

Plant Syst Evol (2010) 288:25–42
DOI 10.1007/s00606-010-0310-5

ORIGINAL ARTICLE

Phylogeny of Salsoleae s.l. (Chenopodiaceae) based on DNA sequence data from ITS, *psbB*–*psbH*, and *rbcL*, with emphasis on taxa of northwestern China

Zhi-Bin Wen · Ming-Li Zhang · Ge-Lin Zhu ·
Stewart C. Sanderson

Received: 15 December 2009 / Accepted: 12 May 2010 / Published online: 27 June 2010
© Springer-Verlag 2010

Abstract To reconstruct phylogeny and verify the monophyly of major subgroups, a total of 52 species representing almost all species of Salsoleae s.l. in China were sampled, with analysis based on three molecular markers (*nrDNA* ITS, *cpDNA psbB*–*psbH* and *rbcL*), using maximum parsimony, maximum likelihood, and Bayesian inference methods. Our molecular evidence provides strong support for the following: (1) Camphorosmeae is nested within Salsoleae s.l. instead of the previously suggested sister relationship. (2) Tribe Salsoleae s.l. is monophyletic and is composed of three monophyletic subunits, Caroxyloneae, the *Kali* clade, and Salsoleae s.str. (3) *Climacoptera* is separated from *Salsola* s.l. It does not form a monophyletic group but is split into two monophyletic parts, *Climacoptera* I and *Climacoptera* II. (4)

Halogeton is clearly polyphyletic, as are *Anabasis* and the genus *Salsola* s.l. (5) *Caroxylon*, *Haloxydon*, *Kali*, and *Petrosimonia* are well-supported monophyletic genera. Additional evidence is needed regarding the monophyly of *Halimocnemis*, which remains unclear.

Keywords Salsoleae s.l. · *Anabasis* · *Climacoptera* · *Halogeton* · Molecular phylogeny · China

Introduction

Chenopodiaceae comprises ca. 110 genera and ca. 1,700 species worldwide (Kadereit et al. 2003), mainly distributed throughout arid, semiarid, saline, and hypersaline ecosystems of temperate and subtropical regions (Pyankov et al. 2001a; Kadereit et al. 2003, 2005; Akhani et al. 2007). The tribe Salsoleae s.l. is one of the largest tribes within Chenopodiaceae, including one-third of the genera currently recognized in the family (Kühn et al. 1993). Native species of Salsoleae are limited to the Old World (Kadereit et al. 2003), except for *Salsola kali* (Wilson 1984) and *Salsola tragus* (Kadereit et al. 2005) in Australia, or *Salsola australis*, which has been merged with *S. tragus* (Borger et al. 2008). Central Asia is one of the distribution centers for Chenopodiaceae (Pyankov et al. 2001a; Kadereit et al. 2005; Akhani et al. 2007), of which northwestern China (e.g., Xinjiang, Gansu, Qinghai, and western Inner Mongolia) represents the largest part (Grubov 1999). This area has sparse annual precipitation, ranging from 200 to 250 mm, and strongly arid or semi-arid and saline soils (Wang 2007). These environments serve as favorable habitats for Salsoleae due to the morphological, anatomical, and physiological characters of these species (Casati et al. 1999; Kadereit et al. 2003).

Z.-B. Wen · M.-L. Zhang (✉)
Key Laboratory of Biogeography and Bioresource in Arid Land,
Xinjiang Institute of Ecology and Geography,
Chinese Academy of Sciences, Urumqi 830011, China
e-mail: zhangml@ibcas.ac.cn

Z.-B. Wen
Graduate University,
Chinese Academy of Sciences,
Beijing 100049, China

M.-L. Zhang
Institute of Botany, Chinese Academy of Sciences,
Beijing 100093, China

G.-L. Zhu
Northwest Normal University, Lanzhou 730070, China

S. C. Sanderson
Shrub Sciences Laboratory, Intermountain Research Station,
Forest Service, U. S. Department of Agriculture,
Ogden, UT, USA

In recent classifications, the subfamily Salsoloideae Ulbr. is virtually synonymous with Spirolobeae (Blackwell 1977; Kühn et al. 1993). Salsoleae was previously treated as a tribe (Meyer 1829; Ulbrich 1934; Iljin 1936; Williams and Ford-Lloyd 1974), and more recently as a monophyletic tribe of subfamily Salsoloideae (Kapralov et al. 2006; Akhani et al. 2007). Monophyly of Salsoleae, Camphorosmeae, and Sclerolaeneae has been supported by molecular data (Kadereit et al. 2003, 2005; Kapralov et al. 2006).

For morphological taxonomy, the classification of Salsoleae has been of two kinds: first, those in which Salsoleae s.l. is classified into subtribes, i.e., Anabaseae and Salsoleae (Meyer 1829; Moquin-Tandon 1840); Sodeae and Anabaseae (Moquin-Tandon 1849); or Sodinae, Anabasiniae, and Halimocnemidae by Ulbrich (1934) and second, those in which subcategories within the tribe are not used (Iljin 1936; Kühn et al. 1993; Grubov 1999; Assadi 2001; Zhu et al. 2003). Considering the morphological differences and the differing systematic treatments, there is a need for molecular evidence to test hypotheses in an attempt to resolve long-standing controversies, clarify phylogenetic relationships, and discern monophyletic lineages.

Salsoleae has previously been studied using molecular methods (Pyankov et al. 2001a; Kadereit et al. 2003, 2005; Kapralov et al. 2006; Akhani et al. 2007). Pyankov et al. (2001a) used ITS sequences from 34 species, with an emphasis on species of *Salsola*. He discovered two separate lineages within Salsoleae s.l., corresponding to the NAD-ME and NADP-ME photosynthetic pathways. The relationship between these and Camphorosmeae was poorly supported [bootstrap value (bt) < 50%].

Analysis of *rbcL* sequences from 12 species likewise suggested that Salsoleae was composed of two lineages, Salsoleae I and Salsoleae II, but these were weakly supported (bt < 50%) (Kadereit et al. 2003). Salsoleae was again placed as sister to Camphorosmeae (bt = 88%), but the sampling was not sufficiently extensive to ensure accuracy.

Based on 132 species, mainly collected from western Asia (e.g., Iran, Turkey), Akhani et al. (2007) used ITS and *psbB-psbH* sequences to explore the phylogeny of Salsoleae. They determined that it was split into two monophyletic tribes (bt = 92%), Salsoleae s.str. and Caroxyloneae, and that Caroxyloneae and Camphorosmeae were both well supported (bt = 94, 97% respectively). They presented a revised classification of Salsoleae s.l. on the basis of their study.

Besides the organization of Salsoleae into subgroups, there remain debatable treatments for some of the genera within it. For instance, *Climacoptera* has been considered a section of *Salsola* (Freitag 1997; Assadi 2001; Zhu et al. 2003) or has been segregated from that genus and given

generic status (Botschantzev 1956; Grubov 1999; Akhani et al. 2007; Takhtajan 2009). It has been shown to form a well supported monophyletic genus with the exclusion of *Climacoptera brachiata* (bt = 100%) (Akhani et al. 2007).

To date, only a few sequences of Salsoleae from northwestern China have been reported (Pyankov et al. 2001a; Kadereit et al. 2003; Akhani et al. 2007). This is a regional gap in data for the molecular phylogeny of Salsoleae, contrasting with coverage in other regions, such as western Asia (Akhani et al. 2007). Utilizing the revised classification of Salsoleae s.l. by Akhani et al. (2007), we sequenced *nrDNA* ITS, *cpDNA psbB-psbH* and *rbcL* spacer, mainly sampling Chinese species, with a focus on the following aims:

- (1) Testing the relationships between Salsoleae and Camphorosmeae
- (2) Reconstructing the phylogeny of tribe Salsoleae and testing the monophyly of the major classification units within the tribe
- (3) Testing the phylogenetic position of *Climacoptera* and its monophyly
- (4) Testing the monophyly of *Halogeton* and *Anabasis*

Materials and methods

Taxon sampling

Fifty-two species of Salsoleae were sampled for this study. This represents almost the total number of species of Salsoleae that occur in China (a total of 64 species in *Flora of China*) (Zhu et al. 2003). Most samples were from field collections from the Province of Xinjiang, China, which were dried by silica gel, with vouchers deposited in XJBI (Herbarium, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, Xinjiang). Other samples came from herbaria: HNWP (Herbarium, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai), PE (Herbarium, Institute of Botany, Chinese Academy of Sciences, Beijing), and SHI (Herbarium, Shihezi University, Shihezi, Xinjiang) (Table 1). The abbreviations of herbaria follow Index Herbariorum (Holmgren and Holmgren 1998) and Index Herbariorum Sinicorum (Fu et al. 1993). Sequences previously reported were also employed (Kadereit et al. 2003, 2005; Akhani et al. 2007). Identification of plant specimens was the responsibility of Professor G. L. Zhu, who has been engaged in Chenopodiaceae taxonomy for many years (Zhu 1996; Zhu et al. 2003). Six species of Suaedoideae and four species of Salicornioideae were chosen as outgroups. New sequences (see accessions HM131608-HM131803 of Table 1) have been deposited in GenBank.

Table 1 Taxa sampled, vouchers, and GenBank accession numbers in this study

Species	Voucher	Source	Genbank Accession Nos.		
			ITS	<i>psbB-psbH</i>	<i>rbcL</i>
Camphorosmeae					
<i>Bassia hyssopifolia</i> (Pall.) O. Kuntze	Akhani et al. (2007)		EF453390	EF453527	–
<i>Camphorosma monspeliaca</i> L.	Kadereit et al. (2003), Akhani et al. (2007)		EF453393	EF453530	AY270071
<i>Chenoleoides tomentosa</i> (Lowe) Botsch.	Kadereit et al. (2003), Akhani et al. (2007)		EF453394	EF453531	AY270076
<i>Kirilowia eriantha</i> Bunge	Akhani et al. (2007)		EF453445	EF453574	–
<i>Pandertia pilosa</i> Fisch. & C. A. Mey.	Kadereit et al. (2003), Akhani et al. (2007)		EF453454	EF453582	AY270114
Salsoleae s.l.					
The Caroxyloneae clade ^a					
<i>Caroxylon</i> Thunb. ^a					
<i>Salsola araneosa</i> Botsch.	Akhani et al. (2007)		EF453461	EF453588	–
<i>Salsola carpatha</i> P. H. Davis	Akhani et al. (2007)		EF453514	–	–
<i>Salsola dzhungarica</i> Iljin	M. L. Zhang; Z. B. Wen; G. L. Zhu 08013 (XJBI)	Urumqi, Xinjiang, China	HM131650	HM131719	HM131783
<i>Salsola glabrescens</i> Burt Davy	Akhani et al. (2007)		EF453479	EF453602	–
<i>Salsola implicata</i> Botsch.	A. J. Qiu & T. Jin 1139 (SHI)	Liumaowan, Shihezi, Xinjiang, China	HM131653	HM131722	HM131785
<i>Salsola micranthera</i> Botsch. 1	M. L. Zhang; Z. B. Wen; G. L. Zhu 08030 (XJBI)	Shanshan, Turfan, Xinjiang, China	HM131656	HM131725	HM131788
<i>Salsola micranthera</i> Botsch. 2	P. Yan 1678 (SHI)	Santai, Bole, Xinjiang, China	HM131657	–	–
<i>Salsola nitrararia</i> Pall.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08097 (XJBI)	Dongdizhen, Qitai, Xinjiang, China	HM131661	HM131729	HM131789
<i>Salsola orientalis</i> S. G. Gmel.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082074 (XJBI)	Fuyun, Altai, Xinjiang, China	HM131662	HM131730	HM131790
<i>Salsola vermiculata</i> L.	Kadereit et al. (2003), Akhani et al. (2007)		EF453501	EF453622	AY270131
<i>Salsola zeyheri</i> (Moq.) Bunge	Akhani et al. (2007)		EF453502	–	–
Climacoptera Botsch.					
<i>Climacoptera I</i>					
<i>Climacoptera affinis</i> (C. A. Mey.) Botsch.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082038 (XJBI)	Hebukesaiter, Tacheng, Xinjiang, China	HM131617	HM131686	HM131753
<i>Climacoptera brachiata</i> (Pall.) Botsch.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08084 (XJBI)	Balikun, Hami, Xinjiang, China	HM131618	HM131687	HM131754

Table 1 continued

Species	Voucher	Source	Genbank Accession Nos.		
			ITS	<i>psbB-psbH</i>	<i>rbcL</i>
<i>Climacoptera</i> II					
<i>Climacoptera crassa</i> (M. Bieb.) Botsch.	Kadereit et al. (2003), Akhani et al. (2007)		EF453401	EF453534	AY270083
<i>Climacoptera ferganica</i> (Drob.) Botsch.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082168 (XJBI)	Xinyuan, Yining, Xinjiang, China	HM131619	HM131688	HM131755
<i>Climacoptera glaberrima</i> Botsch.	Akhani et al. (2007)		EF453396	EF453532	–
<i>Climacoptera iranica</i> U. Prtov	Akhani et al. (2007)		EF453395	–	–
<i>Climacoptera korshinskiyi</i> (Drob.) Botsch.	P. Yan 1237 (SHI)	Sikeshu, Wusu, Xinjiang, China	HM131620	HM131689	–
<i>Climacoptera lanata</i> (Pall.) Botsch.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082086 (XJBI)	Beitun, Altai, Xinjiang, China	HM131621	HM131690	HM131756
<i>Climacoptera longipistillata</i> Botsch.	Akhani et al. (2007)		EF453397	–	–
<i>Climacoptera obtusifolia</i> (Schrenk) Botsch. 1	M. L. Zhang; Z. B. Wen; G. L. Zhu 08120 (XJBI)	Liuynhu, Fukang, Xinjiang, China	HM131622	HM131691	HM131757
<i>Climacoptera obtusifolia</i> (Schrenk) Botsch. 2	M. L. Zhang; Z. B. Wen; G. L. Zhu 082018 (XJBI)	Hutubi, Changji, Xinjiang, China	HM131623	HM131692	HM131758
<i>Climacoptera subcrassa</i> (M. Pop.) Botsch.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08083 (XJBI)	Balikun, Hami, Xinjiang, China	HM131624	HM131693	HM131759
<i>Climacoptera sukaczewii</i> Botsch.	W. Zhai & W. B. Xu 1084 (SHI)	Manasi, Changji, Xinjiang, China	HM131625	HM131694	HM131760
<i>Climacoptera turcomanica</i> (Litv.) Botsch.	Akhani et al. (2007)		EF453399	–	–
<i>Halimocnemis</i> C. A. Mey.					
<i>Halimocnemis azarbaijanensis</i> Assadi	Akhani et al. (2007)		EF453420	EF453551	–
<i>Halimocnemis karelinii</i> Moq.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082032 (XJBI)	Manasi, Changji, Xinjiang, China	HM131627	HM131696	HM131762
<i>Halimocnemis longifolia</i> Bunge	S. P. Yang 2001626 (SHI)	Shihezi, Xinjiang, China	HM131628	HM131697	–
<i>Halimocnemis mollissima</i> Bunge	Akhani et al. (2007)		EF453422	EF453553	–
<i>Halimocnemis purpurea</i> Moq.	Akhani et al. (2007)		EF453426	EF453557	–
<i>Halimocnemis villosa</i> Kar. et Kir.	S. Y. Tang 056 (SHI)	Paotaiuzhen, Shihezi, Xinjiang, China	HM131629	HM131698	HM131763
<i>Kaviria</i> Akhani & E. H. Roalson ^a					
<i>Salsola aucheri</i> (Moq.) Bunge ex Iljin	Akhani et al. (2007)		EF453469	EF453594	–
<i>Salsola vvedenskyi</i> Iljin & M. Popov	Akhani et al. (2007)		EF453462	EF453589	–
<i>Nanophyton</i> Less					
<i>Nanophyton erinaceum</i> (Pall.) Bunge	M. L. Zhang; Z. B. Wen; G. L. Zhu 082160 (XJBI)	Xinyuan, Yining, Xinjiang, China	HM131639	HM131708	HM131773
<i>Ofaiston</i> Raf					
<i>Ofaiston monandrum</i> Moq.	Kadereit et al. (2003), Akhani et al. (2007)		EF453453	EF453581	AY270112

Table 1 continued

Species	Voucher	Source	Genbank Accession Nos.		
			ITS	<i>psbB-psbH</i>	<i>rbcL</i>
<i>Petrosimonia</i> Bunge					
<i>Petrosimonia brachiata</i> Bunge	Akhani et al. (2007)		EF453457	EF453585	–
<i>Petrosimonia glauca</i> Bunge	Akhani et al. (2007)		EF453456	EF453584	–
<i>Petrosimonia gataucescens</i> (Bunge) Iljin	M. L. Zhang; Z. B. Wen; G. L. Zhu 082065 (XJBI)	Fuhai, Altai, Xinjiang, China	HM131640	HM131709	HM131774
<i>Petrosimonia nigdeensis</i> Aellen	Akhani et al. (2007)		EF453458	EF453586	–
<i>Petrosimonia sibirica</i> (Pall.) Bunge	M. L. Zhang; Z. B. Wen; G. L. Zhu 08108 (XJBI)	Wujiagu, Xinjiang, China	HM131641	HM131710	HM131775
<i>Petrosimonia squarrosa</i> (Schrenk) Bunge	M. L. Zhang; Z. B. Wen; G. L. Zhu 082025 (XJBI)	Mosuowan, Shihezi, Xinjiang, China	HM131642	HM131711	HM131776
The <i>Kali</i> clade					
<i>Salsola aperta</i> Pauls.	P. Yan 86301 (SHI)	Shihezi, Xinjiang, China	HM131644	HM131713	–
<i>Salsola arbuscula</i> Pall.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082043 (XJBI)	Hebukesater, Tacheng, Xinjiang, China	HM131645	HM131714	HM131778
<i>Salsola chiwensis</i> Popov	Pyankov et al. (2001a)		AF318642	–	–
<i>Salsola deserticola</i> Iljin	Akhani et al. (2007)		EF453473	EF453598	–
<i>Salsola richteri</i> (Moq.) Kar. ex Litv.	Akhani et al. (2007)		EF453494	EF453616	–
<i>Kali</i> Mill ^a					
<i>Salsola chinghaiensis</i> A. J. Li	Q. Du 0376 (HNWP)	Nuomuhong, Dulan, Qinghai, China	HM131647	HM131716	HM131780
<i>Salsola collina</i> Pall. 1	M. L. Zhang; Z. B. Wen; G. L. Zhu 082122 (XJBI)	Urumqi, Xinjiang, China	HM131648	HM131717	HM131781
<i>Salsola collina</i> Pall. 2	B. Z. Guo 8004 (HNWP)	Xiangride, Dulan, Qinghai, China	HM131649	HM131718	HM131782
<i>Salsola iberica</i> Sennen & Pau	A. R. Li 3184 (HNWP)	ZadaTuolin, Tibet, China	HM131652	HM131721	–
<i>Salsola kali</i> L.	Pyankov et al. (2001a)		AF318646	–	–
<i>Salsola komarovii</i> Iljin	A. R. Li 15910 (HNWP)	Longyashan, Shouwu, Shanxi, China	HM131654	HM131723	HM131786
<i>Salsola paulsenii</i> Litv.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082034 (XJBI)	Shihezi, Xinjiang, China	HM131663	HM131731	HM131791
<i>Salsola pellucida</i> Litv.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082026 (XJBI)	Mosuowan, Shihezi, Xinjiang, China	HM131664	HM131732	HM131792
<i>Salsola praecox</i> Litv.	P. Yan 1521 (SHI)	Shihezi, Xinjiang, China	HM131665	HM131733	HM131793
<i>Salsola monoptera</i> Bunge 1	D. D. Tao 10683 (PE)	Bangchu, Bange, Tibet, China	HM131658	HM131726	–
<i>Salsola monoptera</i> Bunge 2	N. T. Ho 1533 (HNWP)	Jiayixiang, Gonghe, Qinghai, China	HM131659	HM131727	–
<i>Salsola monoptera</i> Bunge 3	Xizang Expedition Team 3770 (HNWP)	Shiquanhe, Ali, Tibet, China	HM131660	HM131728	–

Table 1 continued

Species	Voucher	Source	Genbank Accession Nos.		
			ITS	<i>psbB-psbH</i>	<i>rbcl</i>
<i>Salsola ruthenica</i> Iljin 1	M. L. Zhang; Z. B. Wen; G. L. Zhu 082010 (XJBI)	Yushugou, Changji, Xinjiang, China	HM131667	HM131735	HM131795
<i>Salsola ruthenica</i> Iljin 2	P. Yan & Y. Liu 7081 (SHI)	Yecheng, Kashi, Xinjiang, China	HM131668	HM131736	HM131796
<i>Salsola tragus</i> L. (published under <i>Salsola australis</i> R. Br.)	Pyankov et al. (2001a)		AF318648	–	–
<i>Salsola zaidamica</i> Iljin 1	M. L. Zhang; Z. B. Wen; G. L. Zhu 08068 (XJBI)	Yiwu, Hami, Xinjiang, China	HM131669	HM131737	HM131797
<i>Salsola zaidamica</i> Iljin 2	Q. Du 0239 (SHI)	Tuochangming, Dequanha, Qinghai, China	HM131670	HM131738	–
The Salsoleae s.str. clade ^a					
<i>Salsola divaricata</i> Mass. ex Link	Akhani et al. (2007)		EF453474	EF453599	–
<i>Salsola arbusculiformis</i> Drob.	P. Yan 2774 (SHI)	Tacheng, Xinjiang, China	HM131646	HM131715	HM131779
<i>Salsola laricifolia</i> Turcz. ex Litv.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082125 (XJBI)	Alashankou, Tacheng, Xinjiang, China	HM131655	HM131724	HM131787
<i>Salsola masenderanica</i> Botsch.	Akhani et al. (2007)		EF453504	EF453624	–
<i>Salsola montana</i> Litv.	Akhani et al. (2007)		EF453489	EF453611	–
<i>Anabasis</i> L.					
<i>Anabasis aphylla</i> L. 1	M. L. Zhang; Z. B. Wen; G. L. Zhu 082007 (XJBI)	Xiabahu, Changji, Xinjiang, China	HM131608	HM131677	HM131745
<i>Anabasis aphylla</i> L. 2	P. Yan & J. H. Lu 4278 (SHI)	Wuqia, Atshi, Xinjiang, China	HM131609	HM131678	HM131746
<i>Anabasis brevifolia</i> C. A. Mey.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08036 (XJBI)	Hebukesaiter, Tacheng, Xinjiang, China	HM131610	HM131679	HM131747
<i>Anabasis cretacea</i> Pall.	P. Yan 1665 (SHI)	Santai, Bole, Xinjiang, China	HM131611	HM131680	–
<i>Anabasis elatior</i> (C. A. Mey.) Schischk.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082071 (XJBI)	Fuyun, Altai, Xinjiang, China	HM131612	HM131681	HM131748
<i>Anabasis eriopoda</i> (Schrenk) Benth. ex Volkens	M. L. Zhang; Z. B. Wen; G. L. Zhu 082041 (XJBI)	Hebukesaiter, Tacheng, Xinjiang, China	HM131613	HM131682	HM131749
<i>Anabasis eugeniae</i> Iljin	Akhani et al. (2007)		EF453384	EF453521	–
<i>Anabasis pelliotii</i> Danguy	P. Yan & J. H. Lu 4252 (SHI)	Wuqia, Atshi, Xinjiang, China	HM131614	–	–
<i>Anabasis salsa</i> (C. A. Mey.) Benth.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082099 (XJBI)	Beitun, Altai, Xinjiang, China	–	HM131683	HM131750
<i>Anabasis truncata</i> (Schrenk) Bunge	M. L. Zhang; Z. B. Wen; G. L. Zhu 082055 (XJBI)	Hebukesaiter, Tacheng, Xinjiang, China	HM131615	HM131684	HM131751
<i>Cornulaca</i> Del.					
<i>Cornulaca aucheri</i> Moq.	Akhani et al. (2007)		EF453405	EF453538	–
<i>Cornulaca monacantha</i> Delile	Akhani et al. (2007)		EF453406	EF453539	–
<i>Girgensohnia</i> Bunge					

Table 1 continued

Species	Voucher	Source	Genbank Accession Nos.		
			ITS	<i>psbB-psbH</i>	<i>rbcL</i>
<i>Girgensohnia imbricata</i> Bunge	Akhani et al. (2007)		EF453412	EF453545	–
<i>Girgensohnia minima</i> Korovin	Akhani et al. (2007)		EF453413	EF453546	–
<i>Girgensohnia oppositiflora</i> (Pall.) Fenzl	M. L. Zhang; Z. B. Wen; G. L. Zhu 082148 (XJBI)	Chabuchaer, Yining, Xinjiang, China	HM131626	HM131695	HM131761
<i>Halogeton</i> C. A. Mey					
<i>Halogeton alopecuroides</i> Moq.	Akhani et al. (2007)		EF453430	EF453561	–
<i>Halogeton arachnoides</i> Moq.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08003 (XJBI)	Dabancheng, Urumqi, Xinjiang, China	HM131630	HM131699	HM131764
<i>Halogeton glomeratus</i> (M. Bieb.) C. A. Mey.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08016 (XJBI)	Urumqi, Xinjiang, China	HM131631	HM131700	HM131765
<i>Haloxylon</i> Bunge					
<i>Haloxylon ammodendron</i> (C. A. Mey.) Bunge	M. L. Zhang; Z. B. Wen; G. L. Zhu 082027 (XJBI)	Mosuowan, Shihezi, Xinjiang, China	HM131632	HM131701	HM131766
<i>Haloxylon persicum</i> Bunge ex Boiss. & Buhse	M. L. Zhang; Z. B. Wen; G. L. Zhu 08025 (XJBI)	Cultivated, Turfan Botanical Garden, Xinjiang, China	HM131633	HM131702	HM131767
<i>Horaninovia</i> Fisch. et. Mey.					
<i>Horaninovia platyptera</i> Charif & Aellen	Akhani et al. (2007)		EF453441	EF453572	–
<i>Horaninovia pungens</i> (Gilli) Botsch.	Akhani et al. (2007)		EF453442	–	–
<i>Horaninovia ulicina</i> Fisch. et Mey.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08094 (XJBI)	Qitai, Changji, Xinjiang, China	HM131634	HM131703	HM131768
<i>Ilijin</i> Korov.					
<i>Ilijinia regelii</i> (Bunge) Korov.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08034 (XJBI)	Shanshan, Turfan, Xinjiang, China	HM131635	HM131704	HM131769
<i>Salsola</i> s.str. ^a					
<i>Anabasis setifera</i> Moq.	Akhani et al. (2007)		EF453389	EF453526	–
<i>Fadenia zygophylloides</i> Aellen & C. C. Towns.	Akhani et al. (2007)		EF453513	–	–
<i>Noaea mucronata</i> (Forssk.) Asch. & Schweinf.	Kadereit et al. (2003), Akhani et al. (2007)		EF453452	EF453580	AY270110
<i>Salsola foliosa</i> (L.) Schrad.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08112 (XJBI)	Qitai, Changji, Xinjiang, China	HM131651	HM131720	HM131784
<i>Salsola rosacea</i> L.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082072 (XJBI)	Fuyun, Altai, Xinjiang, China	HM131666	HM131734	HM131794
<i>Salsola soda</i> L.	Akhani et al. (2007)		EF453496	EF453617	–
<i>Seidlitzia florida</i> (M. Bieb.) Bunge ex Boiss.	Akhani et al. (2007)		EF453507	EF453627	–
<i>Sympagma</i> Bunge					
<i>Sympagma regelii</i> Bunge	M. L. Zhang; Z. B. Wen; G. L. Zhu 08023 (XJBI)	Cultivated, Turfan Botanical Garden, Xinjiang, China	HM131676	HM131744	HM131803

Table 1 continued

Species	Voucher	Source	Genbank Accession Nos.		
			ITS	<i>psbB-psbH</i>	<i>rbcL</i>
Outgroup					
Salicornieae					
<i>Kalidium caspicum</i> (L.) Ung.-Stemb.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082166 (XJBI)	Xinyuan, Yining, Xinjiang, China	HM131636	HM131705	HM131770
<i>Kalidium cuspidatum</i> (Ung.-Stemb.) Grub.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08066 (XJBI)	Yiwu, Hami, Xinjiang, China	HM131637	HM131706	HM131771
<i>Kalidium foliatum</i> (Pall.) Moq.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082060 (XJBI)	Fuhai, Altai, Xinjiang, China	HM131638	HM131707	HM131772
<i>Salicornia europaea</i> L.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08075 (XJBI)	Balikun, Hami, Xinjiang, China	HM131643	HM131712	HM131777
Suaedeae					
<i>Borszczowia aralocaspica</i> Bunge	M. L. Zhang; Z. B. Wen; G. L. Zhu 082016 (XJBI)	Hutubi, Changji, Xinjiang, China	HM131616	HM131685	HM131752
<i>Suaeda microphylla</i> (C. A. Mey.) Pall.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08092 (XJBI)	Xidizhen, Qitai, Xinjiang, China	HM131671	HM131739	HM131798
<i>Suaeda paradoxa</i> Bunge	M. L. Zhang; Z. B. Wen; G. L. Zhu 08116 (XJBI)	Liuynhu, Fukang, Xinjiang, China	HM131672	HM131740	HM131799
<i>Suaeda physophora</i> Pall.	M. L. Zhang; Z. B. Wen; G. L. Zhu 08007 (XJBI)	Dabancheng, Urumqi, Xinjiang, China	HM131673	HM131741	HM131800
<i>Suaeda prostrata</i> Pall.	M. L. Zhang; Z. B. Wen; G. L. Zhu 082090 (XJBI)	Beitun, Altai, Xinjiang, China	HM131674	HM131742	HM131801
<i>Suaeda pterantha</i> (Kar. et Kir.) Bunge	M. L. Zhang; Z. B. Wen; G. L. Zhu 082023 (XJBI)	Mostuwan, Shihezi, Xinjiang, China	HM131675	HM131743	HM131802

^a Reference to the revised classification of *Salsola* s.l. by Akhmi et al. (2007)

DNA sequencing

Isolation of total DNA followed the modified 2× CTAB buffer method (Doyle and Doyle 1987). The ITS gene was amplified with primers ITS1 (5'-AGA AGT CGT AAC AAG GTT TCC GTA GC-3') (Kang et al. 2003) and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990). The *psbB-psbH* region was amplified using the primers *psbB-psbH-f* (5'-AGA TGT TTT TGC TGG TAT TGA-3') and *psbB-psbH-r* (5'-TTC AAC AGT TTG TGT AGC CA-3') (Xu et al. 2000). For *rbcL*, two overlapping fragments were amplified using standard primers 1F (5'-ATG TCA CCA CAA ACA GAA ACT AAA GC-3'), 875F (5'-GCA GTT ATT GAT AGA CAG A-3'), 955F (5'-CGT CTA TCT GGT GGA GAT C-3'), and 1460R (5'-CTT TTA GTA AAA GAT TGG GCC GAG-3'). Polymerase chain-reaction (PCR) amplifications followed the procedures described by Kadereit et al. (2003) and Schütze et al. (2003).

PCR products were electrophoresed using a 0.8% agarose gel in a 0.5× TAE (pH 8.3) buffer, then stained with ethidium bromide to confirm a single product, and purified using the PEG precipitation procedure (Johnson and Soltis 1995). Sequencing was performed with an ABI Prism 3730 Genetic Analyzer (Shanghai Sangon Biological Engineering Technology & Service, China).

The ITS sequences were truncated to include only ITS1, 5.8S, and ITS2. The *psbB-psbH* sequences were trimmed to include the 3' end of the *psbB* coding region, the *psbB-psbT* intergenic spacer, the *psbT* coding region, the *psbT-psbN* intergenic spacer, the *psbN* coding region, and the *psbN-psbH* intergenic spacer. The *rbcL* sequences were truncated to start from nucleotide position 64 of the translated region and end with position 1406 (Zurawski et al. 1981). The identification of terminal ends and spacer boundaries of all sequences was based on comparisons with other species of Chenopodiaceae (Pyankov et al. 2001a; Kadereit et al. 2003, 2005; Kapralov et al. 2006; Akhiani et al. 2007). Forward and reverse sequences were aligned using Clustal X (Thompson et al. 1997). Gaps were introduced to the alignment as missing data. Finally, four datasets consisting of ITS, ITS + *psbB-psbH*, *psbB-psbH* + *rbcL* (*cpDNA*), and the three genes combined were assembled.

Maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses were employed for phylogenetic analysis of the four datasets. MP analyses were performed using PAUP* 4.0b1.0 (Swofford 2002). The MP analyses used heuristic searches (1,000 random addition cycles, tree bisection-reconnection, and branch-swapping), and swapping was run to completion for all random addition replicates. Clade support was estimated using 1,000 heuristic bootstrap replicates (100 random

addition cycles per replicate, tree bisection-reconnection branch-swapping; Felsenstein 1985; Hillis and Bull 1993).

The ML analyses were conducted in PHYML v.2.4.3 (Guindon and Gascuel 2003). ML support values were estimated using 1,000 bootstrap replicates in PHYML. Bayesian analyses were conducted using MrBayes, version 3.0b4 (Huelsenbeck and Ronquist 2001; Huelsenbeck and Rannala 2004). Four chains were run (Markov Chain Monte Carlo) by beginning with a random tree and saving a tree after every 100 generations for 1 million generations.

For ML and Bayesian analyses, the appropriate model of DNA substitution was estimated using Modeltest 3.06 (Posada and Crandall 1998). For the ITS dataset, the GTR + I + G model was chosen with the gamma distribution shape parameter set to 1.3504. Base frequencies were set to $A = 0.2114$, $C = 0.2664$, $G = 0.2673$, and $T = 0.2550$. The rate matrix was set to $AC = 1.2082$, $AG = 2.6459$, $AT = 1.7846$, $CG = 0.5266$, $CT = 3.6483$, and $GT = 1.0000$. For ITS + *psbB-psbH* dataset, the GTR + G + I model was chosen with the gamma distribution shape parameter set to 0.7738. Estimated base frequencies were set to $A = 0.2563$, $C = 0.2298$, $G = 0.2270$, and $T = 0.2869$. The rate matrix was set to $AC = 1.0464$, $AG = 2.2013$, $AT = 1.0010$, $CG = 0.6282$, $CT = 3.1537$, and $GT = 1.0000$. For the *cpDNA* dataset, the GTR + G + I model was chosen with the gamma distribution shape parameter set to 0.8461. Estimated base frequencies were set to $A = 0.2867$, $C = 0.1843$, $G = 0.2091$, and $T = 0.3199$. The rate matrix was set to $AC = 1.1044$, $AG = 2.3890$, $AT = 0.3834$, $CG = 0.7923$, $CT = 3.2776$, and $GT = 1.0000$. For the dataset of the three combined genes, the GTR + G + I model was chosen with the gamma distribution shape parameter set to 0.6566. Base frequencies were set to $A = 0.2621$, $C = 0.2146$, $G = 0.2311$, and $T = 0.2902$. The rate matrix was set to $AC = 1.1882$, $AG = 2.3862$, $AT = 0.9805$, $CG = 0.6321$, $CT = 3.8690$, and $GT = 1.0000$.

Results

The results of MP analyses are detailed in Table 2. The MP, ML, and BI analyses all yielded similar topologies for each of the data sets.

ITS analysis

The ML analysis resulted in a single optimal tree ($-\ln L = 12,134.470129$, where $L =$ likelihood). The monophyly of Salsoleae s.l. is strongly supported [bt = 100%, posterior probability (pP) = 99%] (Fig. 1), and the tribe includes three monophyletic clades, Caroxyloneae, Salsoleae s.str., and the *Kali* clade. Camphorosmeae is nested in

Salsoleae s.l., and the monotypic *Sympegma* is sister to Salsoleae s.str. + the *Kali* clade. The monophyly of the genera *Kali*, *Caroxylon*, *Haloxylon*, and *Petrosimonia* are all strongly supported. However, several other genera are not monophyletic, namely, *Anabasis*, *Climacoptera*, *Halimocnemis*, and *Halogeton*. The genus *Salsola* s.l. itself is clearly polyphyletic, with species of *Salsola* present in different clades.

ITS + *psbB-psbH* analysis

The ML analysis of ITS + *psbB-psbH* resulted in a single optimal tree ($-\ln L = 16,941.540684$). Camphorosmeae is again nested in Salsoleae s.l., and Salsoleae s.l. includes three well-supported clades, Caroxyloneae (bt = 91%, pP = 98%), the *Kali* clade (bt = 96%, pP = 99%), and Salsoleae s.str. (bt = 100%, pP = 98%) (Fig. 2). The genus *Sympegma* and the clade Salsoleae s.str. are supported. The *Kali* clade is sister to *Sympegma* + Salsoleae s.str. The Caroxyloneae clade and Camphorosmeae form a supported clade. The monophyly of the genus *Halimocnemis* is poorly supported (bt = 70%, pP = 55%); *Climacoptera* is divided into two monophyletic clades, *Climacoptera* I (bt = 100%, pP = 99%) and *Climacoptera* II (bt = 99%, pP = 99%); and *Climacoptera* I is sister to *Climacoptera* II + *Halimocnemis*.

cpDNA analysis

The ML analysis of *cpDNA* resulted in a single optimal tree ($-\ln L = 8070.098675$). Camphorosmeae is sister to Salsoleae s.l. (bt = 81%, pP = 99%). The monophyly of Salsoleae s.l. is well supported (bt = 97%, pP = 85%). It is composed of three monophyletic clades: Caroxyloneae (bt = 98%, pP = 87%), *Kali* clade (bt = 100%, pP = 98%), and Salsoleae s.str. (bt = 64%, pP = 98%) (Fig. 3). The genus *Sympegma* is sister to Salsoleae s.str. The *Kali* clade and Caroxyloneae clade are supported. The monophyly of *Anabasis* is supported (bt = 79%, pP = 99%). The genus *Halimocnemis* is not monophyletic.

Fig. 1 Maximum likelihood tree derived from ITS sequences. Maximum parsimony bootstrap values are shown above branches, Bayesian posterior probabilities below branches. We follow the revised classification of Salsoleae s.l. by Akhani et al. (2007). A. = *Anabasis*, B. = *Bassia*, Bor. = *Borczowia*, C. = *Climacoptera*, Ca. = *Camphorosma*, Ch. = *Chenoleoides*, Co. = *Cornulaca*, F. = *Fadenia*, G. = *Girgensohnia*, H. = *Halimocnemis*, Halo. = *Halogeton*, Halox. = *Haloxylon*, Ho. = *Horaninowia*, I. = *Iljinia*, K. = *Kalidium*, Ki. = *Kirilowia*, N. = *Nanophyton*, No. = *Noaea*, O. = *Ofaiston*, P. = *Panderia*, Pe. = *Petrosimonia*, S. = *Salsola*, Se. = *Seidlitzia*, Su. = *Suaeda*, Sy. = *Sympegma*

Combined three-gene analysis

The ML analysis of the tree genes combined resulted in a single optimal tree ($-\ln L = 18496.621367$). Salsoleae s.l. forms a monophyly and is composed of three monophyletic clades: Caroxyloneae (bt = 100%, pP = 99%), *Kali* clade (bt = 100%, pP = 99%), and Salsoleae s.str. (bt = 99%, pP = 99%) (Fig. 4). Camphorosmeae is nested in Salsoleae s.l. and sister to the Caroxyloneae clade (bt = 58%, pP = 99%). The genus *Sympegma* is sister to the clade Salsoleae s.str. + *Kali* clade. The monophyly of *Halimocnemis* is supported (bt = 75%, pP = 98%). *Halogeton* is polyphyletic, as is *Anabasis*.

Discussion

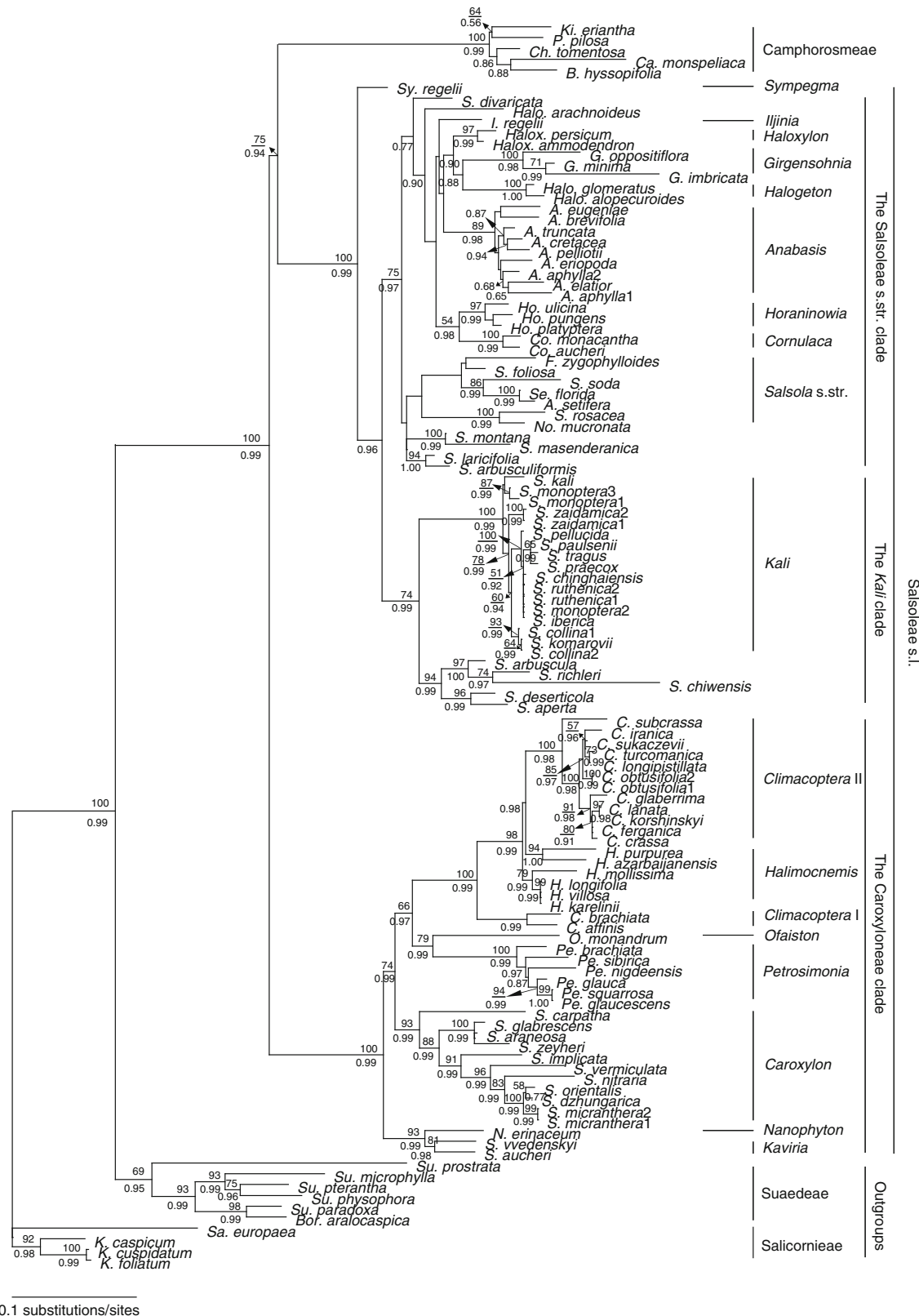
The relationship between Salsoleae s.l. and Camphorosmeae

In the *cpDNA* tree, Camphorosmeae is sister to Salsoleae s.l. (Fig. 3), which is in keeping with the results of previous investigations, a single gene-based study by Kadereit et al. (2003) and a two gene-based study by Akhani et al. (2007), and this has statistical support (bt = 81%; pP = 99%). However, in the other trees, the five genera of Camphorosmeae are nested within Salsoleae s.l. (Figs. 1, 2, 4). We therefore prefer to regard Camphorosmeae as nested within Salsoleae s.l., instead of having a sister relationship as suggested by the previous authors (Kadereit et al. 2003; Akhani et al. 2007). Several morphological characters are

Table 2 Data set and tree statistics from separate MP analyses of ITS, ITS + *psbB-psbH*, *cpDNA* (*psbB-psbH* + *rbcL*), and the three genes combined for Salsoleae

Data set statistics					Tree statistics				
Genic region	Taxa	Aligned length (bp)	Number (%) variable characters	Number (%) parsimony informative characters	Number of shortest trees	Length	CI	RI	RC
ITS	114	685	430 (62.77)	343 (50.07)	145	2,271	0.378	0.802	0.303
ITS + <i>psbB-psbH</i>	114	1,349	655 (48.55)	473 (35.06)	118	2,779	0.412	0.803	0.331
<i>cpDNA</i>	83	2,007	415 (20.68)	251 (12.51)	6	832	0.595	0.832	0.495
Three genes combined	83	2,692	791 (29.38)	562 (20.88)	83	2,623	0.466	0.802	0.374

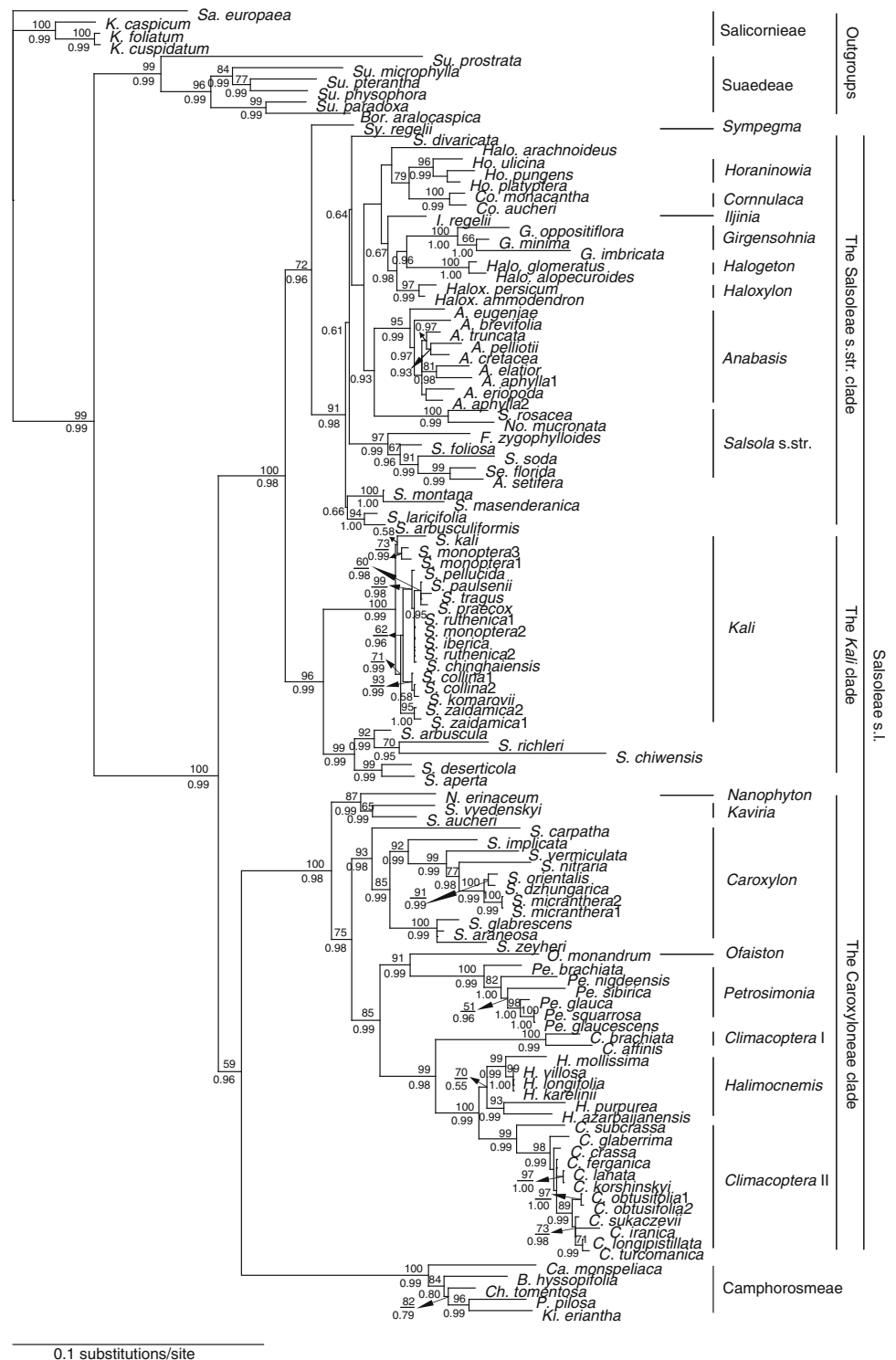
CI Consistency index, RI retention index, RC rescaled consistency



shared between some representatives of Camphorosmeae and Salsoleae (Kadereit et al. 2003; Zhu et al. 2003; Cabrera et al. 2009), such as a hairy plant surface, flat and

dorsiventral cotyledons, and the presence of winglike structures on the fruiting perianth, particularly in *Bassia*, *Kochia*, and *Pandertia* of Camphorosmeae, and in *Salsola*,

Fig. 2 Maximum likelihood tree derived from ITS and *psbB-psbH* sequences. Maximum parsimony bootstrap values are shown above branches, Bayesian posterior probabilities below branches. We follow the revised classification of Salsoleae s.l. by Akhiani et al. (2007). Genus abbreviations as in Fig. 1



Girgensohnia, *Haloxylon*, *Halogeton*, *Ilijinia*, and *Sympegma* of Salsoleae (Zhu et al. 2003). The winged fruiting perianth has been shown to provide some support for the taxonomic implications of the molecular phylogeny of Camphorosmeae (Cabrera et al. 2009). It may be an important shared feature reflecting the close molecular relationship between Camphorosmeae and Salsoleae s.l.

Relationships within the clade Caroxyloneae and the position of *Climacoptera*

Our analyses strongly support three major clades of Salsoleae s.l.: Caroxyloneae, Salsoleae s.str., and the *Kali* clade (Figs. 1, 2, 3, 4), whereas previous molecular studies divided the tribe Salsoleae into two clades

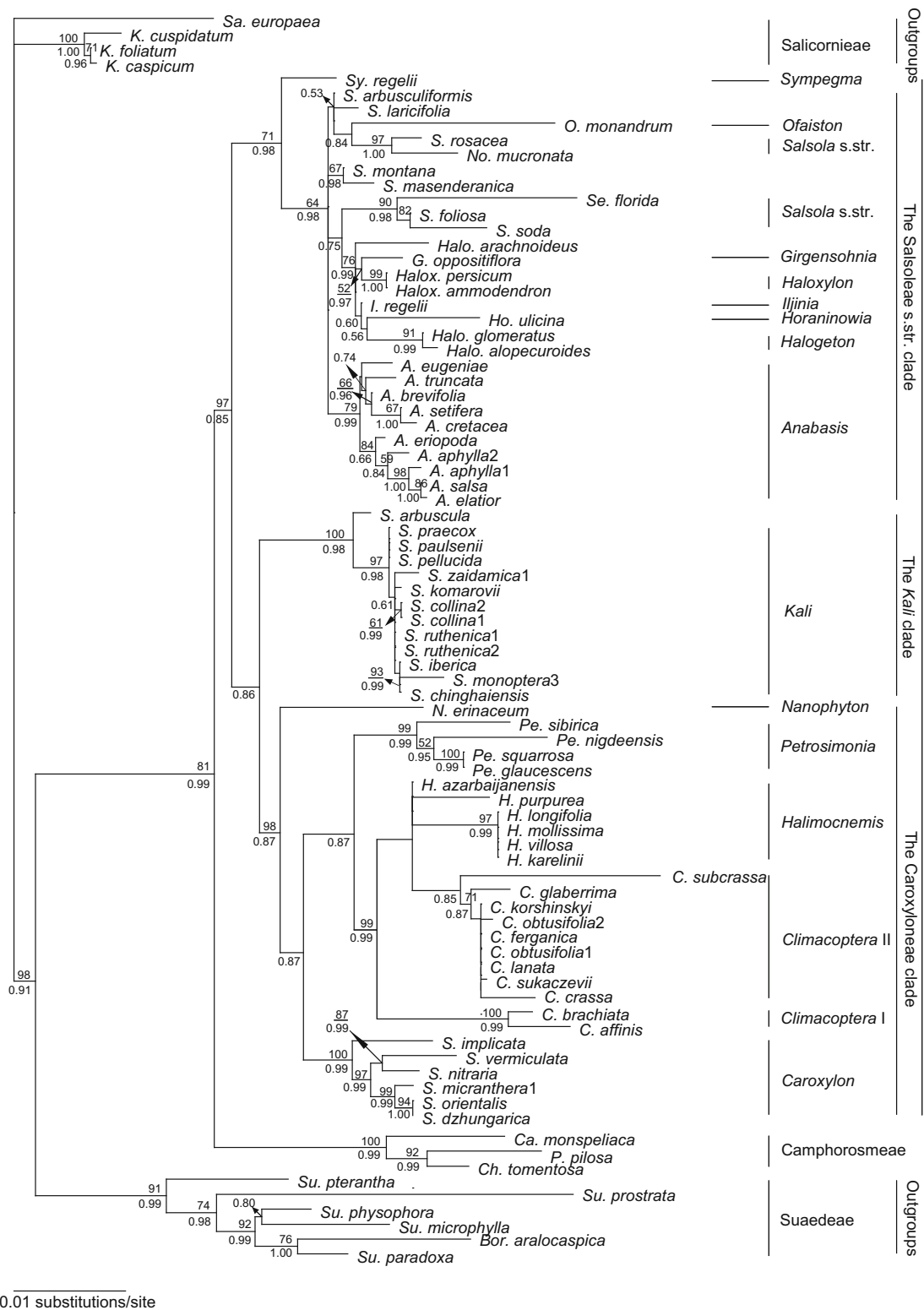


Fig. 3 Maximum likelihood tree derived from *psbB-psbH* and *rbcL* sequences. Maximum parsimony bootstrap values are shown above branches, Bayesian posterior probabilities below branches. We

follow the revised classification of Salsoleae s.l. by Akhani et al. (2007). Genus abbreviations as in Fig. 1

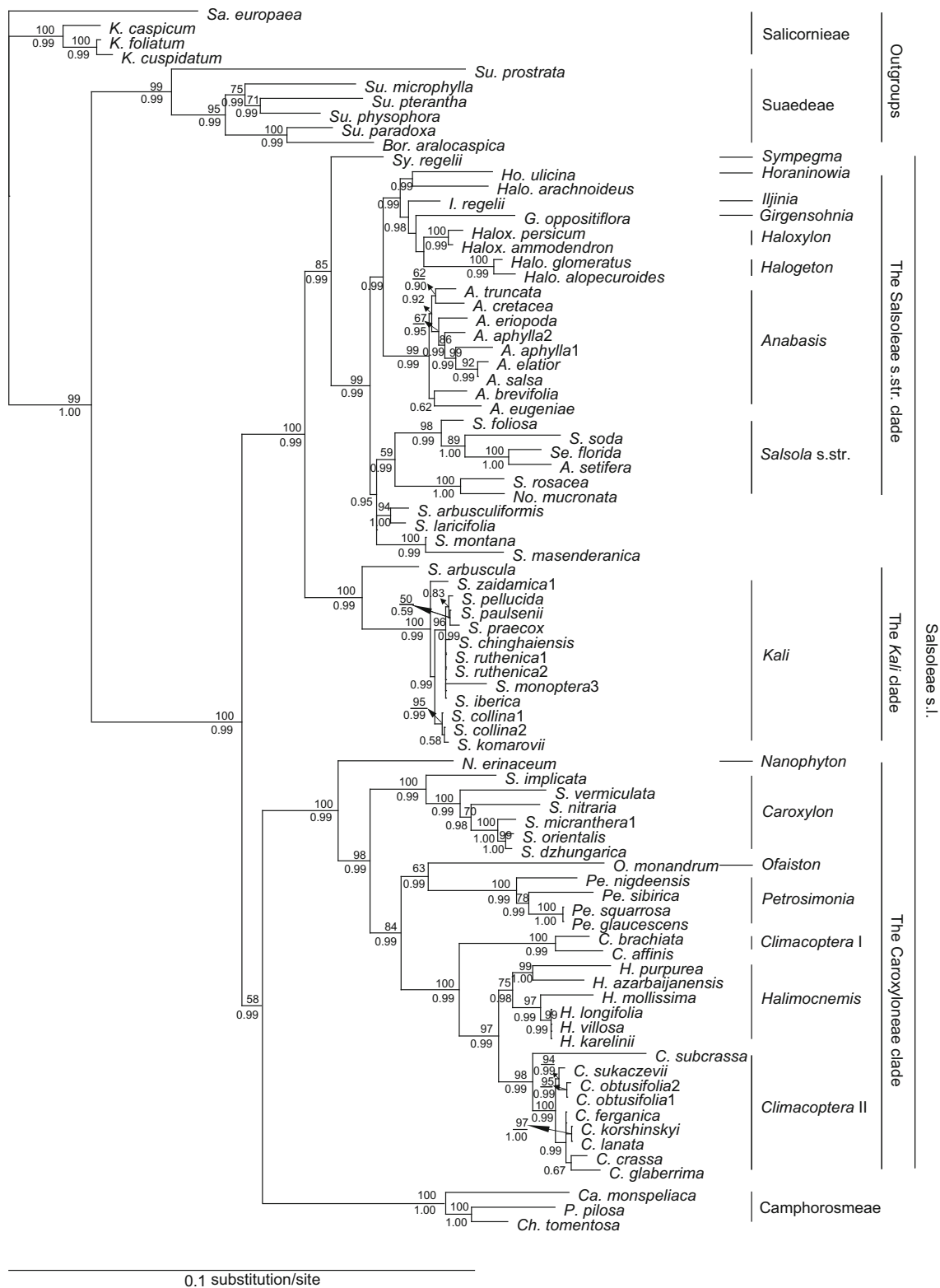


Fig. 4 Maximum likelihood tree derived from ITS, *psbB-psbH*, and *rbcL* sequences. Maximum parsimony bootstrap values are shown above branches, Bayesian posterior probabilities below branches. We

follow the revised classification of Salsoleae s.l. by Akhani et al. (2007). Genus abbreviations as in Fig. 1

Table 3 Morphological and anatomical comparisons between *Climacoptera* I and *Climacoptera* II

Characters	<i>Climacoptera</i> I	<i>Climacoptera</i> II
Habit	Annual	Annual
Leaf phyllotaxy	Alternate or opposite	Alternate
Leaf shape	Semiterete	Semiterete or terete
Leaf base	Not decurrent	Mostly decurrent
Leaf apex	Obtuse	Obtuse
Leaf anatomy	Without hypodermis, one central bundle and secondary bundles peripheral and associated with the Kranz layer	Without hypodermis, one central bundle and secondary bundles subperipheral and not associated with the Kranz layer
Stem	Alternate or opposite	Alternate
Number of flowers per bract	Solitary	Solitary
Number of perianths	Five	Five
Fruiting perianth	Five winged perianths; perianth segments above wing hairy and connate, forming a cone	Five winged perianths; perianth segments above wing hairy or not, reflexed or connate, forming a cone
Seed orientation	Horizontal or vertical	Horizontal
Ecology	Halophytic and xerohalophytic communities	Halophytic and xerohalophytic communities

(Pyankov et al. 2001a; Kadereit et al. 2003; Akhani et al. 2007).

The Chinese species in Caroxyloneae belong to *Caroxylon*, *Petrosimonia*, *Halimocnemis*, the *Climacoptera* I clade, the *Climacoptera* II clade, and the monotypic genus *Nanophyton* (Figs. 1, 2, 3, 4). *Caroxylon* was often regarded as a section of *Salsola* (Ulbrich 1934; Iljin 1936; Botschantzev 1956, 1974; Freitag 1997; Grubov 1999), but also as a genus (Tzvelev 1993; Akhani et al. 2007). Relationships within *Caroxylon* were weakly supported in the previous study (Akhani et al. 2007). Our analyses strongly support the monophyly of *Caroxylon* and relationships within it (Figs. 1, 2, 3, 4). Three primary lineages or clades within *Caroxylon* are clear. *S. carpatha* (a European species, Akhani et al. 2007) is sister to the remaining species of *Caroxylon* (Figs. 1, 2). A small clade consisting of three shrubby species from Africa is sister to the subsequent clade (Figs. 1, 2). Finally, a large clade is composed of Chinese species and *S. vermiculata* (Figs. 1, 2, 3, 4). *S. vermiculata* is restricted to North Africa, but others species are mainly distributed throughout Middle Asia, the Middle East, and Central Asia (Pyankov et al. 2001b; Zhu et al. 2003). These share some morphological characters, such as plants covered with hair, the obtuse apex of the leaves, the expanded leaf base, nondecurrence, and the spicate-paniculate inflorescence (Zhu et al. 2003).

The classification of *Climacoptera* has been a long-standing controversy (Botschantzev 1956; Freitag 1997; Grubov 1999; Assadi 2001; Zhu et al. 2003; Akhani et al. 2007; Takhtajan 2009). Our analyses support the separation of *Climacoptera* from *Salsola* s.l. (Botschantzev 1956; Grubov 1999; Akhani et al. 2007). From field observations,

the winged fruiting perianths of *Climacoptera* are often brightly colored red or purple, which is different from most species of Salsoleae. No data yet suggest whether endozoochorous dispersal might have developed (Sukhorukov 2008). Based on our analysis, *Climacoptera* is composed of two parts, *Climacoptera* I and *Climacoptera* II, but these do not form a monophyletic group (Figs. 1, 2, 3, 4). Morphological evidence for the division of *Climacoptera* into two clades is detailed in Table 3. *Climacoptera* I consists of *Climacoptera brachiata* and *Climacoptera affinis* (Figs. 1, 2, 3, 4), which does not support the previous treatment of *C. brachiata* as a monotypic genus, *Pyankovia* (Akhani et al. 2007). *C. brachiata* and *C. affinis* were placed in *Salsola* by Ulbrich (1934), Iljin (1936), Assadi (2001), and Zhu et al. (2003), and in *Climacoptera* by Botschantzev (1956), Kühn et al. (1993), and Grubov (1999). Several shared morphological features such as habit, leaf shape, leaf anatomy, and the winged fruiting perianth support their close affinity (Table 3). They are dominant summer annuals in Central Asia and Caucasia (Wang 2007; Wei et al. 2008). The monophyly of *Climacoptera* II is strongly supported, as sister to the genus *Halimocnemis* (Figs. 2, 4). *Climacoptera* II is a Eurasian, especially Irano-Turanian group (Akhani et al. 2007). In *Climacoptera* II, *C. subcrassa* is sister to the remaining species of *Climacoptera* II (Figs. 1, 2, 3, 4). A point of morphological evidence supporting this placement of *C. subcrassa* is that perianth segments above the wing are reflexed, forming a star shape (Zhu et al. 2003), which is unlike the other species of *Climacoptera* II.

Our analyses give only uncertain information on the monophyly of *Halimocnemis*, because its monophyly is not

supported in ITS and *cpDNA* trees (Figs. 1, 3), but in ITS + *psbB-psbH* and three-gene analyses, the monophyly of *Halimocnemis* is weakly supported (Figs. 2, 4), similar to a previous study (Akhani et al. 2007), in which a broader circumscription of *Halimocnemis*, including species from *Halanthium*, *Gamanthus*, and *Halotis*, was proposed. According to traditional taxonomy, *Halimocnemis* has ca. 12 species and occurs in the Black Sea region and through the Caspian region to central Asia. It consists of annuals, characterized by having perianth segments connate, with these hardening and forming an urceolate body in fruit, and by inflated bladderlike anther appendages (Zhu et al. 2003). Therefore, *Halimocnemis* should be studied more intensively.

Classification and relationships within the clade Salsoleae s.str. and the polyphyly of the genera *Anabasis* and *Halogeton*

The clade Salsoleae s.str. is strongly supported (Figs. 2, 3). It is mainly composed of *Anabasis*, *Cornulaca*, *Horaninowia*, *Haloxyton*, *Halogeton*, *Iljinia*, and *Salsola* s.str.

Anabasis forms a well supported monophyletic genus with the exclusion of *Anabasis setifera* (Figs. 1, 2, 4), as found by Akhani et al. (2007), even though some of the species of *Anabasis* examined were different. However, the *cpDNA* tree (Fig. 3) supported monophyly including *A. setifera* (bt = 79%; pP = 99%). We cannot be sure of the accuracy of this because only the *psbB-psbH* sequence of *A. setifera* was available from GenBank for *cpDNA* analyses. Additional sequences will be needed to verify the position of *A. setifera* in the *cpDNA* tree. Sukhorukov (2008) showed that *A. setifera* and *A. annua* were separated from the remainder of the genus by a combination of carpological characteristics and chorotype data, while Akhani et al. (1997) merged *A. setifera* and *A. annua*. *Anabasis* comprises ca. 30 species characterized by fleshy annual shoots, opposite leaves and branches, leaves usually reduced or short, and sometimes with blunt spines at their apex, and vertical seeds (Zhu et al. 2003; Sukhorukov 2008). *A. setifera* has linear striated stems, well developed leaves, a colored wing, and a small fruit (2.0–2.5 mm), with a single-layered epidermis, and cylindrical papillae across the entire surface (Sukhorukov 2008). Notably, under different climatic regimes, *A. setifera* exhibits contrasting life forms, annual and perennial, that may depend upon the fluctuation of annual rainfall (Akhani et al. 1997), which may be an important character distinguishing it from other species of *Anabasis*. Combining the morphological characteristics of *A. setifera*, we may give a tentative suggestion that *A. setifera* should be separated from *Anabasis* in order to retain the monophyly of the genus.

Halogeton is not monophyletic (Figs. 1, 2, 3, 4), even though Akhani et al. (2007) determined that *Halogeton* was monophyletic in his analysis of two species, the perennial *H. alopecuroides* and the annual *H. glomeratus*. Based on our results, the annual *H. glomeratus* has a closer relationship with *H. alopecuroides* than does the other annual species *H. arachnoideus*, which is in a separate clade (Figs. 1, 2, 3, 4). *Halogeton* is a minor genus considered to have either one (Grubov 1999), three (Ulbrich 1934; Zhu et al. 2003), or about five species (Akhani et al. 2007), and two life forms (Akhani et al. 2007). The present analyses suggest that *Halogeton* is polyphyletic and do not support the hypothesis that the two life forms form monophyletic sister lineages as suggested by Akhani et al. (2007).

Iljinia is sister to a clade consisting of *Haloxyton*, *Girgensohnia*, and *Halogeton* in the Salsololeae s.str. clade (Figs. 2, 4). A monotypic genus, *Iljinia* is restricted to the Gobi desert in central Asia and consists of extremely xerophytic subshrubs, characterized by alternate leaves, knotless branches, solitary flowers in leaf axils, and a subglobose perianth. It has a -119% carbon isotope ratio, showing a value typical of C₄ species. Both cotyledons and true leaves have a salsoloid mesophyll structure (Pyankov et al. 2001b). The cotyledons have salsoloid anatomy and notably thickened (25–40 μm) periclinal cell walls in the outer epidermis (Sukhorukov 2008), which may help its survival in drought conditions, especially during the period of germination (Butnik 1979; Pyankov et al. 2000).

In the Salsoleae s.str. clade, in addition to C₄ species, C₃ and C₃–C₄ intermediate species also occur. *Salsola arbusculiformis* (a C₃–C₄ intermediate species, Voznesenskaya et al. 2001) and *Salsola laricifolia* [with a -23.1% carbon isotope ratio, typical for C₃ species (Pyankov et al. 2001b), yet according to unpublished data, with a Kranz-like layer] are located together as a sister pair (Figs. 1, 2, 4). Nevertheless, based on a single ITS gene, Pyankov et al. (2001a) placed them in different clades. Their shared morphological characters support the sister pair relationship, in that they consist of small shrubs characterized by semiterete, non-decurrent leaves, with leaves of the older branches often clustered at the apex of dwarf branches, spicate inflorescences, and with the portion of the perianth segments above the wing joined together in a cone (Zhu et al. 2003). Here the placement of *Salsola arbusculiformis* may not provide evidence on whether it is a phylogenetic intermediate between C₃ and C₄.

Salsola foliosa in the Salsoleae s.str. clade seems to have no close relationship to other species in *Salsola* s.str. (Figs. 1, 2, 4). It has been treated as monotypic section *Coccosalsola* (Iljin 1936; Grubov 1999). There is an important morphological evidence for this placement,

possession of a berrylike utricle, which distinguishes it from other species of *Salsola*. It consists of halophytic annuals, mainly distributed in saline soils in deserts or semideserts in North Xinjiang (Zhao et al. 2002).

The monophyly of *Haloxylon* is well supported (Figs. 1, 2, 3, 4), which is in agreement with the results of Akhani et al. (2007). It traditionally comprises *H. ammodendron* and *H. persicum* (Iljin 1936; Grubov 1999). *Haloxylon* is inferred to be derived from *Salsola* (Botschantzev 1969, 1976). The species of *Haloxylon* are tree-like and have a particular photosynthetic organ, the cylindrical shoot (Casati et al. 1999), with C₄ salsoloid anatomy (Voznesenskaya 1976). Nevertheless, the cotyledons are of C₃ photosynthetic type (Pyankov et al. 2001b). Different photosynthetic pathways between leaves and cotyledons as well as elongate roots that have efficient access to underground water (Akhani et al. 2003; Akhani 2004) may enable them to grow well under severe conditions (Casati et al. 1999). These are dominant species in the vegetation of the Junggar Basin (Liu 1995) and have been widely used for sand-binding and afforestation (Liu 1995).

Relationships in the *Kali* clade

The monophyly of the *Kali* clade is well supported (Figs. 1, 2, 3, 4). It is comprised of two parts (Figs. 1, 2, 3, 4), the larger consisting of *Kali*, which Akhani et al. (2007) placed in the Salsoleae s.str. clade of Salsoleae s.l.

Kali is shown to be monophyletic (Figs. 1, 2, 3, 4), in agreement with the results of Akhani et al. (2007), although limited samples were used in their phylogenetic trees. As a group, the genus *Kali* has a series of morphological characters. They are annual herbs, plants papillate, hispid or glabrous, stems with a striped cortex, with the chlorenchyma consisting of palisade and Kranz layers interrupted by longitudinal collenchymatic ridges, leaf apices spine-tipped, flowers solitary, and the fruiting perianth winged or with an irregular process (Rilke 1999; Zhu et al. 2003; Akhani et al. 2007). Species in *Kali* are mainly distributed in continental Asia (from Kazakhstan to Mongolia and within the Iran-Turanian area) (Rilke 1999). In China, the distribution is concentrated in northwestern China, especially in Xinjiang Province (Zhu et al. 2003). Rilke (1999, Table 2) suggested that the center of genetic diversity of *Kali* can be expected in Xinjiang-Dzungaria, where the greatest number of species is found.

Acknowledgments Thanks to Prof. P. Yan for providing Salsoleae s.l. field collections from Xinjiang Province, China, Dr. D. M. Williams (London, UK) for helpful comments on the manuscript, Mrs. Lorraine Williams (London, UK) for improving the English of the manuscript, and two anonymous reviewers for valuable comments on a previous version. This research was funded by the National Basic

Research Program of China (2009CB825104), Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences.

References

- Akhani H (2004) Halophytic vegetation of Iran: towards a syntaxonomical classification. *Ann Bot (Rome)* 4:66–82
- Akhani H, Trimborn P, Ziegler H (1997) Photosynthetic pathways in Chenopodiaceae from Africa, Asia and Europe with their ecological, phytogeographical and taxonomical importance. *Plant Syst Evol* 206:187–221
- Akhani H, Ghabadnejhad M, Hashemi SM (2003) Ecology, biogeography and pollen morphology of *Bienertia cycloptera* Bunge ex Boiss. (Chenopodiaceae), an enigmatic C₄ plant without Kranz anatomy. *Plant Biol* 5:167–178
- Akhani H, Edwards G, Roalson EH (2007) Diversification of the old world Salsoleae s.l. (Chenopodiaceae): molecular phylogenetic analysis of nuclear and chloroplast data sets and a revised classification. *Int J Plant Sci* 168:931–956
- Assadi M (2001) Chenopodiaceae. In: Assadi M, Khatamsaz M, Maassoumi AA (eds) *Flora of Iran*, vol 38. Research Institute of Forests and Rangelands, Tehran, pp 27–65
- Blackwell WH Jr (1977) The subfamilies of the Chenopodiaceae. *Taxon* 26:395–397
- Borger CP, Yan GJ, Scott JK, Walsh MJ (2008) *Salsola tragus* or *S. australis* (Chenopodiaceae) in Australia—untangling taxonomic confusion through molecular and cytological analyses. *Aust J Bot* 56:600–608
- Botschantzev VP (1956) Sbornik rabot po geobotanike, lesovedeniju, paleogeografii floristike: dva novykh roda iz semestva marevykh. In: Akademiku VN, Sukachevu K (eds) *Akademia Nauk SSSR. Izdatel'stvo Akademia Nauk SSSR*, Moscow, pp 108–118
- Botschantzev VP (1969) The genus *Salsola*: a concise history of its development and dispersal (in Russian). *Bot Zhurn* 54:989–1001
- Botschantzev VP (1974) Species subsections *Caroxylon* sections *Caroxylon* (Thunb.) Fenzl generis *Salsola* L. (in Russian). *Nov Sist Vyssh Rast* 11:110–174
- Botschantzev VP (1976) Conspectus specierum sections *Coccosalsola* Fenzl generis *Salsola* L. (in Russian). *Nov Sist Vyssh Rast* 13:74–102
- Butnik AA (1979) Types of development of seedlings of Chenopodiaceae Vent. (in Russian). *Bot Zhurn* 64:834–842 (in Russian)
- Cabrera JF, Jacobs SWL, Kadereit G (2009) Phylogeny of the Australian Camphorosmeae (Chenopodiaceae) and the taxonomic significance of the fruiting perianth. *Int J Plant Sci* 170:505–521
- Casati P, Andreo CS, Edwards GE (1999) Characterization of NADP-malic enzyme from two species of Chenopodiaceae: *Haloxylon persicum* (C₄) and *Chenopodium album* (C₃). *Phytochemistry* 52:985–992
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Freitag H (1997) *Salsola* L. (Chenopodiaceae). In: Rechinger KH (ed) *Flora Iranica*, vol 172. Akademische Druck und Verlagsanstalt, Graz, pp 154–255
- Fu LK, Zhang XC, Qin HN, Ma JS (1993) *Index herbariorum sinicorum* (in Chinese). Chinese Science and Technology Press, Beijing, pp 425–457
- Grubov VI (1999) Chenopodiaceae. In: *Plants of Central Asia*, vol 2. Science Publishers, Enfield, pp 87–133

- Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696–704
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst Biol* 42:182–192
- Holmgren PK, Holmgren NH (1998) (continuously updated) Index herbariorum. <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>
- Huelsenbeck JP, Rannala B (2004) Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Syst Biol* 53:904–913
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755
- Ilijin MM (1936) Chenopodiaceae. In: Siskin BK (ed) Flora SSSR, vol 6 (in Russian). Izdatel'stvo Akademii Nauk SSSR, Leningrad, pp 2–354
- Johnson LA, Soltis DE (1995) Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matK* sequences. *Ann Mo Bot Gard* 82:149–175
- Kadereit G, Borsch T, Weising K, Freitag H (2003) Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C₄ photosynthesis. *Int J Plant Sci* 164:959–986
- Kadereit G, Gotzek D, Jacobs S, Freitag H (2005) Origin and age of Australian Chenopodiaceae. *Org Divers Evol* 5:59–80
- Kang Y, Zhang ML, Chen ZD (2003) A preliminary phylogenetic study of the subgenus *Pogonophace* (*Astragalus*) in China based on ITS sequence data. *Acta Bot Sin* 45:140–145
- Kapralov MV, Akhani H, Voznesenskaya EV, Edwards G, Franceschi V, Roalson EH (2006) Phylogenetic relationships in the Salicornioideae/Suaedoideae/Salsoloideae s.l. (Chenopodiaceae) clade and a clarification of the phylogenetic position of *Bienertia* and *Alexandra* using multiple DNA sequence datasets. *Syst Bot* 31:571–585
- Kühn U, Bittrich V, Carolin R, Freitag H, Hedge IC, Uotila P, Wilson PG (1993) Chenopodiaceae. In: Kubitzki K, Rohwer JG, Bittrich V (eds) The families and genera of vascular plants, vol 2. Springer, Berlin, pp 253–281
- Liu YX (1995) Observations on the formation of Chinese desert floras (in Chinese with English abstract). *Acta Phytotax Sin* 33:131–143
- Meyer CA (1829) Generae Chenopodearum. In: Ledebour CF (ed) Flora Altaica, vol 2. Reimer, Berlin, pp 370–371
- Moquin-Tandon A (1840) Chenopodearum monographica enumeratio. Loss, Paris, p 182
- Moquin-Tandon A (1849) Salsolaceae. In: de Candolle AP (ed) Prodrromus systematis naturalis regni vegetabilis, vol 13. Masson, Paris, pp 41–219
- Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–818
- Pyankov VI, Voznesenskaya EV, Kuz'min AN, Ku MSB, Ganko E, Franceschi VR, Black CC, Edwards GE (2000) Occurrence of C₃ and C₄ photosynthesis in cotyledons and leaves of *Salsola* species (Chenopodiaceae). *Photosynth Res* 63:69–84
- Pyankov VI, Artyusheva EG, Edwards GE, Black CC, Soltis PS (2001a) Phylogenetic analysis of tribe Salsoleae (Chenopodiaceae) based on ribosomal ITS sequences: implications for the evolution of photosynthesis types. *Am J Bot* 88:1189–1198
- Pyankov VI, Ziegler H, Kuz'min A, Edwards G (2001b) Origin and evolution of C₄ photosynthesis in the tribe Salsoleae (Chenopodiaceae) based on anatomical and biochemical types in leaves and cotyledons. *Plant Syst Evol* 230:43–74
- Rilke S (1999) Species diversity and polymorphism in *Salsola* sect. *Salsola* sensu lato (Chenopodiaceae). *Syst Geogr Pl* 68:305–314
- Schütze P, Freitag H, Weising K (2003) An integrated molecular and morphological study of the subfamily Suaedoideae Ulbr. (Chenopodiaceae). *Plant Syst Evol* 239:257–286
- Sukhorukov AP (2008) Fruit anatomy of the genus *Anabasis* (Salsoloideae, Chenopodiaceae). *Aust Syst Bot* 21:431–442
- Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0. Sinauer, Sunderland
- Takhtajan A (2009) Flowering plants, vol 1, 2nd edn. Springer, Berlin
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24:4876–4882
- Tzvelev NN (1993) Notes on Chenopodiaceae of Eastern Europe. *Ukr Bot Zhurn* 50:78–85
- Ulbrich E (1934) Chenopodiaceae. In: Engler A, Prantl K (eds) Die natürlichen Pflanzenfamilien, 2nd edn. Duncker & Humblot, Leipzig, pp 379–584
- Voznesenskaya EV (1976) The ultrastructure of assimilating organs of some species of the family Chenopodiaceae, II (in Russian). *Bot Zhurn* 61:1546–1557
- Voznesenskaya EV, Artyusheva EG, Franceschi VR, Pyankov VI, Kiirats O, Ku MSB, Edwards GE (2001) *Salsola arbusculiformis*, a C₃–C₄ intermediate in Salsoleae (Chenopodiaceae). *Ann Bot* 88:337–348
- Wang RZ (2007) C₄ plants in the deserts of China: occurrence of C₄ photosynthesis and its morphological functional types. *Photosynthetica* 45:167–171
- Wei Y, Dong M, Huang ZY, Tan DY (2008) Factors influencing seed germination of *Salsola affinis* (Chenopodiaceae), a dominant annual halophyte inhabiting the deserts of Xinjiang, China. *Flora* 203:134–140
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322
- Williams JT, Ford-Lloyd BV (1974) The systematics of the Chenopodiaceae. *Taxon* 23:353–354
- Wilson PG (1984) Chenopodiaceae. In: George AS (ed) Flora of Australia, vol 4. Australian Government Publishing Service, Canberra, pp 313–317
- Xu DH, Abe J, Sakai M, Kanazawa A, Shimamoto Y (2000) Sequence variation of non-coding regions of chloroplast DNA of soybean and related wild species and its implications for the evolution of different chloroplast haplotypes. *Theor Appl Genet* 101:724–732
- Zhao KF, Fan H, Ungar IA (2002) Survey of halophyte species in China. *Plant Sci* 163:491–498
- Zhu GL (1996) Origin, differentiation, and geographic distribution of the Chenopodiaceae (in Chinese with English abstract). *Acta Phytotax Sin* 34:486–504
- Zhu GL, Mosyankin SL, Clemants SE (2003) Chenopodiaceae. In: Wu ZY, Raven PH (eds) Flora of China, vol 5. Science Press, Beijing, pp 354–414
- Zurawski G, Perrot B, Bottomley W, Whitfield PR (1981) The structure of the gene for the large subunit of ribulose 1, 5-bisphosphate carboxylase from spinach chloroplast DNA. *Nucleic Acids Res* 9:3251–3270