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RESEARCH ARTICLE

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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 × 7.0–15.0 µm) than *D. glomerata* and *D. musae*. The strain NC14-294 was characterized by smaller conidiogenous cells (4.9–8.8 × 2.0–3.2 µm) 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.

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Didymella chlamydospora; *Microdochium salmonicolor*; novel 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*, *Peyronellaea* 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 β-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 Microdochiaceae 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°16'25.7"N, 128°31'37.3"E) and Pyeongchang (37°37'06.4"N, 128°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 °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 °C for 3–4 days, single colonies were transferred onto new PDA plates to obtain a pure culture, followed by incubation at 25 °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*: NIBRFG0000501933 = 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 °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 °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 on PDA or OA and incubated at 25 °C temperature 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 °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 °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 chlamydos-, cloak, and from Latin - 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

Table 1. List of species used in this study and their GenBank accession numbers for phylogenetic analysis.

Speceis	Strain numbers	GenBank accession numbers ITS	LSU	TUB	RPB2
Didymella acetosellae	CBS 179.97	GU237793	GU238034	GU237575	KP330415
D. aeria	LC:8120	KY742052	KY742206	KY742294	KY742138
D. americana	CBS 185.85	FJ426972	GU237990	FJ427088	KT389594
D. anserina	CBS 360.84	GU237839	GU237993	GU237551	KT389596
D. anserina	CBS 397.65	KT389500	KT389717	KT389797	KT389597
D. anserina	CBS 253.80	KT389498	KT389715	KT389795	KT389595
D. anserina	UTHSC:DI16-255	LT592926	LN907398	LT592995	LT593064
D. arachidicola	CBS 333.75^T	GU237833	GU237996	GU237554	KT389598
D. boeremae	CBS 109942^T	FJ426982	GU238048	FJ427097	KT389600
D. coffeae-arabicae	CBS 123380^T	NR135965	MH874817	FJ427104	KT389603
D. curtisii	PD 92/1460	FJ427041	GU238012	FJ427151	KT389604
D. eucalyptica	CBS 377.91	GU237846	GU238007	GU237562	KT389605
D. exigua	CBS 183.55^T	GU237794	EU754155	GU237525	EU874850
D. gardeniae	CBS 626.68^T	NR135966	GQ387595	FJ427114	KT389606
D. glomerata	CBS 528.66^T	NR158225	MH870525	FJ427124	GU371781
D. herbarum	CBS 615.75	NR135967	EU754186	KF252703	KP330420
D. heteroderae	CBS 109.92^T	FJ426983	GU238002	FJ427098	KT389601
D. lethalis	CBS 103.25	GU237729	GU238010	GU237564	KT389607
D. macrostoma	CBS 482.95	GU237869	GU238099	GU237626	KT389609
D. maydis	CBS 588.69^T	FJ427086	EU754192	FJ427190	GU371782
D. microchlamydospora	CBS 105.95^T	FJ427028	GU238104	FJ427138	KP330424
D. musae	CBS 463.69	MH859353	GU238011	FJ427136	LT623248
D. nigricans	LC:8136	KY742077	KY742231	KY742319	KY742160
D. pinodella	CBS 531.66	FJ427052	MH870528	FJ427162	KT389613
D. pinodes	CBS 525.77^T	GU237883	GU238023	GU237572	KT389614
D. pomorum	CBS 285.76	MH860978	MH872748	FJ427163	KT389615
D. protuberans	CBS 377.93	GU237847	GU238014	GU237565	KT389619
D. sancta	CBS 281.83^T	NR135973	GU238030	FJ427170	KT389623
D. subglomerata	CBS 110.92	FJ427080	GU238032	FJ427186	KT389626
Neoascochyta paspali	CBS 560.81^T	NR135970	GU238124	FJ427158	KP330426
D. chlamydospora	**YW23-14^T**	**MK836111**	**MK836109**	**LC482279**	**LC480708**
Microdochium albescens	CBS 290.79	KP859014	KP858950	–	–
M. bolleyi	CBS 540.92	KP859010	KP858946	–	–
M. citrinidiscum	CBS 109067^T	KP859003	KP858939	–	–
M. colombiense	CBS 624.94^T	KP858999	KP858935	–	–
M. chrysanthemoides	LC5363	KU746690	KU746736	–	–
M. fisheri	CBS 242.91^T	KP859015	KP858951	–	–
M. fisheri	NFCCI 4083	KY777595	KY777594	–	–
M. lycopodinum	CBS 125585	KP859016	KP858952	–	–
M. majus	CBS 741.79	KP859001	KP858937	–	–
M. musae	CBS 143500	MH107895	MH107942	–	–
M. neoqueenslandicum	CBS 108926^T	NR145247	KP858938	–	–
M. nivale	CBS 116205	KP859008	KP858944	–	–
M. novae-zelandiae	CPC 29376	LT990655	LT990627	–	–
M. phragmitis	CBS 423.78	KP859012	KP858948	–	–
M. rhopalostylidis	CBS 145125	MK442592	MK442532	–	–
M. seminicola	CBS 122706	KP859007	KP858943	–	–
M. sorghi	CBS 691.96	KP859000	KP858936	–	–
M. tainanense	CBS 269.76^T	KP859009	KP858945	–	–
M. trichocladiopsis	CBS 623.77^T	KP858998	KP858934	–	–
Humicola olivacea	DTO 319-C7	KX976676	KX976770	–	–
M. salmonicolor	**NC14-294^T**	**MK836110**	**MK836108**	–	–

The isolated strains are indicated in bold of this study.

Resources (NIBR) and Korean Collection for Type Cultures (KCTC), metabolically inactive culture.

Specimen examined: South Korea, Yeongwol, from soil, deposited in NIBR Oct. 2017, H.Y. Jung, (holotype ZEVCFG0000000092, dried and living culture, ex-holotype living culture KCTC 56426).

Ecology and distribution: Seed-, air- and soil-borne saprophyte was reported globally by the various members of this fungus. The strain was isolated from riverside soil in South Korea. The soil was sandy to sandy loam, yellowish brown, medium moisture capacity.

Cultural characteristics: The colonies vary in color depending on the fungal growth on different media. The strain cultured on PDA, MEA, and OA media to study the cultural and morphological characteristics (Figure 1(A–C)). The average fungal growth rates on PDA, MEA, and OA were 60.3–70.0, 52.0–59.0, and 58.0–69.0 mm, respectively, at 25 °C for 7 days. The upper surface of the colonies on PDA were white to olivaceous in the center, with wide olivaceous-colored margins; reverse pale brown to olivaceous green. The colonies on MEA were covered by white aerial mycelia, margin regular, fluffy; reverse became yellowish. And the colonies on OA showed white to olivaceous green in center; reverse olivaceous green to black.

Morphological characteristics: The strain produced a large amount of pycnidia on the surface of the OA media after 3 to 4 weeks (Figure 1(D)). The pycnidia were solitary to aggregate, dark brown to

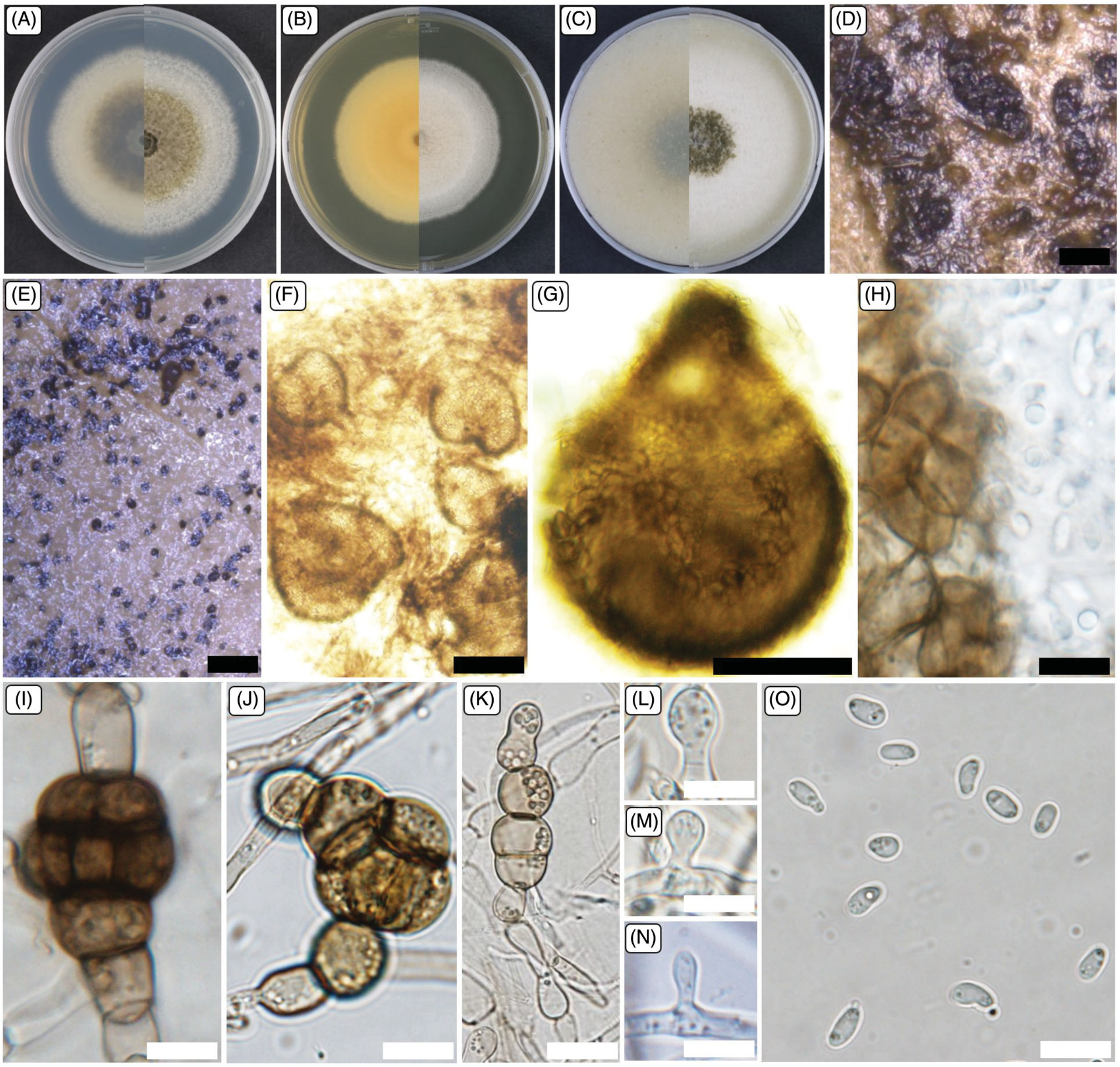

Figure 1. Cultural and morphological characteristics of YW23-14. The colony on potato dextrose agar (PDA) (A); malt extract agar (MEA) (B); oatmeal agar (OA) (C) for 7 days incubation at 25 °C. Pycnidia forming on OA (D); Pycnidia on PDA (E–G); Pycnidial wall (H); Chlamydospores (I–K); Conidiogenous cells (L–N); Conidia (O). Scale bars: D–G = 100 µm; H–O = 10 µm.

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

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. chlamydosporaa* (YW23-14)	93–273 × 68–222	8.0–17.0 × 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. glomerata* (CBS 528.66^{T})	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. herbarum* (CBS 615.75)	130–265 × 120–240	N/A	4.5–6.0 × 2.0–3.0	[6]
6	*D. subglomerata* (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 (−7.0–6.0)×2.2–3.4	[28]
10	*D. heteroderae* (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. tanaceti* (TAS 041-0055)	85–215	N/A	4.0–8.5 × 1.5–3.5	[30]
13	*D. chenopodii* (CBS 128.93)	100–250	Absent	4.0–6.8(−9.8)×1.6–2.6(−4.0)	[31]
14	*D. aeria* (CGMCC 3.18353^{T})	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.

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. glomerata* ((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 × 2.2–4.0 μm) but were almost similar to that of *D. glomerata* ((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 RPB2, 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. pinodella* CBS 110.32, and *D. glomerata* 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 herbicola* (*Phoma herbicola*) B-2-13, *Phoma odoratissimi* CGMCC 3.17502, and *Microsphaeropsis olivacea* CBS 432.71. The RPB2 gene regions revealed similarities with the strains *D. musae* CBS 463.69 (95%), *D. glomerata* 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. glomerata* 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

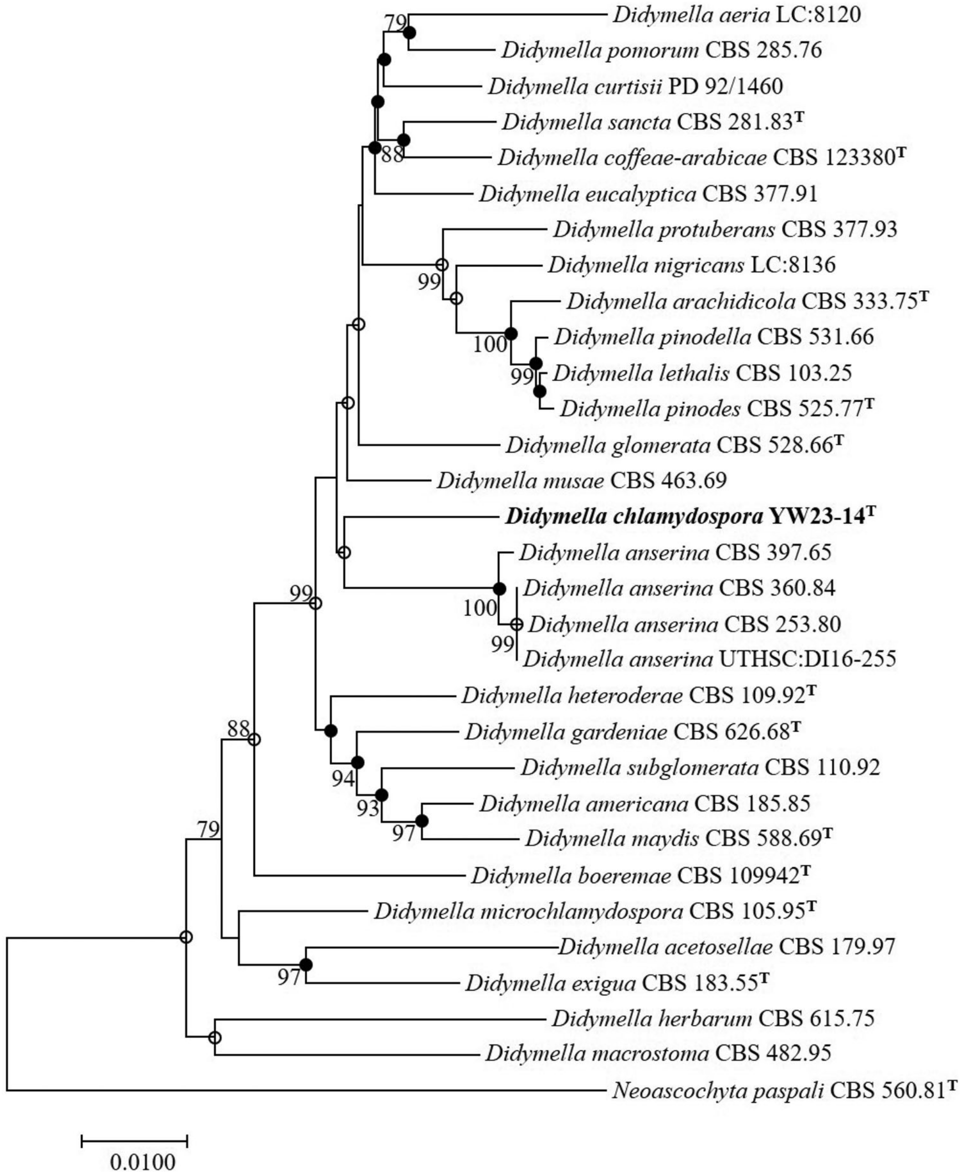

Figure 2. Neighbor-joining phylogenetic tree of YW23-14 based on the combined sequences (ITS + LSU + RPB2 + TUB), showing the relationship between *Didymella chlamydospora* sp. nov. with the closest *Didymella* spp. *Neoascochyta paspali* CBS 560.81T used as an outgroup. The numbers above the branches 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, 0.01 substitutions per nucleotide position.

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 (NIBRFG0000501933 = 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 NIBRFG0000501933, 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,

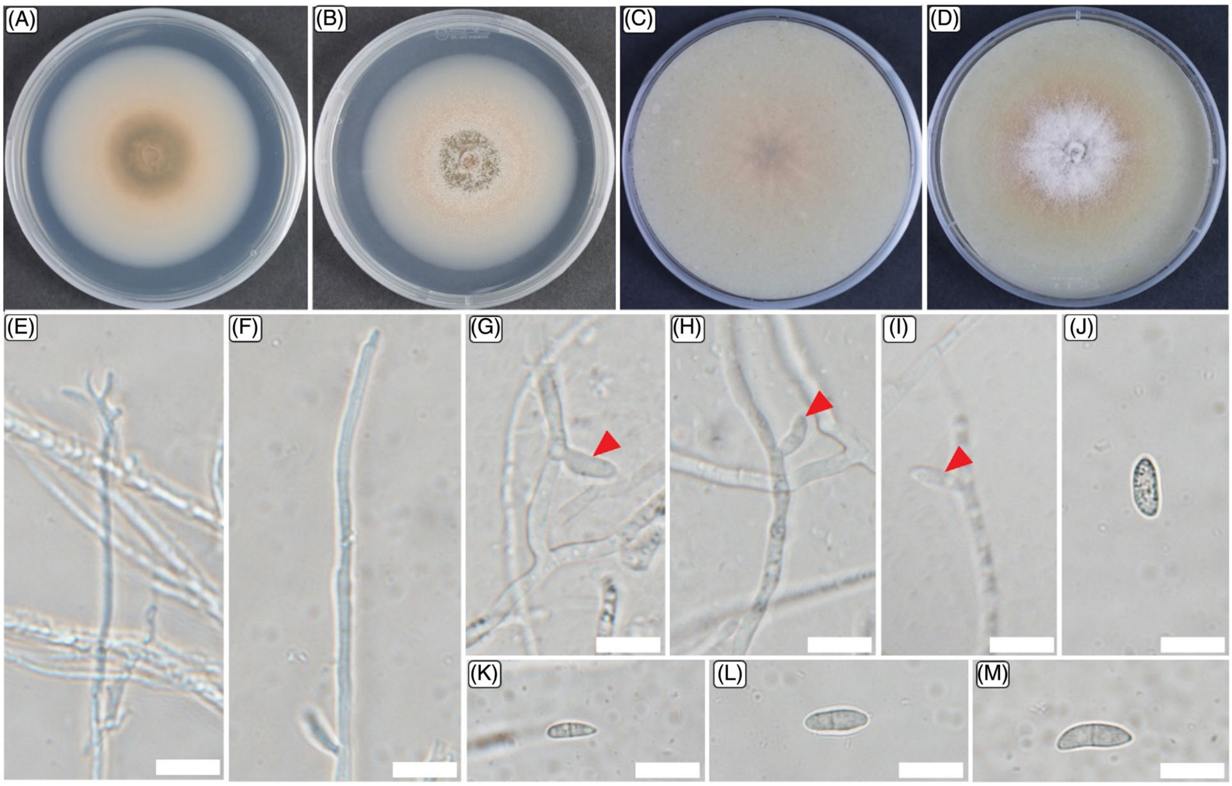

Figure 3. Cultural and morphological characteristics of NC14-294. Colony on potato dextrose agar (A, B) and oatmeal agar (C, D), respectively, at 25 °C in 7 days. Conidiophores (E, F); Conidiogenous cells (G–I); Conidia (J–M). Black arrow indicated conidiogenous cells. Scale bars: E–M = 10 µm.

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 µm. 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 × 2.0–3.2 µm and an average size of 7.7 × 2.6 µm, 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 × 2.40–3.70 µm, and an average size of 9.4 × 2.9 µm; 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 in 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 flocosse, 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 × 2.0–3.2 µm, which is smaller than that of the previously identified *M. phragmitis* (6.0–24.0 × 1.5–3.0 µm) but close to that of *M. lycopodinum* (4.0–12.0 × 2.5–3.5 µm) (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

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 salmonicolora* (NC14-294)	Colonies on PDA were flat, tightly attached with the media, small pallets, 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 umbonate 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, lanose to flocose, 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^T)	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(–23.0)× (2.5–)3.0(–4.0)	[32]
6	*M. trichocladiopsis* (CBS 623.77^T)	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^T)	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^T)	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^T)	Colonies on PDA felty, 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^T)	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.

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^T, 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

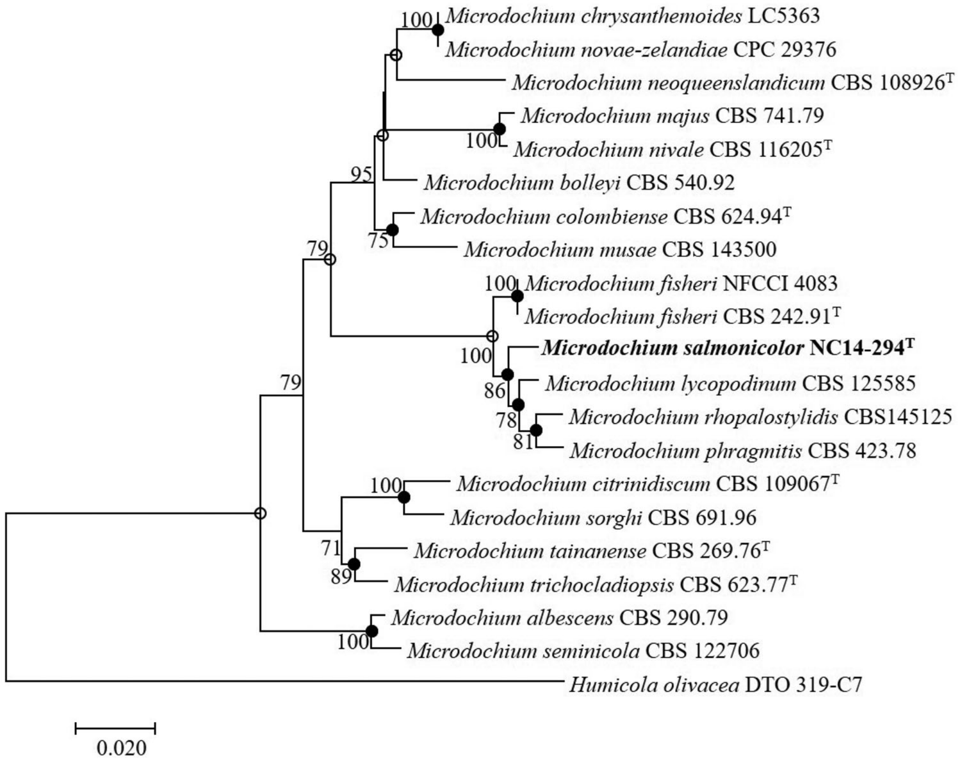

Figure 4. Neighbor-joining phylogenetic tree of NC14-294 based on the combined sequences (ITS + LSU), showing the relationship between *Microdochium salmonicolor* sp. nov. with the closest *Microdochium* spp. *Humicola olivacea* DTO 319-C7 used as an outgroup. The numbers above the branches represent 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.

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 *Neoascochyta 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 which containing 21 strains including the outgroup *Humicola olivacea*. 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).

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 that has been isolated from an estuarine *M. nivale* and that has the potential to control human and animal diseases [43]. Another example is that of an extract of *M. phragmitis* from Antarctic angiosperms, for which the cytotoxic activity against a human tumoral cell line has been reported [44]. Finally, the systematic exploration of *Microdochium* from natural sources is required to evaluate their bioactive potentiality.

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.

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