

Taxonomic Study on the Lichen Genus *Xanthoparmelia* (Ascomycotina, Parmeliaceae) in Korea

Xin Yu Wang, Young Jin Koh and Jae-Seoun Hur*

Korean Lichen Research Institute, Sunchon National University, Sunchon 540-742, Korea

(Received November 17, 2008. Accepted December 13, 2008)

In previous studies investigating the genus *Xanthoparmelia*, thirteen different species have been reported from South Korea alone. However, there currently has been no revisional study performed until now. To explore the genus *Xanthoparmelia*, a phenotypic analysis was performed based on morphological, anatomical and chemical characters, while an investigation of *Xanthoparmelia* phylogeny was based on nuclear ribosomal (nr) DNA ITS sequences. A thorough examination of the specimens deposited in the Korean Lichen Research Institute (KoLRI) confirmed that eight species of *Xanthoparmelia* occur inside South Korea. Our analysis further confirmed the colors of the lower surface and medullar chemistry are important taxonomic characters in *Xanthoparmelia*. This study also presents a detailed description of each species and a key to the genus.

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

The genus *Xanthoparmelia* (Vain.) Hale, comprised of approximately 750 species, constitutes a major part of the family Parmeliaceae Zenker (Blanco *et al.*, 2004). All *Xanthoparmelia* species share key taxonomic characters, including the degree of attachment to the substrate, color of the lower surface (pale brown to ebony black), presence of isidia of different types (cylindrical to globose), shape of the lobes and the medullar chemistry (Hale, 1971; Kurokawa, 1989). The history of *Xanthoparmelia* as an individual genus has been relatively brief, as all *Xanthoparmelia* species were only separated from *Parmelia* as recently as 1974, and few publications on this genus even existed up until 1959. Only since 1964 have virtually all of the 300 or so additional new species of *Xanthoparmelia* been described, with a total of 750 species reported until now. Furthermore, while previous publications on the genus *Xanthoparmelia* exist in South Korea (Park, 1990; Moon, 1999; Kashiwadani *et al.*, 2002; Hur *et al.*, 2005), most of them were floristic surveys and therefore no detailed descriptions were made. These factors support the necessity to continue detailed taxonomic research. Therefore, the aim of this study was to evaluate the importance of taxonomic characters and to investigate previously unreported phenotypic and phylogenetic analyses on *Xanthoparmelia* species in a detailed manner.

Materials and Methods

Phenotypic analysis. A phenotypic analysis based on morphological, anatomical and chemical characters was

performed on fifty-four lichen specimens that were collected from 2003 to 2006 and deposited in KoLRI (Korean Lichen Research Institute). Forty morphological and chemical characters were chosen for the phenotypic analysis (Table 2). Descriptions of the species were based on air-dried specimens which were observed under a stereomicroscope (Nikon SMZ1500). Sections were made with a razor blade and samples were mounted with GAW (glycerol : ethanol : water = 1 : 1 : 1) and observed using a compound microscope (Olympus BX50). Chemical characters were examined by color reaction (KOH, CaCl₂O₂ and *P*-phenylenediamine) and thin layer chromatography (Culberson, 1972) using solvent C (toluene : acetic acid = 170 : 30). Maximum parsimony analysis was performed by PAUP version 4.0b10 (Swofford, 2002). The reliability of the inferred tree was tested by 1000 bootstrap replications with *Flavoparmelia caperata* and *Lecanora muralis* being used as outgroups.

DNA extraction and nrDNA amplification. Sixteen representative specimens (Table 1) were used for DNA extraction. Total DNA was extracted directly from 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 used for amplification were ITS1F (5'-CTTGGTCATTTACAGGAAGTAA-3'; Gardes and Bruns, 1993) and ITS4A (5'-ATTTGAGCTCTTC-CCGCTTCA-3'; White *et al.*, 1990). Previously described conditions by Arup (2002) were used for PCR amplification and cycle sequencing.

*Corresponding author <E-mail : jshur1@sunchon.ac.kr>

Table 1. *Xanthoparmelia* specimens used for ITS sequence analysis

Coll. no.	Species name	Locality	GPS	Alt. (m)
030032	<i>X. claviculata</i>	Mt. Backwoon	N36°57'06.3" E 129°22'54.7"	85
050486	<i>X. claviculata</i>	Mt. Hugseok	N34°41'21.4" E 126°40'51.4"	230
050528	<i>X. claviculata</i>	Mt. Cheonkwan	N34°32'39.8" E 126°56'51.5"	200
040737	<i>X. conspersa</i>	Jeju island	N33°22'10.3" E 126°30'17.0"	1520
061219	<i>X. conspersa</i>	Mt. Joryong	N36°09'14.1" E 127°36'14.8"	663
040173	<i>X. coreana</i>	Mt. Byeon	N35°38'03.4" E 126°34'15.3"	305
050416	<i>X. coreana</i>	Mt. Bugue	N35°47'06.5" E 127°24'59.5"	410
050189	<i>X. hirosakiensis</i>	Mt. Deokyu	N35°50'22.4" E 127°44'48.0"	1550
050205	<i>X. hirosakiensis</i>	Mt. Deokyu	N35°48'15.9" E 127°43'35.9"	1399
061101	<i>X. mexicana</i>	Mt. Joryong	N36°49'08.7" E 128°02'58.6"	967
050465	<i>X. orientalis</i>	Mt. Hugseok	N34°41'21.4" E 126°40'51.4"	203
050490	<i>X. orientalis</i>	Mt. Hugseok	N34°41'21.1" E 126°40'47.5"	230
050397	<i>X. subramigera</i>	Mt. Palgong	N35°36'01.8" E 127°27'57.4"	688
060132	<i>X. subramigera</i>	Mt. Gaya	N35°48'54.4" E 128°07'32.5"	1190
041142	<i>X. tuberculiformis</i>	Mt. Taeback	N37°05'40.4" E 128°56'48.8"	1617
050435	<i>X. tuberculiformis</i>	Mt. Bugue	N35°48'20.6" E 127°23'40.2"	810

Table 2. Forty phenotypic characters chosen for analysis

No. Characters	No. Characters
1. Thallus Foliose	21. Isidia present
2. Thallus subfoliose	22. Isidia absent
3. Thallus crustose	23. Isidia cylindrical
4. Thallus unattached	24. Isidia globose
5. Thallus loosely adnate	25. Medulla white
6. Thallus tightly adnate	26. Medulla pigmentated
7. Thallus saxicolous	27. Lower surface plane
8. Thallus free growing on soil	28. Lower surface canaliculated
9. Lobes subirregular	29. Lower surface pale brown
10. Lobes sublinear to linear	30. Lower surface brown
11. Lobe margin black-rimmed	31. Lower surface black
12. Lobe margin smooth	32. Lower surface rhizinate
13. Lobes plane	33. Lower surface sparsely rhizinate
14. Lobes convoluted	34. Lower surface densely rhizinate
15. Lobes wide	35. Rhizines simple
16. Lobes narrow	36. Rhizines branched
17. Upper surface smooth	37. Usnic acid present
18. Upper surface rugose	38. Fumarprotocetraric acid etc. present
19. Upper surface maculate	39. Slazinic acid etc. present
20. Upper surface emaculate	40. Norlobaridone acid present

Sequencing and phylogenetic analysis. PCR products were sequenced using the ABI 3700 automated DNA Sequencer in NICEM at Seoul National University while Mega3.1 (Kumar *et al.*, 2004) was used for the phylogenetic analysis. Neighbor-joining (Saitou and Nei, 1987) was chosen to construct the phylogenetic tree, using the model kimura 2-parameter. Pairwise deletion was applied to gaps in data, and for a control, the reliability of the inferred tree was tested by 1000 bootstrap replications. *Flavoparmelia caperata* AY581059 and *Lecanora muralis* AF159922 were used as outgroups.

Results and Discussion

Phenotypic analysis. A maximum parsimony tree was performed using PAUP (Swofford, 2002) (Fig. 1) for the phenotypic analysis of *Xanthoparmelia*. Within the *Xanthoparmelia* clade, the species can be separated into two groups that indicate the color of the lower surface is the most important phenotypic character to distinguish between the species. Group I was characterized by a dark brown to black lower surface while the lower surface of group II was characterized by a pale brown color.

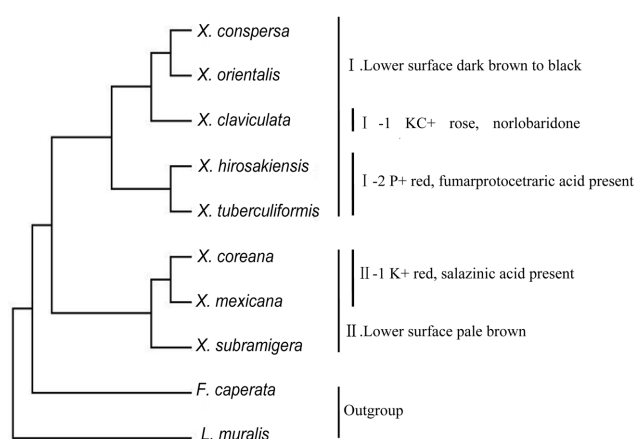


Fig. 1. Maximum parsimony tree of 8 species of *Xanthoparmelia* in Korea; *Lecanora muralis* and *Flavoparmelia caperata* as outgroups. Data matrix has 10 taxa and 40 characters. All characters are of 'unord' type and have equal weight. Character 6 is constant, 11 variable characters are parsimony-uninformative, number of parsimony-informative characters = 23. Tree length = 58, Consistency index (CI) = 0.59, Homoplasy index (HI) = 0.41.

Further classifications of the two groups can be made. In group I, *X. claviculata* contains norlobaridone and thus can be separated from the other four species. *X. hirosakiensis* and *X. tuberculiformis* form an additional small group because of the presence of fumarprotocetraric acid. In group II, *X. coreana* and *X. mexicana* are grouped together because salazinic acid was present in these two species but absent in *X. subramigera*. These classifications support the idea that chemical compound is important, in addition to morphological and anatomical characters,

in providing a framework to separate the different species.

Phylogenetic analysis. The neighbor-joining (NJ) consensus tree (Fig. 2) was constructed by Mega 3.1. Within group I, *X. claviculata* contains the chemical compound norlobaridone and is therefore unique, indicating that the chemical compound is an important character in differentiation between the species. However, there were very few variations (1~2%) in the ITS sequence of *Xanthoparmelia*, and consequently the ITS sequence could not distinguish the species within this particular genus.

The results of phylogenetic and phenotypic trees did not coincide well with each other mainly due to an absence of variation in ITS sequences. However, the presence of norlobaridone in the species *X. claviculata* clearly suggests its uniqueness in the two trees and moreover that chemical compound is a key character in distinguishing between the species. In conclusion, differences in both lower surface color and thallus chemical compound serve as important differentiations in the taxonomy of *Xanthoparmelia* in South Korea.

Taxonomic treatment of the genus. According to the comprehensive analysis, a key to the genus is presented with morphological and chemical characters. Detailed description of each species is also presented.

Key to the genus *Xanthoparmelia* in South Korea

1. Medulla P-, KC+ rose *X. claviculata*
1. Medulla P+, KC- 2
2. Medulla K+ yellow, P+ orange, lower surface black *X. conspersa*
2. Medulla K- or K+ yellow turning red 3
3. K+ yellow turning dark red, P+ yellow to orange

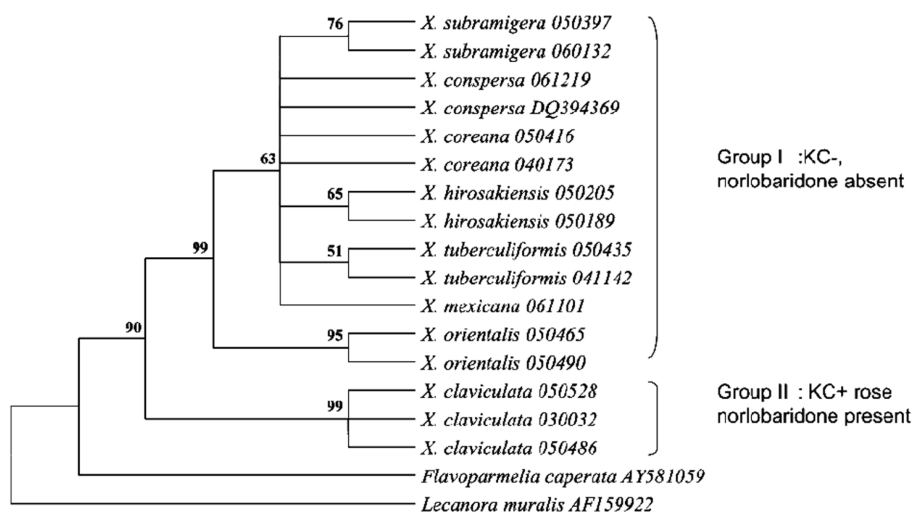


Fig. 2. NJ consensus tree based on nrDNA ITS sequences. Nucleotide: Kimura 2-parameter, pairwise deletion, bootstrap = 1000. The numbers in each node represent bootstrap support values, and the numbers lower than 50 were not shown. (Note: *X. conspersa* DQ3943369 is Hur 040737).

- 4
3. K- (or slowly faint yellow), P+ red 6
4. Lower surface black, isidia subcylindrical, P+ yellow *X. orientalis*
4. Lower surface pale brown 5
5. Lower surface reddish brown, lobes sublinear elongated, P+ yellow *X. coreana*
5. Lower surface ivory brown, lobe apices rounded, P+ orange *X. mexicana*
6. Lower surface pale brown, isidia subglobose *X. subramigera*
6. Lower surface dark brown to black 7
7. Lower surface brown to dark brown, apothecia reddish brown *X. hirosakiensis*
7. Lower surface black, apothecia blackish brown *X. tuberculiformis*

1. *Xanthoparmelia claviculata* Kurok., *J. Jap. Bot.* 64(10): 296 (1989)

Thallus is loosely adnate to moderately adnate; color is yellow-green, saxicolous. Lobes are sublinear, imbricate in the marginal parts, about 1–2 mm wide. Upper surface is slightly shiny and rugose, emaculate, margin smooth and black-rimmed in the tips parts. Isidia are on the upper surface, subglobose and simple, up to 0.01 mm in diameter. Isidia are sometimes branched, with branchlets usually near the base. Medulla is white. The lower surface is brown to dark brown and rugose, and darker in the marginal areas. Rhizines are sparse and simple without branches, while the color is usually darker than the lower surface. Apothecia were not found.

Chemistry: Thallus K-, Medulla K-, C-, KC + rose, P-. Containing norlobaridone (Fig. 4-1) and usnic acid.

Remark: This species can be easily distinguished from other species in South Korea by its unique medullary chemistry. It is a very unique phylogenetic group, and has differences with the groups of other species (Fig. 2).

Specimens examined: Mt. Backwoon, N36°57'06.3", E129°22'54.7", alt. 85m, Hur 030032; Mt. Hugseok, N34°41'21.4", E126°40'51.4", alt. 230m, Hur 050486; Mt. Cheonkwan, N34°32'39.8", E126°56'51.5", alt. 200m, Hur 050528.

2. *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale, *Phytologia* 28: 485 (1974)

Thallus is adnate to loosely adnate, color is dark yellow-green, saxicolous. Lobes are sublinear and very narrow, 0.5–2.5 mm wide. Lacinate is dense in certain areas, lobe margin is smooth and black-rimmed, apices of the lobe are blunt. Upper surface is smooth, emaculate and shiny; moderately isidiate. Isidia are globose to cylindrical, and usually possess dark tips that are 0.1–2 mm in diameter, simple or coralloid branched. Medulla is white. Lower surface is plane, dark brown to black (Fig. 3H); the color

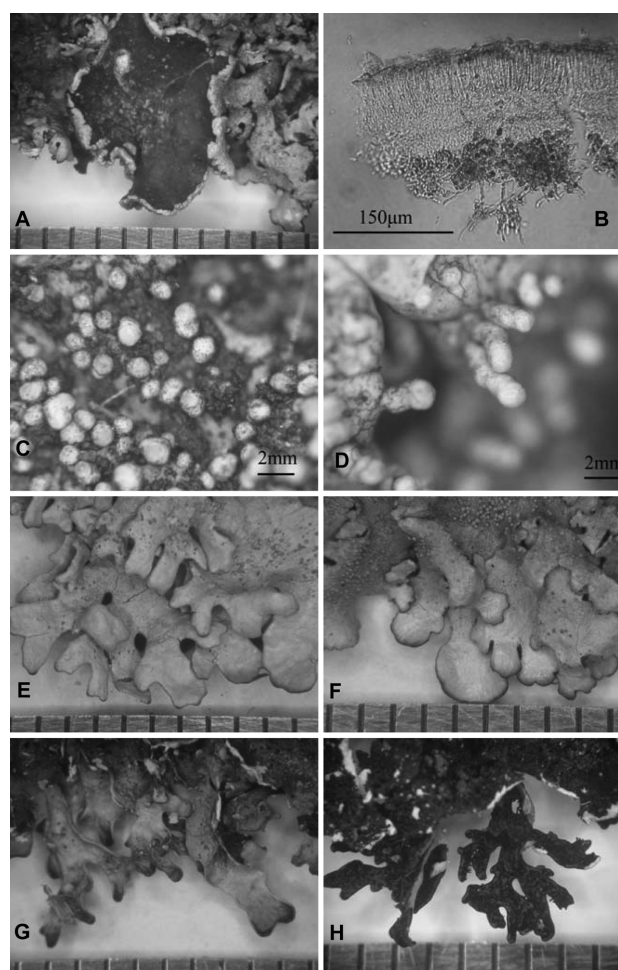


Fig. 3. Morphological characters of *Xanthoparmelia* species. **A.** Apothecia of *X. conspersa* Hur040737; **B.** Vertical section of apothecia Hur040737; **C.** Subglobose isidia of *X. subramigera* Hur050465; **D.** Cylindrical isidia of *X. orientalis* Hur050465; **E.** Sublinear elongated lobe of *X. coreana* Hur050416; **F.** Rotund lobe of *X. mexicana* Hur061101; **G.** Pale brown lower surface of *X. coreana* Hur050416; **H.** Black lower surface of *X. conspersa* Hur040737.

is pale to dark brown in the marginal parts. Rhizines are sparse to moderate, simple, without branches and concolorous with the lower surface. Apothecia (Figs. 3A and 3B) are not common and substipitate; rim is isidiate. Disc is concave, red-brown, 3–8 mm in diameter; spores are fusiform, 9–10 μ m long.

Chemistry: Thallus K-, Medulla K + deep yellow or turning orange; C-, KC-, P + yellow to red orange. Containing stictic, constictic, cryptostictic, usnic acid and varying amounts of norstictic acid.

Remarks: This species is the most common one containing both stictic and norstictic acid along with a black lower surface. It is identical with *X. piedmontensis*, but the latter species has fumarprotocetraric acid (Hale, 1990). This species has been previously documented for its use

in the treatment of venereal disease and snakebites (Brodo *et al.*, 2001).

Specimens examined: Mt. Naejang, N35°29'41.0", E126°52'53.3", alt. 650m, Hur 030617; Jeju island, N33°22'10.3", E126°30'17.0", alt. 1520m, Hur 040737; Mt. Sokri, N36°32'39.7", E127°51'47.8", alt. 700m, Hur 060052; Mt. Joryong, N36°49'04.2", E128°02'53.5", alt. 826m, Hur 061080; Mt. Joryong, N36°09'14.1", E127°36'14.8", alt. 663m, Hur 061219; Mt. Palbong, N36°48'40.7", E126°22'20.6", alt. 194m, Hur 061225.

3. *Xanthoparmelia coreana* (Gyeln.) Hale, *Mycotaxon* 33: 402 (1988)

Thallus is adnate to loosely adnate; color is light yellowish-green, 3~8 cm wide and saxicolous. Lobes are sublinear elongated (Fig. 3E), 1~4 mm wide, almost separate, and with some parts imbricate. The margin of the lobes is smooth and black-rimmed around the apices. Upper surface is shiny and weakly white-maculate. Isidia are moderately to abundantly isidiate, simple and possess a subglobose to cylindrical shape, 0.1~0.2 mm in diameter, usually pale gray tipped. Medulla is white. Lower surface is smooth, chestnut-brown to reddish brown (Fig. 3G), sparsely to moderately rhizinate. Rhizines are simple and 0.3~0.7 mm long, concolorous with the lower surface. Apothecia is lacking.

Chemistry: Thallus K-; medulla K + yellow turning dark red, C-, KC-, P + bright yellow. Containing usnic, salazinic (Fig. 4-2), norstictic and consalazinic acid (trace).

Remark: The species is characterized by a chestnut-

brown to reddish brown lower surface and contains salazinic acid as the major compound. *X. coreana* is very close to *X. mexicana*, but the latter one has a pale brown lower surface and rounded lobes.

Specimens examined: Sorok island, N35°48'11.2", E129°18'47.3", alt. 15m, Hur 030063; Mt. Sobaek, N36°57'27.0", E128°26'40.7", alt. 618m, Hur 030712; Mt. Taebak, N37°06'39.1", E128°55'41.2", alt. 930m, Hur 040079-1; Mt. Byeon, N35°38'03.4", E126°34'15.3", alt. 305m, Hur 040173; Jeju island, N33°33'26.0", E126°43'56.9", alt. 600m, Hur 040891; Wando arboretum, N34°21'10.3", E126°41'10.9", alt. 535m, Hur 050137; Geogum island, N34°25'20.8", E127°08'43.1", alt. 10m, Hur 050212; Mt. Kum, N34°27'28.4", E127°09'48.1", alt. 280m, Hur 050223; Mt. Dalma, N34°22'45.2", E126°35'11.6", alt. 456m, Hur 050342; Mt. Illim, N34°41'17.7", E127°00'57.3", alt. 220m, Hur 050368; Mt. Bugue, N35°47'06.5", E127°24'59.5", alt. 410m, Hur 050416; Mt. Hugseok, N34°41'23.4", E126°41'02.6", alt. 110m, Hur 050448; Mt. Hugseok, N34°41'21.1", E126°40'47.5", alt. 230m, Hur 050497; Mt. Sokri, N36°32'40.9", E127°50'44.0", alt. 615m, Hur 060044; Mt. Gaya, N35°49'00.8", E128°07'33.8", alt. 1250m, Hur 060143; Mt. Hanla, N33°22'20.5", E126°52'42.0", alt. 1m, Hur 061014; Mt. Joryong, N36°49'04.2", E128°02'53.5", alt. 826m, Hur 061077; Mt. Joryong, N36°49'08.7", E128°02'58.6", alt. 967m, Hur 061093; Mt. Cheontae, N36°09'24.9", E127°36'44.0", alt. 306m, Hur 061173; Mt. Cheontae, N36°09'01.6", E127°36'27.2", alt. 541m, Hur 061220; Palbong, N36°48'40.7", E126°22'20.6", alt. 194m, Hur 061228.

4. *Xanthoparmelia hirosakiensis* (Gyeln.) Kurok., *J. Jap. Bot.* 64(10): 289 (1989)

Thallus is adnate to tightly adnate; color is yellowish-green and saxicolous. Lobes are sublinear, long, 1~2.5 mm wide and abundantly branched. Margin of the lobes is smooth and black-rimmed. Upper surface is dull yet sometimes shiny, moderately to abundantly isidiate. Isidia are simple and cylindrically shaped to subglobose, 0.1~0.2 mm in diameter, 0.3~0.8 mm in height, and usually coral-loid branched. Medulla is white. Lower surface is pale-blackish brown to brown. Rhizines are sparse to moderate, concolorous with the lower surface or blackish brown. Apothecia are rarely seen, sessile, 3~7 mm in diameter; disc is weakly concave, reddish brown.

Chemistry: Thallus K-. Medulla K- or slowly faint yellow, C-, KC-, P + orange red. Containing usnic, fumarprotocetraric and protocetraric acid.

Remark: This species is similar with *X. subramigera*, but has a much darker lower surface color in comparison to *X. subramigera*. Fumarprotocetraric acid is the main secondary medullar compounds in this species.

Specimens examined: Mt. Chiak, N37°18'29.8", E128°03'01.7", alt. 1145m, Hur 040606; Mt. Deokyu,

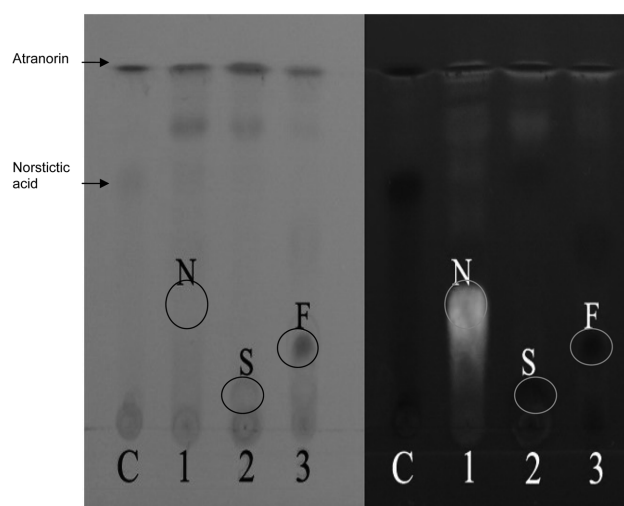


Fig. 4. TLC plate showing the important chemical compound in *Xanthoparmelia* genus identification (Solvent C). 1. *X. claviculata* Hur050528, showing norlobaridone (N); 2. *X. coreana* Hur040173, showing salazinic acid (S); 3. *X. subramigera* Hur050397, showing fumarprotocetraric acid (F).

Table 3. Matrix form of 40 phenotypic characters used in the phenotypic analysis

Character no. Species name	1										2										3										4														
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0					
<i>Xanthoparmelia claviculata</i>	0	1	0	0	1	0	1	0	0	1	1	1	1	0	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	1	0	1	1	0	1	0	1	1	0	1	0	0	1		
<i>X. conspersa</i>	0	1	0	0	1	0	1	0	0	1	1	1	1	0	0	1	1	0	0	1	1	0	?	1	1	0	0	0	0	0	1	1	1	0	1	0	1	0	1	0	0	0			
<i>X. coreana</i>	0	1	0	0	1	0	1	0	0	1	1	1	1	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0			
<i>X. hirosakiensis</i>	0	1	0	0	0	1	1	0	0	1	1	1	1	0	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	1	1	0	1	0	1	0	1	1	0	0			
<i>X. mexicana</i>	0	1	0	0	0	1	1	0	1	0	1	1	1	0	1	0	1	0	?	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0			
<i>X. orientalis</i>	0	1	0	0	1	0	1	0	0	1	1	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1	1	0	1	0	1	0	1	0	1	0			
<i>X. subramigera</i>	0	1	0	0	0	1	1	0	1	0	1	1	1	0	0	1	0	1	1	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	1	0	1	0	1	1	0	0			
<i>X. tuberculiformis</i>	0	1	0	0	1	0	1	0	0	1	1	1	1	0	0	1	1	0	0	1	1	0	1	0	1	0	1	0	0	0	1	1	0	1	1	0	1	1	0	0	1	0	0		
<i>Lecanora muralis</i>	0	0	1	0	0	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	1	0	1	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Flavoparmelia caperata</i>	1	0	0	0	1	0	0	0	0	1	0	1	0	?	1	0	0	1	0	?	0	1	0	0	?	0	0	1	0	0	1	1	1	0	1	0	1	0	1	0	0	0	0		

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

N35°51'24.1", E127°44'53.6", alt. 1695m, Hur 050084; Wando arboretum, N34°21'10.3", E126°41'10.9", alt. 535m, Hur 050141; Mt. Deokyu, N35°50'22.4", E127°44'48.0", alt. 1550m, Hur 050189; Mt. Deokyu, N35°48'15.9", E127°43'35.9", alt. 1399m, Hur 050205; Mt. Joryong, N36°49'08.7", E128°02'58.6", alt. 967m. Hur 061100.

5. *Xanthoparmelia mexicana* (Gyeln.) Hale, *Phytologia* 28: 488 (1974)

Thallus is tightly adnate to adnate on rock; 3–8 cm wide, with thallus color gray-green to olive-green. Lobes are subirregular, crowded, 1.5–4 mm wide and rarely lacinate in the marginal parts. Margin is smooth, apices rotund (Fig. 3F) with black to brownish rim. Upper surface is shiny and continuous or cracked to some extent, without any obvious macula; also moderately to densely isidiate. Isidia are cylindrical to subglobose in shape, 0.1–0.2 mm in diameter, tips are concolorous or brownish. Medulla is white. Lower surface is plain and ivory brown, moderately rhizinate. The rhizines are simple, 0.1–0.6 mm long and dark brown. Apothecia not found.

Chemistry: Thallus K-, Medulla K + yellow turning red, C-, KC-, P + yellow turning red orange. It contains usnic, salazinic norstictic and traces of protocetraric acid.

Remark: Although this species could be mistaken visually with *X. coreana* or *X. subramigera* with a similar lower surface color, these three species remain different in chemical compound composition: *X. mexicana* with protocetraric acid and *X. subramigera* with fumarprotocetraric acid.

Specimens examined: Mt. Jiri, N35°18'14.0", E127°34'10.4", alt. 1430m, Hur 060292; Mt. Joryong, N36°49'08.7", E128°02'58.6", alt. 967m, Hur 061101.

6. *Xanthoparmelia orientalis* Kurok., *J. Jap. Bot.* 64(6): 169 (1989)

Thallus is adnate to loosely adnate; the color is yellowish-green, saxicolous. Lobes are sublinear to subirregular, usually imbricate, 1–2.5 mm wide. Margin is smooth and

black-rimmed near the apices. Upper surface is dull, sparsely to moderately isidiate. Isidia are cylindrical (Fig. 3D) and concolorous with the upper surface, 0.1–0.2 mm in diameter. Medulla is white. Lower surface is blackish brown to black in the center, and the marginal parts are usually brown to dark brown. Rhizines are simple and black, 0.2–0.8 mm long and sparse. Apothecia were previously reported to be rather rare (Kurokawa, 1989) and indeed apothecia were not found in the South Korean specimens.

Chemistry: Thallus K-, medulla K + yellow turning red, C-, KC-, P + bright yellow. Containing usnic, salazinic, consalazinic and traces of norstictic acid.

Remark: This species is characterized by the blackish brown to black lower surface along with a wide brown zone near the lobe apices. It could be confused with *X. coreana*, but the latter species has a much paler lower surface color.

Specimens examined: Mt. Naejang, N35°48'11.2", E129°18'47.3", alt. 685m, Hur 030461; Geogum island, N34°25'20.8", E127°08'43.1", alt. 10m, Hur 050211; Mt. Hugseok, N34°41'21.4", E126°40'51.4", alt. 203m, Hur 050465; Mt. Hugseok, N34°41'21.1", E126°40'47.5", alt. 230m, Hur 050490; Mt. Hugseok, N34°41'21.1", E126°40'47.5", alt. 230m, Hur 050491; Mt. Cheontae, N36°09'24.9", E127°36'44.0", alt. 580m, Hur 061201.

7. *Xanthoparmelia subramigera* (Gyeln.) Hale, *Phytologia* 28: 489 (1974)

Thallus is tightly adnate to adnate; the color is dull yellowish green, saxicolous, 4–7 cm broad. Lobes are subirregular, crowded and imbricate, 0.5–3 mm wide. Lobe margin is smooth with a brownish or blackish rim. Upper surface is continuous, usually cracked in the central parts and slightly maculate. Surface is moderately to densely isidiate. Isidia are subglobose (Fig. 3C), 0.1–0.2 mm in diameter, simple to sparsely branched. Medulla is white. Lower surface is plain, pale brown, sparsely to moderately rhizinate. Rhizines are brown, simple, 0.2–1 mm long.

Pycnidia are rare and laminal. Apothecia were not found.

Chemistry: Thallus K-. Medulla K- or + faint yellow, C-, KC-, P+ orange red. Containing usnic, fumarprotocetraric (Fig. 4-3) protocetraric and traces of succinprotocetraric acid.

Remark: The species is quite similar with *X. hirosakiensis*, but the color of the lower surface is much lighter than *X. hirosakiensis*, and further it has the succinprotocetraric whereas it is absent in *X. hirosakiensis*.

Specimens examined: Mt. Palgong, N35°36'01.8", E127°27'57.4", alt. 688m, Hur 050397; Mt. Gaya, N35°48'54.4", E128°07'32.5", alt. 1190m, Hur 060132; Mt. Jiri, N35°18'24.6", E127°34'21.2", Hur 060307.

8. *Xanthoparmelia tuberculiformis* Kurok., *J. Jap. Bot.* 64(6): 169 (1989)

Thallus is adnate to loosely adnate; color is brownish yellow-green, usually darker in the central parts, saxicolous. Lobes are sublinear to subirregular, 0.5–2 mm wide. Margin is smooth and black or blackish-brown rimmed. Upper surface is dull, emaculate. Moderately to abundantly isidiate. Isidia are simple, subglobose to cylindrically shaped, sometimes coralloid branched with branchlets near the base and less than 0.1 mm in diameter. Medulla is white. Lower surface color is black to blackish brown, moderately rhizinate. Rhizines are simple and black. Apothecia are rare, sessile, 2–6 mm in diameter; disc concave, blackish brown, margin is isidiate.

Chemistry: Thallus K-, medulla K- or slow pale yellow, C-, KC-, P+ orange red. Containing usnic, fumarprotocetraric, protocetraric acid.

Remark: This species is quite similar with *X. hirosakiensis* as both of them have the same chemical compound. However, the lower surface is black in *X. tuberculiformis* while it is brown in *X. hirosakiensis*.

Specimens examined: Mt. Taebaek, N37°05'40.4", E128°56'48.8", alt. 1617m, Hur 041142; Mt. Deokyu, N35°50'19.9", E127°44'43.6", alt. 1440m, Hur 050193; Mt. Bugue, N35°48'20.6", E127°23'40.2", alt. 810m, Hur 050435.

The species not found in this time. There are five additional species of *Xanthoparmelia* previously recorded in the Korean peninsula, but our study failed to report these corresponding specimens. They are *X. botryoides* (Moon, 1999), *X. piedmontensis* (Kashiwadani *et al.*, 2002), *X. plittii* (Ko, 1992), *X. somloensis* (Huneck *et al.*, 1989), *X. subpolyphylloides* (Cho and Lee, 1980).

These particular species are no less important and warrant further discussion, especially considering that the presence of chemical characters distinguish between them as we found in our study. According to Moon (1999), *X. botryoides* was abundant on rock in Mt. Sorak and formerly identified as *X. subramigera* by Park (1990).

Kurokawa (1989) treated *X. botryoides* as a novel species because it contains fumarprotocetraric and succinprotocetraric acid. *X. botryoides* has a dark to blackish brown lower surface and can be easily distinguished from *X. subramigera*, which has a pale brown lower surface. This species is actually very similar with both *X. hirosakiensis* and *X. tuberculiformis* yet can be separated from the latter two species by the presence of succinprotocetraric acid in the medulla, thereby supporting the role of chemical characters in taxonomic identification.

X. piedmontensis contains fumarprotocetraric and usnic acid, and can be further characterized by subglobose to cylindrical isidia. This species was reported by Park (1990) in Korea, but later redetermined to *X. hirosakiensis* by Moon (1990).

X. plittii has cylindrical isidia and a pale brown lower surface, containing stictic, constictic, norstictic and usnic acid. Ko (1992) reported only two members of the *Xanthoparmelia* species, *X. plittii* and *X. piedmontensis*, during the taxonomic analysis of Parmeliaceae and Phyciaceae in Mt. Jiri. At the time, *X. plittii* was identified by the presence of salazinic acid (K+ red) and isidia. However, there have been no reports on this species since, even in Park's (1990) and Moon's (1999) surveys. It is highly possible that two different species, *X. mexicana* or *X. coreana*, were misidentified as *X. plittii* by Ko, since both species are more common in Korea and possess the presence of K+ red and isidia.

X. somloensis is recognized by its loose adnation, generally elongated stenophylloid lobes, its distinct maculation on the upper surface, pale brown lower surface and presence of salazinic acid. This species was reported from North Korea by European lichenologists (Huneck *et al.*, 1989).

X. subpolyphylloides is characterized by its lack of isidia, its pale brown lower surface and presence of usnic, salazinic and consalazinic acid. Cho and Lee (1980) first reported *Parmelia taractica*, which is currently being treated as *X. subpolyphylloides* during studies on Parmeliaceae in Mt. Deokyu area. They described the species as lacking isidia and soredia with color reactions of K+ red and P+ orange. Notably, the color reactions are similar with those of either *X. mexicana* or *X. coreana*. However, both species have isidia thereby differentiating themselves from *X. subpolyphylloides*.

These five species were reported as comprising part of the natural flora of the Korean peninsula without much detailed description. Consequently, they were not traceable due to the lack of voucher specimens and therefore are not included in this paper.

Acknowledgement

This work was supported by a grant from Korea National

Research Resource Center Program (Grant R21-2007-000-10033-0), and also by NON DIRECTED RESEARCH FUND (2005), Sunchon National University, Korea.

References

- Arup, U. 2002. PCR techniques and automated sequencing in lichens. In: *Protocols in lichenology: culturing, biochemistry, ecophysiology and use in biomonitoring*, pp. 392-411. Eds. I. Kranner, R. P. Beckett, and A. K. Varma. Springer-Verlag, New York.
- Blanco, O., Crespo, A., Elix, J. A., Hawksworth, D. L. and Lumbsch, H. T. 2004. A molecular phylogeny and a new classification of parmelioid lichens containing *Xanthoparmelia*-type lichenan (Ascomycota: Lecanorales). *Taxon* 53:959-975.
- Brodo, I. M., Sharnoff, D. S. and Sharnoff, S. 2001. *Lichens of North America*, pp. 735. Yale University Press, New Haven and London.
- Cho, S. S. and Lee, Y. N. 1980. Studies on Parmeliae in Mt. Deogyoo Area. *Kor. J. Mycol.* 8:149-157.
- Culbertson, C. F. 1972. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *J. Chromatography* 72:113-125.
- Ekman, S. 1999. PCR optimization and troubleshooting, with special reference to the amplification of ribosomal DNA in lichenized fungi. *Lichenologist* 31:517-531.
- Gardes, M. and Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes. Application for the identification of mycorrhizae and rusts. *Molecular Ecology* 2:113-118.
- Hale, M. E. 1971. Studies on Parmelia subgenus *Xanthoparmelia* (Lichenes) in South Africa. *Bot. Not.* 124:343-354.
- Hale, M. E. 1990. A synopsis of the lichen genus *Xanthoparmelia* (Vainio) Hale (Ascomycotina, Parmeliaceae). *Smithsonian Contributions to Botany* 74:1-250.
- Huneck, S., Ri, J. D., Ahti, T. and Poelt, J. 1989. Zur Kenntnis der Flechtenflora von Korea. *Herzogia* 8:177-185.
- Hur, J. S., Koh, Y. J. and Harada, H. 2005. A checklist of Korean lichens. *Lichenology* 4:65-95.
- Kashiwadani, H., Moon, K. H., Inoue, M., Thor, G. and Kim, Y. S. 2002. Lichens of the Cheju island, Republic of Korea. I. The macrolichens. In *Proceedings of the 3rd and 4th symposium on collection building and Natural History studies in Asia and the Pacific rim*, pp. 115-135. Eds. T. Kubodera, M. Higuchi and R. Miyawaki. National Science Museum, Tokyo.
- Ko, M. J. 1992. A taxonomic study of family Parmeliaceae and Physciaceae (lichens) in Mt. Chiri. M.S Thesis. Sookmyung Women's University. Seoul, Korea.
- Kumar, S., Tamura, K. and Nei, M. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* 5:150-163.
- Kurokawa, S. 1989. Studies on Japanese species of *Xanthoparmelia* (Parmeliaceae) (1). *J. Jap. Bot.* 64:165-175.
- Kurokawa, S. 1989. Studies on Japanese species of *Xanthoparmelia* (Parmeliaceae) (2). *J. Jap. Bot.* 64:289-298.
- Moon, K. H. 1999. Lichens of Mt. Sorak in Korea. *J. Hattori Bot. Lab.* 86:187-220.
- Park, Y. S. 1990. The macrolichen flora of South Korea. *Bryologist* 93:130-131.
- Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406-425.
- Swofford, D. L. 2002. *PAUP: phylogenetic analysis using parsimony and other methods*. Sinauer Associates, Sunderland, Massachusetts, USA.
- White, T. J., Bruns, T. D., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal DNA genes for phylogenetics. In *PCR Protocols: a Guide to Methods and Applications*, pp. 315-321. Eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White. Academic Press, San Diego, USA.