

Taxonomic Review of the Umbelliferous genus *Sium* L. in Korea: Inferences based on Molecular Data

Byoung-Yoon Lee, Jeongran Lee¹ and Sung-Chul Ko^{2*}

Division of Vascular Plants, National Institute of Biological Resources, Incheon 404-170, Korea

¹National Academy of Agricultural Science, Suwon 441-100, Korea

²Department of Biological Sciences, Hannam University, Daejeon 305-811, Korea

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분자생물학적 자료에 의한 한국산 개발나물속의 분류학적 고찰

이병윤 · 이정란¹ · 고성철^{2*}

국립생물자원관 고등식물연구과, ¹국립농업과학원 농업유전자원센터, ²한남대학교 생명과학과

ABSTRACT: The taxonomy of umbel genus *Sium* L., Apiaceae in Korea was reviewed on the basis of molecular phylogenies derived from sequences of nuclear ribosomal DNA internal transcribed spacer (ITS) regions. The ITS sequence-derived phylogeny indicates that *S. heterophyllum*, endemic to Korea, is identical to *S. tenue*, which is known as endemic to Ussuri regions. Comparisons of sequence pairs across both spacer regions gave divergence values and revealed the identity between *S. tenue* and *S. heterophyllum* on Mt. Moonsoo. On the other hand, the ITS sequences support species delimitation of *S. ternifolium*, as reported recently as a new species that differs from other *Sium* species. The ITS sequence divergence values of 1.4 and 1.6% support species delimitation between *S. serra* and *S. ternifolium*.

Keywords: Apiaceae, Umbelliferae, *Sium ternifolium*, *Sium heterophyllum*

적 요: 핵 리보솜 DNA ITS 구간의 염기서열 정보를 이용하여 한국산 산형과 개발나물속의 분자 계통학적 연구를 수행하였다. ITS 계통수는 한반도 고유종인 대암개발나물(*S. heterophyllum*)이 한반도에 생육이 보고된 바 없는 러시아 우수리지역의 고유종인 *S. tenue*와 동일한 종임을 밝혔다. ITS 1과 ITS 2 구간의 염기서열 비교 시 *S. tenue*와 인천 문수산에 생육하고 있는 대암개발나물이 100% 일치함을 확인할 수 있었다. 또한 ITS 구간의 염기서열은 최근 신종으로 발표된 세잎개발나물(*S. ternifolium*)이 개발나물속의 다른 분류군과 뚜렷하게 구별됨을 지지하고 있었다. 세잎개발나물은 유연관계가 가장 가까운 일본 고유종 *S. serra*의 ITS 염기서열 비교시 1.4와 1.6%의 차이점을 가지고 있음이 확인되어 종으로서의 처리가 적합함을 확인할 수 있었다.

주요어: 산형과, 세잎개발나물, 대암개발나물

The genus *Sium* L. belonging to the tribe Oenanthe Dumort. of the family Apiaceae comprises about 14 species that often live in moist to wet areas (Hardway et al., 2004; Pimenov and Leonov, 1993). Most species of the genus *Sium* are Eurasian, and eastern Asia is the major center of endemism of five species including *S. ninsi*, *S. tenue*, *S. serra*, and *S. suave* (Spalik and Downie, 2006). Among sepceis belonging to the genus *Sium*, four species have been reported in Korea; *S. suave* Walter, *S. ninsi* L.,

S. heterophyllum Y. Lee, and *S. ternifolium* B.Y.Lee & S.C.Ko. (Lee, 1980; Lee, 1993; Lee, 2007; Lee and Ko, 2009).

S. suave is distinguished from *S. ninsi* by the presence of stout rays, seven to eleven leaflets, and broader leaflets (Ohwi, 1965). In addition to these two indigenous species, a couple of *Sium* species endemic to Korea were reported. *Sium heterophyllum* found first in Mt. Daeamsan and Daekwanryoung pass Gangwon-do is allied to *S. ninsi*, but was characterized from the latter by the presence of heterophyllous leaves and by the absence of bulbils on the

*Author for correspondence: kscaa@hannam.ac.kr

axils (Lee, 1993). Another endemic species, *S. ternifolium* was characterized by the presence of only tri-foliated leaflets and the absence of involucre bracts (Lee and Ko, 2009). Although *S. heterophyllum* and *S. ternifolium* were erected as new species, their taxonomic states and ranks within the genus have not been investigated in detail until the recent evolutionary study of *Sium sensu lato* was conducted by Spalik and Downie (2006). The molecular study of *Sium sensu lato* concluded that *S. heterophyllum* was a cultivated form of *S. sisarum* by the common features of tuberous roots (Spalik and Downie, 2006). However, it is not reasonable to make a taxonomic treatment of the Korean endemic species by the experimental results without Korean materials included in the analysis. Taxonomic problems of *S. ninsi* have occurred all the time by recognizing the species as *S. sisarum* by several authors (Hiroe and Constance, 1958; Ohwi, 1965; Lee, 1980; Lee, 1993; Ohba, 1999; Lee, 2006). Furthermore, Lee (2006) treated *S. ninsi* and *S. heterophyllum* as synonyms of *S. sisarum*. The taxonomic problems of Korean species of *Sium* are uncertain with two endemic species of dubious affinity, and their states and phylogenetic relationships are not well recognized. Here, all the possible accessions of Korean taxa of *Sium* and its putative relatives have been investigated in

order to ascertain the taxonomic status and position of two Korean endemic species.

Material and Methods

Nuclear ribosomal DNA internal transcribed spacer (ITS) sequences of *Sium* taxa were obtained in earlier phylogenetic analyses of the *Sium sensu lato* (Spalik and Downie, 2006). Several additional taxa (e.g., *S. heterophyllum*, *S. ninsi*, *S. serra*, and *S. ternifolium*) have been incorporated into the previous data matrix (Table 1). A total of 18 accessions were used for molecular phylogenetic analyses including *Cicuta virosa* as an outgroup. Total genomic DNA was extracted from fresh leaves and herbarium preserved tissue 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

Table 1. 18 accessions of the genus *Sium* and an outgroup examined for nuclear ribosomal DNA internal transcribed spacer sequences (ITS) variation. Accession numbers of newly investigated sequences start with the letter H. Herbarium Acronyms: HNHM = Hannam Natural History Museum, Daejeon; KB = National Institute of Biological Resources, Incheon.

Taxon Name	Source or voucher information	Genbank Ass. No.
<i>Sium ternifolium</i> B.Y.Lee & S.C.Ko	Korea, Chiak-san, <i>S.C.Ko 064185</i> (HNHM)	HQ639018
<i>Sium serra</i> (Franch. & Sav.) Kitag.-1	Japan, Honshu, Spalik and Downie (2006)	DQ005681
<i>Sium serra</i> (Franch. & Sav.) Kitag.-2	Japan, Cultivated, <i>Arai 09082501</i>	HQ639019
<i>Sium sisarum</i> L.-1	Canada, Cultivated, Spalik and Downie (2006)	AY360260
<i>Sium sisarum</i> L.-2	Hungary, Cultivated, Spalik and Downie (2006)	AY360261
<i>Sium sisaroides</i> DC	Turkey, Maras, Spalik and Downie (2006)	DQ005682
<i>Sium ninsi</i> L.-1	Korea, Daeam-san, <i>BYLee 268</i> (KB)	HQ639021
<i>Sium ninsi</i> L.-2	Japan, Honshu, Spalik and Downie (2006)	DQ005679
<i>Sium tenue</i> Kom.	Russia, Siberia, Spalik and Downie (2006)	DQ005704
<i>Sium heterophyllum</i> Y. Lee-1	Korea, Moonsoo-san, <i>BYLee 181</i> (KB)	HQ639022
<i>Sium heterophyllum</i> Y. Lee-2	Korea, Baegwoon-san, <i>BYLee 160</i> (KB)	HQ639023
<i>Sium heterophyllum</i> Y. Lee-3	Korea, Daeam-san, <i>BYLee 220</i> (KB)	HQ639024
<i>Sium frigidum</i> Hand.-Mazz.-1	China, Yunnan, Spalik and Downie (2006)	AF164742
<i>Sium frigidum</i> Hand.-Mazz.-2	China, Yunnan, Spalik and Downie (2006)	DQ005665
<i>Sium suave</i> Walter	Korea, Daeam-san, <i>BYLee 215</i> (KB)	HQ639020
<i>Sium medium</i> Fisch. & C. A. Mey.	India, W Ladakh, Spalik and Downie (2006)	DQ005676
<i>Sium latifolium</i> L.	Denmark, Spalik and Downie (2006)	AY360258
<i>Cicuta virosa</i> L.	Finland, Spalik and Downie (2006)	U78372

analyses of the ITS sequences data 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. All trees were rooted with *Cicuta virosa*, the most closely related genus to the *Sium sensu lato*.

Results

1) ITS sequence analysis

Alignment of all 18 complete ITS 1 and ITS 2 sequences, representing all possible accessions of the genus *Sium* and the outgroup resulted in a matrix of 443 characters. On average, the ITS 1 region was shorter than ITS 2. The length of ITS 1 region in the taxa surveyed ranged from 209 bp in *S. suave*, *S. medium*, and *S. latifolium* to 212 bp in all taxa belonging to *S. ninsi*, *S. tenue*, and *S. heterophyllum* (mean 211 bp). The length of ITS 2 region of the taxa surveyed ranged from 226 bp in *S. suave*, *S. ninsi*, *S. tenue*, *S. heterophyllum*, *S. medium*, and *S. latifolium* to 229 bp in *S. sisarum* and *S. sisaroides* (mean 227 bp). Overall length variation of both spacer regions across

all 18 accessions ranged from 435 bp to 439 bp. These sizes are comparable to those values reported for other Apiaceae (Downie et al., 1998; Lee and Downie, 1999). Of the 443 initial alignment positions, four positions were deleted due to alignment ambiguities. Of the remaining 439 unambiguously aligned positions, 71 (16.2%) were potentially parsimony informative, 336 (76.5%) were constant, and 32 (7.3%) were autapomorphic. Both spacers contributed comparable numbers of informative nucleotide substitutions to the phylogenetic analysis. Values of direct pairwise ITS sequence divergence of the examined 17 accessions of the genus *Sium* (using PAUP's DISTANCE MATRIX option) are presented in Table 2. The sequence divergence values ranged from identity (between *S. tenue* and *S. heterophyllum* at mt. Moonsoo) to 8.3% (between *S. ternifolium* and *S. ninsi*, *S. heterophyllum*). The pairwise nucleotide divergence among *Sium* species and the outgroup varied from 8.8% with *S. medium* to 10.2% with *S. ninsi* or *S. heterophyllum*.

2) Phylogenetic analyses and resolution

The results of the analyses of combined data sets are presented; separate analyses of each spacer region alone was not done. The parsimony analysis of 18 combined ITS sequences using equally weighted character states resulted in two maximally parsimonious trees, and the consensus of these two trees with

Table 2. Pairwise sequence divergence of ITS 1 and ITS regions among representatives of *Sium*. Mean distances (100 × values) are calculated above the diagonal, and actual numbers of unambiguous divergence sites from pairwise sequence comparisons appear below the diagonal. *S. hetero.* = *S. heterophyllum*.

Taxa of <i>Sium</i>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 <i>S. ternifolium</i>	-	1.61	1.38	8.08	7.60	7.37	7.85	8.31	7.85	7.85	8.08	8.31	7.43	8.12	7.14	7.85	8.31
2 <i>S. serra-1</i>	7	-	0.23	7.85	7.14	6.91	7.62	8.08	7.62	7.62	7.85	8.08	7.19	7.89	7.14	7.62	8.08
3 <i>S. serra-2</i>	6	1	-	7.62	6.91	6.68	7.39	7.85	7.39	7.39	7.62	7.85	6.96	7.66	6.91	7.39	7.85
4 <i>S. suave</i>	35	34	33	-	7.16	6.93	7.41	7.87	7.41	7.41	7.64	7.87	6.74	7.44	7.16	1.39	1.85
5 <i>S. sisarum-1</i>	33	31	30	31	-	0.23	6.47	6.93	6.47	6.47	6.70	6.93	6.27	6.96	0.46	6.70	7.16
6 <i>S. sisarum-2</i>	32	30	29	30	1	-	6.24	6.70	6.24	6.24	6.47	6.70	6.27	6.96	0.23	6.47	6.93
7 <i>S. ninsi-1</i>	34	33	32	32	28	27	-	0.46	0.46	0.46	0.69	0.92	6.05	6.74	6.47	6.94	6.94
8 <i>S. ninsi-2</i>	36	35	34	34	30	29	2	-	0.92	0.92	0.92	0.92	6.51	7.21	6.93	7.41	7.41
9 <i>S. tenue</i>	34	33	32	32	28	27	2	4	-	0.00	0.23	0.46	6.05	6.74	6.47	6.94	6.94
10 <i>S. hetero.-1</i>	34	33	32	32	28	27	2	4	0	-	0.23	0.46	6.05	6.74	6.47	6.94	6.94
11 <i>S. hetero.-2</i>	35	34	33	33	29	28	3	4	1	1	-	0.46	6.28	6.98	6.70	7.18	7.18
12 <i>S. hetero.-3</i>	36	35	34	34	30	29	4	4	2	2	2	-	6.51	7.21	6.93	7.41	7.41
13 <i>S. frigidum-1</i>	32	31	30	29	27	27	26	28	26	26	27	28	-	0.69	6.50	6.51	6.98
14 <i>S. frigidum-2</i>	35	34	33	32	30	30	29	31	29	29	30	31	3	-	7.19	7.21	7.67
15 <i>S. sisaroides</i>	31	31	30	31	2	1	28	30	28	28	29	30	28	31	-	6.70	7.16
16 <i>S. medium</i>	34	33	32	6	29	28	30	32	30	30	31	32	28	31	29	-	0.92
17 <i>S. latifolium</i>	36	35	34	8	31	30	30	32	30	30	31	32	30	33	31	4	-

accompanying bootstrap and decay values, and is presented in Fig. 1. The parsimonious tree has a length of 123 steps, consistence index (CI) of 0.935 and 0.912, with and without uninformative characters, respectively, and a retention index (RI) of 0.957. In each of these trees, five major groups of taxa can be discernable; each one with a bootstrap value of 100 and a decay value of more than 6. The first group includes *S. ternifolium* endemic to Korea and *S. serra* endemic to Japan. The second group includes *S. sisarum* and *S. sisaroidesum*, and the third one comprises *S. ninsi*, *S. tenue*, and *S. heterophyllum*. The fourth one is a monotypic clade comprising only accessions of *S. frigidum*. The last one including *S. suave*, *S. medium*, and *S. latifolium* is sister to all other taxa of *Sium* examined in this study.

Discussion

The major objective in carrying out this study was to ascertain the taxonomic states and positions of *S. heterophyllum* and *S. ternifolium*, endemic to Korea. *S. heterophyllum* was recognized by the presence of heterophyllous leaves, that is, heart-shaped leaves at the lower stem and narrowly divided leaf segments at the upper stem (Lee, 1993). On the basis of the ITS-derived phylogeny, *S. heterophyllum* was closely related to *S. tenue*. However, the

phylogeny did not support monophyly of *S. heterophyllum*, revealing no synapomorphic characters. Comparisons of sequence pairs across both spacer regions gave divergence values and revealed identity between *S. tenue* and *S. heterophyllum* at Mt. Moonsoo. Although some variation of sequence divergence between *S. ninsi* and the other two accessions of *S. heterophyllum* were shown from 0.23 to 0.46%, the sequence differences between these two species are quite below those of other taxa belonging to Apiaceae. Among different species of the same genus of Apiaceae, pairwise nucleotide divergence varied in 0.3-0.5% of *Lisaea*, 0.8-1.0% of *Agrocharis*, 2.3-5.9% of *Orlaya*, and 0.8-6.9% of *Torilis* (Lee and Downie, 1999; Lee, 2002). Therefore, *S. heterophyllum* described as a new species by Lee (1993) might be identical to *S. tenue* that was described as an endemic of Ussuri regions (Schischikin, 1950). The taxonomic opinion that *S. heterophyllum* was a cultivated form of *S. sisarum* by the common features of tuberous roots (Spalik and Downie, 2006) might be reconsidered. *S. tenue* was very closely related to *S. ninsi* based on ITS ribosomal DNA phylogenies (Spalik and Downie, 2006). While basal leaves of *S. tenue* are often entire, those of *S. ninsi* are pinnately dissected. Both species have ternate leaves at the upper stem that are shared with *S. serra* and *S. ternifolium*. Both *S. ninsi* and *S. tenue*, however, are almost identical

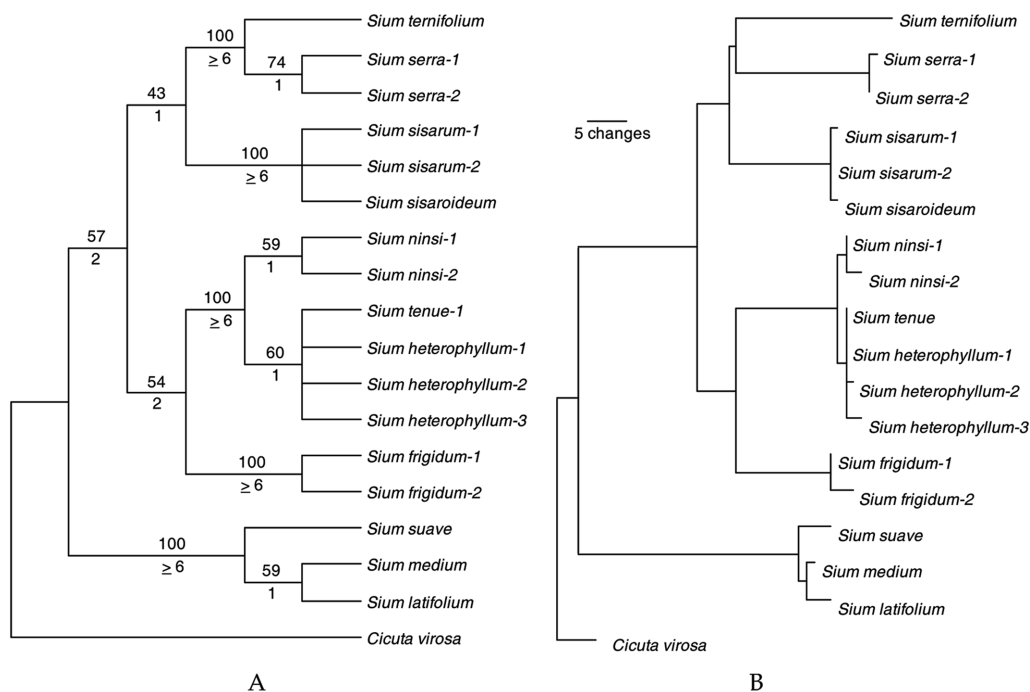


Fig. 1. Cladograms inferred from the analysis of 18 nuclear ribosomal DNA ITS1 and ITS2 sequences from the genus *Sium* and an outgroup. Numbers above nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates; decay values are presented below. A. The strict consensus of two minimal length 123-step trees derived from equally weighted maximum parsimony analysis of combined nuclear rDNA ITS1 and ITS2 sequences (CI's with and without uninformative characters = 0.935 and 0.912, respectively; RI = 0.957); B. one of two minimal length 123-step trees derived from equally weighted maximum parsimony analysis of combined nuclear rDNA ITS1 and ITS2 sequences from 18 accessions of *Sium* and an outgroup.

morphologically with the exception of basal leaf forms and also the low sequence divergence between these two species support their close relationships, ranging from 0.46 to 0.92%. *S. temue* is a probably infraspecific taxon of *S. ninsi* based on low sequence divergence between these two. *S. ninsi* has been treated as a conspecific taxon with *S. sisarum* by several authors (Hiroe and Constance, 1958; Ohwi, 1965; Lee, 1980; Lee, 1993; Ohba, 1999; Lee 2006), but is clearly distinguished from the latter by the absence of axile bulbils on the stem. In several Korean flora, morphological characters of *S. ninsi* were described mixed with those of *S. sisarum* following the Japanese treatments (Lee, 1980; Lee, 2003; Lee 2006). *S. ternifolium* recently reported as a new species is similar to *S. serra* sharing characters such as long-acuminate apex of leaflets, numbers of very slender rays, but identified by the presence of several features including trifoliated leaflets (rarely entire at the basal), and branchless stems (Lee and Ko, 2009). The phylogeny constructed with inclusion of two accessions of *S. serra* and a single accession of *S. ternifolium* revealed the strong monophyly of the clade with 100 of bootstrap values and more than 6 of decay values. The 1.4 and 1.6% of ITS sequence divergence values support the species delimitation between *S. serra* and *S. ternifolium*.

Key to the species of Korean *Sium* L.

1. Stems branched, number of rays 7–12, apex of leaflets acuminate
 2. Roots not thickened, upper cauline leaves dissected pinnately, rays stout 1. *S. suave*
 2. Roots thickened, upper cauline leaves dissected ternately, rays slender
 3. Basal leaves entire 2. *S. temue*
 3. Basal leaves pinnately dissected 3. *S. ninsi*
1. Stems branched rarely, number of rays 2–6, apex of leaflets long acuminate 4. *S. ternifolium*

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Appendix. Specimens examined in the study

1. *S. suave* Walter, Fl. Carol. 115, 1788.

Korean name: Gae-bal-na-mul

Specimens examined. Jeollanam-do: Gokseong-gun, Gonbang-san, 25 Aug. 2005, C.W. Park et al. 2005-352 (KB). Gyeongsangbuk-do: Gyeongju-si, Nam-san, 11 Aug. 2007, H.J. Kim et al. 353 (KB). Gangwon-do: Inje-gun, Daeam-san, 19 Sep. 2009, B.Y. Lee 215 (KB)

2. *S. tenue* Kom. in Bull. Jard. Bot. Petersberg. 16:174, 1916. - *S. heterophyllum* Y. Lee. Korean J. Pl. Taxon. 23: 263-268. 1993.

Korean name: Dae-am-gae-bal-na-mul

Specimens examined. Gangwon-do: Teongwol-eup, Jang-neung wetland, 24 Sep. 2009, B.Y. Lee 240 (KB). Gyeonggi-do: Gimpo-si, Moonsoo-san, 24 Sep. 2009, B.Y. Lee 181 (KB); Suwon-si, Chilbo-san, 4 Sep. 2004, H.I. Lim & H. Choi 0454 (KB). Gyeongsangbuk-do: Gimcheon-si, Gaje-san, 15 May 2004, G.Y. Chung & G.H. Nam s.n. (KB); Sangju-si, 746.3 hill, 28 Sep. 2009, S.C. Ko & D.C. Son s.n. (HNHM); Sangju-si, Poseong-bong, 30 Aug. 2009, S.C. Ko & H.J. Kim s.n. (HNHM); Seongju-gun, Jasan, 16 Oct. 2004, G.Y. Chung & G.H. Nam s.n. (KB); Goryeong-gun, Misoong-san, 21 Oct. 2006, S.H. Cho et al. 3127 (KB). Gyeongsangnam-do: Milyang-si, Hwaak-san, 6 Sep. 1998, M.G. Chung & J.M. Chung 2356 (KB). Incheon metropolitan city: Yeongjong-do, Baegwoon-san, 21 Sep. 2009, B.Y. Lee 160 (KB). Jeollanam-do: Iksan-si, Hamla-san, 31 Aug. 2001, M. Kim 5393 (KB). Ulsan metropolitan city: Seosaeng-myon, Yonggok-san, 8

Oct. 2005, S.H. Cho et al. 1251 (KB).

3. *S. ninsi* L. Sp. Pl. 251, 1753.

Korean name: Gam-ja-gae-bal-na-mul

Specimens examined. Chungcheongbuk-do: Yeongdong-gun, Cheongwan-san, 4 Sep. 2005, J.H. Kim & S.W. Lee 0900 (KB); Eumseong-gun, Deogseong-san, 10 Aug. 2004, J.H. Kim & J.B. Lee 199 (KB). Daejeon metropolitan city: Secheon-dong, Sigjang-san, 14 Sep. 2007, G.H. Tae s.n. (HNHM). Gangwon-do: Inje-gun, Daeam-san, 6. Sep. 2000, K.C. Yang & J.D. Jung s.n. (KB). Gyeonggi-do: Paju-si, 30 Aug. 2006, H.Y. Park & M.N. Kim s.n. (KB); Yangpyeong-gun, Geumwang-san, 9 Sep. 2005, K.T. Yeo & K. Heo 4425 (KB). Gyeongsangbuk-do: Gumi-si, Geomeonjae-san, 3 Oct. 2004, B.U. Oh et al. 203 (KB); Sangju-si, Poseong-bong, 30 Aug. 2009, S.C. Ko & H.J. Kim s.n. (HNHM); Cheongdo-gun, Hwaak-san, 29 Aug. 2007, J.H. Kim & Y.H. Kim s.n. (KB). Gyeongsangnam-do: Yangsan-si, Sinbul-san, 16 Aug. 2003, B.Y. Lee & S.G. Kwon 2118 (KB). Jeollanam-do: Gokseong-eup, Hyeongje-bong, 20 Oct. 2007, H. Kim 3751 (KB); Sooncheon-si, 24 Oct. 1998, H.T. Lim 102411 (KB); Sinan-gun, Imja-do, 30 Aug. 1997, H.T. Im 49551 (KB)

4. *S. ternifolium* B.Y. Lee & S.C. Ko. Korean J. Pl. Taxon. 39: 130-134. 2009.

Korean name: Se-ip-gae-bal-na-mul

Specimens examined. Gangwon-do: Wonju-si, Chiak-san (Holotype of *S. ternifolium* B.Y. Lee & S.C. Ko, 20 Aug. 2009, S.C. Ko 064185 (HNHM); Wonju-si, Chiak-san (Isotype, 20 Aug. 2009, S.C. Ko 064189, 064191 (HNHM); Wonju-si, Chiak-san (Paratype, 20 Aug. 2009, S.C. Ko 064190, 064192, 064193 (HNHM), 064186 (KB).; Won-ju si, Chiak-san, 2 Sep. 2008, S.C. Ko 064183 (KB)