

## A new species of *Saussurea* (Asteraceae) from Tibet and its systematic position based on ITS sequence analysis

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A new species of *Saussurea*, *S. erecta* S. W. Liu, J. T. Pan & J. Q. Liu **sp. nov.**, is described from Tibet. It resembles *S. kingii* but may be distinguished by having distinct stems and glabrous achenes. *Saussurea kingii* was placed in sect. *Pseudoeriacoryne* of subgen. *Eriocoryne*; this section was circumscribed by acaulescence and an inflorescence with congested capitula surrounded by a rosette of leaves. The discovery of *S. erecta* with distinct stems, cauline leaves and corymbose capitula blurred the delimitation of sect. *Pseudoeriacoryne* and suggested that the section may be polyphyletic. Both the close relationship and the significant difference between *S. erecta* and *S. kingii* were confirmed by analyses of nrDNA ITS sequences. The resulting phylogenies based on ITS data further suggest that *Saussurea* sect. *Pseudoeriacoryne*, as traditionally defined, does not constitute a monophyletic group. The rapid radiation and speciation of *Saussurea* in the Qinghai-Tibetan Plateau, as inferred from ITS phylogeny, are discussed. © 2005 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2005, 147, 349–356.

ADDITIONAL KEYWORDS: adaptation – convergent evolution – radiation.

### INTRODUCTION

*Saussurea* DC., one of the largest genera in the Asteraceae with over 300 species (Bremer, 1994), is mainly distributed in temperate regions, the greatest concentration of species being found in subarctic regions of Eurasia (Lipschitz, 1979), especially the Qinghai-Tibetan Plateau (Shi & Jin, 1999). Both morphological and molecular evidence unequivocally place *Saussurea* in the tribe Cardueae Cass. (Bremer, 1994; Garcia-Jacas *et al.*, 2002), but the large size of this genus has deterred attempts to generate a satisfactory infrageneric classification and therefore, the evolutionary history of this genus remains poorly known. A recent infrageneric revision of *Saussurea* provided by Lipschitz (1979) was based exclusively on gross morphology. In this revision, more than 20 sections under six subgenera were acknowledged or established. However, the monophyly of these infrageneric entities has never been tested, especially by molecular methods, because the morphological characters used to circumscribe them in some subgenera and sections seem

likely to have been subject to convergent adaptation. Section *Pseudoeriacoryne* is one such entity, with four species exclusively distributed in the arid Qinghai-Tibetan Plateau: *Saussurea kingii* C. E. C. Fisch., *S. stella* Maxim., *S. thordii* Hemsl. and *S. thomsonii* C. B. Clarke. This section was included in the subgenus *Eriocoryne*, mainly based on its multiple and enlarged inflorescence at the top of the stem. Section *Pseudoeriacoryne* 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, members of sect. *Eriocoryne* have extremely dense trichomes on well developed bracts, which are tightly packed around floral buds. However, acaulescence and congested capitula within a rosette of leaves are found in diverse families and have been shown to be convergent adaptations shared by many alpine plants (Ohba, 1988; Körner, 1999).

During field work for a project on *Saussurea* in 2002, a puzzling specimen was discovered in Tibet that is difficult to ascribe to any extant species or even to a section or subgenus of *Saussurea* according to the key to the infrageneric classification provided by Lip-

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schitz (1979). This suggests that it might represent a new species. The specimen resembles *S. kingii* in being a biennial herb and in having involucre bracts with foliaceous appendages, tomentose stem and leaves, naked receptacle and rugose achenes, which are not found in other species of *Saussurea*. However, the achenes in this specimen are glabrous and it has a distinct stem, cauline leaves, and numerous and corymbose capitula, which conflict with the circumscriptions of both sect. *Pseudoeriocoryne* and subgen. *Eriocoryne*. Further tests are clearly required to determine whether this specimen represents a new species that is closely related to *S. kingii*, or whether it represents a separate lineage in another subgenus. In addition, the discovery of this specimen sheds further doubt on the monophyly of sect. *Pseudoeriocoryne*.

Although relatively short at about 500 bp, the internal transcribed spacers (ITS) of nuclear ribosomal DNA, rapidly evolving regions, have proven phylogenetically informative for investigating the relationships within closed species and the phylogeny of genera (Liu *et al.*, 2002; Bateman *et al.*, 2003; Meerow, Lehmler & Clayton, 2003). In order to evaluate whether this puzzling specimen represents a new *Saussurea* species, and its relationships with other *Saussurea* species, we analysed and compared ITS regions of this specimen, all four species in sect. *Pseudoeriocoryne* and other representative species in *Saussurea*. The analysis was also designed to examine the putative monophyly of sect. *Pseudoeriocoryne*, and relationships of each species in this section with other sections and subgenera of *Saussurea*.

## MATERIAL AND METHODS

Specimens of the new species were first identified using *Flora Reipublicae Popularis Sinicae* (Shi & Jin, 1999), *Qinghai Flora* (Liu, 1996) and *Flora of Tibet* (Liu, Chen & Liang, 1985). All *Saussurea* specimens from Tibet deposited in the herbarium, Northwest Plateau Institute of Biology, the Chinese Academy of Sciences (HNWP) were carefully examined and compared.

The species and origins of the material used for ITS sequencing are listed in Table 1. Total genomic DNA was isolated from silica-dried leaves following the CTAB method of Doyle & Doyle (1987). The ITS regions of nrDNA were amplified with primers 1 and 4 (White *et al.*, 1990). PCR products were purified using a CASpure PCR Purification Kit following the protocol recommended by the manufacturer (Casarray). The sequencing primers were the same as those used for amplifying the sequences. The sequencing reactions were carried out in a Biometra thermocycler using a DYEnamic Dye Terminator Cycle Sequencing Kit (Amersham Biosciences Corp.) following the manufac-

turer's suggested protocol. Sequencing products were separated and analysed on a MegaBACE 500 DNA Analysis System. Both strands of DNA were sequenced through the use of forward and reverse primers.

The ITS sequence alignments were made with Clustal X (Thompson *et al.*, 1997) and refined manually. All boundaries of the sequences were delimited by comparing them with the published sequences of other genera of Asteraceae downloaded from GenBank. We obtained a continuous sequence including ITS1, 5.8S and ITS2 for all newly sequenced species. However, as the sequences from GenBank are mostly devoid of 5.8S, the 5.8S region was excluded in the final ITS matrix. The variations of sampled species within this region are discussed separately. The ITS sequences for the 20 *Saussurea* species reported here have not been recorded before. The sequences of the other genera in the Cardueae, mainly provided by Susanna *et al.* (1995) and Garcia-Jacas *et al.* (2002), were downloaded from GenBank. All the taxa used in the analyses and their GenBank accession numbers are listed in Table 1.

Phylogenetic analyses were performed by PAUP\* 4.0 version b10 (Swofford, 2003) 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 enabled, to search for multiple islands of most parsimonious trees (Maddison, 1991). Bootstrap analyses (Felsenstein, 1985) to assess the relative support for monophyletic groups were calculated from 1000 replicates using a heuristic search with ten random-addition subreplicates, TBR branch swapping and MULPARS in effect.

## RESULTS

A continuous sequence covering ITS1, 5.8S and ITS2 was obtained for all *Saussurea* species. In the 5.8S region, only one and two mutations were found for our new species, *S. erecta*, and *S. stella*, respectively. Therefore, the sequences of this region were trimmed off in the final ITS matrix. The pairwise distances of the investigated species in *Saussurea* are listed in Table 2. The mean pairwise distances within *Saussurea* varied from 1.1% between *S. globosa* and *S. hookeri* to 15.2% between *S. erecta* and *S. amara*. The pairwise distance between *S. erecta* and *S. kingii* is 3.7%. The aligned data sets for 24 taxa of ITS1 and ITS2 combined consisted of 485 positions and included 124 informative characters when gaps were treated as missing. Parsimony analysis produced 16 most parsimonious trees in one island with 423 steps, a consistency index of 0.662, and a retention index of 0.537.

**Table 1.** Origin of material and GenBank accession numbers

Taxon	Origin	Collector	GenBank accession no.
<i>Saussurea</i> DC.			
<i>S. erecta</i> S. W. Liu, J. T. Pan & J. Q. Liu	Sangri, Xizang	J. Q. Liu 1135	AY466430
Subgen. <i>Eriocoryne</i> (DC.) Hook. f.			
Sect. <i>Pseudoeriacoryne</i> Lipsch.			
<i>S. kingii</i> C. E. C. Fisch.	Duilongdeqing, Xizang	J. Q. Liu 1104	AY366311
<i>S. stella</i> Maxim.	Yushu, Qinghai	J. Q. Liu 856	AY366314
<i>S. thomsonii</i> C. B. Clarke	Wulan, Qinghai	S. G. Wu K-784	AY366333
<i>S. thoroldii</i> Hemsl.	Maduo, Qinghai	J. Q. Liu 813	AY366323
Sect. <i>Eriocoryne</i>			
<i>S. depsangensis</i> Pamp.	Chenduo, Qinghai	J. Q. Liu 839	AY366325
<i>S. gnaphalodes</i> (Royle) Sch.-Bip.	Chenduo, Qinghai	J. Q. Liu 837	AY366324
<i>S. medusa</i> Maxim.	Huangyuan, Qinghai	J. Q. Liu 800	AY366321
Subgen. <i>Amphilaena</i> (Stschegl.) Lipsch.			
<i>S. globosa</i> Chen	Jiangda, Xizang	J. Q. Liu 1267	AY366320
<i>S. hookeri</i> C. B. Clarke	Dingqing, Xizang	J. Q. Liu 1255	AY366319
<i>S. obvallata</i> (DC.) Edgew.	Jiangda, Xizang	J. Q. Liu 1265	AY366312
Subgen. <i>Frolovia</i> (DC.) Lipsch.			
<i>S. qinghaiensis</i> S. W. Liu & T. N. Ho	Yushu, Qinghai	Z. D. Wei 22470	AY366350
Subgen. <i>Saussurea</i>			
<i>S. bella</i> Ling	Chenduo, Qinghai	J. Q. Liu 852	AY366326
<i>S. coriacea</i> Y. L. Chen & S. Y. Liang	Nangqian, Qinghai	J. Q. Liu 1008	AY366316
<i>S. katochaete</i> Maxim.	Zhiduo, Qinghai	J. Q. Liu 935	AY366308
<i>S. subulata</i> C. B. Clarke	Maduo, Qinghai	J. Q. Liu 814	AY366348
<i>S. superba</i> Anth.	Qumalai, Qinghai	J. Q. Liu 952	AY366336
<i>S. tatsienensis</i> Franch.	Baiyu, Sichuan	J. Q. Liu 669	AY366306
<i>S. umbrosa</i> Kom.	Huzhu, Qinghai	J. Q. Liu 009	AY366332
Subgen. <i>Theodorea</i> (Cass.) Lipsch.			
<i>S. amara</i> (L.) DC.	Huzhu, Qinghai	J. Q. Liu 002	AY366330
<i>Arctium minus</i> Bernh.	Garcia-Jacas <i>et al.</i> (2002)		AF319049, AF319103
<i>Cirsium arvense</i> (L.) Scop.	Susanna <i>et al.</i> (1995)		L358667
<i>Jurinea humilis</i> DC.	Susanna <i>et al.</i> (1995)		L35868
<i>Onopordum leptolepis</i> DC.	Garcia-Jacas <i>et al.</i> (2002)		AF319086, AF319140

One of these trees is depicted in Figure 1. The informative gaps include 1 bp and 3 bp indels for *S. erecta* and *S. kingii*, 1 bp indels for *S. superba* and *S. amara*, and 1 bp indels for *S. globosa*, *S. obvallata*, *S. katochaete*, *S. depsangensis* and *S. thomsonii*.

## DISCUSSION

The ITS distance, 3.7%, between *S. erecta* and *S. kingii* is larger than that between morphologically distinct species in *Saussurea* (Table 2). In addition, a transition from 'C' to 'T' in the conserved 5.8S region was found for *S. erecta*. Such genetic divergence supports their treatment as two separate species. However, parsimony analysis of ITS data still revealed a strong relationship between them, with 100% bootstrap support. In addition, they share two informative indels (1 and 3 bp) that are absent in the remaining

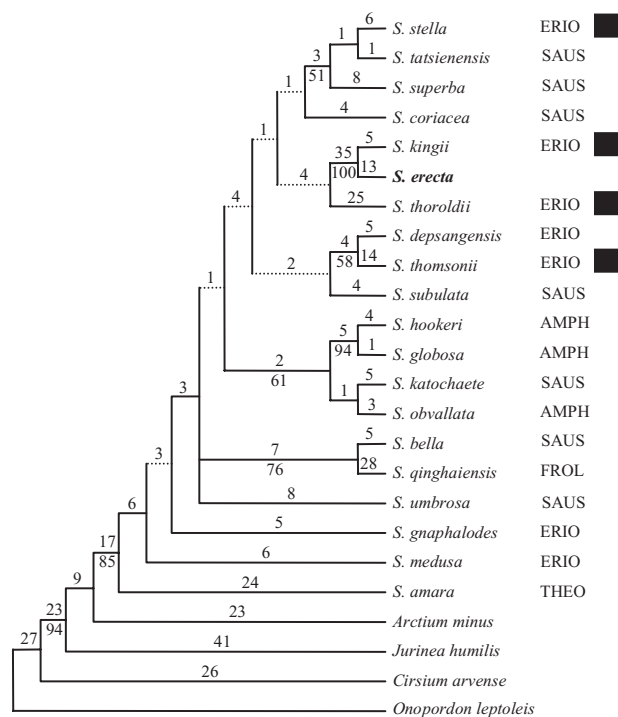
*Saussurea* species. As we noted in the introduction, the close relationship of these two species is further supported by the fact that they are both biennial herbs sharing the following morphological characters: involucre bracts with foliaceous appendages, tomentose stem and leaves, naked receptacle and rugose achenes. This morphological combination was found in none of the other *Saussurea* species distributed in the Qinghai-Tibetan area.

The relationship of the clade comprising *S. erecta* and *S. kingii* to another species of sect. *Pseudoeriacoryne*, *S. thoroldii*, received poor support in both the consensus tree and bootstrap analyses of the ITS data matrix (Fig. 1). A third species of sect. *Pseudoeriacoryne*, *S. stella*, comprised a clade with *S. tatsienensis* and *S. superba*, two species from another subgenus, subgen. *Saussurea*, with a bootstrap support of 51%. The last species of sect. *Pseudoeriacoryne*,

**Table 2.** The pairwise distances of ITS sequences from 20 species of *Saussurea*. Numbers 1–20 are, respectively, 1. *S. kingii*, 2. *S. erecta*, 3. *S. superba*, 4. *S. stella*, 5. *S. tatsienensis*, 6. *S. cortacea*, 7. *S. depsangensis*, 8. *S. thomsonii*, 9. *S. subulata*, 10. *S. thoroldii*, 11. *S. hookeri*, 12. *S. globosa*, 13. *S. katochaete*, 14. *S. obvallata*, 15. *S. umbrosa*, 16. *S. medusa*, 17. *S. gnaphalodes*, 18. *S. bella*, 19. *S. qinghaiensis*, 20. *S. amara*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2	0.037																			
3	0.101	0.125																		
4	0.088	0.103	0.033																	
5	0.088	0.108	0.022	0.015																
6	0.092	0.116	0.031	0.029	0.018															
7	0.097	0.125	0.042	0.042	0.026	0.031														
8	0.119	0.138	0.055	0.057	0.042	0.046	0.040													
9	0.097	0.121	0.040	0.037	0.022	0.026	0.026	0.026	0.042											
10	0.125	0.143	0.077	0.073	0.064	0.068	0.077	0.095	0.068											
11	0.099	0.116	0.051	0.044	0.033	0.037	0.042	0.055	0.037	0.075										
12	0.092	0.114	0.046	0.040	0.029	0.033	0.037	0.055	0.033	0.075	0.011									
13	0.097	0.114	0.044	0.037	0.026	0.026	0.026	0.040	0.053	0.035	0.077	0.024	0.024							
14	0.092	0.114	0.040	0.033	0.018	0.026	0.031	0.031	0.044	0.026	0.073	0.024	0.020	0.018						
15	0.097	0.116	0.048	0.042	0.029	0.035	0.037	0.053	0.033	0.033	0.073	0.037	0.033	0.035	0.024					
16	0.101	0.125	0.055	0.048	0.037	0.042	0.046	0.062	0.037	0.037	0.075	0.037	0.037	0.042	0.033	0.037				
17	0.090	0.110	0.051	0.044	0.033	0.037	0.042	0.062	0.037	0.037	0.070	0.035	0.035	0.033	0.029	0.033	0.026			
18	0.101	0.114	0.057	0.042	0.035	0.040	0.044	0.064	0.040	0.040	0.086	0.046	0.042	0.044	0.031	0.037	0.042	0.040		
19	0.134	0.149	0.101	0.092	0.086	0.090	0.092	0.112	0.090	0.127	0.084	0.088	0.088	0.086	0.081	0.086	0.088	0.075	0.068	
20	0.136	0.152	0.088	0.084	0.077	0.075	0.084	0.099	0.075	0.116	0.079	0.079	0.079	0.073	0.075	0.077	0.075	0.075	0.079	0.121





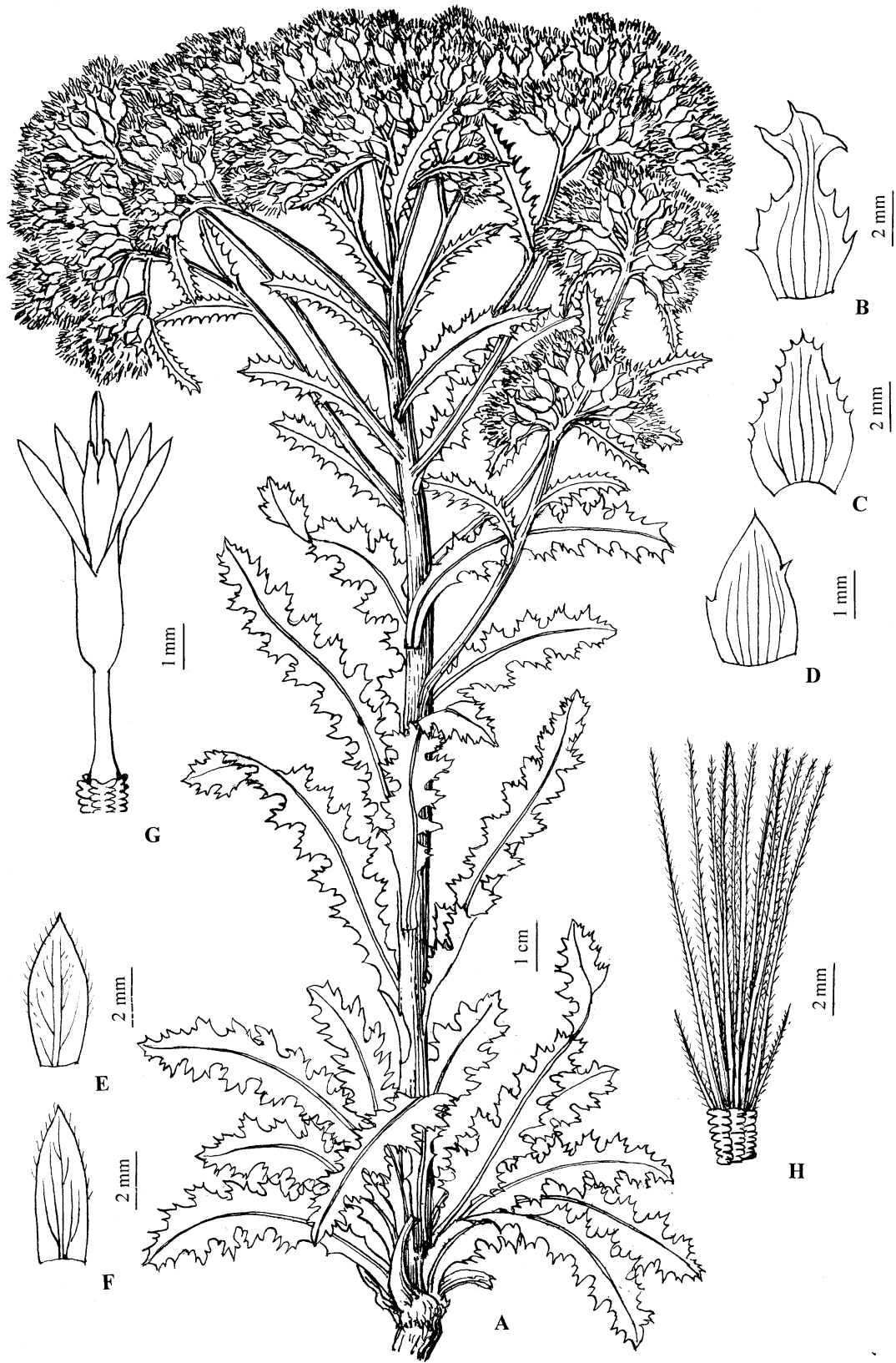
**Figure 1.** One of 16 most parsimonious trees (length = 423; CI = 0.662; RI = 0.537) based on ITS sequence data of some species from *Saussurea* and genera from the Cardueae. The designations ERIO, AMPH, SAUS, FROL or THEO following the species names are abbreviations of *Saussurea* subgen. *Eriocoryne*, subgen. *Amphilaena*, subgen. *Frolovia*, subgen. *Saussurea* and subgen. *Theodorea*, respectively. The taxon in boldface is the new species described in this paper. Numbers above and below branches indicate branch length and bootstrap support. Dashed lines denote branches that collapse in the strict consensus tree. ■ 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.

*S. thomsonii*, is closely related to *S. depsangensis*, a species delimited in sect. *Eriocoryne* with a bootstrap support of 58%. Despite the low support for these relationships and incomplete species sampling in other sections and subgenera, the analyses clearly indicate that sect. *Pseudoeriocoryne* does not constitute a monophyletic group. In fact, the acaulescence and the inflorescence with the congested capitula surrounded by a rosette of leaves, which were used to circumscribe sect. *Pseudoeriocoryne*, are found in diverse families of alpine plants and should surely be treated as convergent adaptations at the family level (Ohba, 1988), resulting from reproductive adaptation to the typical ecological pressures of arid, alpine habitats (Körner, 1999). However, species within a genus sharing these characters in the Qinghai-Tibet Plateau have always

been treated as natural entities, such as *Salix* sect. *Lindleyanae* in Salicaceae (Liu, 1996), *Rhododendron* sect. *Rhododendron* subsect. *Lapponica* in Ericaceae (Fang, 1999), *Androsace* sect. *Chamaejasme* in Primulaceae (Yang & Huang, 1990) and *Saxifraga* sect. *Ciliatae* subsect. *Rosulares* (Pan, 1992). Although the monophyly or polyphyly of sect. *Pseudoeriocoryne* needs further tests, because of the poor bootstrap support within the internal clades constructed by ITS data, our results undoubtedly indicate that it is necessary to explore the origin and monophyly of such entities in other genera and families using molecular evidence, independent of morphology.

In addition to the possible polyphyly of sect. *Pseudoeriocoryne*, our molecular phylogeny also suggests that other infrageneric classifications in *Saussurea* are likely to be unnatural. Our samples covered five of the six subgenera in the morphological classification system of *Saussurea* (Lipschitz, 1979): *Eriocoryne*, *Amphilaena*, *Frolovia*, *Theodorea* and *Saussurea* (Table 1). However, the branches of the phylogenetic tree linking species from one subgenus interweave with those from other subgenera (Fig. 1). For example, *S. bella* of subgen. *Saussurea* is closely related to *S. qinghai* of subgen. *Frolovia*, with a relatively high support of 76%, while *S. tatsienensis* and *S. superba* of this subgenus cluster with *S. stella* of subgen. *Eriocoryne*. However, all of these indications are tentative because the internal clades of ITS phylogeny were poorly resolved.

Although ITS data have proved to be effective in resolving the phylogeny of the tribe Cardueae (Suzanna *et al.*, 1995; Garcia-Jacas *et al.*, 2002), their usefulness in establishing the infrageneric phylogeny of *Saussurea* is extremely limited. Despite this, all investigated *Saussurea* species clustered together as a distinct clade (Fig. 1). Such a distinct lineage, with morphologically diverse species lacking internal grouping, obviously implies that an explosive radiation of this genus occurred in the Qinghai-Tibetan Plateau. Radiative speciation of angiosperms is most frequently recorded from island archipelago biomes, for example *Arygyranthemum* in Macronesia (Francisco-Ortega *et al.*, 1997) and the Hawaiian silverswords (Baldwin & Sanderson, 1998). The radiation of these genera is generally assumed to be driven by the low levels of competition found in newly occupied island habitats and consequently, low levels of genetic mutation (Liem, 1990). However, the mutations in the ITS region between the pairs of *Saussurea* species investigated here ranged from 1.1 to 15.2%, far higher than corresponding figures for genera confined to islands. Nevertheless, most mutations were autapomorphic and were not phylogenetically informative. A similar situation has also been found for *Gentiana* sect. *Chondrophyllae*, which has a similar distribution



**Figure 2.** Holotype of *Saussurea erecta* S. W. Liu, J. T. Pan & J. Q. Liu **sp. nov.**, with details. A, habit of plant; B, abaxial surface of an outer phyllary; C, adaxial surface of a middle phyllary; D–F, inner phyllary; G, floret; H, achene with pappus.

in the Qinghai-Tibetan Plateau. Yan & Küpfer (1997) attributed this pattern to a star phylogeny resulting from simultaneous formation of many species. They also suggested that there is a causal relationship between this pattern and the biennial, herbaceous habit of species in sect. *Chondrophyllae*. However, we propose that the rapid habitat isolation that has occurred during the large-scale uplift of the Qinghai-Tibetan Plateau since the late Pliocene (Shi, Li & Li, 1998) caused this type of star phylogeny and radiation of the species present there. According to this hypothesis, a common ancestor was suddenly isolated during the mountain building due to the rapid uplift of the Plateau, and the simultaneously produced species therefore accumulated autapomorphic mutations, rather than synapomorphic mutations, along nested clades as in a normal gradual isolation of range during the production of new species. In addition, during the climatic oscillations of the Quaternary, the shifts of vegetation in the Qinghai-Tibetan Plateau, alternating between desert-steppes and forests (Tang & Shen, 1996), must have driven migration of the species present there. As a result, on the one hand, there would have been contacts between some isolated species, promoting introgressive hybridization and reticulate evolution among these species, thereby leading to the homogenization of their genetic sequences (Abbott *et al.*, 2003). On the other hand, this climatic process might have intensified the divergence of species that were isolated, but survived *in situ* during the glacial ages (Comes & Kadereit, 1998). Under neither circumstance could low and high sequence divergence produce robust support for internal clades with the infrageneric phylogeny found in the *Saussurea* species presented here.

#### DESCRIPTION

*SAUSSUREA ERECTA* S. W. LIU, J. T. PAN  
& J. Q. LIU SP. NOV. (FIG. 2)

*Type:* China. Sangri, Tibet. On sands, 3650 m, 29°58'N, 91°57'E, 10.ix. 2002, J. Q. Liu 1135.

*Holotype:* HNWP.

*Diagnosis:* Species *S. kingii* C. E. C. Fisch. affinis, sed planta haud diffusa, caule erecto, c. 25 cm alto, acheniis haud arachnoideis, valde distincta.

*Description:* Herbs biennial, c. 25 cm tall. STEM erect, tomentose, 16–17 branched distally, tomentose. LEAVES linear-oblong, 5–8 × 0.8–1.2 cm, both surfaces tomentose and yellow-hyaline-glandular, apex acute, base gradually contracted into petiole 1–2.5 cm, margins subpinnatifidate, lobes denticulate at margin

and teeth cartilaginous-mucronate at apex; bracteal leaves oblanceolate-linear, 8–18 × 2–3 mm, both surfaces tomentose and glandular, apex acute, margins serrate and teeth mucronate at apex. CAPITULA several to numerous, corymbose, supported by bracteal leaves; involucre campanulate, 7–10 mm; phyllaries 3–4-seriate, adaxially tomentose and glandular hairy, abaxially glabrous, outer ones ovate, 6–7 × 3.5–4 mm, margins dark purple, dentate, with appendages at apex, appendages rhomboidal, tridentate, both tomentose and glandular hairy, middle ones ovate, c. 6 × 4 mm, apex acute, margin sparsely dentate and distally dark purple, proximally pale purple, inner ones oblong or lanceolate, 5.5–6 × 2.2–3.5 mm, apex acute, margin distally herbaceous and proximally membranaceous and sparsely dentate or entire; receptacle unpaleaceous. FLORETS pale red-purple, tube 2.5 mm, limb 4.5 mm, 5-lobate, lobes narrow-lanceolate, 3 mm. ACHENES c. 2 mm, transverse-rugose, glabrous, pappus biseriate, white, outer ones bristled, c. 2 mm, sparing, inner ones plumose, 7–9 mm.

The new species differs from *S. kingii* in that the stem is erect (not diffuse) and the achenes are glabrous (not covered with arachnoid hairs).

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#### REFERENCES

- Abbott RJ, James JK, Milne RI, Gillies AC. 2003. Plant introductions, hybridization and gene flow. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* **358**: 1123–1132.
- Baldwin BG, Sanderson MJ. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences, USA* **95**: 9402–9406.
- Bateman RM, Peter MH, Preston JL, Luo YB, Pridgeon AM, Chase MW. 2003. Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Botanical Journal of the Linnean Society* **142**: 1–40.
- Bremer K. 1994. *Asteraceae: cladistics and classification*. Portland: Timber Press.
- Comes HP, Kadereit JW. 1998. The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science* **3**: 432–438.
- Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochemical Bulletin* **19**: 11–15.

- Fang RZ.** 1999. Ericaceae-Rhododendroideae. In: *Flora Reipublicae Popularis Sinicae*, Vol. 57 (1). Beijing: Science Press, 19–213.
- Felsenstein J.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Francisco-Ortega J, Santos-Guerra A, Hines A, Jansen RK.** 1997. Molecular evidence for a Mediterranean origin of the Macaronesian endemic genus *Argyranthemum* (Asteraceae). *American Journal of Botany* **84**: 1595–1613.
- Garcia-Jacas N, Garnatje T, Susanna A, Vilatersana R.** 2002. Tribal and subtribal delimitation and phylogeny of the Cardueae (Asteraceae): a combined nuclear and chloroplast DNA analysis. *Molecular Phylogenetics and Evolution* **22**: 51–64.
- Körner C.** 1999. *Alpine plant life – functional plant ecology of high mountain ecosystems*. Berlin: Springer-Verlag.
- Liem KF.** 1990. Key evolutionary innovations, differential diversity, and symecomorphosis. In: Nitecki M, ed. *Evolutionary innovations*. Chicago: University of Chicago Press, 147–170.
- Lipschitz S.** 1979. *Genus Saussurea DC. (Asteraceae)*. Lenipopoli: Lenipopoli Press.
- Liu JQ, Gao TG, Chen ZD, Lu AM.** 2002. Molecular phylogeny and biogeography of the Qinghai-Tibet Plateau endemic *Nannoglottis* (Asteraceae). *Molecular Phylogenetics and Evolution* **23**: 307–325.
- Liu SW.** 1996. *Saussurea DC.* In: Liu SW, ed. *Qinghai Flora*, Vol. 3. Xining: Qinghai People Press, 443–482.
- Liu SW, Chen YL, Liang SJ.** 1985. *Saussurea DC.* In: Wu ZY, ed. *Flora of Tibet*, Vol. 4. Beijing: Science Press, 311–380.
- Maddison DR.** 1991. The discovery and importance of multiple islands of most-parsimonious trees. *Systematic Zoology* **40**: 315–328.
- Meerow AW, Lehmler DJ, Clayton JR.** 2003. Phylogeny and biogeography of *Crinum* L. (Amaryllidaceae) inferred from nuclear and limited plastid non-coding DNA sequences. *Botanical Journal of the Linnean Society* **141**: 349–363.
- Ohba H.** 1988. The alpine flora of the Nepal Himalayas: an introductory note. In: Ohba H, Malla SB, eds. *The Himalayas plants*, Vol. 1. Tokyo: University of Tokyo Press, 19–46.
- Pan JT.** 1992. Saxifragaceae (1). In: *Flora Reipublicae Popularis Sinicae*, Vol 34 (2). Beijing: Science Press, 1–309.
- Shi YF, Li JJ, Li BY.** 1998. *Uplift and environmental changes of Qinghai-Tibetan Plateau in the late Cenozoic*. Guangzhou: Guangdong Science and Technology Press.
- Shi Z, Jin SY.** 1999. *Saussurea DC.* In: *Flora Reipublicae Popularis Sinicae*, Vol 78 (2). Beijing: Science Press, 1–243.
- Susanna A, Garcia Jacas N, Soltis DE, Soltis PS.** 1995. Phylogenetic relationships in tribe Cardueae (Asteraceae) based on ITS sequences. *American Journal of Botany* **82**: 1056–1068.
- Swofford DL.** 2003. *PAUP\*: phylogenetic analysis using parsimony (\*and other methods)*, Version 4.0b 10. Sunderland, Massachusetts: Sinauer Associates.
- Tang LY, Shen CM.** 1996. Late Cenozoic vegetational history and climatic characteristics of Qinghai-Xizang Plateau. *Acta Micropalaeontologica Sinica* **13**: 321–337.
- Thompson JD, Gibson TJ, Plewinak F, Jeanmougin F, Higgins DG.** 1997. The Clustal–windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876–4882.
- White TJ, Bruns T, Lee S, Taylor JW.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press, 315–322.
- Yan YM, Küpfer P.** 1997. The monophyly and rapid evolution of *Gentiana* sect. *Chondrophyllae* Bunge s.l. (Gentianaceae): evidence from the nucleotide sequences of the internal transcribed spacers of nuclear ribosomal DNA. *Botanical Journal of the Linnean Society* **123**: 25–43.
- Yang YC, Huang RH.** 1990. *Androsace L.* In: Hu QM, ed. *Flora Reipublicae Popularis Sinicae*, Vol. 59 (1). Beijing: Sciences Press, 141–221.