Molecular and Morphological Characterization of Two Novel Species Collected from Soil in Korea

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ABSTRACT

Two fungal species of ascomycetes were discovered during the screening of soil microflora from the Gangwon Province in Korea: Didymella chlamydospora sp. nov. (YW23-14) and Microdochium salmonicolor sp. nov. (NC14-294). Morphologically, YW23-14 produces smaller chlamydospores (8.0–17.0 \times 7.0–15.0 \mu m) than D. glomerata and D. musae. The strain NC14-294 was characterized by smaller conidiogenous cells (4.9–8.8 \times 2.0–3.2 \mu m) compared with the closest strains M. fisheri and M. phragmitis. The detailed descriptions, illustrations, and discussions regarding the morphological and phylogenetical analyses of the closely related species are provided to support the novelty of each species. Thus, YW23-14 and NC14-294 are described here as newly discovered species.

1. Introduction

Didymellaceae, the largest family established in Pleosporales of Ascomycota, with more than 5400 taxon names listed in MycoBank and consisting of three main genera, viz. Ascochyta, Didymella and Phoma, and other allied Phoma-like genera [1,2]. The first generic level of Didymella was used by Saccardo in 1880, with the description of Didymella exigua [3,4]. The limits of Didymellaceae, redefined the genera Epicoccum, Peyronellae and Stagonosporopsis, and established the genus Boeremia and the taxonomic revision of Didymella is necessary, especially because of its phytopathological importance [5]. Recently, a revision has been published under the family of Didymellaceae, encompassing 17 well supported monophyletic clades which were treated as individual genera [6]. The correct species identification in this family has always proven difficult, chiefly relying on morphology and plant host association [5,6]. However, the internal transcribed spacer regions intervening 5.8S nrDNA (ITS), partial 28S large subunit nrDNA (LSU) sequences, and partial regions of RNA polymerase II second largest subunit (RPB2) and \( \beta \)-tubulin (TUB2) genes provide a relatively robust phylogenetic backbone for taxonomic determination [6]. Moreover, the species of Didymellaceae are cosmopolitan and distributed throughout a broad range of environments and most of the members in this family are plant pathogens of a wide range of hosts, mainly causing leaf and stem lesions; some are of quarantine significance [5–8].

The genus Microdochium was introduced with the isolation of species of M. phragmitis from living leaves of Phragmites australis in Germany [9]. Microdochium species are recognized as Fusarium-like fungi, nevertheless, the conidiogenous cells are not phialidic as in true Fusarium species and the conidia have a truncate base rather than ‘foot-cells’. The sexual morphs of Microdochium species are known to reside in Monographella (Amphisphaeriaceae, Xylariales) [10–13]. However, the close affinity of Microdochium to Idriella has been discussed and explored that the genus Microdochium and Idriella are very similar genera which have polyblastic conidiogenous cells and hyaline falcate conidia, with the presence of chlamydospores in culture [14–16]. Nevertheless, morphological and ecological delimitation of Microdochium and Idriella is problematic as well as remains obscure, and taxonomic affinities inferred from molecular data have not yet been established. Furthermore, to accommodate genera like Microdochium, Idriella, and Selenodriella, the taxonomic relationships of Microdochium Syd., Monographella Petr., and Idriella Nelson & Wilh were recently defined based on morphology and DNA sequence data, and introduced a new family Microdochiaeae Hern.-Restr., Crous & Groenew (Sordariomycetes, Xylariales) [17].

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During the recent surveying, two novel fungal species of Ascomycota were collected from soil in Korea. The purpose of this study was to identify the newly discovered fungal species based on morphological and molecular characteristics.

2. Material and methods

2.1. Sample collection and fungal isolation

In 2017, samples were collected in Korea from the riverside and forest soils of Yeongwol (37\(^\circ\)16'25.7"N, 128\(^\circ\)31'37.3"E) and Pyeongchang (37\(^\circ\)37'06.4"N, 128\(^\circ\)33'03.4"E), respectively. The soils were taken randomly from a depth of 10–15 cm using pre-autoclaved sterile spatulas, immediately transferred into sterile plastic bags, and then stored at 4 \(^\circ\)C and then collected soils (1 g) were added to 10 mL of sterile double-distilled water and vortexed gently until dissolved [18]. The solution was serially diluted and then plated on potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates. After incubating the PDA plates at 25 \(^\circ\)C for 3–4 days, single colonies were transferred onto new PDA plates to obtain a pure culture, followed by incubation at 25 \(^\circ\)C. Two strains with different morphology were selected for further molecular analyses. Metabolically inactive and living culture of two strains (Didymella chlamydospora: ZEVCFG0000000092 = KCTC 56426; Microdochium salmonicolor: NIBRFK0000501933 = KCTC 56427) were deposited to the National Institute of Biological Resources (NIBR) and Korean Collection for Type Cultures (KCTC). The fungal strains were maintained in 20% glycerol at −80 \(^\circ\)C for further study.

2.2. Cultural and morphological observations

The cultural and morphological characteristics of YW23-14 and NC14-294 were studied by growing the strains on different media. The strain YW23-14 was transferred onto PDA, oatmeal agar (OA), or malt extract agar (MEA), incubated at 25 \(^\circ\)C for 7–21 days, and then treated with near-ultraviolet (UV) light (12 h light/12 h dark) [19,20]. The strain NC14-294 was transferred onto PDA or OA and incubated at 25 \(^\circ\)C for 7–21 days [17,21]. The fungal growth of each strain was measured, and the colony characters were recorded. The mycological characteristics were observed using a light microscope (BX-50; Olympus, Tokyo, Japan).

2.3. Genomic DNA extraction, PCR amplification, and sequencing

For molecular analyses, the fungal mycelia were grown on PDA plates for 1 week at 25 \(^\circ\)C. Next, total genomic DNA was extracted using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) according to the manufacturer’s instructions and then stored at −20 \(^\circ\)C. The following target genes were amplified for strain YW23-14 according to previous studies: the internal transcribed spacer (ITS) regions, β-tubulin (TUB2), 28S rDNA large subunit (LSU), and the second largest subunit of RNA polymerase II (RPB2) genes [19]. ITS and LSU were used to amplify strain NC14-294 [21]. Next, the amplified PCR products were purified with ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced using Solgent (Daejeon, Korea). The sequence data obtained in this study were adjusted using the SeqMan software (Lasergene, DNASTar Inc., Madison, Wisconsin, USA).

2.4. Molecular phylogenetic analysis

The phylogenetic analyses were conducted using the sequences retrieved from the National Center for Biotechnology Information (NCBI) (Table 1). The ambiguous regions were excluded from the alignments, and the evolutionary relationships with the neighbor-joining algorithm were calculated using Kimura’s two-parameter model [22]. The alignments were manually performed for each gene, and then the sequences were merged by using MEGA7.0 software program. The phylogenetic trees were also inferred by following the maximum likelihood and maximum parsimony algorithms using the software program MEGA7.0 with the bootstrap values based on 1,000 replications [23]. The bootstrap values were considered as significant once equal to or more than 70% [24].

3. Results

3.1. Taxonomical analysis of Didymella chlamydospora YW23-14

3.1.1. Taxonomy

The strain YW23-14 showed distinct morphological characteristics compared with other Didymella species. Therefore, it is proposed as a new species.

**Didymella chlamydospora** K. Das, S.Y. Lee and H.Y. Jung, sp. nov. (Figure 1)

Mycobank: MB 830919

**Etymology:** From Greek chlamydo-, cloak, and spora, spore.

**Typus:** Yeongwol, Korea (37°16'25.7"N, 128°31'37.3"E), isolated from riverside soil. The stock culture (ZEVCFG0000000092 = KCTC 56426) was deposited in the National Institute of Biological...
Cultures (KCTC), metabolically inactive culture. Resources (NIBR) and Korean Collection for Type Cultures (KCTC), ex-holotype living culture KCTC 56426). (holotype ZEVCFG0000000092, dried and living culture, deposited in NIBR Oct. 2017, H.Y. Jung, Hanyang University). The isolated strains are indicated in bold of this study.

The isolated strains are indicated in bold of this study.
black in color with the immersed texture on the surface of medium. The numerous pycnidia were also produced on the surface of agar and the pycnidia were prolific on PDA after 5–6 weeks with the diameter \( (n = 30) \) of 93–273 × 68–222 µm, and with the average diameter 169 × 124 µm (Figure 1(E–G)). The pycnidial wall pseudoparenchymatous, oblong to isodiametric cells, thick-wall, outer wall 2–3-layered, pigmented (Figure 1(H)). The micromorphological structures were analyzed using the culture on OA media. The hyphae were septate, bent, smooth with thin walls, hyaline to pale yellow, subglobose, branched, and width of 2.40–3.60 µm. The chlamydospores were abundant; solitary or in chains; mostly multicellular, but sometimes unicellular; partly short-branched, although sometimes having unbranched chains; usually guttulate; thick-walled; pale brown to brown; globose to subglobose; and with diameters \( (n = 50) \) of 8.0–17.0 × 7.0–15.0 µm and an average size of 12.5 × 11.80 µm (Figure 1(I–K)); Table 2). Conidiogenous cells were phialidic, hyaline, smooth, ampulliform to doliiform, 6.14–11.0 × 3.43–7.4 µm (Figure 1(L–N)). The conidia were unicellular, aseptate, hyaline, and globose to ellipsoidal, with diameters of 4.70–7.40 × 2.2–4.0 µm \( (n = 50) \) and an average size of 6.1 × 2.9 µm (Figure 1(O)).

Note: The shape and size of chlamydospores differed extensively. The YW23-14 strain’s chlamydospores were multicellular or unicellular, with abundant guttules within cells, thick-walled, subglobose globose, and color pale brown to brown. In contrast, the chlamydospores of *D. glomerata* were usually multicellular–dictyosporous, sometimes solitary, smooth then roughened, and dark brown to black, compared with the chlamydospores of the
most closest species of *D. anserina* were absent; but some strains had swollen elements [25]. On the other hand, the chlamydospores of *D. musae* were generally solitary, smooth or irregularly roughened, tanned to dark brown, multicellular-dictyo/phragmosporous, and often discovered to be terminal elements of short lateral branches, sometimes growing as constituent cells [26]. The strain YW23-14 generated numerous smaller-diameter chlamydospores (8.0–17.0 × 7.0–15.0 μm) than the closest known species *D. glomera*ta ((18.0–)30.0–65.0(–80.0) × (12.0–)15.0–25.0(–35.0) μm) and *D. musae* (13.0–45.0(–50.0) × 7.0–20.0(–25.0) μm) (Table 2) [25,26,28]. The conidial size of the strain YW23-14 varied (4.70–7.40 μm) but were almost similar to that of *D. glomera*ta ((3.5–)4–8.5(–10) × 1.5–3.0(–3.5) μm) and *D. musae* (4.0(3.5–)7.0(–8.5) × 2.0–4.0 μm), although larger than that of *D. anserina* (2.4–3.2(–5.5) × 1.8–2.4(–3.0) μm) (Table 2) [25,26,28].

### 3.1.2. Phylogenetic analysis of YW23-14

Through sequence analysis, 621 bp of the ITS region, 761 bp of LSU, 826 bp of RP2B, and 333 bp of TUB2 were obtained. The BLAST search results of ITS sequences in the NCBI database revealed that the strains *Didymella americana* R63-023, *D. pinnello* CBS 110.32, and *D. glomera*ta CBS 127059 each exhibited 99% similarity with the strain YW23-14. The 28S rDNA large subunit (LSU) exhibited 99% similarity with that of strains *Ascochyta herbi*cola (*Phoma herbicola*) B-2-13, *Phoma odoratissim*i CGMCC 3.17502, and *Microsphaeropsis olivace*a CBS 432.71. The RP2B gene regions revealed similarities with the strains *D. musae* CBS 463.69 (95%), *D. glomera*ta UTHSC DI16-205 (94%), *D. anserina* UTHSC DI16-255 (93%), and *D. americana* P-020 (93%). The partial β-tubulin (TUB2) gene was most similar to the strains *P. australis* ICMP 7037 (96%) and *D. americana* MF-010-003 (96%). The maximum likelihood and maximum parsimony trees were also constructed to determine the exact taxonomic position of the strain and indicated the nodes with the filled circles in neighbor-joining phylogenetic tree, whereas, open circles indicate the corresponding nodes with the maximum likelihood or maximum parsimony algorithm (Figure 2). In the phylogram, the strain YW23-14 is placed closely together with *D. anserina* (CBS 253.80, CBS 360.84, CBS 397.65, and UTHSC:DI16-255), *D. musae* CBS 463.69, and *D. glomera*ta CBS 528.66. The phylogenetic analyses revealed, with strong bootstrap support, that YW23-14 belongs to a distinct cluster than the previously identified *Didymella* species. Thus, the neighbor-joining phylogenetic tree supports that the phylogenetic position of the strain YW23-14 is distinct from the other known species of *Didymella* (Figure 2).

### 3.2. Taxonomical analysis of Microdochium salmonicolor NC14-294

#### 3.2.1. Taxonomy

The strain NC14-294 showed distinct morphological characteristics compared with other allied species of

### Table 2. Morphological characteristics of *Didymella chlamydospora* sp. nov. and comparison with the closest species of *Didymella*.

| Sl. No. | Strains name   | Pycnidia (μm) | Chlamydospores (μm) | Conidia (μm) | References |
|---------|----------------|---------------|---------------------|--------------|------------|
| 1       | *D. chlamydospora* (YW23-14) | 93–273 × 68–222 | 8.0–170 × 7.0–15.0 | 4.7–7.4 × 2.2–4.0 | This study |
| 2       | *D. anserina* (CBS 364.91) | 112–136 × 112–176 | Absent | 2.4–3.2(–5.5) × 1.8–2.4(–3.0) | [25] |
| 3       | *D. musae* (CBS 463.69) | 150–200 | 13.0–45.0(–50.0) × 7.0–20.0(–25.0) | 4.0(3.5–)7.0(–8.5) × 2.0–4.0 | [26] |
| 4       | *D. glomera*ta (CBS 328.66) | 100–200 | (18.0–)30.0–65.0(–80.0) × (12.0–)15.0–25.0(–35.0) | (3.5–)4.0–8.5(–10) × 1.5–3.0(–3.5) | [25] |
| 5       | *D. herbaram* (CBS 615.75) | 130–265 × 120–240 | N/A | 4.5–6.0 × 2.0–3.0 | [6] |
| 6       | *D. subglomera*ta (CBS 110.92) | 125–225 | 30.0–65.0 × 15.0–35.0 | (5.0–)7.0–12.0(–15.0) × 2.0–3.5(–4.0) | [25] |
| 7       | *D. prosopidis* (CBS 136550) | up to 200 | 5.0–9.0 | (5.0–)5.5–6.0(–7.0) × (2.5–)3.0(–3.5) | [27] |
| 8       | *D. americana* (CBS 185.85) | 100–220 | 15.0–25.0 | 5.0–8.0(–8.5) × 2.0–3.0(–3.5) | [25] |
| 9       | *D. pinodella* (CBS 319.90) | 96–320 | 8.0–20.0 × 8.0–15.0 | 4.0–6.8(–7.0) × 2.2–3.4 | [28] |
| 10      | *D. heteroderadae* (CBS 875.97) | 70–250 | 5.0–8.0 × 5.0–8.0 | 3.5–7.5(–12.0) × 2.0–3.5(–4.5) | [29] |
| 11      | *D. herbicola* (CBS 629.97) | 120–340 | Absent | 5.0–7.0(–8.5) × 2.0–3.0 | [29] |
| 12      | *D. tanacetae* (TAS 041-0055) | 85–215 | N/A | 4.0–8.5 × 1.5–3.5 | [30] |
| 13      | *D. chenopodi* (CBS 128.93) | 100–250 | Absent | 4.0–6.8(–9.8) × 1.6–2.6(–4.0) | [31] |
| 14      | *D. aeria* (CGMCC 3.18333) | 155–375(–460) × 130–340(–460) | N/A | 3.0–5.0 × 2.0–3.0 | [19] |
| 15      | *D. negriana* (CBS 358.71) | 70–220 | Absent | 4.5–8.5(–10.5) × 2.0–4.0 | [29] |

N/A: not available in previous references.
Microdochium. Therefore, it is described as a new species.

**Microdochium salmonicolor** K. Das, S.Y. Lee and H.Y. Jung, sp. nov. (Figure 3)

MycoBank: MB 830929

**Etymology:** The specific name “salmonicolor” referring to the light salmon color colonies on media.

**Typus:** Pyeongchang, Korea (37°37′06.4″N, 128°33′03.4″E), isolated from forest soil. The stock culture (NIBRFG000501933 = KCTC 56427) was deposited in the NIBR and KCTC, metabolically inactive culture.

**Specimen examined:** South Korea, Pyeongchang, from soil, deposited in NIBR Oct. 2017, H.Y. Jung, (holotype NIBRFG000501933, dried and living culture, ex-holotype living culture KCTC 56427).

**Ecology and distribution:** The different members of this fungi recorded from grasses, cereals, living leaves, roots, and aquatic (marine) environment. The strain isolated from forest soil in South Korea. The soil content plant debris, yellowish brown, lower moisture capacity.

**Cultural characteristics:** The strain was cultured on PDA and OA media to observe the cultural and morphological characteristics (Figure 3(A–D)). On PDA and OA media, the colonies reached 54.0–58.0 and 51.0–56.0 mm, respectively, with a 7-days incubation at 25 °C. The colonies on PDA were flat, tightly attached to the media, aerial mycelium aggregated into slimy masses, small pellets, light salmon to brown in the center with a regular margin, and the reverse color light salmon to dark brown in the center (Figure 3(A,B)). Colonies on OA were flat,
white cottony, rosy buff, margin entire, slightly raised in the center, and tightly attached to the media with several small pellet-like structures on the colony; the reverse color was light salmon to dark brown at the center (Figure 3(C,D)).

**Morphological characteristics:** The micromorphological structures were studied using the cultures on OA medium. The hyphae were hyaline to pale brown, septate, and smooth, with a width of 2.30–3.10 \( \mu \)m. The conidiophores were hyaline to pale brown, septate, branched, and born from the hyphae (Figure 3(E–F)). The conidiogenous cells were subcylindrical to oval, bent in the center, hyaline, tapering towards the edge, (0–1) septate, and had a diameter \( (n = 10) \) of 4.9–8.8 \( \times \) 2.0–3.2 \( \mu \)m and an average size of 7.7 \( \times \) 2.6 \( \mu \)m, with the mycelium reduced to conidiogenous cells that had grown from the hyphae (Figure 3(G–I)). The conidia were hyaline to brown, blunted in both apices, fusiform, clavate, sometimes bent in the middle, (0–1) septate, with dimensions \( (n = 20) \) of 8.0–11.30 \( \times \) 2.40–3.70 \( \mu \)m, and an average size of 9.4 \( \times \) 2.9 \( \mu \)m; sometimes the conidia were produced directly from hyphae (Figure 3(J–M); Table 3). Chlamydospores were not observed.

**Note:** The colonies on PDA were flat, tightly attached to the media, with small pellet-like structures, light salmon to brown color in the center with regular margin, and the reverse color was light salmon to dark brown at the center (Figure 3(A) and Table 3). The closest species, *Microdochium fisheri*, produced colonies that were flat, margin entire, slightly raised to umbonate in the center, and pinkish white with a reverse grayish orange color [21]. *M. lycopodinum* were white cottony, lanose to floccose, buff to rosy buff, and margin effuse on OA media, whereas *M. phragmitis* displayed as floccose, white in the center, sparse aerial mycelia, buff to the periphery, margin effuse, and reverse buff on OA media [17]. The conidiogenous cells of the strain NC14-294 were subcylindrical to oval, bent in the center, tapering towards the edge, hyaline, and had dimensions of 4.9–8.8 \( \times \) 2.0–3.2 \( \mu \)m, which is smaller than that of the previously identified *M. phragmitis* (6.0–24.0 \( \times \) 1.5–3.0 \( \mu \)m) but close to that of *M. lycopodinum* (4.0–12.0 \( \times \) 2.5–3.5 \( \mu \)m) (Table 3) [17]. The conidiogenous cells of *M. phragmitis* were terminal, sympodial, denticulate, hyaline, smooth, cylindrical to clavate, and sometimes navicular. *M. lycopodinum* produced holoblastic conidiogenous cells with percurrent proliferations that were ampulliform to lageniform, and subcylindrical. Regarding *M. fisheri*, the conidiogenous cells were terminal to intercalary, cylindrical to denticulate, and tapering towards the apex, with great variation in length [21]. The comparison with certain species of the genus showing high sequence similarities did not show colonial similarities such as tightly attached with the cultural media, tiny pallets like structures, light salmon to brown in the center with regular margins; reverse

![Figure 3](image-url)
light salmon to dark brown in the center. That’s why, the cultural and morphological characteristics indicate that the Korean strain NC14-294 is distinct from previously known species of Microdochium.

### 3.2.2. Phylogenetic analysis of NC14-294

After the sequence analysis of NC14-294, 578 bp from the ITS regions and 901 bp from the 28S rDNA gene were obtained. According to the BLAST search results, the analysis of the ITS sequences in the NCBI database indicated similarities of 98%, 97%, and 96% with Microdochium lycopodinum CBS 109398, M. phragmitis CBS 423.78, and M. fisheri CBS 242.91\(^\dagger\), respectively. The 28S rDNA large subunit (LSU) showed similarities with those of the strains M. fisheri CBS 242.90 (99%), Arthrobotrys foliicola CBS 242.90 (99%), M. phragmitis CBS 423.78 (98%), and M. lycopodinum CBS 146.68 (98%). The phylogenetic analysis was conducted based on a combination of ITS regions with the partial sequences of the 28S rDNA. The exact taxonomic position of the strain NC14-294 was indicated by the node in the neighbor-joining

### Table 3. Cultural and Morphological characteristics of Microdochium salmonicolor sp. nov. and comparison with the closest species of Microdochium.

| Sl. No. | Strains name | Cultural characteristics | Conidiogenous cells (µm) | Conidia (µm) | References |
|---------|--------------|--------------------------|--------------------------|--------------|------------|
| 1       | Microdochium salmonicolor (NC14-294) | Colonies on PDA were flat, tightly attached with the media, small pellets, light salmon to brown color in center with regular margin; reverse light salmon to dark brown in center. Colonies on OA were flat, white cottony, rosy buff, margin entire, slightly raised in the center, and tightly attached to the media with several small pellet-like structures on the colony; the reverse color was light salmon to dark brown at the center. | 4.9–8.8 × 2.0–3.2 | 8.0–11.3 × 2.4–3.7 | This study |
| 2       | M. fisheri (NFCCI 4083) | Colonies on PDA were flat, margin entire, slightly raised to umbionate center, pinkish white with reverse grayish orange. | N/A | 4.8–12.0 × 1.6–3.6 | [21] |
| 3       | M. lycopodinum (CBS 109399) | White cottony, lanoso to floccose, buff to rosy buff, margin effuse on OA media. PDA: N/A. | 4.0–12.0 × 2.5–3.5 | 8.0–15.5 × 2.5–4.0 | [17] |
| 4       | M. phragmitis (CBS 285.71) | Floccose, white in the center, sparse aerial mycelium, buff to the periphery, margin effuse; reverse buff on OA media. PDA/N/A. | 6.0–24.0 × 1.5–3.0 | 10.0–14.5 × 2.0–3.0 | [17] |
| 5       | M. rhopalostylidis (CPC 34449\(^\dagger\)) | Colonies flat, spreading, with moderate aerial mycelium and smooth, lobate margin, covering dish after 2 wk at 25 °C. On MEA and PDA surface saffron to luteous, reverse sienna; on OA surface umber to saffron. | 4.0–10.0 × 3.0–3.5 | (13.0–16.0–20.0)×(2.5–13.0–4.0) | [32] |
| 6       | M. trichocladiopsis (CBS 623.77\(^\dagger\)) | Colonies on OA flat, lacking aerial mycelium, rosy buff, black near to the inoculum, margin diffuse, reverse similar. | 4.0–37.5 × 2.0–3.0 | 6.0–18.0 × 2.0–3.5 | [17] |
| 7       | M. citrinidiscum (CBS 109067\(^\dagger\)) | Colonies on OA cottony, white, periphery scarce aerial mycelium, saffron, margin diffuse, reverse saffron, no exudate or soluble pigment produced. | 11.0–29.0 × 1.5–2.0 | 7.0–31.0 × 2.0–3.0 | [17] |
| 8       | M. colombiense (CBS 624.94\(^\dagger\)) | Colonies on OA flat, salmon, no exudate or soluble pigment produced, margin diffuse or entire; reverse saffron. | 5.0–11.5 × 2.5–3.5 | 5.0–8.0 × 1.5–2.5 | [17] |
| 9       | M. chrysanthemoides (CGMCC3.17929\(^\dagger\)) | Colonies on PDA felt, compact, erose or dentate, white initially, then becoming yellowish with age. Exudate occasionally appeared on old sporodochia. Reverse yellowish to orange, due to the soluble pigment secreted. | 5.0–12.0 × 3.0–4.5 | 4.5–7.0 × 2.0–3.0 | [33] |
| 10      | M. neoqueenslandicum (CBS 108926\(^\dagger\)) | Colonies on OA center flat, creamy, with concentric rings, peach to salmon, periphery with cottony aerial mycelium, white, margin diffuse, entire. | 4.5–10.0 × 2.0–3.5 | 4.0–9.0 × 1.5–3.0 | [17] |

N/A: not available in previous references.
phylogenetic tree along with the filled nodes in the maximum likelihood and maximum parsimony trees. The corresponding nodes were also recovered using the maximum likelihood or maximum parsimony algorithms, as indicated by the open circles (Figure 4). The analysis of the combined sequences of the phylogenetic tree revealed that the position of NC14-294 was distinct from the other identified species under the genus *Microdochium* (Figure 4).

4. Discussion

For the present study, the following two morphologically different strains were isolated in 2017 from the soil in Yeongwol and Pyeongchang, South Korea: YW23-14 and NC14-294. The strains exhibited morphological differences from each of the previously identified closely related species, as is supported by descriptions of the latter in previous reports (Tables 2 and 3). The phylogenetic relationships between the Korean strain YW23-14 and previously published authentic strains were inferred by using maximum likelihood (ML), neighbor-joining (NJ) and maximum parsimony (MP) analyses of the ITS regions, LSU, RPB2, and TUB2 genes. The boundaries within the *Didymella*, a combined alignment of ITS regions, LSU, RPB2, and TUB2 sequences was created to clarify the species, containing 30 strains (including the outgroup *Neascochyta paspali*). To determine the exact taxonomic position of the strain, phylogenetic analysis was also conducted based on a combination of the sequences with maximum parsimony (tree length = 985, consistency index = 0.43, retention index = 0.57, and composite index = 0.30) (Figure 2). In case of NC14-294, phylogenetic relationships between the isolated Korean strain and previously identified strains were inferred by using ML, NJ, and MP analyses of the ITS regions and LSU. A combined alignment of ITS regions and LSU sequences was created to clarify the species, containing 21 strains including the outgroup *Humicola olivacea* DTO 319-C7. During the analysis of a sequence combination with the maximum parsimony (tree length = 422, consistency index = 0.60, retention index = 0.78, and composite index = 0.56) was also used to determine the precise taxonomic position of the strain (Figure 4). So, the phylogenetic relationship determined through the sequence analyses and indicated that the strains YW23-14 and NC14-294 were distinct from each of those identified species with the strength of the internal branches of the trees as well as the bootstrap values using 1,000 replications (Figures 2 and 4).

![Figure 4. Neighbor-joining phylogenetic tree of NC14-294 based on the combined sequences (ITS + LSU), showing the relationship between *Microdochium salmonicolor* sp. nov. with the closest *Microdochium* spp.*Humicola olivacea* DTO 319-C7 used as an outgroup. The numbers above the branches represent the bootstrap values obtained for 1,000 replicates (values smaller than 70% were not shown). The isolated strain of this study is indicated in bold. Bar 0.02 substitutions per nucleotide position.](image-url)
Previous studies have reported that the genus Didymella is widely distributed in field and ornamental crops, wild plants and most saprobes species are commonly found in living or dead aerial parts of herbaceous, wooden plants [8], and some act as mutualistic endophytes [34]. Didymella pinodes (formerly known Mycosphaerella pinodes) has been reported as the main causal agent of Ascochyta blight, which is one of the most important fungal diseases of the pea (Pisum sativum) worldwide [35,36]. D. tanaceti and D. rosea have been identified as plant pathogens that cause the tan spot in pyrethrum [30]. In addition, D. americana is the causal agent of the foliar disease observed on baby lima bean (Phaseolus lunatus) in fields across western New York State, USA [21]. The recently established family Didymellaceae [2,37] consist of many taxa previously classified in the genus Phoma and their related taxa, and includes many important plant pathogens, some species of which are of quarantine concern [5,7,8].

The members of the genus Microdochium are important plant pathogens, particularly on grasses and cereals. Some of the species of Microdochium are terrestrial cause economic damage to important plants [38,39], nonpathogenic, and also sometimes endophytes [40]. Many species of Microdochium were also identified in the aquatic (marine) environment after evaluating diseased as well as healthy salmon eggs and have been reported as M. lycopodinum and M. phragmitis [41]. The root necrosis and decay of grasses caused by M. bolleyi [42] and M. paspali is responsible for the seashore paspalum disease of Paspalum vaginatum [39]. The study of Microdochium opens a new opportunity to expand the area of research for bioactive compounds such as cyclosporine A, an active compound from Antarctic mon eggs and have been reported as Microdochium salmonicolor [39]. The study of Microdochium opens a new opportunity to expand the area of research for bioactive compounds such as cyclosporine A, an active compound from Antarctic mon eggs and have been reported as Microdochium salmonicolor [39]. The study of Microdochium opens a new opportunity to expand the area of research for bioactive compounds such as cyclosporine A, an active compound from Antarctic mon eggs and have been reported as Microdochium salmonicolor [39]. The study of Microdochium opens a new opportunity to expand the area of research for bioactive compounds such as cyclosporine A, an active compound from Antarctic mon eggs and have been reported as Microdochium salmonicolor [39].

Considering all the aspects of these two new species, the classification and ecology are mostly important, and the potential activities of each of species should be further investigated. According to morphological and phylogenetic analyses, the strains are especially distinct from previously identified strains of the genera Didymella and Microdochium. Thus, these two species are proposed as Didymella chlamydospora sp. nov. and Microdochium salmonicolor sp. nov.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

The authors are grateful to the Ministry of Environment (MOE) of the Republic of Korea for the research on survey data and discovery of indigenous fungal species supported by a grant from the National Institute of Biological Resources (NIBR).

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