Candelaria asiatica, an Ignored New Species from South Korea

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ABSTRACT
The genus *Candelaria* is characterized by a micro-foliose to micro-fruticose thallus and contains eight species, two of which were reported in South Korea. During the excursion of a Korean lichen flora investigation, some suspected *Candelaria concolor* specimens were collected, and their morphological, chemical, molecular phylogenetic, and geographic analyses were conducted. The samples eventually proved to be a new species, *Candelaria asiatica*, which can be recognized by a small, yellow lobate thallus with a pulverulent surface, and a fragile lobe margin with blastidia or phyllidia-like lobules.

1. Introduction
*Candelaria* A. Massal. is a small lichen genus characterized by a micro-foliose to micro-fruticose yellowish thallus and 8- or polyspored asci. This genus comprises eight species: *Candelaria antarctica* (Js. Murray) Poelt, *C. crawfordii* (Müll. Arg.) P.M. Jørg. & D.J. Galloway, *C. concolor* (Dicks.) Arnold, *C. fibrosa* (Fr.) Müll. Arg., *C. fibrosoides* M. Westb. & Frödén, *C. fruticans* Poelt & Oberw., *C. murrayi* Poelt, and *C. pacifica* M. Westb. & Arup, around the world, especially in the South American and African continents [1–3]. Molecular phylogenetic studies on the family Candelariaceae inferred that neither *Candelariella* nor *Candelaria* was monophyletic; the species of *Candelaria* were separated into three different clades [4]. The most well-known and widely distributed species of *Candelaria* are the sor- ediate lichen, *C. concolor* (Dicks.) Arnold, and the esorediate lichen, *C. concolor* (Dicks.) Arnold, and the esorediate lichen, *C. fibrosa* (Fr.) Müll. Arg.; though they are grouped into one clade of *Candelaria* in the strict sense, both species are polyphyletic [4,5].

The type species, *C. concolor*, has been treated as the only common species of *Candelaria* in Europe, until Westberg [5] described a most similar species, *C. pacifica* M. Westb. & Arup from North America. The lichenologist was aware of a situation that the specimens recognized as *C. concolor* may not include only a single species. Thus, after further study, *C. concolor* became a rare lichen in Nordic countries [6], and some specimens collected in Austria, Sweden, and Germany [6–10] under the taxon *C. concolor* agreed with *C. pacifica*. In South Korea, *C. concolor* and *C. crawfordii* have been reported [11–14]. Several specimens of suspected *C. concolor* were collected during a lichen flora survey in the freshwater areas of South Korea, and morphological, chemical, and phylogenetic analyses were conducted. As a result, all the specimens were found to belong to a distinct species different from *C. concolor*.

2. Materials and methods
2.1. Material and morphological studies
Three specimens in this study were collected from South Korea and deposited at Sunchon National University, Korean Lichen Research Institute (KoLRI). Morphological and anatomical characters were recorded under a dissecting microscope (Nikon SMZ 745 T, Tokyo, Japan) and an Olympus BX 50 microscope (Olympus, Tokyo, Japan), and images were captured using an HD-Measure LTHS-300 (Leetech Co., Seoul, South Korea) microscope and a Carl Zeiss MicroImaging with Axio Cam ERc 5 s imaging system (Carl Zeiss MicroImaging, GmbH 37081, Gottingen, Germany). The secondary metabolites were studied using the spot test (K = 10% aqueous KOH solution; C = saturated aqueous Ca(ClO)₂; KC = 10% aqueous KOH solution followed by saturated aqueous Ca(ClO)₂; PD = 5% alcoholic p-phenylenediamine solution) and thin-layer chromatography (TLC) in solvent C [15,16]. UV tests were performed...
using a UV Chamber (CE07 21470) under long (366 nm) wavelength.

2.2. DNA isolation, PCR, DNA sequencing, and sequence alignment

Total genomic DNA was extracted from the newly collected specimens using the NucleoSpin Plant II Kit (Clontech Laboratories, Mountain View, CA) following the manufacturer’s instructions. The internal transcribed spacer (ITS) region and the large subunit of the ribosomal RNA (28S) were targeted via PCR using the primers pairs ITS4/ITS1F [17] and LR0R/LR5 [18,19], respectively. Protocols of PCR amplification were followed [20]. Sequencing was conducted by GenoTech (Daejeon, Korea) and Macrogen (Daejeon, Korea). Newly obtained sequences for the ITS region of Candelaria species (bold in Table 1) were assembled and edited using SeqMan and Mega 7.0 [21], and complemented with publicly available sequences of the family Candelariaceae (Table 1) into a matrix and aligned using Mafft v7.273 [22]. Pycnora xanthococca (Sommerf.) Hafellner was used as an outgroup since Pycnora has been recognized as a possible sister clade to Candelariaceae [23].

2.3. Phylogenetic analysis

The matrix was analyzed under the criterion of maximum likelihood (ML) using RAxML v7.2.6 [24] with the GTR+I+G model. ML bootstrap values were estimated from consensus trees obtained from 1000 nonparametric bootstrapping pseudoreplicates; a clade of bootstrap value >70% indicates crudely supported. Bayesian inference (BI) was performed with MrBayes v3.1.2 [25], applying the best-fitted substitution models (GTR+T+G) based on the Akaike information criterion using jModelTest 3.7 [26]. Bayesian inferences were conducted using four chains and run for 2 million generations. Trees were sampled every 1000 generations with the first 25% of trees discarded. The remaining trees were used to generate a majority-rule consensus tree with posterior probabilities (PP); clades of PP value ≥0.95 were considered as significantly supported.

3. Results and discussion

The matrix contains 40 sequences in total, including 33 taxa of family Candelariaceae; after excluding the

| Table 1. Voucher information of ITS sequences used in this study. |
|---------------------------------------------------------------|
| **Taxon**             | **ITS**              | **Voucher information** | **Location**  |
|-----------------------|----------------------|------------------------|--------------|
| C. asiatica           | MG694269             | D. Liu 171146 (KoLRI)  | South Korea  |
| C. concolor           | EF353205             | Westberg 454 (LD)      | Mexico       |
| C. concolor           | FJ959355             | Krumsvlk s.n. (BG)     | Norway       |
| C. concolor           | GU929921             | Arup L07001 (LD)       | Italy        |
| C. crawfordii         | EF353204             | Moberg & Santesson 8125b (UPS) | China, Yunnan  |
| C. fibrosa            | EF353206             | Worthington 21240 (COLO) | U.S.A. New Mexico |
| C. fibrosa            | GU929923             | Fröden 1670 (LD)       | Argentina    |
| C. fibrosoides        | EF353211             | Fröden 1513 (LD)       | Peru         |
| C. fibrosoides        | EF353212             | Fröden 1512 (LD)       | Peru         |
| C. fruticans          | EF353207             | Lassae s.n. (C)        | Ecuador      |
| C. pacifica           | EF353210             | (John, Lich. Anatol. Exs. no. 16 (ASU) | Turkey |
| C. pacifica           | GU929918             | Westberg 967 (LD)      | U.S.A. California |
| Candelariella aggregata | EF353156           | Westberg 1281 (LD)     | Canada       |
| C. antennaria         | EF353159             | Westberg 1155 (LD)     | U.S.A. Colorado |
| C. aurella            | EF353163             | Westberg 150 (LD)      | U.S.A. Arizona |
| C. biatorina          | EF353164             | Westberg 1181 (LD)     | U.S.A. Colorado |
| C. blastiata          | KO853128             | Davydov 7716 (ALTB)    | Russia       |
| C. borealis           | EF353168             | Westberg 1079 (LD)     | U.S.A. Colorado |
| C. clarkii            | KR052104             | Tripp & D'az 4876 (COLO) | U.S.A. Colorado |
| C. complanata         | EF353173             | Westberg 392 (LD)      | Mexico       |
| C. granuliformis      | GU967376             | Westberg 3128 (LD)     | Sweden       |
| C. kansensis          | EF351581             | Wetmore 55470 (MIN)    | U.S.A. Arizona |
| C. lutella            | EF355182             | Westberg 2808 (LD)     | Norway       |
| C. medians            | EF353184             | Arup L03165 (LD)       | Sweden       |
| C. placodizans        | EF353188             | Westberg 1083 (LD)     | U.S.A. Colorado |
| C. rosulans           | EF353191             | Westberg 1146 (LD)     | U.S.A. Colorado |
| C. spraguei           | EF353194             | Westberg 1037 (LD)     | U.S.A. Colorado |
| C. subdeflexa         | EF353197             | Nash 38631 (ASU)       | U.S.A. Arizona |
| C. vitellina          | EF353199             | Westberg 875 (LD)      | U.S.A. Oregon |
| C. xanthostigma       | EF352502             | Westberg 1122 (LD)     | U.S.A. Colorado |
| Candelina mexicana    | EF355214             | Westberg 388 (LD)      | Mexico       |
| Can. submexicana      | EF355215             | Westberg 408 (LD)      | Mexico       |
| Placomaronea canadalioides | FJ959350     | Fröden 1720 (LD)       | Argentina    |
| P. fuegiana           | FJ959351             | Fröden 1786 (LD)       | Argentina    |
| P. kaemefeltii        | FJ959354             | Fröden 1503 (LD)       | Chile        |
| P. mendaeae           | EF353219             | Westberg 833 (LD)      | U.S.A. Arizona |
| P. minima             | FJ959353             | Fröden 1656 (LD)       | Chile        |
| Pycnora xanthococca   | KF360412             | E. Timdal 11646 (O L 163707) | Norway |

Bold were newly generated in this study, C = Candelaria, Ca. = Candelariella, Can. = Candelina, P. = Placomaronea.
ambiguous sites, 472 sets were reserved. Maximum likelihood and Bayesian analyses based on ITS show similar topology with lower support on the main branches and strong support on the most terminate branches (Figure 1). Both Candelariella and Candelaria are polyphyletic, whereas the genera Candelina and Placomaronea form a single clade, and are complementary sister groups with lower support.

Species of Candelaria separate into three distinct clades and fall into two morphological groups. They are group 1, Candelaria in the strict sense, containing the type species C. concolor, is characterized by a well-developed lower cortex and polysporous asci, our newly described species C. asiatica robustly drops into this clade; group 2 is recognized by lacking a lower cortex and having 8-spored asci, and contains clades Candelaria 2 and Candelaria 3, including C. fibrosoides, C. fruticans, and C. pacifica.

In clade Candelaria 1, neither C. concolor nor C. fibrosa is monophyletic. Specimens of C. concolor collected from Europe form a single clade and are genetically distant from the specimen collected from North America, which is a sister group of C. fibrosa, which was collected from the same continent. In
addition, the specimen of *C. fibrosa* from South America is distant from the specimen collected in New Mexico. *Candelaria asiatica*, with two specimens, forms a robust clade and is the sister group of *C. crawfordii* with lower support, and both of them are collected from East Asia. Unless additional morphological and anatomical characters are found in the future, it is difficult to clarify the relationship among species in the family Candelariaceae at a genetic level up to this study. Otherwise, geography probably plays a significant role in the genetic variation among species of *Candelaria*, which is more or less supported by this study. However, more sequence data and specimens from different continents should be included in order to investigate the natural lineage of Candelariaceae.

**New species**

*Candelaria asiatica* D. Liu & J.-S Hur, sp. Nov., (Figure 2)

Mycobank No.: MB824810

Similar to *Candelaria concolor*, but differs in the lobe tips and margin, which is neither round nor smooth, and are often fragile or have blastidia, which makes them appear sorediate.

**Type:** South Korea, Jeollanam-do, Suncheon-si, Jungang-ro 225, beside the library of Sunchon National University, 34°58'01"N, 127°28'48"E, 47 m,

**Figure 2.** *Candelaria asiatica* (holotype). A, habit; B, lower surface; C, young thalli; D, lobes margin after blastidia or phyllidia-like lobules are dropped; E, F, blastidia or phyllidia-like lobules; G, transverse section of the thallus (Scale bars: A–F = 0.5 mm, G = 40 µm).
Table 2. Morphological difference among the species of Candelaria clade 1.

| Taxon        | Lobe               | Upper surface     | Asexual propagules | Distribution          |
|--------------|--------------------|-------------------|--------------------|-----------------------|
| C. asiatica  | Shallowly to deeply branched, narrow (up to 0.47 mm) | Rough             | Blastidia or phyllidia-like lobules present | South Korea          |
| C. concolor  | Deeply branched, narrow (up to 0.5 mm) | Smooth            | Soredia granular   | Asia, America, and Europe |
| C. crawfordii| Shallowly branched, wider (up to 0.7 mm)     | Smooth            | Soralia labriform, with soredia on the upturned lower surface. | Asia, Australia |
| C. fibrosa   | Wider (up to 2 mm) | Smooth or wrinkled | Soredia absent     | Pantropical region    |

Remarks: Candelaria asiatica is characterized by yellow distinctly foliose to sub-fruticose thallus, usually irregular branched, with a pulverulent surface, slightly upturned and rough lobe tips, and a fragile lobe margin with blastidia or phyllidia-like lobules. Candelaria asiatica resembles C. concolor, but can be distinguished by the rough and pulverulent surface and phyllidia-like lobules, whereas C. concolor has a smooth, flattened surface and thicker thallus [5]. Candelaria asiatica is currently only known from Asia, whereas C. concolor occurs in Europe and America. Furthermore, the cortical hyphae of C. concolor are somewhat gelatinized in Stapper’s figures [9], compared to those of C. asiatica, which are non-gelatinized. Candelaria asiatica differs from C. crawfordii and C. fibrosa by its narrower lobes, and blastidia or phyllidia-like lobules are frequently present on well-developed thallus, especially in old or rostrate individuals, while usually absent on squamulose or young individuals. The differences among the species of Candelaria clade 1 are listed in Table 2.

Specimens examined: South Korea. Jeollanam-do, Suncheon-si, Jungang-ro 225, Sunchon National University, 34°58′01″N, 127°28′48″E, 47 m, on Cerasus sp., 1 Dec 2017, D. Liu 171453; Gangwon-do, Jeongseon-gun, Gohan-eup, 37°11′22″N, 128°53′29″E, 882 m, on a pine tree, together with Normandina pulchella, 22 Sep 2016, D. Liu 171446, Accession number: ITS = MG694269, 28S = MH101754.

Disclosure statement
No potential conflict of interest was reported by the authors.

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