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Taxonomic Position and Affinities of *Isopyrum mandshuricum* within Korean Isopyroideae (Ranunculaceae) Based on Molecular Data

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To examine the taxonomic position and affinities of *Isopyrum mandshuricum* (Ranunculaceae) and related taxa, genetic analysis were carried out on the basis of isozyme patterns and ITS sequences. Molecular data, both isozyme patterns and ITS sequences suggest that *I. mandshuricum* is closely related to *Enemion raddeanum* than to *Semiaquilegia adoxoides*. The estimation of genetic identities by isozyme analysis reveals that *I. manshuricum* is genetically distant from *E. raddeanum*. The phylogenetic tree based on molecular data is rather congruent with the phenogram based on quantitative morphological characteristics, but not consistent with one based on qualitative morphological characteristics. Incongruencies between molecular and qualitative morphological data provide clues to re-evaluate several morphological features.

Isopyrum mandshuricum Kom. (≡*Semiaquilegia mandshurica* Kom.) was described as a new species by Komarov in 1926 (Hill, 1933) and was placed in the tribe Helleboreae (Isopyreae) (Bentham and Hooker, 1873; Buchheim, 1964) or subfamily Isopyroideae (Satake et al., 1983) of Ranunculaceae. On the basis of morphological characteristics of the petal, petal spur, staminode, carpel and fruit, *I. mandshuricum* has been variously treated as a member of *Semiaquilegia* or as *Isopyrum* within Isopyroideae. Four taxa, including *I. mandshuricum*, *Semiaquilegia adoxoides* (DC.) Makino, *Aquilegia buergeriana* var. *oxysepala* (Trautv. et Meyer) Kitamura, and *Enemion raddeanum* Regel, were examined to discuss the taxonomic dispositions of Korean Isopyroideae by Lee and Yeau (1998). In their study, cluster analysis based on quantitative morphological characters resulted in two groups, one containing *I. mandshuricum* and *Enemion raddeanum* Regel, and a second containing *Semiaquilegia adoxoides* and *Aquilegia buergeriana* var. *oxysepala*. The phenetic analysis (Lee and Yeau, 1998) clustered *I. mandshuricum* and *E. raddeanum* together, even though they are different in petal characteristics, an important feature to delimit genera in the family. *Isopyrum mandshuricum* and *E. raddeanum* are distinct from each other in perianth, trichome type of leaves, and inflorescence: *E. raddeanum* has no petal, multicellular trichomes, and umbelliform cyme, while *I. mandshuricum* has a petal, unicellular trichomes, and simple cyme.

Although four taxa of Isopyroideae have been recognized in Korea by the morphological analyses, and the analysis, of quantitative characters indicated that *I. mandshuricum* is more closely related to *Enemion raddeanum* than to *Semiaquilegia adoxoides* (Lee and Yeau, 1998), the taxonomic position of *I. mandshuricum* can not be precisely determined by only four taxa and morphological characters. Therefore, the taxonomic position of *I. mandshuricum* should be estimated by its genetic relationship to the other taxa. In the present study, isozyme patterns and ITS sequences were analyzed to estimate genetic divergence among *I. mandshuricum* and related taxa, and to compare phylogenetic implications by two independent molecular data regarding the taxonomic position and affinities of *I. mandshuricum* within Korean Isopyroideae.

Materials and Methods

Isozyme electrophoresis

A total of 22 populations of four species was examined in this study. Materials were collected from plants in the field or from plants transferred to the greenhouse of Ewha Womans University (Table 1). Four populations of *Aquilegia buergeriana* var. *oxysepala*, five populations of *Enemion raddeanum*, five populations of *Isopyrum mandshuricum* and eight populations of *Semiaquilegia adoxoides* were examined. In most cases young leaves were used as sources of enzymes. The grinding buffer was the same as described by Gottlieb (1981).

Two buffer systems were used for starch gel elec-

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Table 1. Collection data of samples used in electrophoresis and ITS sequence analysis of *Isopyrum mandshuricum* and related taxa of Isopyroideae in Korea

Taxon	Locality	Voucher no	No of population/No of plants
Isopyrum mandshuricum	Mt. Chonma	NSL 13047*	1/ 34
		SHY 140419	1/ 24
	Mt. Whaya	NSL 130420	2/ 90
	Mt. Youngmoon	NSL 130505	1/ 28
Enemion raddeanum	Kohan	NSL E10515*	
	Mt. Odae	NSL E30605	1/ 23
		NSL E40510	4/ 97
E. savilei	N. America	Hodges and Arnold (1994)*	
E. occidentale	N. America	Hodges and Arnold (1994)*	
E. biternatum	N. America	Hodges and Arnold (1994)*	
Dichocarpum stoloniferum	Japan	Hodges and Arnold (1994)*	
Aquilegia buergeriana var. *oxysepala*	Mt. Whaya	NSL A30420*	1/ 27
	Mt. Odae	NSL A30605*	2/ 60
		NSL A40510	1/ 13
A. skinneri	N. America	Hodges and Arnold (1994)*	
Semiaquilegia adoxoides	Jonju	BSK S30513	
	Mt.Naebyun	NSL S30520*	1/ 34
	Jonnam	SHY S30524	2/ 51
	Cheju (Bijarim)	SHY S31028	1/ 22
	Cheju (Samsunghyul)	JHK S40427	1/ 40
	Mt. Durun	JHK S40409	1/ 8
	Japan	Hodges and Arnold (1994)*	2/ 33

*Samples used for ITS sequence analysis.

trophoresis. System I is an electrode buffer composed of 0.5 M Tris, 0.65 M boric acid and 0.02 M EDTA (pH 8.0). This buffer was diluted 1:9 with distilled water to produce the gel buffer.

System II had an electrode buffer of 0.065 M L-histidine and 0.007 M citric acid, adjusted to pH 6.5 with NaOH. The gel buffer was a 1:3 dilution with distilled water of electrode buffer.

Buffer system I was employed to separate alternative forms of the enzymes glutamate dehydrogenase (*Gdh*, EC 1.4.1.2), leusine aminopeptidase (*Lap*, EC 3.4.11.1), phosphoglucomutase (*Pgm*, EC 2.7.5.1), phosphoglucose isomerase (*Pgi*, EC 5.3.1.9) and triosephosphate isomerase (*Tpi*, EC 5.3.1.1). Buffer system II was used to resolve malate dehydrogenase (*Mdh*, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (*6Pgd*, EC 1.1.1.44), and shikimate dehydrogenase (*Skd*, EC 1.1.1.25). Staining methods for all enzyme systems followed Sotis et al. (1983).

Different genetic loci specifying the same enzymes (isozymes) were numbered according to their relative mobilities, for example, the most anodal form designated 1 and the next fastest designated 2. For each locus, the allele encoding the most anodal form was designated *a* with correspondingly slower forms labeled *b, c,* and so forth.

Allelic frequencies were determined for each population. These frequencies were then employed to calculate genetic identities for all pairwise comparisons of populations (Nei, 1972) and gene diversity statistics (Nei, 1973) for each species using GENESTAT (version 3.3., Lewis and Whitkus, 1989).

To certain relationships between populations, phenetic analysis of genetic distances was carried out with NTSYS (version 1.70, Rohlf and Bookstein, 1992).

ITS sequencing

The complete sequences of the nuclear ribosomal ITS region were generated for nine species of the subtribe Isopyroideae (Table 5). Sequences of four ingroup species (*Aquilegia skinnery, Enemion savilei, E. occiddentale, E. biternatum*) and one outgroup taxon (*Isopyrum stoloniferum=Dichocarpum stoloniferum*) were obtained from Hodges and Arnold (1994). *Isopyrum stoloniferum* was chosen as an outgroup because it appeared as a sister group to Aquilegiineae, which includes *Aquilegia, Isopyrum,* and *Semiaquilegia* (Hodges and Arnold, 1994).

Total genomic DNA was isolated from leaf tissue using the CTAB method of Doyle and Doyle (1987), and purified further by ultracentrifugation with CsCl/ethidium bromide gradients (Sambrook et al., 1989). Double-stranded DNAs of the entire ITS region, including the 5.8S coding region, were amplified directly by 30 cycles of symmetric polymerase chain reaction (PCR) using universal primers (White et al., 1990). The initial PCR reaction was 3 min at 95℃ for denaturation, 1 min at 50℃ for annealing, and 1 min at 72℃ for primer extension. The next of 30 cycles consisted of 1 min denaturation at 95℃, 1 min annealing at 50 ℃, and 45 sec extension at 72℃. PCR products were purified by agarose gel electrophoresis with TAE buffer and recovered using glass powder (U.S. Bioclean, U.S. Biochemical).

Double-stranded PCR products were directly sequenced using the Sequenase Version 2.0 (U.S. Biochemical Corp.) dideoxy chain termination method employing two forward primers (ITS3 and ITS5) and two reverse primers (ITS2 and ITS4) (White et al., 1990). Modifications to the Sequenase protocol included denaturation of the double-stranded DNA by boiling the DNA/primer mix for 3 min, followed by snap-chilling

the mixture for 7 min in an ice bath (Winship, 1989). In addition, 1 μl of DMSO was added to both the labeling and termination reactions to reduce the effects of DNA secondary structures (Cosner et al. 1994).

For sequencing, 6% acrylamide gels were used, and electrophoreses were carried out at 1500 V. Gels were fixed for 30 min in 10% acetic acid, transferred to 3-MM Whatmann filter paper, dried under vacumm for 2.5 h at 80℃, and exposed to Kodak XAR x-ray film for 12-72 h.

The boundaries of the ITS regions and rDNA coding regions were identified by comparison to known sequences reported in the previous study (Yokota et al., 1989). Sequences were aligned using the clustal W program (provided by D. Higgins).

Phylogenetic analyses using Fitch parsimony were performed employing PAUP (version 3.1.1; Swofford, 1993) using the branch and bound search option. Bootstrap analysis (Felsenstein, 1985) performed 500 replications to estimate a measure of support for clades. Decay analysis (Bremer, 1988; Donoghue et al., 1992) was also performed to assess the robustness of the monophyletic groups. Trees up to eight steps longer than the shortest tree were examined. Distance matrices were calculated using the DNADIST program of PHYLIP (version 3.52c; Felsenstein, 1993) and nucleotide substitutions (excluding gaps) were estimated using the two parameter method of Kimura (1980). Maximun liklihood phylogeny estimation was explored utilizing the DNAML program in PHYLIP 3.52c. A maximum liklihood tree was inferred using a transition/transversion ratio of 2:0.

Results

Isozyme analysis

Sixteen loci (*Gdh*-1, *G3pdh*-1, *Idh*-1, *Idh*-2, *Lap*-1, *Mdh*-1, *Mdh*-2, *Mdh*-3, *6Pgd*-1, *Gpi*-1, *Gpi*-2, *Pgm*-1, *Skd*-1, *Skd*-2, *Tpi*-1, *Tpi*-2) were scored for over 10 enzyme systems for 584 individuals of 22 populations. The chromosome number of *I. mandshuricum* and *S. adoxoides* is 2n=14, and that of *A. buergeriana* var. *oxysepala* was 2n=20 (Lee, 1967). Chomosome number of *E. raddeanum* is not determined. The number of isozymes for all enzymes, except *G3pdh,* are same as those reported for most other diploid plants. It was reported that *G3pdh* has two isozymes in diploid plants (Crawford, 1990), but only one isozyme was observed in the present study.

Two isozymes for *6Pgd* and *Pgm* were expressed, but only one gene was scored because of its poor activity and/or resolution. Allelic frequencies for all loci are presented in Appendix 1. A single locus, *6Pgd,* was monomorphic in all populations. The number of alleles for polymorphic loci varied from 2.0 to 2.5. Genetic variability for each taxon is presented in Table 2. *Isopyrum mandshuricum* exhibits the lowest value of the mean number of alleles per locus (A), proportion of polymorphic loci (P) and expected heterozygosity, but the highest value for alleles per polymorphic locus.

Total and average within population gene diversity statistics (Nei, 1973) are also lower in *I. mandshuricum* than the other taxa (Table 3). Diversity among population (D_{ST}) of four taxa is similar to gene diversity

Table 2. Proportion of polymorphic loci (P), mean number of alleles per polymorphic locus (A_P), mean number of alleles per locus (A), the expected heterozygosity unbiased for sample size (H_U) for *Isopyrum mandshuricum* and related taxa of Korean Isopyroideae

	A	A_P	P	H_U
ISO[a]	1.1875	2.5000	0.1250	0.0544
AQU[b]	1.4375	2.1667	0.3750	0.1868
ENE[c]	1.6250	2.4286	0.4375	0.1843
SEA[d]	1.4375	2.0000	0.4375	0.1322

[a]*Isopyrum mandshuricum*, [b]*Aquilegia buergeriana* var. *oxysepala*, [c]*Enemion raddeanum*, [d]*Semiaquilegia adoxoides.*

Table 3. Gene diversity statistics for *Isopyrum mandshuricum* and related taxa of Isopyroideae in Korea

	H_S	J_S	H_T	J_T	D_{ST}	G_{ST}
ISO	0.0388	0.9612	0.0719	0.9281	0.0331	0.4602
AQU	0.1094	0.8906	0.1841	0.8159	0.0747	0.4057
ENE	0.0997	0.9003	0.1903	0.8097	0.0906	0.4760
SEA	0.0644	0.9356	0.1345	0.8655	0.0701	0.5215

Acronyms follow Table 2. H_S=gene diversity within populations, J_S=gene identity in populations, H_T=gene diversity in the total population, J_T=gene identity in the total population, D_{ST}=gene diversity between populations, G_{ST}=the coefficient of population differentiation

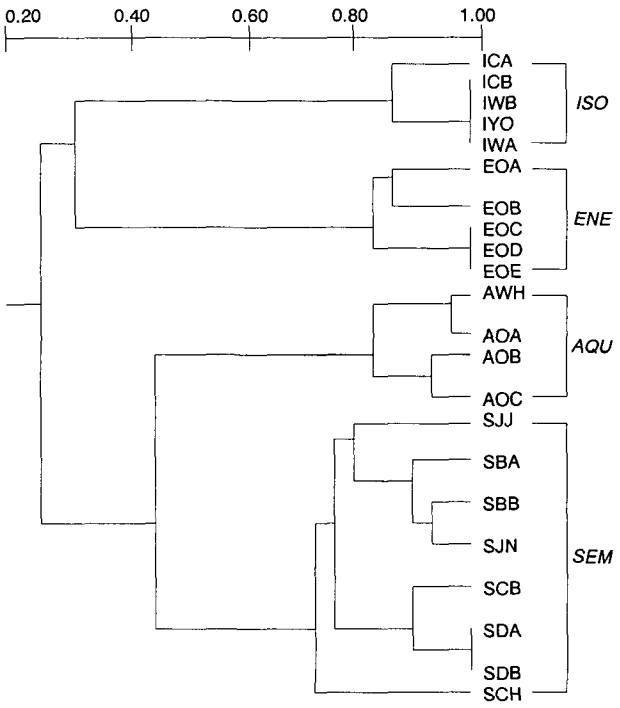

Fig. 1. Phenogram resulting from phenetic analysis based on genetic distance (Nei, 1972) among four taxa of Isopyroideae in Korea. Taxon acronyms follow Table 2; population acronyms ICA, ICB: Mt. Chonma; IWA, IWB: Mt. Whaya, IYO: Mt. Yongmun; AWH: Mt. Whaya; AOA, AOB, AOC: Mt. Odae; EOA, EOB, EOC, EOD, EOE: Mt. Odae; SJJ; Jonju; SBA, SBB: Mt. Naebyun; SJN:Jonnam; SCB: Isl. Cheju (Bizarim), SCS: Isl. Cheju (Samsunghyul), SDA,SDB: Mt. Durun.

Table 4. Genetic identities (above diagonal) and genetic distances (below diagonal) for *Isopyrum mandshuricum* and related taxa of Isopyroideae in Korea

	ISO	*AQU*	*ENE*	*SEA*
ISO		0.3135	0.3400	0.3038
AQU	1.1600		0.3040	0.4203
ENE	1.0788	1.1907		0.2785
SEM	1.1915	0.8667	1.2784	

Acronyms follow Table 2.

in populations (H_S). The coefficient of population differentiation (G_{ST}) for the four taxa was similar and indicates that close to 50% of the total diversity in the four taxa exist among the populations.

Genetic identities (I) and genetic distances (D) (Nei, 1972) among taxa and populations are shown in Table 4 and Appendix 2, respectively. Genetic identities among the four taxa are quite low, around 0.3 to 0.4. In the phenogram based on genetic distances among populations, two major clusters are apparent; one containing *I. mandshuricum* and *E. raddeanum*, and a second with *A. buergeriana* var. *oxysepala* and *S. adoxoides* (Fig. 1).

ITS sequence analysis

Length variation and base composition of ITS region. Complete sequences of the nuclear ribosomal ITS region were generated from four taxa of Korean Isopyroideae. In addition, ITS sequences of five foreign taxa and one outgroup taxon were obtained from Hodges and Arnold (1994). The ITS regions vary in length from 433 bp (*D. stoloniferum*) to 451 bp (*E. biternatum*). The length of ITS1 ranges from 222 bp (*D. stoloniferum*) to 236 bp (*E. biternatum*). The length of ITS2 ranges from 211 bp (*E. raddeanum, D. stoloniferum, I. mandshuricum*) to 215 bp (*E. biternatum*). ITS1 is consistently longer than ITS2. The G+C content in ITS1 varied from 60.7% (138/227) to 63.5% (143/ 225), while ITS2 varied from 59.1% (126/213) to 65.4% (140/214) (Table 5). The aligned sequence length over all taxa is 458 bp; 241 bp in ITS1 and 217 bp in ITS2. Sequence alignment required the introduction of 48 gaps: 37 of which were 1 bp in length, six 2 bp in length and five 3 bp in length (Appendix 3).

Sequence divergence and Phylogenetic analysis of the ITS region. A total 143 variable sites was detected from the ITS regions. More than half (57.3%) of these variable sites (82/143) were in ITS1. Sixty-six sites were phylogenetically informative; 53 sites (65.1% of total informative sites) were in ITS1, and 23 sites (34.7%) in ITS2.

Parsimony analysis of the ITS sequences using equally weighted character states resulted in two equally parsimonious trees of 140 steps. The consistency index (CI) for the trees was 0.814, the retention index (RI) was 0.816, and the homoplasy index (HI) was 0.186 (Fig. 2).

Decay analysis was performed until all nodes collapsed to assess the robustness of each clade. At tree lengths of 141, 142, 143, 144, 145, 146, 147, and 148, there were 9, 15, 34, 78, 123, 205, 359, and 520 equally parsimonious trees, respectively. All internal nodes collapsed at the strict consensus tree of eight equally parsimonious steps longer than the most parsimonious tree. Bootstrap values of internal nodes range from 39% to 99%. Reanalyzing the combined ITS data sets with the 48 gaps scored as present or absent resulted in three minimal length trees of 221 steps each, with a consistency index of 0.814. The topology of two of these trees was identical to the trees in which gaps were excluded. The third tree differs in relationships among the three species, *E. savilei*, *E. occidentale*, and *E. biternatum*.

All trees show two major lineages within Isopyroideae; subtribe Isopyrineae which includes *Isopyrum* and *Enemion*, and subtribe Aquilegiineae which includes *Aquilegia* and *Semiaquilegia*. These two lineages are strongly supported by both bootstrap and decay values.

The maximun likelihood tree (transition/transversion ratio=2.0) and neighbor joining tree (sequence divergence calculated with Kimura-two-parameter method) are identical to one of the trees constructed with equally weighted parsimony (not shown).

Discussion

The levels of genetic variation within a species are affected by many factors, including mating system, mode of reproduction, population size, geographic distribution, and history of genetic drift. Population size and narrow endemism, however, are not solely res-

Table 5. Length and G+C content variation of ITS regions from nine taxa of Isopyroideae

Taxa	ITS1	G+C (%)	ITS2	G+C (%)	Combined	G+C (%)
Enemion savilei	235	62.9	213	64.7	448	63.8
E. occidentale	235	61.7	213	59.1	448	51.5
E. biternatum	236	63.1	215	64.6	451	63.8
E. raddeanum	225	63.5	211	64.9	436	64.2
Dichocarpum stoloniferum	222	63.0	211	60.6	433	61.8
Semiaquilegia adoxoides (JAP)	235	62.9	215	64.1	450	63.5
S. adoxoides (KOR)	227	60.7	214	64.9	441	62.8
Isopyrum mandshuricum	224	62.5	211	64.4	435	63.4
Aquilegia skinneri (AME)	235	61.2	213	64.7	448	62.9
A. buergeriana (KOR1)	234	61.5	214	65.4	448	63.1
A. buergeriana (KOR2)	234	61.1	214	65.4	448	62.9

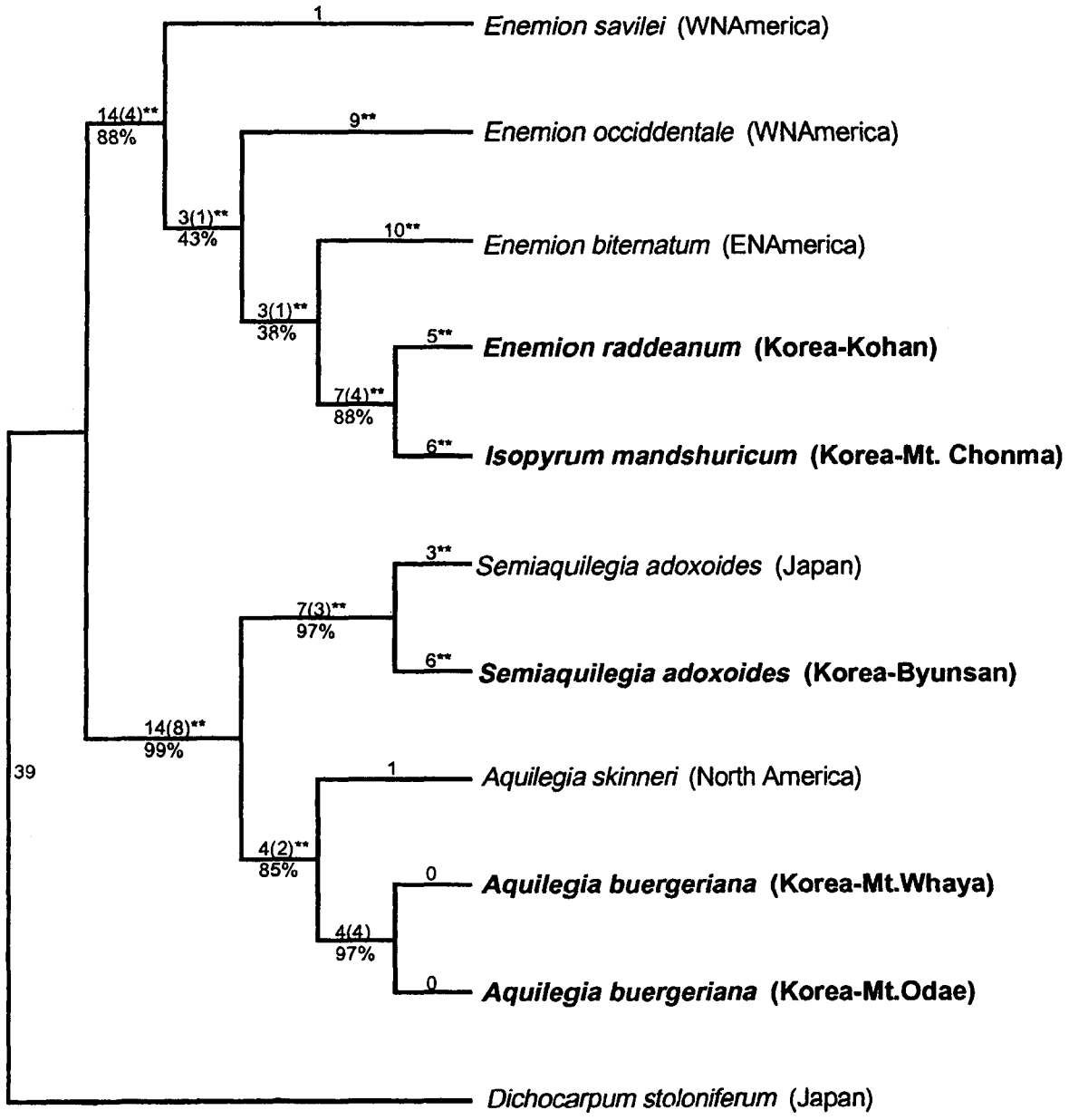

Fig. 2. A Most parsimonious 140-step tree derived from equally weighted parsimonious analysis of combined ITS1 and ITS2 sequences. CI=0.814, RI=0.816, HI=0.186. Numbers above the line are decay values, numbers below branches give the percentage that a group occurred in 500 bootstrap replication. **P<0.01.

ponsible for low levels of genetic variation in species with highly restricted distribution (Vogelmann and Gastony, 1987). Also the low genetic variation of species is caused by a rare and narrow distribution or by an artifact of small sample size. The reason for the reduced genetic variation observed in *I. mandshuricum* seems to be a reflection of a species that has a restricted distribution and reproduces by many small tubers. Gene diversities between populations are greater in all four taxa than diversities within populations and are reflected in the high values of G_{ST}. Total gene diversity is the lowest in *I. mandshuricum*, the geographically restricted and rare species.

Considering that the typical value of conspecific genetic identity is 0.95 (Gottlieb, 1977, 1981; Crawford, 1983, 1990; Giannasi and Crawford, 1986) and congeneric genetic identity falls between 0.65-0.7 (Gottlieb, 1977, 1981; Crawford, 1983, 1990), the observed values of 0.3 to 0.4 in this study fall within the range expected for different genera. The result supports maintaining *I. mandshuricum* as a separate genus from *Enemion* and *Semiaquilegia*. The low genetic identities may have been caused by severe selfing, thus fixing allelic differences. Alternatively, the low

genetic identities may simply reflect the ancient divergence among these taxa. Study of additional species of *Isopyrum* and *Enemion* is needed to address this question. The phenogram based on genetic distance is consistent with the result of the phenetic analysis based on quantitaive morphological characters, but not with the clustering based on qualitative morphological characters such as the presence of petals, types of leaf trichomes and inflorescence (Lee and Yeau, 1998).

In the ITS sequence analysis, two genera, *Aquilegia* and *Semiaquilegia* form monophyletic groups, whereas *Enemion* is paraphyletic. *I. mandshuricum* appears as a sister species to *E. raddeanum*. Even though other species of *Isopyrum* were not included, the ITS phylogeny strongly suggests that this species shows greater affinity to *Enemion* than to *Semiaquilegia*. This implies that *I. mandshuricum* has taxonomic affinity to the genus *Isopyrum* s. lat. rather than *Semiaquilegia*. It means that the presence or absence of petals is not an important character in delimiting the genus *Isopyrum* and supports the classification of Calder and Taylor (1963) that the genus *Enemion*, without petals, included in the genus *Isopyrum*, with petals. The petals of *I. mandshuricum* show the homoplasy with one of *S. adoxoides* and *A. buergeriana* var. *oxysepala*, and the petals of *I. mandshuricum* are considered as a reverse chacracter, because it makes a monophyletic group with *E. savilei* and *E. occiddentale* which lack petals. This cladogram is not consistent with the recognition of *Enemion* as a different genus from *Isopyrum* (Hutchinson and Drummond, 1920; Tamura, 1968; Tamura and Lauener, 1968). Discordance of morphology with molecular data in Ranunculaceae was also suggested by Hoot (1995). Like qualitative morphological characters, isozyme data support the separation of *Enemion* from *Isopyrum* based on the low value of genetic identity. The separation of *Enemion* from *Isopyrum* needs to be studied further and based on a much broader sampling.

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Appendix 1. *Allele frequencies for the 16 putative genetic loci of the populations in Isopyrum mandshuricum and related taxa of Isopyroideae in Korea (ICA, ICB: Mt. Chonma; IWA, IWB: Mt. Whaya, IYO: Mt. Yongmun; AWH: Mt. Whaya; AOA, AOB, AOC: Mt. Odae; EOA, EOB, EOC, EOD, EOE: Mt. Odae; SJJ; Jonju; SBA, SBB: Mt. Naebyun; SJN:Jonnam; SCB: Isl. Cheju (Bizarim), SCS: Isl. Cheju (Samsunghyul), SDA, SDB: Mt. Durun.*

Locus	*Isopyrum mandshuricum*					*Aquilegia buergeriana* var. *oxysepala*				*Enemion raddeanum*					*Semiaquilegia adxoides*							
Allele	ICA	ICB	IWA	IWB	IYO	AWH	AOA	AOB	AOC	EOA	EOB	EOC	EOD	EOE	SJJ	SBA	SBB	SJN	SCB	SCS	SDA	SDB
GDH	24	41	76	14	28	27	31	29	8	30	26	29	23	8	34	39	12	22	37	9	14	19
a	0.000	0.000	0.000	0.000	0.000	0.962	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	1.000	1.000	1.000	1.000	1.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G3PDH	24	41	76	14	28	26	11	29	8	33	27	29	23	8	34	39	12	22	37	9	14	19
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1	24	36	63	14	26	20	17	29	8	20	27	29	23	8	34	25	12	22	37	9	14	19
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-2	24	37	63	14	15	20	17	29	8	20	27	24	23	8	34	25	12	22	37	9	14	19
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LAP	24	37	24	0	23	5	25	29	8	9	27	29	23	8	32	14	8	0	37	9	14	19
a	1.000	1.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000
d	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-1	24	41	76	14	28	26	31	29	8	33	27	29	23	8	34	39	12	22	37	9	14	19
a	0.000	0.000	0.000	0.000	0.000	0.385	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.412	0.500	0.250	0.273	0.000	0.000	0.500	0.500
b	0.000	0.000	0.000	0.000	0.000	0.615	0.984	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.588	0.500	0.750	0.727	1.000	1.000	0.500	0.500
c	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-2	24	41	76	14	28	27	26	29	8	33	27	29	23	8	34	39	12	22	37	9	14	19
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
c	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000
MDH-3	24	41	76	14	28	27	26	29	8	33	27	29	23	8	34	39	12	22	37	9	14	19
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.848	0.852	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.152	0.148	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	0.000	0.000	0.000	0.000	0.000	0.963	0.769	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.862	1.000	0.727	0.000	1.000	0.000	0.000
d	0.000	0.000	0.000	0.000	0.000	0.037	0.231	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138	0.000	0.273	1.000	0.000	1.000	1.000
6PGD	24	0	15	0	0	0	8	29	7	6	27	29	23	8	0	12	0	0	37	9	14	19
a	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	1.000	0.000	1.000	0.000	0.000	1.000	1.000	1.000	1.000
PGI-1	24	30	76	1	25	24	21	29	8	21	16	29	23	8	17	30	0	6	37	9	14	19
a	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.882	0.833	0.000	1.000	1.000	1.000	1.000	1.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.875	1.000	1.000	1.000	0.118	0.167	0.000	0.000	0.000	0.000	0.000	0.000
d	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGI-2	23	41	76	14	15	12	21	29	8	33	24	29	23	8	34	30	8	6	37	9	14	19
a	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.750	1.000	0.957	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM	24	41	64	14	28	24	28	29	8	30	27	29	23	8	34	33	11	22	37	9	14	19
a	0.000	0.000	0.000	0.000	0.000	1.000	0.946	0.500	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.500	0.500	0.000	0.000	0.000	0.000	0.000	0.100	0.621	1.000	1.000	0.500	1.000	1.000	1.000
c	1.000	0.949	0.789	1.000	1.000	0.000	0.000	0.000	0.000	0.817	1.000	1.000	1.000	1.000	0.000	0.379	0.000	0.000	0.500	0.000	0.000	0.000
d	0.000	0.061	0.211	0.000	0.000	0.000	0.000	0.000	0.000	0.183	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SKDH-1	21	18	62	13	7	10	28	29	0	23	17	29	23	8	22	29	5	18	0	0	14	19
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	1.000	1.000
b	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.174	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	0.000	1.000	1.000	1.000	1.000	0.900	0.714	0.500	0.000	0.826	0.176	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
d	0.000	0.000	0.000	0.000	0.000	0.100	0.286	0.000	0.000	0.174	0.824	1.000	0.826	0.750	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SKDH-2	21	18	62	13	7	10	28	29	0	23	17	29	23	8	22	29	5	18	0	0	14	19
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.353	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.000	0.500	0.500
b	0.500	0.500	0.476	0.500	0.500	0.300	0.571	0.500	0.000	0.826	0.647	0.000	0.087	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.500
c	0.500	0.500	0.524	0.500	0.500	0.700	0.429	0.500	0.000	0.174	0.000	1.000	0.913	0.750	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TPI-1	24	41	76	14	28	26	31	29	8	33	27	24	21	8	33	39	12	22	37	9	14	19
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.125	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.963	0.875	1.000	0.750	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI-2	24	41	76	14	28	26	31	29	8	33	27	29	21	8	33	39	12	22	37	9	14	19
a	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	0.000	0.037	0.185	0.143	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	1.000	0.963	0.815	0.857	0.750	0.412	0.154	0.000	0.000	0.000	0.000	0.000	0.000
c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.588	0.846	1.000	1.000	1.000	1.000	1.000	1.000

Appendix 2. Genetic distance among populations of *Isopyrum mandshuricum* and related taxa for the NTSYS (ICA, ICB: Mt. Chonma; IWA, IWB: Mt. Whaya, IYO: Mt. Yongmun; AWH: Mt. Whaya; AOA, AOB, AOC: Mt. Odae; EOA, EOB, EOC, EOD, EOE: Mt. Odae; SJJ; Jonju; SBA, SBB: Mt. Naebyun; SJN:Jonnam; SCB: Isl. Cheju (Bizarim), SCS: Isl. Cheju (Samsunghyul), SDA,SDB: Mt. Durun).

	I. mandshuricum					*A. buergeriana var. oxysepala*				*E. raddeanum*					*S. adxoides*							
	ICA	ICB	IWA	IWB	IYO	AWH	AOA	AOB	AOC	EDA	EDB	EDC	EDD	EDE	SJJ	SBA	SBB	SJN	SCB	SCS	SDA	SDB
ICA																						
ICB	0.0710																					
IWA	0.0699	0.0009																				
IWB	0.0758	0.0000	0.0023																			
IYO	0.0697	0.0000	0.0015	0.0000																		
AWH	1.3832	1.1531	1.1430	1.0827	1.1556																	
AOA	1.2060	1.1994	1.0488	1.1285	1.2019	0.0186																
AOB	1.2834	1.2473	1.2728	1.1761	1.2498	0.1202	0.1083															
AOC	1.2333	1.4417	1.2212	1.3636	1.4454	0.1110	0.0587	0.0362														
EOA	1.2217	1.1969	1.0701	1.1197	1.1923	1.3138	1.0461	1.2843	1.2127													
EOB	1.3970	1.3248	1.4013	1.2786	1.3129	1.3409	1.1816	1.2182	1.2804	0.1193												
EOC	1.0927	1.2488	1.1188	1.1681	1.2391	1.4115	1.1495	1.3994	1.1991	0.1528	0.2003											
EOD	1.0128	1.1902	1.0700	1.1094	1.1811	1.3935	1.1314	1.3442	1.1861	0.1328	0.1886	0.0028										
EOE	1.0684	1.2250	1.0973	1.1410	1.2151	1.3932	1.1139	1.3443	1.2039	0.1133	0.1811	0.0042	0.0033									
SJJ	1.3840	1.3805	1.3726	1.3072	1.3831	0.8900	0.9003	1.0407	0.9169	1.4514	1.3650	1.4982	1.4976	1.4674								
SBA	1.1862	1.3677	1.1934	1.2903	1.3648	0.9341	0.8386	1.0282	0.7688	1.2580	1.3908	1.2668	1.2690	1.2342	0.0211							
SBB	1.5076	1.5040	1.4954	1.4306	1.5067	0.8761	0.8552	0.9089	0.7874	1.5703	1.4750	1.5940	1.5999	1.5513	0.0442	0.0169						
SJN	1.4919	1.4882	1.4797	1.4916	1.4909	0.9086	0.8703	0.8467	0.7224	1.5630	1.5009	1.5867	1.5928	1.5439	0.0523	0.0186	0.0045					
SCB	1.1165	1.2959	1.1281	1.2106	1.2923	0.9911	0.7712	0.7303	0.6202	1.1165	1.3192	1.0972	1.1140	1.0871	0.1389	0.0834	0.1195	0.0769				
SCS	1.0296	1.1749	1.0175	1.0986	1.1787	1.0272	0.8619	1.0773	0.9149	1.2321	1.5047	1.2335	1.2515	1.2233	0.1538	0.1150	0.0934	0.1029	0.1783			
SDA	1.2759	1.4728	1.2681	1.4030	1.4753	1.0917	0.8894	0.8199	0.6549	1.3374	1.5912	1.4695	1.4608	1.3974	0.1444	0.0823	0.1015	0.0633	0.0368	0.1778		
SDB	1.2762	1.4731	1.2684	1.4034	1.4757	1.0920	0.8898	0.8203	0.6550	1.3377	1.5915	1.4698	1.4611	1.3977	0.1448	0.0826	0.1019	0.0637	0.0370	0.1780	0.0000	

Appendix 3. Aligned ITS sequences (ITS 1 and ITS 2) of the internal transcribed spacer region from 9 taxa of Isopyroideae including *Dichocarpum stoloniferum* as an outgroup. An asterisk indicates matched sequences and bar indicates the gaps. Polymorphic sites are represented by following symbols: K=G or T, M=A or C, W=A or T, Y=C or T.

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                ITS 1>
E. savileii     TCGAAACCTGCTCAAGCAGAACGACCCGC-GAACACGT-GAAAACAACCATGTTGGGGACCGGGGAGGGGCGCGAGCCCCCGACCGA--CCCCTTCGCCTAGCCGCGGCTCCGGCC
E. occidden     TCGAAACCTGCTCAAGCAGAACGACCCGC-GAACACGT-GAAAACAACCATGTTGGGGACAGGGGAGGGGCGCGAGCCCCCGACCGA--CCCCTTCGCCTAGCCGTGGCTCCGGTC
E. biternat     TCGAAACCTGCTCAAGCAGAACGACCCGC-GAACACGT-GAAA-CAACCACGTTGGGGACCGGGGAGGGGCGCGAGCCCCCGACCGAACCCCCTTCGCCCAGACGCGGCCCCGGCT
E. raddeanu     TCGAAACCTGCTCAAGCAGAACGACCCGC-GAACACGT-GAAAACAACCATGTCGGGGACCGGGGAGG--CGCGAGCCCCCGACCGA--CCCCTTCGCCTA--CGCCGGCTCGC--
D. stolonif     TCGAAACCTGCTCAAGCAGAACGACCCGC-GAACAAGTCGAAACAAACCACGGCGGGTGCCGGAGAGGGGCGCGAGCCCCCGACCGT--GCCCTCCGCCGTGCCTCGGATC-----
S. adoxo. Ja    TCGAAACCTGCTCAAGCAGA-CGACCCGCCGAACACGT-GAAAACAACCACGCAGGGGTA-GGGGAGGGGCGCGAGCCCCCGACCGA--CCCCTTCGCCGTTCCGCGGCCCCGACC
S. adoxo. Ko    TCGAAACCTGCTCAAGCAGAACGACCCGCTGAACACGT-AAAACAACCAACGCAGGGGTA-GGGGAGGG-TGCGA-CCCCCGACCGA--CCCCTTC--CGGTTGCCGGCCC-----
I. mandshur     TCGAAACCTGCTCAAGCAGAACGACCCGC-GAACACGT-GAAAACAACCATGCTGGGACCGGGGAGGG--CGCGAGCCCCCGACCGA--CCCCTTCGCCTA--CGCCGGCGTCG--
A. skinneri     TCGAAACCTGCTCAAGCAGAACGACCCGC-GAACACGT-GAAAACAACCCTGCAGGGGTCCGGGGAGGGGCGCGAGCCCCCGACCGA--TCCCTTCGTCGTTCCGCGGCCCCGACC
A. buerger1     TCGAAACCTGCTCAAGCAGAACGACCCGC-GAACACGT-GAAAACAACC-TGCAGGGGTCCGGGGAGGGGCGCGAGCCCCCGACCGA--TCCCTTCGTCGTTCCGCGGCCCCGACC
A. buerger2     TCGAAACCTGCTCAAGCAGAACGACCCGC-GAACACGT-GAAAACAACC-TGCAGGGGTCCGGGGAGGGGCGCGAGCCCCCGACCGA--TCCCTTCGTCGTTCCGCGGCCCCGACC
                ********************* ******** ***** ** *** * *  *  *** **  **  **** **********  **** * *       *

E. savileii     GCTTGCGGTCGTCCGTGGATAGGCACAAACAAAAAACCCGGCGCAACTGGCGTCAAGGAAATCTTAGCGGAAACAAGGCGGAGCCCCGCTCGCGGGGCGAAGCCGCGAATCCGAT
E. occidden     GCTTGCGGCCGTCCGTGGATAGGCACAAACACAAA-CCCGGCGCAACTGGCGTCAAGGAAATCTTAGCGGAAACAAGGCGGAGCCCCGCT-GCGGGGCGAAGCCGCGAATCCGAT
E. biternat     GCTTGCGGCCGTCCGTGTATGGGCACAAACAAAAAACCCGGCGCAATTGGCGTCAAGGAAATCTTAGCGGAAACAAGGCGGAGCCCCGCTCGCGGGGCGAAGCCACGAATCCGAT
E. raddeanu     -CGTGCGGCCGTCCGTGGTCGGGCACAAACAAAAAACCCGGCGCAACTGGCGTCAAGGAAATCTTAGCGGAAACAAGGCGGA---CGGCTCGCGGGGCGAAGCCGCGAATCCGAT
D. stolonif     ---------CGTCCGCGGTACGGCACAAACAATAAATCCGGCGCAACTGGCGTCAAGGAAATCTTAGCGGAAACAGGGCGGAGCCCCTCTCGCGGGGCGACGCCAGGAATCCGAT
S. adoxo. Ja    GCATGCGGTCGTCCGCGGTCCGGCACAAAACAAAAACCCGGCGCAACTGGCGTCAAGGAAATCTTAGCGGAAACAGGACCTAGCCCTGCTTGCGGGGCGAAGTCGCGAATCCGAT
S. adoxo. Ko    GCATGCGGTCGTCCGCGGTCCGGCACAAAACAAAAACCCGGCGCAACTGGCGTCAAGGAAATCTTAGCGGAAACAGGACCTAGCCCTGCTTGCGGGGCGAAGTCGCGAATCCGAT
I. mandshur     -CTCGCGGCCGTCCGTGGCTGGGCACAAACAAAAAACCCGGCGCAACTGGCGTCAAGGAAATCTTAGCGGAAACAAGGCGGA---CG-CTCGCGGGGCGAAGTCGCGAATCCGAT
A. skinneri     GCATGCGGTCGTCCGTGGTCCGACACAAACCAAAAACCCGGCGCAATTGGCGTCAAGGAAATCTTAGCGGAAACAAGGCCTAGCCCTGCTTGCGGGGCGAAGTCGCGAATCCGAT
A. buerger1     GCATGCGGTCGTCCGTGGTCCGACACAAACCAAAAACCCGGCGCAATTGGCGTCAAGGAAATTTTAGCGGAAACTAGGCCTAGCCCTGCTTGCGGGGCGAAGTCGCGAATCCGAT
A. buerger2     GCATGCGGTCGTCCGTGGTCCGACACAAACCAAAAACCCGGCGCAATTGGCGTCAAGGAAATTTTAGCGGAAACTAGGCCTAGCCCTGCTTGCGGGGCGAAGTCGCGAATCCGAT
                       ****** *   * *  *****    **  ********* ********************** ***********  * *  *   *  **  ********* * *  *********

                <ITS 1 ITS 2>
E. savileii     ATTTGAAC ACAGCGTCGCCCCC-AA-CCACACCCGTTGTGGGAGGGGAGCGGAGATTGGCCCCCCGATCCCTCGCGGGAACGGTCGGCACAAATGCTGGT-CCCTGGCAGCGGT
E. occidden     ATTTGAAC ACAG-GTCGCCCCC-AA-CCACACC--TTGTGGAAGGGGAGCGGAGATTGGCCCCCCGATCCCTTGCGGGAACGGTCGGCACAAATGCTGGT-CCCTGGCAGCGGT
E. biternat     ATTTGAAC ACAGCGTCGCCCCC-AA-CCACACCCGTTGTGGAGGGGGAGCGGAGATTGGCCCCCCGATCCCTCACGGGAACGGTCGGCACAAATGCTGGTTCCCTGGCAGCGGT
E. raddeanu     ATTTGAAC ACAGCGTCGCCCCCCCAA-CCACACCCGTTGTGGAGGGGGAGCGGAGATTGGCCCCCCGATC--TCGCGGGAACGGTCGGCACAAATGCTGGT-CCCTGGCAGCGGT
D. stolonif     ATTTGAAC ACAGCGTCGCCCTCTAA--CACACCCGTTGTGGGAGGGGAGCGGAGATTGGCCCCCCGAGCCCTCGCGGGCGCGGTCGGCACAAATGCCGGT-CCCTGGCAG-GGT
S. adoxo. Ja    ATTTGAAC ACAGCGTCGCCCCCCAATCCACACTTGTTGTGGGAGGGGAGCGGAGATTGGCCCCCCGAGCCCTCGCGGGCGCGGTCGGCACAAATGTTGGT-CCCTGGCAGCGGT
S. adoxo. Ko    ATTTGAAC ACAGCGTCGCCCCCCAATCCACACTTGTTGTGGGAGGGGAGCGGAGATTGGCCCCCCGAGCCCTCGCGGGCGCGGTCGGCACAAATGTTGGT-CCCTGGCAGCGGT
I. mandshur     ATTTGAAC ACAGCGTCGCCCCC-AA-CCACACCCGTTGTGGAGGGGGAGCGGAGATTGGCCCCCCGATC--TCGCGGGAACGGTCGGCACAAATGCTGGT-CCCTGGCAGCGGT
A. skinneri     ATTTGAAC ACAGCGTCGCCCCCCCAATCCACACTTGTTGTGGGAGGGGGAGCGGAGATTGGCCCCCCGAGCCCTCGCGGGCG--GTCGGCACAAATGCTGGT-CCCTGGCAGCGGT
A. buerger1     ATTTGAAC ACAGCGTCGCCCCCCCAATCCACACTTGTTGTGGGAGGGGAGCGGAGATTGGCCCCCCGAGCCCTCGCGGGCGCGGTCGGCACAAATGTTGGT-CCCTGGCAGCGGT
A. buerger2     ATTTGAAC ACAGCGTCGCCCCCCCAATCCACACTTGTTGTGGGAGGGGAGCGGAGATTGGCCCCCCGAGCCCTCGCGGGCGCGGTCGGCACAAATGTTGGT-CCCTGGCAGCGGT
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Appendix 3. (Continued)

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E.savileii    TGTCGCGGTCAAGTGGTGGCTTCAAATACCATTTCGGTGACCGGTTGGCTCCGCGGCCC-TAGTCGGGACCGAATCGACCCGCAGAGGCCGTTCCGACGGCTTTTCACCCT
E.occidden    TGTCGCGGTCA-GTGGTGGCTTCAAATACCATT-CGGTGACCGGTTTGCTCCGCGGCT----ATTGGGACCGAATCGACCC-CAGAGCCCGTTCCGACGGCATTTCAC---
E.biternat    TGTCGCGGTCAAGTGGTGGCTTCAAATACCATTTCGGTGACCGGTCGGCTCCGCGGCCCCTAGTTGGGACCGAATCGACCCGCAGAGGCCGTTCCGACGGCTTTTCACCCT
E.raddeanu    TGCCGCGGTCAAGTGGTGGCTTCAAATACCATTTCGGTGACCGGTTGCGTCCGCGGCCC-TAGTTGGGACCGAATCGACCCGCAGAGGCCGTTCCGACCGT-TTTCACCCT
D.stolonif    CGCT-CGGTCAAGTGGTGGCTTCAAATACCATTTCGGTGACCG---CGGCCCGCGGCTC-TAGTCGGGACCGAATCGACCCCCAGAGCC-ATTCCGATGGCCCATCACCC-
S.adoxo.Ja    TGCCGCGGTCAAGTGGTGGCTTCAAATACCATTTCGGTAACCGGTTGGCTCCGCGGCCA-TAGTTGGGACCGAATCGACCCGCAGAGGCCGTTTCGACGGGGTTTCACCCT
S.adoxo.Ko    TGCCGCGGTCAAGTGGTGGCTTCAAATACCATTTCGGTGACCGGTTGCGTCCGCGGCCA-TAGTTGGGACCGAATCGACCCGCAGAGGC-GTTCCGACGGCGTTTCACCCT
I.mandshur    AGTCGCGGTCAAGTGGTGGCTTCAAATACCATTTCGGCGACCGGTTGCGTCCGCGGCCC-TAGTTGGGACCGAATCGACCCGCAGACGCCGTTCCGACGCT-CTTCACCCT
A.skinneri    CGCCGCGGTCAAGTGGTGGCTTCAAATACCATTTCGGTGACCGGTTGGCTCCGCG-CCA-TAGTTGGGACCGAATCGACCGCAGAGGCC-GTTCCGACGGCGTTTCACCCT
A.buerger1    CGCCGCGGTCAAGTGGTGGCTTCAAATACCATTTCGGTGACCGGTTGCGTCCGCGGCCA-TAGTTGGGACCGAATCGACCCGCAGAGGC-GTTCCGACGGCGTTTCACCCT
A.buerger2    CGCCGCGGTCAAGTGGTGGCTTCAAATACCATTTCGGTGACCGGTTGCGTCCGCGGCCA-TAGTTGGGACCGAATCGACCCGCAGAGGC-GTTCCGACGGCGTTTCACCCT
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