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Taxonomic Reappraisal on *Suaeda australis* (Chenopodiaceae) in Korea based on the Morphological and Molecular Characteristics

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We used morphological and molecular characteristics to perform a taxonomic reappraisal of *Suaeda australis* (Brown) Moquin-Tandon from Korea. Populations of this species are dispersed at the bottoms of sand zones, and usually exhibit a depressed habit. Except for their total heights and leaf lengths, the morphology of these plants does not differ from that of *S. maritima*. Molecular traits were examined based on ITS and *psb*B-*psb*H spacer region sequences. The former region included ITS-1, 5.8S, and ITS-2, which were 629 nucleotide bases long. Pair-wise distances (*p*-distance) among Korean *Suaeda* species ranged from 1.12 to 17.84. The *psb*B-*psb*H spacer region sequences were 618 nucleotide bases long, and were conserved in the alignment of Korean *Suaeda* species. In our ML and MrBayesian analysis of ITS sequences aligned with other sequences from GenBank, the plants of Korean *Suaeda* made three clades: 1) *S. japonica*; *S. australis*, and *S. maritima*; 2) *S. malacosperma*; and 3) *S. glauca*. However, the *psb*B-*psb*H region sequences could not be resolved among *S. japonica*, *S. maritima*, and *S. australis* from Korea. Molecular and vegetative characteristics indicated that the plants now classified as *S. australis* from Korea should instead be named as *S. maritima* (L.) Dumont.

Key words: Chenopodiaceae, ITS, phylogeny, *psb*B-*psb*H, *Suaeda*

Because of large variations in their morphology and habitats, members of *Suaeda* are considered taxonomically difficult, and approximately 110 species have been described (Seliskar, 1985; Kuhn et al., 1993; Ferren and Schenk, 2003; Shim and Choi, 2004). Species are predominantly halophytes, being found in the salt marshes of semi-desert, desert, and coastal environments. Since the last concise treatment of Chenopodiaceae (Kuhn et al., 1993), which relied on molecular and anatomical characteristics, the genus *Suaeda* has been included in the tribe Suaedeae with *Alexandra*, *Bienertia*, and *Borszczowia*. Schutze et al. (2003) have explained that the phylogenetic trees based on internal transcribed spacer (ITS) and *psb*B-*psb*H sequences of *Suaeda* species correspond to the section defined by Schenk and Ferren (2001), and that ITS sequences make better resolution among species than do *psb*B-*psb*H sequences.

Six species of genus *Suaeda*, – *S. australis* (Brown) Moquin-Tandon, *S. japonica* Makino, *S. heteropetra* Kitagawa, *S. maritima* (L.) Dumont, *S. malacosperma* Hara, and *S. glauca* (Bunge) Bunge – are distributed along the coasts of Korea (Chung, 1992; Chung and Lee, 1995; Lee, 1996; Shim et al., 2001; Shim and Choi, 2004). They occur as dominant or co-dominant species in salt-marsh communities. However, their identification has been confounded because of the large variation in morphological characteristics as a function of habitat. In particular, *S. heteroptera* has been confused with *S. maritima* and has not been further described since the latest report by Park (1974). Chung (1992) also has suggested that using the length and shape of upper leaves, two key characteristics for distinguishing between these species, is too variable when estimating their status. In fact, we confirmed here that no plants classified as *S. heteroptera* are found in Korea, leading us to exclude this species from our current considerations. Lee and Oh (1989) have proposed that habitat-associated morphological differences in *S. japonica*, e.g., leaf shape and branching pattern, originate from genetic variations. Shim and Choi (2004) have used RAPD analysis to show that several predominant DNA fragments can be distinguished among populations of that species. Despite these molecular traits, however, the taxonomic status of *Suaeda* species from Korea remains in dispute, especially for *S. australis* and *S. maritima*.

Relying on ITS and *psb*B-*psb*H sequences from Korean *Suaeda* species, our main objectives in this study were to 1) examine the phylogenetic relationships among *Suaeda* plants collected worldwide and 2) re-appraise the taxonomic status of *S. australis* and *S. maritima* from Korea based on their morphological and molecular characteristics.

MATERIALS AND METHODS

For DNA sequence analysis, we collected 45 specimens of *Suaeda* species along the western and southern coasts of Korea, selecting from mud flats to sand dunes in order to cover the range of morphological types. *Salicornia europaea* Linne also was included as an out-group for our molecular comparisons. All samples were identified based on the description of Chung (1992), and were tagged with species names. Whenever possible, fresh leaf material obtained from plants of each typical habit per species was used directly for DNA isolation. Voucher specimens for this new collection have been deposited in KNU. Their morphological and anatomical characteristics were compared with plants from local populations at 20 sites on the western and southern coasts of Korea. Statistical analysis was performed for those plants gathered from Ganghwa, Gyokpo, Muan,

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and Suncheon.

Fresh or silica-dried leaves of individual plants were ground in liquid nitrogen and total genomic DNA was extracted from 0.1-g powdered samples with either a commercial DNeasy Plant Mini Kit™ (QIAGEN, Gmbh, Hilden, Germany) or the Invisorb Spin Plant Mini Kit™ (Invitek, Verlin-Buch, Germany), according to the manufacturer's instructions. This extracted genomic DNA was stored at –20°C.

Table 1. Plant materials and GenBank accession numbers for DNA sequences determined in this study and used in the phylogenetic analyses. The identification and nomenclature of *Suaeda* species from Korea follows that of Lee (1996) and Schenk and Ferren (2001).

Species and strains	Collection sites	GenBank Accession No.	
		*psb*B-*psb*H	ITS
Suaeda australis (Brown) Moquin-Tandon, SK08	Byunsan, Buan, Korea; 31 July 2005	DQ786354	DQ786335
S. australis (Brown) Moquin-Tandon, SK09	Byunsan, Buan, Korea; 31 July 2005	DQ786355	*
S. australis (Brown) Moquin-Tandon, SK13	Hyungyoung, Muan, Korea; 31 July 2005	DQ786359	DQ786334
S. australis (Brown) Moquin-Tandon, SK14	Hyungyoung, Muan, Korea; 31 July 2005	DQ786360	*
S. australis (Brown) Moquin-Tandon, SK15	Hyungyoung, Muan, Korea; 31 July 2005	DQ786361	*
S. australis (Brown) Moquin-Tandon, SK33	Sangbong, Yeosu, Korea; 1 Augst. 2005	DQ786376	*
Suaeda glauca (Bunge) Bunge, SK35	Sangbong, Yeosu, Korea; 1 Augst. 2005	DQ786387	DQ786331
S. glauca (Bunge) Bunge, SK07	Byunsan, Buan, Korea; 31 July 2005	DQ786390	*
S. glauca (Bunge) Bunge, SK45	Dongeom, Ganghwa, Korea; 30 July 2005	DQ786393	*
S. glauca (Bunge) Bunge, SK05	Dongjin, Gimjea, Korea; 30 July 2005	DQ786392	*
S. glauca (Bunge) Bunge, SK06	Dongjin, Gimjea, Korea; 30 July 2005	DQ786391	DQ786332
S. glauca (Bunge) Bunge, SK16	Hyungyoung, Muan, Korea; 31 July 2005	DQ786389	DQ786333
S. glauca (Bunge) Bunge, SK36	Nongju, Suncheon, Korea; 1 August 2005	DQ786386	*
S. glauca (Bunge) Bunge, SK20	Youngsanggang, Mokpo, Korea; 1 August 2005	DQ786388	*
Suaeda japonica Makino, SK31	Sangbong, Yeosu, Korea; 1 August 2005	DQ786374	DQ786345
S. japonica Makino, SK38	Dangeom, Ganghwa, Korea; 30 July 2005	DQ786379	DQ786346
S. japonica Makino, SK39	Dangeom, Ganghwa, Korea; 30 July 2005	DQ786380	*
S. japonica Makino, SK40	Dangeom, Ganghwa, Korea; 30 July 2005	DQ786381	*
S. japonica Makino, SK41	Dangeom, Ganghwa, Korea; 30 July 2005	DQ786382	DQ786347
S. japonica Makino, SK01	Dongjin, Gimjea, Korea; 30 July 2005	DQ786350	DQ786340
S. japonica Makino, SK02	Dongjin, Gimjea, Korea; 30 July 2005	DQ786351	*
S. japonica Makino, SK03	Dongjin, Gimjea, Korea; 30 July 2005	DQ786352	DQ786341
S. japonica Makino, SK04	Dongjin, Gimjea, Korea; 30 July 2005	DQ786353	*
S. japonica Makino, SK21	Jido, Shinan, Korea; 31 July 2005	DQ786364	*
S. japonica Makino, SK22	Jido, Shinan, Korea; 31 July 2005	DQ786365	DQ786343
S. japonica Makino, SK23	Jido, Shinan, Korea; 31 July 2005	DQ786366	*
S. japonica Makino, SK24	Jido, Shinan, Korea; 31 July 2005	DQ786367	
S. japonica Makino, SK25	Jido, Shinan, Korea; 31 July 2005	DQ786368	DQ786344
S. japonica Makino, SK26	Jido, Shinan, Korea; 31 July 2005	DQ786369	*
S. japonica Makino, SK27	Jido, Shinan, Korea; 31 July 2005	DQ786370	*
S. japonica Makino, SK28	Jido, Shinan, Korea; 31 July 2005	DQ786371	*
S. japonica Makino, SK30	Jido, Shinan, Korea; 31 July 2005	DQ786373	*
S. japonica Makino, SK37	Nongju, Suncheon, Korea; 1 August 2005	DQ786378	*
S. japonica Makino, SK19	Youngsangang, Mokpo, Korea; 31 July 2005	DQ786363	DQ786342
Suaeda malacosperma Hara, SK32	Sangbong, Yeosu, Korea; 1 August 2005	DQ786375	DQ786337
S. malacosperma Hara, SK43	Dongeom, Ganghwa, Korea; 30 July 2005	DQ786384	DQ786339
S. malacosperma Hara, SK44	Dongeom, Ganghwa, Korea; 30 July 2005	DQ786385	*
S. malacosperma Hara, SK17	Hyungyoung, Muan, Korea; 31July 2005	DQ786362	DQ786338
Suaeda maritima (L.) Dumont, SK29	Jido, Shinan, Korea; 31 July 2005	DQ786372	*
S. maritima (L.) Dumont, SK42	Dongeom, Ganghwa, Korea; 30 July 2005	DQ786383	DQ789357
S. maritima (L.) Dumont, SK10	Hyungyoung, Muan, Korea; 31 July 2005	DQ786356	DQ786336
S. maritima (L.) Dumont, SK11	Hyungyoung, Muan, Korea; 31 July 2005	DQ786357	*
S. maritima (L.) Dumont, SK12	Hyungyoung, Muan, Korea; 31 July 2005	DQ786358	*
S. maritima (L.) Dumont, SK34	Sangbong, Yeosu, Korea; 1 August 2005	DQ786377	*
Salicornia europaea Linne, SK18	Jido, Shinan, Korea; 31 July 2005	DQ786349	DQ786348

Primer pairs used for our PCR amplification of the nuclear ribosomal ITS region and the chloroplast *psb*B-*psb*H spacer region sequences were those adopted from the protocol of Schutze et al. (2003). All amplifications were performed in 20 μL volume cocktails of a pre-mixer (*AccuPower*™PCR Pre-Mix; Bioneer, Daejeon, Korea). PCR reactions were run with an initial denaturation at 95°C for 4 min; followed by 33 cycles of annealing at 45 to 55°C for 30 min and extension at 72°C for 2 min; then a final extension at 72°C for 10 min.

PCR products were purified using a High Pure™PCR Product Purification Kit (Roche, Indianapolis, IN, USA), according to the manufacturer's instructions. For all 45 specimens, including the comparison group, we used an ABI PRISM™337 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) to determine the amplified sequences of the forward and reverse strands of ITS, as well as the *psb*B-*psb*H spacer region. The sequences were aligned via PHYDIT (Chun, 1995) and submitted to GenBank under the accession numbers shown in Table 1. These alignments were based on those of the inferred amino acid sequences, and were re-confirmed visually.

All species from Korea were taxonomically re-examined by classical taxonomic methods (Schutze et al., 2003). Herbarium materials as well as plants collected during field work were used. Special emphasis was placed on leaf types, habit, and shape of their reproductive structures.

We used PAUP*4.0b10 (Swofford, 2003) to analyze the data sets for two aligned genes, including sequences from GenBank. *Bassia hyssopifolia* served as one of our out-group species in the phylogenetic analyses. Minimum evolution (ME; Rzhetsky and Nei, 1992) trees were inferred according to the general time reversible (GTR) model (Rodriguez et al., 1990) plus the shape parameter of the gamma distribution (Γ) plus the partitioning of the invariable site (I) model for ITS and *psb*B-*psb*H, as determined by Modeltest 3.06 (Posada and Crandall, 1998). The optimal ME tree was searched heuristically with stepwise addition sequence starting trees and TBR branch swapping. Best-scoring trees were held at

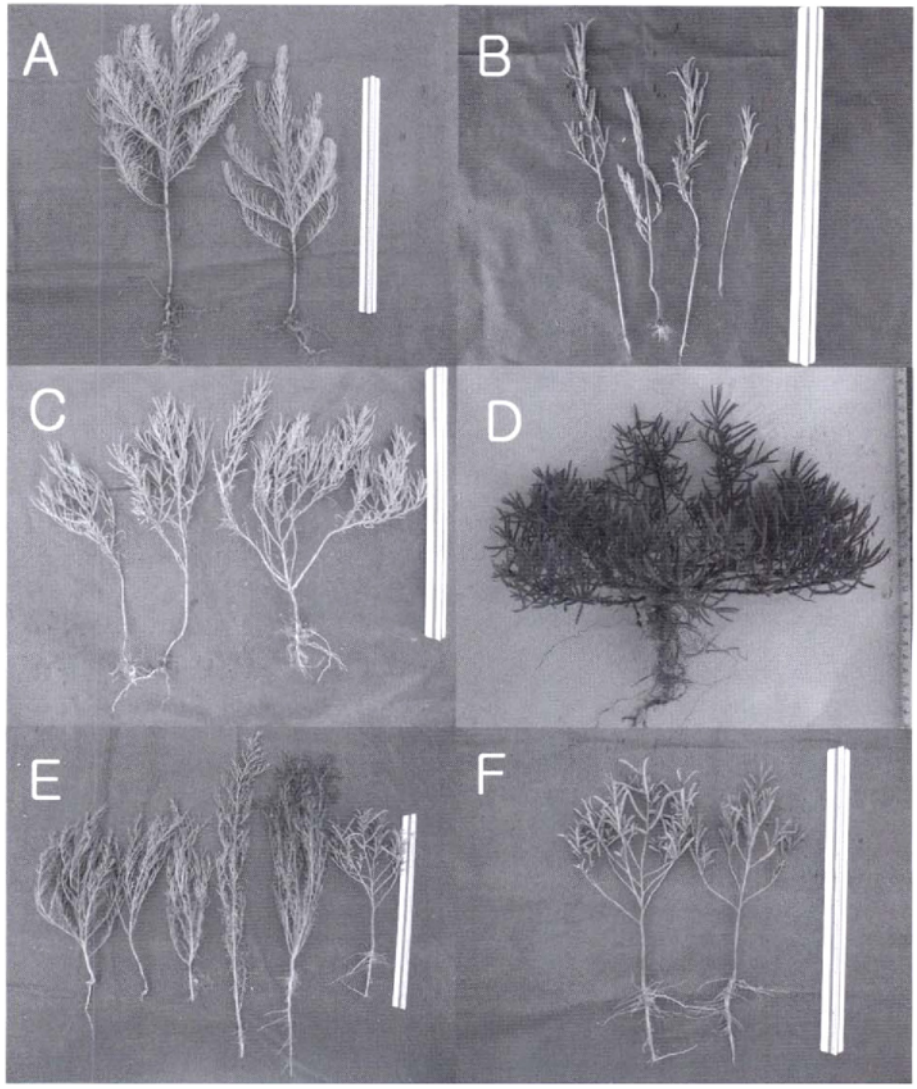

Figure 1. Habits of *Suaeda* species from Korea. **A**, *S. glauca* (Bunge) Bunge from Sangbong, Yeosu; **B**, *S. malacosperma* Hara from Hyungyoung, Muan; **C**, *S. maritima* (L.) Dumont from Hyungyoung, Muan; **D**, *S. australis* (Brown) Moquin-Tandon from Byunsan, Buan; **E,F**, *S. japonica* Makino (E, from Jido, Shinan; F, from Dongjin, Gimjea).

each step. The ME methods used to infer a tree were the same heuristic research conditions as described above. Maximum likelihood analysis also was conducted with that GTR+Γ+I model. Tree likelihoods were estimated using an heuristic search with one random addition sequence replicate and TBR branch swapping. Because of high computational demands, ML bootstrap analyses were conducted with 100 replicates. Bootstrap values (Felsenstein, 1985) also were computed, as implemented in PAUP*, for the ME trees. For that analysis, 2,000 bootstrap data sets were generated from re-sampled data (five random sequence additions for parsimony analysis).

Bayesian analyses (BA) were conducted with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Each analysis was initiated from a random starting tree under the GTR+Γ+I setting; the Bayesian program was set to run four Markov chain Monte Carlo iterations simultaneously for 2,000,000 generations with trees sampled at every 100th generation. Likelihood scores stabilized at approximately 300,000 generations, so the first 3,000 trees were burned. The last 17,000 trees complied with the 50% majority rule consensus tree to obtain Bayesian posterior probabilities (BPP).

RESULTS AND DISCUSSION

Morphological Characteristics

The growth habits of *Suaeda* species from Korea were classified into two types: erect or depressed (Fig. 1). All except *S. australis* showed an erect habit. For example, plants of *S. maritima* live in large, crowded populations along the margins of sand dunes. They are usually exposed to air and fresh water for long periods, which leads to conditions of low salt concentrations. In contrast, plants of *S. australis*, which exhibit a depressed habit, are found in dispersed communities at the bottoms of sand zones (Fig. 2). In an initial report of the habit and growing environment of those two species, Chung (1992) distinguished between the sandy habitat of depressed-type *S. australis* and the erect plants of *S. japonica*.

Our descriptions of the vegetative characteristics of *Suaeda* species, including height, branching pattern, and leaf shapes and arrangement, all agreed with those previously published for Korean specimens (see Chung, 1992; Lee, 1996; Shim et al., 2001). Here, however, we focused on *S. maritima* and *S. australis* so that we could compare their typical habits as a factor in our analyses of the molecular data (Fig. 3). Plants heights for *S. maritima* ranged from 29.84±5.05 cm (n=236) to 41.00±9.56 cm (n=217) versus 15.74±4.96 cm (n=184) to 19.00±6.42 cm (n=169) for *S. australis*. Leaf lengths ranged from 28.71±5.64 mm (n=121) to 42.65±6.25 mm (n=114) for *S. maritima* and from 20.06±10.32 mm (n=94) to 25.89±8.58 mm (n=98) for *S. australis*. The widths of leaves were 2.31±0.58 (n=86) to 2.92±0.62 (n=76) for *S. maritima* and 2.37± 0.28 (n=95) to 2.74±0.32 (n=78) for *S. australis*, and were associated with particular localities. Angle of first branching to the axis was 56.1±0.97° (n=76) for *S. maritima* and 75.8 ±1.65° (n=76) for *S. australis*, which verified that the habit of the latter species was more depressed. Although plant height and leaf length are considered the most important characteristics when distinguishing between these two Korean species, our data showed no significant difference in width between them ($p<0.05$, n=152). Nor did their reproductive characteristics differ ($p<0.05$, n=73 in perianth length; $p<0.05$, n=76 in pistol length; $p<0.05$, n=56 in seed width) (Fig. 4).

Chung (1992) has reported that *S. australis* is distinct from *S. maritima* because of its depressed habit, thicker leaves, and obvious leaf trace below the middle portion of the thallus. In our study, the former species was observed at the bottoms of sand zones where the substrate was very dynamically changed by water currents. We also found that some plants of this species were covered by sand or had been washed away by those currents. Such conditions may have caused them to have depressed thalli. Lee and Oh (1989) have shown that the length and width of leaves for *Suaeda* species is related to salinity in their environment. These plants on coastal margins, where salt concentrations are low, usually have long leaves, compared with the thick and wide leaves on plants growing in a highly saline environment at the bottoms of the coasts.

Characteristics of Sequences

Sequences of the ITS-1, 5.8S, and ITS-2 regions were 629 nucleotide bases long. The aligned sequences of the ITS regions data set, based on 84 taxa and including published sequences, covered 676 bp. They had 352 (52.07%) vari-

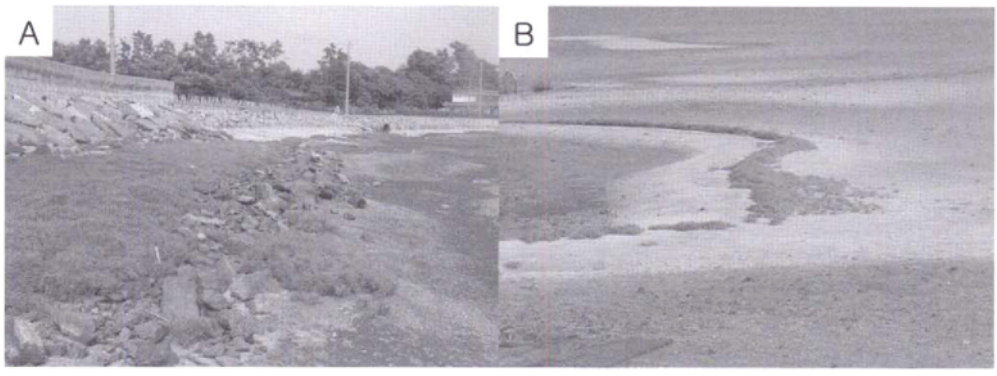

Figure 2. Habitats of *Suaeda* from Hyungyoung, Muan, Korea. **A**: *S. maritima* (L.) Dumont; **B**: *S. australis* (Brown) Moquin-Tandon.

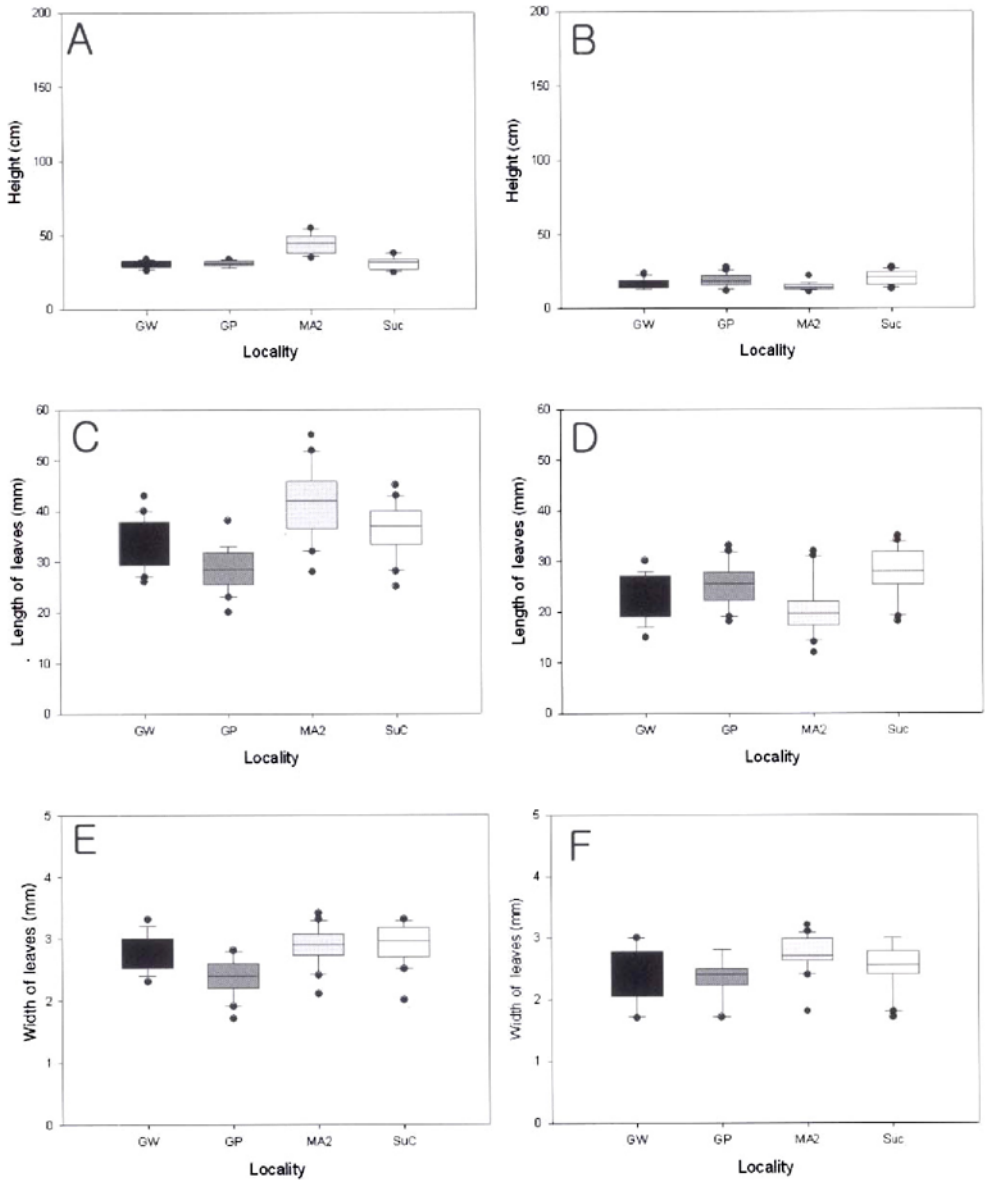

Figure 3. Variation in vegetative characteristics of *Suaeda maritima* (L.) Dumont (**A, C, E**) and *S. australis* (Brown) Moquin-Tandon (**B, D, F**) from Korea. GW: Dongeom population in Ganghwa, GP: Gyokpo population in Buan, MA2: Hyungyoung population in Muan, Suc: Nongju population in Suncheon.

able sites, 285 (42.15%) parsimoniously informative sites, and 59.2% GC contents. Transitions were more common than transversions (Ts/Tv=1.18). When the ITS region sequences of the Korean *Suaeda* species were aligned, we found 90 variable sites out of 651. In particular, *S. glauca* had four distinct sequence blocks. The pair-wise distance (*p*-distance) among Korean species ranged from 1.12 to 17.84, indicating large differences between *S. glauca* and the other three species. However, no difference in "*p*-distance" was identified between *S. maritima* and *S. australis* from Korea, which suggests that their taxonomic status should be re-assessed. In contrast, we found larger "*p*-distances", up to 3.83, between *S. australis* from Korea and *S. australis* from Australia, a result of type and locality. This gap was much larger than that between *S. australis* and *S. malacosperma* from Korea.

The *psb*B-*psb*H spacer region sequences were 618 nucleotide bases long. Aligned sequences of that data set, with 106 published sequences from 62 taxa, spanned 658 bp. They had 159 (24.16%) variable sites, 106 (16.10%) parsimoniously informative sites, and 32.9% GC contents. Transitions were less common than transversions (Ts/Tv=0.91). Except for *S. glauca*, these *psb*B-*psb*H spacer region sequences were conserved in the alignment of the Korean *Suaeda* species, having three deletions comprising five or eight nucleotides and one insertion of nine nucleotides.

The variability of sequences among species (*p*-distance) within that data set was higher than that of the aligned *psb*B-*psb*H spacer region sequences data set (Fig. 5). Although a few variable sites existed among the *Suaeda* species classified in subgenus *Brezia*, no such sites occurred among *S. maritima*, *S. australis*, and *S. japonica* from Korea and Australia.

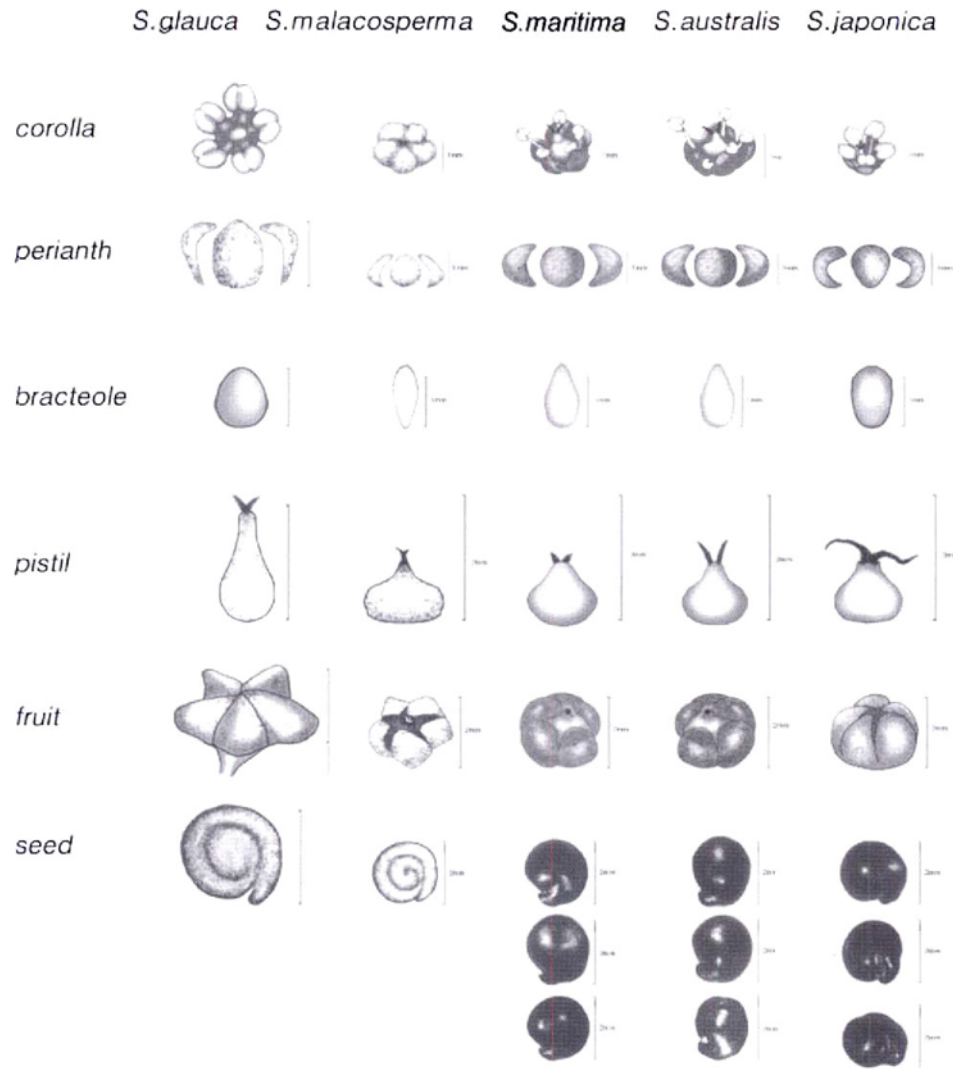

Figure 4. Comparison of floral structures, fruits, and seeds among five *Suaeda* species from Korea.

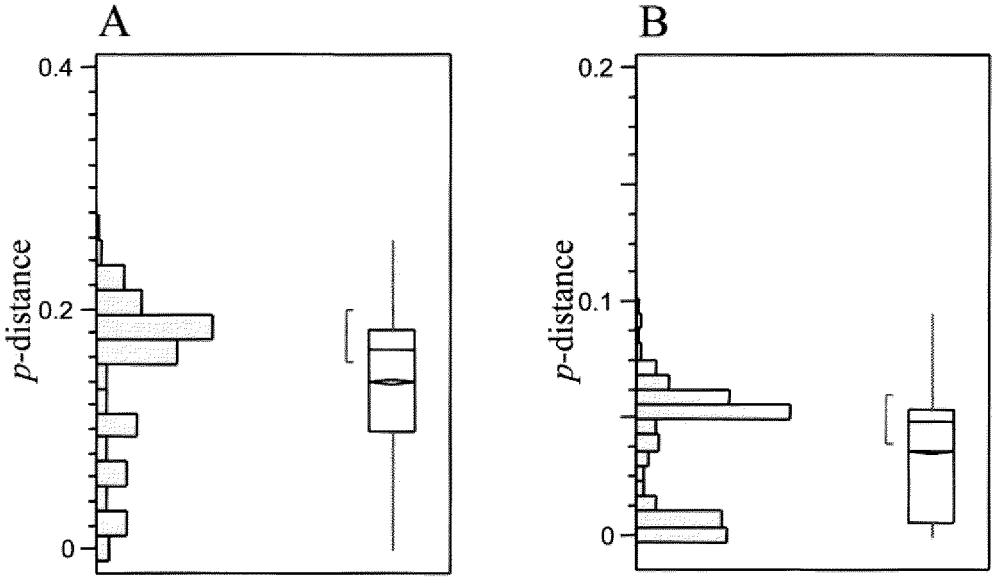

Figure 5. Comparison of pair-wise divergencies (*p*-distances) between ITS sequences (**A**) and plastid *psb*B-*psb*H sequences (**B**). Average *p*-distance for ITS is 0.17659; for *psb*B-*psb*H, 0.035449.

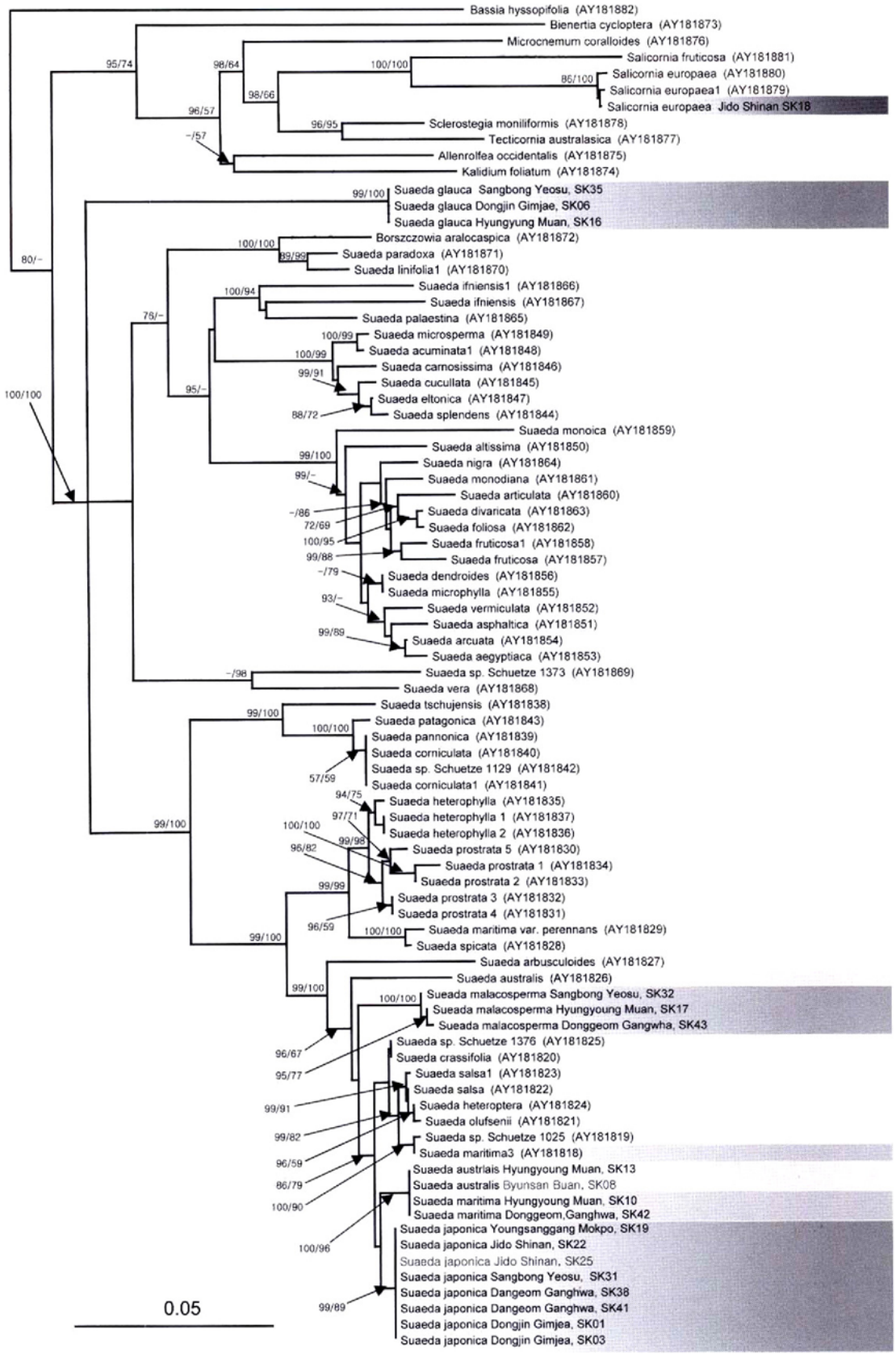

Figure 6. Maximum likelihood tree for *Suaeda* species and related taxa estimated from nuclear rDNA ITS sequence data [GTR+Γ+I model, –Log likelihood = 7016.2656; A–C: 0.9793, A–G: 2.2187, A–T: 1.8577, C–G: 0.3374, C–T: 4.3588, G–T: 1.0000, Γ: 1.3220, I: 0.3356, different nucleotide frequencies (A = 0.2128, C= 0.2951, G = 0.2770, T = 0.2151)]. Bayesian posterior probabilities and bootstrap values shown on branches (BP/ML) from 100 (ML) re-sampling.

Molecular Phylogeny

When the out-group *Bassia hyssopifolia* was included, our phylogenetic analysis of the ITS data matrix generated a minimal tree length of 1,268, with a consistency index (CI) of 0.4850 and a retention index (RI) of 0.8620. In the ML

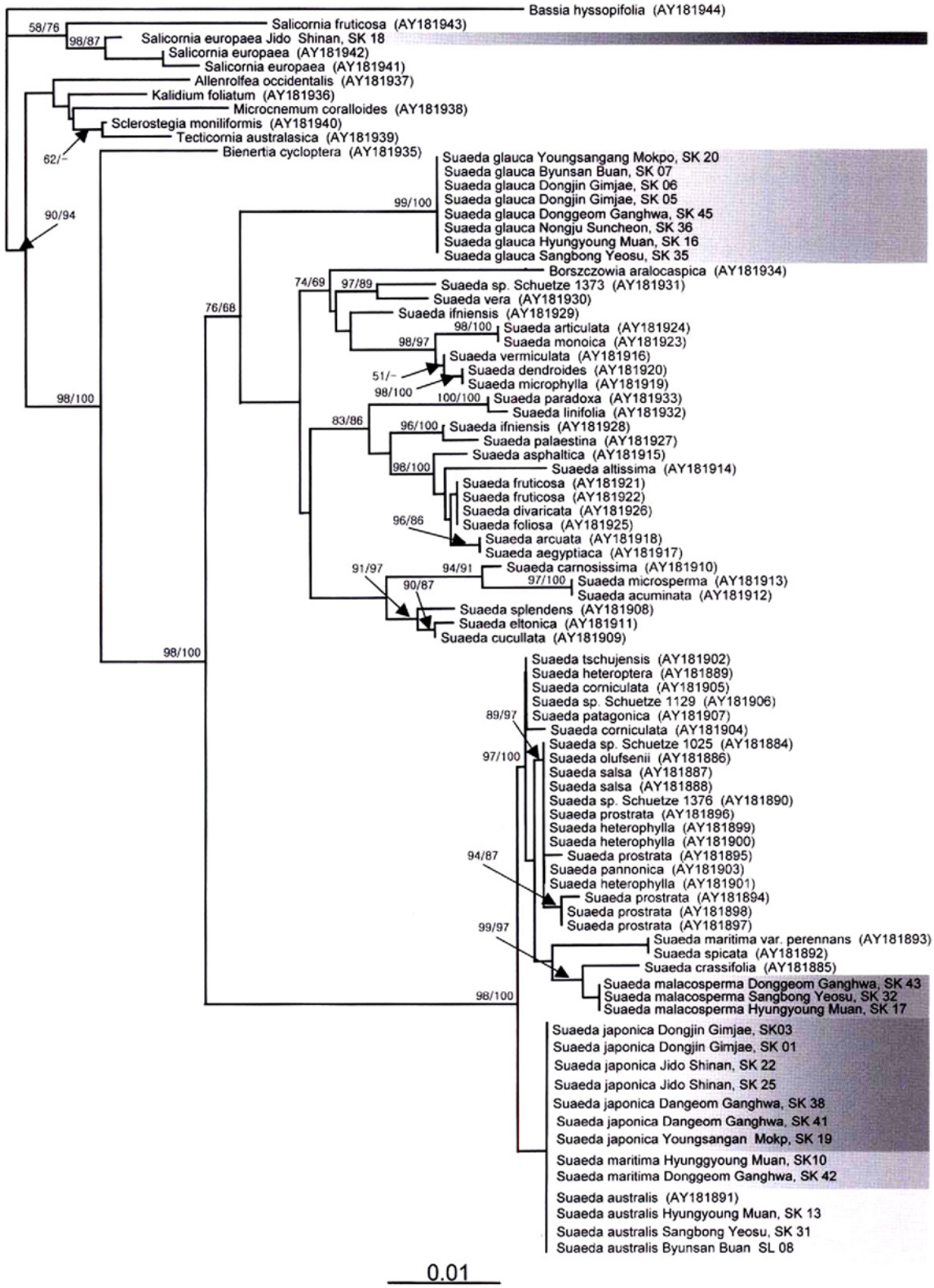

Figure 7. Maximum likelihood tree for *Suaeda* species and related taxa estimated from plastid *psb*B-*psb*H region sequence data [GTR+Γ+I model, model, –Log likelihood = 7094.5854; A–C: 0.8519, A–G: 1.8649, A–T: 1.7755, C–G: 0.3178, C–T: 3.9658, G–T: 1.0000, Γ: 1.6533, I: 0.3493, different nucleotide frequencies (A = 0.2483, C = 0.2565, G = 0.2460, T = 0.2492]. Bayesian posterior probabilities and bootstrap values shown on branches (BP/ML) from 100 (ML) re-sampling.

and MrBayesian analysis, species of Korean *Suaeda* made two clades: 1) *S. japonica*, *S. australis*, *S. malacosperma*, and *S. maritima*; and 2) *S. glauca*. In the former clade, *S. malacosperma* made a primarily out-sister group of *S. japonica*, *S. australis*, and *S. maritima*. Plants of *S. australis* from Australia made a clade distinct from those of the Korean *Suaeda* species, with high bootstrap values and Bayesian posterior probabilities (BPP). Those identified as *S. australis* from Korea were part of a clade with *S. maritima*, and were distinguished from *S. australis* from Australia (Fig. 6). Whereas the Korean species made a large clade with other *Suaeda* species classified in subgenus *Brezia* (Schutze et al., 2003), *S. australis* and *S. maritima* were located heterogeneously in the phylogenetic clade. In particular, *S. australis* from Australia and *S. maritima* from Germany were involved in different clades from those of the Korean plants, which suggested that they have different genetic origins and should have their taxonomic status re-appraised. Schutze et al. (2003) also have demonstrated that *S. maritima* makes a heterogeneous clade composed of other species with the basal clade of *S. australis*.

Although the plants of *S. japonica* were from diverse localities and habitats, e.g., mud plates and artificial dunes at the margins of salt farms, and they had diverse morphologies (height, branching pattern, leaf shape), we found that they made a concrete clade that was separate from other species of *Suaeda*. Likewise, the polymorphism of *S. japonica* from a wide range of environments may not have been fixed by a genetic base.

In their phylogenetic analyses of ITS sequences, Schutze et al. (2003) have reported that all *Suaeda* species can be divided into two clades -- subgenus *Suaeda* and subgenus *Brezia* -- and that the subdivision within the *Suaeda* clade roughly corresponds to the sections previously defined by Iljin (1936) and Schenk and Ferren (2001). The topology of our phylogeny for subgenus *Suaeda*, based on ITS sequences, also agreed with that of Schutze et al. (2003). However, *S. glauca* had no phylogenetic resolution with other *Suaeda* species classified in the subgenera of *Suaeda* and *Brezia*. Although the leaf type from *S. glauca* was the same as from other species of sect. *Schanginia*, their petiolate flower clusters and habit resembled those of *S. altissima* in sect. *Salsina* (Schutze et al., 2003). In that earlier examination, *Suaeda glauca* made an independent clade that showed no resolution with a clade including *Borszczowia aralоc* in sect. *Schanginia* and *S. altissima*. This means that the taxonomic status of *S. glauca* should be re-appraised based on morphological characteristics.

Our phylogenetic analysis of the *psb*B-*psb*H spacer region data matrix, using the out-group *Bassia hyssopifolia*, generated a 263 minimal tree length with a CI =0.7034 and an RI=0.9492. In the ML and MrBayesian analysis, species of Korean *Suaeda* were classified into three clades: 1) *S. japonica*, *S. australis*, and *S. maritima*; 2) *S. malacosperma*; and 3) *S. glauca*. The first is involved in several clades of species classified in subgenus *Brezia* (Schutze et al., 2003), which has three *Suaeda* species from Korea. *S. japonica*, *S. australis*, and *S. maritima* had no resolution among species other than *S. australis* from Australia (Fig. 7). However, *S. malacosperma* could be distinguished from the others, and it made a subclade with *S. crassifolia* from Uzbekistan. Although the clade of *S. glauca* made a basal sister group of *Suaeda* species classified in subgenus *Suaeda* (Schutze et al., 2003), it formed a basal sister clade with a long tree (Fig. 7), which suggested that these probably departed early from common ancestors. The topology for our phylogeny of subgenus *Suaeda*, based on *psb*B-*psb*H region sequences, also agreed with that of Schutze et al. (2003) except for having a very low phylogenetic resolution. The *psb*B-*psb*H region sequences, however, did not explain the phylogenetic relationship of subgenus *Brezia* with *S. japonica*, *S. maritima*, and *S. australis* from Korea.

Since Chung (1992) first described *S. australis* in Korea, its taxonomic status has been in dispute. Morphological differences between it and *S. maritima*, especially in their height and leaf length, may be a function of their natural habitats, e.g., salt concentration or a situation where the substrate becomes covered by sand. Despite those variations, plants of those two species from Korea have identical reproductive structures and the same sequences. Therefore, it is reasonable to classify the plants of *S. australis* from Korea as *S. maritima* (L.) Dumont after we re-examine their holotypes because their sequences differ from those of the European plants.

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LITERATURE CITED

Chun J (1995) Computer-assisted Classification and Identification of Actinomycetes. Ph.D. Thesis, University of Newcastle, England, UK

Chung YJ (1992) A Taxonomic Study of the Korean Chenopodiaceae. Ph.D. Thesis, Sungkyunkwan University, Korea (in Korean)

Chung YJ, Lee ST (1995) Pollen morphology of some Korean Chenopodiaceae. Kor J Plant Taxon 25: 255-276 (in Korean)

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791

Ferren WR, Schenk HJ (2003) *Suaeda* In Flora of North America Editorial Committee, editors. Flora of North America, Volume 4. Oxford University Press, New York.

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian Inference of Phylogeny, version 3.10. Distributed by the author

Iljin MM (1936) Chenopodiaceae. *In* Komarov VL, Shiskin BK, eds, Flora of the USSR. (English translation 1985) Singh/Koeltz, Dehra Dun

Kuhn U, Bittrich V, Carolin R, Freitag H, Hedge IC, Uotila P, Wilson PG (1993) Chenopodiaceae. *In* Kubitzki K, ed, The Families and Genera of Vascular Plants, Ed 2. Springer, Berlin New York London, pp 253-281

Lee KS, Oh KC (1989) Difference of *Suaeda japonica* populations from two different habitats in Sorea, Incheon, Korea. Kor J Ecol 12: 133-144

Lee WC (1996) Lineamenta Flora Korea (II). Academy Press, Seoul

Park MK (1974) Keys to the Herbaceous Plants in Korea (Dictyoledoneae) Chungmusa. Seoul. (in Korean).

Posada D, Crandall KA (1998) MODELTEST: Testing the model of DNA substitution. Bioinformatics 14: 817-818

Rodriguez F, Oliver A, Marin A, Medina JR (1990) The general stochastic model of nucleotide substitution. J Theor Biol 142: 485-501

Rzhetsky A, Nei M (1992) A simple method for estimating and testing minimum evolutionary trees. Mol Biol Evol 9: 945-967

Schenk HJ, Ferren WR (2001) On the sectional nomenclature of *Suaeda* (Chenopodiaceae). Taxon 50: 857-873

Seliskar DM (1985) Morphometric variations of five tidal marsh halophytes along environmental gradients. Amer J Bot 72: 1340-1352

Shim H-B, Choi B-H (2004) RAPD marker variations between and within the species of Korean *Suaeda*. Kor J Plant Taxon 34: 63-74 (in Korean)

Shim H-B, Chung J-Y, Choi B-H (2001) One unrecorded species from Korea: *Suaeda malacosperma* Hara. Kor J Plant Taxon 31: 383-387 (in Korean)

Schutze P, Freitag H, Weising K (2003) An integrated molecular and morphological study of the subfamily Suaedoideae Ulbr. (Chenopodiaceae). Plant Syst Evol 239: 257-286

Swofford D.L. (2003) PAUP. Phylogenetic Analysis Using Parsimony version 4.0b10. Sinauer Associates, Sunderland, MA, USA