Wangsania, a new genus of Apiaceae endemic to Korea

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Wangsania, 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. Wangsania 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 Wangsania. The parsimony analysis of nuclear ribosomal DNA ITS sequences revealed Wangsania 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, Wangsania 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 Peucedanaceae. Six species of Peucedanum, P. coreanum, P. japonicum, P. terebinthaceum, P. elegans, P. hakuanense, 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) confirmed the taxonomy of subfamily Apioideae at the tribal levels and recognized Aciphylleae, Bupleureae, Caraeae, Echinophoreae, Heteromorpheae, Oenantheae, Pleuropermeae, 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-
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 Apioidae 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 taxa investigated 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 221 bp in *Tribulus terrestris* and *Conioselinum vaginatum* (mean 444 bp). These sizes are comparable to the values reported for other Apiaceae (Downie et al., 1998; Lee and Downie, 2000). 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 *Conium maculatum* to 23.4% (between *Pastinaca sativa* and *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

### 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 *Foeniculum vulgare* 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

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**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.

<table>
<thead>
<tr>
<th>Tribe or clade</th>
<th>Taxon</th>
<th>Source or voucher information</th>
<th>Genbank or WIGIS accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aciphylleae</td>
<td>Aciphylleae aerea</td>
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<td>U72377</td>
</tr>
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<td>Aegopodium alpestre</td>
<td>Ansar, Scorak mt. Gangwon-do (July 2. 2011)</td>
<td>WBN0329459</td>
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<td>Caraeae</td>
<td>Carum carvi</td>
<td>Valiejo-Roman et al., 1998</td>
<td>AFO77878</td>
</tr>
<tr>
<td>Caraeae</td>
<td>Grammosciadium macronodon</td>
<td>Downie et al., 2000</td>
<td>AFO73553</td>
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<tr>
<td>Oenantheae</td>
<td>Conium maculatum</td>
<td>Ajani et al., 2008</td>
<td>EU169251</td>
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<tr>
<td>Pleurosperrmea</td>
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<tr>
<td>Pyramidopterpeae</td>
<td>Elaeosticta ramosissima</td>
<td>Degtjareva et al., 2013</td>
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<td>Lee et al., 2004</td>
<td>AY552527*</td>
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<td></td>
<td>Pastinaca sativa</td>
<td>Singi-myeon, Samcheok-si, Gangwon-do (Sep. 19. 2009)</td>
<td>WBN0329464</td>
</tr>
</tbody>
</table>

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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.

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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-

**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 be 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.](image-url)
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. cevaria* 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**


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 1.3-10.2 cm long, II 3.4-5.7 cm long, III 2.6-4.4 cm long, IV 0.5-2.6 cm long; blade 3.7-5.0 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 obtrigilate, apex emarginate, incurved-apex 0.7-0.8 mm long; style filiform, base thickened, stylodium 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, vitiae 1, tiny or indistinct on each of dorsal valleculae, 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**


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); Yeong-wol-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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