Molecular phylogeny and biogeography of three closely related genera, *Soroseris*, *Stebbinsia*, and *Syncalathium* (Asteraceae, Cichorieae), endemic to the Tibetan Plateau, SW China

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Abstract *Soroseris*, *Stebbinsia*, and *Syncalathium* are three genera of the sunflower family (Asteraceae) with restricted distributions on high screes of the Tibetan Plateau. We present a molecular analysis to test the monophyly of the genera, evaluate the phylogenetic relationships and construct their biogeographic diversification history. Nuclear ITS and plastid *trnL-F* and *psbA-trnH* fragments were analyzed with parsimony, Bayesian inference, and relaxed Bayesian dating for all species of *Soroseris*, *Stebbinsia*, and *Syncalathium*. *Stebbinsia* is part of a polytomy with several lineages of *Soroseris*. *Syncalathium* is biphyletic with *Syn. souliei* placed within subtribe Lactucinae and the remaining species close to the *Soroseris-Stebbinsia* clade within subtribe Crepidinae. Bayesian dating based on ITS sequences and using four fossil calibrations suggests that the stem and crown ages of the *Soroseris-Stebbinsia* clade and the two groups of *Syncalathium* are between 8.44 and 1.56 million years. *Stebbinsia* should be treated as a section of *Soroseris* and *Syncalathium souliei* should be excluded from *Syncalathium* and either placed in *Lactuca* s.l. or established as a new genus in Lactucinae. The remaining species are to be treated as *Syncalathium* s.str. in Crepidinae. The diversification of these groups in the Tibetan Plateau is of relatively young age, and can be explained by rapid diversification and radiation of the *Soroseris-Stebbinsia* clade, allopatric speciation within *Syncalathium* s.str. and convergent evolution of *Syncalathium* s.str. and *Syn. souliei*. The speciation events correlated with climatic change and fragmentation of scree habitats during the uplift of the Tibetan Plateau. Possible migration routes in *Syncalathium* s.str. from the northeast to the central and southern part of the Tibetan Plateau are suggested.

Keywords biogeography; phylogeny; *Soroseris*; *Stebbinsia*; *Syncalathium*; Tibetan Plateau

Supplementary Material Figure S1 and Table S1 are available in the free Electronic Supplement to the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

The Tibetan Plateau has the highest mountains and plateaus in the world with an average altitude above 4000 m (Zheng, 1996). The environment in this region is harsh, being associated with high levels of ultraviolet radiation, complex microclimates and strong winds. These environmental features may have had a profound influence on speciation and adaptation of native plants on the plateau (Shih & Chen, 1982). Many specialized plants occurring in similar habitats in this area share particular morphological characteristics such as, e.g., rosette growth forms (Yang & Sun, 2006).

Soroseris Stebbins, *Stebbinsia* Lipschitz, and *Syncalathium* Lipschitz are three closely related small genera from the tribe Cichorieae (also known as Lactuceae Cass.) of the sunflower family, Asteraceae. These genera are all endemic to the Tibetan Plateau (Fig. 1) (Stebbins, 1940; Ying & Zhang, 1994; Liu, 1996; Shih, 1997) and have similar habitats and morphological traits, including almost acaulescent growth habit and an inflorescence with congested capitula surrounded by a rosette of leaves (Fig. 2D–O).

Soroseris is a small genus of six species formerly treated as members of sect. *Glomeratae* under *Crepis* L. (Stebbins, 1940). The genus appears to have affinities to both *Crepis* and *Prenanthes* L. (Babcock, 1936). It is restricted mainly to the

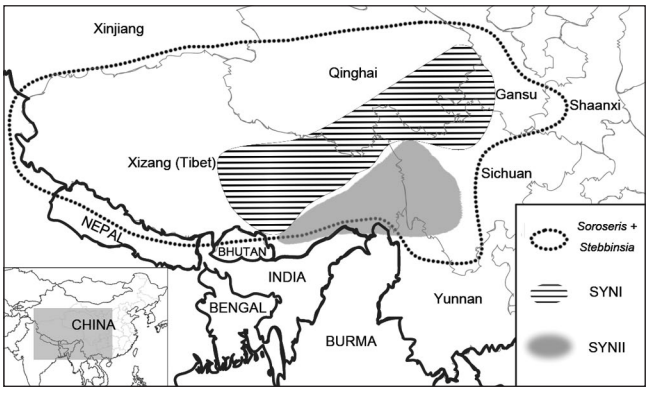

Fig. 1. Distributions of *Soroseris*, *Stebbinsia* and *Syncalathium* (SYNI + SYNII) in the Himalayan-Tibetan region based on field and herbarium collections. SYNI: species of *Syncalathium*, excluding *Syn. souliei*; SYNII: *Syn. souliei*.

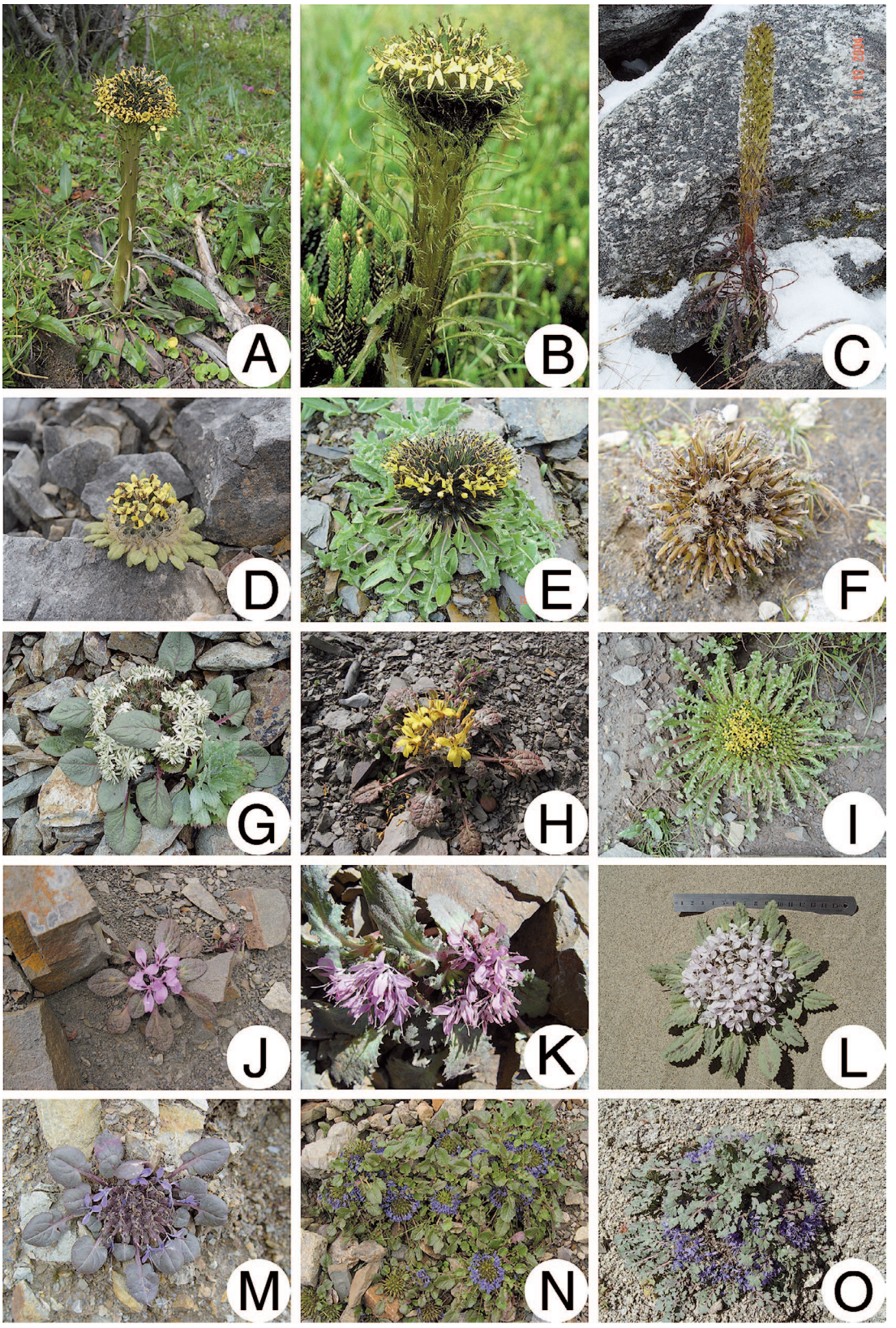

Fig. 2. Selected species of *Soroseris*, *Stebbinsia* and *Syncalathium* in situ. **A,** *Soroseris erysimoides*; **B,** *Sor. hirsuta*; **C,** *Sor. teres*; **D,** *Sor. glomerata*; **E,** *Sor. gillii*; **F,** *Sor. hookeriana*; **G,** *Stebbinsia umbrella*; **H,** *Syncalathium chrysocephalum*; **I,** *Syn. qinghaiense*; **J,** *Syn. kawaguchii*; **K,** *Syn. pilosum*; **L,** *Syn. roseum*; **M–O,** *Syn. souliei* from three different habitats. A, D, H, J, and N photographed by H. Sun; B courtesy of D.E. Boufford; M photographed by Y. Yang; others photographed by J.W. Zhang.

alpine parts of the Sino-Himalayan region of southwestern China. All species occur on alpine screes and sometimes in alpine meadows and thickets at the altitudes ranging from 3000 to 5600 m (Shih, 1997). The monotypic genus *Stebbinsia* was separated from *Soroseris* by Lipschitz (1956) based on *Soroseris umbrella* (Franch.) Stebbins. However, it was still placed in *Soroseris* sect. *Dubyaeopsis* Stebbins by some authors (e.g., Bremer, 1994; Lack, 2007). The genus differs from *Soroseris* in having large, lyrate-pinnatifid (vs. small, entire or pinnatifid) leaves, large involucres with 10–15 (vs. 4–5) inner bracts and 15–40 (vs. 4–6) florets. In addition, the inner bracts of *Stebbinsia* are not thickened at maturity (Stebbins, 1940; Shih, 1997). *Syncalathium* was established by Lipschitz (1956) to include the species formerly placed in *Lactuca* sect. *Aggregatae* Franch. (Franchet, 1895; Stebbins, 1940). Shih (1997) has recently revised *Syncalathium* to contain 8–9 species, each with a relatively restricted and vicarious distribution (Wang & Zhang, 1994; Shih, 1997).

Previous studies on the three genera concentrated mainly on their gross morphology and suggested overall similarity (Stebbins, 1940; Ling, 1965; Shih, 1993, 1997; Yamaji & al., 1996). Recent karyomorphological data (Zhang & al., 2007, 2009) indicate that *Stebbinsia* and *Soroseris* share the same karyotype formula (x = 7m + 1sm) and have the same karyotype asymmetry of 1A. However, the karyotype in *Syncalathium souliei* is x = 3m + 5sm (2A), which is different from that of the other *Syncalathium* species with x = 7m + 1sm (1A) or x = 8m (1A). Therefore, the monophyletic status of the three genera and their relationships need to be tested in a phylogenetic framework. Moreover, the biogeographic diversification of the three endemic genera in the Tibetan Plateau is poorly known.

Phylogenetic and biogeographic analyses have been conducted for some genera of Asteraceae in the Tibetan Plateau. Examples include *Nannoglottis* (Liu & al., 2002), the *Ligularia-Cremanthodium-Parasenecio* complex (Liu & al., 2006), *Saussurea* (Raab-Straube, 2003; Wang & Liu, 2004a,b; Wang & al., 2009), and the *Dolomiaea-Diplazoptilon-Xanthopappus* group (Wang & al., 2007). All these studies have provided useful information for understanding the origin and dispersal of plants on the Tibetan Plateau. For example, studies on Asteraceae (Wang & al., 2007) suggested a continuous origin hypothesis for endemic genera in this area since the middle Miocene.

Several molecular studies concerning the phylogenetic relationships within the subtribe Crepidinae (Cichorieae) have been conducted recently (Enke & Gemeinholzer, 2008; Enke & al., 2008; Kilian & al., 2009). Kilian & al. (2009) analyzed the phylogeny of the tribe Cichorieae and discussed the relationships among the subtribes and genera of the group. These analyses provided a framework for our investigation of the phylogenetic relationships among *Soroseris*, *Stebbinsia*, and *Syncalathium* and their interspecific relationships. The nuclear ribosomal internal transcribed spacer (ITS) has been shown to be a useful source of information for resolving interspecific relationships in the Asteraceae (Baldwin, 1992, 1993; Sang & al., 1994, 1995; Kim & Crawford, 1996; Clevinger & Panero, 2000; Francisco-Ortega & al., 2001; Wang & al., 2009). In addition, the plastid *trnL-F* and *psbA-trnH* regions have been used to reconstruct the phylogeny of some taxa of the family (Kim & al., 2004; Pornpongrungrueng & al., 2007; Wang & al., 2007, 2009; McKenzie & Barker, 2008; Bergh & Linder, 2009).

In the present study we used the nuclear ITS and plastid *trnL-F* and *psbA-trnH* regions to (1) evaluate the monophyly of *Soroseris*, *Stebbinsia*, and *Syncalathium*, (2) reconstruct the phylogenetic relationships among and within the three genera, and (3) assess their biogeographic origin and diversification in the high mountains of the Tibetan Plateau.

■ MATERIALS AND METHODS

Taxon sampling. — We sampled 35 populations representing 23 species of the *Soroseris*, *Stebbinsia*, *Syncalathium* and close relatives in Cichorieae for the combined analysis of nuclear and chloroplast datasets. *Cichorium intybus* was chosen as outgroup according to Kilian & al. (2009). Sequences for other related taxa were obtained from GenBank (see Appendix). All the six species of *Soroseris* were sampled. The monotypic *Stebbinsia* was collected from two localities. For *Syncalathium*, only *Syn. porphyreum* was not included in the study because of the difficulty in obtaining material. *Syncalathium souliei* (including *Syn. orbiculariforme*) was collected from six different localities across its geographic range because this taxon has a different karyotype from other species in the genus. A list of samples, voucher location and GenBank numbers is provided in the Appendix.

DNA extraction, PCR amplification and sequencing. — Genomic DNA was extracted from 20–30 mg of silica-dried leaf tissue, using the Universal Genomic DNA Extraction Kit Version 3.0 (TaKaRa, Dalian, China) following the manufacturer's protocol. The DNA amplifications were performed using TaKaRa *Taq*™ (DR001AM) and a PTC-0200 DNA Engine Peltier thermal cycler (Bio-Rad, California, U.S.A.). The 80 µl volume polymerase chain reactions (PCRs) contained 30–40 ng of template DNAs, 50 mM Tris-HCl, 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 2 mM of each primer and 0.75 U of *Taq* polymerase (TaKaRa, Dalian, China). Amplification and sequencing of the ITS region (ITS1, 5.8S rDNA, ITS2) was performed with primers ITS1 and ITS4 (White & al., 1990). PCR and sequencing of *trnL-F* sequences was performed using the external primers c and f of Taberlet & al. (1991). The primers used for amplifying and sequencing the *psbA-trnH* region were trnH and psbA (Sang & al., 1997). The PCR amplification profiles were identical for all three fragments: one cycle at 94°C for 4 min; 32 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min; and one cycle at 72°C for 7 min.

Amplified products were purified with a QIAquick PCR Purification Kit (BioTeke, Beijing, China), and sequenced using an ABI 3730XL automated DNA sequencer (Applied Biosystems, Foster City, California, U.S.A.). The raw sequence fragments from forward and reverse primers were assembled using Sequencher v.4.1.4 (GeneCodes Corporation, Ann Arbor, Michigan, U.S.A.). All regions were sequenced for both strands where there was an overlap of at least 70%. The sequences were

deposited in GenBank with the accession numbers given in the Appendix. The sequences were aligned with ClustalX v.1.83 (Thompson & al., 1997). Manual adjustments were made using BioEdit v.7.0.5 (Hall, 1999). The indels of *psbA-trnH* (at positions 76–79, 174–184, 257–270, 301–303, 380–383, 396–405, 435–446) and *trnL-F* (at positions 247–256, 306–315, 543–555, 605–608, 874–878) have been coded as one mutational step each. For a broader analysis at the tribal level, sequences of other closely related taxa were downloaded from GenBank (see Appendix).

Phylogenetic analysis. — Parsimony analyses were performed with heuristic searches of 1000 replicates with random stepwise addition using tree bisection-reconnection (TBR) branch swapping, MulTrees and Collapse option selected in PAUP* v.4.0b10 (Swofford, 2002). The gaps caused by mononucleotide repeat units were not considered in the phylogenetic analysis, because their homology can be highly uncertain for these repeated nucleotides (Kelchner, 2000). All characters and character state transformations were weighted equally. The bootstrap probabilities (BP) were calculated from 1000 replicates using a heuristic search with simple addition with the TBR and MULPARS options implemented (Felsenstein, 1985).

Bayesian inference (BI) based on the Markov chain Monte Carlo methods (Yang & Rannala, 1997) was performed using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). A hierarchical likelihood ratio test using log likelihood scores to test the goodness of-fit of nested substitution models was performed as implemented in MrModeltest (Nylander, 2004). The common model GTR+I+G was determined by MrModeltest for the different data partitions in the Bayesian analyses, and the base frequencies were empirically derived. Four simultaneous Monte Carlo Markov chains (MCMCs) were run for 5,000,000 generations and a tree was saved every 1000 generations. The Bayesian trees sampled for the last 3500 trees were used to construct a 50%-majority-rule consensus cladogram. The proportions of bifurcations found in this consensus tree are given as posterior clade probabilities (PP) as an estimator of the robustness of the BI trees. The majority-rule consensus tree was calculated in PAUP*.

To evaluate the congruence of the plastid and nuclear datasets, we first employed the incongruence length difference (ILD) test (Farris & al., 1995). The ILD test was conducted with 100 replicates of heuristic search using TBR branch-swapping with 10 random sequence additions.

Molecular dating and fossil calibration. — To estimate the divergence time of stem and crown lineages in the three genera, we used the ITS sequences because sequences of a wide range of taxa of the tribe are available and only a few plastid sequences of the tribe Cichorieae can be found in GenBank. Taxa from Cichorieae and Liabeae in Asteraceae (see Appendix) were included for the molecular clock calibration. A likelihood ratio test (Felsenstein, 1988) ruled out a global molecular clock ($P < 0.05$) of our datasets. We thus implemented the relaxed Bayesian clock with rates for each branch drawn independently from a lognormal distribution (Drummond & al., 2006). The relaxed molecular clock model, which does not assume a constant rate across lineages, has been used in recent studies to date the dispersal events of a number of plant taxa (e.g., McKenzie & Barker, 2008; Bergh & Linder, 2009).

BEAST v.1.5.4 (http://beast.bio.ed.ac.uk) was used to estimate the divergence times. In order to create input files for BEAST, the BEAUti interface was used, in which a General Time Reversible (GTR) nucleotide-substitution model with Gamma + Invariant sites was applied, with the relaxed molecular clock model and the Birth-Death prior set for branch lengths. Several short BEAST runs were firstly performed to examine the performance of the MCMC. After optimal operator adjustment, as suggested by the output diagnostics, two final BEAST runs each containing 10,000,000 generations were performed and the trees saved every 1000 generations. The log files were then combined to check for convergence towards the same distribution and to ensure adequate sample sizes, and viewed using Tracer v.1.5. After discarding the first 2500 trees as representing the burn-in, the trees and parameter estimates from the two runs were combined. The samples from the posterior were summarized on the maximum clade credibility tree which has the maximum sum of posterior probabilities on its internal nodes (Drummond & al., 2007) using TreeAnnotator v.1.5.4 (Drummond & Rambaut, 2007) with the posterior probability limit set to 0.5 summarizing mean node heights. These were visualized using FigTree v.1.3.1. (http://tree.bio.ed.ac.uk/software/figtree/). Means and 95% higher posterior densities (HPD) of age estimates were obtained from the combined outputs using Tracer. The 95% HPD represents the shortest interval that contains 95% of the sampled values from the posterior (Drummond & al., 2007).

Dating of molecular phylogenies generally requires either a known rate of molecular evolution or reliable calibration points, and time estimation with molecular clocks would be mostly unnecessary if the fossil record provided an accurate and complete representation of evolutionary history (Hedges & Kumar, 2004). More accuracy in the molecular time estimate is obtained with one or more tightly constrained fossil calibrations close to the speciation event, rather than with many calibrations that are poorly constrained (Hedges & Kumar, 2004).

Fossil records of Cichorieae are poor, but there are three different types of microfossils representing echinolophate pollen, i.e., the *Cichorium intybus* type (22.0–28.4 million years [Ma]), the *Scorzonera hispanica* type (ca. 3.4 Ma), and the *Sonchus oleraceus* type (ca. 5.4 Ma) (Hochuli, 1978; Blackmore & al., 1986). Moreover, a reliable achene fossil of *Crepis conyzaefolia* has been dated to the upper Pliocene, and hence we used 2.58–3.60 Ma to constrain the *Crepis* node (Reid & Reid, 1915; Boenigk & Frenchen, 2006; Kemna & Westerhoff, 2007). In the present paper, the stem age of *Cichorium* was constrained as 25.20 ± 3.20 Ma, and the crown ages of *Scorzonera*, *Sonchus* and *Crepis* were constrained as 3.40 ± 1.00 Ma, 5.40 ± 1.00 Ma and 3.09 ± 0.51 Ma, respectively. A recent dating of Asteraceae (Kim & al., 2005) estimated that the stem age of Cichorieae ranged from 28 to 31 Ma based on a slow and a fast rate calibration of *ndhF* from other angiosperm families, and from 24 to 29 Ma based on NPRS dating calibrated with an outgroup fossil. We used a normal distribution with a mean at 28 Ma, the midpoint of Kim & al.'s (2005) dates, and a

standard deviation of 4, as our prior for this node. With secondary calibration dates having several sources of error (see Graur & Martin, 2004), we also ran a separate analysis without the constraint of the stem Cichorieae.

■ RESULTS

Phylogenetic analysis. — The aligned ITS matrix had 683 positions with 358 phylogenetically informative (358/683 = 52.4%) sites for the ITS analysis alone (Fig. 3). The aligned ITS matrix for the combined analysis with chloroplast had 655 positions with 171 phylogenetically informative (171/655 = 26.1%) sites. The *psbA-trnH* dataset was composed of 428 base pairs (bp), 73 of which are parsimony-informative (73/428 = 17.1%). The aligned *trnL-F* dataset contained 882 bp with a lower percentage of potentially parsimony-informative sites (58/882 = 6.6%). Because there is no recombination in the chloroplast DNA, we combined the plastid *psbA-trnH* and *trnL-F* sequences in our analysis (Fig. S1). The combined plastid dataset comprised 37 accessions from ten genera of Cichorieae. The aligned matrix consisted of 1310 positions, including 82 parsimony-uninformative variable sites and 131 potentially parsimony-informative variable sites.

The ILD test suggested significant conflict between the plastid and the ITS datasets ($P = 0.01$). Yet, if *Nabalus tatarinowii* was excluded, the data became combinable ($P = 0.10$). This species was thus excluded when the three datasets were combined. The combined data of the three regions included 35 accessions, representing nine genera of the tribe Cichorieae. The combined nuclear-plastid data (Table S1) consisted of 1965 positions with 157 parsimony-uninformative and 292 potentially parsimony-informative variable sites. The strict consensus tree based on the combined data (Fig. 4) was similar to topologies generated from ITS (Fig. 3) or plastid data (Fig. S1), but had relatively better resolution.

Five subtribes (Chondrillinae, Hypochaeridinae, Lactucinae, Hyoseridinae, Crepidinae) of tribe Cichorieae were resolved in the ITS phylogenetic tree, with low support in the main clades (Fig. 3). The clades of *Soroseris*-*Stebbinsia* (SOR) (BP = 95, PP = 100) and *Syncalathium* s.str. (SYNI) (BP = 94, PP = 100) were nested within the subtribe Crepidinae, and the SYNI clade was close to *Nabalus*, *Hololeion*, and *Dubyaea*, but with poor support (BP < 50, PP < 70). The clade including the six populations of *Syncalathium souliei* (including *Syn. orbiculariforme*) was deeply nested within the subtribe Lactucinae with strong support (BP = 100, PP = 100; SYNII in Fig. 3).

In the combined tree (Fig. 4), all species of *Soroseris* and *Stebbinsia* formed a clade with BP = 100 and PP = 100 (Fig. 4, SOR). All species of *Syncalathium* except *Syn. souliei* (= *Syn. orbiculariforme*) formed a strongly supported clade within the subtribe Crepidinae (the SYNI clade, referred to as *Syncalathium* s.str.; BP = 100, PP = 100; Fig. 4). Two subclades were recognizable within the SYNI clade, one including *Syn. disciforme* and *Syn. qinghaiense* (BP = 100, PP = 100), and the other consisting of the remaining species of SYNI (BP = 100, PP = 100). All populations of *Syncalathium souliei* (including *Syn. orbiculariforme*) constituted a clade with strong support (BP = 100, PP = 100; SYNII in Fig. 4).

Divergence times. — ITS sequences are available from GenBank for many genera of Cichorieae, covering the full morphological diversity of this tribe. Therefore, only the ITS dataset was used to infer the dates of origin for the three genera within the phylogenetic framework at the tribal level. With the root constraint as 28.00 ± 4.00 Ma, the dating suggested that the crown age of *Soroseris*-*Stebbinsia* was about 1.56 Ma (95% HPD: 0.53–2.82 Ma). The diversification of *Syncalathium* s.str. probably occurred around 2.64 Ma (95% HPD: 1.78–4.33 Ma), that of *Syncalathium souliei* at around 3.23 Ma (95% HPD: 1.52–5.30 Ma). Stem ages of the three groups are presented in Table 1. Results without the constraint on the stem Cichorieae (i.e., the root in Fig. 5) showed slightly older ages than those estimated with this calibration point (Table 1).

■ DISCUSSION

***Soroseris* and *Stebbinsia*. —** All species of *Soroseris* and *Stebbinsia* form a clade with strong support (clade SOR, Figs. 3, 4). *Soroseris* is characterized by small, entire or pinnatifid leaves, narrow involucres, and 4–5 inner bracts and florets, the inner bracts becoming spongy and thickened at maturity (Stebbins, 1940; Shih, 1997). *Stebbinsia* was segregated from *Soroseris* based on its larger leaves and greater number of florets (Lipschitz, 1956; Shih, 1997), but is similar to *Soroseris* with possible synapomorphies in its gross morphology and cytology (Stebbins, 1940; Zhang & al., 2007). Our phylogenetic results suggest that *Stebbinsia* is a part of a polytomy with several lineages of *Soroseris* (Figs. 3, 4; Fig. S1), therefore it seems warranted to merge *Stebbinsia* with *Soroseris* as *Soroseris* sect. *Dubyaeopsis*, as originally proposed by Stebbins (1940) and accepted by e.g., Bremer (1994), Lack (2007) and Kilian & al. (2009).

Phylogenetic relationships within the *Stebbinsia*-*Soroseris* group remain uncertain as shown by the polytomy within the clade (Figs. 3, 4). However, there are distinct morphological differences between *Soroseris* and *Stebbinsia* as described in the introduction. In addition, a high level of morphological variation is also found among the species of *Soroseris*. For example, *Sor. teres* has densely long cylindric head whereas the remaining species usually have glomerulate head (Fig. 2C). Furthermore, *Sor. glomerata* has ovate leaves whereas the remaining species usually have lanceolate leaves (Fig. 2D). The unresolved ITS and plastid phylogeny of the SOR lineage may reflect a relatively simultaneous radiation and recent diversification of the species in the Tibetan alpine region and the adjacent areas.

***Syncalathium*. —** As shown in Fig. 3, *Syncalathium* is biphyletic with *Syn. souliei* (including *Syn. orbiculariforme*, SYNII) placed in the subtribe Lactucinae and the SYNI clade (all *Syncalathium* species except *Syn. souliei*) nested in a clade including *Nabalus*, *Hololeion* and *Dubyaea* in the subtribe Crepidinae. The two species of *Syncalathium chrysocephalum* and *Syn. qinghaiense* were formerly treated as *Soroseris chrysocephala* C. Shih and *Sor. qinghaiensis* C. Shih. Liu (1996) and

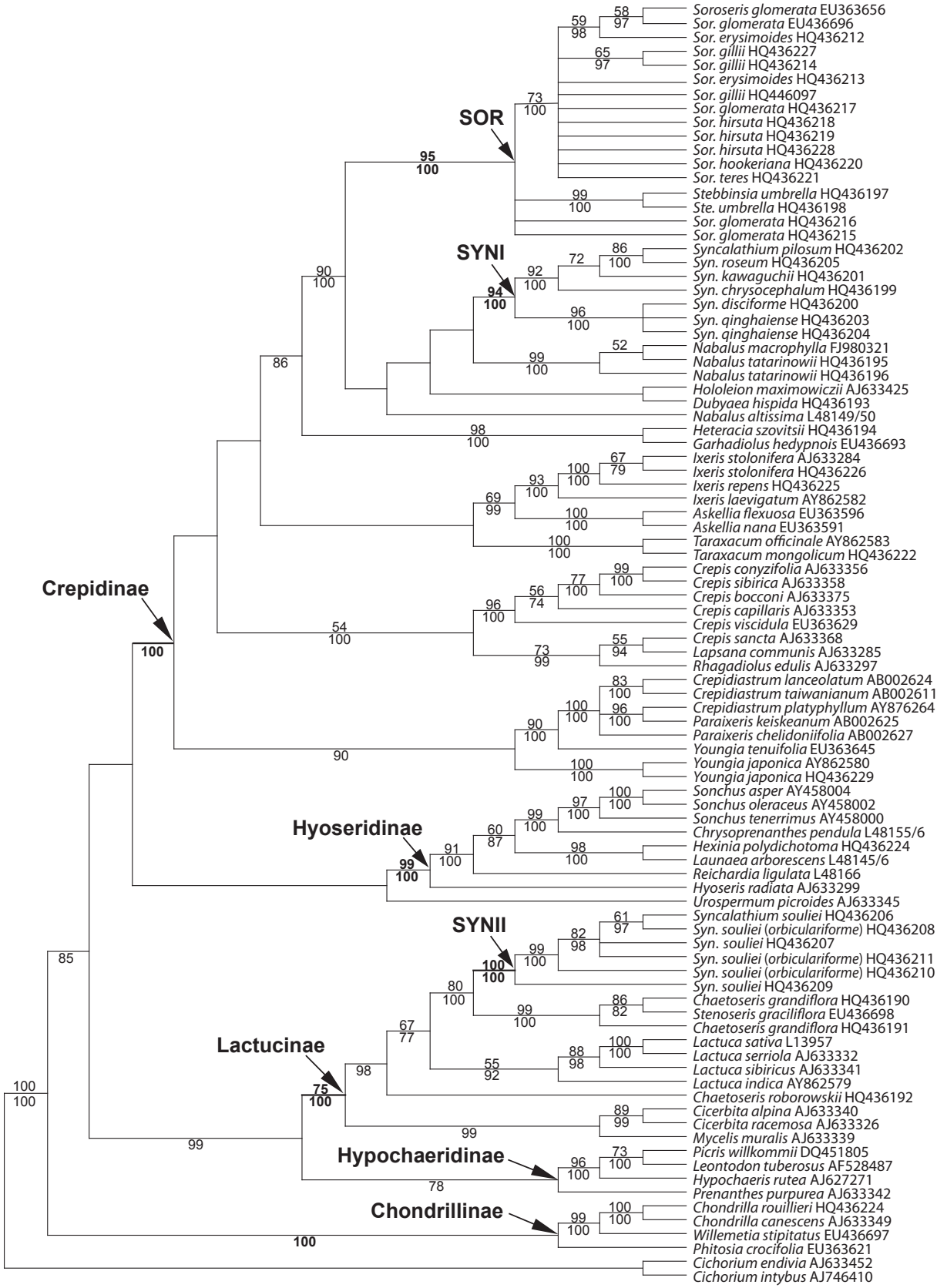

Fig. 3. Strict consensus tree derived from maximum parsimony analysis of nrITS. Tree length = 1912 steps, CI = 0.37, RI = 0.71, and RC = 0.27. Posterior probabilities (>70%) are noted below branches, bootstrap support values (>50%) are indicated above branches. The GenBank accession number follows the species name.

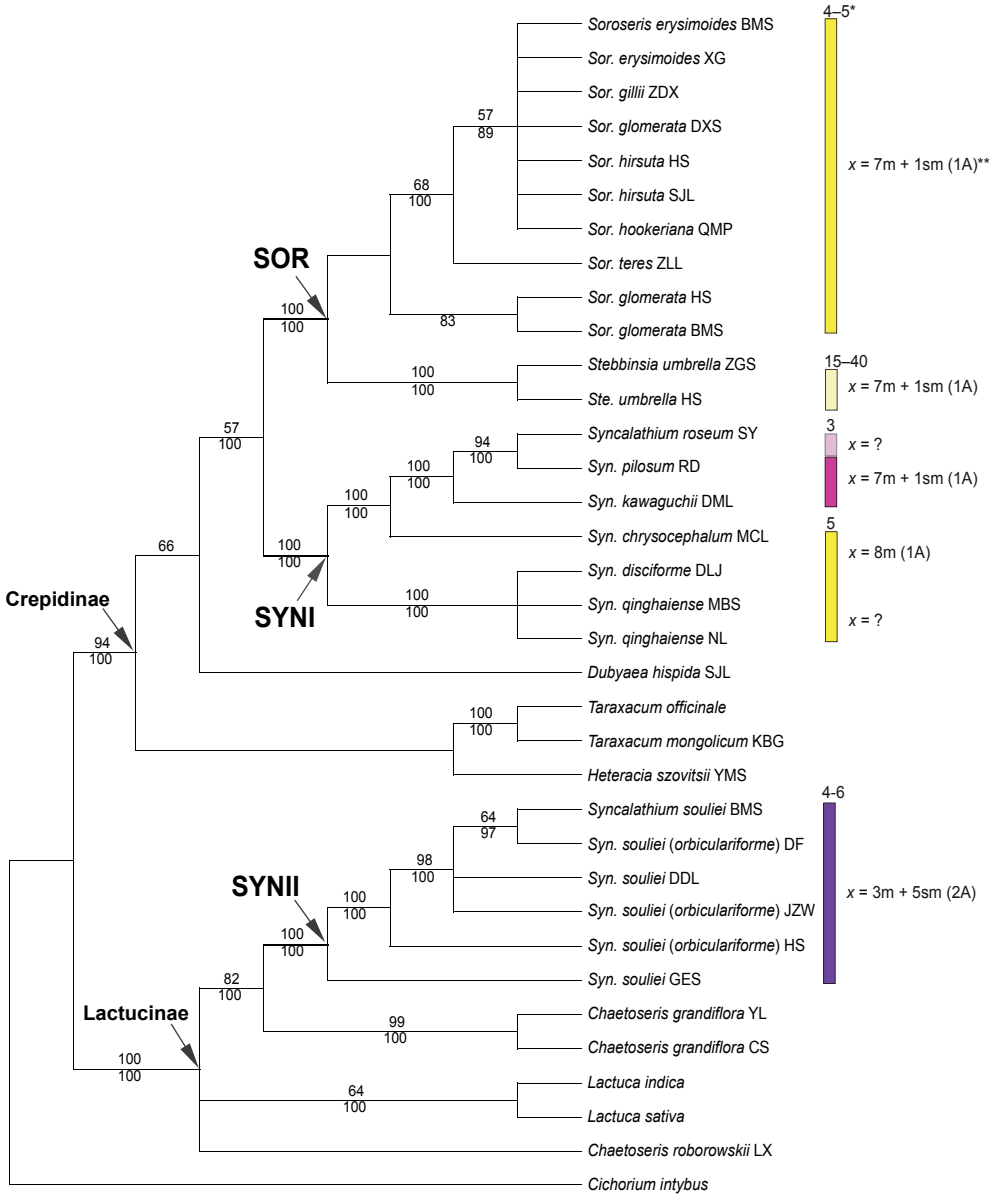

Fig. 4. Strict consensus tree derived from maximum parsimony analysis of the combined nrITS and plastid datasets. Tree length = 774 steps, CI = 0.74, RI = 0.88, and RC = 0.65. Other details are the same as in Fig. 3. The colors of the bars represent floret colors; *number of florets; **karyotype formula (KF) and karyotype asymmetry (KA). The abbreviation of the population's name follows the species name.

Table 1. Divergence age estimates in millions of years before present. Nodes are labeled as in Fig. 5.

		Mean (95% HPD)			
Node	Description	Crown	Stem	Crown	Stem
A	Root node	–	28.00 ± 4.00	–	–
B	*Cichorium* clade	–	25.20 ± 3.20	–	25.20 ± 3.20
C	*Scorzonera* clade	3.40 ± 1.00	–	3.40 ± 1.00	–
D	*Sonchus* clade	5.40 ± 1.00	–	5.40 ± 1.00	–
E	*Crepis* clade	3.09 ± 0.51	–	3.09 ± 0.51	–
SOR	*Soroseris-Stebbinsia*	1.56 (0.53–2.82)	3.79 (1.76–6.24)	1.99 (0.57–3.08)	4.82 (1.97–8.21)
SYNI	*Syncalathium* s.str.	2.64 (1.78–4.33)	4.35 (2.31–6.65)	3.40 (1.30–6.09)	5.50 (2.54–8.92)
SYNII	*Syncalathium souliei*	3.23 (1.52–5.30)	6.69 (3.72–9.85)	4.04 (1.55–6.90)	8.44 (4.13–13.31)

Fig. 5. Chronogram of *Soroseris*, *Stebbinsia*, *Syncalathium* and relatives based on ITS data. Divergence times estimated with BEAST. The tree was rooted using *Liabum* (Liabeae) and calibrated using the midpoint of Kim & al.'s (2005) dates of 28.00 ± 4.00 Ma for the age of the root (node A). Nodes of B, C, D and E were constrained to be 25.20 ± 3.20 Ma, 3.40 ± 1.00 Ma, 5.40 ± 1.00 Ma and 3.09 ± 0.51 Ma based on fossils, respectively. The GenBank accession number follows the species name.

Shih (1997) transferred the two species into *Syncalathium*, respectively, and this treatment is supported by our data (Figs. 3, 4). Within the SYNI clade, species with purple and pink flowers (i.e., *Syn. kawaguchii*, *Syn. pilosum*, *Syn. roseum*) are nested within the group with yellow flowers (*Syn. qinghaiense*, *Syn. disciforme*, *Syn. chrysocephalum*). Morphologically the species with yellow flowers have five florets and the purple ones three, suggesting that a derived condition for three florets and purple (or pink) flowers. Cytologically, the yellow-flowered species have a more symmetrical karyotype (x = 8m) than the purple ones with a karyotype x = 7m + 1sm (Zhang & al., 2007, 2009). Thus, our results are consistent with the view that the symmetrical karyotypes represent more primitive characters than the asymmetrical ones (Levitzky, 1931; Stebbins, 1971).

The SYNII clade includes only *Syncalathium souliei* (including *Syn. orbiculariforme*; Figs. 3, 4). *Syncalathium orbiculariforme* was considered conspecific with *Syn. souliei* by Zhuang (2004), which is supported by our molecular results (Figs. 3, 4). *Syncalathium souliei* (Franch.) Ling was originally described as *Lactuca souliei* by Franchet (1895) (belonging to *Lactuca* sect. *Aggregatae*). Stebbins (1940) already noted the morphological differences between *Lactuca souliei* and the other species, e.g., achenes with one rib on each face and an apex contracted below the pappus disk vs. 5-ribbed and apex not contracted and blue vs. yellow, purple or even pink florets. Ling (1965), however, united it with *Syncalathium* based on the echinolophate pollen and the similarities in gross morphology and the achenes (slightly to strongly compressed, strongly contracted at the apex, 3–5-ribbed). Our analyses suggest that it is more closely related to *Lactuca* s.l. (*Lactuca* s.str., *Stenoseris* and *Chaetoseris*) of subtribe Lactucinae (Fig. 3) and should be separated from *Syncalathium* in the subtribe Crepidinae. Molecular phylogenetic and morphological analyses of the subtribe Lactucinae (N. Kilian, pers. comm.) show *Syncalathium souliei* to be deeply nested within the subtribe Lactucinae. Cytological data also suggest that it is distinct from the other species of *Syncalathium* (Zhang & al., 2007, 2009), with *Syn. souliei* having karyotype x = 3m + 5sm (2A) and the others having either x = 7m + 1sm (1A) or x = 8m (1A). Moreover, they have a segregated distribution, with *Syn. souliei* occurring mainly in northwest Yunnan, southeast Tibet and western Sichuan, and the other species of *Syncalathium* north and west of *Syn. souliei* (Fig. 1). Therefore, we suggest that *Syn. souliei* is separated from *Syncalathium* and either placed back in *Lactuca* s.l. (Lactucinae) or be treated as a new genus in the subtribe.

Biogeographic diversification in the Tibetan Plateau. — *Soroseris*-*Stebbinsia*, *Syncalathium* s.str. and *Syn. souliei* are three well-supported monophyletic groups endemic to high screes of the Tibetan Plateau (Figs. 3, 4). Dating results based on ITS data indicated a recent origin of the three groups in the Tibetan Plateau with their stem ages round 3.79–8.44 Ma (Table 1). The diversification of the extant species of the three groups is also very recent, with estimated ages around 1.56–4.04 Ma from late Pliocene to early Pleistocene and the full range of their 95% HPD being 0.53–6.90 Ma. Therefore, we suggest an origin and diversification of the three endemic groups in this area since the late Miocene.

Molecular calibration of branching time in phylogenetic trees is controversial, and should be treated with caution. However, when paleontological data are lacking, molecular estimates provide the only means of inferring the age of lineages (Li, 1997; Bromham & Penny, 2003). Multiple fossil-based calibration points and multiple molecular markers are important for the accuracy of divergence time estimates. Our estimates were only based on the ITS sequence, but all the other molecular sequences in this study show an overall much lower variation (Table S1), which indicates a recent evolutionary history of the three endemic groups. Therefore, our molecular results suggest recent origins and diversifications of these endemic groups on the Tibetan Plateau, notwithstanding the unavailability of precise dating.

This conclusion also coincides with the recent uplift of the Tibetan Plateau during the Pliocene and the resultant formation of harsh habitats in the region (Shi & al., 1998). Geologic uplift events (first of which began at about 50 Ma) have taken place in the Tibetan Plateau during at least four different periods since the early Miocene, i.e., 22 Ma, 15–13 Ma, 8–7 Ma, and 3.5–1.6 Ma (Harrison & al., 1992; Li & al., 1995; Shi & al., 1998; Guo & al., 2002; Spicer & al., 2003). The origin (3.79–8.44 Ma; Table 1) and diversification (1.56–4.04 Ma; Table 1) of the three endemic lineages likely occurred independently at final two stages of the uplift and formation of the Tibetan Plateau. Geological evidence suggests that the extensive uplifts of the Tibetan Plateau further strengthened the Asian monsoon climate, resulting in rapid expansion of dry and cold habitats in the interior of Asia (An & al., 2001). This scenario also correlates with the current habitat preferences of the studied taxa, as all of them are being distributed in cold and dry alpine meadows, steppe deserts and alpine scree regions. For example, the poor resolution and young divergence ages (1.56–1.99 Ma; Table 1) of SOR may indicate a recent radiation of this group probably triggered by the ecological opportunities provided by largely open and harsh habitats resulting from the more recent uplift of the Tibetan Plateau (i.e., 3.5–1.6 Ma) (Li & al., 1995; Shi & al., 1998). The similar rapid radiation has also been found in other groups of Asteraceae in the Tibetan Plateau, such as *Saussurea* (Wang & Liu, 2004a; Wang & al., 2009), *Ligularia*-*Cremanthodium*-*Parasenecio* complex (Liu & al., 2006) and the *Dolomiaea*-*Diplazoptilon*-*Xanthopappus* group (Wang & al., 2007).

Some authors (Stebbins, 1940; Shih, 1997; Zhuang, 2004) had placed *Syncalathium souliei* in *Syncalathium* on the basis of the similar morphological traits (i.e., the cushion growth with hollow stem, inflorescences with congested capitula, the number of florets, and florets being surrounded by a rosette of leaves; Fig. 2). The calibration times of SYNI and SYNII (Fig. 5; Table 1) are similar, which indicates that *Syncalathium* s.str. and *Syncalathium souliei* may have undergone convergent evolution under climatic changes and similar ecological selection pressures in arid habitats of the Tibetan Plateau (screes, sand slopes), during the uplift of the Plateau. Similar examples of convergence are found in *Androsace* (Wang & al., 2004) and *Rheum* (Wang & al., 2005) from the same region.

The specimen records and field data suggest that the geographic distribution of the species of *Syncalathium* s.str. extend

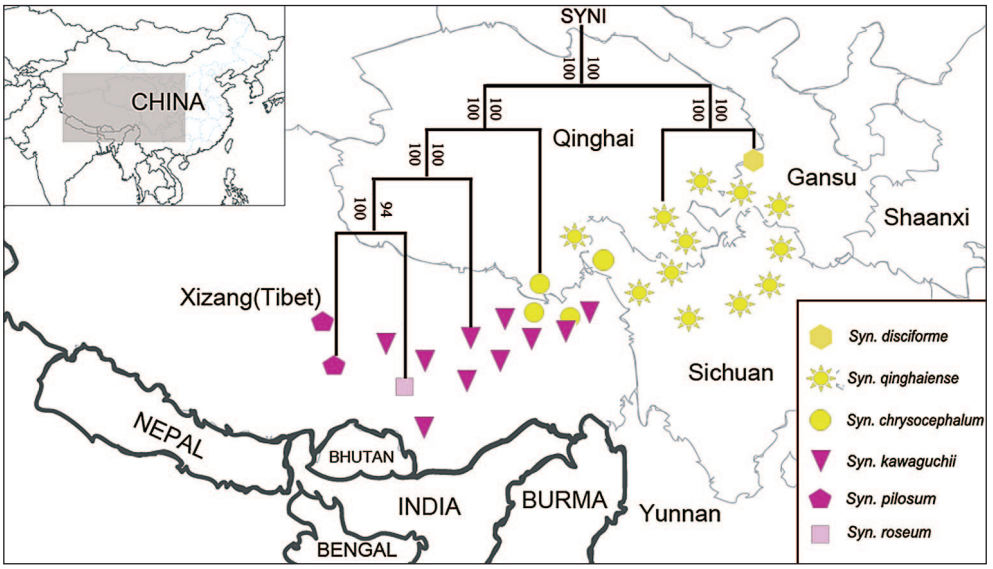

Fig. 6. Dispersal of species of *Syncalathium* (SYNI), excluding *Syn. souliei*. The tree was derived from the combined tree in Fig. 4; the color of each symbol represents floret color.

through Gansu, Qinghai and Sichuan to east and central Tibet, without overlap of species ranges (Wang & Zhang, 1994; Shih, 1997; Zhang, 2009). As shown in Fig. 6, species with five yellow florets (*Syn. qinghaiense*, *Syn. disciforme*, *Syn. chrysocephalum*) are found in the northeastern part of the Tibetan Plateau, while the purple and pink ones (i.e., *Syn. kawaguchii*, *Syn. pilosum*, *Syn. roseum*) occur in the middle and southern parts of the plateau. As described above, species with three purple and pink florets seem to be derived, which is also supported by cytological evidence. We therefore postulate a possible migration route from the northeast (Gansu, Sichuan and Qinghai provinces) to the south and center (Tibet) of the plateau (Fig. 6). This conclusion is consistent with one of the migration routes proposed by Sun (2002) and Shih & Chen (1982) for a number of Tibetan taxa of Asteraceae. Thus, it is feasible to hypothesize that the uplift of the Tibetan Plateau created a change of the climate associated with fragmentation of the scree habitats, resulting in evolution of the endemic *Syncalathium* s.str. through allopatric speciation in the Tibetan Plateau and the adjacent Himalayan region.

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Appendix. Taxa sampled, vouchers and their GenBank accession numbers (ITS/*trnL-F*/*psbA-trnH*). The accession numbers of the taxa for ITS analysis alone and calibration times are shown on the tree (Figs. 3, 5).

Genus, taxon, voucher (herbarium), origin, GenBank accession number for ITS/*trnL-F*/*psbA-trnH*; "–" indicates missing data.

***Soroseris*,** *Sor. erysimoides* (H.-M.) C. Shih, *J.W. Zhang 003* (KUN), China: Yunnan, Baimashan (BMS), HQ436212/HQ436146/HQ436179; *Sor. erysimoides* (H.-M.) C. Shih, *Boufford & al. 39816* (KUN, A), China: Sichuan, Xingu (XG), HQ436213/HQ436147/HQ436180; *Sor. gillii* (S. Moore) Stebbins, *J.W. Zhang 073* (KUN), China: Sichuan, HQ446097/–/–; *Sor. gillii* (S. Moore) Stebbins, *J.W. Zhang 075* (KUN), China: Sichuan, HQ436227/–/–; *Sor. gillii* (S. Moore) Stebbins, *J.W. Zhang 076* (KUN), China: Sichuan, Zheduoshan (ZDS), HQ436214/HQ436148/HQ436181; *Sor. glomerata* (Decne.) Stebbins, *J.W. Zhang 005* (KUN), China: Yunnan, Baimashan (BMS), HQ436216/HQ436150/HQ436183; *Sor. glomerata* (Decne.) Stebbins, *J.W. Zhang 042* (KUN), China: Yunnan, Hongshan (HS), HQ436215/HQ436149/HQ436182; *Sor. glomerata* (Decne.) Stebbins, *J.W. Zhang & Y. Yang 017* (KUN), China: Yunnan, Daxueshan (DXS), HQ436217/HQ436151/HQ436184; *Sor. hirsuta* (Anth.) C. Shih, *J.W. Zhang 008* (KUN), China: Yunnan, Hongshan (HS), HQ436218/HQ436152/HQ436185; *Sor. hirsuta* (Anth.) C. Shih, *J.W. Zhang & T.Y. Tu 138* (KUN), China: Tibet, Sejila (SJL), HQ436219/HQ436153/HQ436186; *Sor. hirsuta* (Anth.) C. Shih, *Boufford & al.* 41254 (KUN, A), China: Tibet, HQ436228/–/–; *Sor. hookeriana* (C.B. Clarke) Stebbins, *J.W. Zhang & T.Y. Tu 213* (KUN), China: Tibet, Qiumupu (QMP), HQ436220/HQ436154/HQ436187; *Sor. teres* C. Shih, *J.W. Zhang 027* (KUN), China: Tibet, Zelila (ZLL), HQ436221/HQ436155/HQ436188; ***Stebbinsia*,** *Ste. umbrella* (Franch.) Lipschitz, *J.W. Zhang & Y. Yang 001* (KUN), China: Yunnan, Zhonggashan (ZGS), HQ436197/HQ436131/HQ436164; *Ste. umbrella* (Franch.) Lipschitz, *J.W. Zhang & Y. Yang 041* (KUN), China: Yunnan, Hongshan (HS), HQ436198/HQ436132/HQ436165; ***Syncalathium*,** *Syn. chrysocephalum* (C. Shih) S.W. Liu, *Boufford & al. 31967* (KUN, A), China: Tibet, Machala (MCL), HQ436199/HQ436133/HQ436166; *Syn. disciforme* (Mattf.) Y. Ling, *J.W. Zhang & W.D. Zhu 07006*, China: Qinghai, Dalijia (DLJ), HQ436200/HQ436134/HQ436167; *Syn. kawaguchii* (Kitam.) Y. Ling, *J.W. Zhang & T.Y. Tu 129* (KUN), China: Tibet, Damala (DML), HQ436201/HQ436135/HQ436168; *Syn. pilosum* (Y. Ling) C. Shih, *J.W. Zhang & T.Y. Tu 220* (KUN), China: Tibet, Rendui (RD), HQ436202/HQ436136/HQ436169; *Syn. qinghaiense* (C. Shih) C. Shih, *Boufford & al. 34442* (KUN, A), China: Sichuan, Nianlong (NL), HQ436204/HQ436138/HQ436171; *Syn. qinghaiense* (C. Shih) C. Shih, *Boufford & al. 39446* (KUN, A), China: Sichuan, Mengbishan (MBS), HQ436203/HQ436137/HQ436170; *Syn. roseum* Y. Ling, *J.W. Zhang & T.Y. Tu 226* (KUN), China: Tibet, Sangye (SY), HQ436205/HQ436139/HQ436172; *Syn. souliei* (Franch.) Y. Ling, *J.W. Zhang 070* (KUN), China: Sichuan, Daofu (DF), HQ436208/HQ436142/HQ436175; *Syn. souliei* (Franch.) Y. Ling, *J.W. Zhang 072* (KUN), China: Sichuan, Jianziwan (JZW), HQ436211/HQ436145/HQ436178; *Syn. souliei* (Franch.) Y. Ling, *J.W. Zhang 074* (KUN), China: Sichuan, Gaoershi (GES), HQ436209/HQ436143/HQ436176; *Syn. souliei* (Franch.) Y. Ling, *J.W. Zhang & T.Y. Tu 098* (KUN), China: Tibet, Dongdala (DDL), HQ436207/HQ436141/HQ436174; *Syn. souliei* (Franch.) Y. Ling, *J.W. Zhang & Y. Yang 002* (KUN), China: Yunnan, Baimashan (BMS), HQ436206/HQ436140/HQ436173; *Syn. souliei* (Franch.) Y. Ling, *J.W. Zhang & Y. Yang 040* (KUN), China: Yunnan, Hongshan (HS), HQ436210/HQ436144/HQ436177; **Other genera:** *Chaetoseris grandiflora* (Franch.) C. Shih, *Boufford & al. 32888* (KUN, A), China: Sichuan, Yele (YL), HQ436190/HQ436124/HQ436157; *Chaetoseris grandiflora* (Franch.) C. Shih, *J.W. Zhang 393* (KUN), China: Yunnan, Cangshan (CS), HQ436191/HQ436125/HQ436158; *Chaetoseris roborowskii* (Maxim.) C. Shih, *Boufford & al. 33832* (KUN, A), China: Tibet, Luoxu (LX), HQ436192/HQ436126/HQ436159; *Chondrilla rouillieri* Kar. & Kir., *J.W. Zhang 341* (KUN), China: Xinjiang, HQ436223/–/–; *Dubyaea hispida* (D. Don) de Candolle, *Tibet-MacArthur 586* (KUN), China: Tibet, Sejila (SJL), HQ436193/HQ436127/HQ436160; *Heteracia szovitsii* Fisch. & C.A. Mey, *J.W. Zhang 302* (KUN), China: Xinjiang, Yaomoshan (YMS), HQ436194/HQ436128/HQ436161; *Hexinia polydichotoma* (Ostenf.) H.L. Yang, *J.W. Zhang 353* (KUN), China: Xinjiang, HQ436224/–/–; *Ixeris repens* (L.) A. Gray, *FOK 079860* (MBK), Japan: Shikoku, HQ436225/–/–; *Ixeris stolonifera* A. Gray, *J.W. Zhang 1024* (KUN), China: Yunnan, HQ436226/–/–; *Nabalus tatarinowii* (Maxim.) Nakai, *Boufford & al. 38449* (KUN, A), China: Sichuan, Muerzhai (MEZ), HQ436195/HQ436129/HQ436162; *Nabalus tatarinowii* (Maxim.) Nakai, *J.W. Zhang & W.D. Zhu 07001* (KUN), China: Sichuan, Tudiling (TDL), HQ436196/HQ436130/HQ436163; *Taraxacum mongolicum* Hand.-Mazz., *J.W. Zhang 392* (KUN), China: Yunnan, Kunming Botanical Garden (KBG), HQ436222/HQ436156/HQ436189; *Youngia japonica* (L.) DC., *J.W. Zhang 388* (KUN), China: Yunnan, HQ436229/–/–; *Cichorium intybus* L., AY504694/AY504776/FJ493262; *Lactuca indica* L., AY862579/GU109288/GU109320; *Lactuca sativa* L., L13957/AP007232/AP007232.