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Phylogeny of Korean *Rubus* (Rosaceae) Based on ITS (nrDNA) and trnL/F Intergenic Region (cpDNA)

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We examined the phylogeny of the genus *Rubus* in Korea using an internal transcribed spacer (ITS) of the nuclear ribosomal DNA and a trnL-trnF (trnL/F) intergenic region of the chloroplast DNA. In all, 21 ingroup species (1.2 kb for each species) were analyzed using parsimony, resulting in 672 aligned sequences from ITS, and 502 bases with trnL/F. Individual and combined analysis of ITS and trnL/F data proved that the genus *Rubus* is a monophyletic group. This phylogeny also substantiated a previous sectional classification scheme rather than a subgenus classification scheme. However, our results did not support the earlier sectional classification by Focke (sect. *Corchorifolii*), but did support the sectional classification of Nakai: sect. *Crataegifolii* (*R. crataegifolius*, *R. takesimensis* and *R. trifidus*), and sect. *Villosii* (*R. corchorifolius*). Most of these species, which are found in Korea and belong to subg. *Idaeobatus*, appeared in two different groups in all data sets. This suggests that this subgenus is a polyphyletic group that has gone through at least two independent evolutionary processes. The taxa, when mapped onto the combined tree, showed that the occurrence of their morphological characters of simple and compound leaves was concurrent in Korean *Rubus*. ITS sequence data were consistent overall with the geographical distribution of each species. Furthermore, the trnL/F sequence data provided phylogenetic information within closely related species.

Keywords: chloroplast DNA (trnL/F), classification, internal transcribed spacer (ITS), phylogeny, *Rubus*

The genus *Rubus* L. belongs to Rosoideae, a subfamily comprising herbs and shrubs in the family of Rosaceae. *Rubus* is distributed worldwide, from the lowland tropics to the subartic region (Thompson, 1995), and is found on all continents except Antarctica (Focke, 1910, 1911, 1914). It exhibits tremendous morphological diversity, with species showing woody, semi-herbaceous, herbaceous, upright, prostrate, or climbing characteristics (Waugh et al., 1990; Alice and Campbell, 1999). This genus is economically and ecologically important as fruit or ornamentals, but can be an invasive weed during early forest succession (Thompson, 1995; Hummer, 1996; Howarth et al., 1997; Alice and Campbell, 1999).

The species circumscription is complicated by agamospermy, polyploidy, and hybridization (Thompson, 1995; Alice and Campbell, 1999). Most of the global taxonomic treatments of *Rubus* implement the Focke monographs (1910, 1911, 1914). Since those early studies, however, its regional variation in taxa morphology has not been researched worldwide, and classification of its species has not been agreed upon by all taxonomists.

Most species of Korean *Rubus* belong to subg. *Idaeobatus*; polyploidy is not prevalent in that geographical region (Thompson et al., 1997). Many taxonomists have suggested different classification systems for that particular genus (Nakai, 1909; Lee WC, 1996). The majority of the recent studies on Korean *Rubus* has been limited to chromosomal research of a few taxa (Iwatsubo and Naruhasi, 1991, 1993; Thompson, 1995). New species have been found only on Geoje Island (*R. longisepalus* Nakai), Jeju Island (*R. hongnoensis* Nakai), and Ulreung Island (*R. takesimensis* Nakai) (Lee, 1979; Lee WC, 1996; Lee YN, 1996).

Using molecular phylogeny, the rbcL gene, ITS region, and ndhF gene markers have been applied in studying phylogenetic relationships at the family level (Morgan et al., 1994; Howarth et al., 1997; Alice and Campbell, 1999; Potter et al., 2000). The non-coding region generally changes more rapidly than does the coding region. The intergenic region, i.e., between the trnL and trnF regions, has a high substitution ratio, and is usually used to reveal phylogenetic relationships at the intraspecific or interspecific level (Taberlet et al., 1991). Therefore, the trnL/F non-coding region of chloroplast DNA (cpDNA) and the internal transcribed spacer region (ITS) of nuclear ribosomal DNA (nrDNA) were selected as molecular markers for this current study.

Based on the rbcL gene phylogeny (Morgan et al.,

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1994), *Rubus* occurs as the sister of *Rosa* L. and *Sanguisorba* L. This relationship is also validated by trnL/F and ITS analyses (Smedmark and Eriksson, 2002). In the phylogeny of Rosoideae based on the latter technique (Eriksson et al., 1998), *Rubus* has also been identified as a sister group of *Geum* L., *Waldsteinia* Wild. and *Fallugia* Endl. Because *Rubus* displays both woody and herbaceous traits, and because *Rosa* represents woody species while *Geum* represents herbaceous species, we selected *Rosa* and *Geum* as the outgroups. This classification system follows that of Focke (1910, 1911, 1914). Therefore, the purpose of our study was to investigate the phylogenetic relationships among all Korean *Rubus*, including the endemic species, and to compare these molecular data with results from previously proposed classification systems (Nakai, 1909; Lee, 1979; Lee WC, 1996; Lee YN, 1996).

MATERIAL AND METHODS

Plant Materials and DNA Extraction

Plant materials for 21 taxa of Korean *Rubus* (Table 1) were transplanted into the experimental garden of

Table 1. Sampling sites for plant materials used in DNA study. Classification system follows that of Focke (1910, 1911).

Taxon		Sources
Subg. *Anoplobatus*		
	R. ribesoideus Matsum.	KN: Keojeo-si, Dundeok-myeon, Beobdong-ri
	R. trifidus Thunb.	JJ: Jejusi Dang-orm (JJ)
Subg. *Cylactis*		
	R. articus L.	Mt. Baekdu (BD)
Subg. *Idaeobatus*		
Sect. *Corchorifolii*	*R. crataegifolius* Bunge	CC: Anmyeon-do, Anmyeon-uep, Sinya-ri, Hangukyakun-meadow (HK) Mt. Baekdu (BD)
	R. corchorifolius L. f.	CC: Anmyeon-do, Anmyeon-uep, Seungjeon-ri, Temple- Songrim
Sect. *Idaeocanthi*	*R. coreanus* Miq.	CN: Mt. Jiri-san, Hwauep Temple
	R. idaeus L.	KW: Taebaec-si, Hwacheon-dong, Ssarijae (TB), Mt. Baekdu (BD)
	R. parvifolius Thunb.	KB: Kyeongsan-si, Hayang-eup, Mt. Muhak (MH), China Yunnam-seong (YN) KN: Geojeo-si Dundeok-myeon, Beobdong-ri (6X) (GJ)
	R. parvifolius var. *taquetii* Nakai	JJ: Seogwipo-si, waterfall of Cheonjiyeon JJ: Jeju-si, Ara-dong, Jeju University
	R. phoenicolasius Maxim	CN: Wando-gun, Wando
	R. schizostylus Le'v.	JJ: Bukjeju-gun, Jocheon-eup, Seonheul-ri, Isidol Pasture
Sect. *Rosoefolii*	*R. croceacanthus* Le'v.	JJ: Seogwiposi, Hawon-dong, Temple-Buphwa
	R. hirsutus Thunb.	KB: Is. Ulreung-do, CN: Yeosu-si, Odong-do
	R. sorbifolius Maxim.	JJ: Jeju-si, Ora-dong, Minoreum
Sect. *Pungentes*	*R. oldhami* Miq.	JJ: Mt. Hanra-san (Yeoungsil →)
	R. hongnoensis Nakai*	JJ: Seogwiposi, waterfall of Cheonjiyeon
	R. longisepalus Nakai*	KN: Geojeo-si, Dongbu-myeon, Mt. Noja
	R. longisepalus var. *tozawai* Nakai*	KN: Geojeo-si, Dongbu-myeon, Mt. Noja
	R. takesimensis Nakai*	KB: Is. Uleung-do, Dodong (DD), Uleung-do, peak of Sungin (SI)
Subg. *Malachobatus*		
	R. buergeri Miq	JJ: Seogwiposi, Tonneko Valley (TK), Jejusi, Dang-orm (DO)
Subg. *Rubus*		
	R. fruticosus L. agg.	KN: Chanwon-si Dond-ep Gaweul-ri Reservoir Junam
Geum japonicum Thunb.		KB: Cheongsong-gun, Mt. Myeonbong
Rosa multiflora Thunb.		KB: Cheongsong-gun, Mt. Myeonbong

KB, Kyeongsangbuk-do; KN, Kyeongsangnam-do; CC, Chungcheongnam-do; KW, Kangwon-do; CN, Cheonranam-do; JJ, Jeju-do.
( ), Abbreviation of population localities. *, These taxa were not mentioned by Focke WO.

the Department of Biology at Kyungpook National University (KNU). Voucher specimens from the original populations were deposited at KNU, and were identified according to their original descriptions and references (Nakai, 1911; Chung, 1957; Lee, 1979; Lee WC, 1996). *Rosa multiflora* Thunb. and *Geum japonicum* Thunb. (Rosaceae) were chosen as the outgroup taxa for our phylogenetic analysis of *Rubus*. Total genomic DNAs were isolated by a modified CTAB method (Doyle and Doyle, 1987) from fresh leaves or tissues stored at -70°C.

PCR Amplification, Purification, and Sequencing of PCR Products

Two non-coding regions of cpDNA and nrDNA were amplified directly by symmetric PCR, using trnF as the universal primer for trnL (Taberlet et al., 1991) and ITS4 for ITS1 (White et al., 1990) (Fig. 1). PCR conditions for trnL/F were one cycle of 95°C for 3 min, 50°C for 1 min, and 72°C for 1 min; followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min (increased by 1 s per cycle); then a final extension of 7 min at 72°C. For the ITS region, the PCR reaction profile was conducted as described above, except that the annealing temperature was 55°C. Amplification was performed in a PE 2400 Amplifier (Perkin Elmer, USA). PCR products were purified with a QIAquick PCR purification kit (QIAgen, USA), and the sequencing reaction used a SequiTherm Excell™-Long-Read™ Cycle sequencing kit-LC (Epicentre Technologies, USA). The sequencing samples were then electrophoresed for 10 h on 6% acrylamide-8 M Urea gels in 1X TBE buffer, using the Automatic Sequencer (Li-COR, USA).

Alignment and Phylogenetic Analyses

Sequences were aligned with the Clustal X program (Thompson et al., 1997). For our phylogenetic tree construction, parsimony analyses were conducted using the PAUP 4.0b10 program (Swofford, 2002). All characters were weighted equally and unordered; gaps were coded as missing data. Heuristic searches were conducted with 'MULPARS', TBR branch swapping, addition sequence simple, collapse of zero-length branches, and 'ACCTRAN' optimization. To obtain bootstrap values, heuristic searches were replicated 1000 times. We also mapped the leaf type (simple or compound) onto our consensus tree using MacClade (Maddison and Maddison, 1992). For the phylogenetic analysis, Genbank was the source of the ITS1, 5.8s gene, and ITS2 sequences of *Geum urbanum* L. (AJ302337), *Rosa multiflora* Thunb. (AB038451), and *Rubus trifidus* Thunb. (AF055738).

RESULTS AND DISCUSSION

trnL/F Region Analysis

The trnL/F data set consisted of 23 taxa with 502

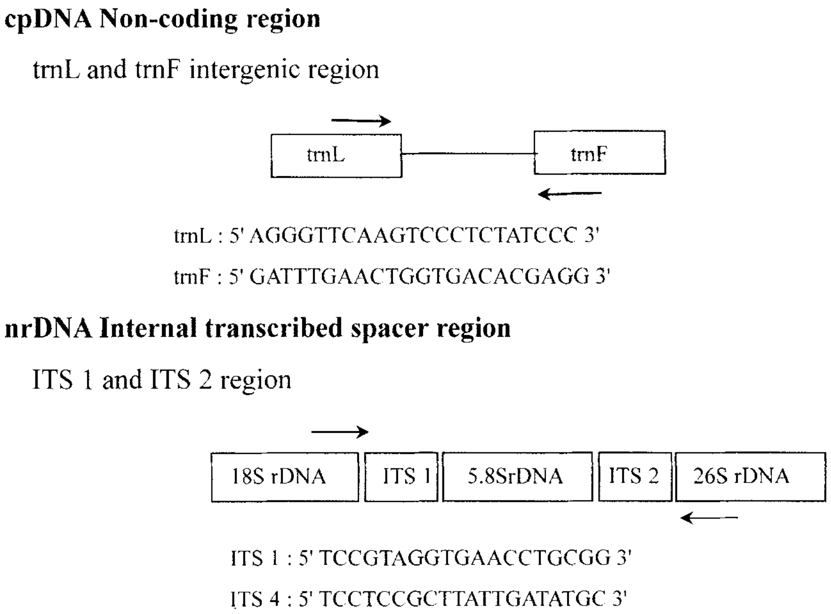

Figure 1. Position, directions, and sequences of primers used to amplify regions.

aligned DNA characters. Parsimony analysis revealed 358 constant, 90 variable, and 55 informative characters. The sequence length of the trnL/F region (Table 2) varied from 408 bases (*Rubus takesimensis* Nakai) to 451 bases (*R. articus* L.). Parsimony analysis produced 48 trnL/trnF trees with 231 steps. The CI and RI parsimonious tree were 0.72 and 0.70, respectively (Fig. 2).

The Korean *Rubus* forms a monophyletic group, segregated from the outgroup with a 96% bootstrap value. This genus contains five clades within the ingroup. Clade I, supported by a 51% bootstrap value, consists of subg. *Idaeobatus* Focke sections *Idaeocanthi* Focke (*R. coreanus* Miq., *R. phoenicolasius* Maxim *R. idaeus* L., *R. parvifolius* Thunb., *R. parvifolius* var. *taquetii* Nakai and *R. schizostylus* Le'v). These species have the morphological characteristics of a corymbose inflorescence and pinnately compound leaves, which are white on the underside. Because seven taxa form polytomies within this clade, we could not derive the relationships of those taxa. Although Clade I is supported by a lower bootstrap value, information about sect. *Idaeocanthi* was not included in the ITS phylogeny.

Clade II, with a 53% bootstrap value, consists of subg. *Cylactis* Focke (*R. articus*), *Malachobatus* Focke

Table 2. Lengths and GenBank accession numbers for trnL-trnF and ITS regions in *Rubus* species and outgroup taxa.

Taxon	trnL-trnF region (cpDNA)		ITS region (nrDNA)	
	Length (in base pairs)	Accession number	Length (in base pairs)	Accession number
R. articus	451	AY818244	628	AY818215
R. buergeri	436	AY818245	627	AY818212
R. coreanus	449	AY818247	627	AY818196
R. corchorifolius	438	AY818235	627	AY818206
R. crataegifolius (HK)	442	AY818229	627	AY818205
″ (BD)	442	AY818242	626	AY818213
R. croceacanthus	437	AY818231	630	AY818209
R. fruticosus	442	AY818246	627	AY818214
R. hongnoensis	437	AY818233	630	AY818211
R. hirsutus	437	AY818230	630	AY818208
R. idaeus (TB)	443	AY837721	627	AY818198
″ (BD)	445	AY818240	625	AY818217
R. longisepalus	449	AY818227	628	AY818203
var. *tozawai*	447	AY818239	626	AY818220
R. oldhami	437	AY818234	627	AY818207
R. parvifolius (MH)	450	AY818222	627	AY818199
″ (YN)	449	AY818241	626	AY818219
″ (6X)	450	AY818224	626	AY818218
var. *taquetii*	450	AY818223	627	AY818200
R. phoenicolasius	449	AY818248	626	AY818197
R. ribesoideus	449	AY818226	627	AY818202
R. schizostylus	450	AY818225	627	AY818201
R. sorbifolius	437	AY818232	629	AY818210
R. takesimensis	408	AY818228	626	AY818216
R. trifidus (JJ)	437	AY818243	627	AY818221
″ (JP)	-	-	627	AF055738
Geum japonicum	440	AY818238	-	-
Geum urbanum	-	-	599	AJ302337
Rosa multiflora	443	AY818237	626	AB038451

( ), Abbreviation of population localities.

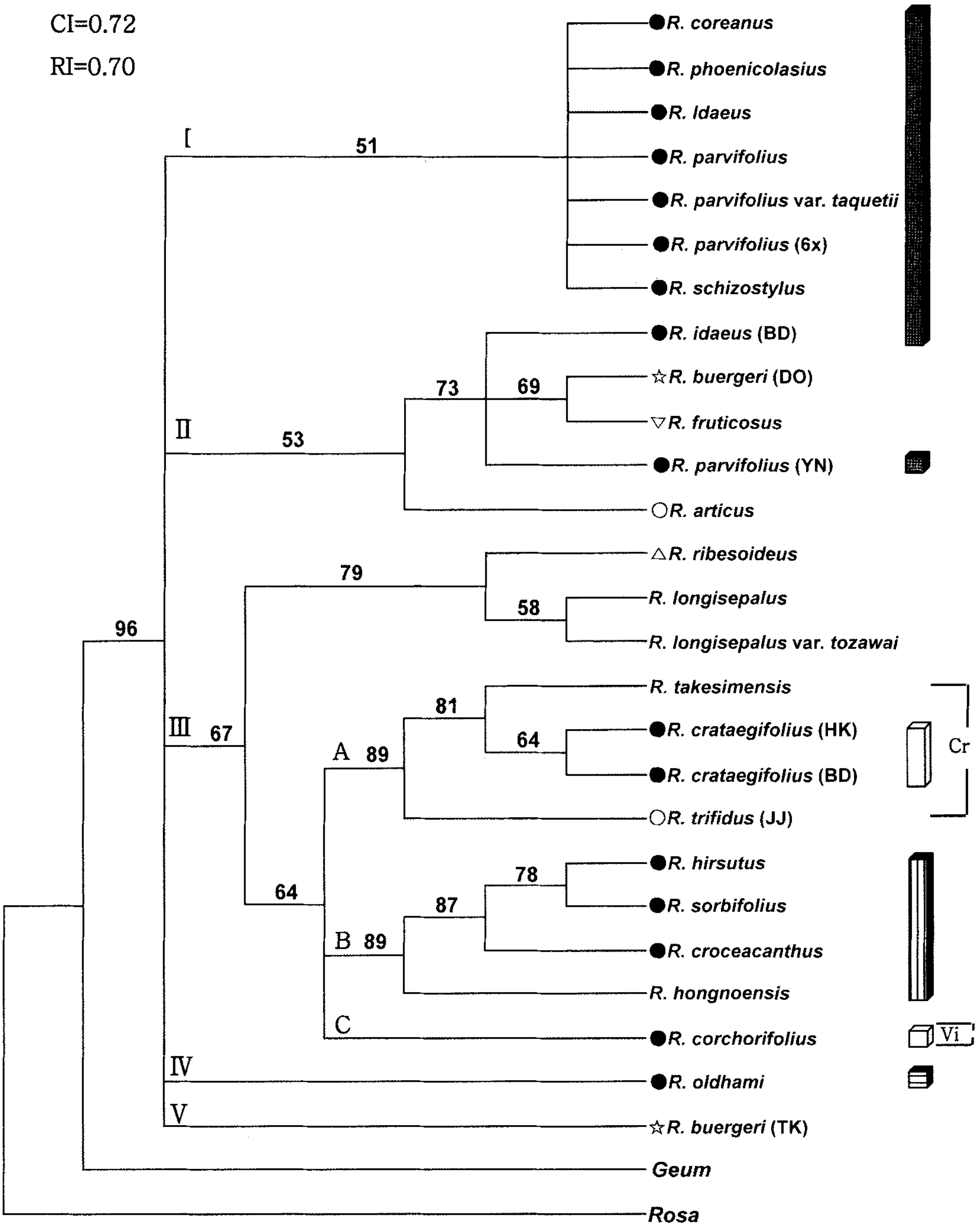

Figure 2. Strict consensus tree of 48 most parsimonious trees of length 231 that contained 26 *Rubus* taxa: trnL/F region. Bootstrap values are marked above branches. Nodes discussed in text are indicated by capital letters. Subg. *Anoplobatus*, ○; *Cylactis*, △; *Idaeobatus*, ●; *Malachobatus*, ★; *Rubus*, ▽; Sect. *Corchorifolii*, □; *Idaeocanthi*, ■; *Rosoefolii*, ▥; *Pungentes*, ▤; Sect. *Crataegifolii* Nakai;]Cr, *Villosii* Nakai;]Vi.

(*R. buergeri* Miq.), *Rubus* Focke (*R. fruticosus* L. agg.) and *Idaeobatus* (*R. idaeus* and *R. parvifolius*, distributed in China). Because this clade contains three different subgenera taxa, no resolution can be made at that level. Moreover, the Chinese taxa of *R. parvifolius* (YN) do not form one clade with the Korean taxa of *R. parvifolius* (4X, 6X), perhaps because of geographical variations and polyploidy level.

Clade III consists of two large groups, with an overall bootstrap value of 67%. Its first group (79% bootstrap value) contains three taxa (*R. ribesoideus* Matsum., *R. longisepalus* and *R. longisepalus* var. *toza-*

wai) of the subg. *Anoplobatus* Focke. Of these, the latter two are found only on Geoje Island, and were first reported by Nakai (Chung, 1943). Because of this more recent discovery, their phylogenetic positions were not included in the studies of Focke (1910, 1911, 1914). These taxa have the morphological characteristics of subg. *Anoplobatus*, e.g., persistent stipules, prickleless stem, and 3 to 5 simple, cleft leaves. Therefore, the morphological and molecular data of *R. longisepalus* and *R. longisepalus* var. *tozawai* suggest that these taxa belong to that subgenus.

The second group in Clade III comprises three subgroups, with an overall bootstrap value of 64%. Subgroup A contains subg. *Anoplobatus* (*R. trifidus*) and subg. *Idaeobatus* (*R. crataegifolius* Bunge and *R. takesimensis*), with an 89% bootstrap value. Alice and Campbell (1999) have also reported that *Rubus trifidus* forms one clade with *R. crataegifolius* of subg. *Idaeobatus* in their ITS phylogeny. *Rubus takesimensis*, found only on Ulreung Island, is an endemic species first reported by Nakai (1918). It is considered the sister species of *R. crataegifolius*, based on morphological characteristics such as simple leaves, deciduous fruits from the calyx, a corymbose inflorescence, prickled peduncle and calyx, and hairless ovary. Nakai proposed a new section, i.e., *Crataegifolii* and assigned *R. crataegifolius* and *R. takesimensis* as members. Our phylogenetic tree, using trnL/F sequences, also supports that classification by Nakai. Subgroup B of Clade III contains subg. *Idaeobatus* sect. *Rosoefolii* Focke (*R. hirsutus* Thunb., *R. sorbifolius* Maxim., *R. croceacanthus* Le'v. and *R. hongnoensis*) with an 89% bootstrap value. *Rubus hongnoensis* is an endemic species on Jeju Island. Nakai (1939) suggested that these four species belong to sect. *Rosoefolii*. Their morphological characteristics include pinnately compound leaves, solitary flowers, having prickles calyx, and a lack of peduncles.

Finally, Subgroup C in the second group of Clade III comprises subg. *Idaeobatus* sect. *Corchorifolii* Focke (*R. corchorifolius* L.f.). Its morphology shows simple leaves, solitary and nodding flowers, fruits deciduous with the calyx, having prickles peduncle and calyx. Focke (1911) assigned *R. corchorifolius* and *R. crataegifolius* to sect. *Corchorifolii*. However, when those two species are compared, *R. crataegifolius* has, except for its simple leaves, a completely different set of morphological traits, manifested by its corymbose inflorescence and deciduous fruits from the calyx. Therefore, Nakai separated this species from sect. *Corchorifolii* and proposed new sect. *Villosii* Nakai that includes *R. corchorifolius*. The results of our trnL/F intergenic region analysis support this re-classification by Nakai.

Clades IV and V contain subg. *Idaeobatus* sect. *Pungentes* Focke (*R. oldhami* Miq.) and subg. *Malachobatus* sect. *Moluccani* Focke (*R. buergeri*), respectively. The former possesses solitary, erect, pinnately compound leaves (5 to 7 leaflets), and a calyx with prickles, while the latter has the morphological characteristics of a shrub species, including deciduous stipules, evergreen, slender stems, scarcely prickly, suborbicular leaves, and a corymb inflorescence.

In the trnL/F tree, most clades are congruent with their sectional classification, although with the taxa of one subgenus forming a clade with other subgenera taxa at the subgenus level. Therefore, our phylogenetic tree, which is derived from analysis of the trnL/F intergenic regions, supports the sectional classification scheme, but does not concur with that scheme at the subgenus level.

ITS Region Analysis

The ITS data set comprised 23 taxa with 672 aligned DNA characters. Parsimony analysis revealed 444 constant, 154 variable, and 232 informative characters. The sequence length ITS region, including 5.8S, varied from 599 bases in *Geum urbanum* to 630 in *Rubus croceacanthus*, *R. hongnoensis* and *R. hirsutus* (Table 2). Six most parsimonious trees were produced with 951 steps. The CI and RI of parsimonious tree were 0.53 and 0.49, respectively (Fig. 3). ITS trees of genus *Rubus* were monophyletic, with 97% bootstrap value support, and shared a similar topology with the trnL-trnF tree.

Clade A contains subg. *Idaeobatus* sect. *Crataegifolii* (*R. crataegifolius* and *R. takesimensis* -- Clade D), and subg. *Anoplobatus* (*R. trifidus* -- Clade E), with an 83% bootstrap value. According to the classification of Focke, *R. trifidus* belongs to subg. *Anoplobatus*. However, that species is not nested with subg. *Anoplobatus* in our ITS tree. Alice and Campbell (1999) also have suggested, based on their own ITS tree, that *R. trifidus* does not belong to subg. *Anoplobatus*. Clade D shows that *R. crataegifolius* is closely related to *R. takesimensis*, with a bootstrap value of 84%. Clade E consists of Korean and Japanese *R. trifidus* species, with a 100% bootstrap value.

Clade B forms two clades (Clades F and G), with a 54% bootstrap value. Clade F includes subg. *Rubus* sect. *Rubus* (*R. fruticosus*). Clade G forms three clades (H, I and J), with a 57% bootstrap value. This clade contains subg. *Anoplobatus* and subg. *Idaeobatus* sec-

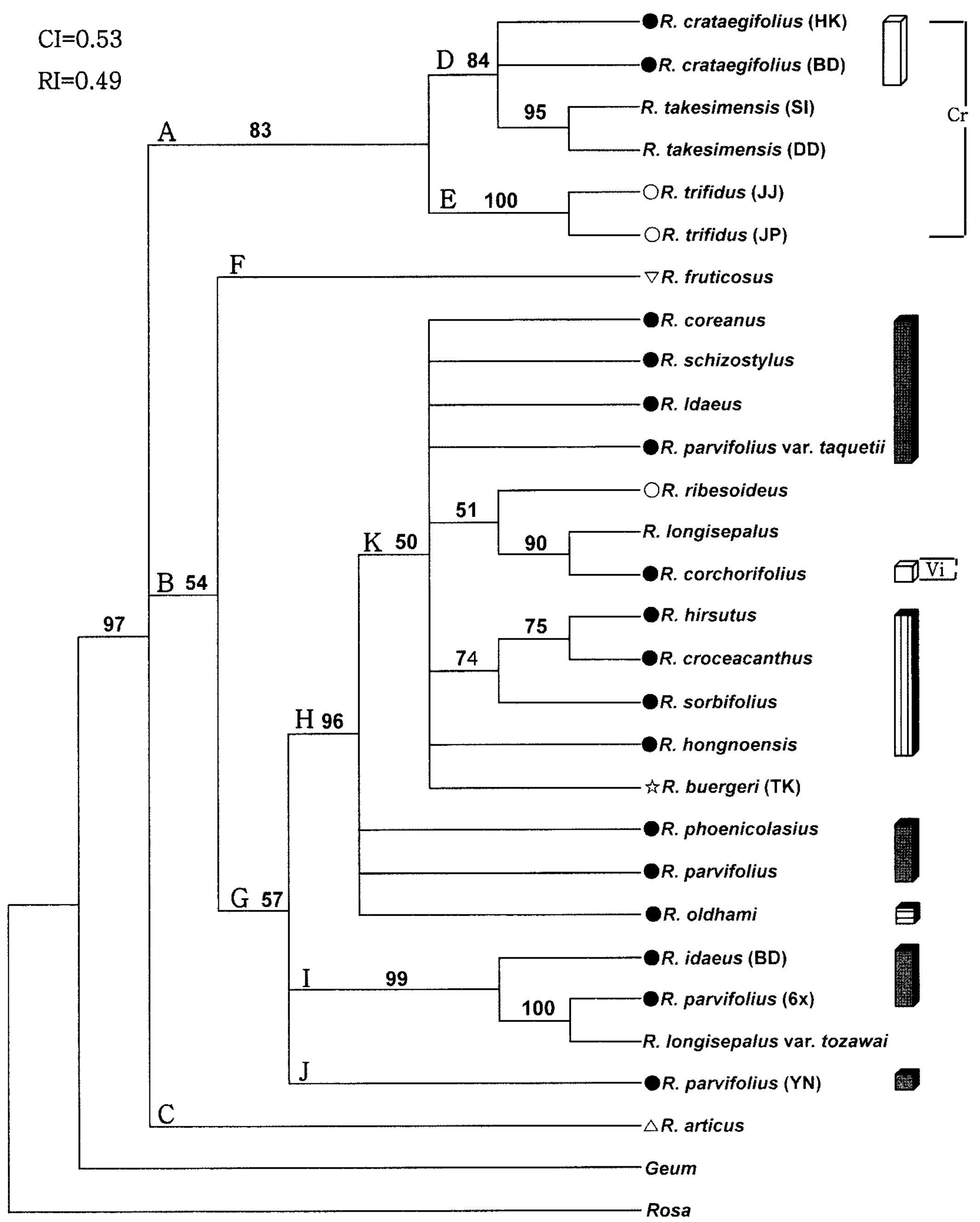

Figure 3. Strict consensus tree of 6 most parsimonious trees of length 951, containing 27 *Rubus* taxa: ITS region. Bootstrap values are marked above branches. Nodes discussed in text are indicated by capital letters. Subg. *Anoplobatus*, ○; *Cylactis*, △; *Idaeobatus*, ●; *Malachobatus*, ★; *Rubus*, ▽; Sect. *Corchorifolii*, ❐; *Idaeocanthi*, ■; *Rosoefolii*, ▥; *Pungentes*, ▤; Sect. *Crataegifolii* Nakai] Cr, *Villosii* Nakai]Vi.

tions *Idaeocanthi*, *Rosoefolii* and *Pungentes*. No resolution was determined here for Clade H, which consists of six taxa of subg. *Idaeobatus*, sect. *Idaeocanthi*; and one species each from sect. *Rosoefolii*, sect. *Pungentes* and subg. *Malachobatus*. All show polytomies. *Rubus corchorifolius*, which belongs to subg. *Idaeobatus* sect. *Corchorifolii*, forms a clade with *R. longisepalus* by a 90% bootstrap value. With regard to the position of *R. corchorifolius*, our ITS tree is not congruent with the trnL/F tree. Species of section *Rosoefolii* (*R. croceacanthus*, *R. hirsutus* and *R. sorbifolius*) are supported by a 74% bootstrap value.

Clade I consists of *R. parvifolius* (6x), *R. longisepalus* var. *tozawai*, and *R. idaeus*, with a 99% bootstrap value. The first two are found only on Geoje Island. The hexaploid *R. parvifolius* forms one clade with *R. longisepalus* var. *tozawai* (100% bootstrap value), but not with other *R. parvifolius* taxa. This demonstrates that ITS phylogeny is congruent with the geographical distribution of this species.

Clade C has a unique Korean herbaceous species, *Rubus articus* of the subg. *Cylactis*. This species, located at the basal clade of the ITS tree, has morphological characteristics that include unarmed plants, flowering stems that arise from aerial shoots, an acuminate apex of leaflets, solitary flowers, and rarely needle-like prickles. It has been suggested that evolution in Rosaceae (Kalkman, 1988) and *Rubus* (Lu, 1983) has proceeded from woody plant to herbaceous plant. However, ITS data analysis by Alice and Campbell (1999) has shown primarily semi-herbaceous occupied basal positions in their ITS data. However, our ITS data analysis also suggests that evolution proceeded from herbaceous to woody, such that the hypotheses of both Kalkman (1988) and Lu (1983) conflict with our current ITS data as well as those of Alice and Campbell (1999).

ITS phylogeny by Alice and Campbell (1999) used 57 taxa from 12 representative subgenera that were suggested by Focke (1910, 1911, 1914). However, that previous analysis sampled only 10 species of subg. *Idaeobatus* from North American and European taxa. In fact, that subgenus consists of 117 species, 80 of which are distributed mainly in East Asia (i.e., China, Japan, Korea and Taiwan). Those East Asian species are lacking in the ITS analysis by Alice and Campbell, making it possible for us to compare only a few taxa with the ITS phylogeny of Alice and Campbell (1999).

Combined Analysis

The combined analysis of ITS and trnL/F sequences resulted in 23 taxa with 1174 aligned DNA characters. Parsimony analysis showed 641 constant, 226 variable, and 305 informative characters, and produced 16 most parsimonious trees with 1227 steps. The CI and RI parsimonious tree were 0.55 and 0.54, respectively (Fig. 4).

We compared the topologies of the combined and the separated data sets. The topology of this combined bootstrap consensus tree is similar to that of the ITS tree (Fig. 3). The monophyly of an ingroup in the combined tree is well supported, with 100% bootstrap values in both the trnL-trnF and ITS tree.

Within the ingroup, the genus *Rubus* forms three main clades (A, B, C). Clade A consists of taxa with simple leaves, while those of Clades B and C have pinnately compound leaves. Lu (1983) considers subg. *Idaeobatus* to be the most primitive group and subg. *Chamaemorus* the most advanced. Investigating that hypothesis, we examined the evolution of leaves from subg. *Idaeobatus*, and found that the morphological characteristic of simple or compound leaf occur at the same time for Clades A and B. The former contains subg. *Idaeobatus* (*R. crataegifolius*, *R. takesimensis* and *R. trifidus*) in both the trnL-trnF and ITS trees. *Rubus trifidus* is the basal clade within Clade A. Previous phylogeny analysis by Alice and Campbell (1999) has shown that *R. trifidus* does not belong to subg. *Anoplobatus*, and Naruhashi (1980) has instead classified that species to subg. *Idaeobatus*, which is closer to *R. crataegifolius*. Our analysis also demonstrated that *R. trifidus* belongs to subg. *Idaeobatus*, thereby agreeing with the conclusions of both Naruhashi (1980) and Alice and Campbell (1999).

Clade B comprises two large groups (D and E). Within Clade D, the species that are found only on Jeju Island form a clade with a low, 55% bootstrap value. Our ITS data analysis results are consistent with the geographical distribution of those species. Clade E, supported by a low, 62% bootstrap value, contains *R. longisepalus* var. *tozawai*, *R. parvifolius* (6x) and *R. idaeus*, the former two being found on Geoje Island. *Rubus parvifolius* (6x) forms one clade with other *R. parvifolius* taxa in the trnL/F tree, while, in the ITS tree, it forms one clade with *R. longisepalus* var. *tozawai*. The trnL/F data show the inheritance of cpDNA from one parental species whereas the ITS data reflect the homogenization of ribosomal DNA from the other parental species. Although we could hypothesize about the origin of hexaploid *R. parvifolius*, based on these results, further analysis is required.

In the two data sets for the trnl/F and ITS trees, there is strong conflict regarding the position of *R. parvifolius* (YN). This may be caused by geographical variations among the Chinese and Korean taxa, or else because polyploidy is found only in the taxa from subg. *Idaeobatus*. Our analysis of the data set shows high resolution in the previous sectional classification scheme but does not support that previous scheme at the subgenus level. Likewise, section *Corchorifolii*, which was suggested by Focke (1911), is not supported in our current study. Instead, the phylogenetic tree derived here supports the sectional scheme of

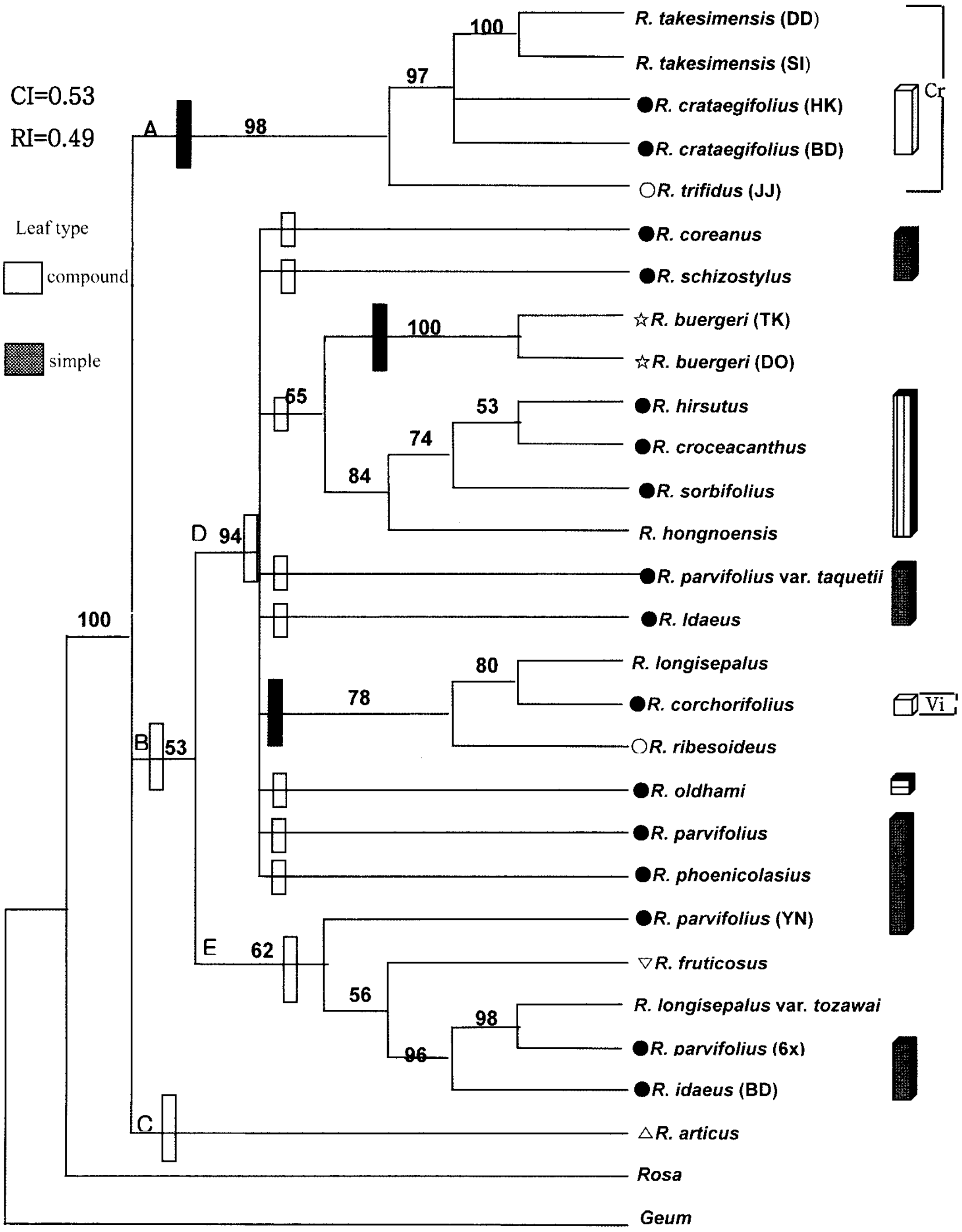

Figure 4. Strict consensus tree of 16 most parsimonious trees of the ITS and trnL/F data set combined. Leaf type mapped onto combined tree. Bootstrap values are marked above branches. Nodes discussed in text are indicated by capital letters. Subg. *Anoplobatus*, ○; *Cylactis*, △; *Idaeobatus*, ●; *Malachobatus*, ★; *Rubus*, ▽; Sect. *Corchorifolii*, □; *Idaeocanthi*, ■; *Rosoefolii*, ▥; *Pungentes*, ▤; Sect. *Crataegifolii* Nakai]Cr, *Villosii* Nakai]Vi.

Nakai: sect. *Crataegifolii* Nakai (*R. trifidus*, *R. crataegifolius* and *R. takesimensis*) and sect. *Villosii* Nakai (*R. corchorifolius*).

The separated and combined analyses of our ITS and trnL/F data suggest that the genus *Rubus* is a monophyletic group. However, trnL/F and ITS phylogeny results indicate that the Korean *Rubus* contains a non-monophyletic subgenus, which is congruent with the ITS phylogeny of Alice and Campbell (1999). Most of the species that are distributed in Korea belong to subg. *Idaeobatus*, a subgenus that can be divided into two groups. The combined data analysis demonstrates that the Korean subg. *Idaeobatus* is polyphyletic, having two apparent lineages (Fig. 4). We can also confirm the phylogenetic position of *R. longisepalus* var. *tozawai* and *R. trifidus* as part of subg. *Anoplobatus* and *Idaeobatus*, respectively. With regard to the position of *R. corchorifolius*, the two data sets (trnL/F and ITS tree) do not agree, perhaps because of the inheritance of cpDNA from one parental species and the homogenization of ribosomal DNA from the other. ITS phylogeny data also do not present any relationship for sect. *Idaeocanthi*. However, even though that section is supported by a lower bootstrap value, the analytical results for the trnL/F intergenic region reveal the monophyletic group characteristic of the subg. *Idaeobatus* sect. *Idaeocanthi* taxa. In this study, the trnL/F analysis produced data not available from our ITS analysis. Furthermore, the trnL/F tree presented more constructive data than did the ITS tree concerning this phylogenetic relationship. Therefore, the use of trnL/F analysis for other genes, e.g., a plastid gene, may further clarify the classification of *Rubus* taxa. Additional research and sampling of the subg. *Anoplobatus* and *Malachobatus* species, as well as that of an additional gene, would reveal the complete phylogenetic relationship within Korean *Rubus*.

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