J. Plant Biol. (2010) 53:94–105
DOI 10.1007/s12374-009-9093-z

ORIGINAL RESEARCH

Phylogenetic Relationship of *Physocarpus insularis* (Rosaceae) Endemic on Ulleung Island: Implications for Conservation Biology

Sang-Hun Oh · Luna Chen · Sung-Hee Kim · Young-Dong Kim · Hyunchur Shin

Received: 18 November 2009 / Revised: 3 December 2009 / Accepted: 10 December 2009 / Published online: 20 January 2010

Abstract Ulleung Island is a biodiversity hot spot harboring approximately 700 species of vascular plants with high number of endemic taxa. *Physocarpus insularis*, one of the 41 endemic species, has an extremely restricted distribution range on the very small, geologically young oceanic island. Phylogenetic relationship of *P. insularis*, however, remained highly controversial whether it is conspecific with *Spiraea chamaedryfolia* or a distinct species in *Physocarpus*, making it difficult to establish necessary conservation programs. We examined comparative morphology of *Physocarpus* and *Spiraea* and reconstructed the phylogeny of the *rbcL*, *matK*, *ndhF*, and *trnL-trnF* regions from the exemplars of Rosaceae. The results strongly supported the placement of *P. insularis* within *Spiraea*. Further phylogenetic analyses of tribe Spiraeeae based on the *trnL-trnF* and internal transcribed spacer data showed that *P. insularis* is closely related to *S. chamaedryfolia*. Morphological analysis revealed that *P. insularis* differs from *S. chamaedryfolia* by having larger leaf blades that are subcordate or truncate at base. Results of this study suggest that *P. insularis* should be recognized as a distinct taxon in *Spiraea* and that conservation efforts on Ulleung Island should focus on protecting its natural environment to conserve evolutionary patterns and processes in addition to specific conservation programs for species in peril.

Keywords Conservation biology · *Physocarpus insularis* · *Spiraea chamaedryfolia* · Ulleung Island

One of the most important requirements in successful conservation programs is a detailed understanding of biology of the subject of conservation. These include taxonomic identity and phylogenetic relationship of the species of interest, accurate nomenclature, biogeography, phylogeography, genetic diversity, demographic dynamics, and ecology. Biodiversity on a small island needs more attention because it is more vulnerable to increased anthropogenic activities and rapid global climatic changes than species on a large island or continent (Rieseberg and Swensen 1996). Endemic species, distributed in only limited areas, are particularly of concern because they do not occur elsewhere.

Ulleung Island, 150 km off the eastern coast of the Korean peninsula, is a small oceanic island that harbors many endemic species, many of which are listed as endangered species (Lee 2008). Of approximately 700 species of vascular plants on the island, 41 species cannot be found elsewhere in the world but Ulleung Island (Nakai 1919; Oh 1978; Lee and Yang 1981; Lee et al. 1997; Lee 1998; Sun and Stuessy 1998; Lim et al. 2003, 2005). These endemic species are phylogenetically dispersed over 36 genera in 25 families, which suggests that each of these species evolved from a founder population of a species without subsequent diverging speciation events, a mode of speciation often referred to as anagenetic speciation (Stuessy et al. 1990). A recent survey demonstrated that Ulleung Island exhibits the highest level of anagenetic

---

S.-H. Oh (✉) · L. Chen
L. H. Bailey Hortorium, Department of Plant Biology,
Cornell University,
412 Mann Library,
Ithaca 14853, USA
e-mail: so253@cornell.edu

S.-H. Kim · Y.-D. Kim
Department of Life Science, Hallym University,
Chuncheon 200-702, South Korea

H. Shin
Department of Biology, Soonchunhyang University,
Asan 336-745, South Korea

speciation among oceanic and continental islands in the Pacific and Atlantic Ocean (Stuessy et al. 2006), illustrating that Ulleung Island is an excellent system to study the early stages of evolution. Numerous systematic and evolutionary studies have investigated a range of biological aspects on the endemic species, encompassing genetic diversity, taxonomic identity, phylogenetic relationship, and the origin of species (Park et al. 1993; Suh et al. 1996; Shin and Choi 1997; Sun et al. 1997; Chung et al. 1998; Sun and Stuessy 1998; Kim et al. 2000, 2002, 2006; Pfosser et al. 2002; Weiss et al. 2002; Woo et al. 2002; Chang and Jeon 2003; Ku et al. 2004; Lim et al. 2006; Ohkawa et al. 2006; Pfosser et al. 2006), which have improved our understanding of the unique biodiversity on Ulleung Island.

Physocarpus insularis (Nakai) Nakai has been considered as one of the rare endemic species on Ulleung Island (Nakai 1918, 1919; Oh 1978; Lee and Yang 1981) and listed as critically endangered species by the Korean government (Lee 2008) because plants of this species can only be found in a small population occupying the rocky slopes in the Do-dong area on the island. The occurrence of a species of *Physocarpus* on this island is very intriguing given the distribution of the members of the genus, which shows intercontinental disjunct distribution pattern between North America and East Asia. In North America, four species (*Physocarpus alternans* (M. E. Jones) J. T. Howell, *Physocarpus capitatus* (Pursh) Kuntze, *Physocarpus malvaceus* (Greene) Kuntze, and *Physocarpus monogynus* (Torr.) J. M. Coult) are distributed in western North America, and one (*Physocarpus opulifolius* (L.) Maxim.) is broadly distributed in eastern North America. In East Asia, *Physocarpus amurensis* (Maxim.) Maxim. grows in thickets in mixed coniferous forests of North Korea, northeastern China, and the Far Eastern Federal District of Russia, and with the exclusion of *P. insularis*, it is the only native species of *Physocarpus* in the region (Oh and Potter 2005).

Interestingly, there have been controversies surrounding the systematics of *P. insularis* whether it should be classified in *Physocarpus* or *Spiraea*. In their morphological study, Kim et al. (2000) determined that *P. insularis* lacks stipules and that its follicular fruits dehisce along ventral suture only, characteristics consistent with the genus *Spiraea*. They further explained that morphology of *P. insularis* do not show the diagnostic characters of *Physocarpus*; instead, it belongs to the range of morphological variation in *Spiraea chamaedryfolia* L. var. *ulmifolia* (Scopoli) Maxim. Subsequently, *P. insularis* was placed under the synonym of *S. chamaedryfolia* var. *ulmifolia*, distributed in Europe and East Asia including the Korean peninsula (Kim et al. 2000). The conclusion of the comparative study by Kim et al. (2000), however, has been seldom appreciated. Floristic studies of vascular plants on Ulleung Island (e.g., Nakai 1919; Oh 1978; Lee and Yang 1981), those in Korea including the most recent treatment (Chung 1957; Lee 1980, 2007), and current conservation efforts (Lee 2008) all have recognized *P. insularis* as a distinct species of *Physocarpus*, endemic on Ulleung Island. By contrast, phylogenetic analysis of tribe Neillieae, which comprises *Physocarpus* and *Neillia*, accepted the results of Kim et al. (2000) and excluded *P. insularis* in their analysis (Oh and Potter 2005). In spite of the conflicting views on the taxonomy of *P. insularis* among the botanists, no study thoroughly examined *P. insularis* using molecular and morphological data to understand whether it is closely related to a species of *Physocarpus* or *Spiraea*.

The accumulated DNA sequence data in public databases that have been generated for reconstructing a phylogeny of Rosaceae provide an opportunity to resolve the debate. Molecular phylogenetic analyses of Rosaceae have identified many strongly supported monophyletic groups within the family such as tribe Neillieae (Morgan et al. 1994; Potter et al. 2002, 2007a). Tribe Spiraeeae to which *Spiraea* belongs is another strongly supported subclade. Both Spiraeeae and Neillieae are members of the subfamily Spiraeoideae, but the two are distantly related tribes. Furthermore, molecular phylogenies of Neillieae (Oh and Potter 2005) and Spiraeeae (Potter et al. 2007b) are studied in detail, which may be useful in specifying to which taxon is *P. insularis* closely related.

The objectives of this study are to elucidate the phylogenetic relationship of *P. insularis* in Rosaceae by using DNA sequences, to evaluate the morphological characters observed in these plants, and to examine the implications for conservation biology of native species on Ulleung Island suggested by these data.

Materials and Methods

Taxon and Gene Sampling for Phylogenetic Analyses

Phylogenetic analyses were employed at two levels: one at the subfamily level and the other at the tribal level. Taxa and genes were included accordingly. For the subfamily level analysis, 32 taxa of Spiraeoideae including *P. insularis* were analyzed along with *Cercocarpus* and *Dryas*, which were used as out-groups. We included all major lineages of Spiraeoideae, including tribes Amygdaleae, Kerrieae, Neillieae, Osmaronieae, Sorbarieae, and Spiraeeae; supertribe Pyrodae; and *Lyonothamnus* (Potter et al. 2007a). Four chloroplast regions (*rbcL*, *matK*, *ndhF*, and *trnL-trnF*) were analyzed in this study, which have shown to provide sufficient information to resolve the groups. All of the sequences except for those from *P. insularis* were taken from previously published studies (Morgan et al. 1994; Potter et al. 2002, 2007a).

Kim (2007) in his recent taxonomic treatment of the Korean *Spiraea* recognized *S. chamaedryfolia* without the variety rank. It is unknown whether or not the circumscription of *S. chamaedryfolia* includes *P. insularis*. Our concept of *S. chamaedryfolia* in this study includes all varieties previously recognized (vars. *chamaedryfolia*, *pilosa*, and *ulmifolia*) but does not include *P. insularis*.

The tribal level analysis included a total of 33 taxa of tribe Spiraeeae, *P. insularis*, and two out-groups. DNA sequences of the chloroplast *trnL-trnF* and the nuclear ribosomal internal transcribed spacer (ITS) regions determined from *P. insularis* and *S. chamaedryfolia* in this study were added to the data generated by Potter et al. (2007b) to further clarify the phylogenetic position of *P. insularis*. The data included eight of nine genera of Spiraeeae with the emphasis of sampling in *Spiraea* (25 species). GenBank accession numbers for the species included in each analysis are summarized in Appendices 1 and 2, but more detailed information about the sources of the materials for the published data can be found in Potter et al. (2007a, b). Fresh materials of *P. insularis* and *S. chamaedryfolia* for DNA sequencing were obtained in Ulleung-gun Agricultural Technology Center on Ulleung Island and Kangwon-do, respectively (Appendix 2). Voucher specimens were deposited in the Hallym University Herbarium (HHU).

DNA Extraction, PCR, and Sequencing

Total DNA was isolated following the methods described in Kim et al. (2009). Sequences of the *matK* and *trnL-trnF* regions were determined in this study using the primers described in Potter et al. (2002). The *ndhF* region was amplified by using primers 972 and 2110R (Olmstead and Sweere 1994) and the *rbcL* region using the primers 1F and 724F (Fay et al. 1997). Primers ITS5a (Stanford et al. 2000) or ITS5 and ITS4 (White et al. 1990) were used for the ITS regions. The target regions were amplified via polymerase chain reaction (PCR) with Phusion High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA) under the following conditions in 25-μl reactions: initial denaturation at 98°C for 1 min 30 s, 20 cycles of 98°C for 10 s, 60°C for 30 s, and 72°C for 2 min, another 20 "touchdown" cycles of 98°C for 10 s, 60°C with 0.5°C decrease per cycle for 30 s, and 72°C for 2 min, followed by final extension at 72°C for 7 min. PCR products were purified with the QIAquick Gel Extraction kit (Qiagen, Valencia, CA, USA) or by using an enzymatic method, which adds 2.5 units of Antarctic Phosphatase and ten units of exonuclease I to the PCR products and incubates the reaction at 37°C for 15 min followed by incubation at 80°C for 15 min. The *matK*, *ndhF*, *trnL-trnF*, and ITS sequences of *P. insularis* were determined using the PCR primers (and nested primers in the case of *matK*) at Cornell University Life Sciences Core Laboratories Center, which uses 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The *rbcL* sequence of *P. insularis* and all sequences of *S. chamaedryfolia* were determined at the DNA sequencing facility at Hallym University. Sequences were edited in Sequencher version 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA).

Phylogenetic Analyses

All four cpDNA regions for the subfamily level phylogeny were combined and simultaneously analyzed. The *trnL-trnF* and ITS regions for the tribal level analysis, however, were not concatenated and separately analyzed. Phylogenetic analyses of the data were conducted with maximum parsimony (MP) and maximum likelihood (ML) methods. All characters were treated as unordered and weighted equally in the MP analyses employed in PAUP* (Swofford 2002). Gaps resulted from multiple alignment of indels were treated as missing data. Heuristic searches were used to find the MP trees with 500 replicates of random taxon addition and tree bisection–reconnection (TBR) branch swapping saving all of the best trees at each step (MulTrees). Branches with a minimum length of zero were collapsed using "amb-" option during the searches in the MP analysis (Nixon and Carpenter 1996). The bootstrap analysis (Felsenstein 1985) with 500 pseudoreplicates was conducted with simple sequence addition and TBR branch swapping. ML analysis was conducted by using the program GARLI version 0.951 (Zwickl 2006). The best-fitting evolutionary model for each data set was determined by the hierarchical likelihood ratio test using Modeltest 3.06 (Posada and Crandall 1998). The selected model for each data set, including substitution model, gamma shape parameter, and proportion of invariable sites, was employed to calculate a likelihood value. All other parameters were estimated from the data. Default values were used for genetic algorithm and other settings. ML bootstrap analyses were conducted with 100 pseudoreplicates by using the same parameters in the program GARLI.

A specific phylogenetic hypothesis that *P. insularis* is a member of *Physocarpus* was tested using the Shimodaira and Hasegawa (1999; SH) test, as implemented in PAUP*. Namely, MP trees constraining *P. insularis* to be sister to *Physocarpus* were generated in the combined Spiraeoideae data using the heuristic search method described previously, and these were evaluated with the original MP trees without constraints. For the SH test, 10,000 bootstrap replicates were resampled using the re-estimated log likelihood (RELL) method.

Morphology

Herbarium specimens from A, BH, CS, DAV, GH, JEPS, LE, MO, NY, PE, POM, and UC were used to investigate the morphology of *Physocarpus* and *Spiraea*. The holotype specimen of *P. insularis* at TI was also examined. Morphometic analysis of *P. insularis* and *S. chamaedryfolia* was conducted using the specimens at BH, HHU, and TI as well as digital images of specimens in the Korea Biodiversity Information System (URL: http://www.nature.go.kr). Leaf blade length, width, and base angle were measured using the image analysis program, ImageJ (Rasband 2007–2009), and the ratio of the leaf length to width was calculated. Measurement unit was calibrated into the metric system with the scale provided in each image. Mature leaves from eight individuals of *P. insularis* and 24 plants of *S. chamaedryfolia* were analyzed (Appendix 3). In four specimens of *S. chamaedryfolia*, we measured two leaves per specimen, one representing a leaf on the fertile branch and the other on the sterile branch. In all other cases, one leaf on either branch type was measured. Of the total of 36 measurements, 26 were made from a leaf on the sterile branch, which grows from the base of the stem during the growing season and does not bear flowers.

Results

Statistics of the final alignments and phylogenetic analyses were summarized in Table 1. Phylogenetic analyses of the concatenated cpDNA regions (*rbcL*, *matK*, *ndhF*, and *trnL-trnF*) from exemplar species of Rosaceae strongly supported (100% bootstrap supports in both MP and ML) that *P. insularis* is more closely related to *Spiraea* than it is to *Physocarpus* (Fig. 1). Forcing *P. insularis* as sister to *Physocarpus* within Neillieae required 190 additional steps, and the SH test indicated that the constrained topology is significantly worse than the original tree ($p<0.0001$).

Comparative morphology showed that *Physocarpus* is readily distinguished from *Spiraea* and that *P. insularis* belongs to *Spiraea* (Table 2). A pair of stipules develops at the base of the leaf in *Physocarpus* and falls off as the leaf matures, but *Spiraea* does not have stipules at all. Stellate hairs are commonly distributed in various parts of body in *Physocarpus*, most notably upper and lower surfaces of the leaves, pedicels, hypanthia, sepals, and carpels, although in some individuals of *P. opulifolius*, surfaces of the floral organs are glabrous. This branched hair is absent in *Spiraea*. Nectaries of *Physocarpus* develop on the adaxial surface of the hypanthium, whereas those of *Spiraea* form conspicuous lobes at the rim of the hypanthium. Carpels of *Physocarpus* are weakly fused at least at base but those of *Spiraea* are free. Follicular fruits of *Physocarpus* become elongated and inflated, as they mature, and fully dehisce along both ventral and dorsal sutures of the carpel, while those of *Spiraea* are slightly enlarged and dehiscent along ventral suture only. The dorsal suture of the follicle of *Spiraea* may open, but it only does at the apex of the carpel not the same length as the ventral suture.

Further phylogenetic analyses of the Spiraeeae based on *trnL-trnF* and ITS data congruently showed that *P. insularis* is nested within *Spiraea* and that it is closely related to *S. chamaedryfolia* with strong bootstrap support (Fig. 2).

Analysis of leaf characters of *P. insularis* and *S. chamaedryfolia* indicated that *P. insularis* could be distinguished from *S. chamaedryfolia* by its larger leaf blades with larger leaf base angles (Table 3, Fig. 3). The blade length on mature leaves varied widely in *S. chamaedryfolia*

Table 1 Summary statistics of data matrices and phylogenetic analyses of the data

	Data matrix		
	Spiraeoideae	Spiraeeae	Spiraeeae
Genic region	*rbcL*, *matK*, *ndhF*, and *trnL-trnF*	*trnL-trnF*	ITS
No. of taxa	33	38	42
No. of characters	5,248	1,065	770
No. of variable characters	1,217	161	323
No. of parsimony-informative characters	717	61	189
No. of MP trees	4	1	54
Length of MP trees	2,040	191	681
CI, excluding autapomorphy	0.6254	0.8333	0.5736
RI	0.8032	0.9352	0.7701
Best-fitting model selected	TVM+I+G	TVM+G	TrN+G
Alpha	0.9279	0.6516	0.4146
Pinvar	0.4436	0	0
$-\ln L$ for ML tree	19,955.769	2,638.068	4,456.544
Tree figure	Fig. 1	Fig. 2a	Fig. 2b

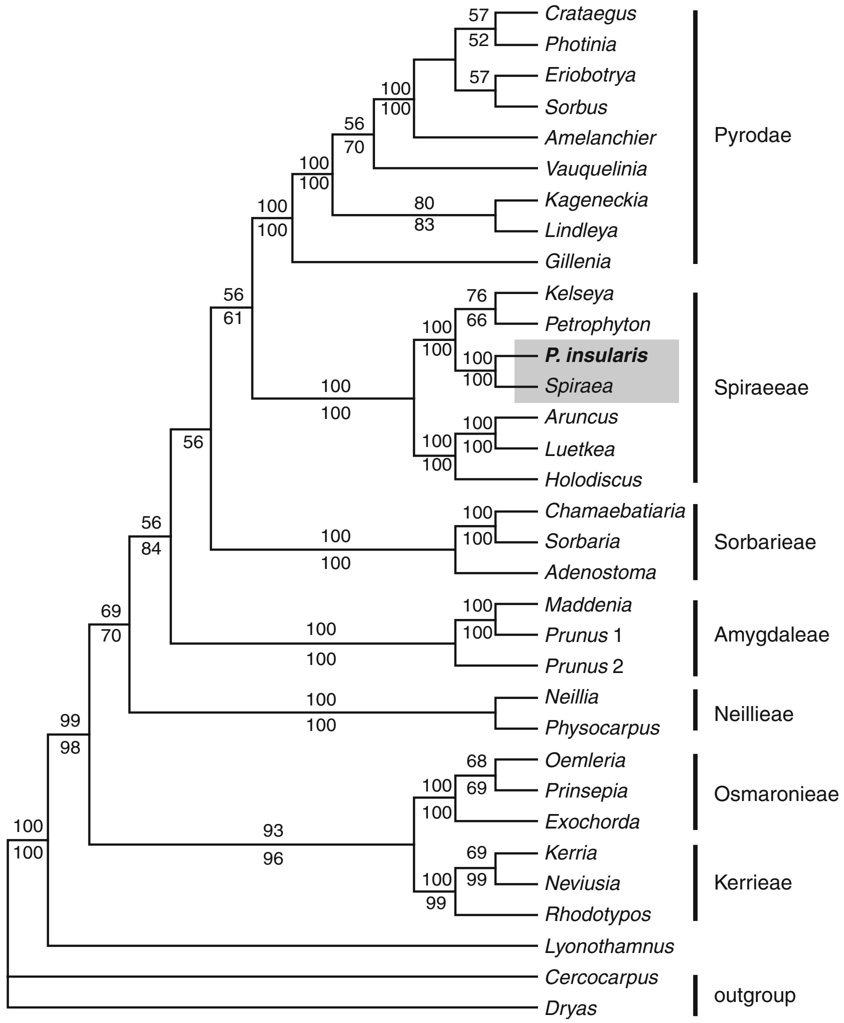


Fig. 1 One of four MP trees, which is identical to the ML tree, from phylogenetic analyses of the combined *rbcL*, *matK*, *ndhF*, and *trnL-trnF* data. Bootstrap values greater than 50% using MP are indicated *above branches* and those based on ML are *below*. Tribal and supertribal classification follows Potter et al. (2007a)

and *P. insularis*, but on average *P. insularis* had longer leaf blades (Table 3). For a given length, *P. insularis* had much wider leaves than *S. chamaedryfolia*, resulting in lower values of the leaf length to width ratio in the insular plants (Fig. 3). The leaves in both *P. insularis* and *S. chamaedryfolia* were ovate with the broadest point located below the middle from the base, but leaves of *S. chamaedryfolia* were more or less elliptical, especially those on the

Table 2 Comparison of selected morphological characters among *Physocarpus*, *Spiraea*, and *P. insularis*

Character	*Physocarpus*	*Spiraea*	*P. insularis*
Leaf structure	Simple	Simple	Simple
Leaf incision	3–5-lobed	Rarely lobed	Unlobed
Stipules	Present (deciduous)	Absent	Absent
Inflorescence	Corymb	Umbel, corymb, compound corymb, panicle	Corymb
Stellate hairs on leaf and inflorescence	Present	Absent	Absent
Nectary lobes at the rim of the hypanthium	Absent	Present	Present
Carpel connation	Connate at least at base	Free	Free
Follicle inflation	Inflated	Not inflated	Not inflated
Follicle dehiscence	Ventral and dorsal sutures	Ventral suture	Ventral suture
Endosperm	Copious	Absent or scarce	Unknown

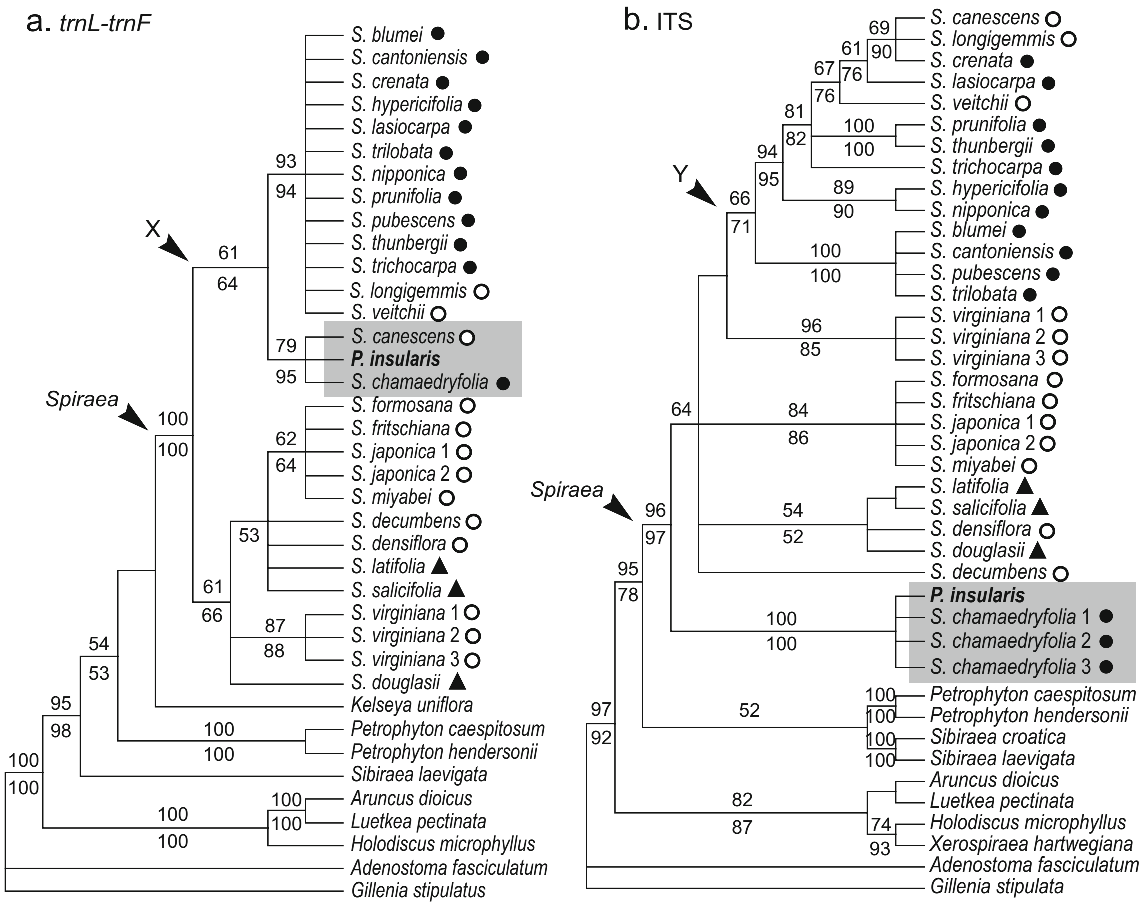


Fig. 2 The single MP tree of tribe Spiraeeae from phylogenetic analyses of the *trnL-trnF* data (**a**) and strict consensus tree of 54 MP trees based on ITS data (**b**). Bootstrap values greater than 50% using MP are indicated *above branches* and those based on ML are *below*. Sectional classification of each species within *Spiraea* (Rehder 1940) is indicated by one of the following symbols: *closed circle*=sect. *Chamaedryon*, *open circle* = sect. *Calospira*, and *closed triangle* = sect. *Spiraea*. The clades, in which species of sect. *Chamaedryon* is nested, are indicated by *X* (**a**) and *Y* (**b**)

flowering branches. Leaf base angle in *P. insularis* was larger than the angle measured in *S. chamaedryfolia* (Fig. 3). The leaf base was subcordate, truncate, or occasionally rounded in *P. insularis*, whereas the homologous structure was shortly attenuate or rounded in *S. chamaedryfolia*. There were no significant differences in floral characters between the two taxa, but *P. insularis* had more stamens than *S. chamaedryfolia*. The Ulleung Island plant had more than 60 stamens per flower, while *S. chamaedryfolia* had 20 to 50 stamens per flower.

Table 3 Measurements of leaf characters of *P. insularis* and *S. chamaedryfolia*

Character	*P. insularis*	*S. chamaedryfolia*
Leaf length (cm)*	4.3–(5.8)–7.8	2.1–(4.4)–7.5
Leaf width (cm)**	3.5–(4.6)–6.1	1.1–(2.3)–3.6
Length/ width ratio**	1.09–(1.28)–1.50	1.45–(1.95)–2.38
Leaf base angle (˚)**	133.5–(178.9)–228.0	74.7–(107.2)–144.0

Mean is indicated in a parenthesis flanked by minimum and maximum values

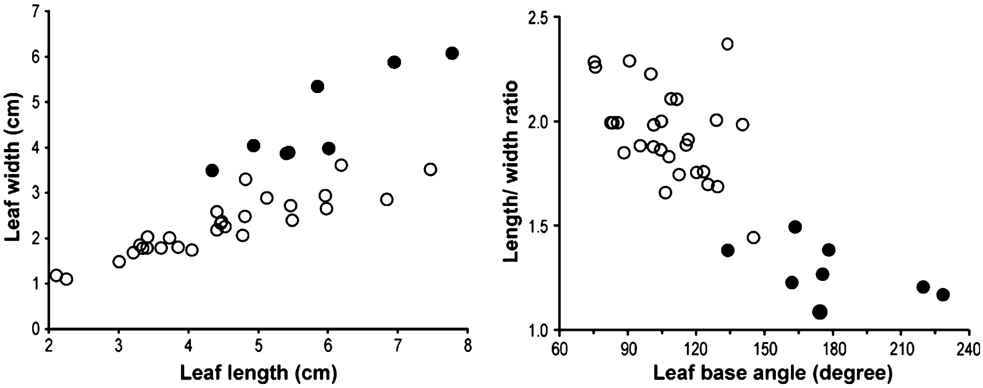
*$p<0.01$; **$p<0.001$

Discussion

Phylogenetic Position of *P. insularis* in Rosaceae

Our molecular phylogenetic analyses and comparative morphology strongly indicate that *P. insularis* is not a species of *Physocarpus* but a member of *Spiraea*. Morphology of *P. insularis* does not have synapomorphies for *Physocarpus* but does possess all the diagnostic characters of *Spiraea* (Table 2), consistent with the observation by Kim et al. (2000). Inflated follicular fruits dehiscent along both ventral and dorsal sutures and stellate hairs in leaves and inflorescence are synapomorphies for *Physocarpus* within

Fig. 3 Scatter plots of leaf characters. Symbols: *closed circle*=*P. insularis*, *open circle*=*S. chamaedryfolia*



Rosaceae (Oh and Potter 2005). Although there is no obvious morphological synapomorphy for *Spiraea*, two features, the lack of stipules and unitegmic ovules, are potential synapomorphies for tribe Spiraeeae (Potter et al. 2007a, b). *Spiraea* can be easily distinguished from other taxa within Spiraeeae by having erect shrubby habit, simple leaves with usually serrate margins, perfect flowers, and free carpels.

Traditionally, fruit type was used as an important character in Rosaceae classification, and shrubs with follicular fruits were once all classified in the genus *Spiraea* (Linnaeus 1753; Cambessèdes 1824; de Candolle 1825). Some species in *Physocarpus* were originally described in *Spiraea*, such as *Spiraea opulifolia* (=*P. opulifolius*). Medikus (1799) segregated *Opulaster* from *Spiraea* based on *S. opulifolia*, and Rafinesque (1838) later independently established *Physocarpus* based on the same species, but *Physocarpus* has been adopted because the earlier name *Opulaster* was invalidly published. When Nakai (1918) described *Opulaster insularis* (he transferred the species to *Physocarpus* in Nakai (1952)), morphological distinction between *Spiraea* and *Physocarpus* was well understood (Maximowicz 1879). Nakai himself knew about the diagnostic characters for the spiraeoid genera and recognized *Physocarpus* as a distinctive taxon having stipulate leaves and inflated follicular fruits dehiscent along both ventral and dorsal sutures (Nakai 1916).

Why, then, did Nakai (1918) classify the insular plant in *Physocarpus* not in *Spiraea*? Apparently, *P. insularis* was described based on a sterile material. Nakai (1918) in his protologue indicated that he only found one individual of the species on Ulleung Island, which did not bear any reproductive organs, and clearly stated that he did not examine any flowers or fruits. Without flowering materials, it is difficult, though not impossible, to identify a plant in Rosaceae. He postulated that the leaves of *P. insularis* are similar to those of *P. monogynus* distributed in western North America (Nakai 1919). Stipules in *Physocarpus* are deciduous such that the mature leaves of *P. monogynus* appear to be very similar to those of *P. insularis*. Both species have ovate leaves with doubly serrate margins. Even so, three- to five-lobed leaves and the presence of stellate hairs on both surfaces of leaves particularly on the vein are another reliable traits to distinguish *Physocarpus* from *Spiraea*. Unfortunately, Nakai did not recognize the taxonomic value of the vegetative characters (Nakai 1916, 1918, 1919). The holotype specimen of *P. insularis* preserved at Tokyo University does not have the diagnostic characters of *Physocarpus* but exhibits those of *Spiraea*: leaves are unlobed and estipulate and do not have stellate hairs.

Phylogenetic Relationship of *P. insularis* Within Spiraeeae

Our molecular analyses of Spiraeeae suggest that *P. insularis* is closely related to *S. chamaedryfolia* (Fig. 2), corroborating Kim et al. (2000). Both species have corymbose inflorescences borne on the lateral branch that develops from the preceding year's stem, two exterior scales in the bud, and ovate leaves with doubly serrate margins.

Morphological analysis indicates that *P. insularis* is distinctive from *S. chamaedryfolia* in having larger leaf blades that are subcordate or truncate at base and more stamens than *S. chamaedryfolia* (Table 3, Fig. 3). A propensity of enlargement of body size is commonly found in endemic species on Ulleung Island, such as *Acer okamotoanum* Nakai, *Hepatica maxima* Nakai, and *Rubus takesimensis* Nakai (Sun and Stuessy 1998), and also widely known on other islands (Carlquist 1965).

The level of genetic divergence between *P. insularis* and *S. chamaedryfolia*, however, is low. The *trnL-trnF* sequences of *P. insularis* and all three accessions of *S. chamaedryfolia* included in this study are identical. In fact, the sequences are identical to the sequence from *Spiraea canescens*. Such low level of molecular divergence of the neutral marker between insular species and its progenitor has also been reported in other endemic species on Ulleung Island. The nucleotide sequence of the *trnL-trnF* region of *A. okamotoanum* distributed on the island is identical to that of the Korean populations of *A. mono* Maxim., a putative progenitor of *A. okamotoanum* (Pfosser et al. 2002). There is only one nucleotide substitution in the same region in each of the following endemic–progenitor species pairs: *Acer takesimense* Nakai–*Acer pseudosieboldianum* (Pax) Kom. (Pfosser et al. 2002) and *Dystaenia takesimana* (Nakai) Kitag.–*Dystaenia ibukensis* (Y. Yabe) Kitag. (Pfosser et al. 2006).

The ITS data, however, show some differences between *P. insularis* and *S. chamaedryfolia*. *Physocarpus insularis* possesses one unique substitution, which distinguishes *P. insularis* from *S. chamaedryfolia*. Four of the five polymorphic sites in *P. insularis* contain a nucleotide not observed in *S. chamaedryfolia* included in the analysis (Table 4). For example, at the position 174, *P. insularis* has an A in addition to a C, while *S. chamaedryfolia* has only a C. This pattern of polymorphism suggests that *P. insularis* might have accumulated genetic changes through mutation and recombination in some of the copies of ribosomal DNA repeats yet to be homogenized within its population ever since the insular plants were established on the island. However, because of the broad geographic distribution of *S. chamaedryfolia* that our study did not cover, we cannot rule out the possibility that the polymorphisms were simply not sampled or even fixed in *S. chamaedryfolia*. It is anticipated that the sequence divergence between the insular plant and its progenitor is low given the recent origin of Ulleung Island at about 1.8 million years before present (Kim 1985), thus much younger age of the colonization and establishment of the spiraeoid plant on the island. Nevertheless, the pattern of variation in our ITS data may not be a negligible level compared with *A. takesimense*, where the endemic species has identical ITS sequence to the widespread *A. pseudosieboldianum* (Suh et al. 1996).

The Origin of *P. insularis*

Physocarpus insularis is the descendant of the established colonists of *S. chamaedryfolia* on Ulleung Island. It is very unlikely that *S. chamaedryfolia* was derived from *P. insularis*. Our molecular data imply that *S. chamaedryfolia*, the progenitor of *P. insularis*, may be of hybrid origin.

Phylogenetic placement of *P. insularis* and *S. chamaedryfolia*, both not included in the previous study of *Spiraea* (Potter et al. 2007b), was strongly conflicted between the chloroplast and nuclear data. In the *trnL*-*trnF* data, the two taxa along with *S. canescens* D. Don were placed within a moderately supported clade (denoted by X in Fig. 2a), in which all taxa of section *Chamaedryon* are nested. Section *Chamaedryon* is characterized by having a simple corymbose or umbellate inflorescence (Rehder 1940). This position of *P. insularis* and *S. chamaedryfolia*, which have corymbose inflorescences, is thus consistent with the pattern where species with simple inflorescences are restricted in the clade. The corresponding clade (denoted by Y in Fig. 2b) was also moderately supported in the ITS tree, except for *P. insularis* and *S. chamaedryfolia*. The *P. insularis*/*S. chamaedryfolia* clade was placed as the first diverging lineage in *Spiraea*, being sister to the remaining species of the genus. *Spiraea canescens* that forms a clade with *P. insularis*/*S. chamaedryfolia* in the cpDNA data was nested within the clade Y in the ITS data (Fig. 2b).

Sampling errors, incomplete lineage sorting, gene duplication/ extinction, and hybridization, or a combination of those may be the source of gene tree conflict (Doyle 1992). Because the strong conflict of the phylogenetic placement of the *P. insularis*/*S. chamaedryfolia* clade is between maternally inherited chloroplast and biparentally transmitted nuclear genomes, it is plausible that the incongruence is associated with hybridization. Hybridization and hybrid speciation are common in *Spiraea* (Rehder 1940) and Rosaceae in general (Potter et al. 2007a). It may be that *S. chamaedryfolia* captured chloroplast DNA from *S. canescens* via repeated hybridization/introgression (Rieseberg and Soltis 1991) or that *S. chamaedryfolia* originated via hybridization between *S. canescens* or its ancestor and unknown species without changing chromosome numbers. Most chromosome counts for *S. chamaedryfolia* have been reported as tetraploid with $2n=36$ (Ceschmedjiev 1983; Starlinger et al. 1994), and *S. canescens* is also a tetraploid with $n=18$ (Sandhu and Mann 1988). The number of chromosomes in *P. insularis* is unknown, however. More complete taxon sampling within *Spiraea* will be needed to draw a robust conclusion about the origin of *S. chamaedryfolia* as well as the cause of the gene tree conflict.

Conservation Implications

Our molecular and morphological data suggest that *P. insularis* should be recognized as a distinct taxon in the

Table 4 Variable sites in the ITS region between *P. insularis* and *S. chamaedryfolia* studied

Taxon	Position in alignment						
	41	174	475	478	538	543	555
P. insularis	T	*A*/C	*G*/T	A/T	C/*T*	*T*	*C*/T
S. chamaedryfolia 1	G	C	T	A	C	C	T
S. chamaedryfolia 2	T	C	T	A	C	C	T
S. chamaedryfolia 3	G	C	T	T	C	C	T

Unique changes in *P. insularis* are indicated with italics genus *Spiraea*. Distribution of *P. insularis* is extremely restricted on Ulleung Island, and the total number of individuals is estimated between 300 and 500 plants (J. Hyun, personal communication). Currently, *P. insularis* is listed in the Rare Plants Data Book in Korea by Forest Service (Lee 2008), but not registered under any categories in the endangered species list maintained by the Ministry of Environment of Korea (URL: http://www.me.go.kr).

Most individuals of *P. insularis* grow in the Do-dong area where a natural habitat of *Abelia insularis* Nakai and *Cotoneaster wilsonii* Nakai has been protected as a Natural Monument of Korea. Population of *P. insularis*, however, is susceptible to soil erosion because they occupy the steep rocky slopes. One of the concerns regarding the conservation of *P. insularis* related to human activities is hybridization. Due to the low genetic divergence between the progenitor and insular species, the progenitor's genome could be assimilated into the insular plants eliminating evolutionary footprints on Ulleung Island, if progenitor species *S. chamaedryfolia* is introduced into Ulleung Island and hybridized with *P. insularis*. Morphological traits analyzed in this study may be utilized to identify the species in a specific conservation program.

At issue is the rank-ordered prioritization of species based on rarity and restricted distribution such as the Red List guidelines (International Union for the Conservation of Nature 2008) for insular taxa, as most endemic species can be listed as threatened species (Martin 2009). All endemic species on Ulleung Island would meet the requirements for the category because the island can be treated as one location (Pfosser et al. 2006) and because the effects of anthropogenic activities have increased (Lim et al. 2008), which raises concerns for the efficacy of conservation programs with the prioritization system alone. But more difficult problems may arise when species delimitation is controversial. For example, implication for conservation programs on *P. insularis* would be sensitive to what taxonomic conclusion is made. If *P. insularis* should be recognized as a distinct taxon in *Spiraea*, as suggested in this study, it would qualify for the "Critically Endangered" category (B1ab) or, conservatively, the "Vulnerable" category (D1) under the Red List guidelines. By contrast, when *P. insularis* is considered as conspecific with *S. chamaedryfolia*, as proposed by Kim et al. (2000), plants recognized as *P. insularis* merely represent the insular population of a broadly distributed species of *Spiraea*; therefore, it has no conservation priority over other threatened species. Species delimitation issue, however, is not confined exclusively to endemic species. It is uncertain about the conservation status on nonendemic native taxa on Ulleung Island that are also distributed elsewhere. Little is known to what extent these native plants may actually be distinct taxa because most current recognition of endemic vs. nonendemic category was primarily derived from floristic surveys and because morphological differentiation of insular plants is weak (Sun and Stuessy 1998). The recent phylogenetic study of *Tsuga* (Havill et al. 2008) shows the possibility that biodiversity on the island may have been underestimated. Plants identified as *Tsuga sieboldii* Carrière on Ulleung Island, which also occurs in Japan, actually form a distinct group more closely related to another Japanese hemlock species *Tsuga diversifolia* (Maxim.) Mast. than to the Japanese *T. sieboldii*, suggesting that the Ulleung Island population of *T. sieboldii* is an evolutionarily significant unit (Moritz 1994; Avise 2005) or a distinct taxon.

The ultimate goal of conservation biology is conservation of overall biodiversity. Ulleung Island is a unique place not only harboring a variety of biogeographic components of East Asia (Oh 1978) but also serving as a model system for an island with the highest level of anagenetic speciation (Stuessy et al. 2006). Furthermore, Ulleung Island is at the early stage of development in the ontogeny of an oceanic island (Stuessy 2007), and new species have been continuously discovered on the island (Lee et al. 1997; Lee 1998; Lim et al. 2003, 2005). If conservation programs focus on only endemic or rare species, it may not secure future biodiversity. One way to overcome this problem is to adopt a system that prioritizes areas for conservation, which also permits to employ specific conservation programs for species under threat of extinction (Avise 1996; Shin and Kim 2002). For example, Ulleung Island may be designated as a biodiversity sanctuary to protect the natural environment and to conserve evolutionary patterns and processes.

Acknowledgements We are grateful to the curators and staff of the aforementioned herbaria, particularly to Dr. Hiroshi Ikeda and Akiko Shimizu of Tokyo University. The authors thank Dr. Jin-O Hyun for valuable discussions. We also thank Jung-Woo Chae (Ulleung-gun Agricultural Technology Center) and Seong-Hyun Cho (Hallym University) to obtain materials of *P. insularis*. This work was supported in part by research funds (to SO) from Cornell University and Boyce Thompson Institute for Plant Research and a research grant (to YK) titled, "Origin of biological diversity of Korea: molecular phylogenetic analyses of major Korean taxa" from the National Institute of Biological Resources, Korea.

Appendix 1

A list of taxa and GenBank numbers used in the phylogenetic analysis of Spiraeoideae. Each entry includes GenBank accession number, in the order, for *matK, ndhF, rbcL*, and *trnL-trnF*. NA means that the particular sequence was not available.

Adenostoma: AF288095, DQ851497, U06790, NA; *Amelanchier*: DQ860450, DQ851498, U06793, DQ863222; *Aruncus*: AF288094, DQ851501, U06794, AF348536. *Cer-*

cocarpus: AF288095, DQ851503, U06796, AF348537; *Chamaebatiaria*: AF288097, DQ851506, U06797, AF348539; *Crataegus*: DQ860458, DQ851511, U06799, DQ863230; *Dryas*: DQ851225, DQ851516, U59818, DQ851231; *Eriobotrya*: DQ860462, DQ851517, U06800, DQ863234; *Exochorda*: AF288100, DQ851519, U06801, AF348542; *Gillenia*: AF288103, DQ851523, DQ250747, AF348554; *Holodiscus*: AF288105, DQ851525, U06807, AF348546; *Kageneckia*: DQ860447, DQ851526, U06808, AF348547; *Kelseya*: DQ851226, NA, NA, NA; *Kerria*: NA, DQ851527, DQ250749, NA; *Lindleya*: DQ860448, DQ851528, U06810, DQ863220; *Luetkea*: DQ851227, NA, DQ250750, DQ851233; *Lyonothamnus*: AF288107, DQ851529, U06811, AF348548; *Maddenia*: DQ851228, NA, NA, AY864827; *Neillia* AF288108, DQ851534, U06813, AF487229; *Neviusia*: AF288109, DQ851535, U06815, AF348550; *Oemleria*: AF288110, DQ851536, U06816, AF348551; *P. insularis*: GU217789, GU217790, GU217791, GU217792; *Petrophyton*: DQ851229, NA, NA, NA; *Photinia*: DQ860470, DQ851539, L11200, DQ863242; *Physocarpus*: AF288112, DQ851540, U06817, AF348553; *Prinsepia*: AF288114, DQ851542, U06819, AF348558; *Prunus* 1: AF288116, DQ851544, U06809, AF348559; *Prunus* 2: AF288115, DQ851543, AF206813, AF348560; *Rhodotypos*: AF288122, NA, U06823, AF348566; *Sorbaria*: AF288125, DQ851553, U06826, AF348569; *Sorbus*: AF288126, DQ851554, U06827, DQ8632470; *Spiraea*: AF288127, DQ851556, L11206, AF348571; *Vauquelinia*: AF288129, DQ851560, U06829, AF348573.

Appendix 2

A list of taxa and GenBank numbers used in the phylogenetic analysis of Spiraeeae. Each entry includes GenBank accession number, in the order, for ITS and *trnL-trnF*. NA means that the particular sequence was not available.

Adenostoma fasciculatum Hook. and Arn.: DQ886358, AF348535; *Aruncus dioicus* (Walter) Fern.: DQ897602, AF196868; *Gillenia stipulata* (Muhl. Ex Willd.) Baillon: DQ811763, AF348554; *Holodiscus microphyllus* Rydb.: DQ897603, DQ897573; *Kelseya uniflora* (Wats.) Rydb.: NA, DQ851232; *Luetkea pectinata* (Pursh) Kuntze: DQ851235, DQ851233; *Petrophyton caespitosum* (Nutt.) Rydb.: DQ851236, DQ851234; *Petrophyton hendersonii* (Canby) Rydb.: DQ897604, DQ897574; *P. insularis* (Nakai) Nakai, Korea, Kyongsangbuk-do, Ulleung-gun, Ulleung-gun Agricultural Technology Center, *Cho 63* (HHU): GU217794, GU217792; *Sibiraea croatica* Degen: AJ876553, NA; *Sibiraea laevigata* (L.) Maxim.: DQ897605, DQ897575; *Xerospiraea hartwegiana* Henrickson: DQ897606, NA; *Spiraea blumei* G. Don.: DQ897607, DQ897576; *S. canescens* D. Don.: DQ897608, DQ897577; *Spiraea cantoniensis* Lour.: DQ897609, DQ897578; *S. chamaedryfolia* L. 1, Korea. Kangwon-do, Taebaek-si, Changjuk-dong, Geom-ryong-so, *Kim and Cho 2009-1*: GU217795, GU217793; *S. chamaedryfolia* 2, Korea. Kangwon-do, Taebaek-si, Changjuk-dong, Geom-ryong-so, *Kim and Cho 2009-2*: GU217796, GU217793; *S. chamaedryfolia* 3, Korea. Kangwon-do, Taebaek-si, Changjuk-dong, Geom-ryong-so, *Kim and Cho 2009-3*: GU217797, GU217793; *Spiraea crenata* L.: DQ897610, DQ897579; *Spiraea decumbens* W. D. J. Koch “White Lace”: DQ897611, DQ897580; *Spiraea densiflora* Nutt. ex Rydb.: DQ886362, AF348571; *Spiraea douglasii* Hook.: DQ897612, DQ897581; *Spiraea formosana* Hayata: DQ897613, DQ897582; *Spiraea fritschiana* Schneid.: DQ897614, DQ897583; *Spiraea hypericifolia* L.: DQ897615, DQ897584; *Spiraea japonica* L. f. 1, DQ897616, DQ897585; *S. japonica* 2 “Neon Flash”: DQ897617, DQ897586; *Spiraea lasiocarpa* Kar. and Kir.: DQ897618, DQ897587; *Spiraea latifolia* (Ait.) Borkh.: DQ897619, DQ897588; *Spiraea longigemmis* Maxim.: DQ897620, DQ897589; *Spiraea miyabei* Koidz.: DQ897621, DQ897590; *Spiraea nipponica* Maxim.: DQ897622, DQ897591; *Spiraea prunifolia* Siebold and Zucc.: DQ897623, DQ897592; *Spiraea pubescens* Turcz.: DQ897624, DQ897593; *Spiraea salicifolia* L.: DQ897625, DQ897594; *Spiraea thunbergii* Siebold ex Blume: DQ897626, DQ897595; *Spiraea trichocarpa* Nakai, DQ897627, DQ897596; *Spiraea trilobata* L.: DQ897628, DQ897597; *Spiraea veitchii* Hemsl.: DQ897629, DQ897598; *Spiraea virginiana* Britton 1: DQ897630, DQ897599; *S. virginiana* 2: DQ897631, DQ897600; *S. virginiana* 3: DQ897632, DQ897601

Appendix 3

Specimens used in leaf character analysis. Specimen images downloaded from Korea Biodiversity Information System are indicated with the accession numbers in the database following herbaria in which the original specimens are deposited.

P. insularis: Korea. *Cho 63* (HHU), *Kim and Han s.n.* (KFI; KNHA200008092158), *Nakai 4344* (TI), *Park s.n.* (SKK; SKKA200010051017), *Sun et al. 3621* (JNU; CBBA 200103231025), *Sun et al. 3621* (JNU; CBBA2001 03221097), *Woo 118* (EWH; ENHA200506152016), *Woo 123* (EWH; ENHA200506152017).

S. chamaedryfolia: Hungary. *Kurimay s.n.* (BH). Japan. *Ohwi s.n.* (BH), *Okuyama s.n.* (BH). Korea. *Cha s.n.* (KUS; KUSA200505232004), *Heo and Koh s.n.* (SKK; SKKA 200508172181), *Im 5042* (CNU; CNNA200009011165), *Kim and Choi s.n.* (KFI; KNHA200008162024), *Kim and Choi s.n.* (KFI; KNHA200008162025), *Kim and Han s.n.* (KFI; KNHA200008162041), *Ko and Kang s.n.* (HNHM;

HNHA200506301013), *Koh s.n.* (KUS; KUSA200508172180), *Lee and Cho 8564* (SNUA; SNFA200008162087), *Lee and Cho 8564* (SNUA; SNFA200008162088), *Lee and Jeon S-3865* (KH; KNKA200607211037), *Lee 8843* (KWNU; KWNA200006291076), *Lee 8845* (KWNU; KWNA200006291078), *Lee 8848* (KWNU; KWNA200006291081), *Lee 8853* (KWNU; KWNA200006291086), *Lee s.n.* (SNUA; SNFA200008162096), *March et al. 7* (SNUA; SNFA200008162086), without collector's name and number (EWH; ENHA200505172001). Romania. *Sagorski s.n.* (BH), *Simkovics s.n.* (BH), *Wolff s.n.* (BH).

References

Avise JC (1996) Toward a regional conservation genetics perspective: phylogeography of faunas in the southeastern United States. In: Hamrick JL, Avise JC (eds) Conservation genetics case histories from nature. Chapman & Hall, New York, pp 431–470

Avise JC (2005) Phylogenetic units and currencies above and below the species level. In: Purvis A, Gittleman JL, Brooks T (eds) Phylogeny and conservation. Cambridge University Press, Cambridge, pp 76–100

Cambessèdes MJ (1824) Monographie des Spirées. Ann Sci Nat 1:352–391

Carlquist S (1965) Island life, a natural history of the islands of the world. The Natural History Press, Garden City

Ceschmedjiev IV (1983) IOPB chromosome number reports LXXX. Taxon 32:506

Chang CS, Jeon JI (2003) Leaf flavonoids in *Cotoneaster wilsonii* (Rosaceae) from the island Ulleung-do, Korea. Biochem Syst Ecol 31:171–179

Chung TH (1957) Korean flora. Shinjisa, Seoul

Chung HG, Chung JM, Chung MG (1998) Allozyme variation in six flowering plant species characterizing Ullung Island, Korea. J Jpn Bot 73:241–247

de Candolle AP (1825) Prodromus Systematis Naturalis Regni Vegetabilis, vol 2. Sumptibus Sociorum Treuttel et Wurtz, Paris

Doyle JJ (1992) Gene trees and species trees: molecular systematics as one-character taxonomy. Syst Bot 17:144–163

Fay MF, Swensen SM, Chase MW (1997) Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). Kew Bull 52:111–120

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Havill NP, Campbell CS, Vining TF, LePage B, Bayer RJ, Donoghue MJ (2008) Phylogeny and biogeography of *Tsuga* (Pinaceae) inferred from nuclear ribosomal ITS and chloroplast DNA sequence data. Syst Bot 33:478–489

International Union for the Conservation of Nature (2008) Guidelines for using the IUCN red list categories and criteria. www.iucnredlist.org/documents/redlist_guidelines_v1223290226.pdf

Kim YK (1985) Petrology of Ulreung volcanic island, Korea, part 1, geology. J Jpn Assoc Mineral Petrol Econ Geol 80:128–135

Kim TJ (2007) *Spiraea*. In: Park CW (ed) Genera of vascular plants of Korea. Academy, Seoul, pp 535–538

Kim CH, Kim TJ, Sun BY (2000) Taxonomic identities of some endemic Korean vascular plants. Korean J Plant Taxon 30:355–361

Kim YS, Chang CS, Shin HT, Kim H, Choi DY (2002) Conservational status of *Cotoneaster wilsonii* on Island Ulleung-do. Korean J Plant Taxon 32:159–175

Kim JS, Yang BH, Chung JM, Lee BC, Lee JC (2006) Genetic diversity and structure of a rare and endemic, spring ephemeral plant *Corydalis filistipes* Nakai of Ullung Island in Korea. J Ecol Field Biol 29:247–252

Kim SY, Kim SH, Shin H, Kim YD (2009) Molecular phylogeny of *Astilbe*: implications for phylogeography and morphological evolution. Korean J Plant Taxon 39:35–41

Ku YB, Oh HK, Kong HY, Suh MH, Lee MH, Trybush S, Cho KH (2004) Genetic diversity and differentiation in remnant populations of *Bupleurum latissimum* Nakai, an endangered endemic plant species to Ulleung Island, Korea. Korean J Biol Sci 8:289–294

Lee TB (1980) Illustrated flora of Korea. Hyangmunsa, Seoul

Lee YN (1998) New taxa on Korean flora (6). Korean J Plant Taxon 28:25–39

Lee S (2007) *Physocarpus*. In: Park CW (ed) Genera of vascular plants of Korea. Academy, Seoul, pp 538–539

Lee BC (2008) Rare plants data book in Korea. Korea National Arboretum, Pocheon

Lee WT, Yang IS (1981) The flora of Ulreung Is. and Dogdo Island. Rep KACN 19:61–95

Lee S, Lee J, Kim S (1997) A new species of *Adenophora* (Campanulaceae) from Korea. J Plant Res 110:77–80

Lim Y, Hyun JO, Shin H (2003) *Aster pseudoglehni* (Asteraceae), a new species from Korea. J Jpn Bot 78:203–207

Lim Y, Hyun JO, Kim YD, Shin H (2005) *Aster chusanensis* (Asteraceae), a new species from Korea. J Plant Biol 48:479–482

Lim Y, Kim YD, Shin H (2006) Lectotypication and identity of *Thymus quinquecostatus* var. *magnus* (Nakai) Kitam. (Labiatae). Korean J Plant Taxon 36:129–136

Lim Y, Na ST, Lee SJ, Cho KH, Shin H (2008) Spatial distribution patterns and implications for conservation of *Scrophularis takesimensis* (Scrophulariaceae), an endangered endemic species on Ulleung Island, Korea. J Plant Biol 51:213–220

Linnaeus C (1753) Species Plantarum. Impensis Laruentii Salvii, Stockholm

Martin JL (2009) Are the IUCN standard home-range thresholds for species a good indicator to prioritse conservation urgency in small islands? A case study in the Canary Islands (Spain). J Nat Conserv 17:87–98

Maximowicz CJ (1879) Adnotationes de Spiraeaceis. Trudy Imperatorskago S-Peterburgskago Botaniceskago Sada 6:105–261

Medikus FK (1799) Beyträge zur Pflanzen-Anatomie. Heinrich Gräff, Leipzig

Morgan DR, Soltis DE, Robertson KR (1994) Systematic and evolutionary implications of *rbcL* sequence variation in Rosaceae. Am J Bot 81:890–903

Moritz C (1994) Defining 'evolutionarily significant units' for conservation. Trends Ecol Evol 9:373–375

Nakai T (1916) Spiraeaceae, Flora Sylvatica Koreana, Pars IV. The Government of Chosen, Seoul, pp 1–29

Nakai T (1918) Notulae ad plantas Japoniae et Koreae XVII. Bot Mag Tokyo 32:103–110

Nakai T (1919) Report on the vegetation of the Island Ooryongto or Dagelet Island, Corea. The Government of Chosen, Seoul

Nakai T (1952) A synoptical sketch of Korean flora. Bull Natl Sci Mus (Tokyo) 31:1–152

Nixon KC, Carpenter JM (1996) On consensus: collapsibility, and clade concordance. Cladistics 12:305–321

Oh SY (1978) Floral studies on the vascular plants of the Dagelet Island. Research Review of Kyungpook National University 25:131–201

Oh SH, Potter D (2005) Molecular phylogenetic systematics and biogeography of tribe Neillieae (Rosaceae) using DNA sequences of cpDNA, rDNA, and *LEAFY*. Am J Bot 92:179–192

Ohkawa T, Kitamura K, Takasu H, Kawano S (2006) Genetic variation in *Fagus multinervis* Nakai (Fagaceae), a beech species endemic to Ullung Island, South Korea. Plant Species Biol 21:135–145

Olmstead RG, Sweere JA (1994) Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Syst Biol 43:467–481

Park CW, Oh SH, Shin H (1993) Reexamination of vascular plants in Ullung Island, Korea II: taxonomic identity of *Acer takesimense* Nakai (Aceraceae). Korean J Plant Taxon 23:217–231

Pfosser MF, Guzy-Wrobelska J, Sun BY, Stuessy TF, Sugawara T, Fujii N (2002) The origin of species of *Acer* (Sapindaceae) endemic to Ullung Island, Korea. Syst Bot 27:351–367

Pfosser M, Jakubowsky G, Schluter PM, Fer T, Kato H, Stuessy TF, Sun BY (2006) Evolution of *Dystaenia takesimana* (Apiaceae), endemic to Ullung Island, Korea. Plant Syst Evol 256:159–170

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818

Potter D, Gao F, Bortiri PE, Oh SH, Baggett S (2002) Phylogenetic relationships in Rosaceae inferred from chloroplast *matK* and *trnL-trnF* nucleotide sequence data. Plant Syst Evol 231:77–89

Potter D, Eriksson T, Evans RC, Oh SH, Smedmark JEE, Morgan DR, Kerr M, Robertson KR, Arsenault M, Dickinson TA, Campbell CS (2007a) Phylogeny and classification of Rosaceae. Plant Syst Evol 266:5–43

Potter D, Still SM, Grebenc T, Ballian D, Bozic G, Franjiae J, Kraigher H (2007b) Phylogenetic relationships in tribe Spiraeeae (Rosaceae) inferred from nucleotide sequence data. Plant Syst Evol 266:105–118

Rafinesque CS (1838) New flora of North America. Philadelphia

Rasband WS (2007–2009) ImageJ. US National Institutes of Health, Bethesda, http://rsb.info.nih.gov/ij/

Rehder A (1940) Manual of cultivated trees and shrubs hardy in North America, exclusive of the subtropical and warmer temperate regions, 2nd edn. Macmillan, New York

Rieseberg LH, Soltis DS (1991) Phylogenetic consequences of cytoplasmic gene flow in plants. Evol Trends Plants 5:65–84

Rieseberg LH, Swensen SM (1996) Conservation genetics of endangered island plants. In: Avise JC, Hamrick JL (eds) Conservation genetics case histories from nature. Chapman & Hall, New York, pp 305–334

Sandhu PS, Mann SK (1988) SOCGI plant chromosome number reports—VII. J Cytol Genet 23:219–228

Shimodaira H, Hasegawa M (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molec Biol Evol 16:1114–1116

Shin H, Choi HK (1997) A taxonomic study on *Thymus* in Korea: numerical analyses of morphological characters. Korean J Plant Taxon 27:117–135

Shin HT, Kim YS (2002) The establishment of conservation area and conservation strategy in Ulleung Island (I)—flora. Korean J Environ Ecol 16:195–216

Stanford AM, Harden R, Parks CR (2000) Phylogeny and biogeography of *Juglans* (Juglandaceae) based on *matK* and ITS sequence data. Am J Bot 87:872–882

Starlinger F, Vitek E, Pascher K, Kiehn M (1994) Neue chromosomenzählungen für die flora Rumäniens. In: Heltmann H, Wendelberger G (eds) Naturwissenschaftliche Forschungen über Siebenbürgen V: Beiträge zur Flora, Vegetation und Fauna von Siebenbürgen, vol 30. Böhlau, Köln, pp 181–194

Stuessy TF (2007) Evolution of specific and genetic diversity during ontogeny of island flora: the importance of understanding process for interpreting island biogeographic patterns. In: Ebach MC, Tangney RS (eds) Biogeogrpahy in a changing world. CRC, Boca Raton, pp 117–133

Stuessy TF, Crawford DJ, Marticorena C (1990) Patterns of phylogeny in the endemic vascular flora of the Juan-Fernandez Islands, Chile. Syst Bot 15:338–346

Stuessy TF, Jakubowsky G, Gomez RS, Pfosser M, Schluter PM, Fer T, Sun BY, Kato H (2006) Anagenetic evolution in island plants. J Biogeogr 33:1259–1265

Suh Y, Cho HJ, Kim S, Park CW (1996) Comparative analysis of ITS sequences from *Acer* species (Aceraceae) in Korea. J Plant Biol 39:1–8

Sun BY, Stuessy TF (1998) Preliminary observations on the evolution of endemic angiosperms of Ullung Island, Korea. In: Ono M, Stuessy TF (eds) Evolution and speciation of island plants. Cambridge University Press, Cambridge, pp 181–202

Sun BY, Kim TJ, Kim CH (1997) A biosystematic study on polyploid populations of the genus *Spiraea* (Rosaceae) in Korea. J Plant Biol 40:291–297

Swofford DL (2002) PAUP* Phylogenetic Analysis Using Parsimony (* and other methods), version 4.0. Sinauer, Sunderland

Weiss H, Sun BY, Stuessy TF, Kim CH, Kato H, Wakabayashi M (2002) Karyology of plant species endemic to Ullung Island (Korea) and selected relatives in peninsular Korea and Japan. Bot J Linn Soc 138:93–105

White TJ, Birns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322

Woo HK, Kim JH, Yeau SH, Lee NS (2002) Morphological and isozyme divergence in Korean *Hepatica* sensu stricto (Ranunculaceae). Plant Syst Evol 236:33–44

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. thesis. The University of Texas, Austin