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Candelariella placodizans (Candelariaceae) reported new to mainland China and Taiwan based on morphological, chemical and molecular phylogenetic analyses

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ABSTRACT: *Candelariella placodizans* is newly reported from China. It was collected on exposed rocks with mosses on the alpine areas of Taiwan and Yunnan Province, China at elevation between 3200-4400 m. Molecular phylogenetic analyses based on ITS rDNA sequences were also performed to confirm the monophyly of the Chinese populations with respect to already existing sequences of the species, and then further to examine their relationships to other members of the genus. An identification key to all 14 known taxa of *Candelariella* in China is provided.

KEY WORDS: China, Distribution, Flora, Identification key, ITS rDNA, Phylogeny, Taiwan, Yunnan.

INTRODUCTION

The genus *Candelariella* Müll. Arg. a member of the family Candelariaceae Hakul. comprises ca. 50 accepted species worldwide (Kirk *et al*., 2008). This genus is distinguished from other genera in the family by the coralloid or squamulose to areolate crustose thallus, the lecanorine or biatorine apothecia, and the simple or one-septate spores with 8 to many spore asci, whereas other genera are characterized by the placodioid to subfoliose thallus for *Candelina* Poelt (3 spp.), the foliose to subfruticose thallus for *Candelaria* A. Massal. (9 spp.), and the squamulose to umbilicate thallus for *Placomaronea* Räsänen (6 spp.) (Westberg *et al*., 2007; Westberg and Frödén, 2007; Westberg *et al*., 2009; Westberg and Arup, 2011). All these genera contain pulvinic acid and its derivatives in the thallus, by which they show the distinctive yellow color.

During the investigation of Candelariaceae specimens preserved in the herbaria of the National Museum of Nature and Science (TNS) and Hiroshima University (HIRO), an interesting *Candelariella placodizans* (Nyl.) H. Magn. (Fig. 1), not reported earlier from China, was found after the critical morphological examinations.

The aim of this study is to confirm that the Chinese material identified as *Candelariella placodizans* was conspecific with those populations already known and to show the monophyly of the Chinese populations with respect to already existing sequences of the species, and then further to examine their relationships to other members of the genus. The identification key to Chinese species of *Candelariella* is also provided.

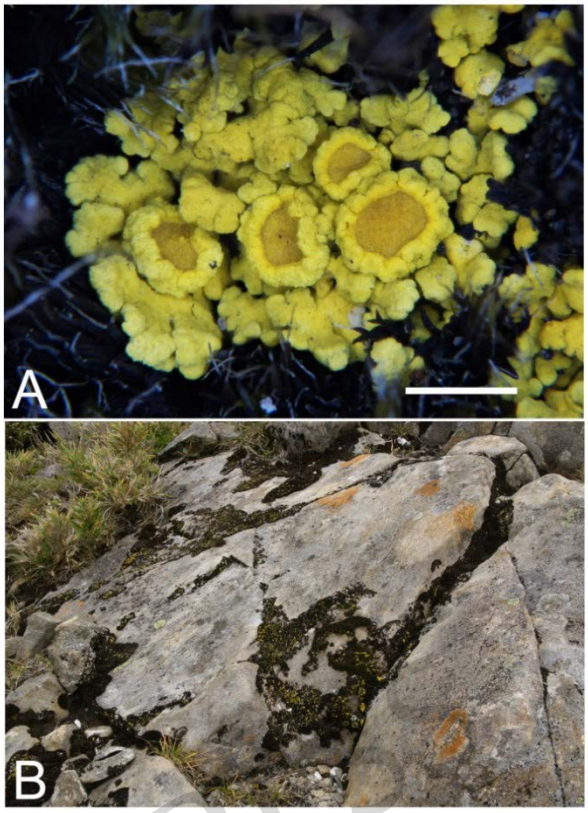


Fig. 1. ***Candelariella placodizans*** collected in Taiwan (Y. Ohmura 7584, TNS). **A.** Thallus. **B.** Habitat where it grows on mosses on rock at ca. 3700 m elev. of Mt. Nanhutashan, Taiwan. Scale = 1 mm.

MATERIALS AND METHODS

The specimens examined are housed in HIRO, TAI and TNS. Morphological observations were made using

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a dissecting microscope (Olympus SZX16) and a bright-field microscope (Olympus BX51). Transverse sections of apothecia and thalli were cut by hand with razor blade and observed after mounting in GAW (glycerin : ethanol : water, 1 : 1 : 1). Measurements are given as (minimum value observed–) range including 85% of the variation (–maximum value observed) with (SD=standard deviation, n=numbers of measurements) following Westberg (2005).

Secondary substances of thallus were examined using the method of thin layer chromatography (TLC) (Culberson and Kristinsson, 1970). Solvent B system (hexane: methyl tert.-butyl ether: formic acid, 140: 72: 18) (Culberson and Johnson, 1982) was used for the TLC analysis.

For molecular-phylogenetic analysis, ITS rDNA sequences of our fresh materials and those retrieved from GenBank were used. One-three unique and appropriate length sequences of every species of *Candelariella* were taken, preferably lacking uncertain nucleotides. This sampling comprised 26 species representing morphological variability within *Candelariella*, ten of which were represented by two or more accessions. The information of the samples with the GenBank accession numbers were shown in Table 1.

DNA extraction and PCR are followed by the method of Frisch and Ohmura (2015). The primer sets were ITS1F (Gardes and Bruns, 1993) and LR1 (Vilgalys and Hester, 1990). PCR cycle condition was followed by Ohmura *et al*. (2006). Sequencing was done on an ABI Prism 3130x genetic analyzer (Applied Biosystems) using the BigDye Terminator ver. 3.1 Cycle Sequencing Kit according to the manufacturer's instructions.

The obtained sequences in this study and the reference sequences from GenBank were aligned by Geneious 6.0 (Biomatters Ltd., New Zealand) and manually optimized. The sequence of *Pycnora xanthococca* (Sommerf.) Hafellner was used as an outgroup since *Pycnora* was suggested as the sister clade to Candelariaceae (Bendiksby and Timdal, 2013). Total alignment sites of 487 bp were used for the maximum likelihood (ML) and Bayesian analyses. Optimal substitution models were inferred separately for ITS1, 5.8S, and ITS2 using PartitionFinder, version 1.1.1 (Lanfear *et al*., 2012).

Bayesian inference with the Markov chain Monte Carlo (BMCMC) method (Larget and Shimon, 1999) was performed using MrBayes 3.2.3 (Ronquist *et al*., 2012). Three parallel analyses were run applying symmetrical model with gamma-distribution (SYM+G) and Kimura 2-parameter with proportion of invariable sites (K80+I) as models of substitution for two partitions: 5.8S and ITS1+ITS2 respectively (last two fragments have the same models of substitutions and were therefore combined). The Bayesian analysis was run for 20,000,000 generations in 6 chains and every 200th generation was sampled. The first 50% of trees were discarded as burn-in and a 50% majority rule consensus tree calculated with the sumt command implemented in MrBayes 3.2.3. Bootstrap support values and BMCMC posterior probability were noted onto the best scoring tree (numbers are shown only when ≥50% in both).

The ML tree and 1000 bootstrap replicates were calculated using RAxML 8.0.26 (Stamatakis, 2014) by raxmlGUI software version 1.3.1 (Silvestro and Michalak, 2012) applying the GTRGAMMA model of substitution, because RAxML do not support SYM and K80 models.

RESULTS and DISCUSSION

Candelariella placodizans (Nyl.) H. Magn.
[MB#365367][Fig. 1A]

Morphology, anatomy and chemistry of Chinese material are consistent with the description of *Candelariella placodizans* provided by Westberg (2007) and the type specimen (lectotype, H-NYL 29138!). The diagnostic features for Chinese materials are 1) the squamulose to granular thallus in which the squamules are 0.25–0.65 mm long (SD=0.12; n=18), 2) the greenish-yellow pulverulent upper surface of thallus, 3) lacking of vegetative propagules, 4) the lecanorine apothecia with (0.4–)0.8–2.0(–2.3) mm diameter (SD=0.5; n=22), 5) the polyspored asci with simple or rarely one-septated, ovoid to narrowly ellipsoid ascospores 7.5–10.0(–12.0) × (4.5–)5.0(–5.3) µm; SD=1.0, 0.1; n=15), 6) the hymenium up to 107.5 µm tall, 7) the occurrence in alpine to subalpine habitats on soil, plant debris, bryophytes and other lichens, and 8) the presence of calycin, pulvinic acid, terpenoids [Rf classes 4–5 (T1), 5 (T2), 5–6 (T3)], and an unidentified substance (Rf class 8, pale, UV_{254nm} + visible, UV_{365nm} + yellow) [Fig. 2]. The range of the apothecia size in Taiwanese material is slightly larger and the hymenium size is slightly taller compared with those of North American materials [apothecia 0.35–1.0(–1.2) mm diameter and hymenium 90–100 µm tall as in Westberg (2007)]. It should be noted that three terpenoids and unidentified substance were constantly detected in all Chinese materials in addition to the known lichen substances of this species. Since these substances have not been reported from other taxa in Candelariaceae, their taxonomic values should be carefully examined in further researches.

Candelariella placodizans has a circumpolar Arctic distribution and also occurs in the alpine belt of the Rocky Mountains in North America above 3000 m elevation (Westberg, 2007). In Asia it is reported from the alpine to subalpine mountains and arctic parts of Ural, Siberia and Russian Far East (Urbanavichus, 2010) and Himalayas above 3900 m elevation (as *C. himalayana* Poelt & Reddi; Poelt and Reddi, 1969; Westberg, 2010) as well as in the mountains in Southwest Asia (Westberg and Sohrabi, 2012). Taiwan is the most south part of distribution area. It

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was collected here at elevation between 3240 to 3710 m. The species could be easily found on mosses over exposed rocks or plant debris in alpine to subalpine areas of Taiwan because of its bright distinctive color (Fig. 1B).

Specimens examined: **CHINA:** Yunnan: Zhongdian Co.: Mt. Xiaoxueshan, 4000-4400 m elev., on mossy rock, 15 Jun. 2004, K. Takahashi 2408 (HIRO). **TAIWAN:** Taichung Co.: around North Peak of Mt. Chilai, Taroko National Park (N24°07′00″, E121°19′55″), over mosses on rock, ca. 3400 m elev., 30 Sep. 2009, Y. Ohmura 7026 (TNS); Shenmajhen Cabin, en route from Shiyuan Yakou to Mt. Nanhu (N24°22′53″, E121°25′35″), on rock with mosses, 3241 m elev., 1 Oct. 2010, Y. Ohmura 7525 (TAI, TNS); main peak of Mt. Nanhu, en route from Shiyan Yakou to Mt. Nanhu (N24°21′38″, E121°26′27″), on mosses, 3712 m elev., 3 Oct. 2010, Y. Ohmura 7584 (TNS); around main peak of Mt. Nanhu, en route from Shiyan Yakou to Mt. Nanhu (N24°21′43″, E121°26′38″), on mosses, 3577 m elev., 3 Oct. 2010, Y. Ohmura 7598 (TAI, TNS).

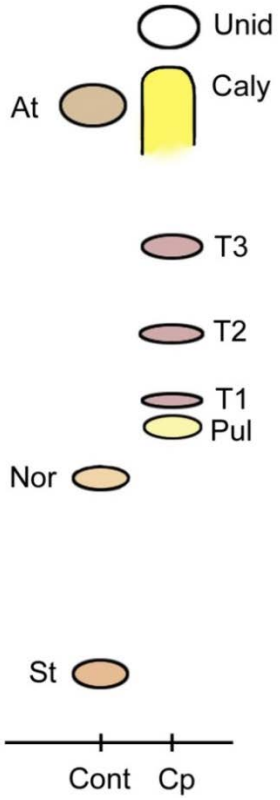


Fig. 2. Schematic illustration for major TLC spots detected from Taiwanese ***Candelariella placodizans*** (solvent B). Cont = control (St = stictic acid as Rf class 2, Nor = norstictic acid as Rf class 4, At = atranorin as Rf class 7); Cp = ***Candelariella placodizans*** (Pul = pulvinic acid, T1, T2, T3 = unidentified terpenoids, Caly = calycin, Unid = unidentified substance).

Molecular phylogenetic analyses

The phylogenetic analyses resulted in the recognition of a monophyletic grouping of all six *Candelariella placodizans* sequences both from North America and from Taiwan with 99/1.00 support values (bootstrap value/BMCMC posterior probability respectively) [Fig. 3]. Taiwanese samples form a monophyletic clade (67/1.00 support values) within *C. placodizans* clade. *Candelariella placodizans* and *C. borealis* M. Westb. formed a sister clade each other (100/1.00) as Westberg *et al*. (2007) also found. The close relationship between these species is well explained by the close morphology and ecological features: the both species have polyspored asci and occur in similar arctic-alpine habitats (Westberg *et al*., 2007). However, *C. placodizans* is distinguished from *C. borealis* mainly by its characteristic greenish-yellow coarsely pulverulent thallus and shape of the squamules.

Key to *Candelariella* species in China

A total of 14 taxa in *Candelariella* are known from China (see Wei, 1991; Abbas *et al*., 2001; Aptroot and Sparrius, 2003; Kurax *et al*., 2010). The identification key to the species is provided below.

1a. Thallus placodioid with granular soredia in the center; continental China …….. *Candelariella medians* (Nyl.) A. L. Sm.
1b. Thallus evenly-crustose: areolate, granulate, subsquamulose, squamulose to lobate ……..…… 2
2a. Thallus sorediate; soredia (–15)30–50(–80) µm diam. ……3
2b. Thallus not sorediate (if granular, then granules larger, ca. 100 µm diam.) ……..……5
3a. Thallus squamulose; squamules plane or ascending from the one-side of margin; soredia (–20)30–50(–60) µm diam., developed on the margin of squamules and dispersed to the lower side; asci 8-spored; habitat corticolous and muscicolous in alpine to subalpine mountains; Taiwan…… *C. sorediosa* Poelt & Reddi
3b. Thallus squamulose to subsquamulose; squamules never ascending from the one-side of margin; soredia (–15)30–50(–80) µm diam., formed on the upper side or the margin of squamules but never spread to the lower side; asci 8-spored or polyspored; habitat corticolous below subalpine area. ……..…….4
4a. Thallus subsquamulose but soon dissolved into entire sorediate crust divided by cracks, yellow, matt surface; soralia discrete, formed on the margin of the squamules and spread onto upper surface; soredia up to 50 µm diam.; asci polyspored; continental China ……..*C. efflorescens* R.C. Harris & W. R. Buck
4b. Thallus clearly-squamulose of effigurated lobes, greenish-yellow, pulverulent surface; soralia central on the thallus, crateriform; soredia 30–80 µm diam.; asci 8-spored; Taiwan…… ……..*C. reflexa* (Nyl.) Lettau
5a. Thallus granulate, well-developed, of evenly dispersed granules (ca. 0.1 µm diam.); asci polyspored; habitat corticolous; continental China ……*C. xanthostigma* (Ach.) Lettau
5b. Thallus not granulate but areolate, subsquamulose, squamulose to lobate; asci 8- or polyspored; habitat on rock, soil, mosses, plant debris, or dead twigs ……..……6
6a. Thallus absent or poorly developed of minute dispersed areoles; habitat on rocks or rarely on dead twigs below subalpine area ……..7
6b. Thallus distinct, usually well-developed, subsquamulose, squamulose to lobate; habitat on different substrates in broad range of elevations ……8
7a. Thallus areolate, often poorly developed of convex areoles, yellow to partly greyish; asci 8-spored; habitat on siliceous rocks or dead twigs; continental China and Taiwan ……*C. aurella* (Hoffm.) Zahlbr.
7b. Thallus absent or poorly developed of minute dispersed areoles, yellow to orange-yellow; asci polyspored; habitat on rocks; continental China ……*C. athallina* (Wedd.) Du Rietz
8a. On mosses, plant debris, soil in alpine to subalpine area…… ……9
8b. On siliceous rocks below subalpine area ……11
9a. Thallus squamulose with the greenish-yellow pulverulent upper surface; asci polyspored ……...*C. placodizans* (Nyl.) H. Magn.
9b. Thallus granulose, squamulose and/or lobate, without the greenish-yellow pulverulent upper surface; asci 8-spored…….10

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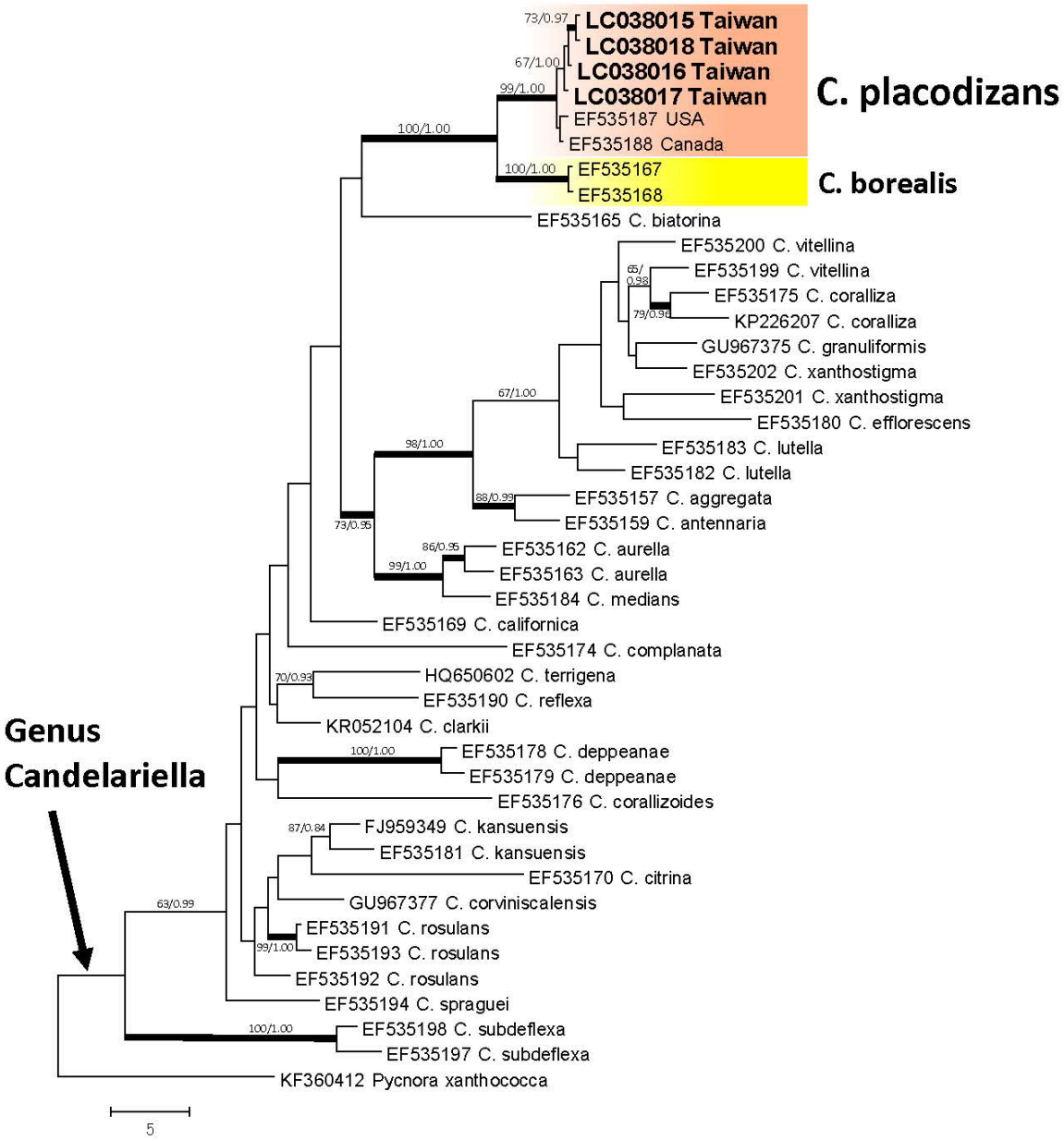


Fig. 3. Phylogenetic relationship between Taiwanese ***Candelariella placodizans*** and the related species based on ITS rDNA sequences. The tree was constructed by ML method, and the reliability of each branch was tested by ML and Bayesian methods. Numbers on tree nodes indicate bootstrap values of ML (left) and BMCMC posterior probabilities (right) (they are shown only when ≥50% and ≥0.50 simultaneously). Thicker branches indicate when the bootstrap value of ML is ≥70% and BMCMC posterior probabilities is ≥0.95 simultaneously. GenBank accession numbers are given as OTU names (see Table 1). ***Pycnora xanthococca*** is used as out group.

10a. Lobes with deeply-incised margin, adpressed to ascending; apothecia with flattened disc and persistent thalline margin; ascospores (13.0–)15.0–18.5 × (4.5–)5.0–6.5 µm, ellipsoid; Taiwan ……..…..……*C. grimmiae* Poelt & Reddi

10b. Lobes with crenulate margin, usually adpressed to the substratum; apothecia with convex disc and indistinct thalline margin when mature; ascospores (9.0–)11.0–15.5(–17.0) × (4.5–)5.0–6.5(–7.0) µm, narrowly-ellipsoid with somewhat attenuate at the ends; Taiwan ……*C. canadensis* H. Magn. (= *C. nepalensis* Poelt & Reddi, see Westberg, 2010)

11a. Asci polyspored; continental China and Taiwan…….. ……*C. vitellina* (Hoffm.) Müll. Arg.

11b. Asci 8-spored ……12

12a. Thallus matt on the surface, orange-yellow, gelatinous epicortex absent; ascospores oblong; continental China…… ……*C. rosulans* (Müll. Arg) Zahlbr. (= *C. oliefera* H. Magn., see Westberg and Sohrabi, 2012)

12b. Thallus shinny on the surface, brownish-yellow, gelatinous epicortex present; ascospores narrowly-ellipsoid; continental China ……..….13

13a. Thallus lobate or squamulose, 0.3–0.5 mm thick; apothecia up to 0.7 mm diam. ……..…… *C. kansuensis* H. Magn.

13b. Thallus squamulose, 0.5–1.0 mm thick; apothecia up to 1.0 mm in diam. ……. *C. kansuensis* f. *frustulenta* H. Magn.

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Appendix 1. Sample numbers and their GenBank accession numbers for the phylogenetic analyses in this study

Species	Locality	Voucher (Herbarium)	GenBank accession number	Reference
Candelariella aggregata	USA: Colorado	Westberg 1080 (LD)	EF535157	Westberg *et al*. (2007)
C. antennaria	USA: Colorado	Westberg 1155 (LD)	EF535159	Westberg *et al*. (2007)
C. aurella	USA: Colorado	Westberg 1053 (LD)	EF535162	Westberg *et al*. (2007)
	USA: Arizona	Westberg 150 (LD)	EF535163	Westberg *et al*. (2007)
C. biatorina	USA: California	Tucker & Bratt 34049 (SBBG)	EF535165	Westberg *et al*. (2007)
C. borealis	Canada: Nunavut	Westberg 2381 (LD)	EF535167	Westberg *et al*. (2007)
	USA: Colorado	Westberg 1079 (LD)	EF535168	Westberg *et al*. (2007)
C. californica	USA: California	Westberg 1244 (LD)	EF535169	Westberg *et al*. (2007)
C. citrina	Mexico: Baja California Sur	Westberg 398 (LD)	EF535170	Westberg *et al*. (2007)
C.clarkii	USA: Colorado	Tripp & D'az 4876 (COLO)	KR052104	Tripp and Lendemer (2015)
C. complanata	Mexico: Baja California Sur	Westberg 383 (LD)	EF535174	Westberg *et al*. (2007)
C. coralliza	Sweden: Oland	Westberg 51 (LD)	EF535175	Westberg *et al*. (2007)
	China: Yanbian Korean Autonomous Prefecture	YK140035	KP226207	Cao *et al*. (2015)
C. corallizoides	Mexico: Baja California Sur	Westberg 341 (LD)	EF535176	Westberg *et al*. (2007)
C. corviniscalensis	USA: Colorado	Morse 15881 & Ladd (S)	GU967377	Westberg *et al*. (2011)
C. deppeanae	USA: Arizona	Westberg 585 (holotype) (LD)	EF535178	Westberg *et al*. (2007)
	Mexico: Chihuahua	Nash 36526 (ASU)	EF535179	Westberg *et al*. (2007)
C. efflorescens	Austria: Steiermark	Arup L97286 (herb. Ulf Arup)	EF535180	Westberg *et al*. (2007)
C. granuliformis	Canada: Nunavut	Mattsson 5209 (UPS)	GU967375	Westberg *et al*. (2011)
C. kansuensis	USA: Arizona	Wetmore 55470 (MIN)	EF535181	Westberg *et al*. (2007)
	USA: Arizona	Westberg s. n. (LD)	FJ959349	Westberg *et al*. (2009)
C. lutella	Norway: Troms	Westberg 2808 (LD)	EF535182	Westberg *et al*. (2007)
	USA: Arizona	Westberg 206 (LD)	EF535183	Westberg *et al*. (2007)
C. medians	Sweden: Skane	Arup L03165 (LD)	EF535184	Westberg *et al*. (2007)
C. placodizans	Canada: Nunavut	Westberg 206 (LD)	EF535187	Westberg *et al*. (2007)
	USA: Colorado	Westberg 1083 (LD)	EF535188	Westberg *et al*. (2007)
	Taiwan: Taichung	Ohmura 7525 (TNS)	LC038015	This study
	Taiwan: Taichung	Ohmura 7026 (TNS)	LC038016	This study
	Taiwan: Taichung	Ohmura 7584 (TNS)	LC038017	This study
	Taiwan: Taichung	Ohmura 7598 (TNS)	LC038018	This study
C. reflexa	Norway: Vestfold	Lindblom & Blom L61 (BG)	EF535190	Westberg *et al*. (2007)
C. rosulans	USA: Colorado	Westberg 1146 (LD)	EF535191	Westberg *et al*. (2007)
	USA: Arizona	Westberg 812 (LD)	EF535192	Westberg *et al*. (2007)
	USA: Colorado	Westberg 199 (LD)	EF535193	Westberg *et al*. (2007)
C. spraguei	USA: Colorado	Westberg 1037 (LD)	EF535194	Westberg *et al*. (2007)
C. subdeflexa	USA: Arizona	Nash 38631 (ASU)	EF535197	Westberg *et al*. (2007)
	USA: Arizona	Westberg 660 (LD)	EF535198	Westberg *et al*. (2007)
C. terrigena	Canada: Nunavut	Lutzoni & Miadlikowska 07.13.03-19 (DUKE)	HQ650602	Schmull *et al*. (2011)
C. vitellina	USA: Oregon	Westberg 875 (LD)	EF535199	Westberg *et al*. (2007)
	Sweden: Oland	Westberg 49 (LD)	EF535200	Westberg *et al*. (2007)
C. xanthostigma	USA: California	Nash 32596 (ASU)	EF535201	Westberg *et al*. (2007)
	USA: Colorado	Westberg 1122 (LD)	EF535202	Westberg *et al*. (2007)
Pycnora xanthococca	Norway	E. Timdal 11646 (O L-163707)	KF360412	Bendiksby and Timdal (2013)

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