

Island-like radiation of *Saussurea* (Asteraceae: Cardueae) triggered by uplifts of the Qinghai–Tibetan Plateau

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Increasing evidence suggests that geological or climatic events in the past triggered the radiative diversification of both animals and plants on islands as well as continents. The Qinghai–Tibetan Plateau (QTP) has been extensively uplifted since the Miocene, but there is little information on possible links between these events and biological diversification in this and adjacent regions. Partly to explore such links, we have examined the diversification of *Saussurea* (Asteraceae: Cardueae), a species-rich genus that is mostly endemic to QTP, but also occurs in arid highlands elsewhere in the Northern Hemisphere. The phylogenetic analyses were conducted on the basis of the nuclear (internal transcribed spacer, ITS) and plastid (*trnL-F* and *psbA-trnH*) sequences from 55 species, representing 19 sections from all six subgenera of *Saussurea*, and species from 15 genera of the Cardueae. The results suggest that the currently circumscribed genus *Saussurea* (*s.l.*) is a polyphyletic group and that five sections should be excluded from the genus. Samples from the other 14 sections (representing five subgenera) clustered as a monophyletic group (here designated the *Saussurea s.s.* lineage, SSSL) with high statistical support. However, none of the analyses (nuclear, plastid or combined) resolved SSSL's infrageneric phylogeny, and the parallel clades of the lineage indicate that island-like adaptive radiation occurred. Furthermore, this radiation appears to have occurred 14–7 Mya, during the period of the major uplift events of QTP. Thus, our results support the hypothesis that geological events may play important roles in driving biological diversification through continental radiation. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **97**, 893–903.

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INTRODUCTION

Globally, biodiversity is highly heterogeneous, and some regions host far greater numbers of species than others (Myers *et al.*, 2000). Furthermore, investigations into the causes of high local biodiversity have provided important insights into the evolutionary pro-

cesses in these regions (Pennington, Cronk & Richardson, 2004; Moore & Donoghue, 2007). Evidence from these studies increasingly suggests that past geological and/or climatic events have triggered the radiative diversification of both animals and plants within islands as well as continents. Hence, plants distributed in such regions may have experienced island-like adaptive radiation within relatively short periods (Richardson *et al.*, 2001; Hughes & Eastwood,

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2006). However, such studies are complicated by the complex taxonomy of species-rich genera, difficulties in collecting experimental materials in the field, and a lack of densely sampled phylogenies.

The Qinghai–Tibetan Plateau (QTP), the largest plateau on Earth, was formed through several uplifting episodes following the collision of India with Asia approximately 40 Mya (Harrison *et al.*, 1992; Shi, Li & Li, 1998; Guo *et al.*, 2002; Spicer *et al.*, 2003; Wang *et al.*, 2008). The uplifting of QTP led to the stable development of the Asian monsoon climate to the south and the aridification of central Asia, making it the most significant geological event of the Miocene period (An *et al.*, 2001; Tapponnier *et al.*, 2001). Plants in QTP are exceptionally diverse (Wang *et al.*, 1993; Wu & Wu, 1996), and species' richness and endemism level are higher than those of any other region of similar latitude (Wu & Wu, 1996; Qian, Ricklefs & White, 2005; Zhang, Ulink & Kadereit, 2007). Furthermore, the high diversity in this region is strongly concentrated in a few genera, especially *Cremanthodium*, *Gentiana*, *Ligularia*, *Pedicularis*, *Rhododendron*, *Rheum* and *Saussurea* (Wu, 1987), and the mechanisms responsible for the establishment of such high diversity have interested botanists for many years (Wulff, 1943; Wu, 1987; Wu & Wu, 1996; Qian *et al.*, 2005). Several previous studies, which focused on a few of these organisms, have suggested that radiative diversification of the above-mentioned genera was triggered by QTP's extensive uplifts, and thus these events may have been responsible for the region's high diversity (Milne, 2004; Wang, Yang & Liu, 2005; Liu *et al.*, 2006). In this study, we aimed to test this hypothesis and elucidate the diversification history of the species-rich genus *Saussurea* (Asteraceae: Cardueae) in QTP through a reconstruction of its phylogeny.

As currently circumscribed, *Saussurea s.l.* comprises 450 species, classified into six subgenera and 20 sections (Lipschitz, 1979). The genus is especially diverse in QTP, where c. 230 mostly endemic species representing 17 sections and all six subgenera have been recorded (Lipschitz, 1979; Rechinger, 1979; Chater & Kitamura, 1982; Liu, 1984, 1985, 1996, 2004; Chen, 1985; Chen & Li, 1994; Hajra, 1995; Wu & Wu, 1996; Shi & Jin, 1999; Saklani & Rao, 2000; Grierson & Springate, 2001). In addition, about 150 species are distributed in Central Asia, Xinjiang, Mongolia, Siberia and the Russian Far East, and about 100 species occur in Eastern Asia (Fig. 1) (Lipschitz, 1979). *Saussurea* species occur in a great variety of habitats, including cold and dry alpine meadows, steppe deserts and scree freeze–thaw regions, although they are mostly found at altitudes higher than 1000 m above sea level (a.s.l.), and sometimes even in areas higher than 5000 m a.s.l. In

addition, the life forms of this genus are extremely diverse. For example, members of *Saussurea* subgenus *Amphilaena*, which are termed 'greenhouse plants', have colourful bracts that are believed to protect their reproductive organs from UV-B radiation damage at very high altitudes (Ohba, 1988). Meanwhile, species of *Saussurea* subgenus *Eriocoryne*, which are called 'cotton' or 'snow-ball' plants, have very dense woolly trichomes, which may protect them from cold at very high altitudes. In addition, some *Saussurea* species that grow in extreme freeze–thaw screes are prostrate, acaulescent and/or cushion-like.

The genus *Saussurea* has been placed in the tribe Cardueae of the family Asteraceae, although its circumscription has not been well addressed (Bremer, 1994; Petit, 1997; Kita *et al.*, 2004; Susanna *et al.*, 2006). Indeed, recent molecular investigations have indicated that *Saussurea* section *Elatae* (*Saussurea* subgenus *Saussurea*), *Saussurea* section *Frolovia* (*Saussurea* subgenus *Frolovia*) and *Saussurea* subgenus *Jurineocera*, all of which lack an outer row of pappus bristles, should be excluded from *Saussurea* (Raab-Straube, 2003; Kita *et al.*, 2004; Wang, Liu & Miehe, 2007). Members of most other *Saussurea* species have two rows of pappus bristles, with an inner row of plumose bristles, which are connate at the base, and an outer row of scabrid bristles, which are free from each other and usually much shorter than the inner ones. However, the monophyly of *Saussurea* requires further testing, as some included species lack an outer row of pappus bristles, for example members of *Saussurea* section *Jacea* (*Saussurea* subgenus *Saussurea*) and *Saussurea* section *Acklandia* (*Saussurea* subgenus *Frolovia*). In addition, the monophyly of most infrageneric groupings, and the phylogenetic relationships between them, are still largely unknown (Wang & Liu, 2004). Following our previous survey of the plastid and nuclear DNA data in the Cardueae (Wang & Liu, 2004), the main objectives of the study presented here are: (1) to increase the number of sampled species to cover most sections in all six *Saussurea* subgenera, thereby rigorously testing the monophyly of this genus, and (2) to unravel the phylogenetic patterns in the lineage of *Saussurea* and examine possible correlations between the diversification history of this genus and QTP uplifts.

MATERIAL AND METHODS

PLANT MATERIAL

Saussurea s.l. contains around 450 species in six subgenera and 20 sections (Lipschitz, 1979). Hence, we included representative species from all of its taxonomic sections, with the exception of *Saussurea* section *Jurineiformes* (*Saussurea* subgenus *Saussurea*), for which the materials used were too poorly preserved to

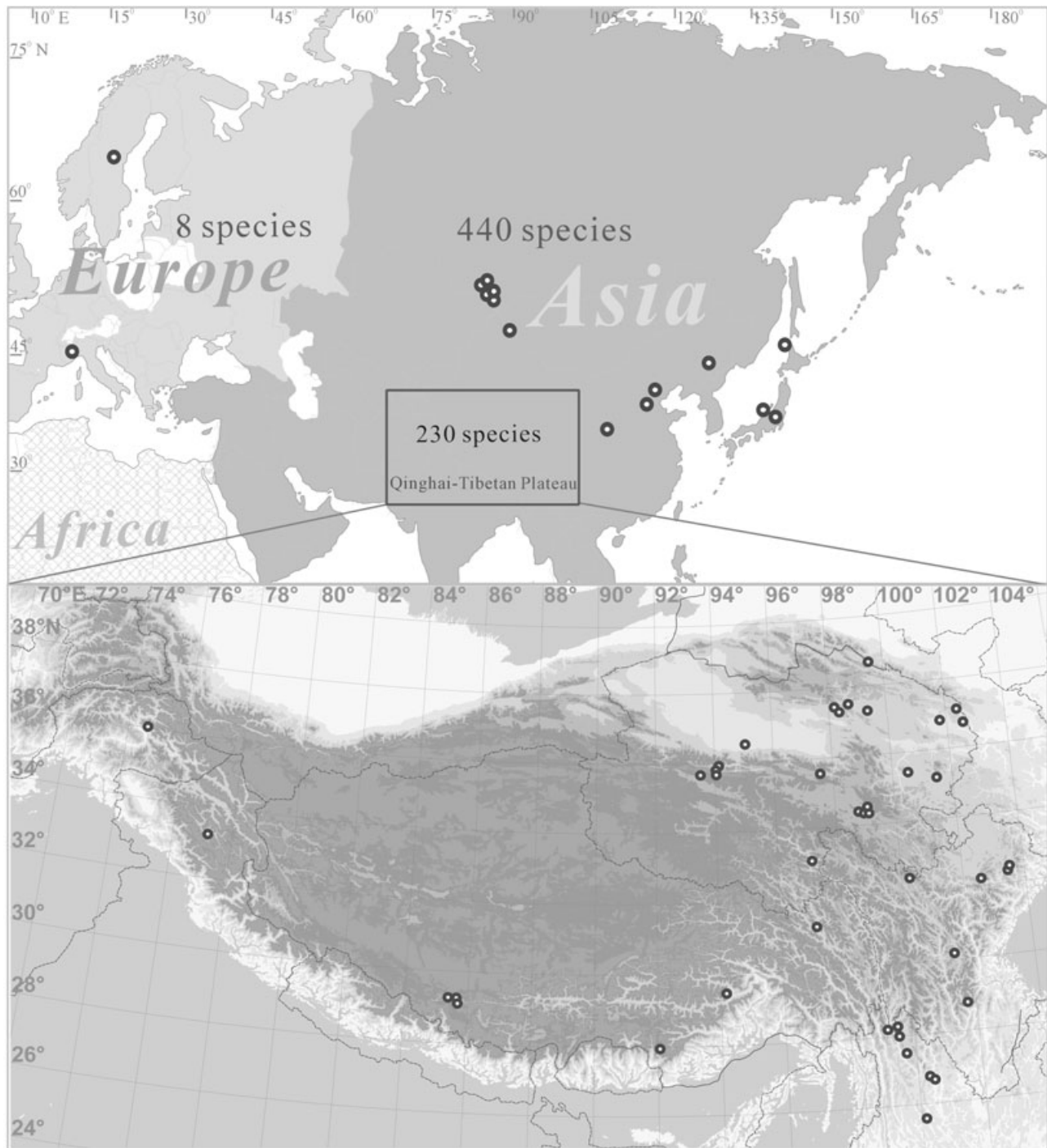


Figure 1. Overview of the distribution of *Saussurea*, with *Saussurea* taxa sampling locations (indicated by circles).

amplify the targeted DNA fragments. In addition, *S. qinghaiensis*, which was recently discovered in QTP and has not yet been assigned to any section, was included as a result of its idiosyncratic morphology. To cover the infrasectional diversity, we selected between one and nine species from each section according to their morphological variance, so that the samples

represented all the groups of species according to our previous morphological examinations. Species-level paraphyly and polyphyly were tested by sampling each species from two or more distinct populations where possible, but little or no variation was found among accessions of all but one species, and the intraspecific accessions grouped together as monophyletic species'

clades in these initial analyses. Thus, only one accession from each of these species was selected for use in further analyses, except that two accessions of *S. katochaete* were included, as two divergent plastid haplotypes and internal transcribed spacer (ITS) types were identified in this species. Overall, 56 individuals from 55 *Saussurea s.l.* species were included in the final phylogenetic reconstruction (Table S2, see Supporting Information), 40 species of which are native to QTP. The remaining species were collected from East Asia (seven species), Central Asia (six species) and Europe (two species; Fig. 1). However, some authors have excluded a few of our selected species from *Saussurea* and have treated them as separate genera, namely *Himalaiella*, *Frolovia* and *Lipschitzella* (Raab-Straube, 2003; Kita *et al.*, 2004). We also included representatives from 15 genera of the Cardueae in our final analyses (Häffner, 2000; Raab-Straube, 2003; Kita *et al.*, 2004), as the phylogenetic relationships between these newly established genera and the extant genera are still unclear, and it is possible that some of them may be united (Wang *et al.*, 2007). Furthermore, *Brachylaena*, *Cousiniopsis* and *Echinops* were used, separately or in combination, as outgroups for the nuclear and plastid sequence analyses. Hence, our analysis included an overall total of 72 species. Leaf samples for most species were taken from the field in 1997 and thereafter stored in silica gel, whereas materials for a small number of species were obtained from herbarium specimens. The origins of the plant samples are listed in Table S1 (see Supporting Information). Voucher specimens were deposited in the herbarium of the Northwest Plateau Institute of Biology, Chinese Academy of Sciences (HNWP).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Total genomic DNA was isolated from accessions collected from the field using the 2× cetyltrimethylammonium bromide (CTAB) method of Doyle & Dickson (1987), and from herbarium specimens using a Plant DNA Isolation Kit (Casarray, Shanghai, China) according to the manufacturer's protocol. The nuclear ITS region and the plastid *trnL-F* and *psbA-trnH* regions were then amplified – using ITS1 and ITS4 (White *et al.*, 1990), *c* and *f* (Taberlet *et al.*, 1991) and *psbA* and *trnH* (Sang, Crawford & Stuessy, 1997) primers, respectively – by polymerase chain reactions and sequenced following the procedures described by Wang *et al.* (2007). In addition, all the newly acquired sequences were archived in GenBank.

DATA ANALYSIS

The analysed datasets consisted of newly acquired sequences of the nuclear ribosomal ITS, plastid *trnL* intron and *trnL-F* intergenic spacer region (*trnL-F*)

and plastid *psbA-trnH* intergenic spacer region (*psbA-trnH*) from 37, 33 and 37 species, respectively, and corresponding sequences for other species downloaded from GenBank (Table S1, see Supporting Information). The sequences were aligned in the ClustalX program (Thompson *et al.*, 1997) using default parameters, and then adjusted by eye where necessary; the sequence boundaries were determined by comparison with published sequences from other Asteraceae genera downloaded from GenBank. Three datasets were then used for phylogenetic analyses. The first comprised nuclear ITS sequences from all 72 accessions, that is sequences from 56 accessions representing 55 species of *Saussurea s.l.*, 15 representing species from other genera of the Cardueae and one from the tribe Tarchonantheae (as an outgroup). These sequences covered both ITS1 and ITS2, but excluded the intervening 5.8S gene, as the sequences downloaded from GenBank did not include this fragment and none of the newly sampled species showed any mutations within it. The second dataset included plastid *trnL-F* and *psbA-trnH* sequences from 41 species of *Saussurea s.l.* and ten species from the other genera of Cardueae. Finally, the third dataset included both nuclear and plastid sequences from species for which both types of sequence were available. *Echinops* of the subtribe Echinopinae was selected as the outgroup for analyses of the second and third datasets according to the ITS analyses and results of previous studies (Petit, 1997; Häffner, 2000; Susanna *et al.*, 2006). The incongruence length difference (ILD) test (Farris *et al.*, 1995) was used to detect potential conflicts between the phylogenetic indications provided by the nuclear and plastid datasets, and to determine whether they could be validly combined. The ILD test was implemented as a partition homogeneity test in the PAUP* program (Sinauer Associates Inc., Sunderland, MA, USA), in which 1000 replicates were each heuristically searched with 100 random sequence additions.

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods were used to analyse the phylogenetic relationships. In the MP analyses, all characters were equally weighted, the gaps were treated as missing characters and heuristic searches were conducted in PAUP* (Swofford, 2003) using 1000 random taxon addition sequences with the tree bisection and reconnection (TBR) branch swapping, MULTREES and COLLAPSE options selected. The robustness of the relationships was assessed by calculating bootstrap percentages (BPs) (Felsenstein, 1985) from 1000 pseudoreplicates using a heuristic search with simple addition and the same options as above. In the ML analyses, the best-fit ML substitution model for each dataset was selected using MODELTEST (Posada & Crandall, 1998), and then these

optimal models were used to parameterize likelihood searches in PAUP*, with the simple addition of sequences, TBR branch swapping, MULTREES and COLLAPSE options selected. The BI analyses were carried out in the MRBAYES program (Ronquist & Huelsenbeck, 2001) using a common GTR + G model, the parameters of which were initially considered to be unknown, and were then estimated during the run. Four chains, each with a different starting seed, were run for two million generations, and the 'temperature' was set to the default value of 0.2. The trees were sampled every 100 generations, and finally PAUP* was used to calculate a majority rule consensus tree from the last 16 001 of the 20 001 trees sampled. The first 4000 trees (burn-in) were excluded to avoid trees that might have been sampled prior to the convergence of the Markov chains. The posterior probabilities (PPs) of each topological bipartition were also estimated according to their frequency across all the 16 001 sampled trees.

The possible evolutionary history of morphological characters within *Saussurea* was explored by mapping nine characters (Table S3, see Supporting Information) onto the ITS topology using the Trace Character function and ACCTRAN optimization within the MacClade program (Maddison & Maddison, 2000). All characters were coded as binary, the character states were unordered and all changes between the states were considered to be equally likely. The molecular clock hypothesis was tested using a likelihood-ratio test (Huelsenbeck & Rannala, 1997) in PAUP* to compare the log-likelihood of the ML trees with and without a molecular clock assumption. As the global molecular clock assumption was rejected, we used r8s software (Sanderson, 2002) to estimate the ML trees' main node divergence ages. Standard deviations for the estimated divergence times were obtained by bootstrapping the dataset 100 times using the SEQBOOT module of the PHYLIP package (Felsenstein, 1989). Branch lengths were then re-estimated for each node under the constrained initial topology in PAUP*. The dating analyses were then repeated for each tree, and node statistics were summarized using the PROFILE command in r8s (Sanderson, 2002). Two points were calibrated based on the fossil pollen records of *Cirsium* (Menke, 1976) and the stem/crown ages of the tribe Cardueae estimated from the molecular data (Kim, Choi & Jansen, 2005).

RESULTS

All the newly acquired sequences included the 5.8S gene, but no mutations were found within this segment and it was not included in any of the previously published sequences. Hence, the 5.8S gene was

omitted from all further analyses. The aligned ITS matrix contained 492 characters, 333 of which were variable, although only 224 were phylogenetically informative when the gaps were treated as missing data. Within the *Saussurea s.l.* and *Saussurea s.s.* lineage (SSSL), 141 of 241 and 99 of 207 variable characters were phylogenetically informative, respectively. MP analyses yielded over 200 000 trees with a length of 1215 steps, a consistency index (CI) of 0.43 and a retention index (RI) of 0.56, and both ML ($-\ln L = 6332.23$) and Bayesian analyses produced largely congruent topologies with the MP analyses, within which nine clades received moderate to high statistical support (Fig. 2). Most of the well-supported groups have been identified previously (Susanna *et al.*, 2006), although our results indicate that *S. forestii* from section *Jacea* (*Saussurea* subgenus *Saussurea*) should be placed among the basal lineages of Cardueae, with a possible close relationship to *Xeranthemum* (Fig. 2). The remaining 54 species of *Saussurea s.l.* clustered into three distinct groups. The first group included *Lipschitzia* and *Himalaiella* (recently separated from *Saussurea*), which formed a well-supported lineage with *Diplazopylon*. The second group showed *S. costus* from section *Aucklandia* (*Saussurea* subgenus *Frolovia*) to be a sister species of *Frolovia frolovii* (PP = 100%, BP = 93%) and closely related to *Dolomiaea souliei*. The third group comprised the remaining species in the well-supported SSSL group (PP = 100%, BP = 73%), which was shown to be a sister group to *Polytaxis* (PP = 100%, BP = 55%). Within SSSL, we identified 24 parallel clades, 15 of which were composed of only one species and nine contained two to six species. Few taxonomic sections as currently circumscribed were discerned (Fig. 2). We also mapped nine traits (Table S3, see Supporting Information) that have been used to circumscribe subgenera or sections on the ITS tree (Fig. 2). Only two characters seem to have evolved once – phyllaries with membranous appendages (used to define *Saussurea* subgenus *Theodorea*) and scapoid or subscapoid herbs [used to define *Saussurea* section *Rosulascentes* (*Saussurea* subgenus *Saussurea*)] – whereas all of the others appear to have had multiple origins within SSSL.

The initial partition homogeneity test showed no incongruence between the two analysed plastid genome fragments ($P = 1.00$), and so the data for these fragments were combined. These combined plastid sequences were 1354 bp in length, with 94 phylogenetically informative base pairs from 134 variable base pairs. Within *Saussurea s.l.*, 66 of 87 variable base pairs were parsimony informative and, within SSSL, 15 of 79 were informative. The three applied methods of phylogenetic reconstruction used recovered topologically congruent trees (Fig. S1,

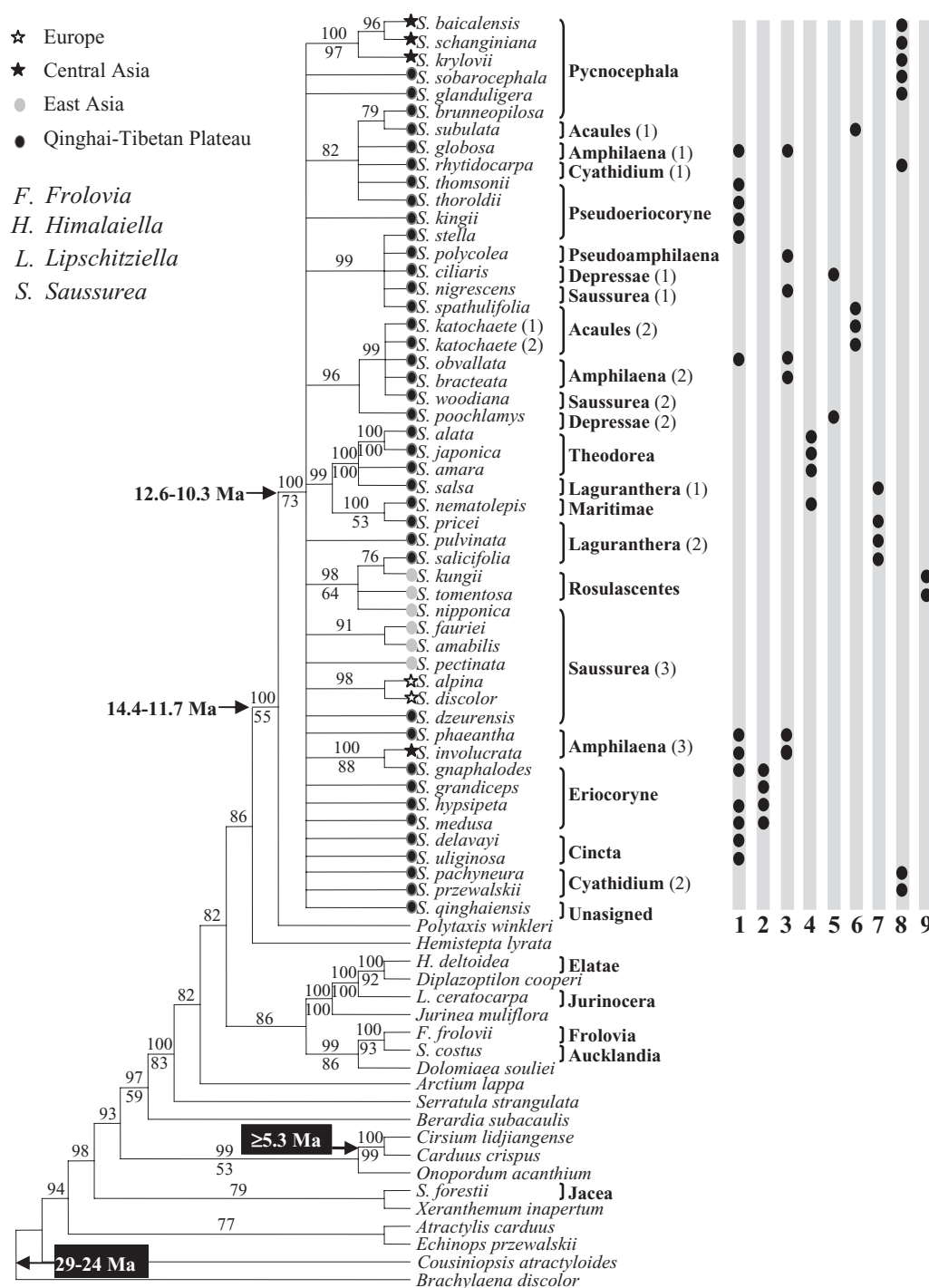


Figure 2. The 50% majority rule consensus tree derived from Bayesian analysis of the nuclear internal transcribed spacer (ITS) dataset. Posterior probabilities (PPs) and bootstrap percentages (BPs) are indicated above and below the branches, respectively. Ages for the calibrated and nodes of interest are shaded. The illustrated morphological characters are: (1) capitula aggregated or not, (2) capitula surrounded by densely lanate subtending leaves or not, (3) capitula supported by membranous uppermost leaves or not, (4) phyllaries with or without membranous appendages, (5) receptacles with or without scales, (6) stems obvious versus absent or rudimentary, (7) roots or rhizomes fibrous to fibrous-lacinate or not, (8) anther-tail cotton-like or ciliate to lacerate, and (9) plant scapoid to subscapoid or not. Species of *Himalaiella*, *Frolovia* and *Lipschitziiella* were included in *Saussurea s.l.*, but are treated here as independent genera. In this figure, *S. polycolea* denotes *S. polycolea* var. *acutisquama* and *S. nipponica* indicates *S. nipponica* var. *hokurokuensis*.

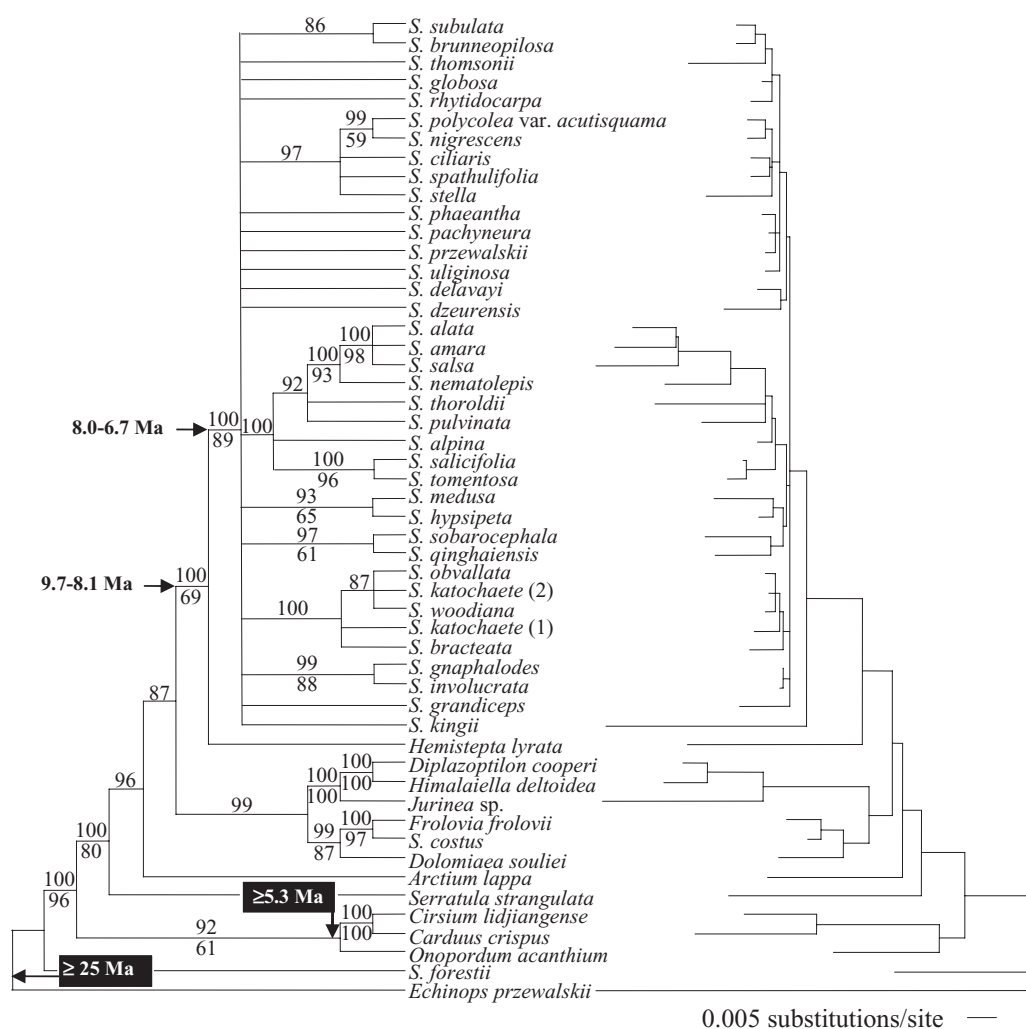


Figure 3. The 50% majority rule consensus tree derived from Bayesian analysis (left) and the maximum likelihood (ML) tree (right) constructed from the analyses of the combined nuclear internal transcribed spacer (ITS) and plastid *trnL-F* and *psbA-trnH* sequences.

see Supporting Information: 107 829 MP trees, length = 178 steps, CI = 0.82 and RI = 0.77; for the ML trees, $-\ln L = 3047.91$). Furthermore, most of the phylogenetic implications from these analyses agreed with those of the ITS data: *S. forestii* was placed at the base of Cardueae, *S. costus* and *F. frolovii* formed a well-supported group (PP = 100%, BP = 66%), and a polytomous group that included representatives of 37 species from 14 sections of five subgenera (PP = 54%, BP < 50%), which corresponded to SSSL, was seen to be a sister group of *Hemistepta* (PP = 100%, BP = 65%). SSSL was composed of 15 parallel clades, only four of which contained more than one species (three to nine species).

Although a few species and clades differed in their phylogenetic positions in the nuclear and plastid trees, the corresponding clusters were poorly supported [Figs 2, S1 (see Supporting Information)]. In

addition, the partition homogeneity test ($P = 0.51$) showed that the nuclear ITS and combined plastid *trnL-F* and *psbA-trnH* datasets were not significantly incongruent, and so they were pooled into a single dataset for further analysis. The resultant combined sequence data matrix was composed of 1846 characters after alignment, 189 of which were variable but uninformative, and 225 were variable and parsimony informative when gaps were treated as missing characters. The topologies of both the ML ($-\ln L = 8219.51$) and 50% Bayesian majority rule consensus trees (Fig. 3) are congruent with the strict consensus tree of the 90 972 MP trees (length = 1023 steps, CI = 0.54, and RI = 0.58), and the phylogenetic relationships indicated by the pooled dataset are largely consistent with those based on the separate datasets. Furthermore, combining the data increased the statistical support for some features, for example

the monophyly of SSSL (PP = 100%, BP = 89%) and its close relationship to *Hemistepta* (PP = 100%, BP = 69%). However, the phylogenetic relationships between the clades of SSSL remained unresolved, despite the increased number of variables available for analysis (Fig. 3).

The likelihood-ratio test strongly rejected the molecular clock assumption for all three sequence matrices (ITS = 6534.36 vs. 6332.23, d.f. = 70, $P < 0.001$; *trnL-F* + *psbA-trnH* = 3047.91 vs. 3096.44, d.f. = 50, $P < 0.001$; *trnL-F* + *psbA-trnH* + ITS = 8219.51 vs. 8281.61, d.f. = 50, $P < 0.001$). Hence, we used the ML phylogram to estimate the diversification ages in SSSL. Fossil records for *Saussurea* and related genera are rare, and the earliest pollen record for this tribe comes from *Cirsium*. This record has been dated to the early Pliocene, and hence we used 5.30 Mya to constrain the *Cirsium* node. In addition, Cardueae have been estimated to have originated between 29 and 24 Mya (Kim *et al.*, 2005). Hence, we used these inferred stem (29–24 Mya) and crown (< 25 Mya) ages to calibrate the ITS and plastid ML phylograms. The results for the separate and combined phylograms differed somewhat (Table S2, see Supporting Information), but in all cases yielded stem/crown ages for SSSL between 14.38 and 6.69 Mya (Fig. 2).

DISCUSSION

CIRCUMSCRIPTION OF *SAUSSUREA* AS A NATURAL GROUP

The only comprehensive revision of *Saussurea* has been provided by Lipschitz (1979), who established six subgenera and 20 sections to accommodate the 390 recognized species. However, three infrageneric groups – *Saussurea* subgenus *Jurinocera*, *Saussurea* section *Frolovia* (*Saussurea* subgenus *Frolovia*) and *Saussurea* section *Elatae* (*Saussurea* subgenus *Saussurea*) – have recently been excluded from this circumscription and instead treated as independent genera: *Lipschitzella*, *Frolovia* and *Himalaiella*, respectively (Kamelin, 1993; Raab-Straube, 2003). Our analyses support the exclusion of these groups, and further indicate that *S. costus*, *S. forestii* and their closely related species should be excluded from *Saussurea* [Figs 2, 3, S1 (see Supporting Information)]. Furthermore, *S. costus* of *Saussurea* section *Aucklandia* (*Saussurea* subgenus *Frolovia*) has a toothed crown atop the achene and a single row of pappus bristles, which are characteristics shared with those species recently placed in the genus *Frolovia* [*Saussurea* section *Frolovia sensu* Lipschitz (Lipschitz, 1979)]. Our results also suggest that all the *Saussurea* section *Aucklandia* species should be united into one genus with the

members of *Frolovia*. However, other molecular evidence suggests that *Frolovia* itself should be reduced into *Dolomiaea* (Wang *et al.*, 2007), and thus the status of *Frolovia* remains uncertain. In addition, *Saussurea* section *Jacea* contains *S. forestii* and four other species that are as yet unexamined (Lipschitz, 1979). The species within this section (all of them occur in the Himalayas) have a single layer of pappus bristles and a dark-brimmed involucre bract. Overall, these data suggest that *S. forestii* does not belong to *Saussurea*, and thus a new genus is required for this species and the others currently placed in *Saussurea* section *Jacea*.

Following the exclusion of the above-mentioned groups, the remaining 14 sections of the five subgenera comprised the moderately supported group of SSSL [Figs 2, 3, S1 (see Supporting Information)]. All members of SSSL share a combination of pappus bristles that is not found in any other members of Cardueae: an inner row composed of plumose bristles that are connate at their base and an outer row with scabrid bristles, which are free of each other and much shorter than those in the inner row. However, the relationships of SSSL to both *Polytaxis* and *Hemistepta* were not well resolved, and require investigation with additional data.

RADIATIVE DIVERSIFICATION OF SSSL

The analyses of both the nuclear and plastid data failed to detect dichotomous relationships among the members of SSSL, and analysis of the combined data (with increased numbers of variables) still yielded 18 parallel clades, many of them unispecific. Such a scenario has been suggested to be indicative of explosive speciation or rapid radiation in a lineage's diversification history (Baldwin & Sanderson, 1998). This type of radiation of species usually occurs in islands, and occasionally in continental regions where major climatic and/or geological oscillations have occurred (Richardson *et al.*, 2001; Hughes & Eastwood, 2006). Furthermore, continental radiation is characterized by a large number of autapomorphic variables accumulating along the parallel branches after the occurrence of simultaneous speciation (Liu *et al.*, 2006). Our data showed that all three of the sampled DNA regions (nuclear ITS and plastid *trnL-F* and *psbA-trnH*) were moderately variable in SSSL, although a few clades were relatively mutation rich. For example, the mutations in the ITS dataset of SSSL occurred in around 42% of the variable base pairs (207 of 492), much higher than the corresponding values reported for radiative genera confined to islands (Baldwin & Sanderson, 1998), although the percentage of informative sites was only 20% (99 of 492). These results suggest that the lack of phylogenetic resolution within

SSSL is not a result of a paucity of mutations, but rather of an evolutionary pattern that is inconsistent with the assumption of a dichotomously branching tree. For instance, sequencing more DNA fragments can satisfactorily resolve phylogenetic relationships in cases in which there are few mutations (Zhang *et al.*, 2006). However, if the latter scenario occurs, the internodes will remain short relative to the average tip nodes within a radiative group, regardless of the number and length of sequences examined, and thus the analysis of more DNA fragments will still fail to resolve the corresponding phylogeny (Murphy *et al.*, 2001). In addition, it should be noted that the species of SSSL from northern and eastern Asia as well as Europe comprise parallel clades with those in QTP, suggesting that they had common origins from a single radiation (Fig. 2). This pattern also differs from previously examined biogeographical disjunctions between Asian and European members of other genera (Zhang *et al.*, 2007).

Our molecular calibration suggests that SSSL began to radiate between 14 and 7 Mya. Although this estimation requires further testing with additional robust phylogenies and more reliable calibration points, it falls between the second and third of four major QTP uplifts believed to have occurred 22–20, 15–13, 8–7 and 3.6–1.8 Mya (Harrison *et al.*, 1992; Shi *et al.*, 1998; Guo *et al.*, 2002; Spicer *et al.*, 2003). Previous analyses have indicated that the radiative diversifications of *Rheum* (Polygonaceae) and the *Ligularia*–*Cremanthodium*–*Parasenecio* complex (Asteraceae) also occurred during these periods (Wang *et al.*, 2005; Liu *et al.*, 2006). Hence, these calibrations suggest that the second and/or third QTP uplifts, which occurred 14–7 Mya, may have played an important role in triggering the explosive speciation of a few plant taxa in QTP. Geological evidence also suggests that the extensive uplifts further strengthened the Asian monsoon climate, resulting in the rapid expansion of cold and dry habitats in the interior of Asia (An *et al.*, 2001). This scenario also correlates with the current habitat preferences of SSSL species, as most are distributed in cold and dry alpine meadows, steppe deserts and snow-line scree regions. Therefore, the SSSL radiation was probably driven by the ecological opportunities, similar to those on islands, provided by largely unoccupied habitats resulting from the extensive QTP uplifts. This hypothesis is strengthened by the fact that the great diversity and evolutionary convergence in life form characteristics of the SSSL species (Fig. 2) are shared by many plant radiations on islands (Emerson, 2002). However, the onset of the diversification of SSSL appears to be more ancient than diversifications recorded on islands, probably because the early QTP uplifts occurred prior to the formation of most islands

in which radiative diversifications have been detailed (Baldwin & Sanderson, 1998). In addition, the unique pappus combinations seen in SSSL probably provided pre-adaptations for the subsequent radiation environment following the QTP uplifts, similar to the key morphological adaptations seen in other radiative genera (von Hagen & Kadereit, 2003). Such combinations presumably promoted the dispersal of achenes in the arid habitats (Bremer, 1994) and probably helped plants of SSSL to successfully compete with other species in the newly created niches. Overall, our results, and previously published data (Richardson *et al.*, 2001; Hughes & Eastwood, 2006), suggest that geological events may have played an important role in generating the current diversity of species through continental radiation in a few taxa and a few regions.

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SUPPORTING INFORMATION

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

Figure S1. The 50% majority rule consensus tree derived from Bayesian analysis (left) and the maximum likelihood tree (right) constructed from combined plastid *trnL-F* and *psbA-trnH* sequence data.

Table S1. List of taxa and sources of plant material, accession numbers in GenBank and voucher specimens deposited in HNWP or references for data from the literature. Species of *Saussurea* arranged according to Lipschitz (Lipschitz, 1979); recent revisions made by Kamelin (Kamelin, 1993) and Raab-Straube (Raab-Straube, 2003) are underlined.

Table S2. Estimates of molecular dating based on nuclear internal transcribed spacer (ITS), plastid *trnL-F* and *psbA-trnH* and the combined nuclear and plastid sequence matrix. SSSL indicates the *Saussurea* s.s. lineage.

Table S3. Statistics of the character state reconstruction.

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