

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

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Three species of *Coccocarpia* have been reported from Korean Peninsular. However, there was no revisional study on this genus before. After careful examination of the specimens deposited in the Korean Lichen Research Institute (KoLRI) and collected from main mountain areas of Korea, two species of *Coccocarpia*, *C. palmicola* and *C. erythroxyli*, have been revealed to occur and confirmed in South Korea. The presence and absence of isidia and apothecia are the most important characters for the South Korean species. We provide the detailed description and illustration of the available two species. A key to the species is also provided.

KEYWORDS: Anatomy, *Coccocarpia*, ITS sequence, Lichen, Morphology, South Korea

The lichen genus *Coccocarpia* Pers. belongs to the family *Coccocarpiaceae* Henssen. The genus was first recognized by Person in 1826. In the end of 19th century, most taxa of *Coccocarpia* were described. Several new species were added in 20th century. The important work in the history of *Coccocarpia* was done by Henssen (1963). After the detailed investigation of the ascocarps, he separated it from *Pannariaceae*. The family contains only one genus and 21 species, widely distributed in tropical and temperate regions. It has the foliose thallus composed of a prominent cortex and white medulla, and most of them have the distinctly foliose and lobate thallus. Most of the species can be distinguished by the presence of the isidia, apothecia and the shape of the isidia. Although there were many reports on the study of *Coccocarpia* (Arvidsson, 1983, 1992; Brodo *et al.*, 2001; Henssen, 1963), almost no expert study on *Coccocarpia* had been conducted in Korea until the macrolichen flora of South Korea was published (Park, 1990). In her paper, 3 species of *Coccocarpia* were reported with brief description of each species and a key to the species. The aim of this study is to evaluate the importance of the taxonomic characters, and to do detailed phenotypic and phylogenetic investigation on the species which have not been done in Korea so far.

Materials and Methods

Morphological and anatomical examination. Fifty-one lichen specimens of *Coccocarpia* from South Korea were examined and are deposited in KoLRI (Korean Lichen Research Institute) (Table 1). External morphologic descrip-

tions were based on the air-dried materials, all of them were observed under the stereomicroscope (Nikon SMZ1500). Sections were made with a razor blade under the stereomicroscope. Samples were mounted with the GAW (glycerol : ethanol : water = 1 : 1 : 1), and observed using compound microscope (Olympus BX50). The chemical characters were examined by medullar color reaction (KOH, CaCl₂O₂, and P-phenylenediamine) and thin layer chromatography (Culberson, 1972).

DNA extraction and nrDNA amplification. Ten representative specimens were used for ITS sequence analysis. Lichen thalli were fractioned with cryo-tissue-crasher (SK200, Tokken, Japan). 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'-CTTGGICATTTACAG-GAAGTAA-3'; Gardes and Bruns, 1993) and ITS4A (5'-ATTTGAGCTCTTCCCGCTTCA-3'; White *et 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 software Mega3.1 (Kumar *et al.*, 2004) was used to do the sequence analyzing. Neighbor-joining was chosen to construct the phylogenetic tree: Gaps data was pairwise deletion, and model was kimura 2-parameter, test of inferred phylogeny was 1000 times of bootstrap. *Leptogium*

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Table 1. Specimens used for taxonomical study of South Korean *Coccocarpia* in this study

Coll. no.	Species	Alt.(m)	GPS	Access Number	Locality ^a
030122	<i>C. palmicola</i>	400	N36°56'48.7" E128°28'03.8"		Mt. Wolchul
030158	<i>C. palmicola</i>	400	N36°57'21.3" E128°26'02.5"		Mt. Cheonchuk
030437	<i>C. palmicola</i>	700	N35°29'46.9" E126°53'40.7"		Mt. Naejang
030609	<i>C. palmicola</i>	600	N37°06'21.7" E128°57'12.1"		Mt. Naejang
030799	<i>C. palmicola</i>	1357	N36°56'48.7" E128°28'03.8"		Mt. Sobaek
040005	<i>C. palmicola</i>	201	N34°59'27.9" E127°20'01.8"		Mt. Jogae
040247-1	<i>C. palmicola</i>	815	N35°16'32.0" E127°33'49.8"		Mt. Jiri
040255	<i>C. palmicola</i>	1370	N35°17'50.5" E127°33'33.7"		Mt. Jiri
040312*	<i>C. palmicola</i>	1650	N35°19'24.0" E127°36'43.5"	EU142937	Mt. Jiri
040705	<i>C. palmicola</i>	975	N33°23'18.1" E126°29'45.1"		Mt. Halla
040971*	<i>C. palmicola</i>	836	N35°20'15.8" E127°41'10.4"	EU142938	Mt. Jiri
040976	<i>C. palmicola</i>	935	N35°20'15.8" E127°41'10.4"		Mt. Jiri
041174	<i>C. palmicola</i>	340	N36°51'37.6" E128°06'13.2"		Mt. Wolak
041208	<i>C. palmicola</i>	835	N36°52'12.7" E128°06'19.9"		Mt. Wolak
041293	<i>C. palmicola</i>	985	N35°04'09.6" E127°39'24.4"		Mt. Baekwoon
041444	<i>C. palmicola</i>	1330	N38°06'40.1" E128°24'33.9"		Mt. Sorak
041489	<i>C. palmicola</i>	1355	N38°07'14.5" E128°23'03.5"		Mt. Sorak
050047	<i>C. palmicola</i>	740	N35°52'31.1" E127°47'02.0"		Mt. Dukyoo
050456	<i>C. palmicola</i>	203	N34°41'21.4" E126°40'51.4"		Mt. Hukseok
050472	<i>C. palmicola</i>	203	N34°41'21.4" E126°40'51.4"		Mt. Hukseok
050514	<i>C. palmicola</i>	456	N34°41'16.7" E126°40'18.1"		Mt. Hukseok
060167	<i>C. palmicola</i>	1125	N35°17'35.6" E127°31'59.8"		Mt. Jiri
060238	<i>C. palmicola</i>	1380	N35°17'43.0" E127°33'00.7"		Mt. Jiri
060355-2	<i>C. palmicola</i>	1120	N35°18'48.9" E127°35'13.5"		Mt. Jiri
060358	<i>C. palmicola</i>	1120	N35°18'48.9" E127°35'13.5"		Mt. Jiri
060450	<i>C. palmicola</i>	895	N35°51'27.5" E127°46'13.3"		Mt. Dukyoo
060525*	<i>C. palmicola</i>	1277	N35°49'43.0" E127°44'26.7"	EU142933	Mt. Dukyoo
060531	<i>C. palmicola</i>	1325	N35°49'41.1" E127°44'27.6"		Mt. Dukyoo
060600	<i>C. palmicola</i>	1101	N35°36'29.2" E127°38'26.1"		Mt. Baekwoon
060621*	<i>C. palmicola</i>	1165	N35°37'08.4" E127°38'06.1"	EU142934	Mt. Baekwoon
060690	<i>C. palmicola</i>	1564	N35°19'40.9" E127°44'15.0"		Mt. Jiri
060722*	<i>C. palmicola</i>	1559	N35°20'03.7" E127°42'52.8"	EU142935	Mt. Jiri
060731*	<i>C. palmicola</i>	1547	N35°20'04.6" E127°42'50.6"	EU142936	Mt. Jiri
040006	<i>C. erythroxyli</i>	201	N34°59'27.9" E127°20'01.8"		Mt. Jogae
040247-2	<i>C. erythroxyli</i>	815	N35°16'32.0" E127°33'49.8"		Mt. Jiri
040727	<i>C. erythroxyli</i>	1280	N33°23'18.1" E126°29'45.1"		Mt. Halla
040743*	<i>C. erythroxyli</i>	1710	N33°21'30.4" E126°31'19.3"	EU142940	Mt. Halla
041267	<i>C. erythroxyli</i>	840	N35°04'17.4" E127°39'27.1"		Mt. Baekwoon
041286	<i>C. erythroxyli</i>	985	N35°04'09.6" E127°39'24.4"		Mt. Baekwoon
041368	<i>C. erythroxyli</i>	910	N38°03'22.9" E128°26'41.0"		Mt. Jumbong
041516	<i>C. erythroxyli</i>	450	N38°11'16.4" E128°21'42.7"		Mt. Sorak
050170	<i>C. erythroxyli</i>	1577	N35°51'10.7" E127°44'56.0"		Mt. Dukyoo
050601	<i>C. erythroxyli</i>	380	N36°24'09.6" E129°10'27.0"		Mt. Juwang
060466*	<i>C. erythroxyli</i>	1590	N35°51'15.1" E127°44'55.5"	EU142932	Mt. Dukyoo
060708	<i>C. erythroxyli</i>	1642	N35°20'01.5" E127°43'01.8"		Mt. Jiri
060772	<i>C. erythroxyli</i>	1202	N35°17'45.1" E127°33'38.5"		Mt. Jiri
060776	<i>C. erythroxyli</i>	1202	N35°17'45.1" E127°33'38.5"		Mt. Jiri
060849*	<i>C. erythroxyli</i>	1413	N35°18'50.1" E127°36'12.9"	EU142931	Mt. Jiri
060997*	<i>C. erythroxyli</i>	1630	N33°21'19.3" E126°30'20.3"	EU142939	Mt. Halla
061078	<i>C. erythroxyli</i>	850	N36°49'04.6" E128°02'52.7"		Mt. Jorung
061217	<i>C. erythroxyli</i>	617	N36°09'21.5" E127°36'19.6"		Mt. Cheontae

^aMt. Baekwoon, 백운산; Mt. Cheonchuk, 천축산; Mt. Cheontae, 천태산; Mt. Dukyoo, 덕유산; Mt. Halla, 한라산; Mt. Hukseok, 흑석산; Mt. Jiri, 지리산; Mt. Jogae, 조계산; Mt. Jorung, 조령산; Mt. Jumbong, 접봉산; Mt. Juwang, 주왕산; Mt. Naejang, 내장산; Mt. Odae, 오대산; Mt. Sobaek, 소백산; Mt. Sorak, 설악산; Mt. Wolchul, 월출산; Mt. Wolak, 월악산.

*Specimens used for DNA sequencing analysis.

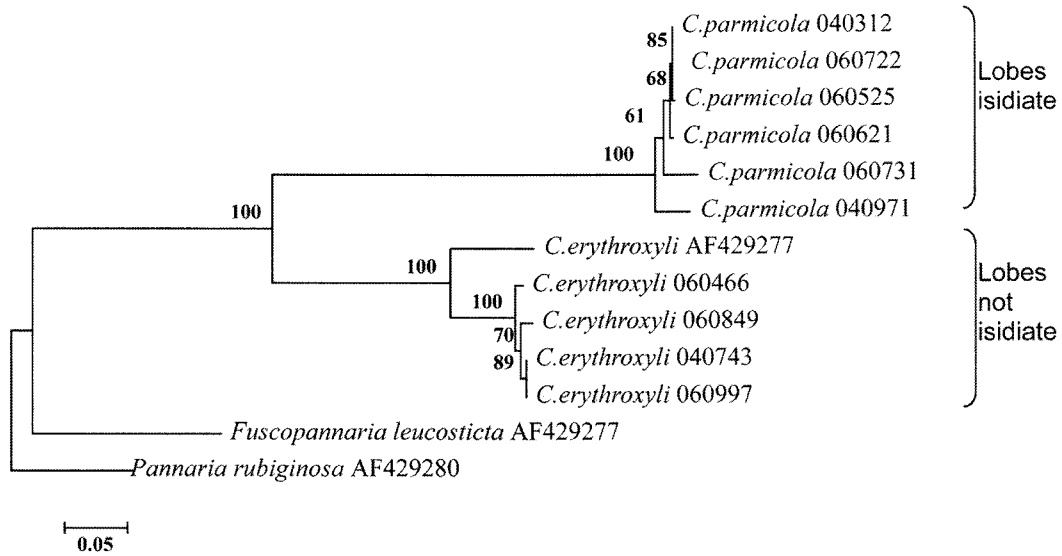


Fig. 1. NJ consensus tree based on nrDNA ITS sequence, numbers in each bootstrap support value. Nucleotide: Kimura 2-parameter, pairwise: deletion, bootstrap = 1000.

lichenoides (GenBank accession number: DQ466041) and *Pannaria rubiginosa* (AF429280) were chosen as the outgroups.

Results and Discussion

Among the 3 species of *Coccocarpia* previously reported in South Korea, only *C. parmicola* (Spreng.) Arv. & D. J. Galloway and *C. erythroxyli* (Spreng.) Swinscow & Krog were confirmed in this study. The main character of *C. parmicola* is the presence of cylindrical isidia, which easily distinguished it from *C. erythroxyli* having no isidia. Presence of apothecia can be an additional character to separate them. Black and biatorine apothecia present in most of *C. erythroxyli* specimens, but absent in *C. parmicola*. The NJ consensus tree constructed by Mega3.1 is shown in Fig. 1. According to the tree, each species finely assembled together and this proved that isidia is the key character to separate the two species.

Taxonomic treatment of the genus. According to the above analysis, a key to the species was made.

Key to *Coccocarpia* species in South Korea

- Isidia present, cylindrical to globose *C. parmicola*
- Isidia absent, biatorine apothecia usually present
..... *C. erythroxyli*

- 한국산 *Coccocarpia* (기와의지) 속 국문 키**
 원통모양의 열아 있음 *C. parmicola* (매화기와의지)
 열아 없음, 검은색의 공모양 자낭반 있음
 *C. erythroxyli* (기와의지)

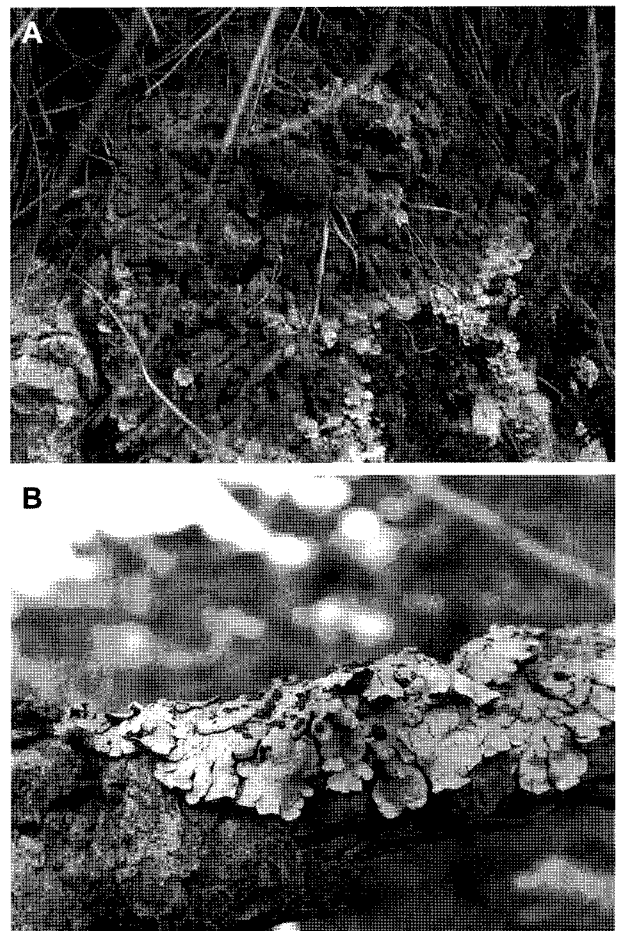


Fig. 2. Habitat of *C. parmicola* (HUR 040427) (A) and *C. erythroxyli* (HUR 030609) (B). Isidia (more dense and dark part) are present at the center of upper surface of *C. parmicola*. Black and biatorine apothecia are clearly shown on the upper surface of *C. erythroxyli*.

Taxonomy

1. *Coccocarpia palmicola* (Spreng.) Arv. & D.J. Gallo-way, *Bot. Notiser* 132: 242 (1979). Fig. 2A, Fig. 3.

External Morphology: Thallus foliose, about 2–8 cm in diameter, color of the thallus include leaden grey, pale grey, bluish grey, and dark brown, darker when wet, color

of the marginal parts usually lighter, sometimes even white (Fig. 2A). Thallus usually loosely attached to the substance. Lobes overlapping and epruinose, most of the lobes flabelliform, sometimes cuneate, very rare linear, 0.1–0.6 cm wide. The surface of the lobes canaliculated frequently, forming radiating lines, seldom flat. The margin of the lobes is deflexed. Sometimes lobe margins

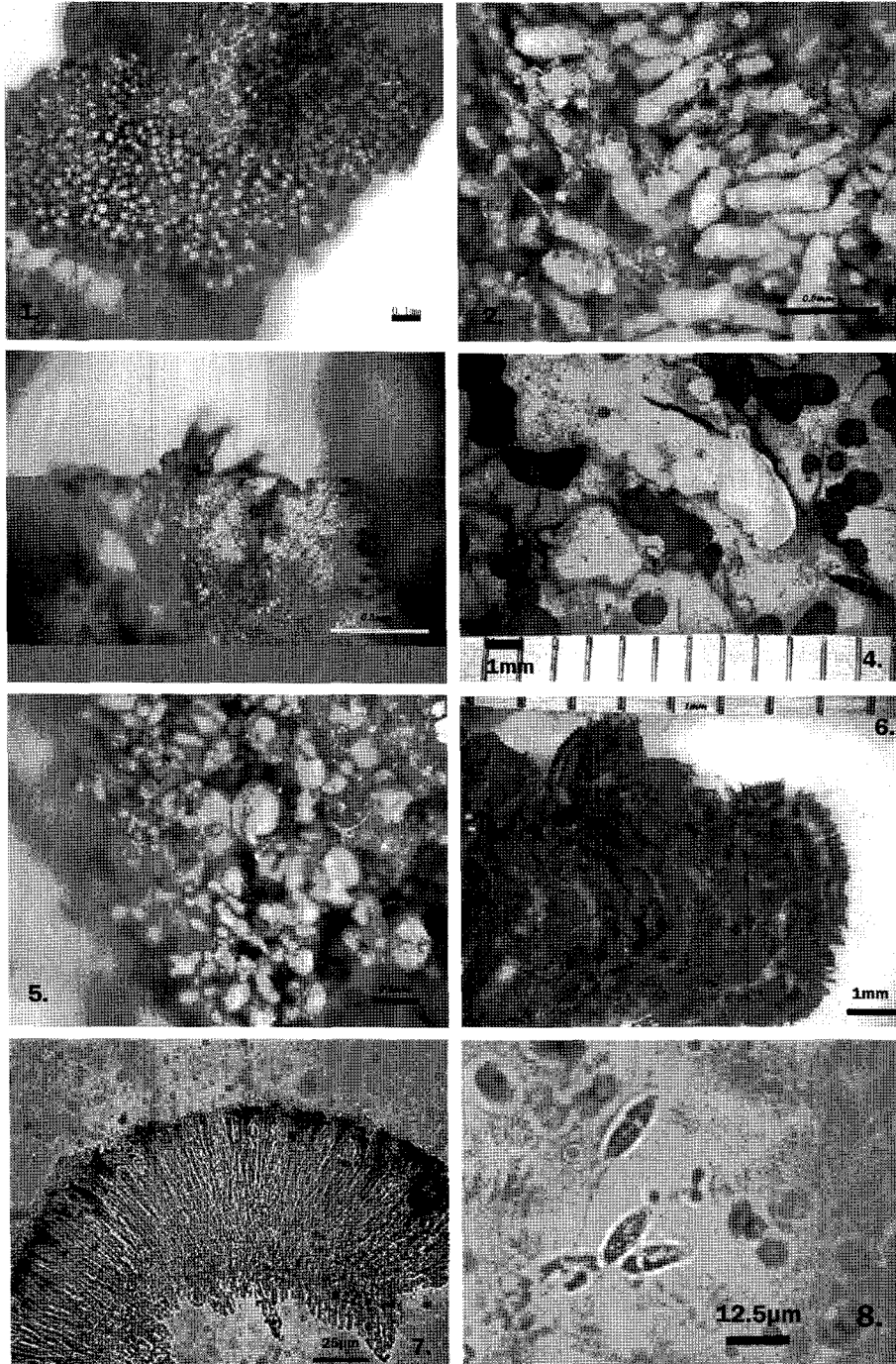


Fig. 3. 1. Globose isidia of *C. palmicola* 030437. 2. Cylindrical isidia of *C. palmicola* 030609. 3. Marginal lobules of *C. erythroxyli* 040005. 4. Apothecia of *C. erythroxyli* 040743. 5. Flabelliform lobules of *C. palmicola* 040255. 6. Rhizines forming concentric lines, *C. palmicola* 040006. 7. Vertical section of apothecia of *C. erythroxyli* 040743. 8. Fusiform spores of *C. erythroxyli* 040743. Scales: 1: 0.1 mm, 2: 0.5 mm, 3: 0.5 mm, 4: 1 mm, 5: 0.2 mm, 6: 1 mm, 7: 25 μm , 8: 12.5 μm .

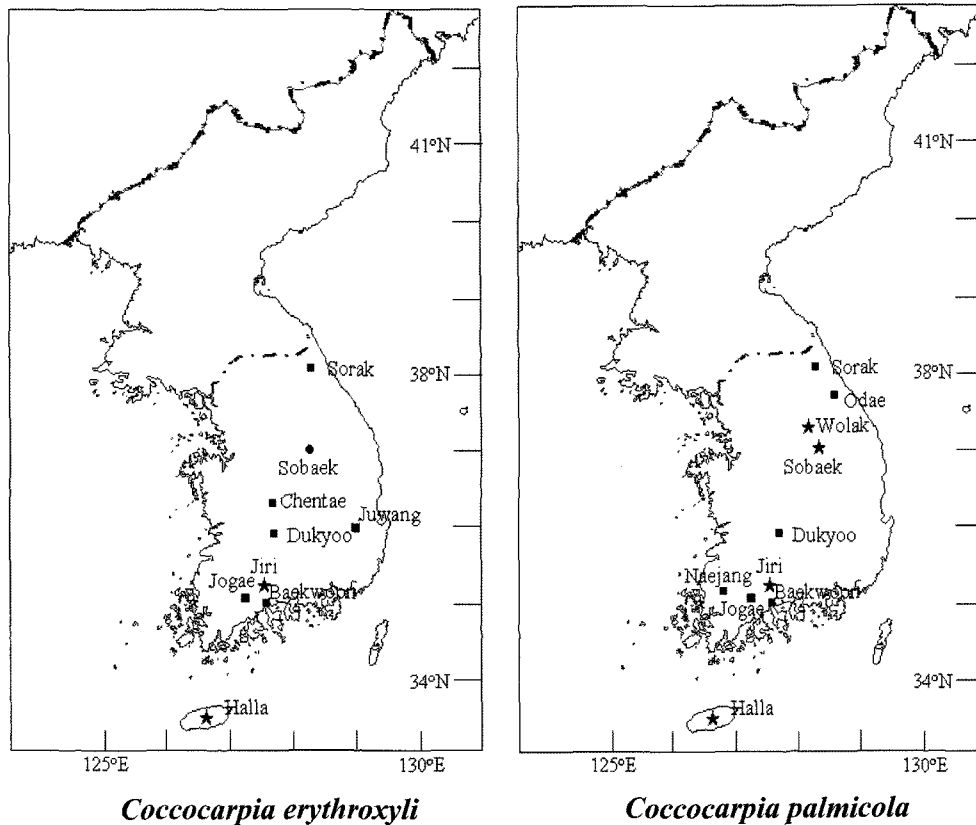


Fig. 4. Distribution of *Coccocarpia* species in South Korean. Circles (●) and squares (■) represent the localities previously (Park, 1990) and newly reported in this study, respectively. The previous records confirmed by newly collected specimens in this survey were indicated as stars (★).

lacinate, apices of the lobes rounded. Second lobes often present. Lobules are rare on the marginal part of the lobes, and more frequently exist in the central parts, among the isidium (Fig. 3-5).

Cortices present. Upper surface with very clear concentric ridges, sometimes glossy, pycnidia sometimes presents on the upper surface. Lower surface with thick hair-like rhizines, usually bluish dark, brown or white, sometimes formed concentric lines (Fig. 3-6), sometimes scattered and forming a dense hypothallus, rhizines often stretched beyond the margins.

Isidia present, with darker color than the thallus or concolorous with thallus, cylindrical and usually coralloid branched (Fig. 3-2), sometimes globose (Fig. 3-1). Soredia absent. Medulla present and the color are always white. Apothecia are nearly not seen.

Chemistry: Thallus K-, P-, C-. KC-; no lichen substance detected.

Habitat: On bark, usually bark of *Quercus*, sometimes on rock.

Distribution in Korea: Mt. Baekwoon, Mt. Dukyoo, Mt. Halla, Mt. Hugseok, Mt. Jiri, Mt. Jogae, Mt. Naejang, Mt. Wolak, Mt. Wulchul, Mt. Sobaek, Mt. Odae, Mt. Sorak (Fig. 4).

Remarks: This species could be characterized by its cylindrical to globose isidia. Rhizines in the lower surface usually are denser, shorter and thicker, rarely forming concentric lines, than the *C. erythroxyli*. Apothecia are not seen.

Representatives of the 33 Specimens examined: Mt. Jiri, N35°17'43.0" E127°33'00.7" alt. 1380 m, on *Quercus* bark, Jae-Seoun Hur 060238. Mt. Dukyoo, N35°51'27.5" E127°46'13.3" alt. 895 m, on *Quercus* bark, Jae-Seoun Hur 060450. Mt. Baekwoon, N35°37'08.4" E127°38'06.1", alt. 1165 m, on bark, Jae-Seoun Hur 060621. Mt. Jogae, N34°59'27.9" E127°20'01.8", alt. 201 m, on rock, Jae-Seoun Hur 040005. Mt. Sorak, N38°07'14.5" E128°23'03.5", alt. 1355 m, on bark + moss + soil, Jae-Seoun Hur 041489. Mt. Wolak, N36°51'37.6" E128°06'13.2", alt. 340 m, on rock, Jae-Seoun Hur 041174. Mt. Hugseok, N34°41'21.4" E126°40'51.4", alt. 203 m, on rock, Jae-Seoun Hur 050456. Mt. Naejang, N35°29'46.9" E126°53'40.7", alt. 700 m, on rock, Jae-Seoun Hur 030437.

2. *Coccocarpia erythroxyli* (Spreng.) Swinscow & Krog, *Norw. J. Bot.* 23: 254 (1976). Fig. 2B, Fig. 3.

External Morphology: Thallus foliose, 2~9 cm in diameter, color varies from brown to grey, usually lead-

grey, sometimes pale, dark or bluish grey, colors darker when the thallus is wet (Fig. 2B). Thallus closely adnate, sometimes loosely attached to the substance. Lobes epruinose and usually weakly branched, secondary lobes often present. Lobes usually narrow cuneate, sometimes overlapping and flabelliform, with round apices, 0.1–0.8 cm wide. Surface of the lobes canaliculated, with or without radiating lines, sometimes flat and even shining. Margins of the lobes are always deflexed. Some of the lobe margins lacinate. Lobules often present, usually round and overlapping in the old parts of the thallus with scalelike shape, sometimes on the edge parts of the lobes (Fig. 3-3), less than 0.1 cm wide.

Cortices present. Upper surface with unclear concentric ridges. Lower surface with hair-like rhizines, color varies from bluish-black to bluish-white. Rhizines usually form concentric lines. Medulla always present and the colors are white. Isidia and soredia absent. Pycnidia present sometimes.

Apothecia (Fig. 3-7) mostly present, biatorine, usually black (Fig. 3-4), sometimes ball-shape and sometimes strongly convex or slightly rose over the surface. Usually with white hairs stretched from the bottom part of the apothecia. Spores fusiform, hyaline, thin-walled and simple, the length is usually about $10\text{--}14 \times 3\text{--}5 \mu\text{m}$ (Fig. 3-8).

Chemistry: Thallus K-, P-, C-. KC-; no lichen substance detected.

Habitat: On the rock surface or bark.

Distribution in Korea: Mt. Baekwoon, Mt. Chentae, Mt. Dukyoo, Mt. Halla, Mt. Jiri, Mt. Jogae, Mt. Juwang, Mt. Sobaek, Mt. Sorak (Fig. 4).

Remarks: The species is similar to *C. palmicola*, but without any isidia, having biatorine apothecia on the upper surface. Rhizines usually sparse, and forming concentric lines. Papilliform pycnidia sometimes present on the upper surface, usually smaller than the apothecia.

Representatives of the 18 Specimens examined: Mt. Jiri, 35°17'45.1" N, 127°33'38.5" E, alt. 1202 m, on *Quercus* bark, Jae-Seoun Hur 060772. Mt. Baekwoon, 35°04'17.4" N, 127°39'27.1" E, alt. 840 m, on rock, Jae-Seoun Hur 041267. Mt. Halla, 33.23'18.1N, 126.29'45.1E, alt. 1280m, on bark. Jae-Seoun Hur 040727. Mt. Sorak, N38°11'16.4" E128°21'42.7", alt. 450 m, on rock, Jae-Seoun Hur 041516.

The species not found in this time

Coccocarpia pellita (Ach.) Müll. Arg. was previously recorded in South Korea (Park, 1990; Hur *et al.*, 2005). *C. pellita* is currently treated as *Umbilicaria polyrrhiza* (L.)

Fr. (www.indexfungorum.org). According to her, this species was not common and most abundant on Mt. Jiri. The only difference between *C. pellita* and *C. palmicola* was the shape of isidia. *C. pellita* had flattened isidia, instead of cylindrical one. There was no chemical difference between two species. *C. palmicola* was also most abundant on Mt. Jiri in her collections which have been deposited in Duke University, USA. However, we have seen no corresponding specimens during our expeditions and, possibly, they are under threaten of extinction.

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