Cryptothecia austrocoreana (Arthoniales, Arthoniaceae), a New Species from South Korea

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Abstract Cryptothecia austrocoreana is a new lichen species from South Korea. The species is characterized by the presence of a heteromorous thallus and faveolate ascigerous area (ascomata) immersed in a slightly raised thallus. The species has muriform ascospores, (4)6–8–spored 8–11 × 3–4 septate, (34)36–48(51) × (17)19–23(25) µm. Atranorin, chloroatranorin, and barbatic acid are present. In the phylogenetic tree, C. austrocoreana belongs to the arthonioid clade in Arthoniaceae.

Keywords Arthoniaceae, Arthoniales, Cryptothecia, New species, South Korea

The genus Cryptothecia is distributed in tropical or subtropical areas worldwide. Approximately 68 species of Cryptothecia have been reported to date, of which 65 were provided with a world key [1, 2]. General morphological characteristics of Cryptothecia are (1) byssoid thallus lacking isidioid outgrowths; (2) medulla I+ blue in patches; (3) trentepohlioid photobiont; (4) broadly clavate to globose thick-walled Cryptothecia-type asci, aggregated in ascigerous areas or loosely dispersed on thallus; (5) muriform ascospores with wavy septa [1]. Cryptothecia is morphologically similar to other genera belonging to Arthoniaceae. A distinguishing character is that Arthonia and Arthothelium have distinct fruiting-bodies, whereas Cryptothecia and Stirtonia lack distinct fruiting-bodies. Additionally, Arthonia and Stirtonia have transversely septated spores, whereas Arthothelium and Cryptothecia have muriform spores [3]. Phylogenetic studies based on DNA sequencing have indicated that Cryptothecia belongs to Arthoniaceae. Furthermore, Cryptothecia is reported to be polyphyletic [4-6].

In South Korea, major genera such as Arthonia and Arthothelium of Arthoniaceae have been studied, but Cryptothecia is undiscovered. The genus Cryptothecia has been studied through a program for the undiscovered taxa of South Korea initiated by the National Institute of Biological Resources. Members of Cryptothecia usually grow on rough bark in shaded and humid subtropical forests. In South Korea, Jeju Island and the southern coasts have a subtropical climate [7]. Cryptothecia was collected from the vicinity of the Seonam Temple, which is located in Jo-gye Mountain Provincial Park in Jeollanam Province. The temple is surrounded by valleys that contain Carpinus tschonoskii, C. laxiflora, Acer pseudosieboldianum, and Meliosma myriantha. The aim of this study was to investigate the phylogenetic position of new species in Arthoniaceae through morphological, chemical, and molecular analysis.

MATERIALS AND METHODS

Morphological and chemical studies. The lichen specimens were collected from Seonam temple, Jeollanam Province, South Korea from 2016 to 2017 and deposited in the Korean Lichen Research Institute (KoLRI) (Fig. 1). External morphological characteristics of lichen specimens were examined using a stereomicroscope (SZX-7; Olympus, Tokyo, Japan). Macro photographs were taken with an Olympus E450 camera using the Quick Photo Camera 2.3 software. The anatomy of the thalli and ascigerous areas was examined using a microscope (Eclipse Ni-U; Nikon, Tokyo, Japan) equipped with a Nikon DS-Filc camera, which was operated using the NIS-Elements BR software. Calcium oxalate crystals were identified using 25% H₂SO₄. Spot tests

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of lichen specimens were conducted using 10% KOH (K), saturated Ca(OCl), (C), 10% KOH followed by saturated Ca(OCl), (KC), and 5% alcoholic p-phenylenediamine (P). Secondary metabolites of lichen specimens were identified using high-performance thin-layer chromatography (HPTLC) and thin-layer chromatography (TLC). HPTLC was performed using solvent systems A, B, and C [8]. TLC was performed using solvent systems A and C per the standard method [9]. Localities from which lichen specimens were collected were mapped using the open source GIS software Quantum GIS 1.7.4 (QGIS).

DNA sequencing and alignment. Genomic DNA was isolated from freshly collected lichen specimens using a NucleoSpin Plant II Kit (MACHEREY-NAGEL, Düren, Germany) according to the manufacturer's protocols. For the mitochondrial small subunit rDNA (mtSSU) region, the primers mtSSU1 (5'-AGCAGTGAGGAATATTGGTC-3') and mtSSU3R (5'-ATGTGGCACGTCTATAGCCC-3') were used. The PCR cycle was followed as described by Zoller et al. [10]. For the RNA polymerase II (RPB2)

Table 1. Voucher and accession numbers for specimens from GenBank

| Species                        | Voucher                  | mtSSU   | RPB2    |
|-------------------------------|--------------------------|---------|---------|
| 1 Arthonia calcarea           | France, Ertz 7540 (BR)   | EU704065| EU704029|
| 2 Arthonia incarnata          | Fonsson FU6271           | -       | KY983983|
| 3 Arthonia didyma             | Belgium, Ertz 7587 (BR)  | EU704047| EU704010|
| 4 Arthonia radiata            | Belgium, Ertz s.n. (BR)  | EU704048| EU704011|
| 5 Arthothelium norvegicum     | USA, McCune 31061 (UPS)  | KJ851003| KJ851114|
| 6 Bromocippinsia cytopsora    | Luxembourg, Diederich 16849 (BR)| JF830771| -       |
| 7 Bromocippinsia cytopsora    | Belgium, Ertz 15244 (BR) | JF830772| -       |
| 8 Chiocodexon natalense       | Uganda, Frisch 11Ug324 (UPS)| KF707647| KF707660|
| 9 Comonarpon cinnabarinae     | Uganda, Frisch 11Ug297 (UPS)| KJ850977| -       |
| 10 Cryptothecia palaeotropica | Uganda, Frisch 11Ug457 (UPS)| KJ850961| KJ851084|
| 11 Cryptothecia assimilis      | Fiji, Lumbsch 19815I (F) | GU327688| -       |
| 12 Cryptothecia punctosorediata| USA, Nelsen 4038 (F)     | JX046450| -       |
| 13 Cryptothecia austrocoreana | Korea, KoLRI No.0411892 | MF769374| 163647  |
| 14 Cryptothecia austrocoreana | Korea, KoLRI No.04721    | MF769375| -       |
| 15 Cryptothecia sp. Ertz 8472 | Rwanda, Ertz 8472 (BR)   | EU704053| -       |
| 16 Cryptothecia sp. Ug1       | Uganda, Frisch 11Ug194 (UPS)| KJ850956| KJ851093|
| 17 Cryptothecia sp. Ug2       | Uganda, Frisch 11Ug18 (UPS)| KJ850955| KJ851092|
| 18 Cryptothecia sp. Ug3       | Uganda, Frisch 11Ug39 (UPS)| KJ850954| KJ851086|
| 19 Cryptothecia subnudulans   | Reunion, v.d.Boom 40613 (hd v.d.Boom)| KJ850952| KJ851087|
| 20 Cryptothecia subnudulans   | Guyana, Joensson Guyana 6a (UPS)| KJ850953| KJ851088|
| 21 Herpothallon inopinatum    | Mexico, Rudolphi 12 (UPS)| KJ850964| KJ857099|
| 22 Herpothallon kigeziense    | Uganda, Frisch 11Ug26 (UPS)| KF707644| KF707654|
| 23 Herpothallon rubrocinctum  | Mexico, Rudolphi 5 (UPS) | KF707643| KF707655|
| 24 Inodera byssaccum          | Japan, Thor 25952 (UPS)  | KJ850962| KJ857089|
| 25 Myriostigma candidum       | Gabon, Ertz 9260 (BR)    | EU704052| EU704015|
| 26 Myriostigma candidum       | Uganda, Frisch 11Ug125 (UPS)| KJ850959| KJ851096|
| 27 Recliningia synescioideae  | Uganda, Frisch 11Ug14 (UPS)| KF707651| KF707656|
| 28 Stirtonia neotropica       | Brazil, Caceres & Aptroot 11112 (ISE)| KP843611| -       |
| 29 Stirtonia sp. Ug1          | Uganda, Frisch 11Ug325 (UPS)| KJ850965| -       |
| 30 Tylophon hibernicum        | France, Diederich 16335 (BR)| JF830779| -       |
| 31 Tylophon hibernicum        | Uganda, Frisch 11Ug220 (UPS)| KJ850966| KJ851097|

mtSSU, mitochondrial small subunit rDNA; RPB2, RNA polymerase II.
region, the primers fRPB2-7cF (5'-ATGGGYAARCAAGC-YATGGG-3') and fRPB2-11aR (5'-GCRTGGATCTTTTCRTCSACC-3') were used. The PCR cycle as described by Liu et al. [11] was followed. Generated sequences were aligned with those of genera belonging to Arthoniaceae selected from GenBank (Table 1). All raw sequences were assembled and edited using BioEdit 7.09 [12].

**Phylogenetic analysis.** The TVM + I + G model as best-fitting the data was determined using Akaike Information Criterion as implemented by jModeltest v2.1.10 for MrBayes [13]. Phylogenies were analyzed using maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference. Bootstrap values were obtained in MEGA 7 using ML and MP methods with 1,000 bootstrap estimates. The ML was analyzed using the GTR + I + G model [14]. Bayesian analyses were conducted with the Metropolis-coupled Markov chain Monte Carlo method using MrBayes v. 3.1.2. In the Bayesian inference, two parallel independent analyses were performed using one cold and three heated chains for 1,000,000 generations, and trees were sampled every 100 generations [15].

**RESULTS AND DISCUSSION**

A phylogenetic tree of the new species was produced by alignment with 28 sequences for mtSSU and 20 sequences for RPB2 selected from GenBank (Table 1). The genera

![Fig. 2. Analysis of phylogenetic relationship among genus Cryptothecia and other related genera based on mitochondrial small subunit sequences. Maximum likelihood (ML), maximum parsimony (MP) bootstrap value ≥70%, and Bayesian posterior probabilities (PP) value ≥95% are shown above the branches. Bootstrap values are shown in the order of ML, MP, and PP in the tree. If the bootstrap value is 100% (ML, MP) or 1.00 (PP), the branch is shown in bold.](image-url)
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Herpothallon, Stirtonia, Myriostigma, Inoderma, and Tylophoron belonging to the cryptothecioid clade were included in the tree. The cryptothecioid clade consists of genera with a byssoid thallus morphologically similar to that of Cryptothecia. Chiodecton natalense (Roccellaceae) was included as an outgroup.

Although Cryptothecia austrocoreana has morphological characteristics indicative of Cryptothecia, it was found to belong to the arthonioid clade in the phylogenetic tree based on mtSSU and RPB2 (Figs. 2 and 3). Previous studies reported that Cryptothecia was polyphyletic and belonged to the cryptothecioid clade [4, 5, 11]. However, this is the first case in which Cryptothecia was found to belong to the arthonioid clade rather than cryptothecioid clade. To date, about 68 species of Cryptothecia have been reported, but only less than 10 species have sequencing data for comparison of relationships. Therefore, we have identified the phylogenetic position of Cryptothecia austrocoreana in Arthoniaceae with related genera. Cryptothecia sequences registered in the NCBI database are the results of RPB2, nuclear large subunit (nLSU), and mtSSU. We also used mtSSU, RPB2, and nLSU for phylogenetic investigation of Cryptothecia austrocoreana, however, we obtained sequences only for mtSSU and RPB2. The sequencing results of Cryptothecia and related genera confirm that Cryptothecia can belong to other groups in addition to the cryptothecioid clade. Further, sequence data of Cryptothecia and minor groups of Arthoniaceae are not sufficiently complete. Thus, a new marker gene that is advantageous for analysis of the Arthoniaceae phylogeny is proposed for further study.

New species.

Cryptothecia austrocoreana J.-J. Woo, L. Lőkös, E. Farkas & J.-S. Hur, sp. nov. (Fig. 4).

MycoBank No.: MB 822507.

Type: South Korea, Jeollanam Prov., Sunchon-si, Seonam Temple, 34°59’33.8” N, 127°20’23.9” E, alt. 160 m, Seonam valley; trees around the valley, on the bark of Meliosma myriantha, 11 Apr 2016, J.-J. Woo, 163647 (holotype: KoLRI 041892, accession number: MF769374); the same locality, 9 Feb 2017, J.-J. Woo, 170597 (KoLRI 044721, accession number: MF769375).

Distribution and ecology: Cryptothecia austrocoreana has been found in the type locality where it grows on the bark of Carpinus tschonoskii and Meliosma myriantha.

Etymology: Name refers to the southern region of South Korea.

Morphology: Thallus crustose, corticolous, ecorticate, byssoid, epithloedal, firmly attached to the substrate, up to 15 cm in diameter, beige to greenish white, lacking soredia and isidia, up to 75 µm thick, heteromorous, with numerous needle-shaped calcium oxalate crystals. Prothallus white, up to 5 mm. The faveolate ascigerous area (ascomata) is mostly spherical and verrucose immersed in a slightly raised thallus. Asci broadly clavate, (4)–8–spored. Paraphyses enclosing the asci. Ascospores hyaline, ellipsoid, muriform,
(34)36–48(51) × (17)19–23(25) µm, 8–11 × 3–4 septate.

**Chemistry:** K+ yellow, C−, KC−, P−, I+ blue in patches (section), UV−. TLC and HPTLC: Atranorin (minor), barbatic acid (major), and chloroatranorin (trace).

**Note:** Atranorin, barbatic acid, and chloroatranorin were detected by HPTLC and TLC in *Cryptothecia austrocoreana*. Only six species (*C. albomaculatella, C. albata, C. aleurina, C. caesioalba, C. fuscopunctata, and C. lunulata*) in *Cryptothecia* contain barbatic acid. *C. fuscopunctata*, which has a lichen substance similar to that in *C. austrocoreana*, differs in the size and shape of spores or the number of septa. In addition, *C. fuscopunctata* has a 135–300 µm thick thallus, which is much thicker than that of *C. austrocoreana*. Table 2 shows a comparison among species containing barbatic acid in the genus *Cryptothecia*. Chloroatranorin was only detected in solvent C in TLC.

![Fig. 4. A, Cryptothecia austrocoreana (holotype), part of thallus and substrate; B, Magnified prothallus; C, Faveolate asigerous area (ascomata); D, Ascus; E, Ascospore (scale bar: B = 2 mm, C = 0.5 mm, D = 20 µm, E = 10 µm).](image)

**Table 2. Comparison of morphological characteristics of species containing a barbatic acid in the genus Cryptothecia**

| Species name          | Ascospore   | Spore size (µm)        | Septation  | Chemistry                  |
|-----------------------|-------------|------------------------|------------|----------------------------|
| *C. austrocoreana*    | (4)6–8-spored | (34)36–48(51) × (17)19–23(25) | 8–11 × 3–4 | Atranorin, Barbatic acid, Chloroatranorin |
| *C. albata*           | 8-spored    | 17–26 × 7              | 7–8 × 1–3  | Barbatic acid, Divaricatic acid, Unknown lichen substance |
| *C. albomaculatella*  | -           | 50–65 × 22–29          | -          | Barbatic acid, Obtusatic acid |
| *C. aleurina*         | 8-spored    | 23–36 × 17–19          | -          | Barbatic acid, Unknown lichen substance |
| *C. caesioalba*       | 6–8-spored  | 50–66 × 20–25          | -          | Barbatic acid, Confluentic acid |
| *C. fuscopunctata*    | (2)4–8-spored | (23)26–36 × 11–14     | 8–10 × 3–5 | Atranorin, Barbatic acid, Barbatic acid, Unknown lichen substances |
| *C. lunulata*         | 8-spored    | 33–56 × 13–17          | -          | Barbatic acid, unknown lichen substances |
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Although Cryptothecia and Arthonia have different spore types, C. austrocoreana is included in arthonioid clade in the phylogenetic trees based on mtSSU and RPB2.

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