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Phylogenetic analyses of *Saussurea* sect. *Pseudoeriocoryne* (Asteraceae: Cardueae) based on chloroplast DNA *trn*L–F sequences

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Abstract

The genus *Saussurea* is distributed mainly in the temperate and subarctic regions of Eurasia and consists of about 300 species classified into six subgenera and 20 sections. Sect. *Pseudoeriocoryne* in the subgenus *Eriocoryne* comprises four species, and is delimited mainly by acaulescence and an inflorescence with congested capitula surrounded by a rosette of leaves. All of these species are endemic to the arid Qinghai–Tibet Plateau. Sequences from the chloroplast DNA *trn*L–F region were obtained for the four species in this section and 26 other species from four subgenera of *Saussurea* to resolve phylogenetic relationships among these species and to determine whether the shared characters that define sect. *Pseudoeriocoryne* are synapomorphic or were acquired by convergent evolution. The resulting phylogenies indicated that *Saussurea* sect. *Pseudoeriocoryne* as traditionally defined does not constitute a monophyletic group and that each of its species belongs to separate clades. Furthermore, none of these species showed a close relationship with the other species of subgenus *Eriocoryne*. Our results further indicated that none of the investigated subgenera are monophyletic, and that species from different subgenera clustered together. All these conclusions are provisional and their confirmation would require stronger phylogenetic support. Two possible explanations are suggested for low sequence divergence, poor resolution of internal clades and clustering of species with the rather distinct morphology of *Saussurea* detected in the present study. The first is rapid radiation and diversification triggered by fast habitat fragmentation due to the recent lifting of the Qinghai–Tibet Plateau and the Quaternary climate oscillations. This could have led to rapid morphological divergence while sequences diverged very little, and also caused the convergent acquisition of similar characteristics in unrelated lineages due to similar selection pressures. The second

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possible explanation is that both introgressive hybridization and reticulate evolution might have caused the transferring of cpDNA sequences between morphologically dissimilar species, thus leading to homogenization of sequences between lineages.

Keywords: *Saussurea*; Sect. *Pseudoeriocoryne*; *trn*L–F; Phylogeny; Rapid radiation; Hybridization

1. Introduction

Convergent evolution is defined as the evolution of similar morphological features in systematically distant taxa, which reflects a common adaptation to a specific type of environment (Rensch, 1959). A well-known example is the cactus-like morphology shared by desert species from unrelated families on different continents. Convergent adaptations among alpine plants have been studied for more than 50 years (Körner, 1999). Five major morphological traits among alpine species have been acquired convergently in many lineages, i.e. cushion form, acaulescence, dwarfism, tussocks and bracts (Ohba, 1988; Körner, 1999). Recently a sixth such trait, i.e. a strong downy indumentum, was recognised by Tsukaya and Tsuge (2001). This trait permits blooming at low temperatures, both for alpine plants and those species that begin to flower in early spring. These types of characteristics are of no systematic significance at or above the family level, because they are found in a variety of families with diverse floral characters and are clearly convergently acquired in many families. Within a genus, however, groups of species that share adaptive traits such as those mentioned above have often been considered to be closely related, and treated as a natural group sometimes recognised at the level of subgenus, section, subsection or series (e.g. *Salix* sect. *Lindleyanae* in Salicaceae, Liu, 1996; *Saxifraga* sect. *Ciliatae* subsect. *Rosulares* in Saxifragaceae, Pan, 1992). However, no attempt has been undertaken to reconstruct the phylogeny of such an entity and test its monophyly. Therefore, the possibility that an adaptation to alpine conditions may be acquired convergently within a single genus also remains untested.

Saussurea DC., comprising over 300 species, is one of the largest genera in the Asteraceae (Bremer, 1994). Both morphological and molecular analyses unanimously placed *Saussurea* in the tribe Cardueae (Bremer, 1994; Garcia-Jacas et al., 2002). *Saussurea* is distributed mainly in the temperate region, with its greatest concentration of species in the subarctic regions of Eurasia, especially the Qinghai–Tibet Plateau and its adjacent areas (Shi and Jin, 1999). The large size of the genus has hampered attempts to generate a satisfactory infrageneric classification of *Saussurea*, and consequently the evolutionary history of this genus remains poorly known. A second problem is the remarkable amount of morphological variation in habit, leaf shape and texture, indument and florescences, both between and within species of *Saussurea*. Moreover, parallel acquisition of morphological characters by species growing in similar arid habitats is presumed to be widespread in this genus as well as others in the Cardueae (Garcia-Jacas et al., 2002). For a highly speciose

genus like *Saussurea*, it is unrealistic to establish a molecular phylogeny including all species because of the difficulty of collecting materials of every species within a short time. One alternative is to subdivide the genus into several small monophyletic groups, and then eventually construct the phylogeny of the whole genus from representatives from each such group. However, many large genera have no modern worldwide monographs, and traditional taxonomic subdivisions often prove to be highly unnatural (Pelser et al., 2002). This makes prior selection of monophyletic subgroups impossible. Therefore, the best way for molecular study of these genera is to start from one small group and establish the systematic position of each species within an enlarged context. The representative of the monophylogenetic groups from such analyses will be further used to investigate the phylogeny in the total genus.

Saussurea sect. *Pseudoeriocoryne* comprises four species: *S. kingii* C.E.C. Fischer, *S. stella* Maxim., *S. thoroldii* Hemsl. and *S. thomsonii* C.B. Clarke (Lipschitz, 1979). This section was included in the subgenus *Eriocoryne* mainly based on the multiple and enlarged inflorescence at the stem top. Sect. *Pseudoeriocoryne* is delimited mainly by acaulescence and an inflorescence with congested capitula surrounded by a rosette of leaves, characters not found in sect. *Eriocoryne*. In addition, except for these four species, other members of sect. *Eriocoryne* have extremely dense trichomes on well-developed bracts, which are tightly packed around floral buds. The four species of sect. *Pseudoeriocoryne* occur almost exclusively in the Qinghai–Tibet Plateau, and display high levels of endemism (Shi and Jin, 1999). Because the monophyly of *Saussurea* has yet to be confirmed by molecular data, the possibility exists that species of sections such as *Pseudoeriocoryne* might be more closely related to other genera of the Cardueae than to other sections of *Saussurea*.

Molecular data, particularly DNA sequences, have greatly contributed to the understanding of the phylogeny, evolution and taxonomy of Asteraceae (Jansen and Kim, 1996). This is especially true for the problematic genera of the family, where morphological data are ambiguous or rendered misleading by parallel evolution (Francisco-Ortega et al., 1997, 2001; Kim et al., 1998; Liu et al., 2002; Panero et al., 1999; Karis et al., 2001; Park et al., 2001). In Asteraceae, the most commonly used DNA sequences for phylogenetic analyses are those from the nuclear ITS and chloroplast DNA *ndh*F and *trn*L–F regions. The *trn*L–F region usually comprises two non-coding sequences, i.e. the *trn*L intron and *trn*L/*trn*F intergenic spacers (Taberlet et al., 1991). Although relatively short at about 800 bp, the *trn*L–F region sequence has proven to be phylogenetically informative from the infrageneric to the tribal level in Asteraceae (Bayer and Starr, 1998; Bayer et al., 2000; Fernández et al., 2001; Liu et al., 2002).

Therefore, data from the *trn*L–F region are ideally suited to examine the monophyly of sect. *Pseudoeriocoryne*, and relationships of its species with other sections and subgenera of *Saussurea* and other genera in the Cardueae. Such a phylogenetic framework is essential to elucidate patterns of evolution in the shared characters of sect. *Pseudoeriocoryne*, i.e. acaulescence and an inflorescence with congested capitula surrounded by a rosette of leaves. It will indicate whether these characters

were convergently acquired, and provide initial insights into the infrageneric classification of *Saussurea*.

2. Materials and methods

2.1. Plant materials

The *trn*L–F sequences for 30 species of *Saussurea* were generated for the present study. All plant material for molecular examination was collected from the Qinghai–Tibet Plateau in 1999–2002. Morphological characateristics were recorded for each species collected, and the infraspecific variation of each character was examined in the field, during fieldwork. The sequences of one species each from 13 other genera in the Cardueae were downloaded from GenBank, having originally been generated by O'Hanlon and Peakall (2000) or Bayer and Starr (1998) (see Table 1 for accession numbers).

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was isolated from silica-dried leaves following the CTAB method of Doyle and Doyle (1987). A sample of about 50 mg of leaf material from each of the specimens was used.

The *trn*L–F regions of cpDNA were amplified with primers c and f (Taberlet et al., 1991). The PCR reaction was performed in a 25 μl volume, containing about 10–40 ng plant DNA, 50 mM Tris–HCI, 1.5 mM MgCl2, 250 μg/ml BSA, 0.5 mM dNTPs, 2 μM of each primer and 0.75 units of Tap DNA polymerase. Initial template denaturation was programmed at 94 °C for 3 min, and then followed by 32 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 90 s, with a final extension step of 72 °C for 7 min.

All double-stranded PCR products were purified using a CASpure PCR Purification Kit following the manufacturer's protocol (Casarray, Shanghai, China). The sequencing primers used for amplifying *trn*L–F were the same as those above for amplifying the sequences. The sequencing reactions were carried out in a Biometra thermocycler using a DYEnamic Dye Terminator Cycle Sequencing Kit (Amersham Biosciences Corporation) following the manufacturer's protocol. Sequencing products were separated and analyzed on a MegaBACE 500 DNA Analysis System. Both strands of DNA were sequenced through the use of forward and reverse primers. All sequences were recorded in both strands with an overlap of at least 80%.

2.3. Sequence alignment, boundary determination and data analysis

The *trn*L–F sequences were aligned using ClustalX (Thompson et al., 1997), and refined manually. The boundaries of these sequences were determined by comparison to the published sequences of other genera of Asteraceae downloaded from GenBank. We recovered a continuous sequence including the *trn*L intron, the *trn*L 3′ and 5′ exons (UAA), and the *trn*L/F intergenic spacer for all newly sequenced

Table 1
Origin of materials and GenBank accession numbers (the infrageneric classification according to Lipschitz (1979))

Taxa	Source	GenBank accession number
Saussurea DC.		
Subgen. *Eriocoryne* (DC.) Hook. f.		
Sect. *Pseudoeriocoryne* Lipsch.		
S. kingii C.E.C. Fischer	Duilongdeqing, Xizang, J.Q. Liu 1104	AY328091
S. stella Maxim.	Yushu, Qinghai, J.Q. Liu 856	AY328110
S. thomsonii C.B. Clarke	Wulan, Qinghai, S.G. Wu K-784	AY328120
S. thoroldii Hemsl.	Maduo, Qinghai, J.Q. Liu 813	AY328103
Sect. *Eriocoryne*		
S. depsangensis Pamp.	Chenduo, Qinghai, J.Q. Liu 839	AY328107
S. gnaphalodes (Royle) Sch.-Bip.	Chenduo, Qinghai, J.Q. Liu 837	AY328106
S. medusa Maxim.	Huangyuan, Qinghai, J.Q. Liu 800	AY328102
Subgen. *Amphilaena* (Stschegl.) Lipsch.		
Sect. *Amphilaena*		
S. bracteata Decne.	Maduo, Qinghai, J.Q. Liu 808	AY328134
S. globosa Chen	Jiangda, Xizang, J.Q. Liu 1267	AY328099
S. hookeri Clarke	Qusong, Xizang, J.Q. Liu 1255	AY328096
S. involucrata (Kar. et Kir.) Sch.- Bip.	Xinjiang, Gift	AY328115
S. obvallata (DC.) Edgew.	Jiangda, Xizang, J.Q. Liu 1265	AY328097
S. tangutica Maxim.	Chenduo, Qinghai, J.Q. Liu 834	AY328105
Sect. *Pseudoamphilaena* Lipsch.		
S. polycolea Hand.-Mazz.	Maduo, Qinghai, J.Q. Liu 824	AY328128
S. polycolea Hand.-Mazz. var. *acutisquama* (Ling) Lipsch.	Zhiduo, Qinghai, J.Q. Liu 931	AY328112
Subgen. *Saussurea*		
Sect. *Acaules* C.B. Clarke		
S. bella Ling	Chenduo, Qinghai, J.Q. Liu 852	AY328108
Sect. *Cyathidium* (Lindl. ex Royle) Ling		
S. ceterach Hand.-Mazz.	Jiacha, Xizang, J.Q. Liu 1159	AY328095
S. coriacea Y.L. Chen et S.Y. Liang	Nangqian, Qinghai, J.Q. Liu 1008	AY328093
S. polypodioides Anth.	Nangqian, Qinghai, J.Q. Liu 1017	AY328094
S. przewalskii Maxim.	Jiangda, Xizang, J.Q. Liu 1266	AY328098
S. rhytidocarpa Hand.-Mazz.	Qumalai, Qinghai, J.Q. Liu 972	AY328114
Sect. *Pycnocephala* Lipsch.		
S. brunneopilosa Hand.-Mazz.	Qumalai, Qinghai, J.Q. Liu 962	AY328113
S. tibetica C. Winkl.	Qumalai, Qinghai, J.Q. Liu 826	AY328116
Sect. *Saussurea*		
S. acuminata Turcz. ex Fisch. et Mey	Huzhu, Qinghai, J.Q. Liu 001	AY328127
S. hieracioides Hook. f.	Qusong, Xizang, J.Q. Liu 1150	AY328117
S. parviflora (Poir.) DC.	Huzhu, Qinghai, J.Q. Liu 006	AY328124
S. superba Anth.	Zhiduo, Qinghai, J.Q. Liu 929	AY328111
S. tatsienensis Franch.	Baiyu, Sichuan, J.Q. Liu 669	AY328118
S. umbrosa Kom.	Huzhu, Qinghai, J.Q. Liu 009	AY328125
Subgen. *Theodorea* (Cass.) Lipsch.		
Sect. *Theodorea*		
S. amara (L.) DC.	Huzhu, Qinghai, J.Q. Liu 002	AY328121

(continued on next page)

Table 1 (*continued*)

Taxa	Source	GenBank accession number
Arctium lappa L.	O'Hanlon and Peakall (2000)	AF129824
Carduus nutans L.	O'Hanlon and Peakall (2000)	AF129825
Cirsium vulgare (Savi) Ten.	O'Hanlon and Peakall (2000)	AF129826
Cousinia hystrix C.B. Clarke	O'Hanlon and Peakall (2000)	AF129827
Cynara humilis Linn.	O'Hanlon and Peakall (2000)	AF129829
Echinops exaltatus Schrad.	Bayer and Starr (1998)	U82030, U82031
Galactites tomentosa Moench.	O'Hanlon and Peakall (2000)	AF129831
Gerbera jamesonii Bolus ex Hook.	Bayer and Starr (1998)	U82036, U82037
Notobasis syriaca Cass.	O'Hanlon and Peakall (2000)	AF129832
Onopordum acanthium L.	O'Hanlon and Peakall (2000)	AF129833
Picnomon acarna (L.) Cass.	O'Hanlon and Peakall (2000)	AF129834
Ptilostemon afer (Jacq.) Greuter	O'Hanlon and Peakall (2000)	AF129835
Silybum marianum (L.) Gaertn.	O'Hanlon and Peakall (2000)	AF129836

species. However, the sequences of other genera in the Cardueae downloaded from Genbank (Table 1) comprised only the *trn*L intron, and the *trn*L/F intergenic spacer. Therefore, in the final *trn*L–F matrix used for phylogenetic analysis, the *trn*L 3′ and 5′ exons (UAA), comprising 49 bp, were trimmed off. These short exons showed no variation within our sampled species and would, therefore, not have contributed any data to the analysis.

Phylogenetic analyses were performed by using PAUP* 4.0b (Swofford, 2000) with all characters unweighted. Heuristic parsimony searches were conducted with 100 replicates of random addition of sequences, in combination with ACCTRAN character optimization and MULPARS + TBR branch-swapping and STEEPEST DESCENT options on to search for multiple islands of most parsimonious trees (Maddison, 1991). Because all gaps with more than 1 bp, which result from one insertion/deletion, will be weighted in one mutation site in the parsimony analysis, we treated all insertions or deletions as missing and rechecked the gap phylogeny information on the most parsimonious tree.

Bootstrap analyses (Felsenstein, 1985) were performed to assess the relative support for monophyletic groups. Bootstrap values were calculated from 1000 replicates by using a heuristic search with 10 replicates random addition with the TBR and MULPARS options on. We also constructed a phylogenetic tree for the data using the neighbor-joining method under Kimura's two-parameter model in both PAUP* 4.0b and MEGA 2.0 (Kumar et al., 2001).

3. Results

The aligned *trn*L–F region data set of 43 taxa consisted of 838 positions, and included 71 variable characters, of which 17 were phylogenetically informative,

when gaps were excluded. The greatest pairwise distance within *Saussurea* was 1.808% (*S. kingii* vs. *S. thomsonii*), but distances of 0.00% were found among all members of each of five groups of species. Members of each group thus had identical sequences to one another, except for some gap differences. These groups were: first, *S. thoroldii*, *S. amara*, *S. acuminata*, *S. umbrosa* and *S. parviflora*; second, *S. stella*, *S. rhytidocarpa*, *S. ceterach*, *S. polypodioides* and *S. coriacea*; third, *S. polycolea* var. *acutisqauma*, *S. superba*, *S. hieracioides* and *S. tatsienensis*; fourth, *S. hookeri*, *S. obvallata*, *S. globosa*, *S. tangutica* and *S. tibetica*; and fifth, *S. polycolea*, *S. depsangensis*, *S. bracteata* and *S. brunneopilosa*. The most distant pairwise distance between *Saussrea* and other genera in the Cardueae is 2.459% (*S. thomsonii* vs. *Echinops exaltatus*) among the aligned 838 sites.

Among sect. *Pseudoeriocoryne*, two species, *S. thoroldii* and *S. stella*, are members of the first and second species groups, respectively, whereas the remaining two species, *S. kingii* and *S. thomsonii*, are the two with the greatest pairwise distance from one another in *Saussaurea*.

Parsimony analysis produced seven most parsimonious trees in one island with 73 steps, all with a consistency index of 0.986 and a retention index of 0.982. One of the trees is depicted as Fig. 1. Monophyly of *Saussurea* sect. *Pseudoeriocoryne* was not supported in any of the seven most parsimonious trees. The neighbor-joining tree (Fig. 2) under Kimura's two-parameter model corresponded well with the parsimony trees in the composition and positions of the major clades, although the exact position of some species differed between the two analyses.

The data matrix included three 1 bp insertions, none of which had phylogenetic significance. One large (18 bp) insertion with the sequence "TATCGAAACTTCATAAAA" in the *trn*L intron was identified for *S. superba*, *S. polycolea* var. *acutisquama*, *S. hieracioides* and *S. tatsienensis*. This insertion unanimously supports their grouping in Fig. 1. Three more deletions ranging from 2 to 4 bp in the *trn*L intron were identified for *Carduus nutans*, *Cirsium vulgare* and *Silybum marianum*. In the *trn*L/F intergenic spacer, *S. thomsonii* was found to have a long (44 bp) deletion with sequence "CTTATCACATGTGATATATATGATACATGTACAAATGAACATCT". Such long deletions are not common in the Asteraceae (Bayer and Starr, 1998). This deletion further supported the isolated position of *S. thomsonii* from the other species of *Saussurea*. *Carduus nutans*, *Cirsium vulgare* and *Silybum marianum* were found to share a 4 bp deletion. Several other deletions from 1 to 9 bp were found for single species, i.e. *Arctium lappa*, *Carduus nutans*, *Echinops exaltatus*, *Gerbera jamesonii*, *Notobasis syriaca* and *Ptilostemon afer*.

4. Discussion

4.1. Systematic implication of trnL–F sequence analyses

A tentative conclusion emerging from the analyses of *trn*L–F sequence data is that *Saussurea* sect. *Pseudoeriocoryne*, as defined by Lipschitz (1979), does not constitute a monophyletic group. The four species that comprise this section share the

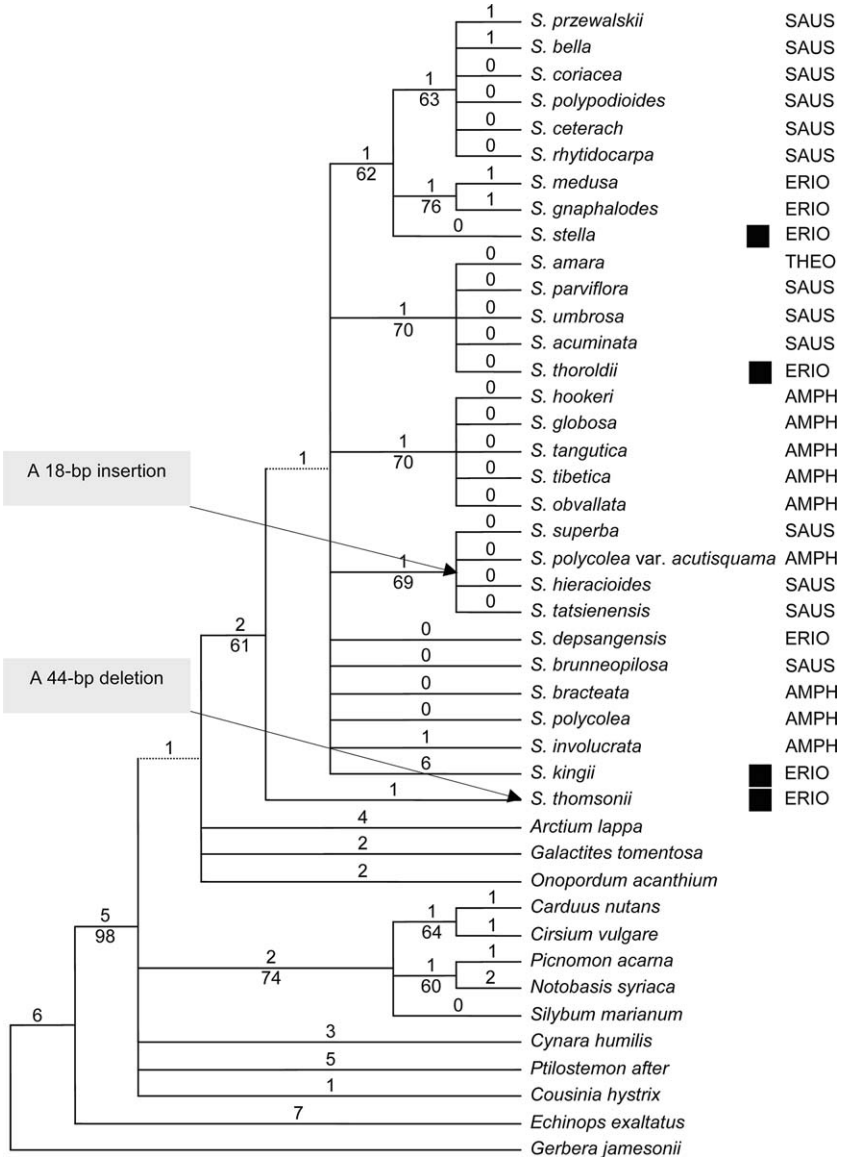

Fig. 1. One of seven equally parsimonious trees (length = 73 steps; CI = 0.986; RI = 0.982) from analysis of *trn*L–F sequence data of sect. *Pseudoeriocoryne* and related species in *Saussurea* and genera in the Cardueae. ERIO, AMPH, SAUS and THEO following each species name are abbreviations of *Saussurea* subgen. *Eriocoryne*, subgen. *Amphilaena*, subgen. *Saussurea* and subgen. *Theodorea*. Numerals above the branches indicate branch length and those below, bootstrap support. Dashed lines denote branches that collapse in the strict consensus tree. Solid bars indicate convergent evolution of the defining characters of sect. *Pseudoeriocoryne*, i.e. acaulescence and an inflorescence with congested capitula surrounded by a rosette of leaves, characters not found in sect. *Eriocoryne*.

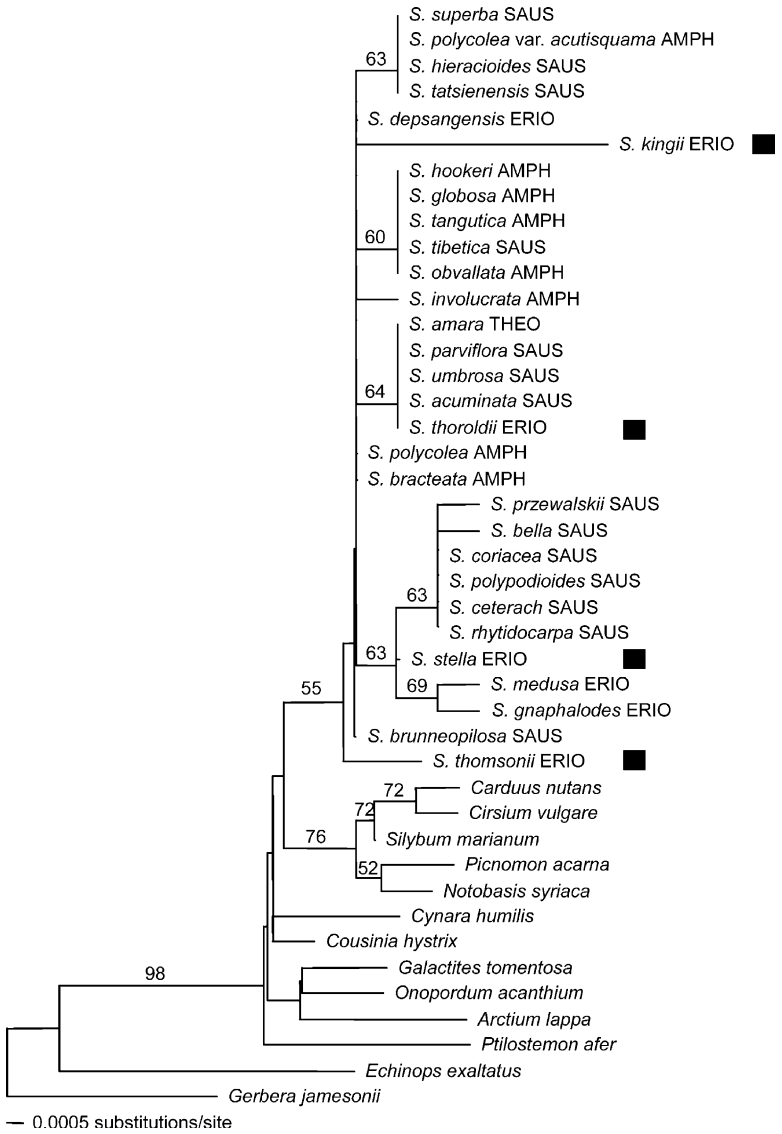

Fig. 2. Neighbor-joining phylogeny under Kimura's two-parameter model, from analysis of *trn*L–F sequence data of sect. *Pseudoeriocoryne* and related species in *Saussurea* and genera in the Cardueae. Numerals above the branches indicate bootstrap support. The remaining explanations are the same as in Fig. 1.

characters of acaulescence and an inflorescence with congested capitula surrounded by a rosette of leaves. However, these four species showed no relationship with one another, and two grouped with other species that lacked these characters while the other two each formed separate clades of their own.

S. stella shared an identical sequence with *S. bella*, *S. ceterach*, *S. coriacea*, *S. globosa*, *S. polypodioides* and *S. rhytidocarpa*. This clade is sister to another comprising *S. medusa* and *S. gnaphalodes*. The second species of sect. *Pseudoeriocoryne*, *S. thoroldii*, has an identical sequence to *S. amara*, *S. acuminata*, *S. parviflora*, and *S. umbrosa*. Though these species form a monophyletic clade in the molecular analysis, no linking morphological character could be found for these five species despite careful inspection of specimens. The third species, *S. kingii*, stands as a separate clade with a long branch. The last species of sect. *Pseudoeriocoryne*, *S. thomsonii*, also has an isolated position in the parsimony analyses (Fig. 1). A unique 44 bp deletion detected in *S. thomsonii* further supported the isolated position for this species in *Saussurea*. All four of the species that comprise sect. *Pseudoeriocoryne* thus have origins separate from one another, some being relatively basal in the phylogeny tree while others occur at the tips among more recently evolved species. Therefore, the morphological characters that are shared by sect. *Pseudoeriocoryne* seem to have evolved independently.

In a recent monograph, Lipschitz (1979) classified *Saussurea* into six subgenera: *Jurinea* (Baill.) Lipsch., *Eriocoryne* (DC.) Hook. f., *Amphilaena* (Stschegl.) Lipsch., *Theodorea* (Cass.) Lipsch., *Frolovia* (DC.) Lipsch. and *Saussurea*. In this study, we sampled representative species from nine sections from four subgenera. The results indicate that none of the subgenera are monophyletic or well circumscribed (Figs. 1 and 2). The three species examined from subgenus *Eriocoryne* sect. *Eriocoryne*, i.e. *S. medusa*, *S. gnaphalodes* and *S. depsangensis*, did not constitute a monophyletic group. The seven species of subgenus *Amphilaena* sampled in the present study were separated among four different clades (Fig. 1).

4.2. What could account for the low sequence divergence, poor resolution of internal clades and clustering of morphologically dissimilar species in Saussurea?

Despite the wide morphological range within *Saussurea*, *trn*L–F sequence divergence was generally very low, ranging from 0.00% in many cases to 1.808% in one case; five groups of species were identified that had identical sequences in each case. Among the few mutations identified, most were autapomorphic in single species, whereas few were synapomorphic and phylogenetically informative. *S. kingii* had accumulated considerably more autapomorphic mutations than any other species, which might result from its biennial habit, as molecular sequences might evolve faster in annual or biennial species than perennials (Gaut et al., 1992, 1993; Wilson et al., 1990).

Both the parsimonious trees and distance trees (Figs. 1 and 2) have rather short internal branches, in contrast with the long terminal branches, which resulted in the poor resolution of the trees. Both trees had short branch lengths between the most recent common ancestor node where diversification began and the branch tips. Because our samples encompassed diverse morphological range in *Saussurea* from nine sections of four subgenera, such a tree topology suggested a recent diversification of *Saussurea* and rapid radiation (Richardson et al., 2001). Similar patterns have been reported for island archipelago biomes, such as *Arygyranthemum*

in Macaronesia (Francisco-Ortega et al., 1997) and silverswords (Baldwin and Sanderson, 1998). The limited sequence variations recovered in these examples are considered indicative of rapid radiation, often hypothesized to have been driven by low levels of competition in newly occupied habitats (Liem, 1990). However, the recent diversity and radiation of *Inga*, a tropical continental tree genus, was suggested to be correlated with the recent major uplifting of the Andes, the bridging of the Isthmus of Panama, and Quaternary climate oscillation (Richardson et al., 2001). The recent uplifting of the Qinghai–Tibet Plateau, and habitat fragmentation during the Quaternary, provided an ideal scenario for rapid radiation and diversification in *Saussurea*.

More than two-thirds of all *Saussurea* species occur in the Qinghai–Tibet Plateau and other parts of Central Asia, indicating that this area might also be the centre of origin of the genus (Lipschitz, 1979). The habitats preferred by most *Saussurea* species are cold and dry alpine meadow, steppe desert and dry slopes. However, geological reconstructions indicate that these arid habitats formed recently during the Pliocene period, as a consequence of the uplifting of the Qinghai–Tibet Plateau (Shi et al., 1998). The first large-scale uplifting of the Qinghai–Tibet Plateau occurred about 3.4 Mya, which was accompanied by the largest glaciers in the Northern Hemisphere (Li et al., 1995). After that, the plateau strongly uplifted again about 2.5 Mya. At this stage, the effects of the greatest Ice Age were felt throughout the Northern Hemisphere and glaciers developed in the major mountain chains of the Qinghai–Tibet area, which continued to exist from the late Pliocene until the Holocene (Fang et al., 1995). The third phase of uplifting occurred about 1.6 Mya. This event led to a colder, drier climate and the formation of the modern river systems in the plateau, and created a much drier climate in Central Asia (Shi et al., 1998). In addition, in response to the global climate oscillations that occurred between the late Pliocene and the Holocene, the vegetation of the Qinghai–Tibet Plateau shifted alternatively between desert-steppes and forests (Tang and Shen, 1996). The rich geological and ecological diversity of the plateau and adjacent areas of Central Asia, together with habitat isolation due to changing climatic conditions during and after the uplift of the plateau, might well have promoted rapid speciation and radiation of *Saussurea* in small, isolated populations. Such a rapid star speciation could have resulted in small numbers of synapomorphic nucleotide substitutions, despite an accumulation of autapomorphic mutations in some long-isolated species, as was found in our data.

As they adapted to the stable aridity of the Qinghai–Tibet Plateau and adjacent areas after the glaciations, species from different lineages might have evolved similar morphology due to similar selection pressures. This would account for the convergent morphology of the species of polyphyletic sections such as *Pseudoeriocoryne*. In fact, the morphological characters that define some of these sections are found in diverse families of alpine plants, and were demonstrated to be of great adaptative value (Ohba, 1988; Körner, 1999). For example, the pubescent bracts that were used to circumscribe sect. *Eriocoryne* are prevalent among alpine species of many genera, and were found to enhance warming, providing a strategy for alpine plants to withstand cold weather during the flowering season (Tsukaya

and Tsuge, 2001). The colorful bracts shared by the members of sect. *Amphilaena* were also found in members of other families, such as *Rheum nobile* of the Polygonaceae. These bracts were revealed to have a similar warming effect as well as protect the reproductive organs from being damaged by UV-B radiation due to the high altitude of the plateau (Terashima et al., 1993; Omori and Ohba, 1996; Omori et al., 2000).

A possible alternative cause of low sequence divergence is cytoplasmic gene flow and chloroplast capture due to ancient or recent hybridization. Ancient introgression could cause homogenization of cpDNA types (Hollingsworth et al., 1999), leading to the low *trn*L–F divergence found here. The identical *trn*L–F region sequences of *S. thoroldii* and four other species and of *S. stella* and five other species might be due to recent hybridization. Our ongoing nuclear ITS DNA fragment analysis places some species with identical *trn*L–F sequence on separate branches. However, as with *trn*L–F sequences, nuclear DNA sequences could not resolve the internal clades of the phylogeny in spite of higher divergence values between species. Most mutations found in ITS sequences were autapomorphic and were not phylogenetically informative (Wang and Liu, unpublished data). In addition, the situation has not been altered by the addition of additional species from our ongoing work, which is expanding sampling of *Saussurea*'s morphological range (including all subgenera) and distribution (out of the Qinghai–Tibet Plateau) for both *trn*L–F and ITS DNA sequences (Wang and Liu, unpublished data). We suggest that both star radiation due to rapid habitat fragmentation and introgression after the rejoining of fragmented habitats might have contributed to the diversity and speciation of *Saussurea*.

Our molecular examinations of other genera, which also have a centre of diversity in the Qinghai–Tibet Plateau, revealed patterns similar to that reported here for *Saussurea*, in *Androsace* (Primulaceae) (Wang et al., in press), *Rheum* (Polygonaceae) (unpublished), *Ligularia* (Asteraceae) (unpublished) and *Cremanthodium* (Asteraceae) (unpublished). Therefore, recent diversification and radiation seems to be a common pattern for those temperate taxa with the greatest diversity of species in the Qinghai–Tibet Plateau and its adjacent areas. For a better understanding and construction of phylogeny in these genera, a more discerning molecular marker will be required. Techniques such as AFLP and RAPD might reveal appropriate levels of variation, but would be ill-suited to phylogeny reconstruction in these groups because they cannot discriminate events such as introgression of germplasms between lineages, and reticulate speciation. Low copy nuclear genes with a fast mutation rate might be more promising for this purpose (Ferguson and Sang, 2001).

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