

Wangsaniana, a new genus of Apiaceae endemic to Korea

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Wangsaniana, a new genus of Apiaceae from limestone areas and typified by *W. insolens* (Kitagawa) B.Y. Lee & J.O. Hyun, is described from Gangwon-do, Korea. *Wangsaniana insolens* was initially treated as *Peucedanum insolens* Kitagawa due to similarity with *Peucedanum cervaria*, but it differs markedly in its unique morphological characters, such as rarely branched roots and a single basal leaf with 3 or -4 ternately dissected leaflets. These characters apparently represent apomorphies for *Wangsaniana*. The parsimony analysis of nuclear ribosomal DNA ITS sequences revealed *Wangsaniana* to be not closely related to either *Peucedanum* or to any of 14 different tribes or clades within subfamily Apioideae.

Keywords: Apiaceae, New genus, *Peucedanum insolens*, *Wangsaniana insolens*

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INTRODUCTION

Peucedanum, Apiaceae, Umbellales occurs in Eurasia and Africa (Pimenov and Leonov, 1993; Spalik *et al.*, 2004). Members of the genus are characterized by flattened fruits without prominent dorsal ridges and with more or less developed lateral wings (Spalik *et al.*, 2004). *Peucedanum* was regarded as an unnatural group and separated into several genera by Pimenov (1987), Frey (1989), and Magee *et al.* (2009). For example, most species of *Peucedanum* in North America are now treated in *Lomatium*. Furthermore, some European members were established as a new genus, *Holandrea*, based on morphological and phytochemical information (Reduron *et al.*, 1997), while two species of *Peucedanum* from Madagascar were described as the new genus, *Billburttia* (Magee *et al.*, 2009). The taxonomic complexity of *Peucedanum* is reflected in molecular evidence that showed the genus was not monophyletic and its members were scattered throughout molecular phylogeny trees (Downie *et al.*, 2001; Spalik *et al.*, 2004). *Peucedanum* was placed within the 'Angelica' clade sensu stricto (Downie *et al.*, 2001), which was traditionally treated as Drude's (1897-1898) tribe Peucedaneae. Six species of *Peucedanum*, *P. coreanum*, *P. japonicum*, *P. terebinthaceum*, *P. elegans*, *P. hakuunense*, and *P. insolens* (Lee *et al.*, 2011) have been treated in Korea. Among them, *P. insolens* is unique in its leaf morphology due to single basal leaf with 3-4

ternately dissected leaflets, and mainly basal peduncles arising from a root crown. We treat these two characters as being unique features that separate it from other species of *Peucedanum*. The goal of our research was to confirm the taxonomic identity and determine the phylogenetic position of *P. insolens* by phylogenetic analyses using nuclear ribosomal DNA ITS sequences.

MATERIALS AND METHODS

The unique morphological characters of *P. insolens* make its position within the genus *Peucedanum* uncertain. We therefore selected representatives of subfamily Apioideae for analysis. On the basis of previous phylogenetic analyses of chloroplast genes (*rbcL*, *matK*), intron (*rpl16*, *rps16*, *rpoC1*) and nuclear ribosomal DNA internal transcribed spacer (ITS) sequences and chloroplast DNA restriction sites, Downie *et al.* (2001) reconfirmed the taxonomy of subfamily Apioideae at the tribal levels and recognized Aciphylleae, Bupleureae, Careae, Echinophoreae, Heteromorphae, Oenantheae, Pleurospermeae, Pyramidopterae, Scandiceae, and Smyrnieae. Downie *et al.* (2001) also recognized seven additional clades that had previously been taxonomically untreated. Among the ten tribes and seven clades, we did not include members of the Echinophoreae due to a lack of molecular information and did not incorporate DNA se-

Table 1. Twenty three accessions of the family Apiaceae examined for nuclear ribosomal DNA internal transcribed spacer (ITS) sequences variation. Accessions from NCBI Genbank were started with single or two letters while newly investigated sequences started with letters of WIGIS (Wildlife Integrated Genetic Information System; National Institute of Biological Resources, Korea) or WBN (WIGIS Barcode Numbers). Asterisk (*) means that the DNA sequences were submitted to the NCBI Genbank, but their publication could not be checked by authors of the current paper.

Tribe or clade	Taxon	Source or voucher information	Genbank or WIGIS accession number
Aciphyllaeae	<i>Aciphylla aurea</i>	Michell <i>et al.</i> , 1998	U72377
Careae	<i>Aegopodium alpestre</i>	Ansan, Seorak mt. Gangwon-do (July 2. 2011)	WBN0329459
	<i>Carum carvi</i>	Valiejo-Roman <i>et al.</i> , 1998	AF077878
	<i>Grammosciadium macrodon</i>	Downie <i>et al.</i> , 2000	AF073553
Oenantheae	<i>Conium maculatum</i>	Ajani <i>et al.</i> , 2008	EU 169251
Pleurospermeae	<i>Pleurospermum yunnanense</i>	Zhou <i>et al.</i> , 2008	EU236202
Pyramidoptereae	<i>Elaeosticta ramosissima</i>	Degtjareva <i>et al.</i> , 2013	HM229376
Scandiceae	<i>Scandix australis</i>	Wojewodzka <i>et al.</i> , 2008	FJ415104*
	<i>Daucus carota</i> L.	Lee <i>et al.</i> , 2004	AY552527*
Smyrnieae	<i>Smyrnum olusatrum</i>	Downie <i>et al.</i> , 1998	U30594
Angelica clade	<i>Angelica dahuria</i>	Saryeoni forests, Jeju-do (Aug. 10. 2011)	WBN0329461
	<i>Peucedanum terebinanthemum</i>	Daekwanryoung, Gangwon-do (Sep. 8. 2009)	WBN0329462
	<i>Peucedanum insolens</i>	Singi-myeon, Samcheok-si, Gangwon-do	WBN0329463
Apium clade	<i>Foeniculum vulgare</i>	Tabanca <i>et al.</i> , 2005	AY581806
	<i>Anethum graveolens</i>	Reduron <i>et al.</i> , 2009	GQ148795
	<i>Petroselinum crispum</i>	Reduron <i>et al.</i> , 2009	GQ148798
Arracacia clade	<i>Arracacia aegopodioides</i>	Danderson <i>et al.</i> , 2009	GQ862378*
	<i>Rhodosciadium purpureum</i>	Danderson <i>et al.</i> , 2011	JQ305012*
Conioselinum clade	<i>Conioselinum vaginatum</i>	Zhou <i>et al.</i> , 2009	FJ385041
Heracleum clade	<i>Heracleum moellendorffi</i>	Lee <i>et al.</i> , 2012	WIGIS004981
	<i>Pastinaca sativa</i>	Ajani <i>et al.</i> , 2008	EU169305
Komarovia clade	<i>Komarovia anisosperma</i>	Valiejo-Roman <i>et al.</i> , 1998	AF077897
Pimpinella clade	<i>Pimpinella brachycarpa</i>	Daeamsan, Gangwon-do (Sep. 19. 2009)	WBN0329464

quences from the tribes Bupleureae and Heteromorpheae because of their unique morphological features. We therefore chose 22 representative taxa from seven tribes and seven clades within the Apioideae as proposed by Downie *et al.* (2001) to clarify the relationships of *P. insolens* (Table 1). The ITS sequences of 16 taxa were obtained from Genbank, seven additional taxa were sequenced in this study. Total genomic DNAs from the seven taxa were extracted from fresh leaves and tissue from herbarium specimens using the modified CTAB procedure of Doyle and Doyle (1987). Double stranded DNAs of the complete ITS regions in each genome were amplified by the polymerase chain reaction (PCR) 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, 2002). Phylogenetic 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. A bootstrap analysis was done using 100 resampled data sets. All trees were rooted with *Pleurospermum yunnanense*, revealed as the most basal species within the Apioideae (Downie *et al.*, 2001).

RESULTS

1) ITS sequence analysis

Among 23 complete ITS sequences, the ITS 2 region was shorter than ITS 1. The length of ITS 1 region in the taxa investigated ranged from 211 bp in *Foeniculum vulgare* to 221 bp in *Pimpinella brachycarpa* and *Pastinaca sativa* (mean 216 bp). The length of ITS 2 region of the taxa surveyed ranged from 217 bp in *Elaeosticta ramosissima* to 225 bp in *Cicuta maculatum* (mean 222 bp). Overall length variation of both spacer regions across all 23 accessions ranged from 432 bp to 444 bp. These sizes are comparable to the values reported for other Apiaceae (Downie *et al.*, 1998; Lee and Downie, 1999). Of the 656 initial alignment positions, 73 positions were deleted due to alignment ambiguities. Of the remaining 583 unambiguously aligned positions, 195 (33.4%) were potentially parsimony informative, 288 (49.4%) were constant, and 100 (17.2%) 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 23 accessions (using PAUP's DISTANCE MATRIX option) are presented in Table 2. The sequence divergence values ranged from 2.2% (between *Foeniculum vulgare* and *Anethum graveolens*) to 23.4% (between

Table 2. Pairwise sequence divergence of ITS 1, ITS 2, and 5.8S ribosomal DNA regions among taxa included in the study. 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 the strict consensus 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
1 <i>Foeniculum vulgare</i>	–	2.21	6.31	8.36	12.34	13.27	11.05	12.69	12.71	12.31	11.19	15.86	15.57	13.94	15.50	12.22	18.35	22.66	16.57	17.84	14.26	16.48	14.76
2 <i>Anethum graveolens</i>	12	–	6.64	8.27	12.75	13.10	10.93	11.81	12.20	11.81	10.52	15.50	15.21	13.41	15.33	12.08	18.52	21.85	17.13	18.20	14.66	15.93	14.60
3 <i>Petroselinum crispum</i>	34	35	–	8.70	12.48	12.66	10.82	12.64	12.85	12.45	11.15	14.68	14.95	13.35	14.31	12.17	18.88	22.06	17.07	17.42	14.39	16.45	14.73
4 <i>Comoselinum vaginatum</i>	45	45	47	–	9.41	9.43	6.84	7.37	7.38	7.55	6.26	11.99	12.05	11.00	12.33	10.78	15.79	19.33	14.52	15.78	11.87	14.12	12.43
5 <i>Heraclenum moellendorffii</i>	66	69	67	51	–	4.79	9.43	10.54	10.00	10.72	9.80	14.97	14.71	12.15	16.51	13.19	17.35	21.64	15.51	16.00	14.33	15.48	14.71
6 <i>Pastinaca sativa</i>	71	71	68	51	26	–	10.02	12.78	11.69	12.22	10.93	15.52	14.71	13.83	16.32	15.42	18.18	21.78	17.19	16.51	16.38	17.62	17.10
7 <i>Conium maculatum</i>	59	59	58	37	51	54	–	9.98	10.00	10.54	9.06	14.07	13.17	12.84	14.41	11.71	16.07	21.37	15.67	16.69	13.80	15.42	13.91
8 <i>Arracacia aegopodioides</i>	68	64	68	40	57	69	54	–	5.15	6.06	4.77	16.08	15.61	12.71	16.10	13.56	16.94	20.26	16.04	18.45	14.31	16.38	14.31
9 <i>Rhodosciadium purpureum</i>	68	66	69	40	54	63	54	28	–	6.25	5.33	15.18	15.08	13.48	16.88	13.80	18.07	21.60	17.19	18.30	14.89	16.04	14.71
10 <i>Angelica dahurica</i>	66	64	67	41	58	66	57	33	34	–	4.59	15.89	15.79	12.52	16.29	14.33	18.62	20.63	16.79	19.20	14.12	16.01	14.49
11 <i>Peucedanum terebinthaceum</i>	60	57	60	34	53	59	49	26	29	25	–	13.30	12.82	10.28	14.04	12.29	17.31	19.88	15.85	17.13	12.26	14.33	12.82
12 <i>Carum carvi</i>	85	84	79	65	81	84	76	87	82	86	72	–	14.99	13.94	14.89	16.11	20.59	23.37	20.26	18.72	16.85	17.06	15.74
13 <i>Grammosciadium macrodon</i>	83	82	80	65	79	79	71	84	81	85	69	27	–	12.20	15.52	15.95	19.73	22.67	19.48	17.82	16.76	15.58	14.63
14 <i>Aegopodium alpestre</i>	74	72	71	59	65	74	69	68	72	67	55	75	66	–	13.80	13.43	17.79	21.64	16.66	16.94	15.35	15.08	14.31
15 <i>Elaeosticta ramosissima</i>	82	82	76	66	88	87	77	86	90	87	75	80	84	74	–	14.98	18.19	24.39	17.97	18.37	16.69	17.04	15.70
16 <i>Peucedanum insalens</i>	65	65	65	58	71	83	63	73	74	77	66	87	86	72	80	–	16.63	21.60	14.52	16.91	12.12	13.38	11.87
17 <i>Daucus carota</i>	98	100	101	85	93	98	86	91	97	100	93	111	106	95	97	89	–	20.59	16.79	17.66	16.41	16.48	15.77
18 <i>Scandix australis</i>	121	118	118	104	116	117	115	109	116	111	107	126	122	116	130	116	111	–	19.88	22.28	18.28	18.88	18.85
19 <i>Smyrniolum olusatrum</i>	88	92	91	78	83	92	84	86	92	90	85	109	105	89	96	78	90	107	–	16.16	13.61	15.64	13.03
20 <i>Komarovia anisosperma</i>	94	97	92	84	85	88	89	98	97	102	91	100	95	90	97	90	94	119	86	–	14.87	14.76	14.74
21 <i>Aciphyllyla aurea</i>	76	79	77	64	77	88	74	77	80	76	66	91	90	82	89	65	88	98	73	79	–	11.75	8.56
22 <i>Pimpinella brachycarpa</i>	88	86	88	76	83	95	83	88	86	86	77	92	84	81	91	72	89	102	84	79	63	–	6.22
23 <i>Pleurospermum yunnanese</i>	79	79	79	67	79	92	75	77	79	78	69	85	79	77	84	64	85	102	70	79	46	34	–

Elaeosticta ramosissima and *Scandix australis*). The pairwise nucleotide divergence between *Peucedanum insolens* and other accessions varied from 10.8% with *Conioselinum vaginatum* to 21.6% with *Scandix australis*. The divergence between *Peucedanum terebinthaceum* and *P. insolens* was 12.3%.

2) Phylogenetic analyses and resolution

The results of the analyses of combined data sets are below; separate analyses of each spacer region were not done. The parsimony analysis of 23 combined ITS sequences and 5.8S ribosomal DNAs using equally weighted character states resulted in three maximally parsimonious trees. The consensus of the three trees with accompanying bootstrap values is presented in Fig. 1. The most parsimonious tree had a length of 790 steps, a consistency index (CI) of 0.560 and 0.462, with and without uninformative characters, respectively, and a retention index (RI) of 0.477. In each of the trees, two major groups of taxa were discernable. The first group in-

cluded five clades of *Apium*, *Conioselinum*, *Heracleum*, *Angelica*, *Arracacia*, and the tribes Oenantheae, Careae, and Pyramidoptereae. *P. insolens* was basal to the first group. The second group comprised the two tribes Scandiceae and Smyrnieae, and the *Komarovia* clade; tribes Aciphyllae and Pleurospermeae were sister to this group.

DISCUSSION

The major objective in carrying out this study was to ascertain the taxonomic status and position of *P. insolens*, a species endemic to Korea. *P. insolens* has a unique leaf morphology that was not observed in other species subfamily Apioideae. A single basal leaf with 3 or 4-ternate dissection is well developed with a petiole 11.0-21.5 cm long arising from the root crown while cauline leaves conspicuously reduced. The long, cylindrical root and solitary stem are unusual and diagnostic characters for identifying *P. insolens*. In transverse section the fruit

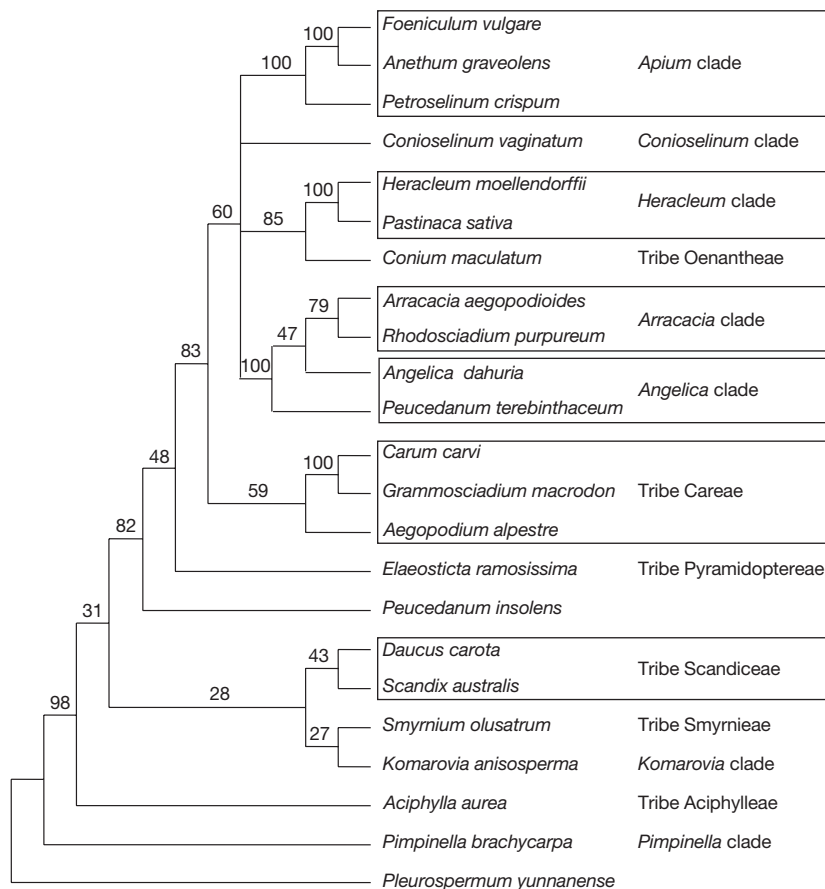


Fig. 1. Strict consensus of three minimal length 790-step trees derived from equally weighted maximum parsimony analysis of combined nuclear rDNA ITS and 5.8S sequences (CI's with and without uninformative characters = 0.560 and 0.462, respectively; RI = 0.477). Numbers above nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates.

of *P. insolens* is strongly compressed dorsally and homo-mericarpic. On the basis of the above morphological characteristics on specimens collected in the Deogwoo area in Gangwon-do Korea, Kitagawa (1972) proposed the new species, *Peucedanum insolens*. Kitagawa (1972), also, considered *Peucedanum cervaria* to be closely related to *P. insolens* in that both species share the similar characters of leaves in 3-ternate to pinnate dissection and the presence of small cauline leaves. *Peucedanum cervaria* and *P. insolens* share such features as the unequal base of the leaflets and scarcely developed dorsal ribs on the mericarps, but differs in several features (Tutin *et al.*, 1968). For example, *P. cervaria* has a root stock with fibers, terminal and lateral inflorescences, and numerous bracts; *P. insolens* does not have a fibrous rootstock at the stock, only a terminal inflorescence, and no (or rarely 1-3) bracts. Furthermore, their geographic distributions are different; *P. cervaria* ranges from central Europe to Ukraine while *P. insolens* is restricted to limestone areas in Korea. Traditionally, *Peucedanum* has been delimited by laterally expanded wings on the fruits, which was considered to be an adaptation for wind dispersal and likely evolved independently and locally in several umbellifers (Theobald, 1971; Lee, 2002; Spalik *et al.*, 2004; Liu *et al.*, 2006). Therefore, it is not surprising that *P. insolens* is placed distantly from other members of *Peucedanum*. The strict consensus of maximum parsimonious trees (MP) revealed that *P. insolens* was not closely related to any of the tribes included in the current study. The pairwise distance of *P. insolens* from each clades and tribes ranged from 10.8% (*Conioselinum* clade) to 21.6% (tribe Scandiceae). These values were much higher than the intergeneric pairwise distance within clades (2.2-6.6% in *Apium* clade, 4.6% in *Angelica* clade, 5.2% in *Arracacia* clade). Thus, the evidence that *P. insolens* is distinct from other tribes and clades of Apioideae is conclusive. The ITS polymorphisms in intraspecific levels of the family Apiaceae have been so few that even weak bootstrap support for the taxonomic position has not misled phylogenetic inferences (Chung *et al.*, 2005; Spalik and Downie, 2006; Winter *et al.*, 2008). Therefore, extremely high pairwise distance of ITS and strong bootstrap values support separation of *P. insolens* from other taxa of apioides. Comparisons of *P. insolens* with the other tribes or clades clearly indicate that it represents a distinct new genus or even higher taxonomic placement (e.g., subtribe or tribe) easily recognized by current molecular phylogenies and unique morphological characters such as rarely branched roots, a single basal leaf with 3-4 ternately dissected leaflets and fruit anatomy. The new combination for *P. insolens* is made to establish the genus *Wangsania* B.Y. Lee & J.O. Hyun (Fig. 2).

TAXONOMIC TREATMENT

Wangsania B.Y. Lee & J.O. Hyun, gen. nov. TYPE: *Peucedanum insolens* Kitagawa [= *Wangsania insolens* (Kitagawa) B.Y. Lee & J.O. Hyun].

Herbs perennial. Roots rarely branched, long cylindrical, 10-50 cm long and 1-2.5 cm in diameter, thickest at base, wrinkled horizontally. Stems absent or solitary, 9.9-22.5 cm tall, slender, glabrous. Leaves basal, single or rarely two, 4-ternately dissected; petiole 15.9-27.5 cm long, arising from root crown; leaflets 3-10 mm long, 3-4 mm wide, petiolulate at all branch levels; petiolule I 6.3-10.2 cm long, II 3.4-5.7 cm long, III 2.6-4.4 cm long, IV 0.5-2.6 cm long; blade 3.7-5 cm long, 1.8-3.2 mm wide, base oblique, margins serrate; cauline leaves absent or conspicuously reduced, petiole 0.3-4.7 cm long. Inflorescences terminal, compound umbels; rays 8-10, 3-5 cm long, unequal in length, glabrous; bracts absent or 1-3, filiform to subulate, 2-7 mm long, 0.2-0.5 mm wide, margins membranous; bracteoles absent or 1-4, subulate to linear, 3-6 mm long, 0.2-0.7 mm wide; pedicels 10-25, 2-10 mm long, unequal in length. Flowers bisexual; calyx teeth triangular, persistent, base fleshy, green; petals white, elliptic to oval, or obtriangulate, apex emarginate, incurved-apex 0.7-0.8 mm long; style filiform, base thickened, stylopodium conical. Fruit oval, 7-8 mm long, 5 mm wide; mericarps strongly compressed dorsally; dorsal ridges filiform, not winged; lateral ridges extended into broad wings, 1 mm wide, vittae 1, tiny or indistinct on each of dorsal valliculae, 2 on commissural face.

Etymology

The genus name is derived from the place of discovery of the type species, Wangsan-myeon, Gangwon-do, Korea.

Distribution

Wangsania insolens is endemic to limestone areas in Gangwon-do, Chungcheongbuk-do, and Gyeongsangbuk-do, Korea.

Phenology

Flowering in July-September; fruiting in August-October.

Selected specimens examined

KOREA. Gangwon-do: Hadong-myeon, Daeya-ri, Gajaegol, elev. 219 m, 27 Jul. 2010, Nam K. H. *et al.* SHY902 (2 sheets) (KB); Jeongseon-gun, Buk-myeon, Goyang-ri, Mt. Ballon, elev. 984 m, 6 Sep. 2012, Kim J. H. & Kim Y. R. SHY3-1932 (KB); Jeongseon-gun, Buk-myeon, Goyang-ri, Mt. Sangjeongbawi, elev. 984 m, 11 Oct. 2012, Kim J. H. & Kim J. S. SHY3-2284 (KB);

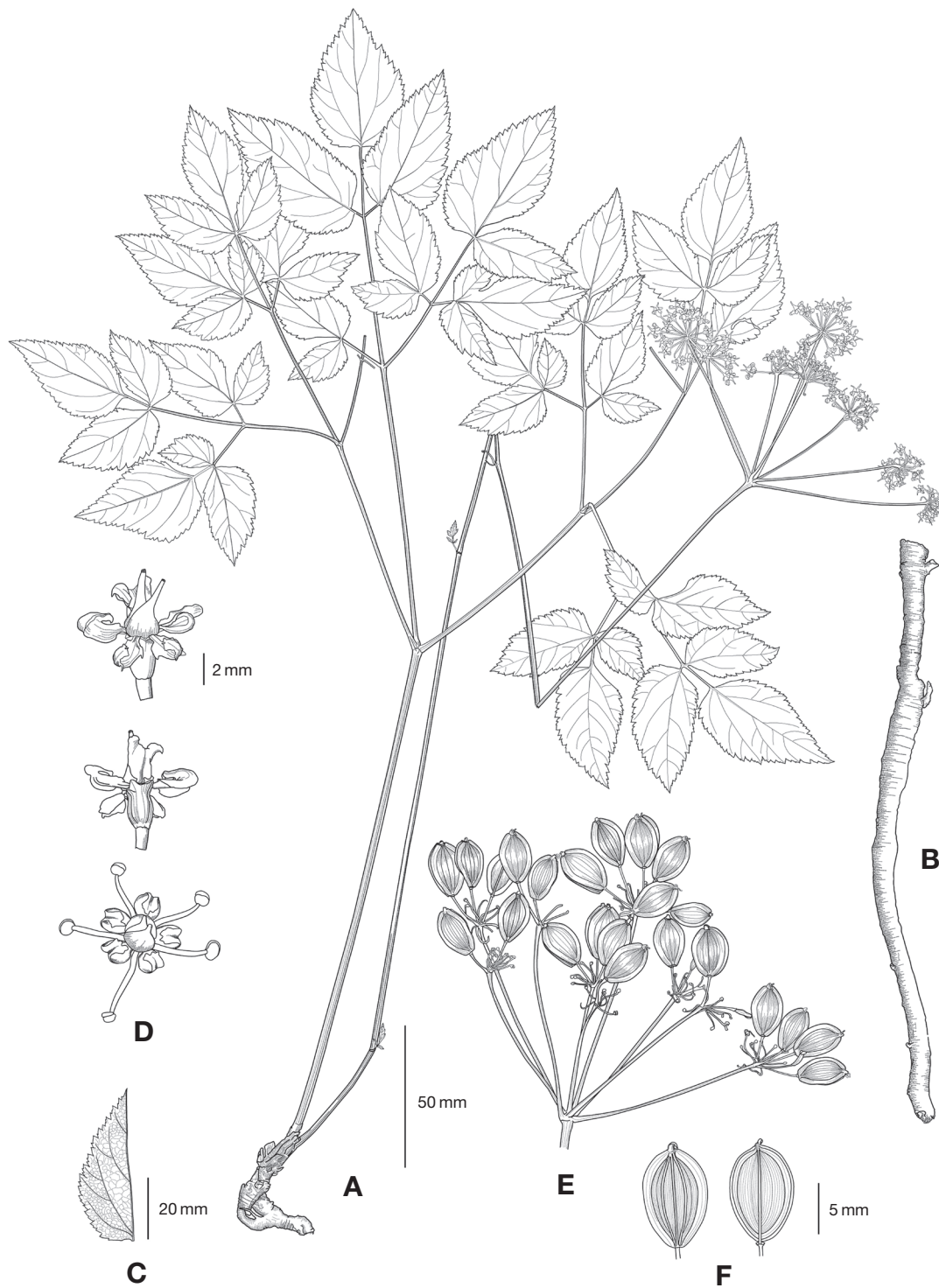


Fig. 2. *Wangsanias insolens* (Kitagawa) B.Y. Lee & J.O. Hyun. A. Habit: a basal leaf, single, 4-ternately dissected, petiolulated leaflets arising from the root stock, a cauline leaf absent or conspicuously reduced if present. B. Root: a rarely branched and long cylindrical shape. C. half of a leaflet. D. individual flowers. E. Inflorescence. F. Fruits.

Jeongseon-gun, Dong-myeon, Hwaam-ri, elev. 652 m, 10 Oct. 2010, Hyun J. O. NAPI-J20111611 (KB); Jeongseon-gun, Jeongseon-eup, Deogu-ri, Deoksangi valley, elev.

341 m, 11 Oct. 2012, Nam K. H. & Kim Y. R. SHY3-2255 (KB); Yeongwol-gun, Donghae-si, Cheongok-dong, Cheongok cave, elev. 50 m, 24 Aug. 2011, Nam G. H. &

Kim J. H. SHY2-1190 (KB).

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