RESEARCH ARTICLE

The position of two recently described endemic taxa in the system of the genus *Saussurea* (Asteraceae)

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Citation: Yusupovsky DY, Pyak EA (2024) The position of two recently described endemic taxa in the system of the genus *Saussurea* (Asteraceae). Acta Biologica Sibirica 10: 1479–1486. https://doi.org/10.5281/zenodo.14286645

Abstract

Two new species of the genus *Saussurea*, endemic to the Chuya Steppe, wast intermountain depression in the south-east of the Russian part of the Altai Mountains, have recently been described on a morphological and phylogenetic basis. *Saussurea draconis* was found to be closely related to *S. salsa* and *S. turgaiensis* from *S.* sect. *Laguranthera*. *Saussurea* × *magica* was found to originate from the hybridisation between *S. amara* and *S. daurica*, belonging to *S.* sect. *Theodorea* and *S.* sect. *Laguranthera*, respectively. The polyphyly of the latter has been repeatedly demonstrated by the results of molecular phylogenetic analyses, and the position of the species of ser. *Salsae* (to which *S. salsa*, *S. turgaiensis* and *S. daurica* belong) in this section raises, in particular, obvious doubts. This prompted us to undertake an investigation of the aforementioned species, with a view to clarifying their systematic position, based on an analysis of the ITS1-5.8S-ITS2, *rsp*16-trnQ, *ndh*F-rpl32, *ycf*4-*cem*A regions and a comparative morphological analysis. The results of this investigation led to the conclusion that the position of *S.* × *magica* in *S.* subgen. *Theodorea* sect. *Theodorea* is confirmed. *Saussurea salsa*, *S. daurica*, *S. turgaiensis*, *S. kaschgarica* and *S. pseudosalsa*, i.e. species of the ser. *Salsae*, as well as *S. draconis*, were transferred from *S.* subgen. *Saussurea* sect. *Laguranthera* to *S.* subgen. *Theodorea* sect. *Theodorea*.

Keywords

Altai Mountains, taxonomic position, *Theodorea*, *Salsae*, *Saussurea draconis*, *Saussurea* × *magica*

Introduction

Recently, *S. draconis* Yusupovsky & E. Pjak was described as a new species, and *S. × magica* Yusupovsky & E. Pjak was described as a new nothospecies originated from the hybridisation between *S. amara* (L.) DC. and *S. daurica* Adams, based on phylogenetic and morphological data (Yusupovsky and Pyak 2024). As a result of this work, the systematic position of both the new species and the new nothospecies remained unclear. *Saussurea draconis*, which is closely related to *S. salsa* Spreng. and *S. turgaiensis* B. Fedtsch., was provisionally placed in S. subgen. *Saussurea* DC. sect. *Laguranthera* (C. A. Meyer ex Endlicher) Lipsch. However, the polyphyly of this section and the close affinity of the species belonging to *S.* sect. *Laguranthera* ser. *Salsae* Lipsch. to the species belonging to *S.* subgen. *Theodorea* (Cass.) Lipsch. sect. *Theodorea* (Cass.) Lipsch., has already been demonstrated previously, as shown by the results of molecular analyses by Raab-Straube (2003), Kita et al. (2004), Wang et al. (2009), Xu et al. (2019). *Saussurea × magica* was preliminary placed in *S.* subgen. *Theodorea* sect. *Theodorea* on the basis of the presence of membranous purple appendages on the phyllaries, which is the main diagnostic morphological feature of the section, but the parental species of *S. × magica*, i.e. *S. amara* and *S. daurica*, belong to the sections *Theodorea* and *Laguranthera*, respectively. This highlights the necessity for a molecular and morphological investigation of this group to accurately determine the systematic position of the recently described species.

Materials and methods

To study the systematic position of these taxa, we included DNA sequences of 51 species from *S.* subgen. *Theodorea* sect. *Theodorea* (16 of 22 species), *S.* subgen. *Saussurea* sect. *Laguranthera* (21 species), *S.* subgen. *Saussurea* (2 species from *S.* sect. *Rosulascentes* (Kitam.) Lipsch., 2 species from *S.* sect. *Lagurostemon* (Cass.) DC., 2 species from *S.* sect. *Strictae* C. B. Clarke, 1 species from *S.* sect. *Gymnocline* Franch., 3 species from *S.* sect. *Saussurea*), *S.* subgen. *Eriocoryne* (DC.) Hook. f. sect. *Eriocoryne* (Wall. ex DC.) Hook. f. (2 species) and *S.* subgen. *Amphilaena* (Stschegl.) Lipsch. sect. *Amphilaena* (Stschegl.) Lipsch. (2 species) to construct phylogenetic networks and midpoint-rooted phylogenetic trees. In total, 120 sequences were downloaded from GenBank and 36 sequences were generated for this study. All voucher specimens (see Suppl. material 1: Table 1) were deposited in the P.N. Krylov Herbarium of Tomsk State University (TK), NCBI GenBank accession numbers are listed in Suppl. material 2: Table 2. The morphological analyses were based on both field observations and herbarium specimens, either collected by the authors or previously deposited in the herbaria (TK, MW, LE). Detailed floral morphology was observed from dried herbarium material using a Zeiss Stemi 508 stereo microscope (Carl Zeiss, Oberkochen, Germany).

Total genomic DNA was extracted from silica gel dried young leaves using a commercially available kit, D-Plants-250 (Biolabmix, Novosibirsk, Russia). All amplification primers and detailed amplification protocol are presented in the previous study (Yusupovsky and Pyak 2024). Neighbor-net phylogenetic networks based on ITS1-5.8S-ITS2 and concatenated ITS1-5.8S-ITS2 and *rsp*16-*trn*Q + *ndh*F-*rpl*32 + *ycf*4-*cem*A data sets were constructed in SplitsTree 4 (Huson and Bryant 2006) using uncorrected Hamming distances. Phylogenetic trees were constructed using the Bayesian and Maximum likelihood methods based on ITS1-5.8S-ITS2 data set. Bayesian analyses were conducted using Mr.Bayes 3.2.6 (Ronquist et al. 2012) with four simultaneous Markov Chain Monte Carlo (MCMC) of 1000000 generations, with one tree sampled every 500 generations until the average deviation of split frequencies reached a value below 0.01. The optimal evolutionary model was identified using MrModelTest 2.3 (Nylander 2004) and PAUP (Swofford 2003), in accordance with AIC, which was determined to be GTR+G. Maximum likelihood tree was reconstructed using IQ-TREE 1.6.12 (Nguyen et al. 2015) with the best-fit model TNe+G4 calculated using ModelFinder (Kalyaanamoorthy et al. 2017). Branch support was obtained through the using of 100000 ultra-fast bootstrap replicates (Hoang et al. 2018). Reconstructed phylogenetic trees were drawn in the FigTree 1.4.4 (Rambaut 2012).

Result

The topologies of the ITS1-5.8S-ITS2 phylogenetic trees yielded by Maximum likelihood and Bayesian analyses were found to be congruent. Consequently, only the Maximum likelihood tree is presented (Fig. 1B). The phylogenetic analysis revealed that *S. salsa*, *S. turgaiensis*, *S. kaschgarica* Rupr., *S. pseudosalsa* and *S. daurica*, which belong to ser. *Salsae* (Lipschitz 1979), as well as *S. draconis* and *S. × magica*, form a strongly supported clade (PP = 1, BS = 100) with all species of sect. *Theodorea* included in the analysis, with the exception of *S. nematolepis* Ling, *S. pseudomalitiosa* Lipsch. and *S. chingiana* Hand.-Mazz., resulted to be sister to the species of sect. *Laguranthera* (PP = 0.99, BS = 82). Possible network-like evolutionary relationships among the studied species, reconstructed based on the ITS1-5.8S-ITS2 marker, are in agreement with the results of the ML and BA analyses about the main groups (Fig. 1A). The combined ITS1-5.8S-ITS2 and *rsp*16-*trn*Q + *ndh*F-*rpl*32 + *ycf*4-*cem*A data sets yielded a splits graph that did not show incongruence between the nuclear and chloroplast datasets, indicating a lack of conflict in the position of *S. draconis*, *S. × magica*, *S. salsa*, *S. turgaiensis*, *S. kaschgarica*, *S. pseudosalsa* and *S. daurica* within sect. *Theodorea* (Fig. 2). The obtained splits graph confirms the close affinity of *S. draconis*, *S. × magica* and the species belonging to ser. *Salsae* with *S. amara*, *S. japonica* (Thunb.) DC., *S. pinnatidentata* Lipsch., *S. pulchella* (Fisch.)

Fisch., *S. paradoxa* Lipsch., *S. ceterachifolia* Lipsch., *S. laciniata* Ledeb., *S. runcinata* DC., *S. robusta* Ledeb. belonging to sect. *Theodorea*. This group is well separated (BS = 99.9) from all other species belonging to sect. *Laguranthera* included in the study.

Comparative morphological analysis of the included species revealed that the representatives of sect. *Theodorea* possess a complex morphology of vegetative and generative organs. *Saussurea laciniata*, *S. ramosa* Lipsch., *S. ceterachifolia*, *S. runcinata*, *S. paradoxa*, *S. alata* DC. are characterised by the presence of fleshy leaves, phyllaries with a small and membranous purple appendage at the apex and few to many stems. *Saussurea salsa*, *S. turgaiensis*, *S. kaschgarica*, *S. pseudosalsa* and *S. draconis* are also characterised by the presence of fleshy leaves, few to many stems (solitary to few in *S. pseudosalsa*), but their phyllaries are without a membranous appendage at the apex. *Saussurea robusta* and *S. nematolepis* have non fleshy leaves, solitary stem, and the phyllaries without membranous appendage, but apically subulate and reflexed. *Saussurea japonica* (Thunb.) DC., *S. pulchella*, *S. amara*, *S. pseudomalitiosa* and *S. chingiana* are characterised by non fleshy leaves, solitary stem and phyllaries with a small and membranous purple appendage. *Saussurea prostrata* C. Winkl., *S. pinnatidentata* Lipsch. and *S. × magica* possess fleshy leaves, solitary stem (solitary to few in *S. prostrata*) and phyllaries with a small and membranous purple appendage. All the mentioned species have straw-coloured pappus (*S. daurica* is characterised by white pappus, but populations with straw-colored pappus are found in nature, *S. × magica* is characterised by straw-colored below middle and white above middle pappus) and anther basal appendages with lacerate tails. Morphological comparisons with the species belonging to sect. *Laguranthera* are presented in Figure 1C.

Discussion

The position of the species belonging to ser. *Salsae* in sect. *Theodorea* is supported by both molecular and comparative morphological analyses. *Saussurea salsa*, *S. turgaiensis*, *S. kaschgarica*, *S. pseudosalsa*, *S. daurica*, i.e. 5 of 6 species of ser. *Salsae*, as well as recently described *S. draconis*, need to be transferred in sect. *Theodorea*. The provisional placement of *S. × magica* in sect. *Theodorea* by Yusupovsky and Pyak (2024) is confirmed. *Saussurea famintziniana* Krasn., the only species of ser. *Salsae* which was not included in the phylogenetic analyses, requires further special study to establish its systematic position, we prefer to leave it in sect. *Laguranthera* until more data is available.

The monophyly of subgen. *Theodorea* remains unconfirmed even after the transfer of the aforementioned taxa, as the position of three species endemic to China, *S. nematolepis*, *S. pseudomalitiosa* and *S. chingiana*, remains unclear and requires detailed analysis. Furthermore, five species (*S. ladyginii* Lipsch., *S. chinnampoensis*

H.Lév. & Vaniot, *S. tsoongii* Y.S. Chen, *S. jurineoides* H.C. Fu, *S. malitiosa* Maxim.) grown predominantly in China remain unstudied. This underscores the necessity for further research and complete revision of the subgenus. According to the results of comparative morphological analysis, the presence of appendages, which have traditionally been used as the main diagnostic character of the section, cannot be employed as an independent, but only in combination with other vegetative and generative organ characteristics, such as leaf texture, colour of the pappus and anther appendages. Moreover, habitat is found to be very important for differentiating the sections *Laguranthera* and *Theodorea*, thus, representatives of the first section occur mainly on rocky, stony and clay steppe slopes, representatives of the second section occur mainly in intermountain basins, preferring moist solonetz steppe habitats.
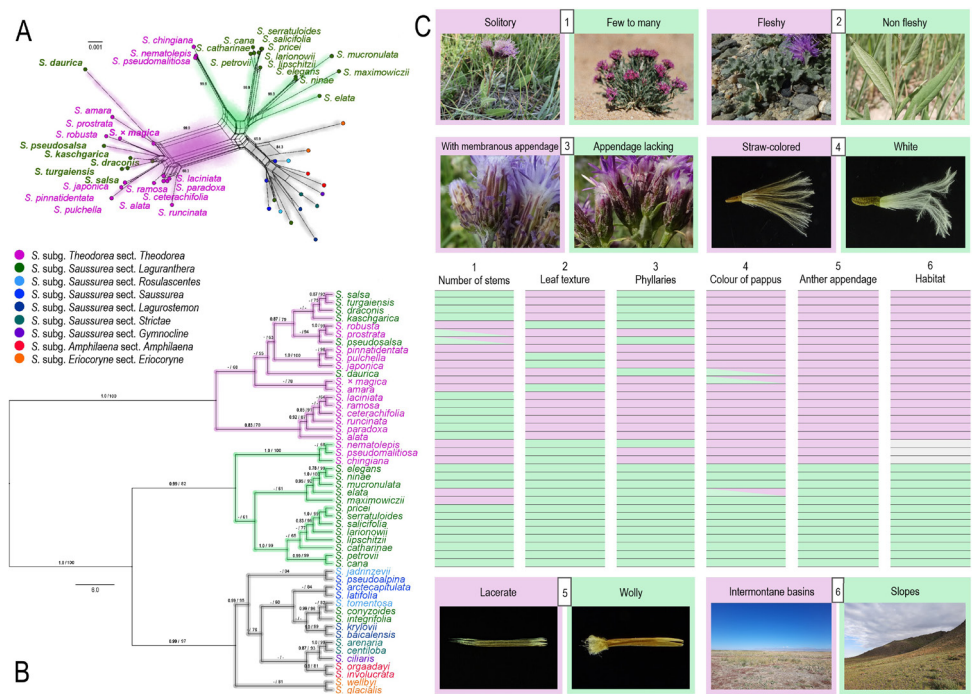

Figure 1. Maximum likelihood phylogenetic trees and NeighbourNet phylogenetic networks of *Saussurea* species. **A** – Phylogenetic network inferred from ITS1-5.8S-ITS2 sequence data with bootstrap values greater than 50% given for the main clusters; **B** – Phylogenetic tree based on ITS1-5.8S-ITS-2 sequence data. Numbers above branches represent posterior probability greater than 0.70 and ML bootstrap values greater than 50%. Colours highlight the corresponding clades in trees and clusters in networks; **C** – Comparison of morphological features and habitat, grey colour – no data.
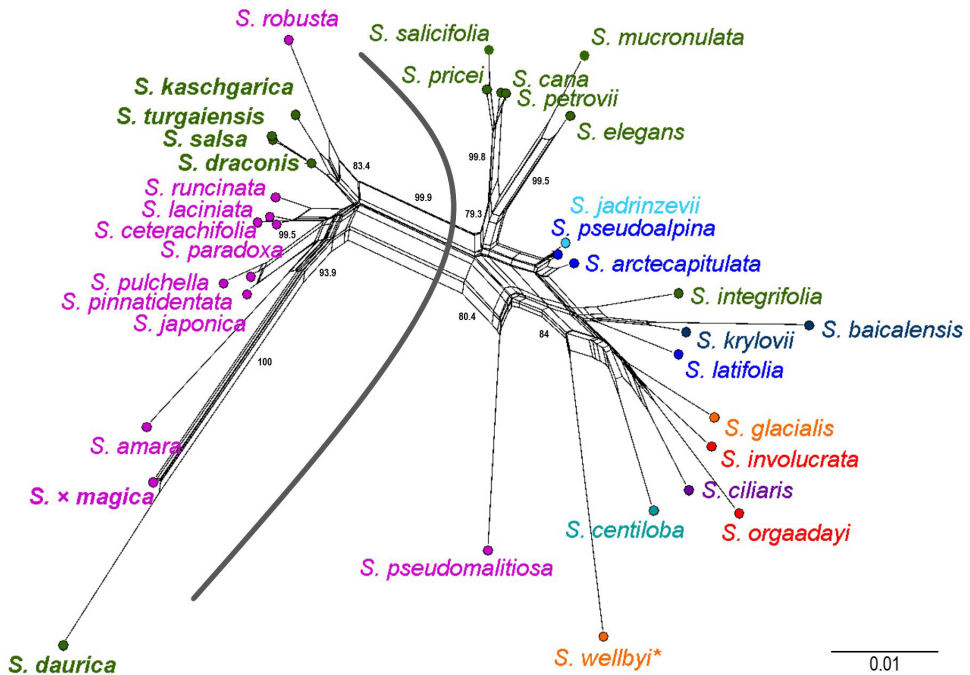
Figure 2. NeighbourNet phylogenetic network, based on combined ITS1-5.8S-ITS2 and *ndh*F-*rpl*32 + *rsp*16-*trn*Q + *ycf*4-*cem*A sequence data, with bootstrap values greater than 75% given for the main clusters.

Acknowledgements

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Supplementary material 1

Table 1. NCBI GenBank accession numbers, all newly generated sequences are in bold

Authors: Daniil V. Yusupovsky, Elizaveta A. Pyak
Data type: table

Link: http://journal.asu.ru/biol/article/view/16509/14075

Supplementary material 2

Table 2. Origins of plant materials used in the study

Authors: Daniil V. Yusupovsky, Elizaveta A. Pyak
Data type: table
Link: http://journal.asu.ru/biol/article/view/16509/14076