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RESEARCH ARTICLE

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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.

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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 sorediate 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

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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
Candelaria asiatica	**MG694269**	**D. Liu 171446 (KoLRI)**	**South Korea**
C. asiatica	**MG694270**	**D. Liu 171454 (KoLRI)**	**South Korea**
C. concolor	EF535205	Westberg 454 (LD)	Mexico
C. concolor	FJ959355	Krumsvik s.n. (BG)	Norway
C. concolor	GU929921	Arup L07018 (LD)	Italy
C. concolor	GU929922	Arup L07001 (LD)	Italy
C. crawfordii	EF535204	Moberg & Santesson 8125b (UPS)	China. Yunnan,
C. fibrosa	EF535206	Worthington 21240 (COLO)	U.S.A. New Mexico
C. fibrosa	GU929923	Frödén 1670 (LD)	Argentina
C. fibrosoides	EF535211	Frödén 1513 (LD)	Peru.
C. fibrosoides	EF535212	Frödén 1512 (LD)	Peru.
C. fruticans	EF535207	Lassøe s.n. (C)	Ecuador
C. pacifica	EF535210	[John, Lich. Anatol. Exs. no. 16] (ASU)	Turkey
C. pacifica	GU929918	Westberg 967 (LD)	U.S.A. California
Candelariella aggregata	EF535156	Westberg 1281 (LD)	Canada
Ca. antennaria	EF535159	Westberg 1155 (LD)	U.S.A. Colorado
Ca. aurella	EF535163	Westberg 150 (LD)	U.S.A. Arizona
Ca. biatorina	EF535164	Westberg 1181 (LD)	U.S.A. Colorado
Ca. blastidiata	KX853128	Davydov 7716 (ALTB)	Russia
Ca. borealis	EF535168	Westberg 1079 (LD)	U.S.A. Colorado
Ca. clarkii	KR052104	Tripp & D'az 4876 (COLO)	U.S.A. Colorado
Ca. complanata	EF535173	Westberg 392 (LD)	Mexico
Ca. granuliformis	GU967376	Westberg 3128 (LD)	Sweden
Ca. kansuensis	EF535181	Wetmore 55470 (MIN)	U.S.A. Arizona
Ca. lutella	EF535182	Westberg 2808 (LD)	Norway
Ca. medians	EF535184	Arup L03165 (LD)	Sweden
Ca. placodizans	EF535188	Westberg 1083 (LD)	U.S.A. Colorado
Ca. rosulans	EF535191	Westberg 1146 (LD)	U.S.A. Colorado
Ca. spraguei	EF535194	Westberg 1037 (LD)	U.S.A. Colorado
Ca. subdeflexa	EF535197	Nash 38631 (ASU)	U.S.A. Arizona
Ca. vitellina	EF535199	Westberg 875 (LD)	U.S.A. Oregon
Ca. xanthostigma	EF535202	Westberg 1122 (LD)	U.S.A. Colorado
Candelina mexicana	EF535214	Westberg 388 (LD)	Mexico
Can. submexicana	EF535215	Westberg 408 (LD)	Mexico
Placomaronea candelarioides	FJ959350	Frödén 1720 (LD)	Argentina
P. fuegiana	FJ959351	Frödén 1786 (LD)	Argentina
P. kaernefeltii	FJ959354	Frödén 1503 (LD)	Chile
P. mendozae	EF535219	Westberg 833 (LD)	U.S.A. Arizona
P. minima	FJ959353	Frödén 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*.

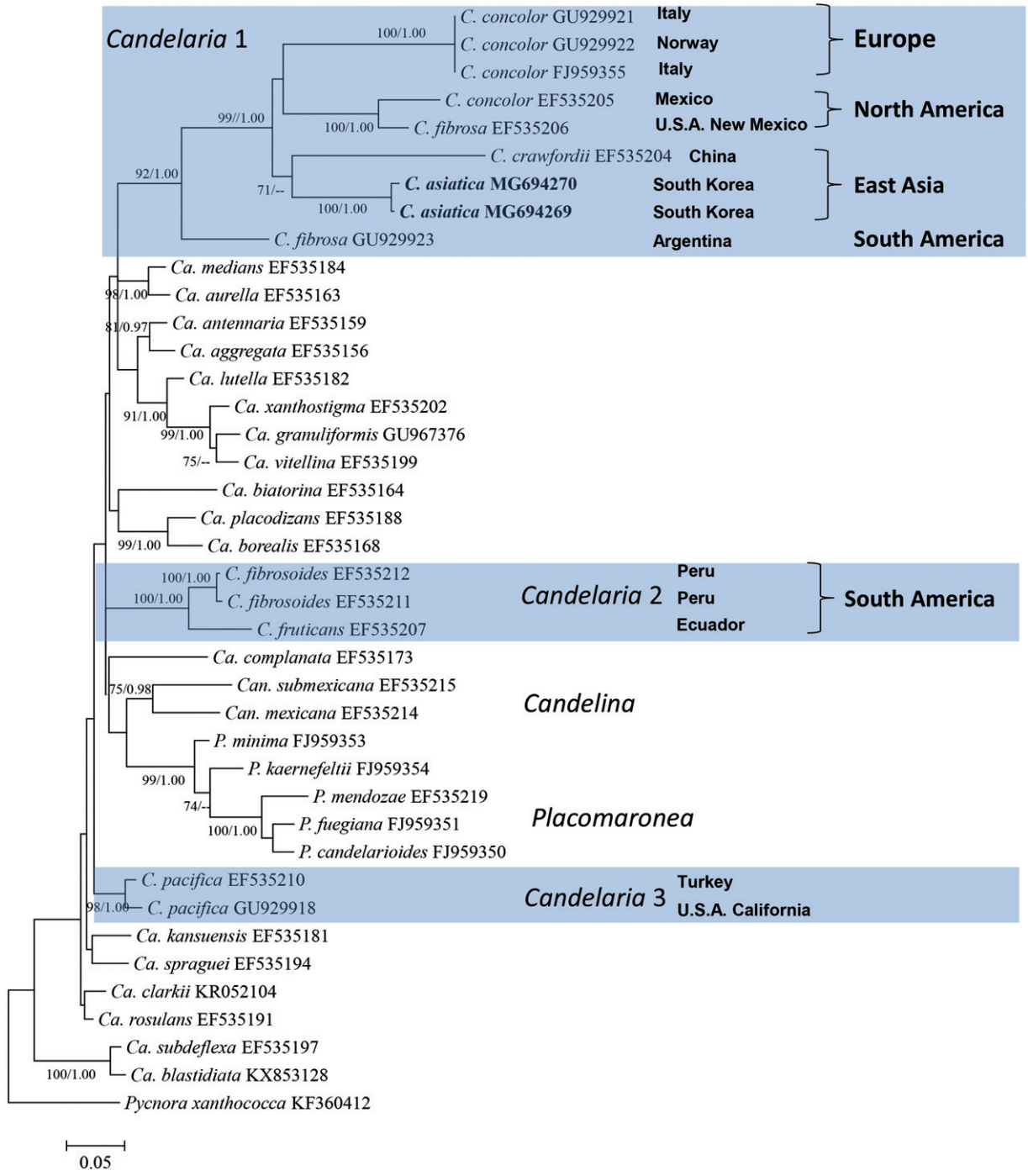

Figure 1. Most likely phylogenetic relationships among species of *Candelaria* inferred from ITS (nrDNA) sequences. Nodes supported by ML bootstrap values >70% and Bayesian posterior probabilities >0.95 are noted on the branches, and bootstrap values are erased on the lower supported clades. Clades of the genus *Candelaria* are highlighted in blue color.

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

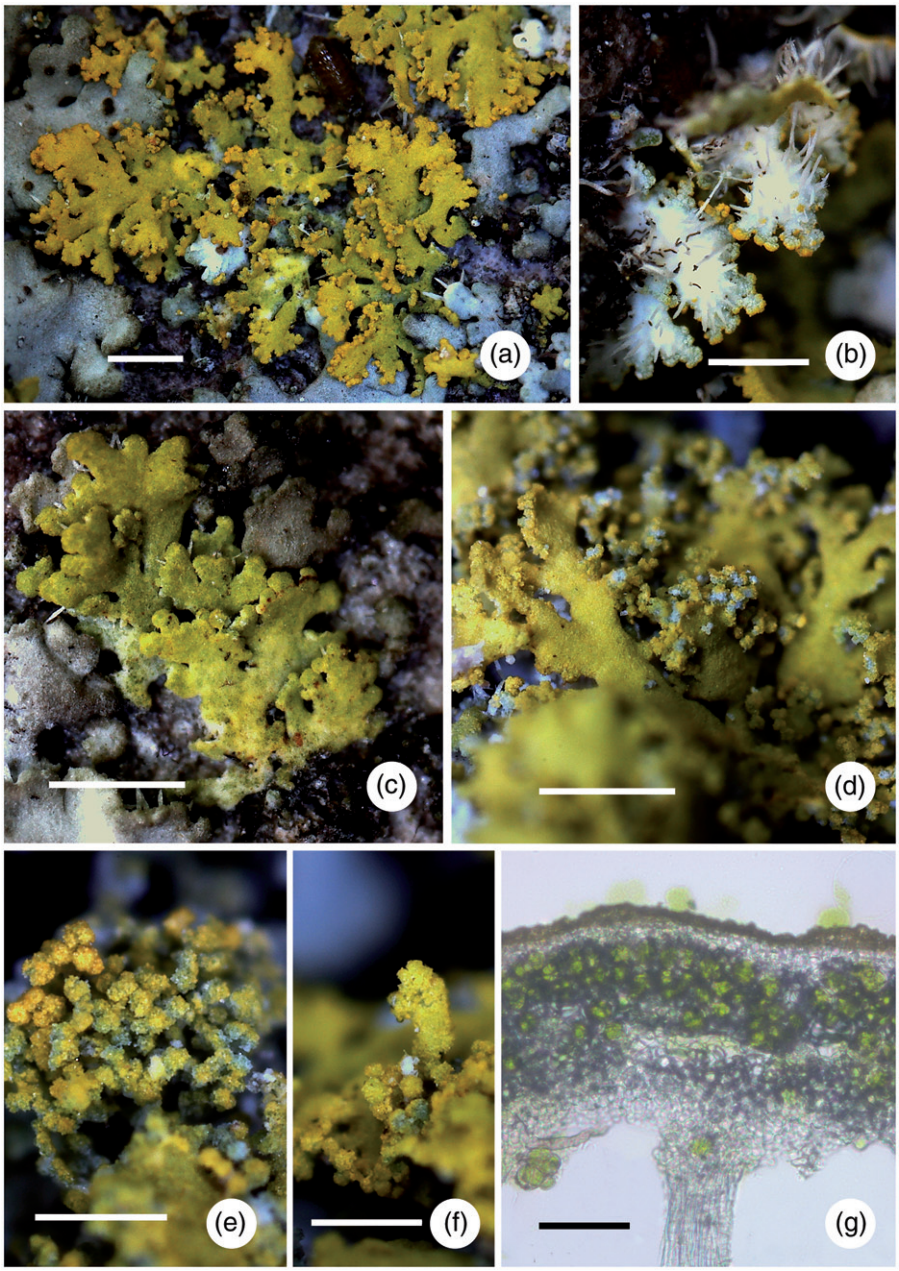

Figure 2. *Candelaria asiatica* (holotype). A, habit; B, lower surface; C, young thalli; D, lobes margin after blastidia or phyllidia-like lobules are dropped; E, F, blastidia or phyllidia-like lobules; G, transverse section of the thallus (Scale bars: A–F = 0.5 mm, G = 40 μm).

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,

Table 2. Morphological difference among the species of *Candelaria* clade 1.

Taxon	Lobe	Upper surface	Asexual propagules	Distribution
C. asiatica	Shallowly to deeply branched, narrower (up to 0.47 mm)	Rough	Blastidia or phyllidia-like lobules present	South Korea
C. concolor	Deeply branched, narrower (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

on *Cerasus* sp. or lichenicolous, 30 Nov 2017, D. Liu 171454 (holotype, KoLRI!). Accession Number: ITS = MG694270, 28S = MH101755.

Etymology: The epithet "*asiatica*," from Asia, refers to the geographic distribution of this species.

Morphology: Thallus corticolous or lichenicolous, scattered, mini squamulose to small foliose or sub-fruticose, 0.2–1.2 (–2.5) cm across, adnate, irregular, or rosettes, usually aggregates into extensive colonies covering the substrate; lobes dorsiventral, linear, irregular branched (2–4 times), 0.1–0.47 mm, adnate to erect, lobe tip slightly upturned, crenate; lobe surface color various, yellow to greenish yellow in the center, then becoming bright yellow toward the lobe tip, not smooth and pulverulent; margins usually have blastidia or phyllidia-like lobules (lower cortex poor development, Figure 2(C)), 40–120 µm wide (Figure 2(D,E,F)), loosely adnate lobe tips, which makes the thallus margin or tips pseudocyphellae-like, blastidia and lobulates absent when the colonization is very small or young; thallus 100–130 µm thick; upper cortex (pseudocortex) distinct, 8–16 µm thick, cortex cells 4–7 µm wide; algae chlorococcoid, very unevenly distributed below the upper cortex; medula white but not well-developed; the lower cortex is usually present at the center of the thallus, lacking at lobe tip; lower surface white, covered with white rhizines; apothecia and pycnidia not seen.

Chemistry: K–, KC–, C–, PD–; calycin and pulvinic acid as major substances.

Ecology: *Candelaria asiatica* was found growing on *Cerasus* sp., together with or directly on *Physciella melanchra* (Hue) Essl., and under open areas.

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 rosulate 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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