the biogeographical history of Saxifragaceae based on 51 taxa and found that multiple radiations had occurred in East Asia and western North America. However, the distribution of the most recent common ancestor (MRCA) of Saxifraga and that of Saxifragaceae could not be reconstructed unambiguously and might have covered either or both of these areas. Also, their study did not focus on Saxifraga specifically and did not delineate the QTP as a separate area in biogeographical analyses. Therefore, the role of the QTP and surrounding mountain chains in the diversification of Saxifraga remains unclear.

By presenting a comprehensive approach to reconstruct the biogeographical history of Saxifraga based on 420 Saxifragaceae taxa, we aim to answer the following questions: (1) When did Saxifragaceae, Saxifraga and, in particular, sections Ciliatae and Porphyrion evolve and diversify? (2) What is the biogeographical history of Saxifraga, and did the QTP region act as a source area for regional and/or global Saxifraga diversity? (3) Is there a temporal correlation between the uplift of the QTP and diversification? (4) Which roles did immigration and in situ radiation play for establishing Saxifraga’s diversity in the QTP region?

**MATERIALS AND METHODS**

**Samples and sequences**

Our dataset consists of 321 ingroup taxa representing more than ~60% of extant Saxifraga species covering all sections recognized by Tkach et al. (2015). In addition, we included 99 outgroup taxa covering all remaining genera of Saxifragaceae (except for the monotypic South American narrow endemic Hieronymusia, probably closely related to Suksdorffia; Engler, 1918), as well as representatives of all other families of the Saxifragaceae alliance (Saxifragaceae, Grossulariaceae, Iteaceae including Pterostemonaceae; Fishbein et al., 2001), and for rooting purposes one distant Saxifragales taxon, Penthorum chinense. We collected samples (28 in total) during field campaigns to Western China and Nepal (2011, 2012 and 2014). Leaves were dried in silica gel and vouchers were
Divergence time estimation

Divergence time estimation was carried out in BEAST 1.8.2 (Drummond et al., 2012) using four fossil-informed constraints. There are currently no well-documented fossils in Saxifragaceae, and thus fossil constraints were limited to the outgroup. For all fossils, the lowest bounds of the most recent stratigraphic age estimate were used as offset for node age prior distributions. Stratigraphic ages were transformed to absolute ages (and vice versa) according to Cohen et al. (2013, updated). We constrained the Ribes crown at 14.5 Ma using the age of fossilized leaves of Ribes webbii from the Stewart Valley flora, Nevada, USA, ascribed to Ribes subgroup Calobrya (Hermsen, 2005). We followed Zhu et al. (2013) in their use of the Ribes leaf fossils from the Republic flora of the Okanagan Highlands, USA, but decided for a slightly younger, more conservative age estimate complying with Moss et al. (2005) and set the Ribes stem node constraint to minimally 48.9 Ma (Hermsen, 2005). In accordance with Deng et al. (2015) and Zhu et al. (2013), we used the age of *Itea* fossil pollen from the Republic flora of the Okanagan Highlands to constrain the MRCA of *Itea* and *Pterostemon* at 49 Ma as recommended by Hermsen (2013). Finally, we used the age of fossils of *Divisestylus* from the Raritan Formation of New Jersey, USA (Hermsen et al., 2003). Even though cladistic analyses placed this genus as sister to Iteaceae (Hermsen et al., 2006), *Divisestylus* was originally considered to bear morphological resemblance to both, Saxifragaceae and Iteaceae (Hermsen et al., 2003). We therefore chose a conservative approach and used this fossil to constrain the crown of the Saxifragaceae alliance at 89 Ma.

Analyses were carried out using either one common or two separate uncorrelated log-normal (UCLN) clock models for nuclear and plastid data, and both the Yule tree prior and the Birth Death tree prior with incomplete sampling (BDinc) were tested for the dataset. Node age prior distributions were always log-normal. No specific information was available from the fossil record to aid in specifying the shape of the log-normal prior distributions. We, thus, used conservative settings ($m = 1.5$, $SD = 1.0$) to accommodate a relatively high degree of uncertainty.

All analyses were run for 100 million generations, sampling every $10,000^{th}$ generation. Three or more runs each were combined using LogCombiner 1.8.2 (part of the BEAST package) after evaluating effective sample size values, visual inspection for convergence in Tracer 1.6 (Rambaut et al., 2014) and removal of appropriate burn-in (varying proportions, on average less than 20%) to sample the stationary phase of each run. Maximum clade credibility (MCC) trees were constructed in TreeAnnotator 1.8.2 (part of the BEAST package) and edited in FigTree 1.4.2 (Rambaut, 2014). Bayesian model selection was performed using the posterior simulation-based analogue of Akaike information criterion through Markov chain Monte Carlo (AICM; Baele et al., 2012) implemented in Tracer 1.6.

Ancestral range estimation

We delineated nine geographical areas for ancestral range estimation based on extant distribution patterns, climatic zones and historical geographical barriers to dispersal: (A) Europe to Ural Mountains and the northern parts of the Middle East, including the mountains of northwest Africa and the island of Madeira; (B) northern Asia spanning the entirety of Siberia including Kamchatka and Sakhalin; (C) North America comprising Canada, Greenland, the USA and Mexico; (D) QTP in combination with the Hengduan Mountains, the Himalayas, the Karakorum Range, Pamir, the Tian-shan and the Altai Mountains; (E) East Asia including other regions of China, Taiwan, Mongolia and the Korean peninsula; (F) Japan; (G) Southeast Asia, including the mountains of Myanmar, Laos, Vietnam and northern Thailand; (H) Southeast Africa; (I) eastern and southern parts of South America (incl. Patagonia, Terra del Fuego and Falkland Islands) (Fig. 1, Appendix S1.4). Saxifraga comprises several taxa with broad circumboreal distribution ranges; thus,
ancestral ranges were constrained to add up to a maximum of six areas. Finally, we defined four time slices in accordance with global tectonic events with potential relevance to the distribution of Saxifraga (> 70 Ma, 70–35 Ma, < 35–6 Ma, < 6–0.5 Ma, Appendix S1.4). Dispersal multipliers were adjusted for each time slice (Appendix S1.5).
Figure 1 Ancestral range estimation and biogeographical scenarios for Saxifraga. (a) Area delineation. (b) Ancestral range estimation based on DEC++ model implemented in BioGeoBEARS. Analysis performed on maximum clade credibility tree from divergence dating analysis (BEST) using two separate uncorrelated log-normal clock models for nuclear and plastid data under the Yule tree prior. Node support values (posterior probabilities) are presented for all nodes. 95% highest posterior density (HPD) intervals are indicated by grey bars at each node. Cumulative probabilities for estimated ancestral ranges are shown by boxes on each node (see Appendix S1.6 for a detailed description of the visual representation of results from biogeographical analyses). Colours as in (a). Clades are collapsed to aid legibility. (c) Biogeographical scenarios for sections of Saxifraga that have experienced dispersal into and/or out of the QTP region. Colours as in (a).

We used the R (R Core Team, 2015) package BioGeoBEARS (Matzke, 2013, 2014) for ancestral range estimation on the MCC chronogram from the beast run with the lowest AICM value. We tested the implemented biogeographical models DEC, DIVALIKE and BAYAREALIKE [the latter two being (simplified) likelihood implementations of the processes assumed by DIVA and BAYAREA], each of them with and without the additionally implemented J parameter modelling jump dispersal (Matzke, 2013). A posteriori model testing via the Akaike information criterion (AIC) was performed in BioGeoBEARS.

RESULTS

Divergence time estimation

Maximum likelihood and BI yielded highly congruent tree topologies with strong overall node support in particular at the backbone (Appendix S2.1). While we confirmed previously established intra- and interclade relationships and monophyly of sections, our study provided no further major taxonomical findings (Appendix S2.2).

Bayesian model selection via AICM clearly indicated better model fit of the analyses using separate clock models for nrDNA and ptDNA data (Appendix S2.3). Overall, the analyses under the Yule tree prior yielded the best model fit. Thus, we report results (median age and 95% highest posterior density intervals) from those analyses here. However, it should be noted that age estimates from analyses using the BDinc tree prior were noticeably younger towards the tips of the tree compared to results from Yule tree prior analyses (Appendix S2.4).

The stem of Saxifragaceae was estimated to 84 (74–93) Ma (Table 1). Stem Saxifraga was dated to 74 (64–83) Ma. The MRCA of the earliest diverging Saxifraga sections, Heterisia and Irregulares, appeared relatively shortly thereafter at 62 (52–72) Ma. Sections Saxifragella [53 (45–62) Ma], Ciliatae [41 (34–49) Ma], Pseudocymbalaria [40 (29–50) Ma] and Cymbalaria [38 (31–45) Ma] were the next oldest groups, all dating back to the Eocene. During the Oligocene, several sections evolved, including Bronchiales [40 (29–50) Ma], Saxifraga [(31 (26–38) Ma], Cotylea and Mesogynae [both, 24 (18–31) Ma], while sections Porphyrion [22 (17–28) Ma], Gymnoptera [19 (14–25) Ma], Ligulatae and Trachyphyllum [both, 14 (10–19) Ma] originated in the Miocene. Radiations within sections occurred during the Oligocene and Miocene, with the exception of Trachyphyllum and Cotylea which diverged very recently during the Pleistocene.

Ancestral range estimation

Biogeographical analyses were conducted on beast results. Overall, AIC values showed that the DEC++ model for biogeographical reconstruction yielded the best model fit (Appendix S3.1). Results from this analysis will thus be presented and discussed here.

The common ancestors of Saxifragaceae and Saxifraga originated in North America with high probability (Fig. 1, Appendices S3.2 & S3.3). Early diversification of Saxifraga likely took place in North America giving rise to sections Heterisia and Irregulares and in a combined area of North America, South America, and northern Asia giving rise to section Saxifragella. Consecutively, range shifts led to a large northern hemisphere distribution range comprising North America, northern Asia, and from the Middle Eocene onward, Europe. The area of origin and diversification was unambiguously estimated to be Europe for sections Cymbalaria, Saxifraga, Cotylea, Gymnoptera, Trachyphyllum, Ligulatae and Porphyrion. Of these sections, only Ligulatae, Porphyrion and Saxifraga exhibited dispersal events to other delineated areas (Appendix S3.3). The stem of section Mesogynae was estimated to have originated in Europe, but most probable ancestral areas for the crown group included Europe, northern Asia, North America, the QTP, East Asia and Japan. The MRCA of section Ciliatae most likely lived in a large area spanning northern Asia and the QTP region, possibly including North America. Consecutively, the section diversified in the QTP region followed by independent dispersal events, mostly to neighbouring regions, in several taxa. Sections Pseudocymbalaria and Bronchiales both originated in northern Asia: they diversified in situ, or in northern Asia and/or North America, respectively. Finally, section Irregulares originated in North America along with section Heterisia before spreading to either Japan or the QTP region and diversifying there.

DISCUSSION

Because Saxifraga is an almost ubiquitous component of the temperate flora, studying the spatio-temporal evolution of this genus might provide insight in the evolution of temperate biomes. Our study shows how floristic exchanges between temperate regions, characterized by punctual explosive radiations, may have fostered the establishment of mountainous hotspots of biodiversity, such as those surrounding the QTP. The vast majority of studies investigating the origin of
Table 1 Median node age estimates and corresponding 95% highest posterior density (HPD) intervals for selected Saxifragaceae nodes according to BEAST analysis using two separate UCLN clock models for nuclear and plastid data under the Yule tree prior. Node support (posterior probability, pp) indicated for each node. ***: ≥ 0.98 pp; **: ≥ 0.94–0.97 pp, *: ≥ 0.90–0.94 pp, (-): < 0.9 pp.

<table>
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<th>Node Description</th>
<th>Median Age (Ma)</th>
<th>95% HPD (Ma)</th>
<th>pp</th>
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<td>83.96</td>
<td>74.35–92.85</td>
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<tr>
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<td>73.63</td>
<td>63.88–82.78</td>
<td>***</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Saxifraga crown</td>
<td>61.75</td>
<td>52.05–71.81</td>
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<td>52.51</td>
<td>44.60–61.83</td>
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<td>40.92</td>
<td>34.48–48.55</td>
<td>***</td>
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<td>26.21</td>
<td>19.95–34.00</td>
<td>***</td>
</tr>
<tr>
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<td>40.01</td>
<td>29.02–52.09</td>
<td>**</td>
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<tr>
<td>sect. Pseudocymbalaria crown</td>
<td>17.83</td>
<td>9.49–27.36</td>
<td>***</td>
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<td>40.01</td>
<td>29.02–50.30</td>
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<td>2.43</td>
<td>0.47–5.44</td>
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Tibetan biota have either focused on endemic clades or have suffered from biased sampling towards Tibetan taxa (Favre et al., 2015). With an extensive sampling across the entire distribution range of Saxifraga, our study includes the most complete sequence dataset to date for Saxifraga, which represents a good base for further molecular dating and biogeographical analyses.

Our phylogenetic reconstruction of Saxifragaceae corroborates previous phylogenetic findings (Appendix S2.2; Deng et al., 2015; Tkach et al., 2015). Furthermore, our study complements the results of Gao et al. (2015) regarding three monophyletic clades within section Ciliatae (Appendix S2.1). Some Ciliatae subgroups exhibited an apparent lack of phylogenetic signal, likely due to extremely rapid speciation. For these clades, methods targeting genetic variation of intricate species complexes might be required to improve phylogenetic resolution.

Divergence time estimation

Our divergence time estimates are substantially older than those reported in some previous studies investigating Saxifragales and Saxifragaceae. Recently, Gao et al. (2015) published a median age estimate for stem Saxifraga of 38.45 Ma, which was congruent with the mean age of 38.37 Ma reported by Deng et al. (2015) and with c. 44 Ma estimated by Zhu et al. (2013). Discrepancies to our study [Saxifraga stem 74 (64–83) Ma] might stem from limited taxon coverage (63, 42 and 51 taxa, respectively) and a comparatively small number of fossil calibrations in those studies, factors that have been shown to bias age estimates towards younger ages under some circumstances (Linder et al., 2005; Sauquet et al., 2012). In addition, the choice of fossils and their placement on the phylogeny are known to have dominant effects on divergence date estimation (Ho & Duchêne, 2014). For instance, Jian et al. (2008) and Magallón et al. (2015), using similar fossil calibration schemes as this study, published age estimates concordant to ours (stem Saxifraga c. 65 Ma; stem Saxifragaceae 86 Ma, respectively). While we applied the utmost care in choosing fossils and their placement on the topology, designing a calibration scheme is admittedly subjective and further work will be needed to reconcile age estimates in this group.

In general, older age estimates for Saxifraga and Saxifragaceae might translate into more realistic mean ITS clock rates (substitution rate per time). Gao et al. (2015) found the mean ITS clock rate was c. 8.6 × 10−3 substitutions per site per million years across Saxifragaceae, thus exceeding other available estimates for the ITS region in flowering plants ranging from 6.06 ± 0.15 × 10−3 (Sang et al., 1994) to 3.5 × 10−4 (Suh et al., 1993). In contrast, the clock rate estimate for the ITS region of Saxifraga of 1.72 × 10−3 published by Richardson et al. (2001) is likely an underestimation, as it would suggest Saxifragaceae to be 200 Myr old (Gao et al., 2015). The results of our analyses are intermediate between these two extremes (3.63 × 10−3 under the Yule tree prior; 5.03 × 10−3 under the Bdinc tree prior).

Early biogeographical history of Saxifraga

Our results indicate that Saxifraga and Saxifragaceae originated in North America. This result is partly consistent with the findings of Deng et al. (2015) who reported ancestral ranges for Saxifragaceae and Saxifraga to be western North America and/or Asia. From North America, Saxifraga expanded its range towards South America and northern Asia, the latter most likely reached by crossing the Bering land bridge (BLB).

Range expansion to South America resulted in the formation of a single species, Saxifraga bicuspidata. To explain a similar disjunct distribution pattern in Chrysosplenium (Saxifragaceae), Soltis et al. (2001) suggested ancient long-distance dispersal events, or stepwise dispersal along the Western Cordillera southwards with subsequent extinction in
Central America. *Saxifraga* species are typically dispersed by seed over short distances (Oliver *et al.*, 2006), but long-distance dispersal has been shown for some species, potentially via strong winds, herbivores or migrating birds (Gabrielsén *et al.*, 1997). For instance, *Saxifraga magellanica* from South America is nested within the almost exclusively European section *Saxifraga* which would seem to indicate a long-distance dispersal event. However, this pattern might be challenged if additional South American species of section *Saxifraga* (*S. adenodes*, *S. bousingsaultii* and *S. pavonii*, unavailable for this study) were included. Similarly, stepwise dispersal seems plausible in the case of *S. bicuspidata*; however, considering the relatively long isolation of the South American continent, this possibility depends on the time frame of the dispersal events which could not be inferred from our analysis.

The BLB, connecting North America and northern Asia, likely played a significant role in the biogeographical history of *Saxifraga* by facilitating dispersal between the continents at different points in time (e.g. 65.5 Ma, 58 Ma and from 56 Ma onwards; Brikiatis, 2014). In *Saxifraga*, this dispersal route played a significant role for initial range expansion to Asia and for further dispersal throughout the Northern Hemisphere. Dispersal from North America to Asia (including the QTP) across the BLB is less often recovered than vice versa (Wen *et al.*, 2010), yet, the case of *Saxifraga* is not unique. Similar patterns were suggested, for example, for two other members of the Saxifragaceae alliance, Grossulariaceae and Iteaceae, both of which likely colonized Asia from their original ranges in North America, most likely via the BLB (Schultheis & Donoghue, 2004; Hermens, 2013). In addition, rare cases of dispersal from North America to the QTP region specifically have been recovered in other groups such as *Picra* (Ran *et al.*, 2015). This suggests that New World to Old World dispersal via the BLB and northern Asia was a recurrent event, relevant to the establishment of biodiversity hotspots in the vicinity of the QTP.

**The role of the QTP for the diversification of *Saxifraga***

*Saxifraga* did not originate in the QTP region. Instead, members of sections *Irregulares*, *Mesogyne*, *Porphyrian*, *Saxifraga* and the ancestor of section *Ciliatae* colonized the region at least seven times independently. This pattern is different from other large alpine genera, such as *Gentiana* (Favre *et al.*, 2016) and *Rhodiola* (Zhang *et al.*, 2014), which originated in the QTP region. Other groups (e.g. *Hyoscymaeae* of Solanaceae, *Tu et al.*, 2010), although not formally tested by biogeographical analyses, seem to have originated in more distant regions and subsequently colonized the QTP region, similar to *Saxifraga*.

Our results clearly demonstrate the role of the QTP as a regional species sink (i.e. experiencing colonization from other areas) during the early evolution of *Saxifraga*. The colonization of the QTP region by section *Ciliatae* [possibly as early as 41 (34–49 Ma)] represents one of the oldest regional immigration events within *Saxifraga*. It coincides with the time frame for which elevation of 4000 m or more has been reconstructed for southern Tibet (Renner, 2016). This suggests that *Saxifraga* could have colonized the QTP region as a pre-adapted and cold-tolerant lineage.

Two main lineages underwent rapid radiations following their colonization of the QTP: sections *Ciliatae* and *Porphyrian*. The former counts 175 species of which more than 150 occur in the QTP and its flanking regions in China and Nepal (with > 70% endemism) and its explosive radiation took place 7 (5–10) Ma (within subsection *Hirculoideae*). *Porphyrian* comprises 90 species with c. 40 species occurring in the QTP and neighbouring Chinese and Nepalese mountain ranges (also with > 70% endemism) and its rapid radiation (within subsection *Kabschia sensu* Tkach *et al.* (2015)) occurred roughly in the same time frame [6 (4–8) Ma] (Pan *et al.*, 2001; Akiyama & Gornall, 2012). These time frames correspond to the timing of radiation of other alpine taxa occurring in the QTP region (Hughes & Atchison, 2015). Younger than the major uplift of the QTP proper (Eocene to Oligocene) and of the Himalayas (Early to Middle Miocene, Favre *et al.*, 2015), the timing of diversification of these lineages coincides with the orogenesis of the Hengduan Mountains (i.e. Late Miocene or Pliocene; Favre *et al.*, 2015). These mountains represent the most diverse area for *Saxifraga* within the QTP region (Xu *et al.*, 2014), and in situ radiation of section *Ciliatae* was most likely promoted by the island-like distribution of montane and alpine habitats resulting from surface uplift. In fact, the Hengduan Mountains are among the most rugged mountains of the world and such physiographic complexity seems to be a key factor promoting rapid radiations via allopatric speciation, as well as the evolution of narrow endemics (Hughes & Atchison, 2015; Hughes *et al.*, 2015). Topographic complexity might also buffer extinction during climate change (e.g. Miocene cooling, Quaternary climate oscillations) through the possibility of vertical displacement (Lancaster & Kay, 2013). Radiation of *Saxifraga* within the QTP region thus seems to be caused by the same factors suggested for other alpine plant radiations (Hughes & Atchison, 2015). In summary, high species diversity of *Saxifraga* in the QTP region was shaped by a combination of immigration of ancestral lineages followed by local rapid radiation and species persistence in the course of mountain building at the flanks of the plateau. However, species-rich radiations in mountains are certainly the result of a more complex interplay between several abiotic and biotic factors. Further studies regarding the role of climate modifications, biotic interactions (e.g. plant-pollinator interaction) and ecological opportunity (e.g. niche evolution, key innovations) are needed to complete the picture of species diversification in this region.

Regional and global *Saxifraga* diversity was affected by several independent dispersal events out of Tibet: at least 10 times in section *Ciliatae* and once in section *Irregulares*. Most of these dispersal events led to the colonization of areas
adjacent to the QTP, such as East Asia or Southeast Asia (e.g. *S. filicaulis, S. brachypoda*), whereas others resulted in widespread species distributions across the Northern Hemisphere (e.g. *S. hirculus, S. flagellaris*), mostly throughout the last 2 Myr. Similar patterns of predominant diversification in the QTP region followed by dispersal to adjacent regions have also been observed in other genera (see Liu et al., 2014 and references therein). Besides acting as a species sink, the QTP and its mountain ranges, therefore, also constituted a source of *Saxifraga* diversity, in particular during the Pliocene and Pleistocene and at the regional scale.

Overall, the biogeographical history of *Saxifraga* is inconsistent with the ‘out-of-QTP’ hypothesis postulating the QTP and adjacent areas as geographical origin of the majority of northern temperate plants (Wen et al., 2014). In fact, our study reveals that dispersal between the QTP and neighbouring regions is not unidirectional as assumed by the ‘out-of-QTP’ hypothesis. Rather, biotic interchange, for example, between alpine (QTP region) and arctic (northern Asia) areas might have contributed to the assembly of biotas on either side.

**CONCLUSIONS**

We found that *Saxifraga* originated outside the QTP, but diversified there before having colonized the area multiple times independently. The uplift of the Hengduan Mountains, resulting in high topographical complexity, likely has influenced the rapid diversification of *Saxifraga* in the QTP region. Following the diversification of the genus in the region of the QTP, some lineages dispersed out of Tibet. At different points in time, the QTP region, thus, represented either a sink or a source for the diversity of *Saxifraga*, for example, with major dispersal directions from and to the Asian Arctic, and to adjacent areas in East and South Asia. Therefore, our study shows that a combination of immigration events, recent *in situ* radiation and lineage persistence, has contributed to the establishment of high biodiversity levels of *Saxifraga* in the QTP region. In the future, more studies should investigate widespread taxa not restricted to the QTP and its surroundings to conclusively unravel the overall role of the QTP region for the diversification of plants.

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**SUPPORTING INFORMATION**

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

**Appendix S1** Extended material and methods.

**Appendix S2** Taxonomic considerations and detailed results of divergence dating.

**Appendix S3** Detailed results of ancestral range estimation.
This research is part of Jana Ebersbach’s doctoral dissertation. She studies *Saxifraga* as part of her research and education programme at Leipzig University. Her research focuses on systematics and biogeography of *Saxifraga*, with particular focus on the region of the Qinghai-Tibet Plateau.

Author contributions: A.F., A.N.M.-R. and J.E. conceived the ideas. J.E., A.F. and I.M. collected samples in the field and J.E. and I.M. analysed the data. H.S. provided plant material and supported fieldwork logistics. N.T., M.R., M.H.H. provided sequence data, plant specimens and taxonomical assistance. J.E., A.F., I.M. and A.N.M.-R. were involved in interpreting the results. J.E. wrote the first draft of the manuscript and all authors contributed to writing the final version. A.N.M.-R., together with A.F., recruited the financial support for this study.

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