The Lichen *Dirinaria picta* New to South Korea

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Abstract Two species of the lichen genus *Dirinaria* (Tuck.) Clem. are recognized in the South Korean lichen mycota, viz: *D. applanata* and *D. picta*. Descriptions of each species with their morphological, anatomical, and chemical characteristics together with molecular analysis and a key to known *Dirinaria* species are presented. *D. picta* is recorded from South Korea for the first time.

Keywords *Dirinaria*, Key, New record, Physciaceae, South Korea

The genus *Dirinaria* (Tuck.) Clem. was segregated as a new section from the genus *Pyxine* by Tuckerman [1] based on the apothecial and thallus characteristics. Later in 1909, section *Dirinaria* was changed to genus *Dirinaria* by Clements [2]. According to his explanation, the genus *Dirinaria* is distinguished from the closely related genera *Physcia* (Schreb.) Michx and *Pyxine* Fr. by the presence of a thalline exciple and a black hypothecium. According to Awasthi [3] and Elix [4], genus *Dirinaria* has the following main characteristics: Very closely appressed foliose thallus with irregular to radiating, discrete or confluent, eciliate lobes; white, grey-white or bluish grey, pruinose or epruinose upper surface with soredia, or isidia and whitish to pale yellow medulla. In addition, this genus has *Lecanora*-type, clavate asci with unitunicate, brown, 1-septate spores. *Dirinaria* is a pantropical and subtropical genus. Some species extend to temperate regions [4]. Monographic studies on this genus were conducted by Swinscow and Krog [5], Awasthi [3], Kalb [6], and Elix [4]. To date, c. 36 species have been reported world-wide [4]; of these, only one species, *Dirinaria applanata* (Fée) D. D. Awasthi, has been reported so far from South Korea. As many *Dirinaria*

species have been reported world-wide, and no recent revisionary work has been conducted in this group, there must be a possibility of finding more species in South Korea. Therefore, the aim of this study was to identify the presence of other *Dirinaria* species in the collection of South Korean lichen species, which are deposited in the Korean Lichen Research Institute (KoLRI) using taxonomic, chemical, and molecular analysis.

The study was based on the specimens deposited in KoLRI. The lichen samples were identified using stereo and light microscopes: a dissecting microscope (NIKON SMZ645; Nikon, Tokyo, Japan) was used for identification of morphological characteristics of the thallus, reproductive structures, color, size, and shape, while a compound microscope (ZEISS Scope. A1; Zeiss, Oberkochen, Germany) was used for studying the anatomy of thalli. Spot test reactions were performed on thallus under a compound microscope. Chemicals were extracted in analytical grade acetone using 0.1 g of the thallus in a 1 mL eppendorf tube. Thin layer chromatography (TLC) was then performed using a glass plate coated with TLC Silica gel 60, in solvent system A (toluene : dioxin : acetic acid = 180 : 45 : 5) [7]. All examined localities of specimens were mapped using open source GIS software Quantum GIS 1.7.0 (QGIS). Voucher specimens have been deposited in the herbarium of the Lichen & Allied Bio-resource Center at the KoLRI, Suncheon National University, South Korea. Total DNA was extracted directly from the thalli of the selected specimens according to Ekman [8] and was purified using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The nuclear ribosomal RNA gene region, including internal transcribed spacers 1 and 2 and the 5.8S subunit (internal transcribed spacer [ITS]), was amplified using the primers ITS1F [9] and LR5 [10]. Amplification was performed on a Takara JP/TP600 PCR machine (Takara Bio Inc., Otsu, Japan). One initial cycle of 5 min at 94°C was followed by 30 cycles of the following steps: 30 sec at 94°C, 39 sec at 57°C

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and 1 min at 72°C. Amplifications ended with a final cycle at 72°C for 10 min. PCR products were then sent to the sequencing facilities of the Genotech Cooperation, Seoul, South Korea for cleaning and sequencing. Sequencing was performed using the fluorescent marker BigDye and an ABI 3730xl sequencing machine (Applied Biosystems, Carlsbad, CA, USA). Alignment was performed for the South Korean materials (GenBank Nos. KF290487, KF290488, KF290489, and EU670223) and, seven additional ITS sequences of *Dirinaria* from GenBank, using BioEdit software [11]. Ambiguous regions were delimited [12] and excluded from the alignment. Three species (*Parmelia saxatilis*, *Heterodermia obscurata*, and *H. speciosa*) were selected as out-groups based on BLAST comparison in GenBank. Phylogenetic relationships between taxa were investigated using MEGA 5.1 software [13]. The dataset was analyzed using the maximum likelihood (ML) method and support values were obtained using a bootstrap analysis of 1,000 pseudoreplicates.

According to Hur *et al.* [14], *Physcia picta* (Sw.) Nyl., which was reported by Park [15], has been synonymized by *Dirinaria applanata*. The current study including molecular and taxonomic data confirmed the presence of another species, *Dirinaria picta* (Sw.) Schaer. ex Clem., thus, there are two currently known species of genus *Dirinaria* from South Korea. The constructed ML tree (Fig. 1) shows that all South Korean *Dirinaria* species clustered within a different group (boot-strap 44%). Both *D. applanata* and *D. picta* are sister to each other within this group

Fig. 1. Phylogenetic relationships between *Dirinaria* species recorded from South Korea (A) and some other regions of the world (B, Spain; C, Germany; D, Cambodia; E, India; F, out group). This phylogeny was obtained using the marker internal transcribed spacer and a maximum likelihood criterion using the software MEGA 5.1. * Selected species for the current study.

(boot-strap > 98%). The selected species of *Dirinaria* for the current study are marked with an astral sign.

Key to the known species of *Dirinaria* in South Korea

- 1. Lobes folded, somewhat convex, glued together; apices flabellate; thallus longitudinally plicate and rugose *D. applanata*

Fig. 2. Habit (A) and closed-up of the upper surface (B) of *Dirinaria applanata*; habit (C) and close-up of the upper surface (D) of *D. picta* (scale bars: A, C = 1 cm, B, D = 2 mm).

1a. Lobes slightly flat, overlapping; apices not flabellate; thallus not longitudinally plicate or rugose *D. picta*

***Dirinaria applanata* (Fée) D. D. Awasthi**, J. Indian Bot. Soc. 49: 135 (1970).

Thallus 5~6 cm wide, completely appressed to the substrate. Lobes radiating, contiguous, longitudinally plicate and rugose, plane to convex, 1~2 mm wide. Upper surface whitish grey to greenish grey, maculate, pruinose, sorediate; Soralia laminal, farinose. Medulla white. Lower surface black. Apothecia not seen. Conidia bacilliform, $3\sim4 \times 1.0 \mu\text{m}$ (Fig. 2).

Chemistry: Cortex K+ yellow, C–, KC–, P+ yellow; medulla K–, C–, KC–, P–; TLC: atranorin, divaricatic acid, unknown 1, unknown 2 (Fig. 3).

Ecology and distribution: In South Korea, it occurs on bark (*Pinus* sp. and Cherry sp.), or rocks from coastal areas to sub montane forests (Fig. 4). According to Elix [4], this species spreads in pantropical to subtropical regions and often extends into temperate zones.

Fig. 3. Thin layer chromatography profile of *Dirinaria* species in a solvent system A. 1, *D. applanata* with atranorin (a), unknown 1 (b), unknown 2 (c) and divaricatic acid (d); 2, control [*Lethariella cladonioides* (Nyl.) Krog] with norstictic acid (e), atranorin; 3, *D. picta* with atranorin, unknown 1, unknown 2, and divaricatic acid.

Fig. 4. Distribution of *Dirinaria* species in South Korea, *D. applanata* (●), *D. picta* (☆).

Remarks: *D. applanata* is characterized by longitudinally plicate and rugose, folded lobes with flabellate apices, farinose soredia, and the presence of divaricatic acid in the medulla. This species very closely resembles *D. picta*, however, the latter has somewhat flat, overlapping lobes. According to Brodo *et al.* [16], distinguishing between these two species is very difficult.

Selected specimens examined: Jindo Co., Sea side, on rock, $34^{\circ}32'9.19''$ N, $126^{\circ}18'6.94''$ E, elev. 2 m, 3 Jun 2011, X. Y. Wang & J. A. Ryu, 110476 (GenBank No. KF290487); Cheongsan Island, on rock, $34^{\circ}12'2.18''$ N, $126^{\circ}54'3.57''$ E, elev. 5 m, 23 Jun 2011, X. Y. Wang & J. A. Ryu, 110562 (GenBank No. KF290489).

***Dirinaria picta* (Sw.) Schaer. ex Clem.**, in Clements & Shear, Gen. Fung., Edn 2 (Minneapolis): 323 (1931).

Thallus 2~7 cm wide, tightly adnate. Lobes flat, radiating, contiguous, not longitudinally plicate, slightly overlapping, 0.5~1.0 mm wide; apices discrete. Upper surface grey to bluish grey, pruinose, sorediate. Soralia laminal, globose, capitate; soredia farinose. Medulla white. Lower surface black in the center, paler toward the lobe tips. Apothecia not seen. Conidia bacilliform, $3\sim4 \times 1 \mu\text{m}$ (Fig. 2).

Chemistry: Cortex K+ yellow, C–, KC–, P+ yellow; medulla K–, C–, KC–, P–; TLC: atranorin, divaricatic acid, unknown 1, unknown 2 (Fig. 3).

Selected specimens examined: Mt. Cheongwan, on bark, $34^{\circ}32'39.8''$ N, $126^{\circ}56'51.5''$ E, elev. 200 m, 7 Oct 2005, J. S. Hur, 050536 (GenBank No. EU670223); Jeungdo Island, on

rock, 35°57'8.87" N, 126°07'9.93" E, elv. 5 m, 1 Jun 2011, J. S. Hur, 110348 (GenBank No. KF290488).

Ecology and distribution: In South Korea, it occurs on bark (*Pinus* sp.), or rocks from coastal areas to sub montane forests (Fig. 4). According to Elix [4], this species spreads in pantropical to subtropical regions and often extends into temperate zones.

Remarks: This species is characterized by the presence of flat, overlapping lobes, globose, capitate soralia with farinose soredia and by the presence of divaricatic acid. *D. applanata* differs in having glued, folded lobes without overlapping.

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