

Taxonomic review of the umbelliferous taxa *Heracleum moellendorffii* complex in Korea based on molecular phylogenies of nuclear ribosomal ITS sequences

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Taxonomy of umbelliferous taxa *Heracleum moellendorffii* complex has been unclear in their species delimitation in the far-eastern Asian regions. In both Korea and China *Heracleum moellendorffii* was adopted for their description while *H. sphondylium* was chosen to describe Japanese *Heracleum*. From Genbank accessions, taxa collected from Kamtchatka and Promorskiy, Russia were defined as *H. maximum*, endemic taxon to North America. In this study, we reviewed the taxonomy of *Heracleum moellendorffii* complex in Korea and neighboring countries on the basis of molecular phylogenies derived from sequences of nuclear ribosomal DNA internal transcribed spacer (ITS) regions. From three Korean accessions of *Heracleum* investigated in this study, two types of ITS sequences were obtained; two accessions were related to Chinese *H. moellendorffii* var. *moellendorffii* and North American *H. maximum* without forming a clade while the other one was identical to accession from *H. maximum* from Primorskiy, Russia. In the other hand, Japanese *H. moellendorffii* (= *H. sphondylium* ssp. *sphondylium* var. *nipponicum* in the flora of Japan) was closely related to *H. maximum* accessions from Korea and Russia, not nested within the clade comprising several subspecies of *H. sphondylium*. In order to delimit species boundaries among putatively closely related *Heracleum* species in far-eastern Asian countries, more samples and much more rapidly evolved DNA regions must be investigated with interpretation of morphological and anatomical features.

Keywords: Apiaceae, *Heracleum maximum*, *Heracleum moellendorffii*, Umbelliferae

INTRODUCTION

The genus *Heracleum* L. belongs to the family Apiaceae (=Umbelliferae), and is distinguished from other umbel genera by characteristics such as fruit vittae with short and clavate shapes, not extending to the base of fruit, markedly enlarged bifid outer petals, thickened lateral mericarp wings with rigid margins (She *et al.*, 2005). The genus *Heracleum* comprises about 65 to 120 species that often live in open forest areas (Pimenov and Leonov, 1993; Lee, 2007). Most species of the genus *Heracleum* are Eurasian with the Sino-Himalayan and Caucasus regions considered to be major centers of biological diversity (Pimenov and Leonov, 1993; Ma *et al.*, 2005; Yu *et al.*, 2011). Numbers of Korean *Heracleum* taxa were different depending upon taxonomist's opinions. Lee (1980) and Lee (2007) reported just single species, *H. moellendorffii* while Lee (1996) recognized two varieties of *H. moellendorffii* Hance; var. *moellendorffii* and var. *subbipinnatum* (Franchet) Kitagawa (= *H. moellendorffii* for. *angustum*). However, Im (1998) reported three taxa vege-

tated in Korea, two varieties of *H. moellendorffii* Hance (var. *moellendorffii*, var. *subbipinnatum* (Franchet) Kitagawa) and *H. dissectum* Ledeb. While the number of *Heracleum* taxa in Korea were counted by three (Im, 1998; She *et al.*, 2005), just one species, was described in other Korean flora (Lee, 1980; Lee, 2007) and recognized in both Korean plant names index (<http://www.nature.go.kr/kpni>) and National list of species of Korea (Vascular plants) (Lee *et al.*, 2011). *Heracleum moellendorffii* is distributed widely in Asia and has long been regarded as a taxonomically complex with three varieties commonly recognized: var. *moellendorffii*, var. *subbipinnatum* (Franchet) Kitagawa, var. *paucivittatum* R.H. Shan & T.S. Wang. *Heracleum moellendorffii* var. *moellendorffii* can be distinguished from var. *subbipinnatum* by the presence of broadly ovate leaves in outline, with ultimate segments broad to ovate (Pu and Watson, 2005). In the other hand, var. *subbipinnatum* is characterized by elliptic leaves in outline, with ultimate segments ovate to lanceolate. Although three varieties of *H. moellendorffii* have been suggested, their taxonomic status and positions have not been investigated in detail until the recent phylogenetic and

biogeography studies of Chinese *Heracleum* were conducted by Yu *et al.* (2011). *Heracleum dissectum* reported from Korea is distinguished from *H. moellendorffii* by the presence of basal and lower leaves with 1-2 pinnate dissection, triangular but minute calyx teeth, vallicular vittae extended to 2/3 the length of mericarps (Pu and Watson, 2005). In the far-eastern Asian countries, there have been taxonomic problems in describing the same or similar species of *Heracleum*. In both Korean and Chinese flora, *Heracleum moellendorffii* was adopted for their description while *H. sphondylium* or *H. lanatum* (= *H. maximum*) was chosen to describe Japanese *Heracleum moellendorffii* (Ohba, 1999; Takahashi, 2009). Taxa collected in Kamtchatka and Promorskiy, Russia were defined as *H. maximum*, endemic taxon to North America (data were obtained from the Genbank accessions). Through recent field surveys on Daeam-san in Korea, we found a taxon of *Heracleum* that was quite different morphologically in radical leaves from other Korean *Heracleum* taxa. This species that grows in the partly shady mountain

woodlands of the mountain has similar leaf characteristics with *H. maximum* in deeply-divided lobes of upper radical leaves. Here, all the possible accessions of *Heracleum moellendorffii* and its allied taxa have been investigated in order 1) to ascertain their taxonomic status and positions by comparing putatively related species in neighboring regions, 2) to identify a *Heracleum* taxon collected in Daeamsan, and 3) to record them in the national species list in Korea. To achieve these objectives, we used the internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA) which have been a particularly valuable marker for phylogenetic interpretation at interspecific level among Apiaceae taxa (Downie *et al.*, 1998; Lee, 1998; Lee and Downie, 1999; Zhou *et al.*, 2008; Lee *et al.*, 2010; Yu *et al.*, 2011).

MATERIALS AND METHODS

Nuclear ribosomal DNA internal transcribed spacer

Table 1. Thirty accessions of the genus *Heracleum* and outgroups examined for nuclear ribosomal DNA internal transcribed spacer sequences (ITS) variation. Ingroup taxa were all *Heracleum*, and newly investigated sequences start with letters of WIGIS (Wildlife Integrated Genetic Information System; National Institute of Biological Resources, Korea). Acronyms: Genbank Acc. No.=Genbank accession numbers. A taxon collected from Daeamsan was preliminary treated as *H. maximum* while *H. lanatum* ssp. *moellendorffii* collected in Japan (obtained from Genbank) was treated as *H. moellendorffii* var. *moellendorffii* due to a synonym treatment.

Taxon name	Source or voucher information	Genbank/WIGIS Acc. No.
<i>H. dissectifolium</i> K.T. Fu	China, Sichuan, Danba, Zhake Village	FJ812141
<i>H. dissectum</i> Ledeb.	China, Xinjiang, Aertai	GU967800
<i>H. fargesii</i> Boiss.	China, Sichuan, Nanchuan	FJ812114
<i>H. forrestii</i> Wolff	Unknown; Paik and Watson (2007)	EU185688
<i>H. franchetii</i> M. Hiroe	China, Sichuan, Xiaojin, Mengbi Shan	FJ812124
<i>H. hemsleyanum</i> Diels	China, Sichuan, Xiaojin, Balangsan	FJ812111
<i>H. maximum</i> W. Bartran	USA, Colorado	EU594924
<i>H. maximum</i> W. Bartran	Russia, Kamtchatka	EU594922
<i>H. maximum</i> W. Bartran	Russia, Primorskiy Kray	EU594923
<i>H. maximum</i> W. Bartran	Korea, Daeamsan	WIGIS004982
<i>H. moellendorffii</i> Hance		
var. <i>moellendorffii</i>	Korea, Gyeonggi-do, Cheonggye-san	WIGIS004981
var. <i>moellendorffii</i>	Korea, Gangwon-do, Taebaek-si	WIGIS004980
var. <i>moellendorffii</i>	China, Sichuan, Xiaojin, Balangsan	FJ812137
var. <i>moellendorffii</i>	China, Shaanxi, Meishan, Taiba Shan	FJ812127
var. <i>moellendorffii</i>	Japan, Honshu	EU594925
var. <i>subbipinnatum</i> (Franch.) Kitagawa	Unknown; Yu <i>et al.</i> (2009)	FJ812134
var. <i>subbipinnatum</i> (Franch.) Kitagawa	China, Gansu, Lianlu, Lianhua Shan	FJ812131
<i>H. souliei</i> Boiss.	China, Sichuan, Kangding-Hailuo gou	FJ812112
<i>H. sphondylium</i> L.		
ssp. <i>montanum</i> (Schleich. ex Gaudin.) Briq.	France, Luchon	EU594907
ssp. <i>sibiricum</i> (L.) Simonkai	Poland, Zyzniewo	EU594910
ssp. <i>sphondylium</i>	Poland, Wigry NP.	EU594906
ssp. <i>sphondylium</i>	Poland, Wigry NP.	EU594905
ssp. <i>sphondylium</i>	France	EU169286
ssp. <i>transsilvanicum</i> (Schur) Brummit	Romania, Fagars	EU594914
<i>H. wenchuanense</i> F.T. Pu et X.J. He	China, Sichuan, Wenchuan, Balang Shan	FJ812122
<i>H. wolongense</i> F.T. Pu et X.J. He	China, Sichuan, Wenchuan, Balang Shan	FJ812142
<i>A. dahurica</i> (Fisch. ex Hoffm.) Benth. & Hook.	Korea, Gywonggi-do, Gwangdeog-san	JN603213
<i>A. decursiva</i> Benth. & Hook.	Korea, Chilbosan	JN603215
<i>A. gigas</i> Nakai	Korea, Daegwanryeong	JN603232
<i>A. polymorpha</i> Nakai	Korea, Chiag-san	JN603221

(ITS) sequences of *Heracleum* taxa were obtained in earlier phylogenetic and biogeographic analyses of the Chinese *Heracleum* (Yu *et al.*, 2011). In order to confirm taxonomic positions of three Korean *Heracleum* taxa, we have incorporated ITS sequences of above taxa as well as several accessions of *H. sphondylium* found in Japan. And also, several accessions of *Heracleum* from Genbank have been incorporated into the data matrix (Table 1). A total of 30 accessions were used to reconstruct molecular phylogenies including four taxa of *Angelica* as outgroups. Total genomic DNA was extracted from fresh leaves and

herbarium preserved tissues using the modified CTAB procedure of Doyle and Doyle (1987). Double stranded DNAs of the complete ITS regions in each genomic DNA were PCR (polymerase chain reaction)-amplified using primers “ITS 5” and “ITS 4” (White *et al.*, 1990). Details of the amplification reactions, purification, and alignment were the same as described in Lee (1998). Pairwise nucleotide differences of unambiguously aligned positions were determined from the distance matrix option in Phylogenetic Analysis Using Parsimony (PAUP*, Swofford, 2000). Phylogenetic analyses of the ITS sequences data

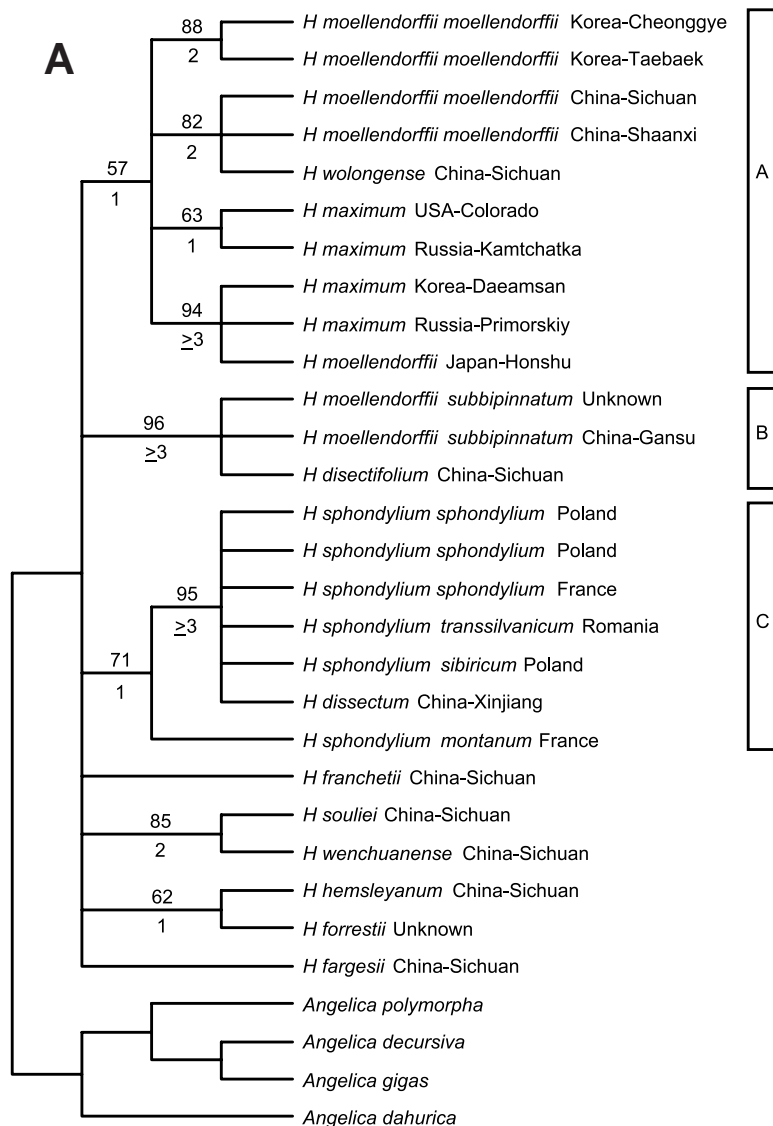


Fig. 1. Cladograms inferred from the analysis of 29 nuclear ribosomal DNA ITS1 and ITS2 sequences from the genus *Heracleum* and an outgroup. (A) The strict consensus of two minimal length 138-step trees derived from equally weighted maximum parsimony analysis of combined nuclear rDNA ITS and 5.8S sequences (CI's with and without uninformative characters=0.91 and 0.89, respectively; RI=0.95). Numbers above nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates; decay values are presented below. (B) The Maximum likelihood tree using a transition/transversion rate ratio of 1.5. Branch lengths are proportional to the number of expected nucleotide substitutions per site. Boxes A, B, and C indicate clades *H. maximum-moellendorffii*, *H. subbipinnatum*, and *H. sphondylium*, respectively.

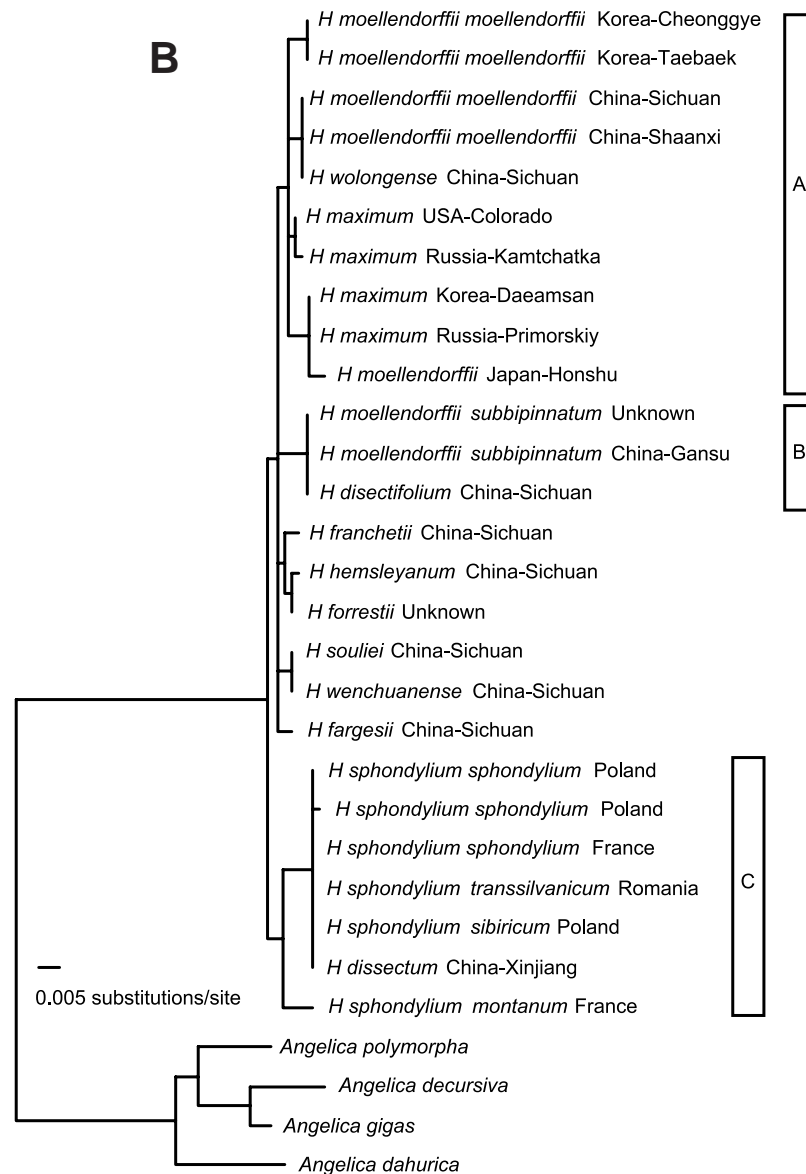


Fig. 1. Continued.

sets were carried out using the heuristic search strategies of PAUP*. All searches were conducted with 100 random-addition replicates using tree bisection-reconnection (TBR) branch swapping. In order to identify weakly supported nodes, decay analyses (Bremer, 1988) were conducted until tree storage memory was exhausted. A bootstrap analysis was done using 100 resampled data sets. In addition to parsimony analysis, maximum likelihood phylogeny estimation using the ITS sequences was explored utilizing PAUP*. A minimum likelihood tree was inferred using a transition/transversion rate ratio of 1.5, General Search Option. Empirical base frequencies were derived from the sequence data and used in the maximum likelihood calculations.

RESULTS

1. ITS sequence analysis

Alignment of all 30 complete ITS 1, 5.8S ribosome, and ITS 2 sequences, representing all possible accessions of the genus *Heracleum* and outgroups resulted in a matrix of 604 characters. Overall length of both ITS spacer regions across all 26 ingroup accessions is 602 bp with the exception of 603 bp in *H. franchetii*. These sizes are comparable to those values reported for other Apiaceae (Downie *et al.*, 1998; Lee and Downie, 1999; Lee *et al.*, 2010). Of the 608 unambiguously aligned positions, four bp were deleted from the data matrix due to alignment

Table 2. Pairwise sequence divergence of ITS 1, ITS 2, and 5.8S ribosomal DNA regions among representatives of *Heraclium*. Mean distances (100 × values) are calculated above the diagonal, and actual numbers of unambiguous divergence sites from pairwise sequence comparisons appear below the diagonal. The orders of taxa listed on the table were followed from those of the Maximum likelihood tree shown in the Fig. 1.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		
1 <i>H moell</i> Cheonggye	–	0.00	0.67	0.67	0.67	0.67	0.50	0.67	0.83	0.83	1.17	1.17	1.17	1.17	1.00	1.00	0.83	0.83	0.83	0.83	1.17	1.83	1.67	1.67	1.67	1.67	1.83	
2 <i>H moell</i> Taebaek	0	–	0.67	0.67	0.67	0.50	0.67	0.83	0.83	1.17	1.17	1.17	1.17	1.00	1.00	0.83	0.83	0.83	0.83	0.83	1.17	1.83	1.67	1.67	1.67	1.67	1.83	
3 <i>H moell moell</i> Sichuan	4	4	–	0.00	0.00	0.50	0.67	0.83	0.83	1.17	1.17	1.17	1.17	1.00	1.00	0.83	0.83	0.83	0.83	0.83	1.83	2.00	1.83	1.83	1.83	1.83	1.83	
4 <i>H moell moell</i> Shaanxi	4	4	0	–	0.00	0.50	0.67	0.83	0.83	1.17	1.17	1.17	1.17	1.00	1.00	0.83	0.83	0.83	0.83	0.83	1.83	2.00	1.83	1.83	1.83	1.83	1.83	
5 <i>H wolongense</i>	4	4	0	0	–	0.67	0.83	0.83	0.83	1.17	1.17	1.17	1.17	1.00	1.00	0.83	0.83	0.83	0.83	0.83	1.83	2.00	1.83	1.83	1.83	1.83	1.83	
6 <i>H maxim</i> Colorado	3	3	3	3	3	–	0.17	0.67	0.67	1.00	1.00	1.00	1.00	0.83	0.83	0.67	0.67	0.67	0.67	0.67	1.67	0.83	1.67	1.67	1.67	1.67	1.67	
7 <i>H maxim</i> Kamtchatka	4	4	4	4	4	1	–	0.83	0.83	1.17	1.17	1.17	1.17	1.00	1.00	0.83	0.83	0.83	0.83	0.83	1.83	2.00	1.83	1.83	1.83	1.83	1.83	
8 <i>H maxim</i> Daeamsan	5	5	5	5	5	4	5	–	0.00	0.33	1.33	1.33	1.33	1.17	1.17	1.00	1.00	1.00	1.00	2.00	2.17	2.00	2.00	2.00	2.00	2.00	1.67	
9 <i>H maxim</i> Primorskiy	5	5	5	5	5	4	5	0	–	0.33	1.33	1.33	1.33	1.17	1.17	1.00	1.00	1.00	1.00	2.00	2.17	2.00	2.00	2.00	2.00	2.00	1.67	
10 <i>H moell</i> Honshu	7	7	7	7	7	6	7	2	2	–	1.67	1.67	1.67	1.50	1.50	1.33	1.33	1.33	1.33	2.17	2.33	2.17	2.17	2.17	2.17	2.17	2.00	
11 <i>H moell sub</i> Unknown	7	7	7	7	7	6	7	8	8	10	–	0.00	0.00	1.17	1.17	1.00	1.00	1.00	1.00	2.00	2.17	2.00	2.00	2.00	2.00	2.00	2.00	
12 <i>H moell sub</i> Gansu	7	7	7	7	7	6	7	8	8	10	0	–	0.00	1.17	1.17	1.00	1.00	1.00	1.00	2.00	2.17	2.00	2.00	2.00	2.00	2.00	2.00	
13 <i>H dissectifolium</i>	7	7	7	7	7	6	7	8	8	10	0	0	–	1.17	1.17	1.00	1.00	1.00	1.00	2.00	2.17	2.00	2.00	2.00	2.00	2.00	2.00	
14 <i>H franchetii</i>	6	6	6	6	6	5	6	7	7	9	7	7	7	–	0.67	0.50	0.83	0.83	0.50	1.83	2.00	1.83	1.83	1.83	1.83	1.83	1.83	
15 <i>H hemsleyanum</i>	6	6	6	6	6	5	6	7	7	9	7	7	7	4	–	0.17	0.83	0.83	0.83	1.50	1.67	1.50	1.50	1.50	1.50	1.50	1.50	
16 <i>H forrestii</i>	5	5	5	5	5	4	5	6	6	8	6	6	6	3	1	–	0.67	0.67	0.67	1.67	1.83	1.67	1.67	1.67	1.67	1.67	1.67	
17 <i>H souliei</i>	5	5	5	5	5	4	5	6	6	8	6	6	6	5	4	4	–	0.00	0.67	1.67	1.83	1.67	1.67	1.67	1.67	1.67	1.67	
18 <i>H wenchuanense</i>	5	5	5	5	5	4	5	6	6	8	6	6	6	5	4	4	0	–	0.67	1.67	1.83	1.67	1.67	1.67	1.67	1.67	1.67	
19 <i>H fargesii</i>	5	5	5	5	5	4	5	6	6	8	6	6	6	5	4	4	4	4	–	1.67	1.83	1.67	1.67	1.67	1.67	1.67	1.67	
20 <i>H sph sph</i> Poland	10	10	11	11	11	10	11	12	12	13	12	12	12	11	9	10	10	10	10	10	–	0.17	0.00	0.00	0.00	0.00	0.00	1.33
21 <i>H sph sph</i> Poland	11	11	12	12	12	11	12	13	13	14	13	13	13	12	10	11	11	11	11	11	1	–	0.17	0.17	0.17	0.17	0.17	1.50
22 <i>H sph sph</i> France	10	10	11	11	11	10	11	12	12	13	12	12	12	11	9	10	10	10	10	10	0	1	–	0.00	0.00	0.00	0.00	1.33
23 <i>H sph trans</i> Romania	10	10	11	11	11	10	11	12	12	13	12	12	12	11	9	10	10	10	10	10	0	1	0	–	0.00	0.00	0.00	1.33
24 <i>H sph sib</i> Poland	10	10	11	11	11	10	11	12	12	13	12	12	12	11	9	10	10	10	10	10	0	1	0	0	–	0.00	0.00	1.33
25 <i>H dissectum</i> China	10	10	11	11	11	10	11	12	12	13	12	12	12	11	9	10	10	10	10	10	0	1	0	0	0	–	0.00	1.33
26 <i>H sph montana</i> France	11	11	11	11	11	10	11	10	10	12	12	12	12	11	9	10	10	10	10	10	8	9	8	8	8	8	8	–

problems. Of the remaining 604 positions, 85 (14.1%) were potentially parsimony informative, 490 (81.1%) were constant, and 29 (4.8%) were autapomorphic. Values of direct pairwise ITS sequence divergence of the examined taxa of the genus *Heracleum* ranged from identity (between two accessions of *H. moellendorffii* var. *moellendorffii* from Korea, between two accessions of *H. maximum* from Korea and Russia, between two accessions of *H. moellendorffii* var. *subbipinnatum*, and among four accessions of subspecies of *H. sphondylium*, ssp. *transsilvanicum*, ssp. *sibiricum*, ssp. *sphondylium* to 2.33% between *H. sphondylium* ssp. *sphondylium* (EU594905) and *H. moellendorffii* from Japan (Table 2).

2. Phylogenetic analyses and resolution

The parsimony analysis of 30 combined ITS and 5.8S ribosomal sequences using equally weighted character states resulted in six maximally parsimonious trees, and the consensus of these trees with accompanying bootstrap and decay values, and is presented in Fig. 1A. In the consensus of these trees, three major groups of taxa can be discernable; but these groups are unresolved with other clades or species, *H. fargesii* and *H. franchetii*. The first group, “A clade” (supported with 57% of bootstrap values), includes five accessions of *H. moellendorffii*, four accessions of *H. maximum* from Korea, Russia, U.S.A., and *H. wolongense*. The second group, “B clade” (supported with 96% of bootstrap values and decay values of more than or equal to three), includes two accessions of *H. moellendorffii* var. *subbipinnatum*, and *H. dissectifolium*. The third group, “C clade” (supported with 71% of bootstrap values), comprises several subspecies of *H. sphondylium* and *H. dissectum*. The Maximum likelihood tree with Ts/Tv rate ratios of 1.5 was topologically congruent to the parsimony trees (Fig. 1B). The clades recognized in the parsimony analyses were shown in the maximum likelihood tree: clades A, B, other accessions, and clade C.

DISCUSSION

The genus of *Heracleum* has a wide distribution in Europe and Asia, and has long been problematic in generic delimitation with other Umbelliferous genera (e.g., *Pastinaca*, *Peucedanum*) with strongly dorsally depressed fruits (Pu and Watson, 2005). The features of vittae such as oblanceolated (=clavated) shapes, usually shorter than the length of the mericarp, are the characteristics to separate the genus from other genera (Ohba, 1999; She *et al.*, 2005; Lee, 2007; Yu *et al.*, 2011). As Pu and Watson (2005) suggested, however, these unique characters do not support inclusion of all the Chinese *Heracleum* into the generic boundaries. Their suggestions were consis-

tent with results of molecular phylogenetic studies which have shown the genus to be non-monophyletic (Downie *et al.*, 1998; Zhou *et al.*, 2008). Moreover, infraspecific levels of several species of the genus (i.e., *H. moellendorffii* and *H. sphondylium*) were recognized and the species can be consequently divided into several subspecies or varieties (Ohba, 1999; Pu and Watson, 2005). The major objective in carrying out this study was to ascertain the taxonomic states and positions of all the possible accessions of *Heracleum moellendorffii* and its allied taxa comparing putatively related species in neighboring regions. The scientific name, *Heracleum moellendorffii* was adopted to describe both Korean and Chinese taxa (Lee, 1980; She *et al.*, 2005; Lee, 2007) while *H. sphondylium* or *H. lanatum* (= *H. maximum*) was chosen to describe Japanese *Heracleum moellendorffii* (Ohba, 1999; Takahashi, 2009). Furthermore, collections obtained from Kamtchatka and Promorskiy, Russia were defined as *H. lanatum* (= *H. maximum*), endemic taxon to North America (data were obtained from the Genbank accessions). Before more discussions, we first comprised all the above taxa and defined as *Heracleum moellendorffii* complex. *Heracleum moellendorffii* was divided into three different varieties on the basis of shapes in pinnation patterns and segment forms of basal leaves, and the presence or absence of dorsal vittae (Pu and Watson, 2005). However, the seven accessions of *H. moellendorffii* investigated in here failed to construct a monophyletic clade while *H. dissectifolium*, *H. maximum*, and *H. wolongense* were included within the clade “A” which was the same phylogeny suggested by Yu *et al.* (2011). Moreover, two accessions of *H. moellendorffii* var. *subbipinnatum* were not nested within the clade. Two accessions of Korean *H. moellendorffii* were identical in ITS and 5.8S ribosomal DNA sequences, but did not form a clade together with Chinese *H. moellendorffii*. No inclusion of Korean *H. moellendorffii* into Chinese ones was reflected by the ITS sequence differences between these two; four nucleotide differences and 0.67 percent of sequence divergences. The second objective of the study was to identify a *Heracleum* taxon collected in Daeamsan, Korea. Although accessions of Korean *H. moellendorffii* var. *moellendorffii* had identical ITS sequences, another accession collected from Daeamsan, interestingly, was identical to Russian (Primorskiy) *H. maximum* (= *H. lanatum*), endemic to North America, in its ITS sequence. Therefore, the Daeamsan accession might be conspecific to *H. maximum* in terms of the current molecular phylogenies. *H. maximum* is commonly called as ‘Cow Parsnip’ due to its big sizes in leaf, height, and strong scented features (Constance, 1993). However, some Eurasian taxa of the genus show unclear phylogenetic relationships, and their taxonomic status as a species are unstable as well. In the flora of Japan, *H. lanatum* (= *H. maximum*) was described as a synonym of *H. sphon-*

dylium ssp. *montanum* following Mabberley (1987)'s treatment (Ohba, 1994; 1999). However, *H. maximum* and *H. sphondylium* ssp. *montanum* failed to construct a monophyletic clade in this study reflecting that each of these two taxa might be an independent species. Considering 1.67-1.83% divergence in their ITS and 5.8S ribosomal DNA sequences between the two clades of *H. maximum* and their phylogenetic placements, they need to be treated as distinct species from *H. sphondylium*, not a infraspecific taxon of it. Although more samples are needed to clarify Japanese *Heracleum moellendorffii* complex, an Genbank accession from Japan might be treated as conspecific to or infraspecific taxon of *H. maximum*, which is suggested by small sequence divergence (two nucleotides) with Korean and Russian accessions of *H. maximum* and strong bootstrap value (94%) and decay values (≥ 3). The second clade "B" comprising two accessions of *H. moellendorffii* var. *subbipinnatum* and *H. dissectifolium* was reflected by the identity of their ITS sequences and the shared morphological characters such as two-pinnated basal leaves, absence of bracts, lanceolate ultimate segments, solitary vittae below each furrow, clavated vittae extending to 1/2 length of mericarp, as were described in the flora of China (Pu and Watson, 2005). From the current molecular phylogenetic study, *H. moellendorffii* var. *subbipinnatum* could be treated as an independent species separated from *H. moellendorffii*. The third clade "C" comprises several subspecies of *H. sphondylium* and *H. dissectum*. Taxonomy of *H. moellendorffii* has been often complicated with *H. sphondylium* which was reported only to be found in Japan within East Asian regions (Ohba, 1994; 1999). For example, *H. barbatum* ssp. *moellendorffii*, *H. lanatum* ssp. *moellendorffii*, *H. moellendorffii* var. *akasimontanum*, *H. moellendorffii* var. *tsurugisanense* were all described as subspecies of *H. sphondylium* (Ohba, 1999). While morphological differences between *H. moellendorffii* and *H. sphondylium* were not clear, the current ITS sequences-derived phylogeny supports the separation between these two taxa reflected by sequence divergences ranging from 1.67 to 2.33 percent. Considering that each of *H. fargesii*, *H. forrestii*, *H. hemsleyanaum* and *H. wenchuanense* was kept to remain as a species (Pu and Watson, 2005) with even below the 1.0% in the ITS sequence divergence value among themselves, we can support separation of the ssp. *montanum* from groups of *H. sphondylium* by the sequence divergence values (1.33-1.50%). Therefore, we have suggested that *H. sphondylium* ssp. *montanum* be treated taxonomically as an independent taxon, *H. montanum* Schleich. ex Gaudin. When focused on Korean accessions, we figured out that accessions of *H. moellendorffii* collected from Cheonggyesan and Taebaek were not different from Chinese accessions of *H. moellendorffii* var. *moellendorffii*. Secondly, the *Heracleum* sp.

collected from Daeamsan, Korea was identified to *H. maximum*. However, the monophyly of accessions of *H. maximum* was not supported from the current molecular phylogenies, with pairwise sequence divergences ranged from identity to 0.83 percent. In terms of non-monophyly and sequence divergences, intraspecific taxonomy of *H. maximum* might be understood when their genetic diversity of the east Asian countries were investigated along with geographical distributions. In conclusion, quite differences in the ITS sequences of the Korean *Heracleum* from those of neighboring regions such as China and Japan require much more works and samplings to be done to delimit their species boundaries.

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