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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

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ABSTRACT. The Chenopodiaceae includes taxa with both C_3 and C_4 photosynthesis with diverse kinds of Kranz anatomy and single-celled C_4 species without Kranz anatomy; thus, it is of key importance for understanding evolution of C_4 photosynthesis. All of the C_4 genera except *Atriplex*, which belongs to Chenopodioideae, are in the Salicornioideae / Suaedoideae / Salsoloideae s.l. (including Camphorosmeae and Sclerolaeneae) clade. Our study focused on the relationships of the main lineages within this clade with an emphasis on the placement of the single cell functioning C_4 genus *Bienertia* using maximum parsimony, maximum likelihood, and Bayesian inference phylogenetic analyses of the nuclear ribosomal ITS and five chloroplast DNA regions (*atpB-rbcL, matK, psbB-psbH, rbcL,* and *trnL-trnF*). Further we provide a detailed phylogeny of *Alexandra* and *Suaeda* based on ITS, *atpB-rbcL,* and *psbB-psbH.* Our molecular data provide strong statistical support for the monophyly of: (1) a Salicornioideae / Suaedoideae / Salsoloideae s.l. clade; (2) a Salicornioideae / Suaedoideae clade; (3) the subfamilies Salicornioideae, Suaedoideae (including *Bienertia*) and Salsoloideae s.l.; (4) the tribes Suaedeae, Salsoleae, and Camphorosmeae; (5) the Salicornieae if Halopeplideae is included; and (6) *Suaeda* if *Alexandra* is included. *Alexandra lehmannii* is therefore reclassified as ***Suaeda lehmannii*** and a new section of *Suaeda* is created, section ***Alexandra.*** There are four independent origins of C_4 photosynthesis within the Suaedoideae including two parallel origins of Kranz C_4 anatomy (in *Suaeda* sections *Salsina* s.l. and *Schoberia*) and two independent origins of C_4 systems without Kranz anatomy (in *Bienertia* and in *Suaeda* section *Borszczowia*).

KEYWORDS: *Alexandra*, Amaranthaceae, Chenopodiaceae, generic circumscription, photosynthetic pathway evolution, *Suaeda*.

The Chenopodiaceae Vent., sometimes treated as a part of the Amaranthaceae Juss. sensu lato (s.l.; Angiosperm Phylogeny Group 1998; 2003: Judd et al. 2002), are among the most diverse lineages (~110 genera and >1400 species; Kühn et al. 1993) of the core Caryophyllales (Cuénoud et al. 2002). The Chenopodiaceae includes species primarily of temperate and subtropical regions commonly dominating salt-marshes, deserts, and semi-deserts. The family possesses the highest diversity of photosynthetic organ anatomy among the angiosperms and this diversity is primarily linked to the multiple origins of C_4 photosynthesis (Akhani et al. 1997; Kadereit et al. 2003). The Chenopodiaceae have more C_4 taxa (45 genera and ~550 species; Sage 2001) than any other family of dicots and, to our present knowledge, they are the only angiosperm family that includes terrestrial C_4 plants lacking Kranz anatomy. Instead, some species have a functional C_4 photosynthetic mechanism within a single cell rather than the typical dual-cell C_4 system (Voznesenskaya et al. 2001; Edwards et al. 2004; Akhani et al. 2005). Members of the family with this distinctive carbon fixation mechanism include *Bienertia cycloptera* Bunge ex Boiss., *B. sinuspersici* Akhani, and *Suaeda aralocaspica* (Bunge) Freitag & Schütze (=*Borszczowia aralocaspica* Bunge).

Organization of Chenopodiaceae genera into subfamilies and tribes has been a source of confusion since the early 1800s (Blackwell 1977). The first division of the Chenopodiaceae into groups was by Meyer (1829) who used seed structure to separate species with exalbuminous seeds and a spiral embryo from those with albuminous seeds and a peripheral embryo. Since Meyer's work (1829), these two major subcategories of the family—Cyclolobeae (embryo annular, endosperm usually present) and Spirolobeae (embryo spirally coiled, endosperm usually lacking)—have been employed by most authors creating classifications within the Chenopodiaceae, including Moquin-Tandon (1840, 1849), Watson (1874), Bentham and Hooker (1880), Volkens (1893), Rendle (1925), Ulbrich (1934), Iljin (1936), Williams and Ford-Lloyd (1974), and Blackwell

(1977). Though the exact rank of the Cyclolobeae and Spirolobeae has differed depending on the author (referenced in Blackwell 1977), the Spirolobeae is virtually equal to the subfamily Salsoloideae Ulbr. in most recent classifications (e.g., Williams and Ford-Lloyd 1974; Blackwell 1977; Kühn et al. 1993) while the Cyclolobeae is equal to the subfamily Chenopodioideae sensu Blackwell (1977) or the subfamilies Chenopodioideae, Salicornioideae Ulbr., and Polycnemoideae Ulbr. sensu Kühn et al. (1993). Based on taximetric data, Scott (1977a, b) considered the leafy Cyclolobeae to be the Chenopodiaceae s. s., while he treated the stem-succulent taxa as the Salicorniaceae and the Spirolobeae as the Salsolaceae. The two molecular phylogenetic studies with taxa sampled from across the whole family (*rbcL,* Kadereit et al. 2003; *ndhF,* Pratt 2003) showed that the Cyclolobeae and Spirolobeae were partially polyphyletic groups. The Spirolobeae (the Salsoleae Moq. and Suaedeae Moq.) and four tribes of the Cyclolobeae (the Camphorosmeae Endl., Halopeplideae Ulbr., Salicornieae Dumort., Sclerolaeneae A.J.Scott) formed a monophyletic clade (bootstrap [bs] = 98% for *rbcL* phylogeny and 99% for *ndhF* phylogeny; Kadereit et al. 2003; Pratt 2003). Thus, molecular data suggest the parallel evolution of spirally twisted embryos in the Salsoleae and Suaedeae (Kadereit et al. 2003; Pratt 2003). The presence of water storage tissue in photosynthetic organs is one of the few characters that is shared by almost all representatives of this clade but almost absent in the rest of the Chenopodiaceae; so, we will further refer to this clade as the succulent clade. The representatives of the Spirolobeae and Cyclolobeae intermingle within the succulent clade: the Salsoleae, Camphorosmeae, and Sclerolaeneae form one monophyletic lineage whereas the Halopeplideae, Salicornieae, and Suaedeae group together in another monophyletic lineage (Kadereit et al. 2003; Pratt 2003).

In both *rbcL* and *ndhF* based phylogenies only the monophyly of the Camphorosmeae + Sclerolaeneae clade (bs = 97% and 100%, respectively) and the monophyly of the Camphorosmeae + Sclerolaeneae + Salsoleae clade (bs = 88% and 100%, respectively) have strong statistical support (Kadereit et al. 2003; Pratt 2003). The monophyly of the Halopeplideae + Salicornieae clade and the Halopeplideae + Salicornieae + Suaedeae clade were not well supported (bs = 55% and 54%) in the study of Kadereit et al. (2003), whereas they were both strongly supported (bs = 100%) in the study of Pratt (2003). However, only two and four taxa from these clades were used in the last work. In both studies, there was no statistical support for the monophyly of the Salsoleae (Kadereit et al. 2003; Pratt 2003). Like the molecular results, morphological characters of taxa within the succulent clade do not give a clear picture of their phylogenetic relationships.

Significant morphological variation within the succulent clade and deficiency of morphological markers that support phylogenetic relationships among major clades call for an extensive molecular investigation within this clade. It was shown that the analyses based on single gene or small number of genes provided insufficient evidence for establishing or refuting phylogenetic hypotheses (Rokas et al. 2003). For this reason, results from the first single gene molecular studies (Kadereit et al. 2003; Pratt 2003) need to be tested by more robust analyses of concatenated data sets of multiple genes.

The prominent trait of the succulent clade is that it contains all Chenopodiaceae genera with C_4 species except for *Atriplex* L. (and possibly *Axyris*; Akhani et al. 1997). In *Atriplex*, C_4 species are all rather similar in leaf anatomy and possess only NAD-ME C_4 photosynthesis (Osmond et al. 1980), while in the succulent clade both NAD-ME and NADP-ME C_4 photosynthetic types are present and these show outstanding diversity of photosynthetic anatomy including Kranz and single-cell C_4 functioning systems variously present in leaves, cotyledons, and stem cortex. Both C_4 genera without Kranz anatomy (*Bienertia* Bunge ex Boiss. and *Borszczowia* Bunge) belong to the tribe Suaedeae sensu Kühn et al. (1993), but their taxonomic position has been changed or questioned after recent molecular phylogenetic studies. *Borszczowia* was submerged into *Suaeda* Scop. and given sectional rank based on the ITS, chloroplast *atpB-rbcL* and *psbB-psbH* phylogenies obtained for the Suaedeae (Schütze et al. 2003), while *Bienertia* showed conflicting relationships, being sister to *Suaeda* in the *atpB-rbcL* and *psbB-psbH* phylogenies (Schütze et al. 2003) but sister to the Salicornioideae in the ITS (Schütze et al. 2003) and *rbcL* (Kadereit et al. 2003) phylogenies; the monotypic tribe Bienertieae Ulbr. was maintained (Schütze et al. 2003), which is better supported by topologies obtained from *matK/trnK* sequences (Müller and Borsch 2005).

The Suaedeae sensu Kühn et al. (1993) equals the Suaedoideae Ulbr. sensu Schütze et al. (2003) and comprises the halophytic leaf-succulent genera *Alexandra*, *Bienertia*, and *Suaeda* (including *Borszczowia*), which are important components of littoral and inland saline and alkaline habitats, particularly in arid and semi-arid vegetation zones. This group includes C_3, Kranz-C_4, and single-cell C_4 species and thus mirrors in microcosm the significant part of C_4 diversity observed in the Chenopodiaceae. However, *Suaeda* is known to be taxonomically very difficult (Akhani and Podlech 1997; Schenk and Ferren 2001) and though recent work of Schütze et al. (2003) cleared up some questions of Suaedeae relationships, many questions remain. Among these are the evolution of the C_4 syndrome within the Suaedeae, the clarification of the phylogenetic position of *Alexandra* and *Bienertia*, and the veri-

fication of the generic position of *Suaeda kossinskyi* Iljin, which has been transferred to *Bienertia* by Tzvelev (1993).

This study presents maximum parsimony, maximum likelihood, and Bayesian inference analyses of the nuclear ribosomal DNA internal transcribed spacer region (ITS) and five chloroplast DNA sequences (*atpB-rbcL*, *matK*, *psbB-psbH*, *rbcL*, and *trnL-trnF*). These analyses are used to address five primary goals: (1) define the relationships within the succulent clade (comprising the Camphorosmeae, Halopeplideae, Salicornieae, Salsoleae, Sclerolaeneae, Suaedeae) and to test the monophyly of its major classification units; (2) clarify the position of *Bienertia* within the succulent clade; (3) test the monophyly of genus *Suaeda* with respect to the monotypic genus *Alexandra*; (4) test whether *Suaeda kossinskyi* belongs to *Suaeda* or *Bienertia*; and (5) test the hypothesis of four independent origins of C_4 photosynthesis within the Suaedoideae.

Materials and Methods

Sampling Design. For DNA isolation we used both herbarium material (collections of H. Akhani, Department of Biology, Tehran University, Iran; Washington State University [WS] Pullman, USA; V. L. Komarov Botanical Institute RAS, St. Petersburg, Russia [LE]) or, in a few cases, plant material fixed in CTAB in the field and living plants from the Washington State University greenhouses. Species and voucher information is presented in Appendix 1.

For the phylogenetic analyses of the Salsoloideae/Salicornioideae/Suaedoideae relationships with emphasis on the position of *Bienertia*, we used representatives of all tribes that belong to these subfamilies in all classifications (1 genus of Halopeplideae; 5 genera of Salicornieae; 4 genera of Salsoleae, 3 genera of Suaedeae; sensu Kühn et al. 1993), as well as representatives from two tribes moved to Salsoloideae s.l. in recent molecular work (3 genera of Camphorosmeae and 1 genus of Sclerolaeneae; Kadereit et al. 2003). *Suaeda* was represented by species from four sections (*Borszczowia*, *Brezia*, *Salsina*, *Schoberia*) and both known species of *Bienertia* (*B. cycloptera* and *B. sinuspersici*; Akhani et al. 2005) were included. Representatives of three tribes of Chenopodioideae s.l. (2 genera of Atripliceae; 1 genus of Chenopodieae; 1 genus of Corispermeae) were included as outgroups (Appendix 1). In addition to new sequences of ITS, *atpB-rbcL*, *matK*, *psbB-psbH*, and *trnL-trnF* added by this study, sequences of these genes were extracted from GenBank from the studies of Schmitz-Linneweber et al. (2001), Cuénoud et al. (2002), Schütze et al. (2003), Shepherd et al. (2004), and Müller and Borsch (2005). Sequences of *rbcL* gene were taken from the studies of Hudson et al. (1990), Schmitz-Linneweber et al. (2001), and Kadereit et al. (2003). Twenty-three of the taxa included in these analyses were a combination of sequences from the same taxa but from the different sources. However, in 9 cases all six genes were not available from a single species, therefore gene copies from closely related species within a genus or section of a genus were concatenated to represent the genus as a whole. While this is not optimal, this set of analyses was focused on determining the relationships among tribes and subfamilies, and therefore combining sequences of different closely related species within a genus is expected to have no effect on recovery of phylogenetic relationships at these higher levels (Appendix 1). The combined dataset for 27 species/species combinations and six genes had 8.6% missing data.

In separate analyses of relationships within the Suaedeae tribe we include the monotypic *Alexandra lehmannii* and 44 species of *Suaeda*, representing all *Suaeda* sections and about half of the recognized species in the genus (Appendix 1). Four species from outside tribe Suaedeae were included as outgroups (*Bienertia cycloptera* Bunge ex Boiss., *Kalidium caspicum* Ung.-Sternb., *Salsola canescens* (Moq.) Boiss., and *S. kali* L.; Appendix 1). In addition to our new ITS, *atpB-rbcL* and *psbB-psbH* sequences, previously reported sequences from GenBank were used from the study of Schütze et al. (2003). In the ingroup, there were five taxa (11%) where we combined our sequences for the same species with those of Schütze et al. (2003). Sequences previously reported for *S. moquinii* (Torr.) Greene and *S. intermedia* S.Watson were combined as these names are currently considered to be included within *S. nigra* (Raf.) J.F.Macbride (Ferren and Schenk 2003). Three species with only one of the three gene sequences available (*S. arbusculoides* L.S.Sm., ITS; *S. monodiana* Maire, ITS; and *S. gracilis* Moq., *psbB-psbH*) were analyzed only in single gene analyses and not included in the combined analyses to minimize the effects of missing data. The combined three-gene data set for the 42 ingroup species had 5.4% missing data.

New sequences have been deposited in GenBank (accessions DQ499332 to DQ499442). The data matrix and resultant trees have been deposited in TreeBASE (accession: S1527).

DNA Sequencing. DNA was isolated using a modified 2× CTAB buffer method (Doyle and Doyle 1987). DNA regions were gathered for two different multigene data sets. For analyses of the Salsoloideae/Salicornioideae/Suaedoideae relationships (hereafter referred to as the subfamily data set) the nrDNA ITS spacer region and cpDNA *rbcL*, *matK*, *psbB-psbH*, *atpB-rbcL*, and *trnL-trnF* regions were gathered. For the analyses of relationships within the Suaedeae tribe (hereafter referred to as the Suaedeae data set), the nrDNA ITS spacer region and cpDNA *psbB-psbH* and *atpB-rbcL* spacer regions were gathered. All regions were combinations of previously published sequences (Appendix 1) and newly sequenced samples except for the *rbcL* sequences, which were all previously published. Templates of the nrDNA ITS region were prepared using the primers ITS5HP (5′-AGG TGA CCT GCG GAA GGA TCA TT-3′; Suh et al. 1993) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′; White et al. 1990). Polymerase chain-reaction (PCR) amplifications follow the procedures described by Roalson et al. (2001). The chloroplast *psbB-psbH* spacer 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). The chloroplast *atpB-rbcL* spacer region was amplified using the primers ''atpB-rbcL-f'' (5′-GAA GTA GTA GGA TTG ATT CTC-3′) and ''atpB-rbcL-r'' (5′-CAA CAC TTG CTT TAG TCT CTG-3′; Xu et al. 2000). The chloroplast *matK* region was amplified using the primers ''ACmatK500F'' (5′-TTC TTC TTT GCA TTT ATT ACG-3′; Hilu et al. 2003) and ''trnK2R'' (5′-AAC TAG TCG GAT GGA GTA G-3′; Johnson and Soltis 1995). The chloroplast *trnL-trnF* spacer region was amplified using the primers ''trnLc'' (5′-CGA AAT CGG TAG ACG CTA CG-3′) and ''trnLf'' (5′-ATT TGA ACT GGT GAC ACG AG-3′; Taberlet et al. 1991). Polymerase chain-reaction (PCR) amplifications follow the procedures described by Johnson and Soltis (1995), Zimmer et al. (2002), or Schütze et al. (2003), depending on the gene region amplified.

The PCR products were electrophoresed using a 0.8% agarose gel in a 0.5× TBE (pH 8.3) buffer, and subsequently stained with ethidium bromide to confirm a single product and purified using the PEG precipitation procedure (Johnson and Soltis 1995).

Sequencing was performed using an ABI Prism 3730 Genetic Analyzer. Direct cycle-sequencing of purified template DNAs followed manufacturer's specifications, using the PRISM® Dye-Deoxy™ Terminator Kit (PE Biosystems) or the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems).

The two ITS sequencing primers provide sequences for overlapping fragments that collectively cover the entire spacer and 5.8S rDNA regions along both strands. The two *psbB-psbH*, two *atpB-rbcL*, two *matK*, and two *trnL-trnF* sequencing primers provide near complete overlap along both strands. Sequencing of ITS and each chloroplast region used the same primers as for amplification.

Automated DNA sequencing chromatograms were proofed, edited, and contigs were assembled using Sequencher 4.0 (Gene Codes Corporation, Inc.). The ITS sequences were truncated to in-

clude only ITS1, 5.8S, and ITS2. The *atpB-rbcL* sequences were truncated to include only the spacer between these genes. The *psbB-psbH* sequences were truncated 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 *matK* sequences covered the 3′ end of the *matK* coding region. The *trnL-trnF* sequences covered the *trnL* intron, *trnL* exon 2, and *trnL-F* intergenic spacer. Identification of the terminal ends and spacer boundaries of ITS1, 5.8S, ITS2, the *atpB-rbcL* spacer, the *psbB-psbH* gene region, the *matK* gene region, and the *trnL-F* spacer region was based on comparisons with other species of Chenopodiaceae (Cuénoud et al. 2002; Schütze et al. 2003; Shepherd et al. 2004). Sequences were aligned by eye (*matK* and *rbcL*) or using Clustal × (Thompson et al. 1997) with the following parameters: pairwise comparisons-gap opening penalty: 10.00, gap extension penalty: 1.00; and multiple comparisons- gap opening penalty: 10.00, gap extension penalty: 1.00. The resultant alignment was then checked by eye for necessary minor corrections to the alignment. Alternate alignment parameters did not result in significantly different topologies (results not shown). Seven gaps from the *atpB-rbcL* region, six gaps from the *psbB-psbH* region and one gap from the ITS region have been coded as binary characters and included in the Suaedeae parsimony analyses. Inclusion or exclusion of gaps did not change the maximum parsimony topology, but did increase branch support for some branches (data not shown).

Maximum Parsimony Analyses. In the subfamily maximum parsimony (MP) analyses, each individual sequence region of 6 genes, individual truncated data sets for ITS and *atpB-rbcL* with extremely variable regions removed, the 6-gene combined data set, and the 6-gene combined data set using the truncated versions of ITS and *atpB-rbcL* were each analyzed. For the Suaedeae MP analyses, each individual sequence region (3), the 3-gene combined data set, and the 3-gene plus coded gaps data set were each analyzed. All analyses were performed using PAUP* 4.0b10 (Swofford 2001). Subfamily analyses used either branch-and-bound (*psbB-psbH, atpB-rbcL* truncated, *matK,* and *trnL-F*) or heuristic (all others) searches (ACCTRAN, 1000 random addition cycles, TBR branch swapping). Suaedeae analyses were performed using heuristic searches (ACCTRAN, 100 random addition cycles, TBR branch swapping). The total number of trees swapped per random addition replicate was constrained to 10,000 in the *atpB-rbcL* region Suaedeae analysis and the *psbB-psbH* region Suaedeae analysis due to a large number of equally parsimonious trees. Swapping was run to completion for all random addition replicates. Clade support was estimated using 100 heuristic bootstrap replicates (100 random addition cycles per replicate, TBR branch swapping; Felsenstein 1985; Hillis and Bull 1993). The total number of trees swapped per random addition replicate in all of the Suaedeae bootstrap analyses was constrained to 10,000 due to a large number of trees.

Maximum Likelihood Analyses and Tests of Alternative Topologies. Maximum likelihood (ML) analyses of the 6-gene subfamily data set and 3-gene Suaedeae data set were performed using PAUP* 4.0b10 (Swofford 2001). Heuristic searches were employed (TBR branch swapping). Clade support was estimated using 100 heuristic bootstrap replicates (10 random addition cycles and 100 total rearrangements per replicate, TBR branch swapping; Felsenstein 1985; Hillis and Bull 1993). Subfamily ML analyses employed the general time reversable (GTR) model with proportion of invariant sites (I) and gamma shape (G) parameters and empirical base frequencies (six substitution types: A/C- 0.9993, A/G- 1.3381, A/T- 0.4823, C/G- 0.7391, C/T- 1.9689, G/T- 1.0000; I = 0.3222; G = 0.7910; A- 0.3106, C- 0.1718, G- 0.1951, T- 0.3225). Suaedeae ML analyses employed a four rate class transition (TIM) model with proportion of invariant sites (I) and gamma shape (G) parameters and empirical base frequencies (four substitution types: A/C- 1.0000, A/G- 1.3647, A/T- 0.6371, C/G- 0.6371, C/T- 2.5453, G/T- 1.0000; I = 0.3185; G = 0.7002; A- 0.2944, C- 0.1837, G- 0.1955, T- 0.3264). These models were chosen based on the results of analysis using DT_ModSel (Minin et al. 2003). The DT_ModSel analysis uses a Bayesian information criterion to select a model using branch-length error as a performance measure in a decision theory framework that also includes a penalty for model overfitting. Four alternative topologies for the Suaedeae data set were tested using the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) in a maximum likelihood framework employing constraint options implimented in PAUP*: monophyly of *Suaeda* + *Borszczowia* minus *Alexandra*, monophyly of *Suaeda* + *Alexandra* minus *Borszczowia*, monophyly of *Suaeda* section *Salsina* excluding species traditionally placed in sections *Immersa* and *Limbogermen*, and monophyly of Kranz-C_4 type *Suaeda* species (not including species with C_4 function in a single-cell). The SH analysis was run with 10000 RELL bootstraps (one-tailed). Tests were done comparing the RELL bootstraps to full optimization analyses and no significant difference was found in the two methods (data not shown).

Bayesian Inference Analyses. Bayesian inference analysis of the 6-gene subfamily data set and 3-gene Suaedeae data set were performed using MrBayes v.3.0 (Huelsenbeck and Ronquist 2001). Ten million generations were run with four chains (Markov Chain Monte Carlo), and a tree was saved every 100 generations. Priors included a separate model for each gene allowing up to six substitution types and rates following a gamma distribution for each gene. In the subfamily data set analysis, a parameter for invariant sites was included for the gene regions ITS and *rbcL*. For the Suaedeae data set analysis, an invariant sites parameter was included for the *psbB-psbH* region. This model was chosen based on the results of analysis using DT_ModSel for each individual gene (see ML methods above; Minin et al. 2003). For each gene in the subfamily data set the DT_ModSel results were: ITS, SYM+I+G (six rate classes following a gamma distribution and invariant sites); *atpB-rbcL,* TVM+G (five rate classes following a gamma distribution); *psbB-psbH,* K81uf+I+G (three rate classes following a gamma distribution and invariant sites); *matK,* TVM+G (as above); *rbcL,* TIM+I+G (four rate classes following a gamma distribution and invariant sites); and *trnL-F,* K81uf+G (three rate classes following a gamma distribution). For each gene in the Suaedeae data set the DT_ModSel results were: ITS, SYM+G (six rate classes following a gamma distribution); *atpB-rbcL,* K81uf+G (as above); and *psbB-psbH,* K81uf+I+G (as above). Since MrBayes only allows the choices of 1, 2, or 6 rate categories, all of the 3+ rate category submodels of the general time reversible model were placed in the six rate class. Majority rule consensus trees of the trees sampled in Bayesian inference analyses yielded probabilities that the clades are monophyletic (Lewis 2001). The trees from the MrBayes analysis were loaded into PAUP*, discarding the trees sampled during the ''burnin'' of the chain (Huelsenbeck and Ronquist 2001; the first 20,000,000 generations) to only include trees after stationarity was reached. Posterior probability values (pp) are presented on the single ML topologies.

Light Microscopy. Leaf samples were fixed at 4°C in 2% (v/v) paraformaldehyde and 1.25% (v/v) glutaraldehyde in 0.05 M PIPES buffer, pH 7.2. The samples were dehydrated with a graded ethanol series and embedded in London Resin White (LR White, Electron Microscopy Sciences, Fort Washington, PA, USA) acrylic resin. Cross sections were made on a Reichert Ultracut R ultramicrotome (Reichert-Jung GmbH, Heidelburg, Germany). Semithin sections were stained with 1% (w/v) Toluidine blue O in 1% (w/v) Na2B4O7.

RESULTS

Figure 1 shows examples of species in the succulent clade of Chenopodiaceae from natural habitats. These are members of subfamily Salicornioideae (1A showing a *Salicornia* dominated community, and 1B *Kalidium capsicum*), and from subfamily Suaedoideae including *Bienertia sinuspersici* (1C), *Suaeda fruticosa* (1D), *S. microsperma* (1E), *S. linifolia* (1F), *S. physophora* 1G), and from subfamily Salsoloideae, *Girgensohnia opposi-*

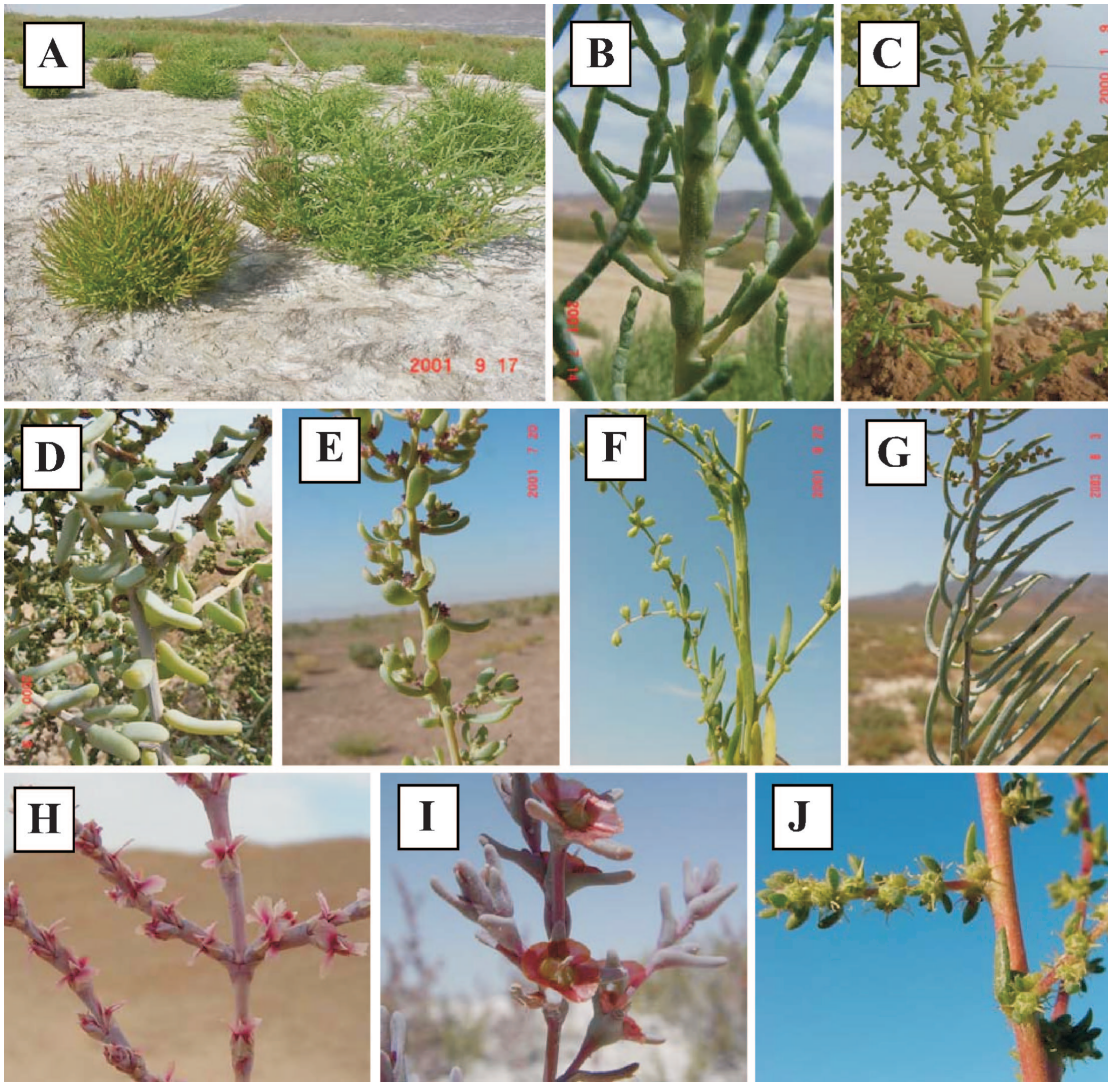

FIG. 1. Representatives of major lineage of the succulent clade of Chenopodiaceae. A. *Salicornia* dominated communities (*S. persica* Akhani among others) in Gavkhooni hypersaline wetland in Esfahan Province, Central Iran. B. *Kalidium caspicum* (L.) Ungern-Sternb., subfamily Salicornioideae C. *Bienertia sinuspersici* Akhani, subfamily Suaedoideae, in salty, sandy soils along the Kol river in Hormozgan province, S. Iran. D. *Suaeda fruticosa* Forssk. ex J.F.Gmelin, subfamily Suaedoideae, a widely distributed C_4 species in warm salt deserts of the Middle East to North Africa, Khuzestan Province, S. Iran. E. *Suaeda microsperma* (C.A. Mey.) Fenzl, subfamily Suaedoideae, a C_4 annual characterized by flat leaves and long caducous bristle, Semnan Province, Central Iran. F. *Suaeda linifolia* Pallas subfamily Suaedoideae, a C_3 species characterized by flat oblong leaves and paniculately branched inflorescence born of the petiole. G. *Suaeda physophora* Pallas subfamily Suaedoideae, a shrubby C_3 species distributed in the cold temperate salt deserts in Central Asia and Northeast and Northwest Iran. H. *Girgensohnia oppositiflora* (Pallas) Fenzl, subfamily Salsoloideae, tribe Salsoleae, a C_4 species with opposite branches and slightly succulent leaves, winged fruiting perianths inhabiting moderate salty and non-salty dry disturbed soils from Yazd Province, Central Iran. I. *Climacoptera crassa* (M. Bieb.) Botsch,.subfamily Salsoloideae, tribe Salsoleae, a widely distributed C_4 species in Central and Minor Asian and Iranian salines with succulent leaves and winged fruiting perianths, Tüz Guli Lake, Central Anatolia. J. *Bassia hyssopifolia* (Pall.) O. Kuntze, subfamily Salsoloideae, tribe Camphorosmeae, salty disturbed soils between Tehran and Semnan. All pictures by H. Akhani.

tiflora (1H), *Climacoptera crassa* (1I), and *Bassia hyssopifolia* (1J).

Characteristics of the fifteen aligned matrices and the resultant MP analyses are detailed in Table 1. Due to space constraints, these trees are not presented here but are available on request from the corresponding author.

All individual analyses of the subfamily data sets resulted in similar topologies that generally differ only in the level of resolution found and branch support as

TABLE 1. Subfamily and Suaedeae analysis results. Unaligned sequence lengths are not given for concatenated data sets. Abbreviations are as follows: Sf. = subfamily analysis, Su. = Suaedeae analysis, ITS = internal transcribed spacer region (including the 5.8S rRNA), *atpB* = *atpB-rbcL* spacer region, *matK* = *matK-trnK* gene region, *trnL* = *trnL-trnF* spacer region, *psbB* = *psbB-psbH* spacer region, G = gaps included, T = truncated, # Var. Char. = number of variable characters, # PI Char. = number of potentially parsimony informative characters, CI = consistency index (Kluge and Farris 1969), RI = retention index (Farris 1989), and RC = rescaled consistency index (Farris 1989).

	# Taxa	Aligned Length	# Var. Char.	# PI Char.	Ingroup Unaligned Length	Uncorrected Pairwise Divergence	# Gaps (Length Range)	Tree Length	# Trees	CI	RI	RC
Sf. ITS	25	704	384	295	602–644	1.0–26.5	97 (1–21)	1386	4	0.501	0.520	0.261
Sf. ITS (T)	25	625	312	230	553–573	1.0–24.2	65 (1–21)	1032	8	0.519	0.537	0.279
Sf. *atpB*	24	890	455	207	504–800	0–27.1	94 (1–337)	843	121	0.740	0.639	0.473
Sf. *atpB* (T)	24	818	388	156	504–734	0–27.1	79 (1–265)	623	4	0.793	0.691	0.548
Sf. *matK*	24	1245	492	261	1172–1236	1.1–13.4	28 (1–10)	895	2	0.699	0.668	0.467
Sf. *psbB*	27	748	196	94	651–688	0.5–8.1	62 (1–15)	316	174	0.744	0.716	0.532
Sf. *trnL*	25	1409	502	242	935–1096	0–28.4	133 (1–238)	788	1	0.813	0.757	0.616
Sf. *rbcL*	23	1343	222	116	1343	0.6–4.5	0	397	24	0.622	0.624	0.388
Sf. All	27	6339	2251	1215	—	1.1–15.9	414 (1–337)	4662	1	0.658	0.615	0.405
Sf. All (T)	27	6188	2112	1099	—	1.1–15.3	367 (1–265)	4088	1	0.680	0.635	0.432
Su. ITS	49	686	365	250	621–635	0–20.7	56 (1–9)	1053	2	0.522	0.788	0.435
Su. *atpB*	42	920	316	142	523–795	0–19.3	74 (1–200)	479	2382	0.802	0.869	0.697
Su. *psbB*	47	675	123	77	609–637	0–6.3	35 (1–23)	188	8587	0.734	0.912	0.670
Su. All	47	2281	798	462	—	0–18.0	165 (1–200)	1730	252	0.632	0.806	0.510
Su. All + G	47	2295	802	476	—	0–18.0	—	1747	252	0.634	0.812	0.515

measured by bootstrap analysis. When the strict consensus topologies of the complete and truncated ITS and *atpB-rbcL* data sets are compared, the ITS complete analysis results in a more resolved strict consensus topology with higher bootstrap support for several branches. This suggests that while alignment might be difficult, the more variable ITS regions are contributing branching structure that is lost in the analysis of the truncated data set, and these branches are congruent with the topologies found with other genes. Alternatively, the *atpB-rbcL* region truncated data set strict consensus tree is more resolved than the complete data set consensus tree, suggesting that the excluded sections might be contributing homoplasy to the analysis and confounding relationships. When all genes are combined, either with the complete ITS and *atpB-rbcL* region data sets or with the truncated data sets, a single tree is found in both cases and nearly every branch in the tree has strong support. These two topologies differ in only a single branch. In the complete data matrix *Salsola kali* L. and *Girgensohnia oppositiflora* (Pall.) Fenzl form a grade leading to a clade of *Salsola canescens* (Moq.) Boiss., *Petrosimonia glauca* (Pall.) Bunge, and *Climacoptera crassa* (M. Bieb.) Botsch., whereas these two species form a clade sister to the *Salsola canescens*, *Petrosimonia*, and *Climacoptera* clade in the truncated data set analysis. Neither of these branches has strong support.

The succulent clade is monophyletic (maximum parsimony bootstrap [mpbs] = 100%). Well-supported branches are found for the monophyletic Suaedeae (mpbs = 100%) sister to *Bienertia* (mpbs = 71%). The Salicornioideae is monophyletic (mpbs = 100%), but within this subfamily, the Salicornieae is paraphyletic with regards to the Halopeplideae. The Suaedoideae (Suaedeae + *Bienertia*) + Salicornioideae clade (mpbs = 100%) is sister to a Salsoloideae clade (mpbs = 100%). Within the monophyletic Salsoloideae clade (mpbs = 98%), monophyletic Salsoleae and Camphorosmeae (including Sclerolaeneae) clades are found (mpbs = 87% and 100%, respectively).

Maximum likelihood analysis of subfamily relationships using the all gene complete data set recovers a single phylogenetic hypothesis (-lnL = 32895.30085) very similar to the topology found in the MP analyses and with strong support for most branches as measured by ML bootstrap (mlbs) and Bayesian inference posterior probabilities (pp; Fig. 2). The only differences between the ML and MP results are the branching orders of *Salsola kali* and *Girgensohnia*, and *Panderia* and *Camphorosma*, both of which are branches with low support in both analyses.

Evaluation of relationships within the Suaedeae by MP analysis of the individual ITS, *atpB-rbcL,* and *psbB-psbH* data sets showed largely congruent strict consensus trees with most differences associated with poorly supported branches in one or more analyses, such as the different placements of the "*S. ekimii*" + *S. vera* Forssk. ex J.F.Gmelin clade. Analyses of the combined data set of all gene regions and this data set with gaps coded as binary characters resulted in the same strict consensus, but with slightly different branch support as measured by bootstrap values (further reference to mpbs1 = all data and mpbs2 = all data plus coded gaps). Most of the branches show strong support and this topology is congruent with the single topology

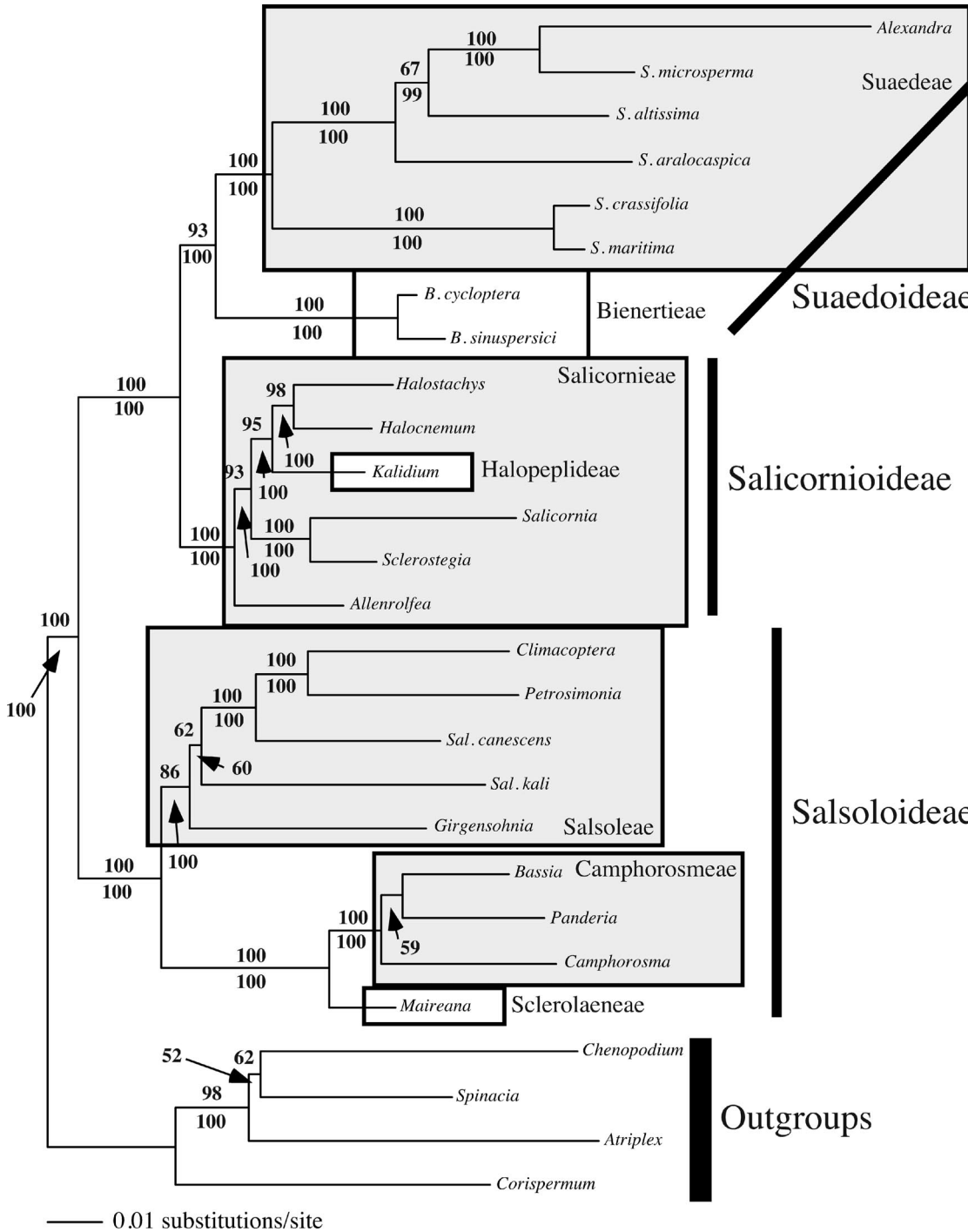

FIG. 2. The single maximum likelihood phylogram (-lnL = 32895.30085) based on combined complete ITS, *atpB-rbcL*, *matK*, *psbB-psbH*, *trnL-trnF*, and *rbcL* sequence. Numbers above branches refer to bootstrap percentages and those below branches refer to Bayesian inference posterior probabilities. Abbreviations are as follows: *B.* = *Bienertia*, *Sal.* = *Salsola*, and *S.* = *Suaeda*.

(Fig. 3; -lnL = 12514.28030) resulting from the ML analysis of the combined gene data set which is similarly well supported by ML bootstrap and Bayesian posterior probabilities. These topologies suggest well supported monophyly of (1) the two *Suaeda* subgenera *Brezia* (Moq.) Freitag & Schütze (mpbs1/mpbs2/mlbs/pp = all 100%) and *Suaeda* (100/99/100/100), (2) sections *Brezia* (Moq.) Volk. (all 100), *Salsina* Moq. sensu Schütze et al. (2003; all 100), *Schanginia* (C.A.Mey) Volk. (all 100), *Suaeda* (all 100), *Physophora* Iljin (95/87/98/100), and *Schoberia* (C.A.Mey) Volk. (95/93/93/100). The section *Salsina* sensu Schenk and Ferren (2001) is paraphyletic with regards to the monotypic section *Immersa* Townsend and section *Limbogermen* Iljin and is sister to the monotypic section *Macrosuaeda* Tzvel. (all 100). The monotypic section *Borszczowia* (Bunge) Freitag & Schütze is sister to section *Schanginia* (all 100). The genus *Suaeda* is paraphyletic with regards to the monotypic genus *Alexandra*, which is sister to section *Schoberia* (all 100). The *Suaeda* sections *Physophora*, *Salsina* s.l., and *Schoberia* and the genus *Alexandra* form a monophyletic clade (74/89/82/100).

Four alternative topologies of relationships within Suaedeae were tested here in comparison with the ML hypothesis: monophyly of *Suaeda* + *Borszczowia* minus *Alexandra*, monophyly of *Suaeda* + *Alexandra* minus *Borszczowia*, monophyly of *Suaeda* section *Salsina* excluding species traditionally placed in sections *Immersa* and *Limbogermen*, and monophyly of Kranz-C_4 type *Suaeda* species (not including species with C_4 function in a single-cell). In all four cases, the constrained ML topology was significantly worse than the ML tree (all $P < 0.05$). This allows us to statistically exclude (given the data) the possibility that *Suaeda* excluding *Alexandra* and *Borszczowia* is monophyletic, that section *Salsina* excluding *Limbogermen* is monophyletic, and that there was a single origin of Kranz C_4 in *Suaeda*.

Examples of the diversity in leaf anatomy in subfamily Suaedoideae are shown in Figs. 4A–H. This includes *Bienertia cycloptera* in tribe Bienertieae with Bienertioid leaf anatomy, a species with single-cell functioning C_4 photosynthesis (4A), *Suaeda aralocaspica* in section *Borszczowia*, another single cell C_4 species, with Borszczowioid leaf anatomy (4B), *S. cochlearifolia* and *S. eltonica* in section *Schoberia* with Conospermoid leaf anatomy (4C, D), *S. arcuata* in section *Salsina* with *Suaedoid* type leaf anatomy (4E), *S. heterophylla*, a C_3 species, in section *Brezia* with austrobassioid type anatomy (4F), *S. linifolia* in section *Schanginia* a C_3 species (4G), and *S. physophora* a C_3 species in section *Physophora* (4H).

Discussion

Monophyly of the Succulent Clade. The combined analysis closely resembles the clades found in previous single gene-based studies (Kadereit et al. 2003; Pratt 2003; Müller and Borsch 2005) but gives much stronger statistical support and better resolution to infraclade lineages. The monophyly of the succulent clade is strongly supported, lending further support to the non-monophyly of the Spirolobeae and Cyclolobeae. We agree with the conclusion of Kadereit et al. (2003) that the Spirolobeae and Cyclolobeae subdivisions are artificial. Our molecular data give strong statistical support for independent origins of spirally twisted embryos in the Salsoleae and Suaedoideae from plesiomorphic annular embryos. However, the nature of embryo shape in Chenopodiaceae needs to be reevaluated in a broader context to consider the seed structure and dispersal mechanism. The embryos of many Salsoleae we have studied (Akhani, Ghaffari, and Doulatyari, unpublished data) are indeed well developed cotyledon leaves which are packed into winged or unwinged indurated perianths (Fig. 1H, I). These are covered by a thin membranous testa. In contrast, in Suaedeae the embryos are much smaller and strongly packed into an indurate testa. It is interesting that in both species of *Bienertia* both types of indurated testa and non-indurated scarious testa have been observed.

***Relationships within the Salicornioideae / Suaedoideae Clade and the Position of* Bienertia.** Salicornioideae and Suaedoideae comprise taxa of dramatically different morphology but close ecological characteristics, as they are mostly obligate hygrohalophytes. Along salinity and moisture gradients, the members of Salicornioideae often associate with C_3 *Suaeda* in the relatively wetter habitats, but with decreasing water availability the C_4 *Suaeda*, *Bienertia*, and several species of Salsoleae dominate in most saline vegetation zones of SW Asia (Akhani 2004). The cartaceous seed testa may be an important shared feature between Salicornioideae and Suaedoideae. Except *Halosarcia indica* (Willd.) P.G.Wilson, all other Salicornioideae possess C_3 photosynthesis and comprise taxa with reduced leaves and articulated stems (Fig. 1A, B); the Suaedoideae have nearly equal numbers of C_3 and C_4 taxa with well-developed leaves (Fig. 1A–G). These subfamilies traditionally have been regarded as distant from each other because of numerous morphological differences (see detailed discussion in Kadereit et al. 2003). However, monophyly of the Salicornioideae / Suaedoideae clade was suggested by previous molecular studies (Kadereit et al. 2003; Pratt 2003) although without statistical support for this clade, nor for its relationship to other lineages in the family. Our analyses give strong statistical evidence for the monophyly of (1) the Salicornioideae / Suaedoideae clade (Fig. 2; mpbs/mlbs/pp = 100/100/100), (2) the Salicornioideae and Suaedoideae (including *Bienertia*) subfamilies (Fig. 2; 100/100/100 and 71/93/100, respectively) and (3) the Bienertieae and Suaedeae tribes (Fig. 2; 100/

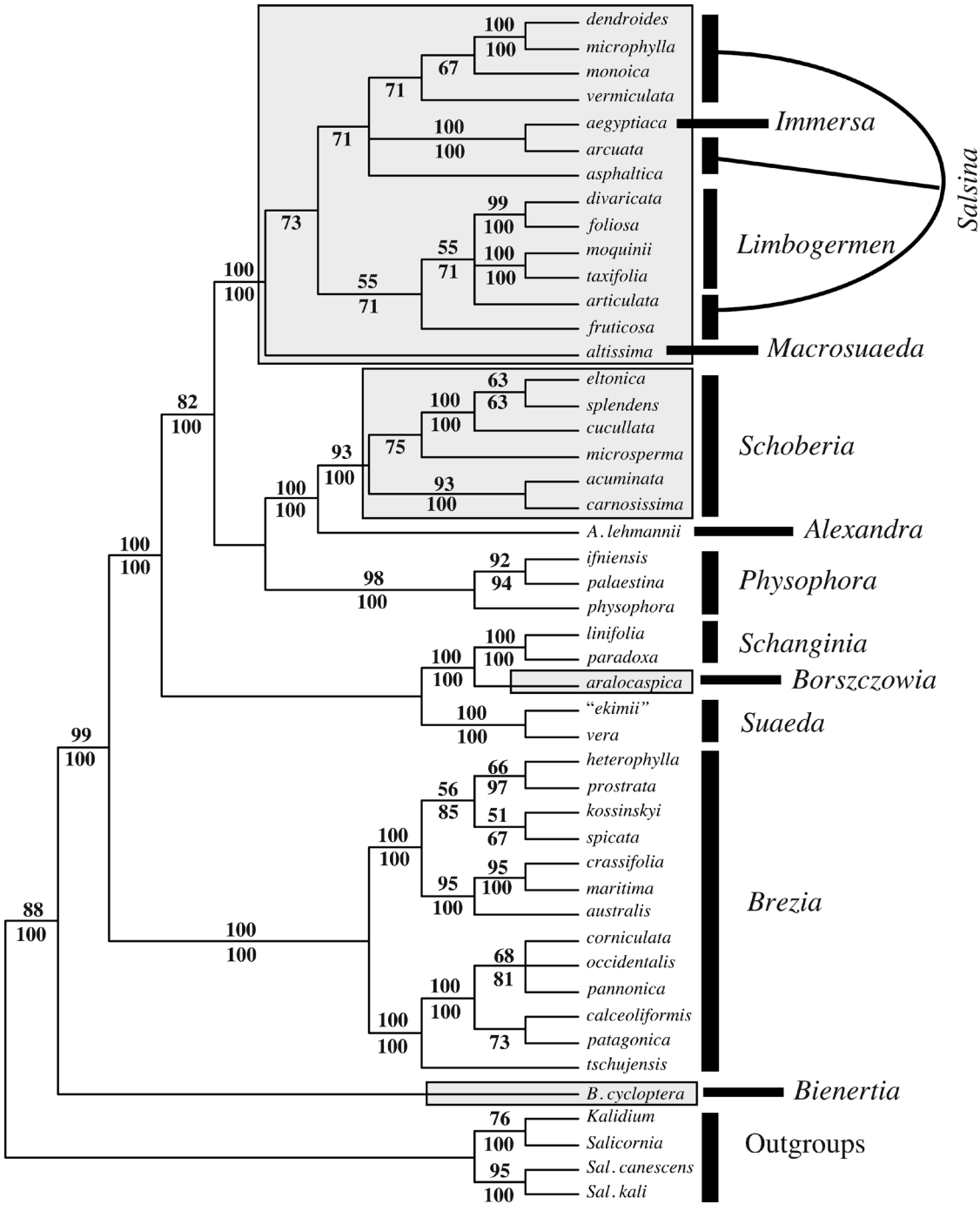

FIG. 3. The single maximum likelihood phylogram (-lnL = 12514.28030) of tribe Suaedeae based on combined complete ITS, *atpB-rbcL*, and *psbB-psbH* sequence. Numbers above branches refer to bootstrap percentages and those below branches refer to Bayesian inference posterior probabilities. Gray boxes denote C_4 clades/lineages (both Kranz C_4 and single-cell C_4). Abbreviations are as follows: *A.* = *Alexandra*, *B.* = *Bienertia*, and *Sals.* = *Salsola*. All names without generic designation refer to *Suaeda* species.

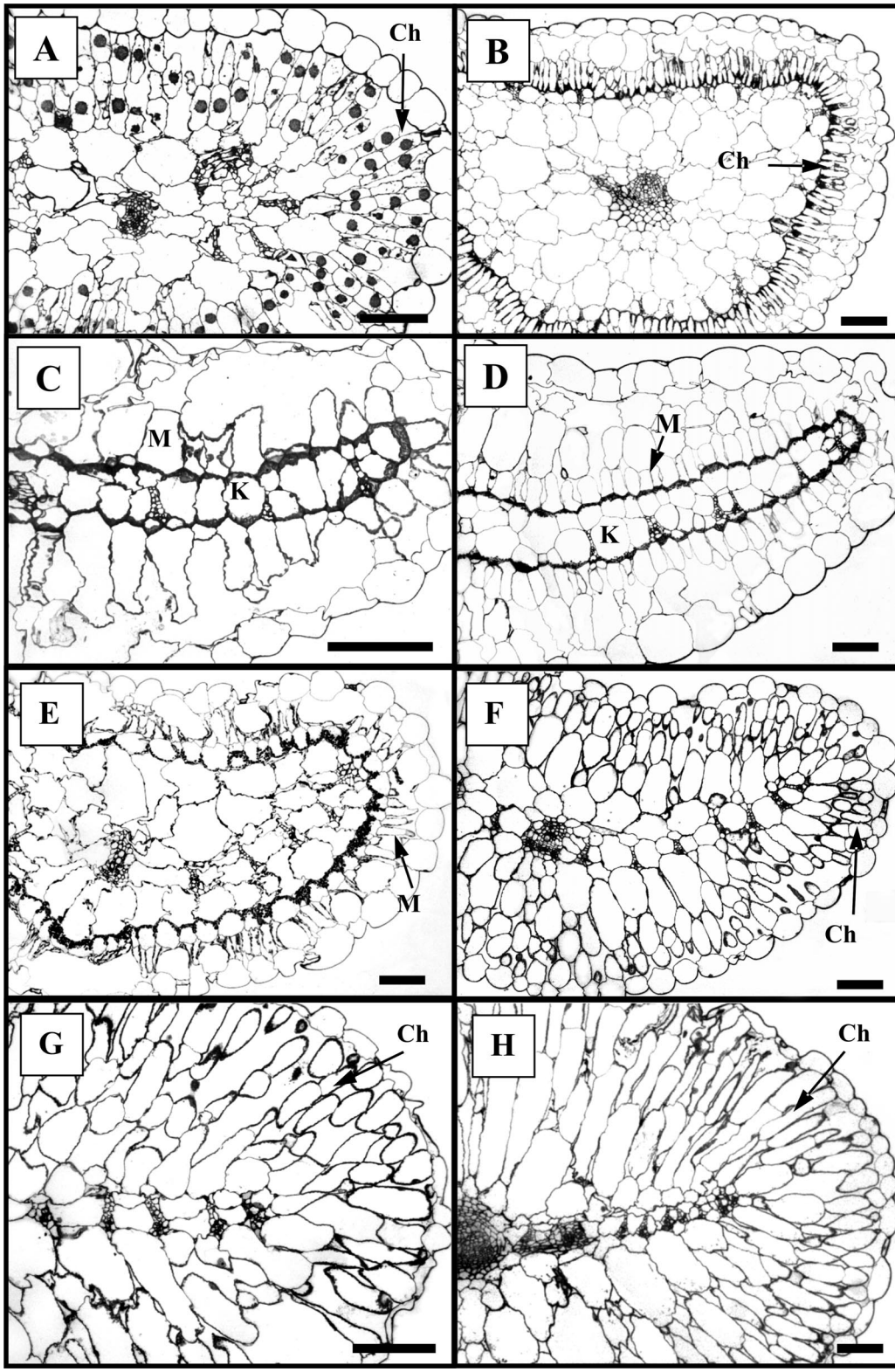

FIG. 4. Light microscopy of leaf cross-sections of representative Suaedoideae species. A. *Bienertia cycloptera* (single-cell C_4 species). B. *Suaeda* (*Borszczowia*) *aralocaspica* (single-cell C_4 species), C. *Suaeda cochlearifolia* (Kranz C_4), D. *S. eltonica* (Kranz C_4), E. *S. arcuata* (Kranz C_4), F. *S. heterophylla* (C_3), G. *S. linifolia* (C_3), H. *S. physophora* (C_3). For classification of *Suaeda* species according to sections see Fig. 8, and for additional information on leaf structure see Schütze et al. (2003). Photosynthetic tissue, Ch = chlorenchyma cells; for species with Kranz anatomy, M = mesophyll cells, K = Kranz sheath cells. Scale bar = 100 μm.

100/100 for both). The subfamilies and tribes of Kadereit et al. (2003) are upheld.

Tribe Bienertieae includes two *Bienertia* species with C_4 photosynthesis lacking Kranz anatomy (Voznesenskaya et al. 2002; Edwards et al. 2004; Akhani et al. 2003, 2005) and with morphological characters (vesicular hairs, fleshy bracteoles, flattened seeds, connate and circularly winged tepals, elongated spike-like inflorescence and low pollen pore numbers) unique in the Salicornioideae / Suaedoideae clade. In previous work, this lineage has had an ambiguous phylogenetic position (Kadereit et al. 2003; Schütze et al. 2003). In our multi-gene analyses Bienertieae is sister to Suaedeae forming a monophyletic Suaedoideae. There are likely more species in this clade yet to be recognized (H. Akhani, unpubl. data).

In our combined analyses the Salicornieae is paraphyletic with respect to the Halopeplideae (Fig. 2). As several members of the Halopeplideae have leaves (species in *Halopeplis*, *Kalidiopsis*, and *Kalidium*) this might suggest multiple losses of leaves in the Salicornioideae or a regain of leaves in the Halopeplideae. This will need to be explored with more extensive sampling within these tribes.

Relationships within the Salsoloideae s.l. Salsoloideae s.l. (Salsoleae, Camphorosmeae, and Sclerolaeneae) includes the largest number of genera within the Chenopodiaceae and largest number of C_4 species in the family. Our sampling design does not cover all main lineages within Salsoloideae but the topology obtained requires comment. The well-supported monophyly of (1) the Salsoleae / Camphorosmeae / Sclerolaeneae clade (Fig. 2; mpbs/mlbs/pp = 98/100/100) and (2) the Camphorosmeae / Sclerolaeneae clade (Fig. 2; 100/100/100) are in agreement with those of past studies (Kadereit et al. 2003; Pratt 2003). Previous studies have not provided support for the monophyly of the Salsoleae (Kadereit et al. 2003), but the analyses here support its monophyly well (87/86/100).

Phylogenetic Patterns of Diversification in* Suaeda *and the Inclusion of* Alexandra *in* Suaeda *s.l. Our findings closely resemble the results in the study by Schütze et al. (2003), and they give stronger statistical support and resolution to infraclade lineages. The topologies obtained suggest well-supported monophyly of (1) the two *Suaeda* subgenera *Brezia* (Moq.) Freitag & Schütze and *Suaeda* (Fig. 3; mlbs/pp = 100/100 and 100/100, respectively), (2) sections *Brezia* (Moq.) Volk. (100/100), *Salsina* Moq. sensu Schütze et al. (2003; 100/100), *Schanginia* (C.A.Mey) Volk. (100/100), *Suaeda* (100/100), *Physophora* Iljin (98/100), and *Schoberia* (C.A.Mey) Volk (93/100). The section *Salsina* sensu Schenk and Ferren (2001) is paraphyletic with regards to the monotypic section *Immersa* Townsend and the monophyletic section *Limbogermen* Iljin and is sister to the monotypic section *Macrosuaeda* Tzvel. Thus our results support submersion of *Immersa*, *Limbogermen*, and *Macrosuaeda* into *Salsina* s.l. (Schütze et al. 2003). The former monotypic genus *Borszczowia* has been previously reclassified as *Suaeda* section *Borszczowia* (Bunge) Freitag & Schütze (Schütze et al. 2003) and is supported here as the sister to section *Schanginia*. This species, with its C_4 photosynthesis occurring within a single cell, is positioned between C_3 section *Schanginia* and the C_3 shrubby section *Suaeda*, suggesting that this type of photosynthesis likely evolved from C_3 ancestors rather than from a C_4 ancestor with Kranz anatomy.

Suaeda kossinskyi Iljin was moved to *Bienertia* by Tzvelev (1993; *Bienertia kossinskyi* (Iljin) Tzvel.) based on perianth structure. However, whereas *Bienertia* is a species having single-cell C_4 photosynthesis, *S. kossinskyi* Iljin has been shown to be a C_3 species with typical astro-bassioid leaf anatomy (Carolin et al. 1975; Freitag and Stichler 2002). Our phylogenetic analyses confirm that *S. kossinskyi* is a member of *Suaeda* section *Brezia*, not a species of *Bienertia* as proposed by Tzvelev (1993; Fig. 3).

The endangered monotypic genus *Alexandra*, an endemic of the Aral region of Central Asia is investigated here for the first time using molecular markers. *Alexandra* was described more then century ago and since then was treated as a separate genus of the Suaedeae in all classifications (Iljin 1936; Kühn et al. 1993). In the recent reevaluation of the Suaedoideae *Alexandra* was treated as a separate genus related to *Suaeda* section *Schanginia* based on morphological data (Schütze et al. 2003). Our analyses showed that *Suaeda* is paraphyletic with regards to *Alexandra*, which is placed sister to section *Schoberia* (mpbs1/mpbs2/mlbs/pp = 100/100/100/100). The *Suaeda* sections *Physophora*, *Salsina* s.l., and *Schoberia* and the genus *Alexandra* together form a monophyletic clade. There is also morphological evidence for the placement of *Alexandra* as a sister taxon to section *Schoberia*. The flat leaves with hyaline margins and spike-like inflorescences are similar to several species of that section (Fig. 1D). Additionally, the longitudinal outgrowth found in the perianth of *Alexandra* and glossy smooth seeds are further characters shared between this and most members of section *Schoberia*. However, *Suaeda* section *Schoberia* are more highly branched and have smaller leaves than *Alexandra*. Anatomically the members of section *Schoberia* are interesting because of their unique Kranz cells positioned in the central parts of leaves and having centrifugally positioned chloroplasts (Fig. 4C& 4D). *Alexandra* is a C_3 plant with a carbon isotope value of −26.69‰ to −27.05‰ in the leaves (Akhani and Ziegler, unpubl. data; Kapralov et al., unpubl. data). Interestingly, this C_3 species is closely allied to the *Suaeda* C_4 section *Schoberia*, while the C_4 *Borszczowia* is phylogenetically more closely related to the C_3 *Suaeda* section *Schangi-*

nia. *Suaeda* is here recircumscribed to include *Alexandra* in the monotypic *Suaeda* section *Alexandra*.

Suaeda section **Alexandra** (Bunge) Kapralov, Akhani & E. H.Roalson sect. nov.—TYPE: *Alexandra lehmanii* Bunge. 1 sp.; Central Asia. Basionym: *Alexandra* Bunge, *Linnaea* xvii. 120 (1843).

Suaeda lehmannii (Bunge) Kapralov, Akhani & E.H.Roalson comb. nov. Basionym: *Alexandra lehmannii* Bunge, *Linnaea* 17: 120. 1843.—TYPE: In deserto Aralensi, *Lehmann s.n.* (holotype LE).

Our analyses provide statistical support for the monophyly of (1) the Salicornioideae / Suaedoideae / Salsoloideae s.l. clade and (2) the Salsoloideae s.l. subfamily (including the Camphorosmeae and Sclerolaeneae) found in previous works (Kadereit et al. 2003; Pratt 2003). This is the first study to provide strong statistical support for the monophyly of (1) the subfamily Salicornioideae, (2) the subfamily Suaedoideae (including *Bienertia*), and (3) the Salicornioideae / Suaedoideae clade. Previous studies have suggested these groups were monophyletic but weakly supported (Kadereit et al. 2003; Schütze et al. 2003). Our molecular data strongly support the placement of (1) the Halopeplideae tribe within the Salicornieae tribe and (2) the monotypic *Alexandra* within *Suaeda* as the sister taxon to section *Schoberia*. There is statistical support for four independent origins of C_4 photosynthesis within the Suaedoideae including two independent origins of Kranz C_4 anatomy (in the *Suaeda* sections *Salsina* s.l. and *Schoberia*) and two independent origins of non-Kranz C_4 systems (in *Bienertia* and *Suaeda* section *Borszczowia*), making this relatively small subfamily a unique model for investigation of C_4 syndrome evolution.

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APPENDIX 1. Specimens and sequences used for phylogenetic analyses of the succulent clade and tribe Suaedeae. Voucher specimens for new data are housed at the Tehran University, Iran (HB. Akhani), unless stated otherwise. Collections from the Marion Ownbey Herbarium, Washington State University are denoted as WS. The nomenclature of tribes and subfamilies follows Kühn et al. (1993). The nomenclature of *Suaeda* sections follows Schenk & Ferren (2001) and Schütze et al. (2003). Suaedeae analyses samples of *Alexandra* and outgroup specimen information are provided under ''Succulent Clade Analyses.'' Gene abbreviations are as follows: I = nrDNA internal transcribed spacers, A = *atpB-rbcL* spacer, M = *matK,* P = *psbB-psbH* spacer region, R = *rbcL,* and L = *trnL-trnF* spacer region.

Succulent Clade Analyses: Chenopodioideae: Atripliceae *Atriplex patula* L., U.S.A., Ohio, Henry Co., Marion Twp., 14/9/1996, *M. A. Vincent & T. G. Lammers 7518* (WS), I- DQ499332, A- DQ499360, P- DQ499416, L- DQ499379; Cuénoud et al. (2002), M- AY042550, Hudson et al. (1990), R- X15925; *Spinacia oleracea* L., Schmitz-Linneweber et al. (2001), A- AJ400848, P- AJ400848, R- AJ400848, L- AJ400848; **Chenopodioideae: Camphorosmeae:** *Bas-*

sia hirsuta (L.) Asch., Müller & Borsch (2005), M- AY514831, *B. hyssopifolia* (Pall.) Kuntze, U.S.A., Nevada, Hot Creek Range, Box Canyon, 6150 ft, 29/8/1987, *A. Tiehm 11569* (WS), I- DQ499333, A- DQ499361, P- DQ499417, L- DQ499380, *B. sedoides* (Pall.) Asch., Kadereit et al. (2003), R- AY270063; *Camphorosma lessingii* Litw., Uzbekistan, Bukhare region, Kyzyl-Kum station, Pyankov s.n. (no voucher), I- DQ499334, A- DQ499362, P- DQ499418, L- DQ499381, *C. monspeliaca* L., Müller & Borsch (2005), M- AY514829, Kadereit et al. (2003), R- AY270071; *Panderia pilosa* Fisch. & C.A.Mey., Iran, Tehran, 35 km E Eshtahard, ca. 8 km W Mardabad, Rude Shur, 1163 m, 8/12/2000, *H. Akhani 1451,* I- DQ499335, P- DQ499419, L- DQ499382, Kadereit et al. (2003), R- AY270114; **Chenopodioideae: Chenopodieae:** *Chenopodium botrys* L., *R. R. Halse* 6418 (WS), I- DQ499336, A- DQ499363, P- DQ499420, L- DQ499383, Müller & Borsch (2005), M- AY514835, Kadereit et al. (2003), R- AY270080; **Chenopodioideae: Corispermeae:** *Corispermum filifolium* C.A.Mey., Kadereit et al. (2003), R- AY270084, *C. ladakhianum* Grey-Wilson & Wadhwa, no voucher, M- AY514837, *C. pacificum* Mosyakin, U.S.A., Washington, Snake River, ca. 235 m, 1/10/2001, *P. F. Zika 16656* (WS), I- DQ499337, A- DQ499364, P- DQ499421, L- DQ499384; **Chenopodioideae: Sclerolaeneae:** *Maireana brevifolia* (R. Br.) P.G.Wilson, Kadereit et al. (2003), R- AY270106, *M. campanulata* P.G.Wilson, Australia, South Australia, Lake Eyre, 16/8/1991, *F. J. Badman 4884* (WS), I- DQ499338, A- DQ499365, P- DQ499422, *M. sedifolia* (F.Muel.) P.G.Wilson, Cuénoud et al. (2002), M- AY042613, *M. triptera* (Benth.) P.G.Wilson, Shepherd et al. (2004), L- AY603736; **Salicornioideae: Halopeplideae:** *Kalidium caspicum* Ung.-Sternb., Iran, Semnan, 38 km E of Khors towards Chajam, S of Kavire Haj Ali Qoli, 1098 m, 14/11/2002, *H. Akhani 16497,* I- DQ499339, M- DQ499401, P- DQ499423, L- DQ499385, Kadereit et al. (2003), R- AY270102, *K. foliatum* (Pall.) Moq., Schütze et al. (2003), A- AY181809; **Salicornioideae: Salicornieae:** *Allenrolfea occidentalis* (S. Watson) Kuntze, U.S.A., Idaho, Cassia Co., Raft River Flat, 6/7/1950, *G. Zappettini & K. Morton s.n.* (WS), I- DQ499340, P- DQ499424, Schütze et al. (2003), A- AY181910, Kadereit et al. (2003), R- AY270052, *A. vaginata* (Griseb) Kuntze, Müller & Borsch (2005), M- AY514828; *Halocnemum strobiliaceum* (Pall.) M.Bieb., Iran, Semnan, Alborz Mountains, 67 km W Damghan in the road towards Cheshmeh Ali, high saline soils, 1822 m, *H. Akhani & Salimian 15330,* I- DQ499341, A- DQ499366, P- DQ499425, L- DQ499386, Müller & Borsch (2005), M- AY514842, Kadereit et al. (2003), R- AY270094; *Halostachys belangeriana* (Moq.) Botsch., Iran, Semnan, SW of Touran Protected Area, 8 km after Razeh towards Sahl, along dry river, around Cheshmeh Morra (spring), degraded *Tamarix* stand, 1227 m, 14/11/2002, *H. Akhani 16493,* I- DQ499342, M- DQ499402, P- DQ499426, L- DQ499387; *Salicornia dolichostachya* Moss., Kadereit et al. (2003), R- AY270125, *S. europaea* L., Schütze et al. (2003), A- AY181814, P- AY181941, *S.* sp., Iran, Tehran, ca. 60 km W Tehran, Rude Shur near Mardabad, 1160 m, 10/10/2003, *H. Akhani 17315,* I- DQ499343, M- DQ499403, L- DQ499388; *Sclerostegia moniliformis* P.G.Wilson, Schütze et al. (2003), I- AY181878, A- AY181813, P- AY181940, Kadereit et al. (2003), R- AY270133, Shepherd et al. (2004), L- AY603747; **Salsoloideae: Salsoleae:** *Climacoptera crassa* (M.Bieb.) Botsch., Kazakhstan, 973 km, KL 14, Safronova, 30/09/2002, no voucher, I- DQ499344, M- DQ499404, P- DQ499427, L- DQ499389, Kadereit et al. (2003), R- AY270083; *Girgensohnia oppositiflora* (Pall.) Fenzl, Iran, Yazd, ca. 27 km W Taft towards Abarkuh, disturbed soils around the road, 2179 m, 18/9/2001, *H. Akhani & Ghobadnejhad 15701,* A- DQ499367, M- DQ499405, P- DQ499428, L- DQ499390, Kadereit et al. (2003), R- AY270087; *Petrosimonia glauca* (Pall.) Bunge, Iran, E Azerbaijan, 13 km E Maman, near salt mine (Maadan-e Namak-e Maman), 1378 m, 2/9/2001, *H. Akhani & Ghobadnejhad 15535,* I- DQ499345, A- DQ499368, M- DQ499406, P- DQ499429, L- DQ499391, *P. nigdensis* Aellen, Kadereit et al. (2003), R- AY270116; *Salsola canescens* (Moq.) Boiss. (section *Caroxylon*), Iran, Tehran, N Tehran, Golabdareh, 3/9/1998, *H. Akhani 13185,* I- DQ499346, A- DQ499369, M- DQ499407, P- DQ499430, Kadereit et al. (2003), R- AY270127; *S. kali* L. (section *Kali*), Pyankov et al., 2001, I- AF318646, Pyankov et al. (2001) leaf material (no voucher), A- DQ499370, P- DQ499431, L- DQ499392, Müller & Borsch (2005), M- AY514843, Kadereit et al. (2003), R- AY270129; **Salsoloideae: Suaedeae:** *Alexandra lehmannii* Bunge, Kazakstan, Dzhambul, Czu river, in Ulanbeli salt marshes, 22/8/1969, *Diomina 5171b* (LE), I- DQ499347, A- DQ499371, M- DQ499408, P- DQ499432, L- DQ499393; *Bienertia cycloptera* Bunge, cultivated at Washington State University from material originally collected from Iran, Tehran, margin of Kavir Protected Area, Mobarakieh, 821 m, 16/10/2000, *H. Akhani 14386,* I- DQ499348, A- DQ499372, M- DQ499409, P- DQ499433, L- DQ499394, Kadereit et al. (2003), R- AY270066; *B. sinuspersici* Akhani, Iran, Khuzestan, 17 km N of Bandare Mahshahr, 28 m, 31/10/2003, *H. Akhani 17433,* I- DQ499349, A- DQ499373, M- DQ499410, P- DQ499434, L- DQ499395; *Suaeda aralocaspica* (Bunge) Freitag & Schütze (section *Borszczowia*), Kazakstan, no voucher, I- DQ499350, A- DQ499374, M- DQ499411, P- DQ499435, L- DQ499396, Kadereit et al. (2003), R- AY270068; *S. crassifolia* Pall. (section *Brezia*), Schütze et al. (2003), I- AY181820, A- AY181760, P- AY181885, Iran, E Azerbaijan, between Mayan Sofla and Qezel Dizel, 6 km E of Qezel Dizel, saline flats around the road, 1335 m, 5/9/2001, *H. Akhani & Ghobadnejhad 15588,* M- DQ499412, L- DQ499397, Kadereit et al. (2003), R- AY270136; *S. maritima* (L.) Dumort., sensu Akhani & Podelch (1997) (section *Brezia*), Schütze et al. (2003), I- AY181818, A- AY181758, P- AY181883, Iran, E Azerbaijan, 10 km SW Sarab in the road towards Asbforoushan, salty soils along the Talkheherud and surrounding saline flats, 1700 m, 8/9/2001, *H. Akhani & Ghobadnejhad 15627,* M- DQ499413, L- DQ499398, Kadereit et al. (2003), R- AY270137; *S. altissima* (L.) Pall. (section *Salsina*), Schütze et al. (2003), I- AY181850, A- AY181785, P- AY181914, Kadereit et al. (2003), R- AY270135, *S. fruticosa* Forssk. ex J.F.Gmelin (section *Salsina*), Iran, Khorassan, 110 km SE Sarbisheh, near Mahirud, 2/9/2003, *H. Akhani & M. R. Joharchi 17310,* M- DQ499414, L- DQ499399; *S. microsperma* (C.A.Mey.) Fenzl (section *Schoberia*), Schütze et al. (2003), I- AY181849, P- AY181913, Iran, Khorassan, 39 km N Gonabad, along Kal-Shur river, 31/8/2003, *H. Akhani & M. R. Joharchi 17240,* A- DQ499375, M- DQ499415, L- DQ499400.

Suaedeae Analyses: ***Brezia*** *S. arbusculoides* L.S.Sm., Schütze et al. (2003), I- AY181827; *S. australis* (R.Br.) Moq., Schütze et al. (2003), I- AY181826, A- AY181766, AY181891; *S. calceoliformis* (Hooker) Moq., U.S.A., Nevada, Spring Valley, Baking Powder Flat, 9/8/1985, *A. Tiehm 10096* (WS), I- DQ499351, A- DQ499376, P- DQ499436; *S. corniculata* (C.A.Mey.) Bunge, Schütze et al. (2003), I- AY181841, A- AY181780, P- AY181905; *S. crassifolia* Pall., Schütze et al. (2003), I- AY181820, A- AY181760, P- AY181885; *S. heterophylla* (Kar. & Kir.) Bunge, Schütze et al. (2003), I- AY181837, A- AY181776, P- AY181901; *S. maritima* (L.) Dumort., Schütze et al. (2003), I- AY181818, A- AY181758, P- AY181883; *S. kossinskyi* Iljin, Kazakhstan, Ulken-Kul, *Yakubov 202* (LE), I- DQ499352, A- DQ499377, P- DQ499437; *S. occidentalis* (S.Watson) S.Watson, U.S.A., Nevada, Elko Co., 5340 ft, 220/8/1986, *A. Tiehm 10897* (WS), I- DQ499353, P- DQ499438; *S. pannonica* (Beck) Graebn., Schütze et al. (2003), I- AY181839, A- AY181778, P- AY181903; *S.* aff. *patagonica* Speg., Schütze et al. (2003), I- AY181843, A- AY181782, P- AY181907; *S. prostrata* Pall., Schütze et al. (2003), I- AY181834, A- AY181773, P- AY181898; *S. spicata* (Willd.) Moq., Schütze et al. (2003), I- AY181828, A- AY181767, P- AY181892; *S. tschujensis* Lomonosova & Freitag, Schütze et al. (2003), I- AY181838, A- AY181777, P- AY181902; ***Immersa***: *S. aegyptiaca* (Hasselq.) Zohary, Schütze et al. (2003), I- AY181853, A- AY181788, P- AY181917; ***Limbogermen***: *S. divaricata* Moq., Schütze et al. (2003), I- AY181863, A- AY181797, P- AY181926; *S. foliosa* Moq., Schütze et al. (2003), I- AY181862, A- AY181796, P- AY181925; *S. nigra* (Raf.) J.F.Macbride (as *S. moquinii* (Torr.) Greene), Schütze et al. (2003), I- AY181864, A- AY181798; *S. nigra* (Raf.) J.F.Macbride, U.S.A., Nevada, Humboldt Co., 7/7/1964, *N. H. Holmgren & J. L. Reveal 1320* (WS), P- DQ499439; *S. taxifolia* (Standley) Standley, no voucher, I-

DQ499354, A- DQ499378, P- DQ499440; ***Macro suaeda***: *S. altissima* (L.) Pall., Schütze et al. (2003), I- AY181850, A- AY181785, P- AY181914; ***Salsina***: *S. arcuata* Bunge, Schütze et al. (2003), I- AY181854, A- AY181789, P- AY181918; *S. articulata* Aellen, Schütze et al. (2003), I- AY181860, A- AY181795, P- AY181924; *S. asphaltica* Boiss., Schütze et al. (2003), I- AY181851, A- AY181786, P- AY181915; *S. dendroides* (C.A.Mey.) Moq., Schütze et al. (2003), I- AY181856, A- AY181791, P- AY181920; *S. fruticosa* Forssk. & J.F.Gmelin, Iran, Khorassan, 110 km SE Sarbisheh, near Mahirud, 2/9/2003, *H. Akhani & M. R. Joharchi 17310,* I- DQ499355, Schütze et al. (2003), A- AY181793, P- AY181922; *S. microphylla* Pall., Schütze et al. (2003), I- AY181855, A- AY181790, P- AY181919; *S. monodiana* Maire, Schütze et al. (2003), I- AY181861; *S. monoica* Forssk. & J.F.Gmelin, Schütze et al. (2003), I- AY181859, A- AY181794, P- AY181923; *S. vermiculata* Forssk. & J.F.Gmelin, Schütze et al. (2003), I- AY181852, A- AY181787, P- AY181916; ***Physophora***: *S. physophora* Pall., Iran, Khorassan, Golestan National Park, 6 km E of Cheshm-e-Khan, 15/10/2003, *H. Akhani s.n.,* I- DQ499356, Schütze et al. (2003), A- AY181802; *S. ifniensis* Caball., Schütze et al. (2003), I- AY181866, A- AY181800, P- AY181928; *S. palaestina* Eig. & Zohary, Schütze et al. (2003), I- AY181865, A- AY181799, P- AY181927; ***Schanginia***: *S. linifolia* Pall., Iran, Khorassan, Golestan National Park, near Cheshme Khan, 14/10/2003, *H. Akhani s.n.,* I- DQ499357, Schütze et al. (2003), A- AY181805, P- AY181932; *S. paradoxa* Bunge, Schütze et al. (2003), I- AY181871, A- AY181806, P- AY181933; ***Schoberia***: *S. acuminata* (C.A.Mey.) Moq., Iran, Khuzestan, 35 km W of Ahvaz on the road towards Bandare Mahshahr, 34 m, 31/10/2003, *H. Akhani 17427,* I- DQ499358, Schütze et al. (2003), P- AY181912; *S. carnosissima* Post, Schütze et al. (2003), I- AY181846, A- AY181783, P- AY181910; *S. cucullata* Aellen, Schütze et al. (2003), I- AY181845, P- AY181909; *S. eltonica* Iljin, Schütze et al. (2003), I- AY181847, A- AY181784, P- AY181911; *S. gracilis* Moq., Iran, E Azerbaijan, ca. 3 km S Eskanlu towards Safarlu, along Iran-Azerbaijan border, 266 m, 4/9/2001, *H. Akhani & Ghobadnejhad 15571,* P- DQ499441; *S. microsperma* (C.A.Mey.) Fenzl, Iran, Khorassan, 39 km N Gonabad, along Kal-Shur river, 31/8/2003, *H. Akhani & M. R. Joharchi 17240,* I- DQ499359, P- DQ499442; *S. splendens* (Pourr.) Gren. & Godr., Schütze et al. (2003), I- AY181844, P- AY181908; ***Suaeda***: *S. vera* Forssk. & J.F.Gmelin, Schütze et al. (2003), I- AY181868, A- AY181803, P- AY181930; *S.* ''*ekimii*'', Schütze et al. (2003), I- AY181869, A- AY181804, P- AY181931.