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 Peninsula. 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; Hessen, 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 descriptions 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, CaCl₂, 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 DNasey 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'-CTTGG TCA TTT ACAG-GA AG T AA -3'; Garde and Bruns, 1993) and ITS4A (5'-ATTGAGCTCTTCCCGCTTCA-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*
Table 1. Specimens used for taxonomical study of South Korean Coccoecarpi in this study

| Coll. no. | Species          | Alt.(m) | GPS                     | Access Number | Locality  |
|-----------|------------------|---------|-------------------------|----------------|-----------|
| 030122    | C. palmicola     | 340     | N36°51'37.6" E128°06'13.2" | EU142937       | Mt. Wolak  |
| 040120    | C. palmicola     | 935     | N36°52'12.7" E128°06'19.9" | Mt. Wolak      |
| 041174    | C. palmicola     | 385     | N36°54'09.6" E127°39'24.4" | Mt. Baekwoon   |
| 050047    | C. palmicola     | 740     | N37°03'15.4" E128°32'03.5" | Mt. Sonak      |
| 050147    | C. palmicola     | 203     | N37°04'21.4" E128°34'51.4" | Mt. Hulseo     |
| 060167    | C. palmicola     | 162     | N37°17'35.6" E127°31'39.8" | Mt. Jiri       |
| 060238    | C. palmicola     | 1380    | N37°19'43.0" E127°33'00.7" | Mt. Jiri       |
| 060335    | C. palmicola     | 501     | N37°18'48.9" E127°31'13.5" | Mt. Jiri       |
| 060535*   | C. palmicola     | 1277    | N37°19'43.0" E127°44'26.7" | Mt. Dukyoo     |
| 060531    | C. palmicola     | 1325    | N37°19'41.1" E127°44'27.6" | Mt. Dukyoo     |
| 060600    | C. palmicola     | 1101    | N37°19'32.9" E127°38'26.1" | Mt. Baekwoon   |
| 060621*   | C. palmicola     | 1165    | N37°19'30.4" E127°38'06.1" | Mt. Baekwoon   |
| 060690    | C. palmicola     | 1564    | N37°19'40.9" E127°44'15.0" | Mt. Jiri       |
| 060722*   | C. palmicola     | 1559    | N37°20'03.7" E127°42'52.8" | Mt. Jiri       |
| 060731*   | C. palmicola     | 1547    | N37°20'04.6" E127°42'30.6" | Mt. Jiri       |
| 060006    | C. erythroryli   | 201     | N37°29'57.9" E127°01'01.8" | Mt. Jiri       |
| 040247-2  | C. erythroryli   | 815     | N37°16'32.0" E127°33'49.8" | Mt. Jiri       |
| 040727    | C. erythroryli   | 1280    | N37°21'31.8" E126°29'45.1" | Mt. Jiri       |
| 040743*   | C. erythroryli   | 1710    | N37°21'30.4" E126°31'19.3" | Mt. Jiri       |
| 061267    | C. erythroryli   | 840     | N37°04'17.4" E127°39'27.1" | Mt. Dukyoo     |
| 061286    | C. erythroryli   | 985     | N37°04'09.6" E127°39'24.4" | Mt. Dukyoo     |
| 041368    | C. erythroryli   | 910     | N37°03'22.9" E128°26'41.0" | Mt. Jumbong    |
| 041516    | C. erythroryli   | 450     | N37°11'16.4" E128°21'42.7" | Mt. Sonak      |
| 050750    | C. erythroryli   | 1577    | N37°51'10.7" E127°34'56.0" | Mt. Dukyoo     |
| 050601    | C. erythroryli   | 380     | N36°24'09.6" E125°01'27.0" | Mt. Jiri       |
| 060466*   | C. erythroryli   | 1590    | N36°51'15.1" E127°44'55.5" | Mt. Jiri       |
| 060708    | C. erythroryli   | 1642    | N35°20'01.3" E127°43'30.1" | Mt. Jiri       |
| 060772    | C. erythroryli   | 1202    | N35°17'45.6" E127°33'38.5" | Mt. Jiri       |
| 060776    | C. erythroryli   | 1202    | N35°17'45.1" E127°33'38.5" | Mt. Jiri       |
| 060849*   | C. erythroryli   | 1413    | N35°18'50.1" E127°36'12.9" | Mt. Jiri       |
| 060997*   | C. erythroryli   | 1630    | N35°21'19.3" E126°30'20.3" | Mt. Jiri       |
| 061078    | C. erythroryli   | 850     | N36°49'04.6" E128°02'52.7" | Mt. Jorung     |
| 061217    | C. erythroryli   | 617     | N36°09'21.5" E127°36'19.6" | Mt. Cheonbuk   |

*Mt. Baekwoon, 북웅산; Mt. Cheonbuk, 천북산; Mt. Cheonta, 천타산; Mt. Dukyoo, 두교산; Mt. Halla, 화라산; Mt. Hulseo, 호서산; Mt. Jiri, 지리산; Mt. Jorung, 조용산; Mt. Jumbong, 정봉산; Mt. Juwang, 주왕산; Mt. Naejang, 내장산; Mt. Odae, 오대산; Mt. Solbok, 소복산; Mt. Sonak, 송악산; Mt. Wolchul, 월출산; Mt. Wolak, 월악산.

*Specimens used for DNA sequencing analysis.
**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. palmicola* (Spreng.) Arv. & D. J. Galloway and *C. erythroxyl* (Spreng.) Swinscow & Krog were confirmed in this study. The main character of *C. palmicola* is the presence of cylindrical isidia, which easily distinguished it from *C. erythroxyl* having no isidia. Presence of apothecia can be an additional character to separate them. Black and biatorine apothecia present in most of *C. erythroxyl* specimens, but absent in *C. palmicola*. 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. palmicola*
Isidia absent, biatorine apothecia usually present
.............................................................. *C. erythroxyl*

한국산 *Coccocarpia* (개화기와지의) 속 국문 키
원통모양의 열아 있음 ........... *C. palmicola* (개화기와지의)
열아 없음, 검은색의 공모양 자생한 있음
.............................................................. *C. erythroxyl* (개화기와지의)

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

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

1. *Coccocarpia palmicola* (Spreng.) Arv. & D.J. Galloway, *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

![Figures 1-8](image-url)

**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.
Laciniate, 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). Soreidia 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. Backwoon, Mt. Dukyo, Mt. Halla, Mt. Hugseok, Mt. Jiri, Mt. Jogae, Mt. Naejang, Mt. Wolak, Mt. Wulchul, Mt. Sobaeck, Mt. Odae, Mt. Sonok (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. Dukyo, 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. Jl 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 laciniate. Lobules of ten 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 10~14 × 3~5 µm (Fig. 3-8).

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

Habitat: On the rock surface or bark.

Distribution in Korea: Mt. Backwoon, Mt. Chentae, Mt. Dukyoo, Mt. Halla, Mt. Jiri, Mt. Jogae, Mt. Juwang, Mt. Sobak, 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 rock, Jea-Seoung Hur 041267; Mt. Halla, 33°38′5″ N, 126°29′45.1″ E, alt. 1280m, on bark, Jae-Seoung Hur 040727; Mt. Sorak, N38°11′16.4″ E128°21′42.7″, alt. 450 m, on rock, Jae-Seoung Hur 041516.

The species not found in this time

Coccocarpia pellita (Ach.) Mill. 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 threat of extinction.

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References

Arup, U. 2002. PCR techniques and automated sequencing in lichens. Pp. 392-411. In Knapp, I., Beckett, R. P. and Varma, A. K. Eds. Protocols in lichenology: culturing, biochemistry, exophyiology and use in biomonitoring. Springer-Verlag, New York.

Arvidsson, L. 1983. A monograph of the lichen genus Coccocarpia. Opera Bot. 67: 1-96.

Arvidsson, L. 1992. Flora of Australia 54: 152-159.

Brodo, I. M., Shamoff, S. D. and Shamoff, S. 2001. Lichen of North America. Yale University Press, New Haven and London, pp. 279-280.

Cullerson, C. F. 1972. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. Journal of Chromatography 72: 113-125.

Ekman, S. 1999. PCR optimization and trouble shooting 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 basidiomyctes. Application for the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118.

Henssen, A. 1963. Eine Revision der Flechtenfamilien Lichinaeae und Ephebaceae. Symbolae Bot. Upsalienses 18: 90.

Hur, J-S., Koh, Y. J. and Harada, H. 2005. A Checklist of Korean Lichens. Lichenology 4: 75.

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.

Park, Y. S. 1990. The Macrolichen Flora of South Korea. The Bryologist 93: 105-160.

White, T. J., Bruns, T. D., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal DNA genes for phylogenetics. Pp 315-321. In Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. Eds. PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego, USA.