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

Abstract

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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 *Lipschitzziella* are resurrected. A synopsis of these three genera is presented and the new combinations *Himalaiella abnormis*, *H. afghana*, *H. albescens*, *H. auriculata*, *H. chenopodiifolia*, *H. chitralica*, *H. deltoidea*, *H. foliosa*, *H. heteromalla*, *H. nivea*, *H. peguensis*, *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 (Lipšic 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 involucre 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. *Jurincera*, the three of *S.* sect. *Jurineiformes* and two of *S.* sect. *Elatae* have been placed alternatively in *Jurinea* or *Saussurea*. *S.* subg. *Jurincera* has been assigned generic rank as *Lipschitzia* (Kamelin 1993). *S.* sect. *Frolovia* was raised to generic rank as *Frolovia* (Lipšič 1954), but later again sunk in *Saussurea*, as *S.* subg. *Frolovia*, by the same author (Lipšič 1961, 1962, 1979). In recent taxonomic literature (Čerepanov 1995, Hajra 1988, 1995, Krasnoborov 1997, Kamelin 1999), a wide circumscription of *Saussurea* was adopted, and neither *Frolovia* nor *Lipschitzia* 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 1. Infrageneric classification of the genus *Saussurea* DC. with approximate species numbers (in brackets) according to Lipšič (1979).

<i>Saussurea</i>		
subg. <i>Jurincera</i> (Baill.) Lipsch.	(2)	
subg. <i>Eriocoryne</i> (DC.) Hook. f.	(26)	
sect. <i>Eriocoryne</i>		(17)
sect. <i>Pseudoeriacoryne</i> Lipsch.		(4)
sect. <i>Cincta</i> Lipsch.		(5)
subg. <i>Amphilaena</i> (Stschegl.) Lipsch.	(24)	
sect. <i>Amphilaena</i>		(19)
sect. <i>Pseudoamphilaena</i> Lipsch.		(5)
subg. <i>Theodorea</i> (Cass.) Lipsch.	(20)	
sect. <i>Theodorea</i>		(16)
sect. <i>Maritimae</i> (Nakai) Lipsch.		(4)
subg. <i>Frolovia</i> (DC.) Lipsch.	(6)	
sect. <i>Aucklandia</i> (Falc.) O. Hoffm.		(2)
sect. <i>Frolovia</i>		(4)
subg. <i>Saussurea</i>	(316)	
sect. <i>Depressae</i> C. B. Clarke		(10)
sect. <i>Jurineiformes</i> (Lipsch.) Lipsch.		(3)
sect. <i>Jacea</i> Lipsch.		(5)
sect. <i>Acaules</i> C. B. Clarke		(13)
sect. <i>Laguranthera</i> (C. A. Mey. ex Endl.) Lipsch.		(54)
sect. <i>Elatae</i> Hook. f.		(12)
sect. <i>Pycnocephala</i> Lipsch.		(19)
sect. <i>Cyathidium</i> (Lindl. ex Royle) Ling		(36)
sect. <i>Rosulascentes</i> (Kitam.) Lipsch.		(15)
sect. <i>Saussurea</i>		(149)

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 Lipšić (1979), this feature consistently characterises three of the generic subdivisions: *S.* subg. *Frolovia* (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 *Carduinae*, 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 parphyly 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_{UAA}* gene, the *trnL* intron, the *trnL-trnF* intergenic spacer and a part of the *trnF_{GAA}* gene), the primers *trnL1* (c) (CGA AAT CGG TAG ACG CTA CG) and *trnFr* (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.

Species	Voucher specimen	EMBL (ITS1)	EMBL (ITS2)	EMBL (trnL-trnF)
<i>Saussurea amara</i> (L.) DC.	Russia, Altai Republic, <i>Raab-Straube 020383</i> (B)	AJ606173	AJ606213	AJ606157
<i>Saussurea apus</i> Maxim.	China, Qinghai, <i>Raab-Straube, Smalla & Sun 1280</i> , cultivated at BGBM, Berlin, acc. no. 021-04-01-10, <i>Raab-Straube</i> (B)	AJ606186	AJ606226	AJ605579
<i>Saussurea baicalensis</i> (Adams) B. L. Rob.	Russia, Altai Republic, <i>Raab-Straube 020356</i> (B)	AJ606207	AJ606245	AJ606150
<i>Saussurea bracteata</i> Decne.	China, Xizang, G. & S. <i>Miehe 9639/18</i> (GOET)	AJ606185	AJ606225	–
<i>Saussurea ceratocarpa</i> Decne.	India, Himachal Pradesh [seed exchange], cultivated at BGBM, Berlin, acc. no. 172-01-96-50, <i>Häffner</i> (B)	AJ606170	AJ606210	AJ606138
<i>Saussurea chionophora</i> Hand.-Mazz.	China, Yunnan, <i>Raab-Straube, Smalla & Sun 433</i> (B)	–	–	AJ606161
<i>Saussurea columnaris</i> Hand.-Mazz.	China, Yunnan, <i>Raab-Straube, Smalla & Sun 494</i> (B)	AJ606187	AJ606227	AJ606152
<i>Saussurea controversa</i> DC.	Russia, Altai Republic, <i>Raab-Straube 020126</i> (B)	AJ606178	AJ606218	AJ606166
<i>Saussurea davurica</i> Adams	Russia, Altai Republic, <i>Raab-Straube 020348</i> (B)	AJ606174	AJ606214	AJ606163
<i>Saussurea deltoidea</i> (DC.) Sch. Bip.	China, Sichuan [seed exchange], cultivated at BGBM, Berlin, acc. no. 014-21-99-10, <i>Raab-Straube</i> (B)	AJ606169	AJ606209	AJ606137
<i>Saussurea discolor</i> (Willd.) DC.	Italy, Piemont, <i>Thiv 3527</i> (B)	AJ606177	AJ606217	AJ606167
<i>Saussurea erubescens</i> Lipsch.	China, Qinghai, <i>Ho, Bartholomew & Gilbert 687</i> (MO 4648745)	AJ606201	AJ606240	AJ606153
<i>Saussurea frolovii</i> Ledeb.	Russia, Altai Republic, <i>Raab-Straube 020140</i> (B)	AJ606171	AJ606211	AJ606139
<i>Saussurea globosa</i> F. H. Chen	China, Sichuan, <i>Raab-Straube, Smalla & Sun 957</i> (B)	AJ606202	AJ606241	AJ606151
<i>Saussurea graminea</i> Dunn	China, Sichuan, <i>Raab-Straube, Smalla & Sun 975</i> (B)	AJ606203	AJ606242	–
<i>Saussurea hieracioides</i> Hook. f.	China, Gansu, <i>Raab-Straube, Smalla & Sun 1292</i> , cultivated at BGBM, Berlin, acc. no. 021-07-01-10, <i>Raab-Straube</i> (B)	AJ606200	AJ606239	AJ606141
<i>Saussurea hookeri</i> C. B. Clarke	China, Sichuan, <i>Raab-Straube, Smalla & Sun 1108</i> (B)	AJ606195	AJ606234	AJ606159
<i>Saussurea iodostegia</i> Hance	China, Beijing Shi, <i>Raab-Straube & Steinhof 2001-20</i> (B)	AJ606198	AJ606237	AJ606148

<i>Saussurea japonica</i> (Thunb.) DC.	China, Beijing Shi, Raab-Straube & Steinhof 2001-17 (B)	AJ606172	AJ606212	AJ606154
<i>Saussurea krylovii</i> Schischk. & Serg.	Russia, Altai Republic, Raab-Straube 020316 (B)	AJ606205	AJ606243	AJ606165
<i>Saussurea laniceps</i> Hand.-Mazz.	China, Yunnan, Raab-Straube, Smalla & Sun 655 (B)	AJ606176	AJ606216	–
<i>Saussurea leucophylla</i> Schrenk	Russia, Altai Republic, Raab-Straube 020189 (B)	AJ606204	–	AJ606160
<i>Saussurea linearifolia</i> Ludlow	Nepal, Einarsson & al. 3069 (UPS 200035)	AJ606180	AJ606220	AJ606155
<i>Saussurea longifolia</i> Franch.	China, Yunnan, Yu T. T. 12171 (KUN)	AJ606191	AJ606230	–
<i>Saussurea multiensis</i> Hand.-Mazz.	China, Sichuan, Raab-Straube, Smalla & Sun 877 (B)	AJ606179	AJ606219	AJ606143
<i>Saussurea obvallata</i> (DC.) Sch. Bip.	China, Sichuan, Raab-Straube, Smalla & Sun 1185 (B)	AJ606181	AJ606221	AJ606147
<i>Saussurea orgadaya</i> Khanm. & Krasnob.	Russia, Altai Republic, Raab-Straube 020375 (B)	AJ606208	AJ606246	AJ606149
<i>Saussurea pachyneura</i> Franch.	China, Yunnan, Raab-Straube, Smalla & Sun 495 (B)	AJ606196	AJ606235	AJ606162
<i>Saussurea phaeantha</i> Maxim.	China, Sichuan, Harry Smith 11962 (UPS 200024)	AJ606194	AJ606233	–
<i>Saussurea pilinophylla</i> Diels	China, Sichuan, Raab-Straube, Smalla & Sun 1209 (B)	AJ606183	AJ606223	AJ605578
<i>Saussurea polycolea</i> Hand.-Mazz.	China, Yunnan, Raab-Straube, Smalla & Sun 390 (B)	AJ606190	–	AJ606146
<i>Saussurea pricei</i> N. D. Simpson	Russia, Altai Republic, Raab-Straube 020231 (B)	AJ606175	AJ606215	AJ606168
<i>Saussurea pubifolia</i> S.W. Liu & T. N. He	China, Xizang, Qinghai-Tibet Expedition Team 750735 (KUN 729593)	AJ606189	AJ606229	–
<i>Saussurea salwinensis</i> J. Anthony	China, Yunnan, Raab-Straube, Smalla & Sun 641 (B)	–	–	AJ606164
<i>Saussurea schanginiana</i> (Wydler) Fisch. ex Herder	Russia, Altai Republic, Raab-Straube 020117 (B)	AJ606206	AJ606244	AJ606158
<i>Saussurea stella</i> Maxim.	China, Yunnan, Raab-Straube, Smalla & Sun 533 (B)	AJ606197	AJ606236	AJ606142
<i>Saussurea tangutica</i> Maxim.	China, Xizang, G. & S. Miede 9494/00 (GOET)	AJ606193	AJ606232	AJ606156
<i>Saussurea tatsienensis</i> Franch.	China, Sichuan, Raab-Straube, Smalla & Sun 953 (B)	AJ606188	AJ606228	AJ606140
<i>Saussurea tunicata</i> Hand.-Mazz.	China, Xizang, Miede & Wündisch 94-64-7 (GOET)	AJ606184	AJ606224	AJ605580
<i>Saussurea veitchiana</i> Drum. & Hutch.	China, Hubei, Sino-American Expedition 162 (KUN 729988)	AJ606199	AJ606238	–
<i>Saussurea velutina</i> W. W. Sm.	China, Yunnan, Raab-Straube, Smalla & Sun 800 (B)	AJ606182	AJ606222	AJ606145
<i>Saussurea weitsteiniana</i> Hand.-Mazz.	China, Yunnan, Raab-Straube, Smalla & Sun 801 (B)	AJ606192	AJ606231	AJ606144

Table 3. Origin of plant material of *Saussurea* species used for morphological analysis of achene and pappus microstructure.

<i>Saussurea alpina</i> (L.) DC.	Austria, Kärnten, <i>Vogt 15869 & al.</i> (B) Russia, Altai Republic, <i>Raab-Straube 020383</i> (B)
<i>Saussurea ceratocarpa</i> Decne.	India, [Himachal Pradesh], <i>Lace 1789</i> (B)
<i>Saussurea chenopodiifolia</i> Klatt	Afghanistan, Parwan, <i>Neubauer 4482</i> (B)
<i>Saussurea deltoidea</i> (DC.) Sch. Bip.	India, Darjeeling, <i>Ern 3428</i> , cultivated at BGBM, Berlin, acc. no. 001-28-78-10, <i>Schwerdtfeger 10760</i> (B)
<i>Saussurea frolovii</i> Ledeb.	Russia, Altai Republic, <i>Ždanova & al.</i> 13.8.1987 (B)
<i>Saussurea heteromalla</i> (D. Don) Hand.-Mazz.	Pakistan, Northern Areas, <i>Nüsser 1053</i> (B)
<i>Saussurea japonica</i> (Thunb.) DC.	China, Beijing Shi, <i>Raab-Straube & Steinhof 2001-17</i> (B)
<i>Saussurea medusa</i> Maxim.	China, Sichuan, <i>Raab-Straube, Smalla & Sun 1026</i> (B)
<i>Saussurea obvallata</i> (DC.) Sch. Bip.	China, Sichuan, <i>Raab-Straube, Smalla & Sun 1183</i> (B)

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 *trnL1* [c] / *trnFr* [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 *trnL-trnF* region, it was necessary to sequence additionally with the primer *trnL2r* (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.

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

Alignment and phylogenetic analysis

The ITS (ITS1 and ITS2, omitting the 5.8S rRNA) and *trnL-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.

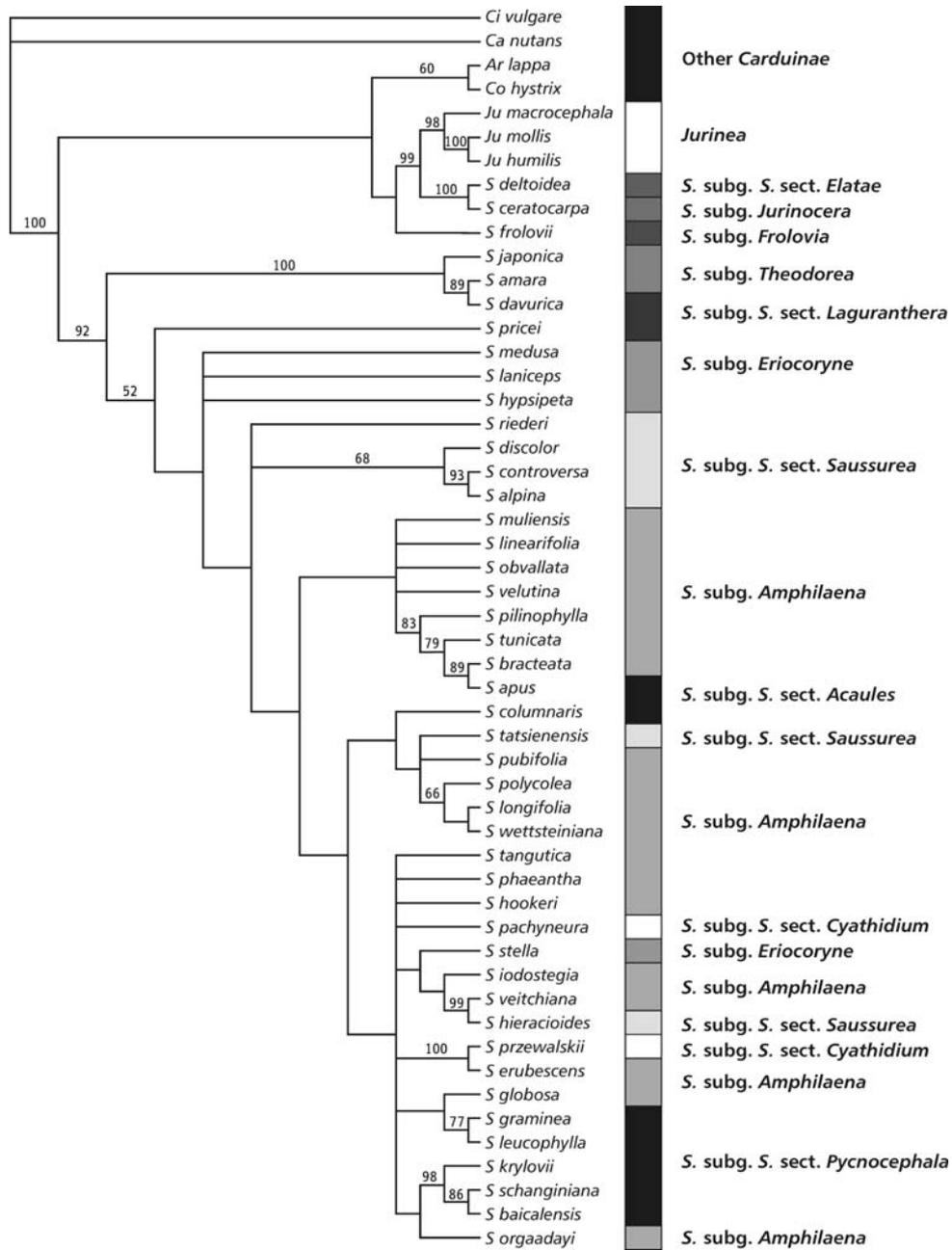


Fig. 1. Strict consensus tree of 3184 equally most parsimonious trees obtained in a heuristic search with 100 random addition sequence replicates. Numbers above branches represent percentages from a bootstrap analysis with 1000 replicates (indicated only when >50 % and branches identical with consensus 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 *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 *Saussurea*. Only 42 of 45 analysed species form a monophyletic lineage with good bootstrap support (92 %). Three *Saussurea* species appear among the outgroup taxa *Arctium*, *Cousinia* and *Jurinea*. A clade consisting of *S. deltoidea* and *S. ceratocarpa* (representing *S. sect. Elatae* and *S. subg. Jurinocera*) receives 100 % support from bootstrap analysis. This clade is sister to the genus *Jurinea*, which is very well resolved as monophyletic, too (bootstrap value 98 %). The sister group relationship between *Jurinea* and *S. subg. S. sect. Elatae* and *S. subg. Jurinocera* receives 99 % support. *S. frolovii* (representing *S. subg. Frolovia*) is sister to the monophyletic group formed by *Jurinea*, *S. subg. S. sect. Elatae* and *S. subg. Jurinocera*, but without support from bootstrap analysis.

The *Saussurea* clade consists of 42 species from *S. subg. Theodorea*, *S. subg. Eriocoryne*, *S. subg. Amphilaena* and *S. subg. Saussurea* (excluding *S. sect. Elatae*). Within this clade, the two analysed members of *S. subg. Theodorea*, *S. japonica* and *S. amara*, together with *S. davurica* from *S. subg. S. sect. Lagurathera*, form a monophyletic group (bootstrap support 100 %). *S. amara* is sister to *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. *S. subg. S. sect. Saussurea*, including the European *S. alpina* and *S. discolor* and the Siberian *S. controversa*, form a moderately supported clade (bootstrap value 68 %). *S. controversa* and *S. alpina* are sister taxa (93 % bootstrap support). The Chinese species from this section (*S. hieracioides* and *S. tatsienensis*) are not part of this clade.

Saussurea pilinophylla, *S. tunicata* and *S. bracteata* (*S. subg. Amphilaena* sect. *Amphilaena*) form a monophyletic, well supported group (bootstrap support 83 %) together with *S. apus* (*S. subg. S. sect. Acaules*, 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 *S. tunicata* (bootstrap support of 79 %).

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

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

The alignment of the *trnL-trnF* region was remarkably uniform. Sequences of the *trnL-trnF* region were almost identical in all analysed species, including the outgroup genera *Carduus*, *Cirsium*, *Arctium*, *Cousinia* and *Jurinea*, and yielded only 12 informative sites in an alignment of

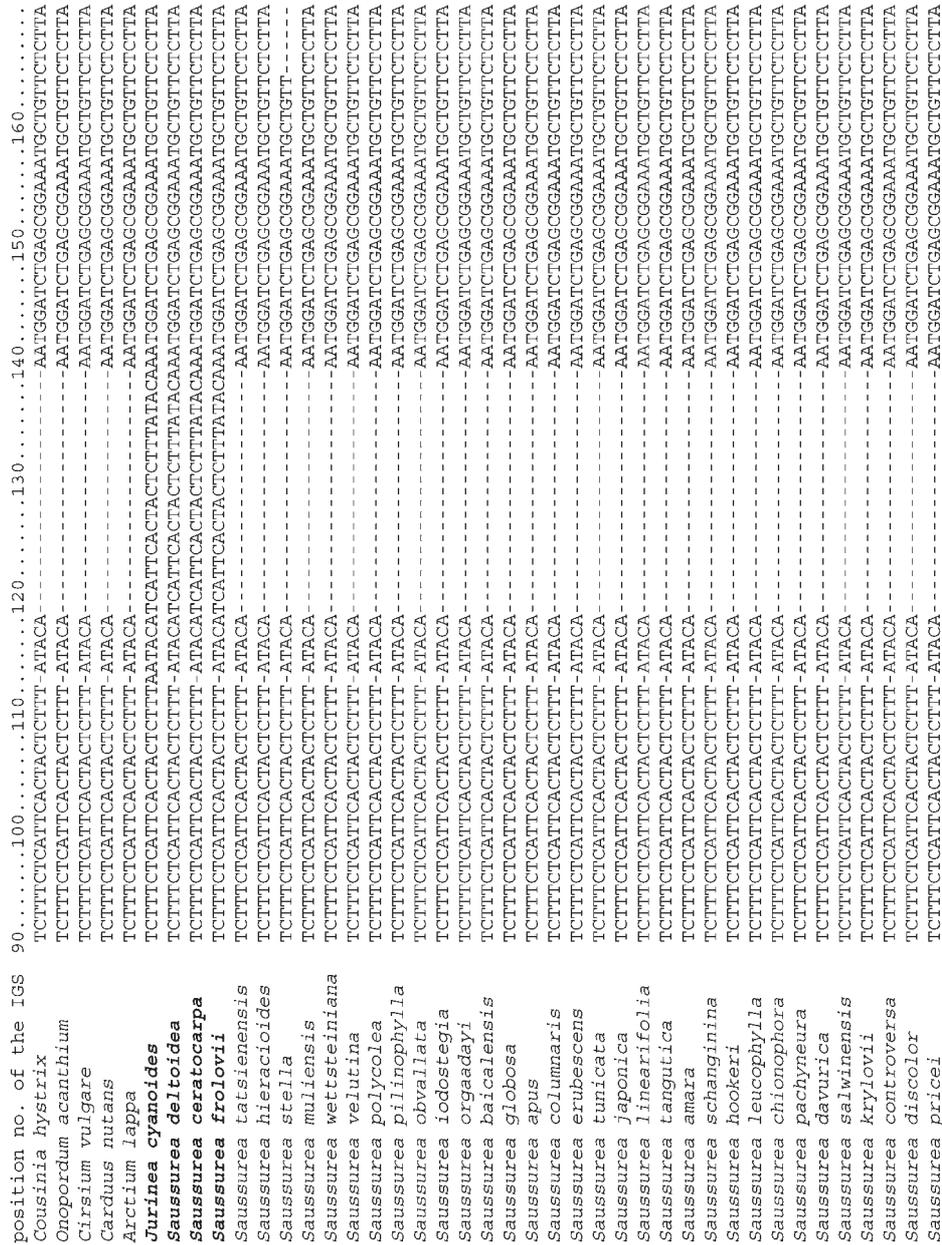


Fig. 2. Part of the alignment of the *trnL-trnF* intergenic spacer (IGS). – Species with 21 bp insertion in bold.

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, obovoidal 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 *Carduinae*. Therefore, it seems perfectly justified to treat *S.* subg. *Jurinocera* as a genus of its own, an idea already informally expressed by Lipšič (1979: 42: "An genus proprium novum?"). Kamelin (1993) described the genus *Lipschitzziella*

Kamelin with two species, *L. carducephala* (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*; achene 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 (Lipšic 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 Lipšic' 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*, *Lipschitzella* and *Frolovia*

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

≡ *Saussurea* sect. *Elatae* Hook. f., Fl. Brit. India 3: 373. 1881, p.p., excl. *S. affinis* Spreng., *S. jacea* C. B. Clarke and *S. denticulata* Wall. ex C. B. Clarke. – Type (Kitamura 1969): *Saussurea heteromalla* (D. Don) Hand.-Mazz. ≡ *Himalaiella heteromalla* (D. Don) Raab-Straube

Himalaiella abnormis (Lipsch.) Raab-Straube, **comb. nova** ≡ *Saussurea abnormis* Lipsch. in Bot. Žurn. 56: 826-827. 1971.

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

= *Jurinea amplifolia* subsp. *remotiloba* Bornm. in Feddes Repert. Spec. Nov. Regni Veg. Beih. 108(1): 55. 1938 ≡ *Saussurea amplifolia* (Bornm.) Rech. f. in Kongel. Danske Vidensk. Selsk. Biol. Skrift. 8(2): 170. 1955, p.p.

Himalaiella albescens (DC.) Raab-Straube, **comb. nova** ≡ *Aplotaxis albescens* DC., Prodr. 6: 540. 1838 ≡ *Saussurea albescens* (DC.) Sch. Bip. in Linnaea 19: 330. 1846.

Himalaiella auriculata (DC.) Raab-Straube, **comb. nova** ≡ *Aplotaxis auriculata* DC., Prodr. 6: 541. 1938 ≡ *Saussurea auriculata* (DC.) Sch. Bip. in Linnaea 19: 331. 1846.

Himalaiella chenopodiifolia (Klatt) Raab-Straube, **comb. nova** ≡ *Saussurea chenopodiifolia* Klatt in Sitzungsber. Math. Phys. Cl. Königl. Bayer. Akad. Wiss. München 8(1): 92. 1878.

Himalaiella chitralica (Duthie) Raab-Straube, **comb. nova** ≡ *Saussurea chitralica* Duthie in Ann. Roy. Bot. Gard. (Calcutta) 9: 45, t. 57. 1901.

= *Jurinea amplifolia* var. *silvatica* Bornm. in Feddes Repert. Spec. Nov. Regni Veg. Beih. 108(1): 54. 1938

= *Jurinea amplifolia* var. *agrestis* Bornm. in Feddes Repert. Spec. Nov. Regni Veg. Beih. 108(1): 54. 1938

= *Saussurea amplifolia* (Bornm.) Rech. f. in Kongel. Danske Vidensk. Selsk. Biol. Skrift. 8, 2: 170. 1955, p.p.

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

Himalaiella foliosa (Edgew.) Raab-Straube, **comb. nova** ≡ *Aplotaxis foliosa* Edgew. in Trans. Linn. Soc. London 20: 77. 1846 ≡ *Saussurea foliosa* (Edgew.) Hook. f., Fl. Brit. India 3: 373. 1881, non Ledeb. 1829 ≡ *Theodorea stracheyana* Kuntze, Revis. Gen. Pl. 1: 368. 1891 ≡ *Saussurea stracheyana* (Kuntze) Lipsch. in Sist. Zametki Mater. Gerb. Krylova Tomsk. Gosud. Univ. Kuybyševa 84: 26. 1967.

Himalaiella heteromalla (D. Don) Raab-Straube, **comb. nova** ≡ *Cnicus heteromallus* D. Don, Prodr. Fl. Nepal.: 166. 1825 ≡ *Saussurea heteromalla* (D. Don) Hand.-Mazz., Symb. Sin. 7(4): 1152. 1936.
= *Aplotaxis candicans* DC., Prodr. 6: 540. 1838 ≡ *Saussurea candicans* (DC.) Sch. Bip. in Linnaea 19: 330. 1846.

Himalaiella nivea (DC.) Raab-Straube, **comb. nova** ≡ *Aplotaxis nivea* DC., Prodr. 6: 541. 1838 ≡ *Saussurea nivea* (DC.) Sch. Bip. in Linnaea 19: 331. 1846, non Turcz. 1837 ≡ *Saussurea deltoidea* var. *nivea* (DC.) Hook. f., Fl. Brit. India 3: 375. 1881.
= *Saussurea crispa* Vaniot in Bull. Acad. Int. Géogr. Bot. 12: 21. 1903.

Himalaiella peguensis (C. B. Clarke) Raab-Straube, **comb. nova** ≡ *Saussurea peguensis* C. B. Clarke, Comp. Ind.: 235. 1876 ≡ *Saussurea deltoidea* var. *peguensis* (C. B. Clarke) Hook. f., Fl. Brit. India 3: 375. 1881.

Lipschitziella Kamelin in Opred. Rast. Sred. Azii 10: 371, 632. 1993 ≡ *Saussurea* subg. *Stephanodontos* Lipsch. in Trudy Moskovsk. Obšč. Isp. Prir. 3: 178. 1960. – Type (Kamelin 1993): *Lipschitziella carduicephala* (Iljin) Kamelin
= *Jurinea* sect. *Jurinocera* Baill., Hist. Pl. 8: 81. 1886 ≡ *Saussurea* subg. *Jurinocera* (Baill.) Lipsch., Rod Saussurea DC.: 42. 1979. – Type (Baillon 1886): *Saussurea ceratocarpa* Decne.

Lipschitziella carduicephala (Iljin) Kamelin in Opred. Rast. Sred. Azii 10: 371, 632. 1993 ≡ *Jurinea carduicephala* Iljin in Acta Horti Bot. Tadshik. 1(1): 64. 1933 ≡ *Saussurea carduicephala* (Iljin) Iljin in Lipsch. in Trudy Moskovsk. Obšč. Isp. Prir. 3: 178. 1960.

Lipschitziella ceratocarpa (Decne.) Kamelin in Opred. Rast. Sred. Azii 10: 632. 1993 ≡ *Saussurea ceratocarpa* Decne. in Jacquemont, Voy. Inde 4: 93, t. 101. 1843 ≡ *Jurinea ceratocarpa* (Decne.) Benth. in Benth. & Hook., Gen. Pl. 2: 474. 1873.

Frolovia (DC.) Lipsch. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 16: 461. 1954 ≡ *Aplotaxis* [unranked] *Frolovia* DC. in Prodr. 6: 538. 1838, p.p., excl. *A. roylei* DC., *A. uniflora* DC., *A. cespitosa* DC., *A. nepalensis* (Spreng.) DC., *A. leontodontoides* DC. and *A. ? pungens* DC. ≡ *Saussurea* sect. *Frolovia* (DC.) Kitam. in Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, Biol. 13: 141. 1937, sensu Lipšić 1979 ≡ *Saussurea* subg. *Frolovia* (DC.) Lipsch. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 21: 370. 1961, p.p., excl. *S. costus* (Falc.) Lipsch. and *S. laneana* W. W. Sm. – Type (Lipšić 1979): *Saussurea frolovii* Ledeb. ≡ *Frolovia frolovii* (Ledeb.) Raab-Straube

Frolovia asbukinii (Iljin) Lipsch. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 16: 462. 1954 ≡ *Saussurea asbukinii* Iljin in Bot. Žurn. 27: 144. 1942.

Frolovia frolovii (Ledeb.) Raab-Straube, **comb. nova** ≡ *Saussurea frolovii* Ledeb., Icon. Pl. 4: 16, t. 352. 1833 ≡ *Aplotaxis frolovii* (Ledeb.) 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. Tadžik. SSR 10: 161, t. 22. 1991 ≡ *Saussurea gilesii* Hemsl. in Hooker's Icon. Pl. 18 [= ser. 3, 8, 2]: ad t. 1736. 1888.

Frolovia gorbunovae (Kamelin) Raab-Straube, **comb. nova** ≡ *Saussurea gorbunovae* Kamelin in Turczaninowia 2(4): 25. 1999.

Frolovia sulcata (Iljin) Lipsch. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 16: 462. 1954 ≡ *Saussurea sulcata* Iljin in Bot. Mater. Gerb. Glavn. Bot. Sada RSFSR 3(26): 101. 1922.

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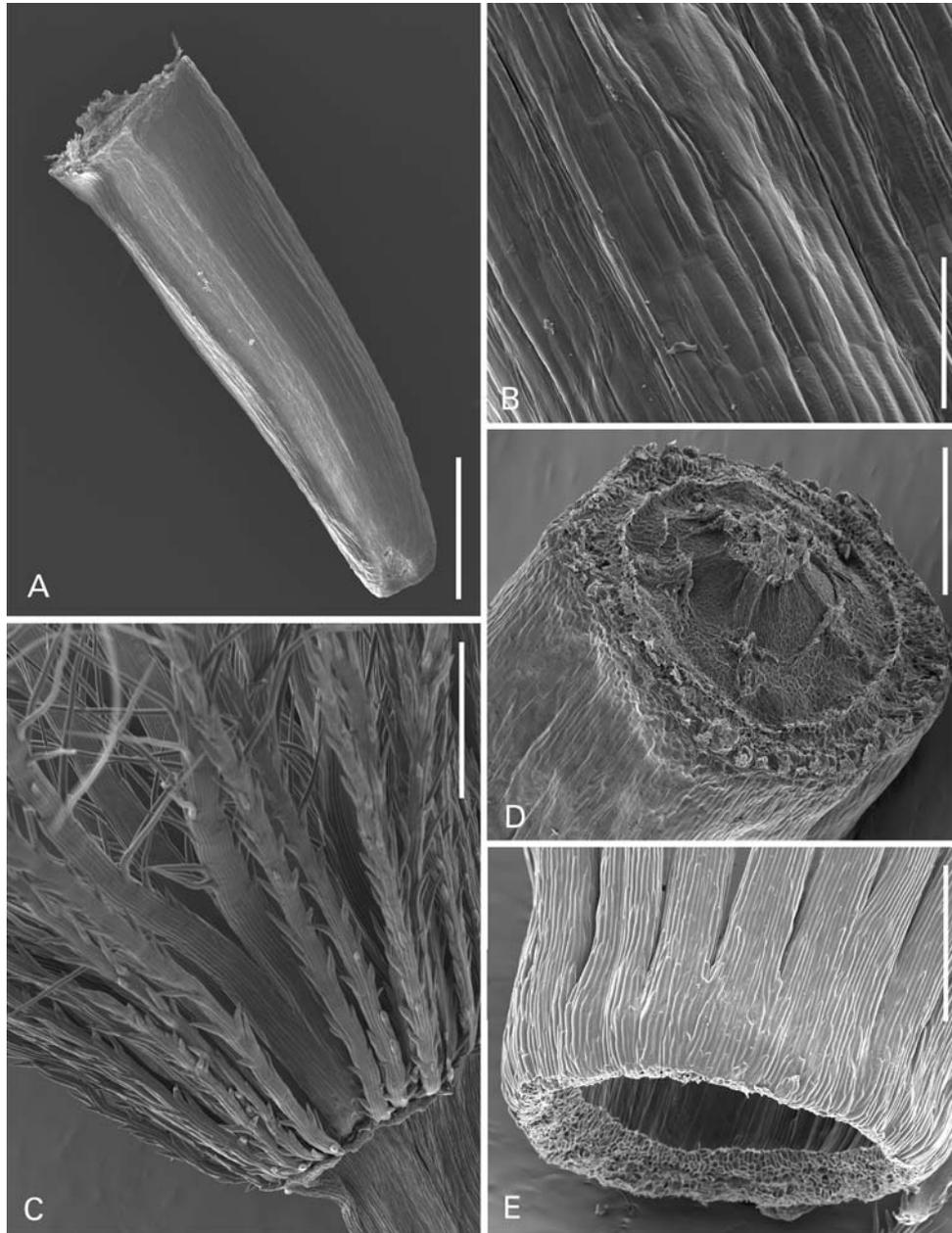


Fig. 3. *Saussurea alpina*, scanning electron micrographs of achenes and pappus – A: overview; B: surface; C: pappus detail; D: apical region; E: base of inner pappus (A-B, D-E from Vogt 15869 & al; C from Raab-Straube 020383). – Scale bars A = 1 mm, B = 0.09 mm, C = 0.5 mm, D = 0.4 mm, E = 0.4 mm.

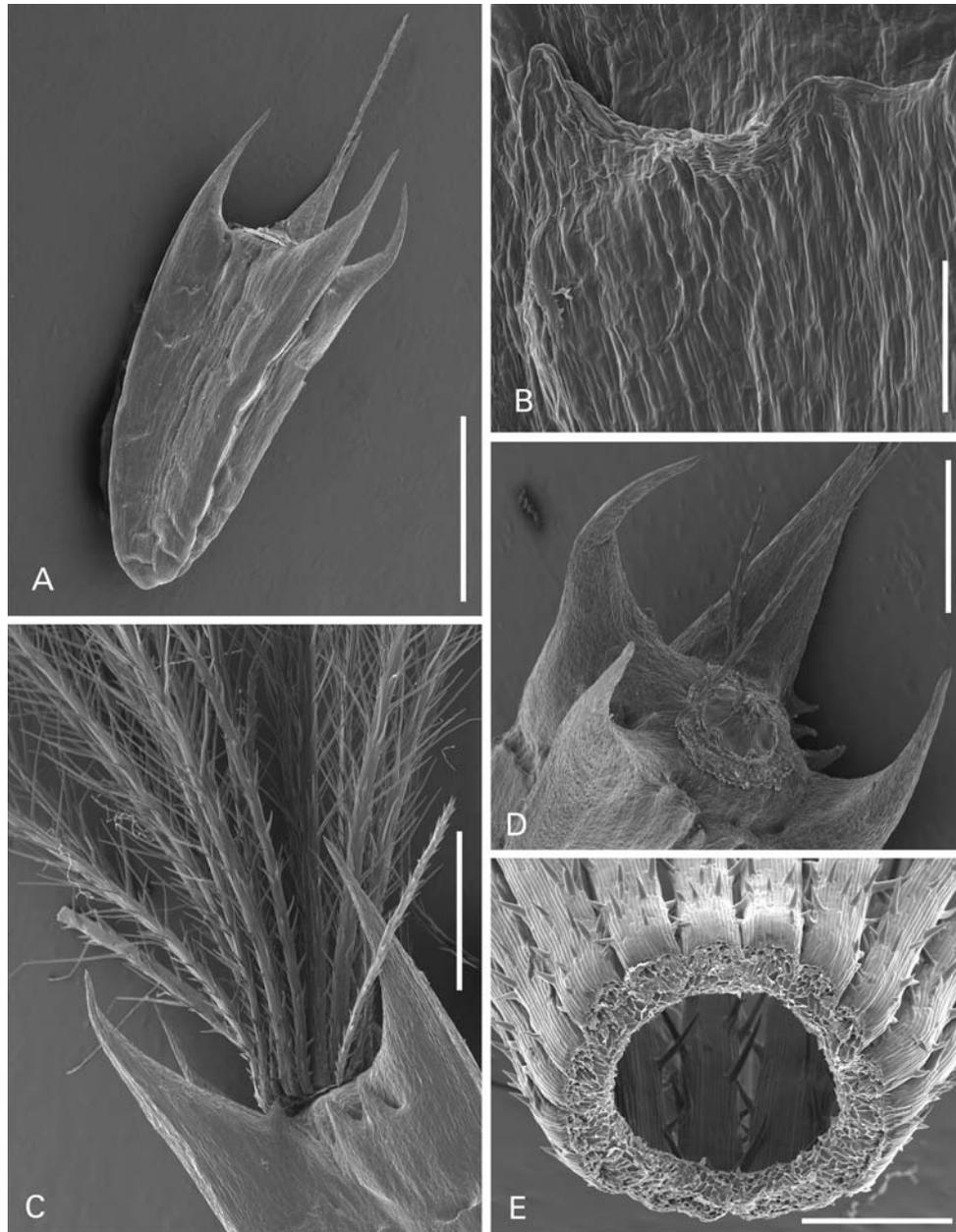


Fig. 4. *Lipschitzziella ceratocarpa*, scanning electron micrographs of achenes and pappus (from *Lace 1789*) – A: overview; B: surface; C: pappus detail; D: apical region; E: base of inner pappus. – Scale bars A = 2 mm, B = 0.09 mm, C = 0.9 mm, D = 0.8 mm, E = 0.3 mm.

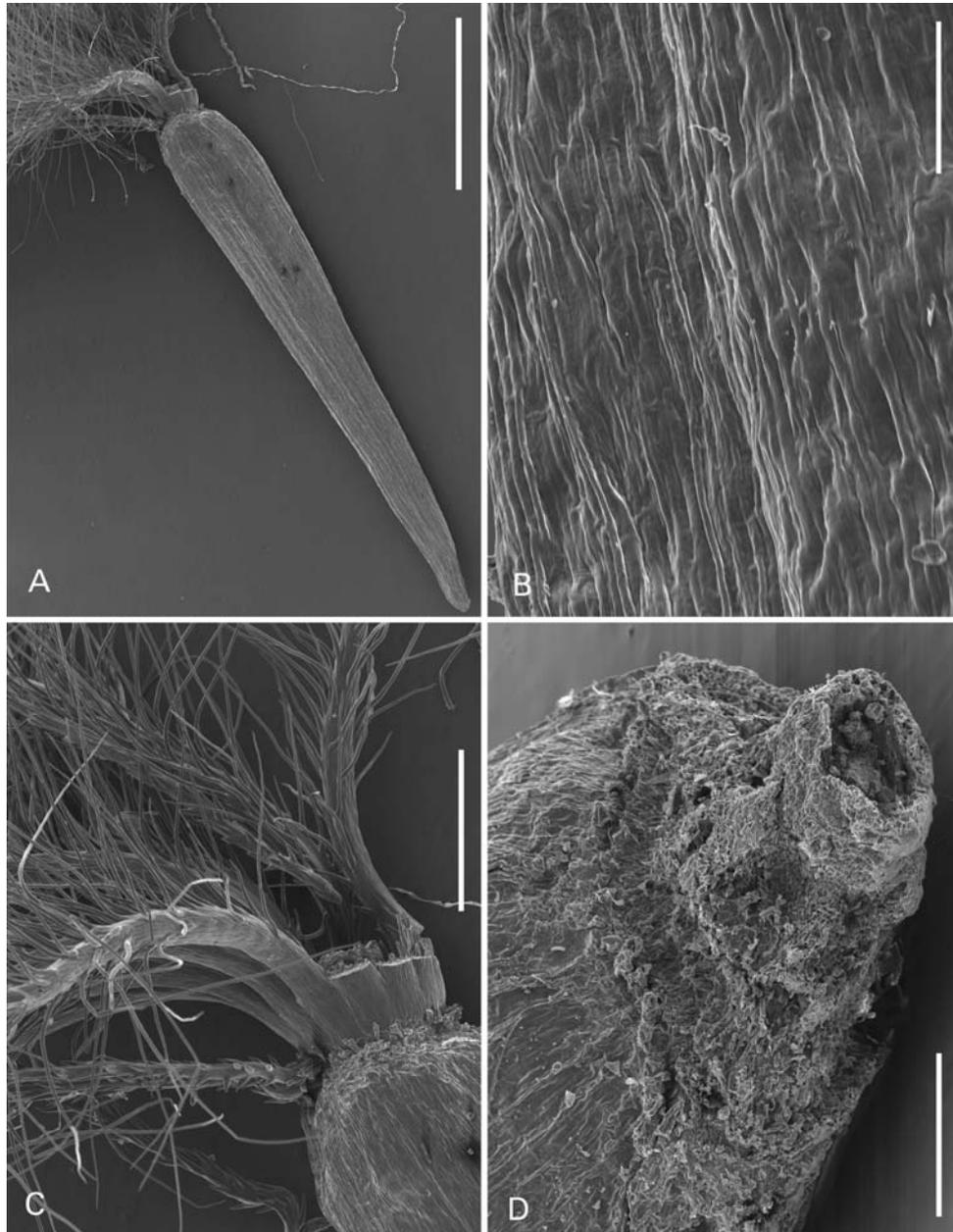


Fig. 5. *Saussurea medusa*, scanning electron micrographs of achenes and pappus (from Raab-Straube, Smalla & Sun 1026) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 3 mm, B = 0.09 mm, C = 0.8 mm, D = 0.3 mm.

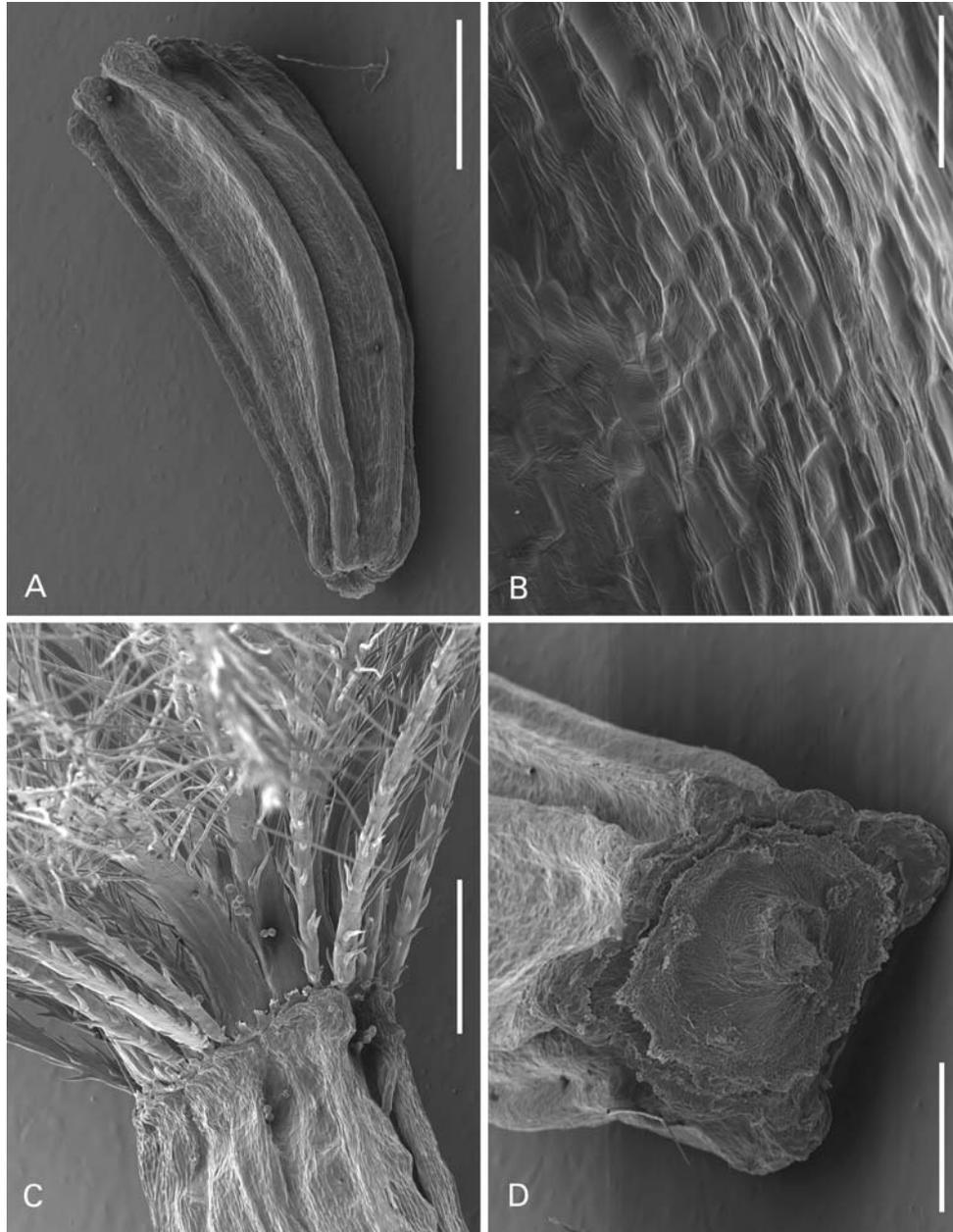


Fig. 6. *Saussurea obvallata*, scanning electron micrographs of achenes and pappus (from Raab-Straube, Smalla & Sun 1183) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 1 mm, B = 0.09 mm, C = 0.7 mm, D = 0.5 mm.

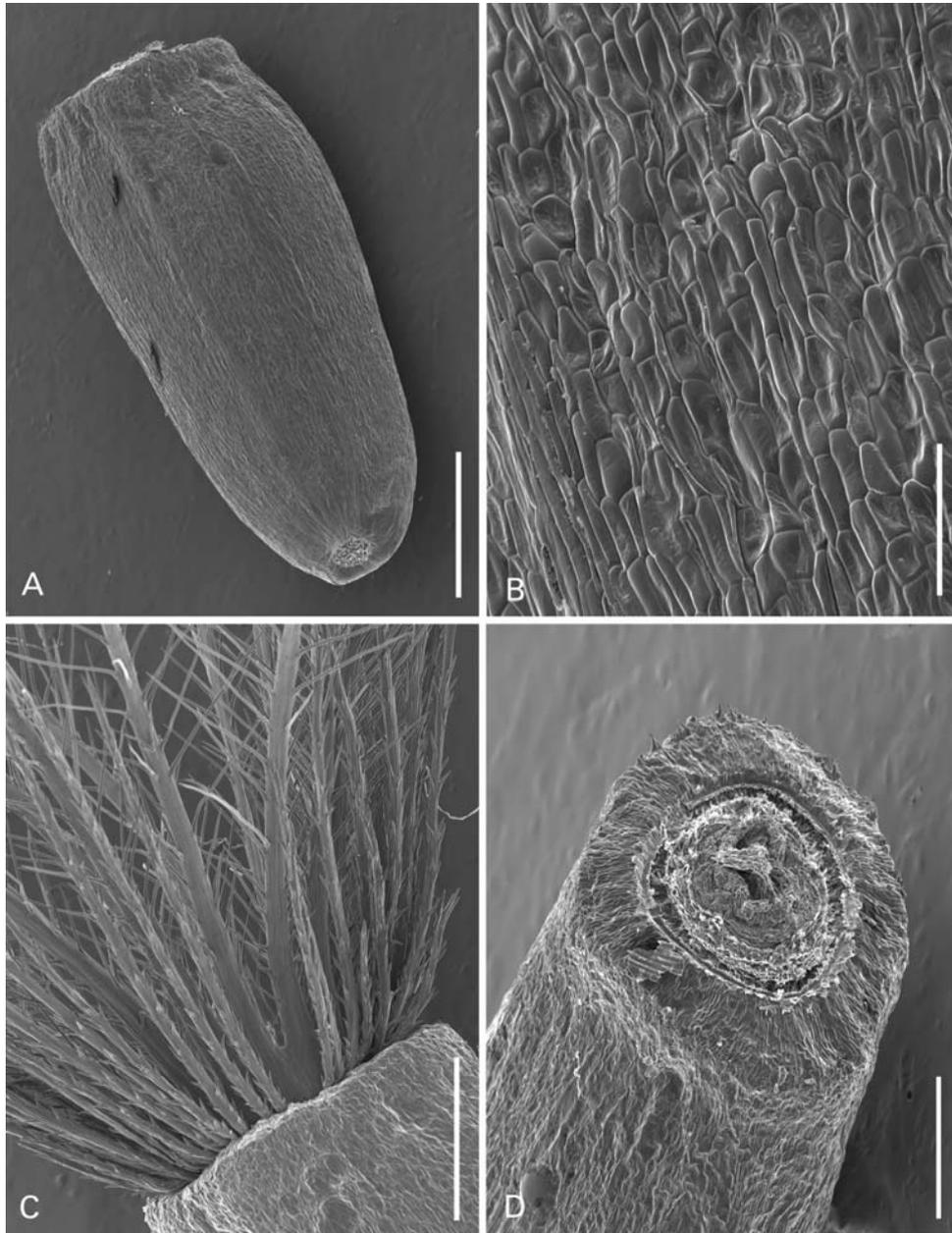


Fig. 7. *Saussurea japonica*, scanning electron micrographs of achenes and pappus (from Raab-Straube & Steinhof 2001-17) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 0.9 mm, B = 0.09 mm, C = 0.6 mm, D = 0.4 mm.

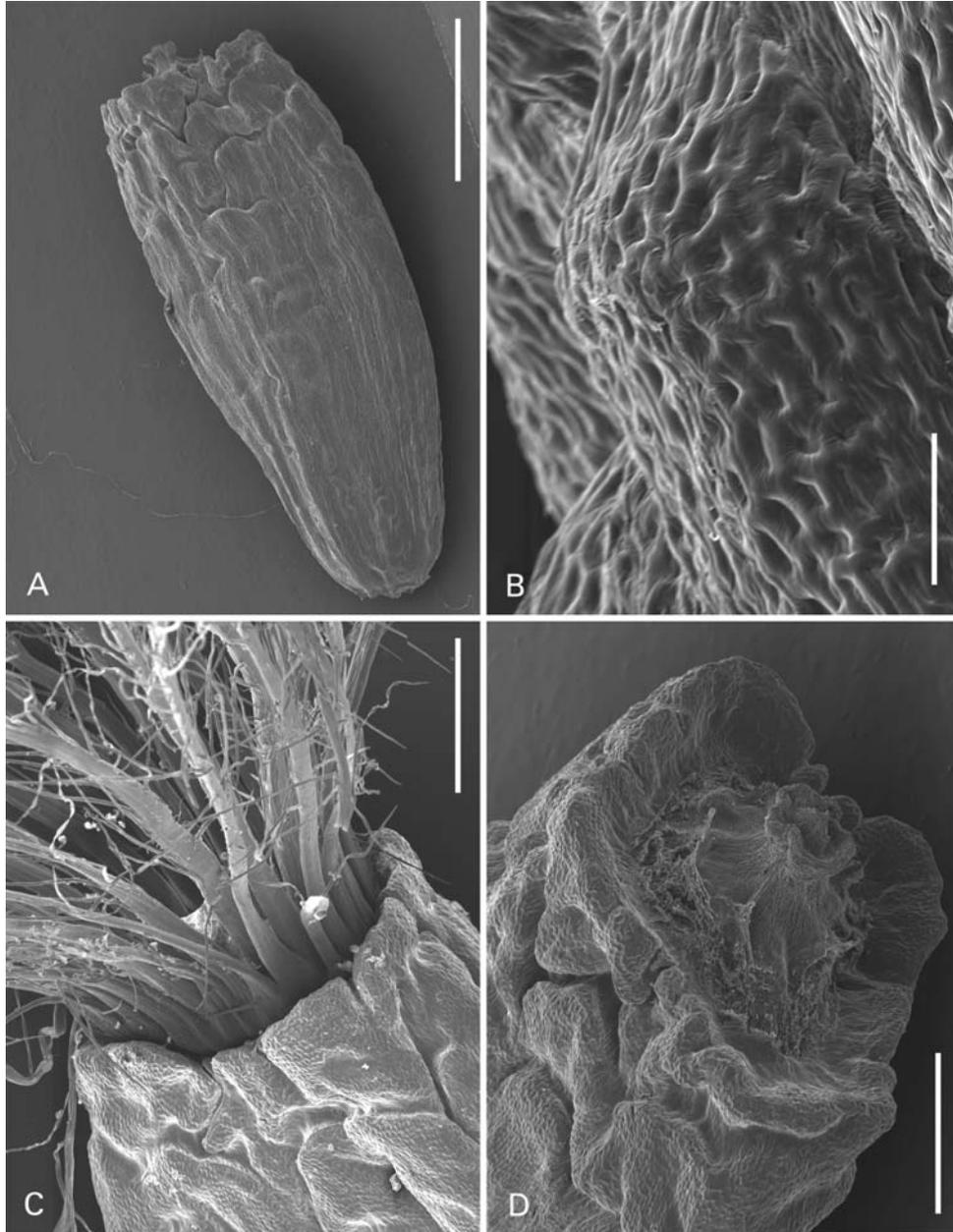


Fig. 8. *Frolovia frolovii*, scanning electron micrographs of achenes and pappus (from Žanova & al., 13.8.1987) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 2 mm, B = 0.09 mm, C = 0.8 mm, D = 0.7 mm.

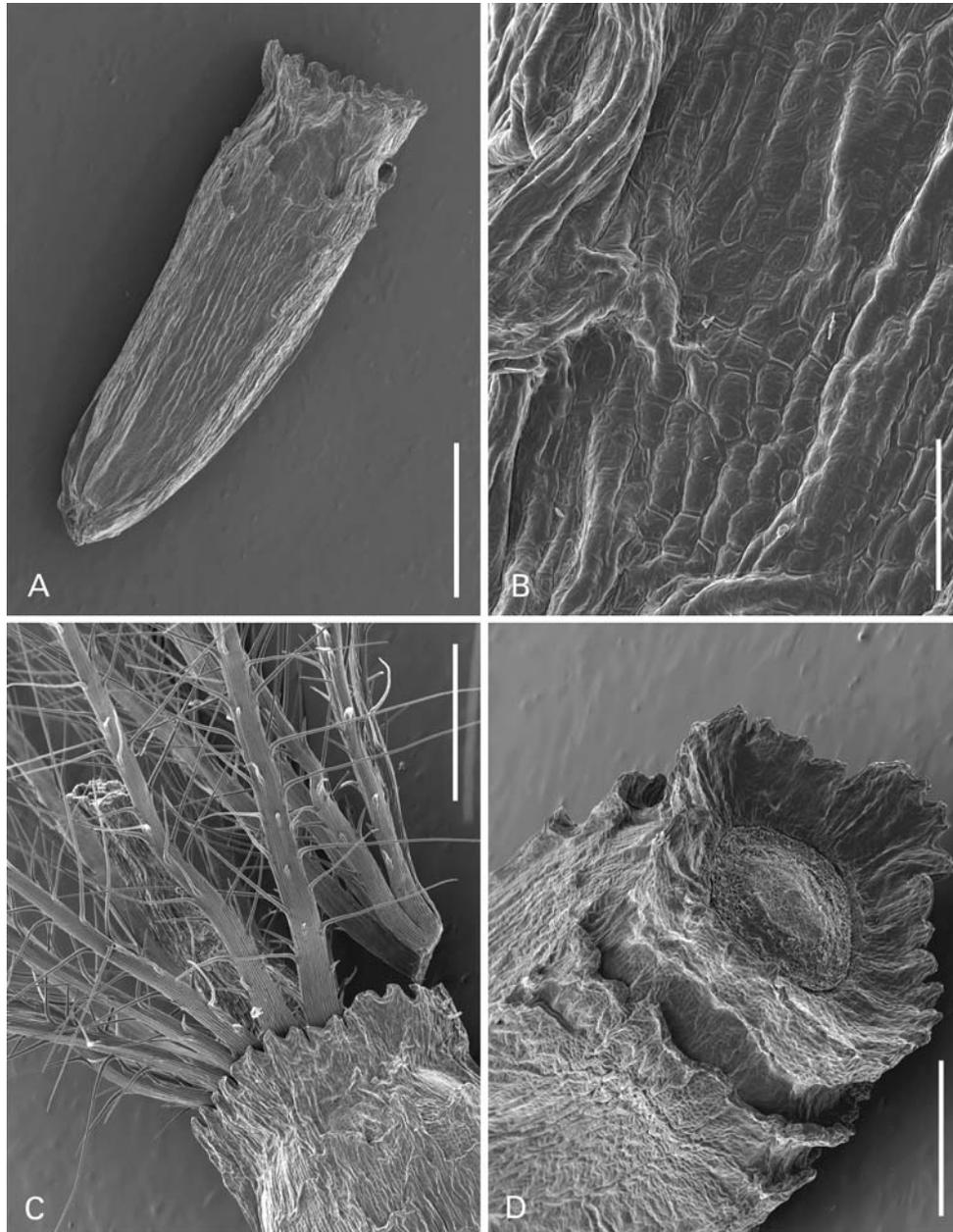


Fig. 9. *Himalaiella deltoidea*, scanning electron micrographs of achenes and pappus (from BGBM acc. no. 001-28-78-10) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 1 mm, B = 0.09 mm, C = 0.6 mm, D = 0.5 mm.

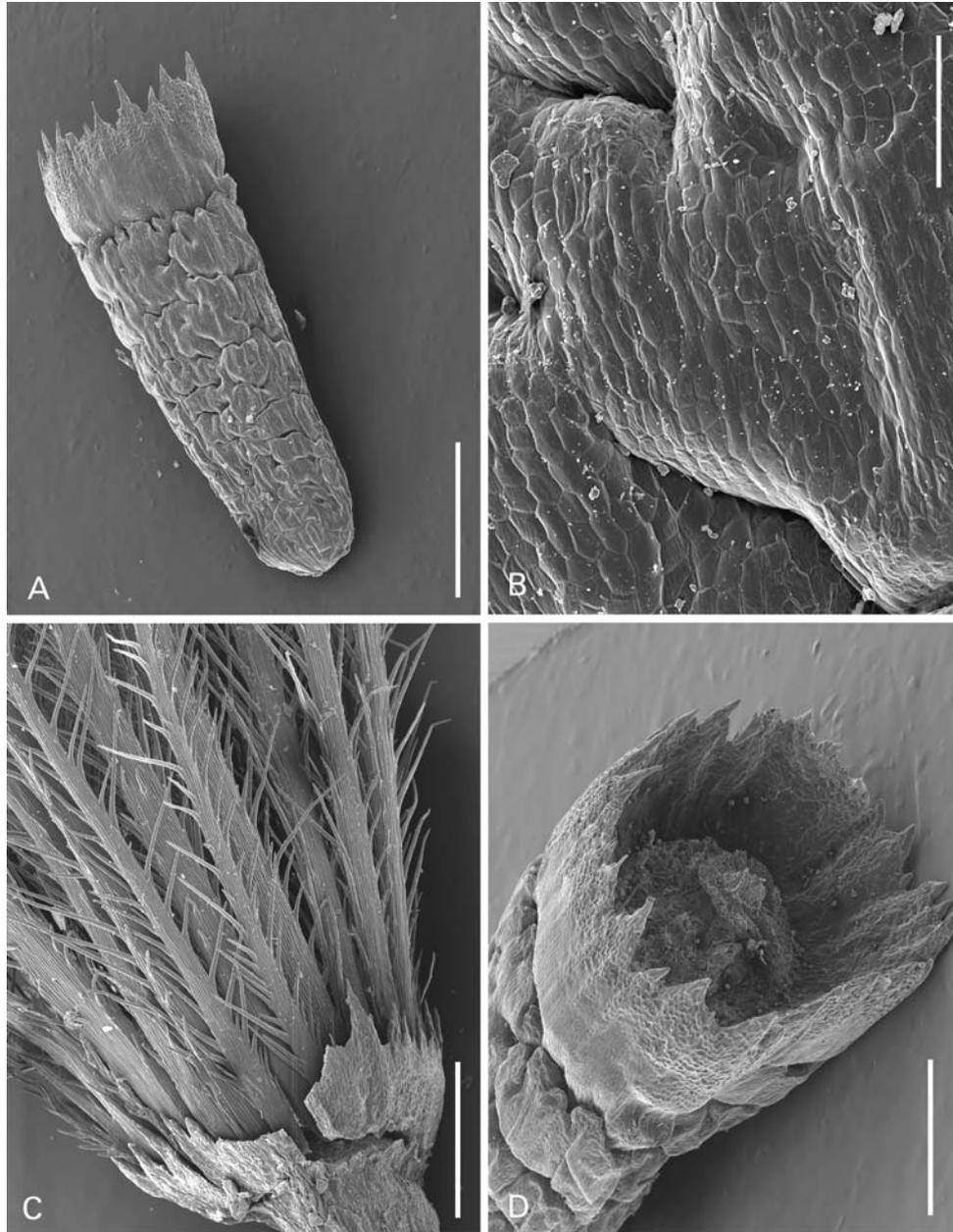


Fig. 10. *Himalaiella chenopodiifolia*, scanning electron micrographs of achenes and pappus (from *Neubauer 4482*) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 1 mm, B = 0.09 mm, C = 0.5 mm, D = 0.5 mm.

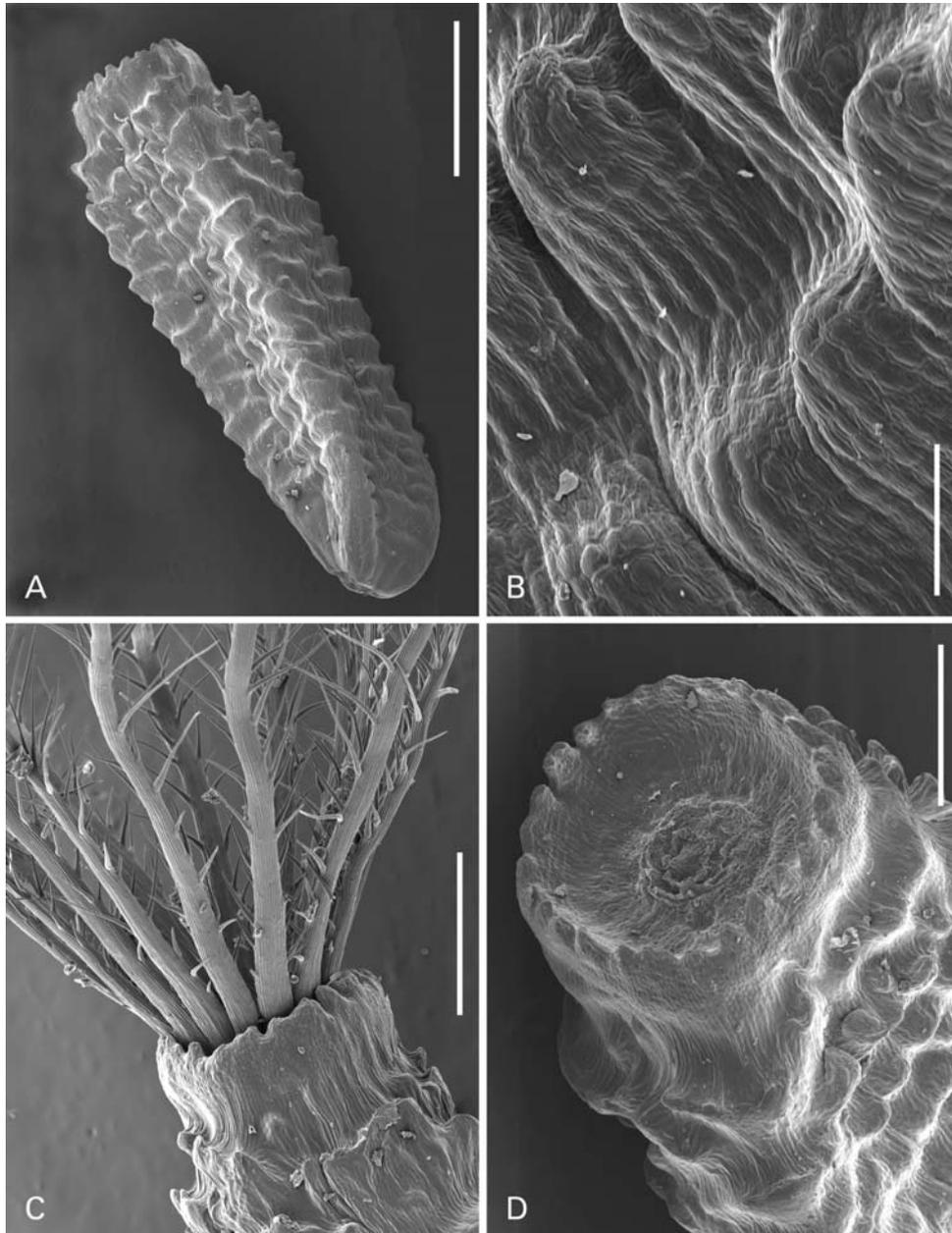


Fig. 11. *Himalaiella heteromalla*, scanning electron micrographs of achenes and pappus (from Nüsser 1053) – A: overview; B: surface; C: pappus detail; D: apical region. – Scale bars A = 1 mm, B = 0.09 mm, C = 0.6 mm, D = 0.4 mm.