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Taxonomic Study on the Lichen Genus *Cetrelia* (Lecanorales, Ascomycota) in South Korea

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Seventy-two lichen specimens of *Cetrelia* collected in South Korea since 2003 were examined by both phenotypic and phylogenetic analyses. The phenotypic analysis was based on morphological and chemical characters, and the phylogenetic analysis was based on nrDNA ITS sequences. The result suggested that the presence and absence of isidia, soredia, lobules and medullar reaction C+ or C– are the important characters in the taxonomy of this genus. Four species of *Cetrelia*, *C. chicitae*, *C. braunsiana*, *C. japonica*, and *C. pseudolivetorum* have been identified in this study. Description of each species is presented with morphological and chemical characters. A key to the *Cetrelia* species is also presented.

KEYWORDS: ***Cetrelia*, ITS sequences, Lichen, Phenotypic analysis, Phylogenetic analysis**

Cetrelia W.L. Culb. & C.F. Culb. belongs to the lichenized ascomycete family *Parmeliaceae* Zenker, cetrarioid genera. The name of *Cetrelia* is a fanciful concoction from *Cetraria* and *Parmelia*, in which most of the species had previously been placed. Culberson and Culberson (1968) combined some species from the old genus *Cetraria* and some species from *Parmelia* into the new genus *Cetrelia* based on morphological and chemical characters. The main characters of this genus are the broad lobe, the presence of laminal pseudocyphella, and the production of aromatic compounds such as orcinol-type depsides or depsidones. Although there were many reports on the study of *Cetrelia* (Beguinot 1982; Chen 1986; Culberson and Culberson 1968; Elix 1994; Harada 1996; Lai 2001), almost no expert study on *Cetrelia* had been conducted in Korea until the macrolichen flora of South Korea was published (Park, 1990). In her paper, 6 species of *Cetrelia* were reported with brief description of each species and a key to the genus. However, there are still some problems such as ambiguous characters which made it difficult to differentiate species and to do the corresponding taxonomic work. According to the most newly published checklist of Korean lichens (Hur *et al.*, 2005), there were 8 species of *Cetrelia* recorded in Korean peninsula. The aim of this study was to evaluate the importance of different taxonomic characters which have not been carefully examined by phenotypic and phylogenetic analyses so far.

Materials and Methods

Phenotypic analysis. Seventy-two lichen specimens of *Cetrelia* from South Korea were examined and are deposited in KoLRI (Korean Lichen Research Institute). The gross morphology and anatomy of the specimens were examined by the dissecting microscope (Nikon SMZ 1500) and compound microscope (Olympus BX50). The chemical characters were examined by medullar color reaction and thin layer chromatography (Culberson, 1972; White and James, 1985). Nineteen morphological and chemical characters were chosen for the phenotypic analysis (Table 1, 2). Maximum parsimony analysis was performed by PAUP version 4.0b10 (Swofford, 2002), with

Table 1. Nineteen phenotypic characters chosen for phenotypic analysis of *Cetrelia* genus in this study

No.	Characters
1	Thallus lobes tiled
2	Thallus lobes erect
3	Upper surface pale brownish or tan
4	Upper surface reddish brown
5	Spinule present on the marginal upper surface
6	Upper surface pseudocyphellate present
7	Lower surface pseudocyphellate present
8	Lobules present
9	Lobules flat
10	Lobules thick similar with isidia
11	Isidia present
12	Soredia present
13	Medullar reaction KC+
14	Alectoronic acid present
15	α-Collatolic acid
16	Rhizines present
17	Rhizines black
18	Medullar reaction C+
19	Olivetoric acid present

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Table 2. Matrix form of 19 phenotypic characters used to phenotypic analysis

Species \ Character No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
C. japonica	1	0	1	0	0	1	0	1	1	0	0	0	0	0	0	1	1	0	0
C. pseudolivetorum	1	0	1	0	0	1	0	1	0	1	0	1	0	0	0	1	1	1	1
C. chicitae	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	1	1	0	0
C. braunsiana	1	0	1	0	0	1	0		0	0	1	0	1	1	1	1	1	0	0
Cetraria islandica	0	1	0	1	1	0	1	0	0	0	0	0	?	?	?	0	0	0	0

Note: '1' indicates positive, '0' indicates negative, '?' indicates unknown.

Cetraria islandica as the out group.

DNA extraction and nrDNA amplification. Twenty lichen thalli were fractioned with cryo-tissue-crasher (SK200, Tokken, Japan) (Table 3). Total DNA was extracted directly from whole thalli according to Ekman (1999) with DNeasy Plant Mini Kit (QIAGEN, Germany), then purified by PCRquick-spin™ PCR Product Purification Kit (iNtRON Biotechnology, INC.). The nrDNA ITS region (ITS1-5.8S-ITS2) was amplified by PCR. Primers for amplification were: ITS1F (5'-CTTGGTCATTTACAGGAAGTAA-3'; Gardes and Bruns, 1993) and ITS4A (5'-ATTTGAGCTCTTCCCGCTTCA-3'; White *el al.*, 1990). Previously described conditions by Arup (2002) have been used for PCR amplification and cycle sequencing.

Sequencing and phylogenetic analysis. PCR products were sequenced by ABI 3700 automated DNA Sequencer in NICEM at Seoul National University. The phylogenetic analysis was executed by the Software Mega2 (Kumar *et al.*, 2001). Kimura 2-parameter model was taken, and gaps were retained initially while being excluded in the pairwise distance estimation. The neighbor joining (NJ) (Saitou and Nei, 1987) method was used in constructing the phylogenetic tree and the reliability of the inferred tree was tested by 1,000 bootstrap relications. *Cetralia islandica* (Genbank accession no.: EF373567) was used as the outgroup.

Table 3. Twenty specimens used in phylogenetic analysis

Collection no.	Species name	Accession no.	Locality
040988	*C. japonica*	EU142918	Mt. Jiri
041594	*C. japonica*	EU142924	Mt. Sorak
030818	*C. japonica*	EU142920	Mt. Sobeak
040344	*C. japonica*	EU142915	Mt. Jiri
030789	*C. japonica*	EU142919	Mt. Sobeak
040760	*C. japonica*	EU142916	Mt. Halla
040790	*C. japonica*	EU142923	Mt. Halla
030397	*C. japonica*	DQ394377	Mt. Taebaek
060656	*C. japonica*	EU142925	Mt. Jiri
060812	*C. japonica*	EU142926	Mt. Jiri
060828	*C. japonica*	EU142927	Mt. Jiri
060350	*C. japonica*	EU142928	Mt. Jiri
060317	*C. chicitae*	EU142914	Mt. Jiri
041282	*C. braunsiana*	EU142917	Mt. Baekwoon
040416	*C. braunsiana*	EU142913	Mt. Odea
040425	*C. braunsiana*	DQ394376	Mt. Odea
061074	*C. pseudoolivetorum*	EU142929	Mt. Jiri
060718	*C. pseudoolivetorum*	EU142930	Mt. Jiri
030784	*C. pseudoolivetorum*	EU142922	Mt. Sobeak
050176	*C. pseudoolivetorum*	EU142921	Mt. Dukyoo

Results and Discussion

Phenotypic analysis. Phenotypic analysis was performed by morphological and chemical characters (Table 1 and 2). Maximum parsimony analysis showed that *Cetrelia* was divided into two sections (I and II), according to the presence or absence of lobules, indicating that this character is the most important character to distinguish the species (Fig. 1).

The results also indicated that either morphological characters or chemical characters were not enough to distinguish the species in *Cetrelia* and thus they should be considered together. For example, in section I, both *C. pseudolivetorum* (Asahina) W.L. Culb. & C.F. Culb. and *C. japonica* (Zahlbr.) W.L. Culb. & C.F. Culb. are lobu-

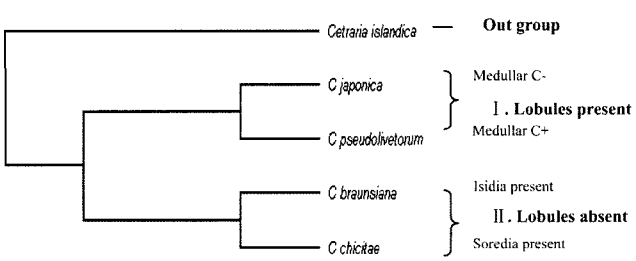

Fig. 1. UPGMA tree of 4 species of *Cetrelia* in South Korea, and *Cetraria islandica* as outgroup. Data matrix has 5 taxa, 19 characters. All characters are of type 'unord', all characters have equal weight, among which 15 variable characters are parsimony-uninformative, number of parsimony-informative characters = 4. Tree length = 19, consistency index (CI) = 1, homoplasy index (HI) = 0, CI excluding uninformative characters = 1, HI excluding uninformative characters = 0, retention index (RI) = ,1, rescaled consistency index (RC) = 0.

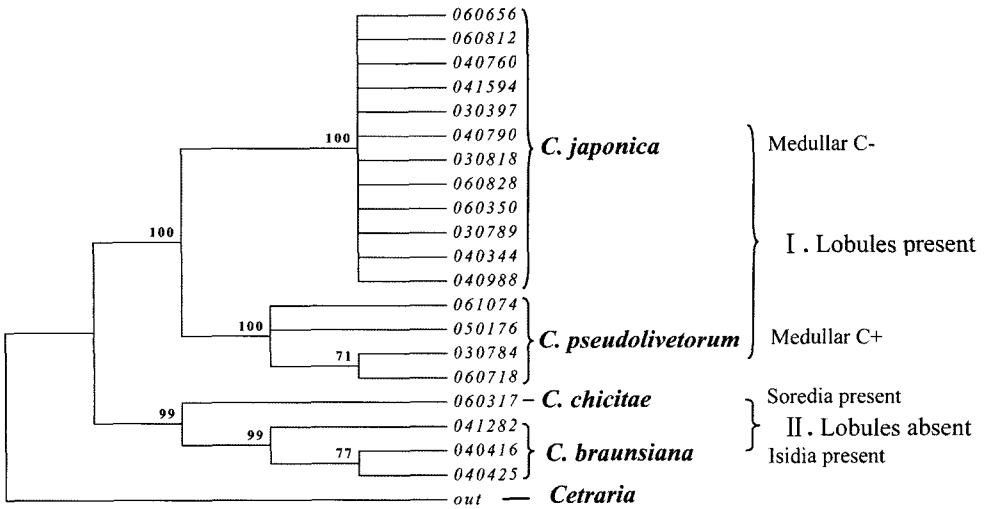

Fig. 2. NJ consensus tree based on nrDNA ITS sequences. Nucleotide: Kimura 2-parameter, pairwise deletion, bootstrap = 1000. The numbers in each node represents bootstrap support value, and the numbers lower than 50 were not shown.

late and that is why they are easily wrong in brief morphological identification. Medullar color reaction of C is further significant to distinguish them. Olivetoric acid (C+ reaction) was only present in *C. pseudolivetorum* but was absent in *C. japonica* (Fig. 3). In section II, *C. chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb. and *C. braunsiana* (Müll. Arg.) W.L. Culb. & C.F. Culb. produce almost the same chemical compounds of alectoronic acid and α-collatolic acid. It is difficult to distinguish them by medullar color reaction or TLC, but they are morphologically very different, *C. chicitae* is marginal soridate while *C. braunsiana* is isidate.

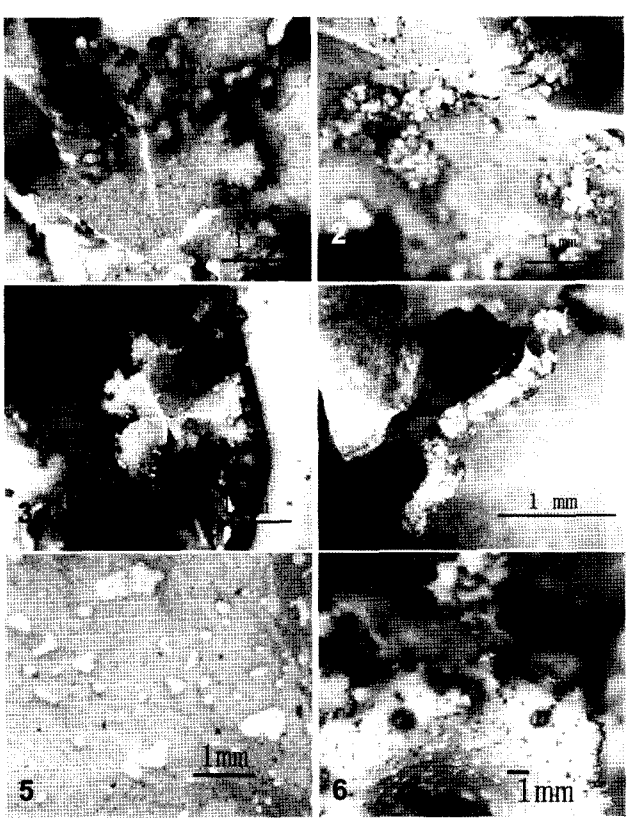

Fig. 3. 1. Isidia-like lobules of *C. pseudolivetorum* 030784; 2. Isidia of *C. braunsiana* 040425; 3. Lobules of *C. japonica* 050267: **a**. pycnidia; 4. Soredia of *C. chicitae* 060317; 5. Pseudocyphella on upper surface of *C. japonica* 050267; 6. Acervulus of *C. japonica* 050314.

Phylogenetic analysis. The NJ consensus tree constructed by Mega2 is shown in Fig. 2. According to the tree, each species finely assembled together and this proved that the taxonomy of *Cetrelia* based on morphological and chemical characters is reliable.

Besides, all tested specimens were divided into two sections (I and II) according to the presence or absence of lobules, indicating that this character is very important in the taxonomy of *Cetrelia*, which is accordant with the phenotypic analysis (Fig. 1).

In conclusion, according to the comprehensive analysis of phenotypic and phylogenetic analysis, four characters are thought to be important in the taxonomy of *Cetrelia* and they are listed as follows: presence or absence of lobules, presence or absence of soredia, presence or absence of isidia, medullar reaction C– or C+. Among these four species, *C. japonica* and *C. pseudolivetorum* have closer relationship whereas *C. chicitae* and *C. braunsiana* are closely related.

In this paper, only ITS region was involved in phylogenetic anlysis, so, not all characters could be well evaluated. The amount of examined specimens is limited and only 4 species had been identified from South Korean materials. Some characters which are not considered to distinguish these four species might be significant to distinguish them from other species. Therefore, more corre-

sponding work needs to be done in the future.

Taxonomic treatment of the genus. According to the above comprehensive analysis, a key to the genus is presented with morphological and chemical characters. Only representative specimens were cited when the number is large.

Key to *Cetrelia* species in South Korea

1. Thallus sorediate or isidiate, not lobulate.
   2. Thallus sorediate, medullar reaction C– ..... *C. chicitae*
   2. Thallus isidiate, medullar reaction C– ..... *C. braunsiana*
1. Thallus neither sorediate nor isidiate, but lobulate
   3. Thallus with dorsiventral flat lobules, medullar reaction C– ..... *C. japonica*
   3. Thallus with isidioid thick lobules, medullar reaction C+ pink or red ..... *C. psuedolivetorum*

한국산 *Cetrelia*(매화칼퀴지의) 속 국문 키

1. 분아나 열아 있으나 열편은 없음
   2. 분아 있음, 수층 C 반응 없음 ..... *C. chicitae*(분칼퀴지의)
   2. 열아 있음, 수층 C 반응 없음 ..... *C. braunsiana*(옷칼퀴지의)
1. 분아나 열아는 없고 열편 있음
   3. 납작한 형태의 열편, 수층 C 반응 없음 ..... *C. japonica*(작은잎매화칼퀴지의)
   3. 원통형의 두꺼운 열편, 수층 C 반응 있음(분홍 또는 붉은색) ..... *C. pseudolivetorum*(둥근잎매화칼퀴지의)

Taxonomy

1. *Cetrelia chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb., *Contrib. U.S. Natl. Herb.* 34 (7): 504-505, 1968

External morphology: Thallus medium, about 8.5 cm broad, lobes 0.8~1.8 cm broad. Upper surface ashy olive-green, margins somewhat rolled and downward; sorediate along margins (Fig. 3-4), pseudocyphellate, pores small, punctiform to enlongate but rarely exceeding 1mm. Lower surface jet-black, margins brown or colored like upper surface, rhizines to 1 mm or less, black, tips white. Medulla white. Pycnidia and apothecia not present in Korean specimens.

Chemistry: Thallus medullar C–, K–, KC+ pink, P–, contains atranorin, alectoronic, α-collatolic acid, 4-O-methylphsodic, and physodic acid (Fig. 4).

Distribution: Mt. Jiri (Fig. 5).

Habitat and ecology: Alt. 1620 m; on trunk of *Abies*.

Remarks: This species is rare and easily distinguished from other Korean species by its marginal soredia. It might be morphologically mistaken with *C. japonica* having poorly developed lobules, but they can be separated from each other by chemical analysis.

One specimen examined: 060317 Jae-Seoun Hur, June 17, 2006.

2. *Cetrelia braunsiana* (Müll. Arg.) W.L. Culb. & C.F. Culb. *Contrib. U.S. Natl. Herb.* 34 (7): 493-498, 1968

External morphology: Thallus medium to large, 5~17 cm broad, lobes 0.5~0.7 cm broad. Granular or coralloid isidia finely or poorly developed along the margins or on the upper surface (Fig. 3-2), upper surface ashy-green, tan or uniformly brownish in some old herbarium specimens. Margins ascendant or downward. Pseudocyphel-

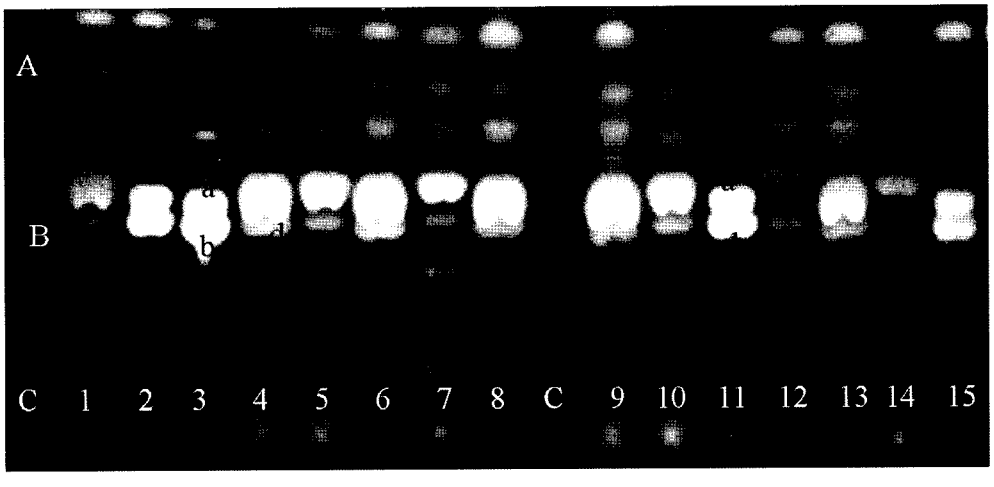

Fig. 4. TLC plate for compounds known in *Cetrelia* under UV light, developed with solvent B' system. Lane C refers to control (Artranorin and norstictic acid); Lane 1, 4, 5, 6, 7, 8, 9, 10, 13 and 14 are *C. japonica*; Lane 2, 11 and 15 are *C. braunsiana*; Lane 3 is *C. chicitae*; Lane 12 is *C. pseudolivetorum*. Spot A: atranorin, B: norstictic acid, **a**: α-collatolic acid, **b**: alectoronic acid, **c**: microphyllinic acid, **d**: 4-O-demethylmicrophyllinic acids, **e**: olivetoric acid.

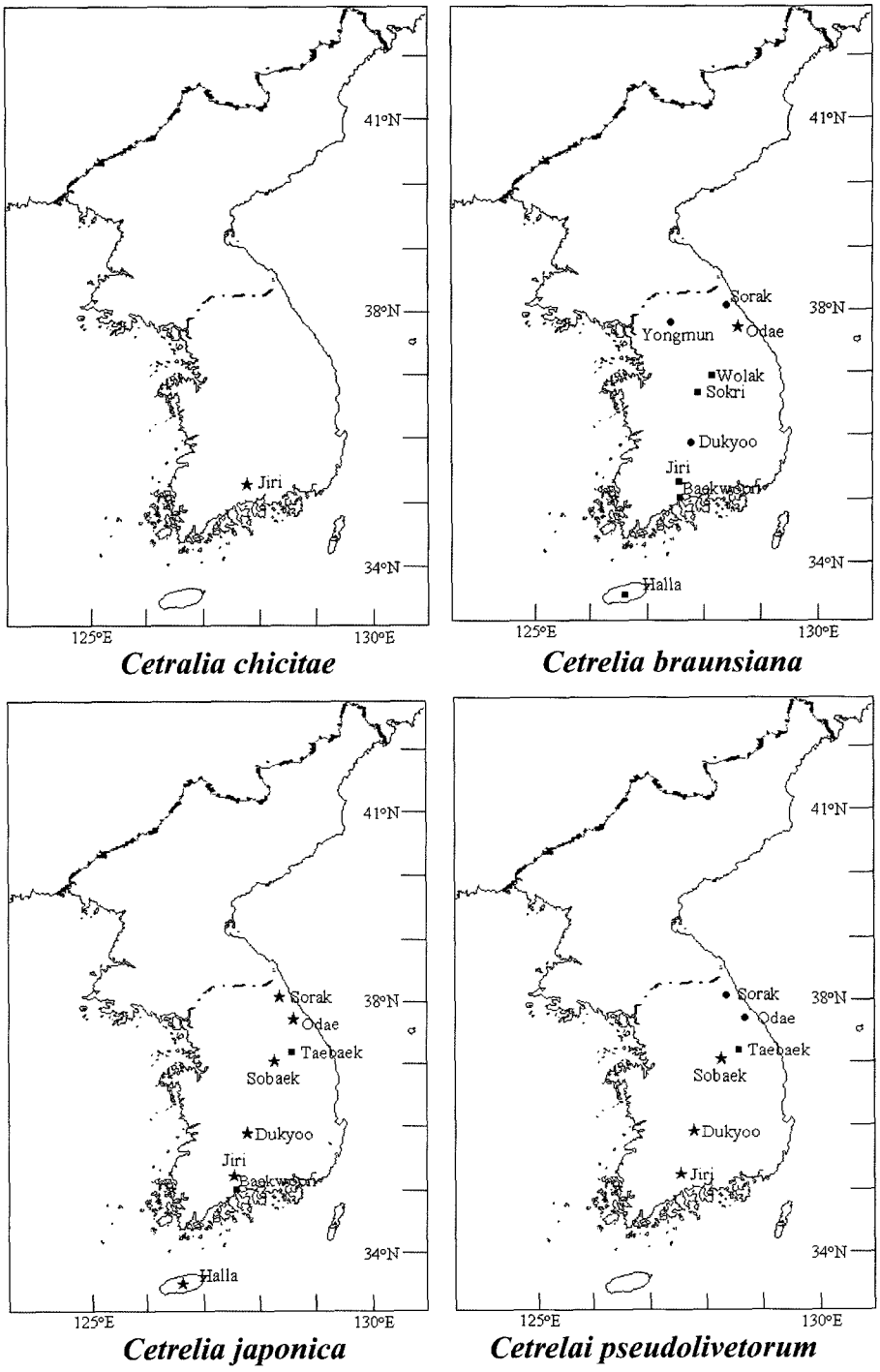

Fig. 5. Distribution of *Cetrelia* species in South Korean. Circles (●) and squares (■) represent the localities previously (Park, 1990) and newly reported in this study, respectively. The previous records confirmed by newly collected specimens in this survey were indicated as stars (★).

late, pores small, punctiform to irregular but rarely exceeding 1mm. Lower surface black, margins brown or grayish like the color of upper surface, rhizines black, about 1 mm. Apothecia not seen, pycnidia present in some specimens, limited on the tips of isidia.

Chemistry: Thallus medullar C–, K–, KC+ pink, P–, contains atranorin, alectoronic, α-collatolic acid, 4-O-methylphsodic, and physodic acid (Fig. 4).

Distribution: Mt. Baekwoon, Mt. Halla, Mt. Jiri, Mt. Juhul, Mt. Odae, Mt. Sokri, Mt. Wolak (Fig. 5).

Habitat and ecology: Alt. 510~1700 m; on trunk of *Abies*, *Betula*, *Pinus* and *Quercus*; sometimes on rock.

Remarks: Morphologically *C. braunsiana* is similar to *C. pseudolivetorum* because of the isidia-like lobules of *C.*

pseudolivetorum, but chemically they are quite different. Medullar color reaction of *C. braunsiana* is C–, whereas *C. psuedolivetorum* is C+.

Representatives of 14 specimens : 040425 Jae-Seoun Hur, May 7, 2004.

3. *Cetrelia japonica* (Zahlbr.) W.L. Culb. & C.F. Culb. *Contrib. U.S. Natl. Herb.* 34 (7): 511-513, 1968

External morphology: Thallus medium to large, 6~16 cm broad, lobes 0.5~1 cm broad, tips pruinose occasionally, the margins densely fringed with multi-branched lobules (Fig. 3-3), lobules narrow or broadly expanded and lobe-like, flat. But in some specimens, lobules poorly developed, unbranched and flat. Upper surface greenish-gray to yellowish-green, or tan in some old herbarium specimens. The margins ascendant or downward. Pseudocyphellae conspicuous, punctiform to elongate smaller or about 1 mm (Fig. 3-5). Lower surface black, margins brown or grayish like the color of upper surface, rhizines black, tips white sometimes, about 1 mm, but in some specimens, rhizine absent or few. Apothecia not seen, pycnidia black, about 0.1 mm broad, the ostiole prominent, terminal, or absent, acervulus present occasionally (Fig 3-6), laminal, about 1 mm broad. Conidia 1.25 × 5 μm, rod shaped, the ends somewhat enlarged.

Chemistry: Thallus medullar C–, K–, KC+ pink or KC–, P–, contains atranorin, microphyllinic, 4-O-methylolivetoric, and 4-O-demethylmicrophyllinic acids (Fig. 4).

Distribution: Mt. Baekwoon, Mt. Dukyoo, Mt. Halla, Mt. Jiri, Mt. Jumbong, Mt. Odae, Mt. Sobaek, Mt. Sorak, Mt. Taebaek (Fig. 5).

Habitat and ecology: Alt. 450~1900 m; on trunk of *Abies*, *Acer* and *Quercus*; occasionally on rock with mosses.

Remarks: It may be confused with *C. pseudolivetorum* without a chemical test if the lobules are not well developed. The lobules tend to be flatter and more dorsiventral in *C. japonica* than in *C. pseudolivetorum*. This species is the most common species in South Korea.

Representatives of 52 specimens : 041594 Jae-Seoun Hur, October 12, 2004.

4. *Cetrelia pseudolivetorum* (Asahina) W.L. Culb. & C.F. Culb. *Contrib. U.S. Natl. Herb.* 34 (7): 519-521, 1968

External morphology: Thallus medium to large, 5~15 cm, lobes 0.5~1.5 cm broad, isidia-like dorsiventral dissected lobules present on margin of the lobes or on the upper surface (Fig. 3-1). Upper surface grayish or uniformly light brownish or tan in old herbarium specimens, smooth or becoming cracked, psuedocyphellate small to medium, about 0.5 mm broad, punctiform or slightly elongate. Lower surface black, margins brown or grayish like the color of upper surface, rhizines black. Apothecia not seen. Pycnidia present on the tips of lobules, few or abundant.

Chemistry: Thallus medullar C+ pink or red, K–, KC+ pink to red, P–, contains atranorin, olivetoric acid, anziaic acid as an accessory (Fig. 4).

Distribution: Mt. Dukyoo, Mt. Jiri, Mt. Sobaek, Mt. Taebaek (Fig. 5).

Habitat and ecology: Alt. 1300~1550 m; mostly on rock: sometimes on trunk of *Quercus*.

Remarks: *C. pseudolivetorum* is very close to *C. japonica* when it has well developed dorsiventral lobules, and close to *C. braunsiana* when it has isidia-like lobules, but their chemistry is very different.

Representatives of 5 specimens: 030784 Jae-Seoun Hur, October 2, 2003.

The species not found in this time

Two more species of *Cetrelia monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. and *C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. were previously recorded in South Korea (Park, 1990). These were rarely found even 20 years ago and all her collections were deposited in Duke University, USA. However, we have seen no corresponding specimens during our expeditions and, possibly, they are under threaten of extinction. *C. monachorum* is currently treated as synonymy of *C. olivetorum*. This species is easily distinguished from *C. japonica* and *C. pseudolivetorum* because of having soredia. It is not confused with *C. chicitae* and *C. braunsiana* because of C+ reaction due to the presence of olivetoric acid.

Cetrelia isidiata (Asahina) W.L. Culb. & C.F. Culb. and *Cetrelia nuda* (Hue) W.L. Culb. & C.F. Culb. were floristically reported in Korean peninsula without detailed description. These were not traceable due to lack of voucher specimens at this moment, so they are not included in this paper.

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