Phylogenetic relationships in Saussurea (Compositae, Cardueae) sensu lato, inferred from morphological, ITS and trnL-trnF sequence data, with a synopsis of Himalaiella gen. nov., Lipschitziella and Frolovia

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Source: Willdenowia, 33(2) : 379-402
Published By: Botanic Garden and Botanical Museum Berlin (BGBM)
URL: https://doi.org/10.3372/wi.33.33214
Phylogenetic relationships in *Saussurea* (*Compositae, Cardueae*) sensu lato, inferred from morphological, ITS and trnL-trnF sequence data, with a synopsis of *Himalaiella* gen. nov., *Lipschitziella* and *Frolovia*

Abstract


DNA sequences from the nuclear ribosomal ITS and from the chloroplast trnL-trnF regions of 47 species of all six subgenera and 13 sections of *Saussurea* were analysed. The cladogram resulting from ITS sequence data indicates that *Saussurea* in its present circumscription is paraphyletic. S. sect. *Elatae* and S. subg. *Jurinocera* together are sister to the genus *Jurinea*. The affinities of S. subg. *Frolovia* are unclear, while representative species of the remaining four subgenera and ten sections form a strongly supported monophyletic group. Within this clade, the delimitation of S. subg. *Eriocoryne* and S. subg. *Amphilaena* against S. subg. *Saussurea* is not evident. The trnL-trnF intergenic spacer sequences show a remarkable synapomorphy, a 21bp insertion, for *Jurinea cyanoides*, S. deltoidea, S. ceratocarpa and S. frolovii, supporting the paraphyly of *Saussurea*. These results correspond to differences in morphological characters of achenes and pappus, which also suggest that S. sect. *Elatae*, S. subg. *Jurinocera* and S. subg. *Frolovia* are not closely related to the remainder of the genus. Consequently, S. sect. *Elatae* is separated as the new genus *Himalaiella*, and the small generic segregates *Frolovia* and *Lipschitziella* are resurrected. A synopsis of these three genera is presented and the new combinations *Himalaiella abnormis*, *H. afghana*, *H. albescens*, *H. auriculata*, *H. chenioides*, *H. chitralica*, *H. deltoidea*, *H. foliosa*, *H. heteromalla*, *H. nivea*, *H. peguenensis*, *Frolovia frolovii* and *F. gorbunovae* are validated.

Introduction

The genus *Saussurea* DC. (*Compositae, Cardueae*), in its presently accepted wide circumscription, has its centre of diversity in E Asia and comprises approximately 400 species (Lipić 1979). In China it is one of the largest genera of *Compositae*, with over 250 species in total (Shih & Jin 1999), more than 100 of which are reported from the Hengduan mountain range alone (Chen &
Li 1994). Most species are small to medium-sized perennials, often inhabiting high mountains up to the very limit of flowering plant life. The current classification of the genus is based on a wide syndrome of morphological characters, including life span, growth form, leaf shape and indumentum, number, size and shape of the capitula and the synflorescence, receptacular bristles, shape of the involucral bracts, as well as flower, achene and pappus morphology. The genus is presently divided into six subgenera with a total of 20 sections (Table 1). S. subg. Saussurea, with 10 sections and more than 300 species, is by far the largest.

For most species described in Saussurea, their inclusion in this genus is uncontroversial. However, there are a few whose position within Saussurea is in doubt. The two species of S. subg. Jurinocera, the three of S. sect. Jurineiformes and two of S. sect. Elatae have been placed alternatively in Jurinea or Saussurea. S. subg. Jurinocera has been assigned generic rank as Lipschitziella (Kamelin 1993). S. sect. Frolovia was raised to generic rank as Frolovia (Lipsč 1954), but later again sunk in Saussurea, as S. subg. Frolovia, by the same author (Lipsč 1961, 1962, 1979). In recent taxonomic literature (Čerepanov 1995, Hajra 1988, 1995, Krasnobarov 1997, Kamelin 1999), a wide circumscription of Saussurea was adopted, and neither Frolovia nor Lipschitziella were accepted.

The structure of the pappus of Saussurea has confused botanists working on the classification of the genus since 1833, when A. P. de Candolle split off Aplotaxis, named, as the name implies, for its “single row” of pappus bristles. Aplotaxis was merged again with Saussurea in 1846 by C. H. Schultz (“Bipontinus”), who argued that the allegedly simple pappus of Aplotaxis was

<table>
<thead>
<tr>
<th>Table 1. Infrageneric classification of the genus Saussurea DC. with approximate species numbers (in brackets) according to Lipsč (1979).</th>
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</thead>
<tbody>
<tr>
<td><strong>Saussurea</strong></td>
</tr>
<tr>
<td>subg. Jurinocera (Baill.) Lipsch.</td>
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<tr>
<td>subg. Eriocoryne (DC.) Hook. f.</td>
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<tr>
<td>sect. Eriocoryne</td>
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<tr>
<td>sect. Pseudoeriocoryne Lipsch.</td>
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<tr>
<td>sect. Cincta Lipsch.</td>
</tr>
<tr>
<td>subg. Amphilaena (Stschegl.) Lipsch.</td>
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<tr>
<td>sect. Amphilaena</td>
</tr>
<tr>
<td>sect. Pseudoamphilaena Lipsch.</td>
</tr>
<tr>
<td>subg. Theodorea (Cass.) Lipsch.</td>
</tr>
<tr>
<td>sect. Theodorea</td>
</tr>
<tr>
<td>sect. Maritimae (Nakai) Lipsch.</td>
</tr>
<tr>
<td>subg. Frolovia (DC.) Lipsch.</td>
</tr>
<tr>
<td>sect. Aucklandia (Falc.) O. Hoffm.</td>
</tr>
<tr>
<td>sect. Frolovia</td>
</tr>
<tr>
<td>subg. Saussurea</td>
</tr>
<tr>
<td>sect. Depressae C. B. Clarke</td>
</tr>
<tr>
<td>sect. Jurineiformes (Lipsch.) Lipsch.</td>
</tr>
<tr>
<td>sect. Jacea Lipsch.</td>
</tr>
<tr>
<td>sect. Acaules C. B. Clarke</td>
</tr>
<tr>
<td>sect. Laguranthera (C. A. Mey. ex Endl.) Lipsch.</td>
</tr>
<tr>
<td>sect. Elatae Hook. f.</td>
</tr>
<tr>
<td>sect. Pycnocephala Lipsch.</td>
</tr>
<tr>
<td>sect. Cyathidium (Lindl. ex Royle) Ling</td>
</tr>
<tr>
<td>sect. Rosulascentes (Kitam.) Lipsch.</td>
</tr>
<tr>
<td>sect. Saussurea</td>
</tr>
</tbody>
</table>
an artefact due to the falling off, or simply overlooking, of the outer pappus bristles. Neither Clarke (1876) nor Hooker (1881) did accept Aplotaxis in their authoritative treatments of Saussurea, so that Aplotaxis has remained in synonymy up to the present day. But even though it is true that the outer row of pappus bristles falls off easily and is often missing in herbarium specimens, there are also species of Saussurea where the outer pappus is lacking altogether. As pointed out by Lipić (1979), this feature consistently characterises three of the generic subdivisions: S. subg. Frorovia (6 species), S. sect. Jurineiformes (3 species), and S. sect. Elatae (12 species, 6 of them previously placed in Aplotaxis).

It is well known that achene and pappus provide useful characters for delimitation of Compositae genera (Dittrich 1970, Bremer 1994). Saklani & al. (2000) studied the achene micromorphology of 23 Indian species of Saussurea, but did not attempt to correlate their results with the accepted infrageneric classification. In her genus-level study of the Cardinae, Häffner (2000) suggested that Saussurea might be paraphyletic, based on the morphological characters of 13 species. My own studies encompass the morphological investigation of pappus and achenes of representative species of all six subgenera, and of all available species of S. sect. Elatae. In addition, molecular sequence data from nuclear ribosomal RNA (the ITS region) and from the chloroplast genome (the trnL-trnF region) were used to test the possible paraphyly of the genus.

Material and methods

Plant material

Plants for this study were collected during expeditions to the Hengduan Mountains (Southwestern China), to the Dongling Mountain near Beijing (Northern China) and to the Altai Mountains (Southern Siberia, Russia). For the molecular study, 42 species of the genus Saussurea, representing all six subgenera and 13 of the 20 sections were chosen (Table 2). Focus is on S. subg. Amphilaena consisting of 25 species, 19 of which have been included in the analysis. For DNA extraction, fresh collected leaves were dried and stored in silica-gel. Additional leaf and fruit material was taken from plants raised from seeds collected in the field or obtained via seed exchange and cultivated in the Botanic Garden Berlin-Dahlem. Dried leaf material of S. discolor was provided by M. Thiv (Stuttgart). Leaf material for DNA extraction was taken with kind permission of the curators from herbarium specimens from B, GOET, KUN, MO and UPS. For micromorphological studies of achenes and pappus, one species from each subgenus and three species of S. sect. Elatae were chosen (Table 3). Voucher specimens are deposited in the Herbarium of the Botanic Garden and Botanical Museum Berlin-Dahlem (B).

DNA extraction, PCR and sequencing

Total cell DNA was extracted from dry plant leaf tissue using the DNeasy Plant MiniKit (QIAGEN) according to the manufacturer’s protocol.

The primers ITS1-P1 (GGA AGT AAA AGT CGT AAC AAG G) and ITS2-P4 (TCC TCC GCT TAT TGA TAT GC) (White & al. 1990) were used for amplification of the whole internal transcribed spacer region of the ribosomal nuclear DNA (including ITS1, 5.8S rRNA and ITS2). In cases where the whole region could not be amplified, ITS1 and ITS2 were amplified separately, using the primers ITS1-P1 and ITS1-P2 (CTC GAT GGA ACA CGG GAT TCT GC) for ITS1, and ITS2-P3 (GCA TCG ATG AAG AAC GCA GC) and ITS2-P4 for ITS2, respectively. Still better results could be obtained for ITS2 when using the primer pair ITS2-D (CTC TCG GCA ACG GAT ATC TCG) and ITS2-SR (CTT AAA CTC AGC GGG TAG TCC C).

To obtain amplification products of the trnL-trnF region of the chloroplast DNA (including a part of the trnL-GAAA gene, the trnL intron, the trnL-trnF intergenic spacer and a part of the trnF-GAAA gene), the primers trnL1 (c) (CGA AAT CGG TAG ACG CTA CG) and trnF1 (f) (ATT TGA ACT GGT GAC ACG AG) (Taberlet & al. 1991) were used.
Table 2. Origin of plant material (and herbarium where voucher is deposited) of *Saussurea* species used for DNA sequencing and accession numbers of the EMBL database.

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher specimen</th>
<th>EMBL (ITS1)</th>
<th>EMBL (ITS2)</th>
<th>EMBL (trnL-trnF)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Saussurea amara</em> (L.) DC.</td>
<td>Russia, Altai Republic, <em>Raab-Straube</em> 020383 (B)</td>
<td>AJ606173</td>
<td>AJ606213</td>
<td>AJ606157</td>
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<tr>
<td><em>Saussurea baicalensis</em> (Adams) B. L. Rob.</td>
<td>Russia, Altai Republic, <em>Raab-Straube</em> 020356 (B)</td>
<td>AJ606207</td>
<td>AJ606245</td>
<td>AJ606150</td>
</tr>
<tr>
<td><em>Saussurea bracteata</em> Decne.</td>
<td>China, Xizang, G. &amp; S. Miehe 9639/18 (GOET)</td>
<td>AJ606185</td>
<td>AJ606225</td>
<td>–</td>
</tr>
<tr>
<td><em>Saussurea ceratocarpa</em> Decne.</td>
<td>India, Himachal Pradesh [seed exchange], cultivated at BGBM, Berlin, acc. no. 172-01-96-50, Höffner (B)</td>
<td>AJ606170</td>
<td>AJ606210</td>
<td>AJ606138</td>
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<tr>
<td><em>Saussurea chionophora</em> Hand.-Mazz.</td>
<td>China, Yunnan, <em>Raab-Straube</em> Smalla &amp; Sun 433 (B)</td>
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<td>–</td>
<td>AJ606161</td>
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<tr>
<td><em>Saussurea columnaris</em> Hand.-Mazz.</td>
<td>China, Yunnan, <em>Raab-Straube</em> Smalla &amp; Sun 494 (B)</td>
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<td>AJ606227</td>
<td>AJ606152</td>
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<td><em>Saussurea controversa</em> DC.</td>
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<td>AJ606218</td>
<td>AJ606166</td>
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<td><em>Saussurea davarica</em> Adams</td>
<td>Russia, Altai Republic, <em>Raab-Straube</em> 020348 (B)</td>
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<td><em>Saussurea discolor</em> (Willd.) DC.</td>
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<td>AJ606217</td>
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<td><em>Saussurea erubescens</em> Lipsch.</td>
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<td>AJ606240</td>
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<td><em>Saussurea frolovii</em> Leleb.</td>
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<td>AJ606234</td>
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<td><em>Saussurea iodostegia</em> Hance</td>
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<td>AJ606237</td>
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<tr>
<td>Species</td>
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<td><strong>Saussurea japonica</strong> (Thunb.) DC.</td>
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<td><strong>Saussurea krylovii</strong> Schischk. &amp; Serg.</td>
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<td><strong>Saussurea laniceps</strong> Hand.-Mazz.</td>
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<td><strong>Saussurea leucophylla</strong> Schrenk</td>
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<td><strong>Saussurea lineartifolia</strong> Ludlow</td>
<td>Nepal, Einarsson &amp; al. 3069 (UPS 200035)</td>
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<td><strong>Saussurea longifolia</strong> Franch.</td>
<td>China, Yunnan, Tu T. T. 12171 (KUN)</td>
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<tr>
<td><strong>Saussurea miluensis</strong> Hand.-Mazz.</td>
<td>China, Sichuan, Raab-Straube, Smälla &amp; Sun 877 (B)</td>
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<tr>
<td><strong>Saussurea ovbollata</strong> (DC.) Sch. Bip.</td>
<td>China, Sichuan, Raab-Straube, Smälla &amp; Sun 1185 (B)</td>
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<td><strong>Saussurea orguadanxi</strong> Khunn. &amp; Krasnob.</td>
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<td><strong>Saussurea pachyneura</strong> Franch.</td>
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<td><strong>Saussurea phaeantha</strong> Maxim.</td>
<td>China, Sichuan, Harry Smith 11962 (UPS 200024)</td>
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<td><strong>Saussurea pilanophylla</strong> Diels</td>
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<td><strong>Saussurea polycolea</strong> Hand.-Mazz.</td>
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<td><strong>Saussurea pricei</strong> N. D. Simpson</td>
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<td><strong>Saussurea pulifolia</strong> S.W. Liu &amp; T. N. He</td>
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<td><strong>Saussurea salwinensis</strong> J. Anthony</td>
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<tr>
<td><strong>Saussurea schaginiana</strong> (Wydler) Fisch. ex Herder</td>
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<td><strong>Saussurea stella</strong> Maxim.</td>
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<td><strong>Saussurea taisienensis</strong> Franch.</td>
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<td><strong>Saussurea tunicata</strong> Hand.-Mazz.</td>
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<td><strong>Saussurea veitchiana</strong> Drumm. &amp; Hutch.</td>
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<td><strong>Saussurea velutina</strong> W. W. Sm.</td>
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<td><strong>Saussurea wetstteiiana</strong> Hand.-Mazz.</td>
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<td>AJ606192</td>
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</table>
In all cases, polymerase chain reaction (PCR) was conducted in an Eppendorf Mastercycler gradient with an initial denaturation phase of 94 °C for 2 min, followed by 36 cycles of 94 °C for 20 s; annealing at 42 °C for 45 s, extension at 72 °C for 1 min (for \textit{trn}L1 \[c\] / \textit{trn}Fr \[f\]: 2 min) and final extension for 10 min. PCR products were purified with either MinElute PCR Purification Kit (QIAGEN) or Montage PCR Centrifugal Filter Devices (MILLIPORE).

For cycle sequencing, the same primers were used as for amplification, sequencing from both ends of the amplification products. For some PCR products of the \textit{trn}L-\textit{trn}F region, it was necessary to sequence additionally with the primer \textit{trn}L2r \(d\) (GGG GAT AGA GGG ACT TGA AC; Taberlet & al. 1991) in order to get a readable sequence at the beginning of this region. Sequencing was performed with an automatic sequencer (Beckman Coulter CEQ 8000 Genetic Analysis System) following the manufacturer’s protocols.

\textit{TrnL-}\textit{trnF} sequences of \textit{Arctium lappa} L. (EMBL acc. no. AF129824), \textit{Carduus nutans} L. (AF129825), \textit{Cirsium vulgare} (Savi) Ten. (AF129826), \textit{Cousinia hystrix} C. A. Mey. (AF 129827), \textit{Jurinea cyanoides} DC. (JCY404231), \textit{Onopordum acanthium} L. (AF129833) were taken from the EMBL database. ITS sequences from the EMBL database of the following species were included in the analysis: \textit{Arctium lappa} (ALITS12RR), \textit{Carduus nutans} (AF443678), \textit{Cirsium vulgare} (AF443715), \textit{Jurinea humilis} DC. (JHITS1A), \textit{J. macrocephala} DC. (AF319081, AF319135), \textit{Saussurea alpina} (L.) DC. (AF319091, AF319145), \textit{S. hypsipeta} Diels (AF257788), \textit{S. medusa} Maxim. (AF257787), \textit{S. przewalskii} Maxim. (AF257786). ITS sequences of \textit{Cousinia hystrix} and \textit{Saussurea riederi} Herd. were taken from Häffner & Hellwig (1999).

### Alignment and phylogenetic analysis

The ITS (ITS1 and ITS2, omitting the 5.8S rRNA) and \textit{TrnL-}\textit{TrnF} sequences were edited with Chromas, Version 1.45 (McCarthy 1996) and aligned by hand, using the alignment editor ALIGN by Hepperle (2003). Aligned sequences were analysed with PAUP* (Swofford 2001). All characters were weighted equally. Gaps were treated as missing data, but coded as binary characters and appended to the data matrix. Maximum parsimony analysis was performed conducting a heuristic search with 100 random addition sequence replicates, TBR (tree-bisection-reconnection) algorithm, MULTrees on and steepest descent option not in effect. To assess the stability of the resulting clades, a bootstrap analysis with 1000 replicates, with an enforced maximum of 10 000 trees per replicate, was conducted (simple addition sequence, TBR algorithm, MULTrees on and steepest descent not in effect). Bootstrap values of the resulting 50 % majority rule consensus tree (not shown) are indicated on the ITS consensus tree (Fig. 1) when their value was higher than 50 % and the branching pattern was congruent in both trees.
Fig. 1. Strict consensus tree of 3184 equally most parsimonious trees obtained in a heuristic search with 100 random addition sequence replicates. Numbers above branches represent percentages from a bootstrap analysis with 1000 replicates (indicated only when >50% and branches identical with consensus tree).
Morphological analysis

Saussurea achenes with pappus were collected in the field or taken from herbarium specimens. They were first examined and measured using a Wild M3C stereomicroscope (Heerbrugg). For scanning electron microscopy, they were mounted on sample plates and coated with 40 nm gold/palladium in a Low Voltage Cool Sputter Coater (EMITECH K 550). SEM was conducted with a Philips SEM 515.

Results

Molecular analysis of ITS and \textit{trnL-trnF} sequence data

The alignment of ITS1 and ITS2 consists of 490 sites and 17 binary coded gap characters. 187 characters are parsimony informative and 76 are variable, but parsimony uninformative. In the heuristic search, 3184 equally most parsimonious trees with 606 steps length, a consistency index (CI) = 0.55 and a retention index (RI) = 0.68 were found.

The strict consensus tree (Fig. 1) clearly shows that ITS sequence data do not support monophyly of the genus \textit{Saussurea}. Only 42 of 45 analysed species form a monophyletic lineage with good bootstrap support (92 %). Three \textit{Saussurea} species appear among the outgroup taxa \textit{Arctium}, \textit{Cousinia} and \textit{Jurinea}. A clade consisting of \textit{S. deltoidea} and \textit{S. ceratocarpa} (representing \textit{S. sect. Elatae} and \textit{S. subg. Jurinocera}) receives 100 % support from bootstrap analysis. This clade is sister to the genus \textit{Jurinea}, which is very well resolved as monophyletic, too (bootstrap value 98 %). The sister group relationship between \textit{Jurinea} and \textit{S. subg. Elatae} and \textit{S. subg. Jurinocera} receives 99 % support. \textit{S. frolovi} (representing \textit{S. subg. Frolovia}) is sister to the monophyletic group formed by \textit{Jurinea}, \textit{S. subg. Elatae} and \textit{S. subg. Jurinocera}, but without support from bootstrap analysis.

The \textit{Saussurea} clade consists of 42 species from \textit{S. subg. Thedorea}, \textit{S. subg. Eriocoryne}, \textit{S. subg. Amphilaena} and \textit{S. subg. Saussurea} (excluding \textit{S. sect. Elatae}). Within this clade, the two analysed members of \textit{S. subg. Thedorea}, \textit{S. japonica} and \textit{S. amara}, together with \textit{S. davurica} from \textit{S. subg. S. sect. Laguranthera}, form a monophyletic group (bootstrap support 100 %). \textit{S. amara} is sister to \textit{S. davurica} (bootstrap support 89 %). Most of the upper clades receive no support from bootstrap analysis and many polytomies occur, due to very similar sequences in the ITS region. Nevertheless, some branching patterns in the tree are quite clear. \textit{S. subg. Saussurea}, including the European \textit{S. alpina} and \textit{S. discolor} and the Siberian \textit{S. controversa}, form a moderately supported clade (bootstrap value 68 %). \textit{S. controversa} and \textit{S. alpina} are sister taxa (93 % bootstrap support). The Chinese species from this section (\textit{S. hieracioides} and \textit{S. tatrienensis}) are not part of this clade.

\textit{Saussurea pilinophylla}, \textit{S. tunicata} and \textit{S. bracteata} (\textit{S. subg. Amphilaena} sect. \textit{Amphilaena}) form a monophyletic, well supported group (bootstrap support 83 %) together with \textit{S. apus} (\textit{S. subg. S. sect. Aculeus}), a section of unclear affinities. The latter two species are sister taxa with bootstrap support of 89 %, the clade formed by these two is sister to \textit{S. tunicata} (bootstrap support of 79 %).

\textit{Saussurea polycolea}, \textit{S. longifolia} and \textit{S. wettsteiniana}, all members of \textit{S. subg. Amphilaena}, make up a moderately supported clade (bootstrap support 66 %). \textit{S. veitchiana} is sister to \textit{S. hieracioides} (99 % bootstrap support), and \textit{S. przewalkii} and \textit{S. erubescens} are sister taxa with 100 % bootstrap support.

Most Siberian members of \textit{Saussurea} sect. \textit{Pycocephala} (\textit{S. baicalensis}, \textit{S. krylovii} and \textit{S. schanginiana}) also form a well supported monophyletic group (98 % bootstrap support), while the Chinese member of this section (\textit{S. graminea}) is sister to the likewise Siberian \textit{S. leucophylla} (bootstrap support 77 %).

The alignment of the \textit{trnL-trnF} region was remarkably uniform. Sequences of the \textit{trnL-trnF} region were almost identical in all analysed species, including the outgroup genera \textit{Carduus}, \textit{Cirsium}, \textit{Arctium}, \textit{Cousinia} and \textit{Jurinea}, and yielded only 12 informative sites in an alignment of
Fig. 2. Part of the alignment of the trnL-trnF intergenic spacer (IGS). – Species with 21 bp insertion in bold.
857 base pairs length (813 characters were constant, 32 were variable but parsimony uninformative). Therefore, no cladogram based on trnL-trnF sequences was calculated. However, the trnL-trnF intergenic spacer provides one highly informative character. A 21 bp insertion (Fig. 2) is shared by Jurinea cyanoides, Saussurea ceratocarpa, S. deltoidea and S. frolovii. Since this insertion is never present in other members of Saussurea, nor in any sample from the outgroups, it gives strong support for a common origin of these species, which represent the genus Jurinea and Saussurea sect. Elatae, S. subg. Jurinocera and S. subg. Frolovia.

Morphology of achenes and pappus

The consistently homomorphic achenes of Saussurea are straight or slightly incurved, cylindrical, obvoidal or obconical in shape. They may be rounded, striate, distinctly ribbed or four- to five-angled, and isodiametric to dorsiventrally flattened. Variable characters include the shape of the detachment area and apical region as well as the surface pattern. The surface is usually smooth, but in some species it is rugose, squamose or minutely aculeate. The pericarp is sometimes elongated into an irregularly dentate crown or into long horns. The pappus typically consists of one inner row of 14 to 16 dorsiventrally flattened, plumose, 10-20 mm long bristles, which are connate into a ring and fall off as a whole, and an outer row of scabrid, shorter, terete bristles falling off individually. Details can be described as follows.

Saussurea (subg. Saussurea sect. Saussurea) alpina – Fig. 3

Saussurea alpina, which provides the type of the generic name, has narrowly cylindrical, 3.6–4.8 mm long, 0.8–1.2 mm broad achenes with longitudinal ribs (Fig. 3A). The pericarp is smooth (Fig. 3B). The apex is truncate and lacks a pericarp crown (Fig. 3D). The pappus consists of an inner row of laterally fused, flattened, plumose bristles (Fig. 3C, E) and an outer row of individually deciduous, terete, scabrid shorter bristles (Fig. 3C).

Saussurea (subg. Saussurea sect. Elatae) deltoidea, chenopodiifolia and heteromalla – Fig. 9–11

The achenes of the investigated species of this section (Fig. 9A, 10A, 11A) are typically 3–4 mm in some species (S. albescens) up to 6.5 mm long and c. 1 mm broad, obconical, four-angled and isodiametric. Their surface is strongly rugose (S. chenopodiifolia, Fig. 10B), muricate (S. heteromalla, Fig. 11B) or squamulose near the apex (S. deltoidea, Fig. 9B). The pericarp is always elongated beyond the apical plate forming an irregularly dentate crown (Fig. 9D, 10D, 11D). The SEM micrographs of Saklani & al. (2000) show this type of crown in additional members of the same section: S. abnormis (op. cit. fig. 1A), S. albescens (op. cit. fig. 1C, 1D), S. auriculata (op. cit. fig. 1E) and S. stracheyana (op. cit. fig. 5G). Lipšič reports such a crown from S. afghana, too (Lipšič 1979: 141, t. XXVI); it is also known from S. chitralica (Duthie 1901: t. 57). The pappus always consists of a single row of laterally fused, flattened, plumose bristles; an outer row of individually deciduous bristles is completely lacking (Fig. 9C, 10C, 11C).

Saussurea (subg. Jurinocera) ceratocarpa – Fig. 4

The achenes are isodiametric, obconical, four-angled, 3.8–4.2 mm long and 1.6–1.8 mm broad (Fig. 4A). The four angles are elongated into four acute, up to 1 mm long horns, which suggest zoochory as dispersal mode (Fig. 4D). The surface of the achenes is squamulose or aculeate in the upper half (Fig. 4B). The pappus (Fig. 4C, E) is biseriate, consisting of an inner row of connate, plumose bristles and an outer row of individually deciduous, short, scabrid bristles. However, the inner bristles are not fused laterally as in the typical Saussurea pappus, but fixed basally on a deciduous ring of tissue (Fig. 4E).

Saussurea (subg. Eriocoryne) medusa – Fig. 5

The achenes are 8–10 mm long, 1.3–1.7 mm broad, narrowly obconical, slightly triangulate and flattened (Fig. 5A). The surface is smooth to minutely rugulose (Fig. 5B). The apex of the achene is rounded (Fig. 5D), the disc and the style basis are indurate and sclerenchymatous. The pappus
(Fig. 5C) is of the common *Saussurea* type with plumose, laterally fused inner and scabrid, shorter outer bristles. Unlike in most *Saussurea* species, the inner pappus is not deciduous but persistent (anemochory).

**Saussurea** (subg. *Amphilaena*) *obvallata* – Fig. 6
The achenes are straight or slightly curved inwards, distinctly ribbed, 4-4.8 mm long and 1.3-1.6 mm broad (Fig. 6A). The surface is smooth (Fig. 6B), the apex truncate without any pericarpal crown (Fig. 6D). In all investigated taxa of *S*. subg. *Amphilaena*, the pappus (Fig. 6C) is of the common *Saussurea* type.

**Saussurea** (subg. *Theodorea*) *japonica* – Fig. 7
The achenes are obovoidal, 3.4-4.4 mm long and 1.6-1.8 mm broad, almost terete, only slightly flattened, striate but without distinct ribs (Fig. 7A); their surface is smooth (Fig. 7B). The pericarp is truncate at the apex and no crown is present (Fig. 7D). The pappus is the typical biseriate *Saussurea* pappus (Fig. 7C).

**Saussurea** (subg. *Frolovia*) *frolovii* – Fig. 8
The achenes are obovoidal, 7-8 mm long, 2.4-3 mm broad and isodiametric (Fig. 8A). Their surface is distinctly rugose (Fig. 8B) with strongly thickened epidermal cell walls. The pericarp extends well above the apical plate, forming an irregular cup-shaped crown (Fig. 8D). The pappus consists of one single row of laterally fused, flattened, plumose bristles (Fig. 8C).

**Discussion**

The molecular data strongly indicate that *Saussurea* in its current circumscription is paraphyletic, a hypothesis already made by Häffner (2000) and corroborated by the new morphological data from achenes and pappus. Evolution of the nuclear ribosomal ITS region and the chloroplast *trnl-trnf* region including a 21 bp insertion in *Saussurea* sect. *Elatae*. *S*. sect. *Frolovia* and *S*. subg. *Jurinocera* suggest that these taxa are more closely related to the genus *Jurinea* than to the bulk of *Saussurea*. Consequently, to obtain a more natural classification and to circumscribe *Saussurea* as a monophyletic taxon, they have to be excluded from *Saussurea*. They also differ from *Jurinea*, especially in pappus structure, and should therefore not be included in this genus (as has been done earlier with some of their species). Morphologically, all three taxa are well characterized.

*Saussurea* sect. *Elatae* differs from *Saussurea* sensu stricto and from *S*. subg. *Jurinocera* by the uniseriate pappus and the pericarp crown, which has been observed in nine of the twelve species. For molecular and morphological reasons, it seems most justified to exclude *S*. sect. *Elatae* from *Saussurea* and place it into a genus of its own. The name *Aplotaxis* (Candolle 1833: 330) cannot be applied, because it is based on Lessing’s (1832) monotypic genus *Eriostemon* and its single species *E. taraxacifolium* (D. Don) Less. = *Saussurea nepalensis* Spreng. = *Aplotaxis nepalensis* (Spreng.) DC. This species is not a member of *S*. sect. *Elatae* but of *S*. sect. *Cyathidium*. Its pappus is sometimes described as double (Chater & Kitamura 1982: 37, Grierson & Springate 2001: 1446), sometimes as simple (Hajra 1995: 207), the achenes are smooth and a pericarp crown has not been observed. Since no other generic name is available to accommodate the species of *S*. sect. *Elatae*, I propose here the new genus *Himalaiella*, most of which species occur in the Himalayas and adjacent high mountains.

*Saussurea* subg. *Jurinocera* can easily be distinguished from all *Saussurea* species by the long, sharp horns at the apex of the achene. I agree with Häffner (2000: 35), who regards these horns as an independent formation that is not homologous with the pericarp crown. Although molecular results show a close relationship of this group to *S*. sect. *Elatae*, especially the pappus and achene morphology are unique within *Carduiinae*. Therefore, it seems perfectly justified to treat *S*. subg. *Jurinocera* as a genus of its own, an idea already informally expressed by Lipić (1979: 42: “An genus proprium novum?”). Kamelin (1993) described the genus *Lipschitziella*...
Kamelin with two species, *L. carduicephala* (Iljin) Kamelin and *L. ceratocarpa* (Decne.) Kamelin, a concept that is consistent with molecular and morphological data from this study and which is followed here.

My results from ITS and trnL-trnF sequences show that *Saussurea frolovii* is not part of *Saussurea sensu stricto*, but that it is close to *Jurinea*, *S. sect. Elatae* and *S. subg. Jurinocera*; achenes and pappus morphology are similar to those of *S. sect. Elatae*. Shih & Jin (1999), taking into account the similarity between these taxa, included the Chinese species of *S. sect. Elatae* in *S. subg. Frolovia*. A part of *S. subg. Frolovia* has occasionally (Lipšić 1954, Černeva 1962, Šaripova 1991, Kamelin & Kovalevskaja 1993) been treated as a genus of its own, *Frolovia* (DC.) Lipsch. Kamelin (1999) discusses the status of this group again, now accepting Lipšić’ latest approach, which includes *Frolovia in Saussurea*, but noting that the composition of the group is still in doubt. According to my results, *Frolovia* (DC.) Lipsch. should be resurrected as a distinct genus. To accommodate the recently described *Saussurea gorobunovae* Kamelin in this genus, the appropriate new combination is validated below.

The remaining four subgenera, *Saussurea subg. Theodorea*, *S. subg. Eriocoryne*, *S. subg. Amphilaena* and *S. subg. Saussurea*, together are monophyletic. Molecular results suggest that limits between these taxa will have to be redefined. For instance, *S. subg. Theodorea* may be merged with *S. sect. Laguranthera*, Euro-Siberian species of *S. sect. Saussurea* may be separated from Chinese members of this section and circumscription of *S. subg. Amphilaena* may be changed. However, more detailed studies using different molecular markers and including additional taxa are needed to further clarify relationships within *Saussurea s. str.*

**Taxonomic synopsis of Himalaiella, Lipschitziella and Frolovia**

**Himalaiella** Raab-Straube, *gen. nov.*


**Himalaiella afghana** (Lipsch.) Raab-Straube, *comb. nova* = *Saussurea afghana* Lipsch. in Bot. Zurn. 60: 1446. 1975.


Himalaiella deltoidea (DC.) Raab-Straube, **comb. nova** = Aplotaxis deltoidea DC., Prodr. 6: 541. 1838 = Saussurea deltoidea (DC.) Sch. Bip. in Linnaea 19: 331. 1846.


Frolovia frolovii (Ledeber.) Raab-Straube, **comb. nova** = Saussurea frolovii Ledeb., Icon. Pl. 4: 16, t. 352. 1833 = Aplotaxis frolovii (Ledeber.) DC., Prodr. 6: 538. 1838 = Frolovia ledebouriana Lipsch. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 16: 461. 1954, nom. illeg. Note. – Under the present Code (Greuter & al. 2000), there is no alternative to naming this taxon Frolovia frolovii, which is not a tautonym, because the epithet does not repeat exactly the genus name (Art. 23. 4 ICBN). No other epithet is available.
**Frolovia gilesii** (Hemsl.) B. A. Scharip. in Fl. Tadzik. SSR 10: 161, t. 22. 1991 ≡ *Saussurea gilesii* Hemsl. in Hooker’s Icon. Pl. 18 [= ser. 3, 8, 2]: t. 1736. 1888.


**Acknowledgements**

My warmest thanks to Sun Hang (Kunming), who made our joint expedition to the Hengduan Mountains (SW China) possible. I thank many other colleagues who have assisted in fieldwork: Milan Smalla, Wieland Peschel (both Berlin), Monika Steinhof (Bremen), Qin Hai-ning (Beijing), Irena Čuhina, Irina Pauftova, Tamara Smekalova (all St Petersburg), Alexander Šmakov and Maxim Kuzev (Barnaul). I thank Jana Bansemer for technical assistance in the DNA lab, Monika Lüchow and Jeannette Ueckert for SEM assistance, Michael Rodewald for digital image processing and Michael Meyer and his staff for taking constant care of my living plant collection in the Botanic Garden Berlin-Dahlem. For fruitful discussion and encouragement, I would like to thank Werner Greuter, who also read an earlier version of this manuscript, Norbert Kilian (both Berlin), Christoph Oberprieler (now Regensburg) and Ihsan Al-Shehbaz (Saint Louis, Missouri). For translation of literature from Russian, I thank Juri Roskov (Reading). The plant collecting expedition to Southwest China was funded by the National Geographic Society (Grant # 6851-00) and by an additional grant from the DAAD, the expedition to Siberia by the Mattfeld-Quadbeck-Stiftung, Berlin. A part of this study was supported by a NaFoG grant from the Land Berlin.

**References**

Clarke, C. B. 1876: *Compositae* indicae, descriptae et secum genera Benthamii ordinatae. – Calcutta.

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Fig. 3. *Saussurea alpina*, scanning electron micrographs of achenes and pappus – A: overview; B: surface; C: pappus detail; D: apical region; E: base of inner pappus (A-B, D-E from *Vogt 15869 & al*; C from *Raab-Straube 020383*). – Scale bars A = 1 mm, B = 0.09 mm, C = 0.5 mm, D = 0.4 mm, E = 0.4 mm.
Fig. 4. Lipschitziella ceratocarpa, scanning electron micrographs of achenes and pappus (from Lace 1789) – A: overview; B: surface; C: pappus detail; D: apical region; E: base of inner pappus. – Scale bars A = 2 mm, B = 0.09 mm, C = 0.9 mm, D = 0.8 mm, E = 0.3 mm.
Fig. 5. *Saussurea medusa*, scanning electron micrographs of achenes and pappus (from Raab-Straube, Smalla & Sun 1026) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 3 mm, B = 0.09 mm, C = 0.8 mm, D = 0.3 mm.
Fig. 6. *Saussurea obvallata*, scanning electron micrographs of achenes and pappus (from Raub-Straube, Smalla & Sun 1183) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 1 mm, B = 0.09 mm, C = 0.7 mm, D = 0.5 mm.
Fig. 7. *Saussurea japonica*, scanning electron micrographs of achenes and pappus (from Raab-Straube & Steinhof 2001-17) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 0.9 mm, B = 0.09 mm, C = 0.6 mm, D = 0.4 mm.
Fig. 8. Frolovia frolovii, scanning electron micrographs of achenes and pappus (from Zdanova & al., 13.8.1987) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 2 mm, B = 0.09 mm, C = 0.8 mm, D = 0.7 mm.
Fig. 9. *Himalaiella deltoidea*, scanning electron micrographs of achenes and pappus (from BGBM acc. no. 001-28-78-10) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 1 mm, B = 0.09 mm, C = 0.6 mm, D = 0.5 mm.
Fig. 10. *Himalaiella chenopodiifolia*, scanning electron micrographs of achenes and pappus (from Neubauer 4482) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 1 mm, B = 0.09 mm, C = 0.5 mm, D = 0.5 mm.
Fig. 11. *Himalaiella heteromalla,* scanning electron micrographs of achenes and pappus (from Nüsser 1053) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 1 mm, B = 0.09 mm, C = 0.6 mm, D = 0.4 mm.