First Records of Rare Ascomycete Fungi, *Acrostalagmus luteoalbus, Bartalinia robillardoides*, and *Collariella carteri* from Freshwater Samples in Korea

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**ABSTRACT**

The distribution and occurrence of rare ascomycete fungi within freshwater samples in Korea was investigated. Three rare fungal strains, CNUFC-YR537-1, CNUFC-CNUP1-1, and CNUFC-NDR3-1, were isolated using serial dilution method. On the basis of their morphological characteristics and phylogenetic analysis of their internal transcribed spacer regions and 28S rDNA sequences, the three isolates were identified as *Acrostalagmus luteoalbus, Bartalinia robillardoides, and Collariella carteri*, respectively. To our knowledge, these are the first reports of rare genera *Acrostalagmus, Bartalinia, and Collariella* from Korea, and the first reports of *A. luteoalbus, B. robillardoides, and C. carteri* from freshwater samples.

**1. Introduction**

Freshwater fungi have an important role in the decomposition of organic matter in ecological systems, by virtue of producing enzymes that break down wood and producing bioactive compounds against pathogenic bacteria, fungi, and nematodes [1–4]. There are more than 622 species (170 genera) of Ascomycetes; more than 226 species of Hyphomycetes (55 genera); and 183 species of Trichomycetes (3 orders), while little is known about the Basidiomycetes and Zygomycetes [3].

Sordariomycetes is one of the largest classes of Ascomycetes, with six subclasses, 32 orders, 105 families, and 1,331 genera [5]. Its species are characterized by nonlichenized, flask-shaped fruiting bodies (perithecia) and unitunicate asci [6]. They can be found in soil, dung, leaf litter, fresh water, plants, arthropods, and mammals [3,5,7,8].

The genus *Acrostalagmus*, which belongs to the class Sordariomycetes, order Hypocreales, and family Hypocreaceae, was established by Corda (1838) [9], with the type species *A. cinnabarinus* Corda. The type belonging to this genus are characterized as verticillate conidiohapes, with hyaline, egg-shaped conidia formed singly [10]. Members of *Acrostalagmus* are found in saffron soil, needle mushroom, vermicompost, and branches of cacao [11–14]. They are also known for their ability to produce a variety of secondary metabolites. To date, 27 species belonging to this genus are known, according to the Index Fungorum (www.indexfungorum.org).

The genus *Bartalinia*, which belongs to the class Sordariomycetes, order Anphiphaeriales, and family Bartaliniaceae, was established by Tassi (1900) [15], with the type species *Bartalinia robillardoides*. It is characterized by the production of fusiform conidia with an acute or blunt apex and having three to four septate. Members of the genus have frequently been isolated from the leaves, stems of medicinal plants, or dead aerial spines of *Rosa canina* L. [16–19]. *B. robillardoides* has been reported to produce taxol, an anticancer drug [20]. Several types of compounds have been investigated from *B. robillardoides* strain LF550, such as three new chloroazaphilones named helicusin E, isochromophilone X, and isochromophilone XI, which expressed antimicrobial activity towards *Bacillus subtilis, Staphylococcus lentus, Candida albicans, Trichophyton rubrum*, and *Septoria tritici*, and also inhibited PDE4 and AChE [21]. Currently, there are 17 accepted species in this genus [22].

The genus *Collariella*, which belongs to the class Sordariomycetes, order Sordariales, and family Chaetomiaceae, was introduced by Wang et al. [23]. Based on phylogenies derived from ITS, LSU rDNA, rpb2, and tub2 sequence data combined with morphological observations, seven species have been recognized [23]. Currently, nine species have been...
registered in Index Fungorum. The species belonging to this genus are characterized by the production of a dark collar-like apex around the ostiolar pore of the ascomata [23]. They are found in soil, dust, and air [23–25]. Some of them have been reported to produce a variety of metabolites, including chaetoquadrin E, cochlidiolin B, prenisatin, and SB236049/SB236050/SB238569 [23].

In Korea, many studies on the fungal diversity of various habitats have been conducted, although the occurrence of freshwater fungi remains poorly understood. Until now, only five new species and eight new records from freshwater habitat have been reported in Korea [19,26–30].

During our collection from freshwater samples, three rare species were found in Korea: *A. luteoalbus*, *B. robillardoides*, and *C. carteri*.

2. Materials and methods

2.1. Isolation of fungal strains from freshwater samples

Freshwater samples were collected from a branch stream of the Nakdong river located in Gyeongsangbuk-do, the Yeongsan river located in Gwangju, and a pond located in the Chonnam National University Arboretum, Gwangju, Korea. The samples were transported in sterile 50-mL Falcon tubes and stored at 4°C until examination. Serial dilution methods were employed using potato dextrose agar (PDA), malt extract agar (MEA), and corn meal agar (CMA). The media and method used were based on the protocol described by Wanasinghe et al. [19] and Nguyen et al. [31]. The plates were incubated at 25°C for 3–7 days. To isolate pure cultures, individual colonies with various morphologies were picked up, transferred to PDA, and subcultured until pure mycelia were obtained. All pure isolates, including those of *A. luteoalbus*, *B. robillardoides*, and *C. carteri*, were stored in 20% glycerol at −80°C at the Chonnam National University Fungal Collection (CNUFC), Gwangju, Korea. *A. luteoalbus*, *B. robillardoides*, and *C. carteri* strains isolated in our study were designated CNUFC-YR537-1 and CNUFC-YR537-2, CNUFC-CNUP1-1 and CNUFC-CNUP1-2, CNUFC-NDR3-1 and CNUFC-NDR3-2, respectively. Strains CNUFC-YR537-1, CNUFC-CNUP1-1, and CNUFC-NDR3-1 were also deposited at the Culture Collection of the Nakdonggang National Institute of Biological Resources (NNIBR, Sangju, Korea).

2.2. Morphological studies

For detailed morphological studies, CNUFC-YR537-1, CNUFC-CNUP1-1, and CNUFC-NDR3-1 strains were cultured on PDA, MEA, and CMA. The plates were incubated at 25°C in the dark for 7 d. Samples were mounted in lactophenol solution (Junsei Chemical Co. Ltd., Tokyo, Japan) and observed under an Olympus BX51 microscope with DIC optics (Olympus, Tokyo, Japan). For scanning electron microscopy (SEM), samples were prepared as described previously [32].

2.3. DNA extraction, polymerase chain reaction (PCR), and sequencing

Strains CNUFC-YR537-1, CNUFC-CNUP1-1, and CNUFC-NDR3-1 were grown on PDA, covered with cellophane at 25°C for 4–5 days. Genomic DNA was extracted using the Solg TM Genomic DNA prep. Kit (Solgent Co. Ltd., Daejeon, South Korea). The internal transcribed spacers (ITS1, and ITS2) and 5.8S region of the ribosomal DNA were amplified with the primer pair ITS1 and ITS4 [33]. The large subunit of 28S rDNA was amplified with the primer pair LROR and LR5F [34]. The PCR amplification mixture (total volume, 20 μL) was comprised of 2 μl of the fungal DNA template (10 ng), 1.5 μl of each primer (5 pM/μl), 1 μl Accupower® PCR premix (Bioneer Corp., Daejeon, South Korea) containing Taq DNA polymerase, dNTPs, buffer, and tracking dye, and 14 μl sterile water. The amplification parameters were as follows: an initial denaturation step at 95°C for 5 min, followed by 35 thermal cycles with denaturation at 94°C for 30 s, annealing at 55°C (ITS) or 52°C (28S) for 30 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The PCR products were purified using the Accuprep® PCR Purification Kit (Bioneer Corp.). DNA sequencing was performed in an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA).

2.4. Phylogenetic analysis

The fungal sequences obtained from the GenBank database (Table 1) were aligned using Clustal_X v.1.83 [35] and edited with Bioedit v.5.0.9.1 [36]. Phylogenetic trees based on the ITS rDNA and 28S sequences were constructed using the neighbor-joining method in MEGA 6 [37]. *Colletotrichum boninense*, *Glomerella cingulate*, *Microascus trigonosporus*, and *Pestalotiopsis malayana* were used as outgroups. The reliability of internal branches was assessed using the p-distance substitution model with 1000 bootstrap replications. Sequence data were compared with similar sequences available in the GenBank databases using BLASTn.
### 3. Results

#### 3.1. Phylogenetic analysis

A BLAST search of ITS sequences via the NCBI database indicated that the isolates CNUFC-YR537-1, CNUFC-CNUP1-1, and CNUFC-NDR3-1 most closely resembled *A. luteoalbus* (GenBank accession no. KP050692), *B. robillardoides* (GenBank accession no. KJ710460), and *C. carteri* (GenBank accession no. KX976747), with 99.6% (542/544 bp), 99.6% (539/541 bp), and 99.8% (499/500 bp) homology, respectively.

### Table 1. Sequences used in this study and GenBank accession numbers.

| Taxon name                      | Collection no. (Isolate no.) | GenBank accession no. |
|---------------------------------|-----------------------------|-----------------------|
| **ITS**                         |                             |                       |
| Acremonium alcalophilum         | CBS 114.92                  | JX158421              |
| Acrostalagmus annulatus         | DAOM212126                  | GU180632              |
| Acrostalagmus luteoalbus        | V209                        | KJ443275              |
| Acrostalagmus luteoalbus        | V208                        | KJ443274              |
| Acrostalagmus luteoalbus        | V207                        | KJ443273              |
| Acrostalagmus luteoalbus        | DO137                       | KP050692              |
| Acrostalagmus luteoalbus        | CBS 194.87                  |                       |
| Acrostalagmus luteoalbus        | CNUFC-YR537-1               | MH482849              |
| Acrostalagmus luteoalbus        | CNUFC-CNUP1-1               | MH482850              |
| Bartalina robillardoides        | CBS 122705 (T)              | KJ710460              |
| Bartalina robillardoides        | CNUFC-CNUP1-2               | MH482847              |
| Bartalina robillardoides        | CNUFC-CNUP1-1               | MH482848              |
| Bartalina robillardoides        | CNUFC-CNUP1-2               | MH482854              |
| Bartalina laurina               | HKUCC 6537                  | AF405302              |
| Bartalina pondoensis            | A1S1-D17                    | KJ767127              |
| Bartalina pondoensis            | WSD118                     | KU536132              |
| Bartalina pondoensis            | CLBSS                       | KU645988              |
| Bartalina pondoensis            | SOU-QU20                    | KU945962              |
| Broomella rosae                 | MFLU 16-0244                | MG828990              |
| Broomella vitalbae              | MFLUCC 14-1000              | KP757754              |
| Collariella bostrychodes        | CBS 596.83                  | KJ976642              |
| Collariella bostrychodes        | DTO 324-H3                  | KJ976644              |
| Collariella bostrychodes        | CBS 163.73                  | KJ976641              |
| Collariella gracilis            | CBS 249.75                  | KJ976649              |
| Collariella gracilis            | CBS 146.60 (T)              | KJ976648              |
| Collariella carteri             | CBS 128.85 (T)              | KJ976647              |
| Collariella carteri             | CNUFC-NDR3-1                | MH482845              |
| Collariella carteri             | CNUFC-NDR3-2                | MH482852              |
| Collariella causiformis         | CBS 792.83 (T)              | KJ976646              |
| Collariella quadrangulata       | CBS 152.59                  | KJ976651              |
| Collariella quadrangulata       | CBS 142.58                  | KJ976650              |
| Collariella robusta             | CBS 551.83                  | KJ976652              |
| Collariella robusta             | CBS 508.84                  | KJ976653              |
| Collariella virescens           | CBS 148.68 (T)              | KJ976654              |
| Collariella virescens           | CBS 547.75                  | KJ976655              |
| Colletotrichum boninense        | 270                         | FJ224116              |
| Dysthiopsis lakefasiensis       | HKUCC 7303                  | AF452047              |
| Glomerella cingulata            | FAU 533                     | AF543786              |
| Microascus trigonosporus        | CBS 218.31 (T)              | LM652443              |
| Neotragacteles endophylica      | EML-ASS-1                   | KJ443277              |
| Neotragacteles endophylica      | EML-ASS-2                   | KJ443265              |
| Pestalotiopsis malayana         | CBS 102200                  | KM199306              |
| Phlegmocinulum uniforme         | CBS 131312                  | JQ044445              |
| Sordariomyces trionii           | MAG1                        | KJ443277              |
| Sordariomyces trionii           | MAG3                        | KJ443279              |
| Sordariomyces magadi            | MAG2                        | KJ443278              |
| Truncatella angustata           | UCD2157OR                   | FJ794472              |
| Truncatella angustata           | ICMP 7062                   | AF382383              |
| Truncatella hartigi             | CBS 118145                  | DQ278912              |
| Truncatella helichrysi          | CBS 123029                  | DQ278912              |
| Hyalotiella spartii             | MFLUCC 15-0002              | KP757753              |
| Truncatella restionacearum      | CBS 118150                  | DQ278914              |
| Truncatella resinosinae         | CMW 18755                   | DQ278929              |
| Verticillium albo-atum           | CBS 130.51                  | DQ825977              |
| Verticillium dahilae            | 76 Greece                   | AF104926              |
| Verticillium dahilae            | ATCC 16535                  |                       |
| Verticillium zaregansianum       | V204                        | KJ443270              |
| Verticillium zaregansianum       | V203                        | KJ443269              |
| Verticillium zaregansianum       | V202                        | KJ443268              |
| Zetiasplozna acaciae            | CPC 23421                   | KJ869206              |

Bold letters indicate isolates and accession numbers determined in our study.

ATCC: American Type Culture Collection, Manassas, VA, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CNUFC: Chonnam National University Fungal Collection, Gwangju, South Korea; CPC: Culture collection of Pedro Crous, housed at CBS; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; EML: Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, South Korea; ITS: internal transcribed spacer; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; T: ex-type strain.
no. KJ443145), B. robillardoides (GenBank accession no. KJ710438), and C. carteri (GenBank accession no. KX976742) showed 100% (850/850 bp), 99.8% (871/873 bp), and 100% (487/487 bp) homology with the 28S sequence of the isolates CNUFC-YR537-1, CNUFC-CNUP1-1, and CNUFC-NDR3-1, respectively.

On the basis of the ITS and 28S sequence analysis, the isolates CNUFC-YR537-1, CNUFC-CNUP1-1, and CNUFC-NDR3-1 were identical to A. luteoalbus, B. robillardoides, and C. carteri, respectively (Figures 1–3).

3.2. Taxonomy

3.2.1. Taxonomy of CNUFC-YR537-1

A. luteoalbus (Link) Zare, W. Gams & Schroers, Mycological Research 108 (5): 581 (2004) (Table 2, Figure 4).
**Sporotrichum vile** P. Karst., Hedwigia 30: 303 (1891).

**Description:** Colonies of the strain grew slowly on PDA, appearing dull orange to orange-brown, and reaching 24–26 mm in diameter after 7 days of incubation at 25°C. The reverse of the colonies were orange, appearing a lighter orange in the centers. Conidiophores were erect, pale orange-brown, measured 33–121 × 2.0–4.0 μm, and repeatedly verticillately branched in one to several orders. Phialides were flask-shaped, hyaline, and measured 10–29 × 2.0–3.5 μm. Conidia were oval, pale reddish, and measured 3.5–6.2 × 2.2–2.9 μm.

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### 3.2.2. Taxonomy of CNUFC-CNUP1-1

*B. robillardoides* Tassi, Bollettino del Laboratorio de Orto Botanico Reale Universita Siena 3: 5 (1900) (Table 3, Figure 5).

≡Seimatosporium robillardoides (Tassi) Arx, The genera of fungi sporulating in pure culture: 224 (1981).

**Description:** Colonies of the strain grew rapidly on PDA, were greyish, and reached 80–83 mm in diameter after 14 d culture at 25°C. Conidiomata were globose or subglobose, appearing black to brownish black, and measured 276.7–482.2 × 228.1–364.8 μm. Conidiophores were reduced to conidiogenous cells,
which were hyaline, cylindrical to subcylindrical, ampuliform, measured 8.4–11.3 × 2.5–3.8 μm, and were formed from the inner cells of the peridial wall. Conidia were subcylindrical to slightly curved fusoid, basal cell obconic with a truncate base, 4-septate, and measured 18.3–24.2 × 3.0–3.8 μm. The apical appendage was 12.4–18.2 μm long and three-branched. The basal appendage was 3.8–7.3 μm long, single, unbranched, filiform, and excentric.

3.2.3. Taxonomy of CNUFC-NDR3-1

C. carteri X. Wei Wang, Houbraken & Samson, Studies in Mycology 84: 179 (2016) (Table 4, Figure 6).
Colonies of this strain grew moderately slowly on PDA, reaching 35–37 mm in diameter after 7 d incubation at 25°C. The colony color was pale yellowish and then became greenish/olivaceous with age. Ascomata were globose to subglobose, and measured 81–364.5 × 278–247.9 μm. Ascomatal hairs were 3–5 μm diameter at the base, tapering and fading towards the tip. Asci were fasciculate, clavate, or slightly fusiform, contained seven to eight ascospores, and measured 16.5–31.5 × 7.8–10.8 μm. Ascospores were brown, limoniform, usually biapiculate at both ends, and measured 5.0–6.7 × 4.5–5.5 μm.

**4. Discussion**

The use of DNA sequences has dramatically increased the number of fungal species being recognized and identified [38,39]. Especially the ITS-5.8S rDNA sequences have become important features for the rapid identification of fungi [40,41]. The LSU region on its own or in combination with the ITS region is also valuable in identifying fungi at the intermediate taxonomic level [41,42].

Phylogenetic analysis based on ITS and 28S sequences showed that CNUFC-YR357-1 and CNUFC-YR357-2 strains were clustered within the same clade as *A. luteoalbus* from NCBI (Figure 1). The results of our molecular data analysis were consistent with the phylogeny presented by Grum-Grzhimaylo et al. [43]. The morphological characteristics of the *A. luteoalbus* isolate studied were generally similar to those previously described by Zare et al. [10], who transferred this species to genus *Acrostalagmus* from genus *Verticillium*, based on their molecular studies. Species of *A. luteoalbus* have been reported to produce two new indole diketopiperazines, namely luteoolbusins 1 and 2, along with eight previously known ones (3–10). These compounds

**Figure 4.** Morphology of *A. luteoalbus* CNUFC-YR357-1. (A), Colonies in corn meal agar (CMA). (B), Colonies in potato dextrose agar (PDA). (C–E), Conidiophores bearing conidia. (F,G), Conidiophores and phialides. (H), Conidia (scale bars D–H = 20 μm).

**Table 3.** Morphological characteristics of CNUFC-CNUP1-1 compared to *B. robillardoides* reference strain.

| Characteristics     | Present isolate                                      | *B. robillardoides* |
|---------------------|-----------------------------------------------------|---------------------|
| Conidiogenous cell  | Cylindrical to subcylindrical, ampulliform, measured 8.4–11.3 × 2.5–3.8 μm | Ampulliform, hyaline, thin-walled, smooth, measured 4–8 × 3–4.5 μm |
| Conidia             | Subcylindrical to slightly curved fusoid, 4-septate, measured 18.3–24.2 × 3.0–3.8 μm | Subcylindrical, 4-septate, smooth, slightly constricted at the septa, measured (19–) 21–24(–27) × 3–4 μm |
| Apical appendage    | Three-branched, 12.4–18.2 μm long                    | Three-branched, measured (15–) 16–20(–22) μm long |
| Basal appendage     | Single, unbranched, filiform, excentric, 3.8–7.3 μm long | Single, unbranched, filiform, flexuous, excentric, 4–7 μm long |

*From the description by Crous et al. [16].
were evaluated for their cytotoxic activities against SF-268, MCF-7, NCI-H460, and HepG-2 cell lines, and compounds 1–5 showed significant cytotoxicity against all four cancer cell lines [44]. Two new epipo-lythiodioxopiperazines, named chetracins E and F (1 and 2), along with the previously known chetracin C, exhibited cytotoxicity against the five tested cancer cell lines at the low-micromolar or nanomolar IC50 values isolated from \textit{A. luteoalbus} [45]. A novel alkali-tolerant rhamnosidase was isolated from the soil fungus \textit{A. luteoalbus} from Argentina [46].

Our analyses of ITS and 28S sequences showed that the strains CNUFC-CNUP1-1 and CNUFC-CNUP1-2 clustered with \textit{B. robillardoides} CBS 122705 (type species) (Table 4). The CNUFC-CNUP1-1 isolate was morphologically most similar to \textit{B. robillardoides}, as described by Crous et al. [16]. \textit{B. robillardoides}, \textit{B. laurina}, and \textit{B. rosicola} have 4-septate conidia. However, there is significant morphological variation in the conidial measurement; \textit{B. robillardoides} has a conidia length/width ratio of 6.4 [16]; \textit{B. laurina} has a conidia length/width ratio of 7.4 [47]; \textit{B. rosicola} has a conidia length/width ratio of 3.8 [19]. Some \textit{Bartalinia} species have been recorded as plant pathogens that cause diseases on economically important crops [22]; while some species, such as \textit{B. robillardoides}, are well known for their secondary metabolites used in pharmaceutical industry. Hence, it is necessary to explore the biodiversity of \textit{Bartalinia} species in Korea.

Phylogenetic analysis based on ITS and 28S sequences showed that CNUFC-NDR3-1 clustered within the same clade as \textit{C. carteri} CBS 128.85 (type species) (Figure 3). The morphological features of our isolates corresponded well with the description of \textit{C. carteri} by Wang et al. [23]. However, our observations

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Table 4. Morphological characteristics of CNUFC-NDR3-1 compared to \textit{C. carteri} reference strain.

| Characteristics       | Present isolate                  | \textit{C. carteri}\textsuperscript{a}              |
|-----------------------|-----------------------------------|----------------------------------------------------|
| Ascomata              | Globose to subglobose, 81–364.5 × 78–247.9 μm | Globose to subglobose, 175–320 × 110–220 μm       |
| Ascomatal hair        | 3–5 μm diameter at the base, tapering and fading towards the tips | 4–8.5 μm near the base, tapering and fading towards the nearly hyaline tips |
| Asci                  | Fasciculate, clavate or slightly fusiform, seven to eight ascospores, measured 16.5–31.5 × 7.8–10.8 μm | Fasciculate, clavate or slightly fusiform, eight ascospores, measured 17–26 × 8.5–11.5 μm |
| Ascospores            | Brown, limoniform, usually biapiculate at both ends, measured 5.0–6.7 × 4.5–5.5 μm | Olivaceous when mature, limoniform, usually biapiculate at both ends, bilaterally flattened, measured (4.5–5–6(–7.5)) × 4.4–5 μm |

\textsuperscript{a}From the description by Wang et al. [23].
showed that the CNUFC-NDR3-1 isolate produces seven to eight ascospores per ascus, whereas the ascus of *C. carteri* CBS 128.85 produces eight ascospores, as described by Wang et al. [23]. Our results suggest that different isolates of the same species of *C. carteri* have different number of ascospores. Eight ascospores in each ascus are frequently occurring in comparison to seven ascospores (rare). This species is also showed to produce metabolite such as cochlodinin B and prenisatin [23].

This study significantly improved our understanding of the rare ascomycete genera *Acrostalagmus*, *Bartalinia*, and *Collariella* in freshwater from Korea. There are numerous unknown freshwater derived species still awaiting description. In addition, three species obtained from this study may potentially be highly valuable. Thus, the potential biological activities of *A. luteoalbus*, *B. robillardoides*, and *C. carteri* should be further studied.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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