MYCOBIOLOGY
2021, VOL. 49, NO. 2, 105–121
https://doi.org/10.1080/12298093.2020.1862472

RESEARCH ARTICLE

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Six Newly Recorded Fungal Taxa from Freshwater Niche in Korea

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ABSTRACT

Six interesting fungal strains were isolated during a survey of fungal diversity associated with freshwater; these strains were designated as CNUFC YJW2-22, CNUFC MSW11-6-2, CNUFC HRS5-3, CNUFC MSW242-6, CNUFC DMW2-2, and CNUFC CPWS-1. Based on a polyphasic approach including phylogenetic analyses of internal transcribed space (ITS), large subunit (LSU), beta-tubulin (BenA), and calmodulin (CaM) gene sequences, morphological analyses, the six strains were found to be identical to *Acremonium guillematii*, *Cadophora novi-eboraci*, *Lectera nordwiniana*, *Mycoarthris corallina*, *Talaromyces siamensis*, and *Tetracladium globosum,* respectively. To our knowledge, these are the first records of the rare *Lectera*, *Mycoarthris*, and *Tetracladium* genera in Korea, and the first reports of *A. guillematii*, *C. novi-eboraci*, *L. nordwiniana*, *M. corallina*, *T. siamensis*, and *Te. globosum* in a freshwater environment.

ARTICLE HISTORY
Received 20 September 2020
Revised 2 December 2020
Accepted 6 December 2020

KEYWORDS
Ascomycetes; freshwater; morphology; phylogeny; taxonomy

1. Introduction

Freshwater fungi are a diverse group of organisms best known for being ecologically important decomposers in stream ecosystems. The group is classified into families of various orders, belonging to the classes Ascomycetes, Basidiomycetes, Chytridiomycetes, Zygomycetes, and Glomeromycetes [1–4]. Goh and Hyde [1] reported more than 600 species of freshwater fungi. Further, the number of new taxa isolated from freshwater environments is rapidly increasing due to global contribution of reliable and complete sequencing data.

The genus *Acremonium* was classified by Link (1809) [5] and includes some of the most simply structured of all filamentous anamorphic fungi [6]. *Acremonium* is characterized by simple verticillate conidiophores; solitary, aculeate phialides; and unicellular conidia produced in mucoid heads or unconnected chains. Species of *Acremonium* are common in substrates such as soil and plant debris [7,8]; while some species cause important diseases in plants [9,10], others are agents of opportunistic infection in humans [11,12]. *Acremonium* comprises more than 100 species; however, only seven species have previously been reported in Korea: *A. strictum*, *A. acutatum*, *A. zonatum*, *A. sclerotigenum*, *A. varicolor*, *A. persicinum*, and *A. tubakii* [13,14].

The genus *Cadophora* was first described by Lagerberg and Melin in 1927 [15] with *C. fastigiata* as a type species, classified based on the production of single-phialide conidiophores with distinct flask-shaped, hyaline collarettes [15,16]. *Cadophora* species have been isolated from plants [16], soil [17], and decaying wood [18,19]. Currently, the genus *Cadophora* comprises 17 species, of which two have been reported in Korea: *C. malorum*, and *C. fastigiata* [20,21].

The genus *Lectera* was described by Cannon in 2012 in order to reclassify *Volutella colletotrichoides* J.E. Chilton. Cannon [22] transferred *V. colletotrichoides* to the genus *Lectera* from the genus *Volutella* based on morphological and molecular research, and renamed the species *Lectera colletotrichoides*. The species of this genus are commonly found in soil and plants such as wheat, chili, and kidney bean [23]. Some species of this genus are known to be plant pathogens, and *L. colletotrichoides* is pathogenic to legumes [22]. Currently, there are eight accepted species in this genus, according to the Index Fungorum (www.indexfungorum.org).

The genus *Mycoarthris* was established by Marvanová in 2002. This genus contains only one species, *Mycoarthris corallina*, belonging to the Calloriaceae family. It is also the type species of this genus and was isolated from foam in a stream in the United Kingdom [24]. This species possesses chains of rod-like arthroconidia [24]. There are no published reports of this genus in Korea.

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The genus *Talaromyces* was established by Benjamin (1955) [25] to classify a teleomorph of *Penicillium,* with *Talaromyces vermiculatus* (=*T. flavus*) as the type species. These species are characterized by cleistothecial or gymnothecial ascomata, unitunicate 8-spored asci, and unicellular ascospores with or without equatorial crests. *Talaromyces* species have been isolated from indoor air, dust, soil, clinical samples, plants, seeds, leaf litter, honey, pollen [26,27]. More than 150 *Talaromyces* species have been described worldwide [28]. In Korea, only nine species of *Talaromyces*, belonging to sections *Talaromyces* and *Trachyspermi*, have been well described [29–34].

The genus *Tetracladium* was described by De Wild in 1893 [35] with *Te. marchalianum* as a type species, and it is common in aquatic habitats. Nineteen years later, Grove [36] transferred *Titaea maxilliformis* and *Tridentaria setigera* to genus *Tetracladium* as *Te. maxilliforme* and *Te. setigerum*, respectively. Later, *Te. apiense*, *Te. furcatum*, *Te. breve*, *Te. palmatum*, and *Te. nainitalense* were added to the genus [37,38]. Recently, *Te. ellipsoideum*, *Te. globosum*, and *Te. psychrophilum* described by Wang et al. [39] were isolated from soil in China. Currently, 11 valid species of *Tetracladium* are known according to the Index Fungorum, most of which produce distinctive, characteristically-shaped conidia [40].

Freshwater fungi play an important role in decay of wood and leafy materials in aquatic ecosystem as well as in occurrences of plant and animal diseases, and some of them have potential to produce various antibiotics and enzymes [41,42]; however, only a limited number of research on freshwater fungi has been carried out in Korea [33,43–46]. In particular, few reports of aquatic hyphomycetes (also known as freshwater hyphomycetes or Ingoldian fungi) in Korea exist [47].

The aim of the present study was to isolate and describe the six newly-recorded fungal taxa from freshwater niche using morphological and molecular analyses: *A. guillematii*, *C. novi-eboraci*, *L. nordwiniana*, *M. corallina*, *T. siamensis*, and *Te. globosum*.

2. Materials and methods

2.1. Isolation of fungal strains

Water samples were collected from the Wonhyo valley in Gwangju, the Jukrim reservoir in Yeosu, the Hwangnyong river in Gwangju, pond at Ming Ok Heon garden in Damyang, and pond located in the Chonnam National University Arboretum, Gwangju, Korea. These samples were transported to the laboratory in sterile 50-mL conical tubes and were stored at 4 °C until examination. Fungal strains were isolated by a serial dilution plating method. For dilution, 1 mL of each freshwater sample was mixed with 9 mL sterile distilled water, and the solution was shaken for 8 min. Then, serial dilutions were made ranging from 10^{-1} to 10^{-4}. A 100 µL aliquot of each dilution was spread onto potato dextrose agar (PDA: 39 g of potato dextrose agar in 1 L of deionized water; Becton, Dickinson, and Co., Sparks, MD, USA) supplemented with the antibiotic neomycin (final concentration 50 ppm). The petri plates were incubated at 20–25 °C for 7–14 days. Pure isolates were obtained by selecting individual colonies of varying morphologies, transferring them to PDA plates, and subculturing until pure mycelia were obtained. For stock storage, the pure isolates were maintained in PDA slant tubes and 20% glycerol at −80 °C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea. The strains CNUFC MSW11-6-2, CNUFC MSW242-6, CNUFC YJW2-22, CNUFC HRS5-3, CNUFC DMW2-2, and CNUFC CPWS-1 were also deposited at the Culture Collection of the Nakdonggang National Institute of Biological Resources (NNIBR, Sangju, Korea).

2.2. Morphological examination

To observe morphology, the CNUFC MSW11-6-2, CNUFC MSW242-6, CNUFC YJW2-22, and CNUFC HRS5-3 strains were cultured on PDA, malt extract agar (MEA; malt extract 20 g, agar 20 g in 1 L of deionized water), and oatmeal agar (OA; oat meal 30 g, agar 15 g in 1 L of deionized water). CNUFC DMW2-2 was culture on czapek yeast autolysate agar (CYA), yeast extract sucrose agar (YES), and Blakeslee's malt extract agar [48]. CNUFC CPWS-1 was culture on PDA. All plates were incubated at 25 °C in the dark for 13–14 days, except for CNUFC CPWS1 was incubated at 20 °C. Samples were mounted in lactophenol solution (Junsei Chemical Co. Ltd., Tokyo, Japan) or distilled water before being observed under an Olympus BX51 and BX53 microscope with DIC optics (Olympus, Tokyo, Japan).

2.3. DNA extraction, PCR, and sequencing

Genomic DNA was extracted directly from the mycelia and spores of fungal isolates using the Solg Genomic DNA Prep Kit (Solgent Co., Ltd., Daejeon, South Korea). The internal transcribed spacer (ITS) region, large subunit (LSU), beta-tubulin (BenA), and calmodulin (CaM) genes were amplified using the primer pairs ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [49], LROR (5′-ACCCGCTGAACTTAAGC-3′) and LR5F

(5′-GCTATCCTGAGGGAAAC-3′) [50], Bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) [51], and CF1 (5′-GCCGACTCTTTGACY GARGAR-3′) and CF4 (5′-TTTYTGCATCATRAGYTGGAC-3′) [52], respectively. The PCR products were purified using the Accuprep PCR Purification Kit (Bioneer Corp.). Sequencing was performed using the same PCR primers and was run on the ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.4. Phylogenetic analysis

Fungal strain sequences were initially aligned using Clustal X v.2.0 [53], and the alignment was edited using BioEdit v.7.2.6 [54]. Phylogenetic analyses were conducted using MEGA 7 [55], and phylogenetic trees were made based on the maximum likelihood (ML) method and the Kimura 2-parameter model. The reliability of internal branches was assessed using the p-distance substitution model with 1,000 bootstrap replications. The sequences of CNUFC MSW11-6-2, CNUFC MSW11-6-2-1, CNUFC MSW242-6, CNUFC MSW242-8, CNUFC YJW2-22, CNUFC YJW2-22-1, CNUFC HRS5-3, CNUFC HRS5-3-1, CNUFC DMW2-2, CNUFC DMW2-2-1, CNUFC CPWS-1, and CNUFC CPWS-2 were deposited in the NCBI database under the accession numbers shown in Table 1.

3. Results

3.1. Phylogenetic analysis

The ITS sequence analysis by the Basic Local Alignment Search Tool (BLAST) indicated that the CNUFC MSW11-6-2, CNUFC MSW242-6, CNUFC YJW2-22, CNUFC HRS5-3, CNUFC DMW2-2, and CNUFC CPWS-1 isolates were 99.7% (584/586 bp), 99.8% (416/417 bp), 98.8% (513/519 bp), 99.1% (520/525 bp), 99.4% (516/519 bp), and 100% (417/417 bp) related to *C. novi-eboraci* (GenBank accession no. KM497036), *M. corallina* (GenBank accession no. AH009124), *A. guillematii* (GenBank accession no. AB828055), *L. nordwiniana* (GenBank accession no. MK047462), *T. siamensis* A3S2-40 (GenBank accession no. KJ767055), and *Te. globosum* MY25 (GenBank accession no. JX029133), respectively. The 28S sequence of CNUFC YJW2-22 and CNUFC HRS5-3 showed similarities of 99.4% (774/779 bp), and 99.7% (846/849 bp) with *A. guillematii* CBS 767.69 (GenBank accession no. MH871190), and *L. nordwiniana* CBS 144922 (GenBank accession no. MK047513). BLASTn analysis of BenA of CNUFC DMW2-2 and CNUFC MSW11-6-2 showed similarities of 100% (399/399 bp), and 99.5% (607/610 bp) with *T. siamensis* CBS 475.88 (GenBank accession no. JX091379), and *C. novi-eboraci* GLMC 274 (GenBank accession no. MN232975). Similarity, BLASTn analysis of CaM of CNUFC DMW2-2 showed similarities of 99.6% (470/472 bp) with *T. siamensis* DTO 269I3 (GenBank accession no. KJ775428). Based on the ITS, LSU, BenA, and a combined (ITS + BenA + CaM) trees, CNUFC YJW2-22, CNUFC MSW11-6-2, CNUFC HRS5-3, CNUFC MSW242-6, CNUFC DMW2-2, and CNUFC CPWS-1 isolates were identical to *A. guillematii*, *C. novi-eboraci*, *L. nordwiniana*, *M. corallina*, *T. siamens*, and *Te. globosum*, respectively (Figures 1–6).

3.2. Taxonomy

3.2.1. Taxonomy of CNUFC YJW2-22

Acremonium guillematii Gams, Cephalosporium-artige Schimmelpilze: 56 (1971) [56] (Figure 7).

Description: Colonies grew slowly on 2% MEA in the dark at 25 °C, reaching 23–26 mm in diameter over 14 days and were ivory to intense yellow with a flat, velvety texture. The reverse side of the colonies was intense yellow. Conidophores were solitary, sparsely branched, and orthotropic with slender phialides, and measured 11.5–31.5 × 1.3–2.7 µm. Conidia were globose, subglobose, ellipsoidal or ovoid, and measured 1.6–3.7 × 1.5–2.5 µm.

3.2.2. Taxonomy of CNUFC MSW11-6-2

Cadophora novi-eboraci Travadon, Lawr, Roon-Lath, Gubler, Wilcox, Rolsh & Baumgartner, Fungal Biology 119: 61 (2015) [16] (Figure 8).

Description: Colonies grew slowly on 2% MEA in the dark at 25 °C, reaching 13–14 mm in diameter over 13 days, and appeared deep orange with a flat texture and smooth margins. The reverse side of each colony was deep orange. Conidiophores were simple or branched, hyaline, and measured 8.3–69 × 1.5–3 µm. Conidiogenous cells were terminal or lateral, commonly solitary, obclavate, ampulliform, subcylindrical, flexuous, and measured 4–15 × 1–3 µm. Collarettes were short, flaring, cup–-shaped, parallel, and measured 1.5–2 × 1–1.8 µm. Conidia were ovoid, cylindrical to oblong, elliptical, and measured 3–6 × 1–4 µm.

3.2.3. Taxonomy of CNUFC HRS5-3

Lectera nordwiniana Crous, Luangsa-Ard, Wingfield, et al., Persoonia 41: 349 (2018) [57] (Figure 9).

Description: Colonies grew slowly on PDA in the dark at 25 °C, reaching 21–23 mm in diameter over 14 days, were beige to bright salmon and were slightly elevated. The reverse sides of the colonies were beige to bright salmon in color. Conidiomata

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

		GenBank accession No.			
Taxon name	Collection No.	ITS	LSU	BenA	CaM
Acremonium blochii	CBS 993.69	HE608636	HQ232000		
A. breve	CBS 150.62^{T}	MH424706	HQ232005		
A. camptosporum	CBS 756.69	MH859414	HQ232008		
A. chrysogenum	CBS 779.69	MH859424			
A. chrysogenum	CBS 401.65	MH858636			
A. chrysogenum	CBS 144.62		HQ232017		
A. curvulum	CBS 430.66	MH424698	HQ232026		
A. e.g.yptiacum	CBS 114785^{T}	MH424616	FN706550		
A. exiguum	CBS 587.73		HQ232035		
A. flavum	CBS 316.72	MH860487			
A. flavum	CBS 142.71	MH860039			
A. flavum	CBS 596.70		HQ232037		
A. gamsii	CBS 726.71^{T}	MH860311	HQ232040		
A. guillematii	TAMA0164	AB828056			
A. guillematii	TAMA0163	AB828055			
A. guillematii	CBS 767.69		MH871190		
A. guillematii	CBS 766.69^{T}		HQ232042		
A. guillematii	**CNUFC YJW2-22**	**MW020709**	**MW023395**		
A. guillematii	**CNUFC YJW2-22-1**	**MW020710**	**MW023396**		
A. minutisporum	CBS 147.62		HQ232061		
A. nigrosclerotium	CBS 154.72^{T}	MH860423	HQ232069		
A. persicinum	CBS 310.59^{T}		HQ232077		
A. pinkertoniae	CBS 157.70^{T}	MH859534	HQ232089		
A. potronii	CBS 379.70	AY632655	HQ232096		
A. psammosporum	CBS 590.63		HQ232100		
A. radiatum	CBS 142.62^{T}	MH858125	HQ232104		
A. roseolum	CBS 289.62^{T}	MH858153	HQ232123		
A. rutilum	JCM23088	NR_077124	AB540506		
A. sclerotigenum	CBS 124.42^{T}	MH856101	HQ232126		
A. spinosum	CBS 136.33^{T}		HQ232137		
A. tubakii	CBS 790.69	MH859429			
A. vitellinum	CBS 792.69		HQ232151		
Botryotinia fuckeliana	LGM002	KC683713			
Cadophora fastigiata	CBS 307.49			KM497131	
C. finlandia	CBS 444.86	AF486119			
C. finlandica	CBS 444.86^{T}	AF486119		KM497130	
C. gregata	ATCC 11073^{T}	U66731		MF677920	
C. interclivum	BAG4^{T}	MF979577			
C. luteo-olivacea	U5	KM497041		KM497122	
C. luteo-olivacea	CBS 141.41^{T}	AY249066		KM497133	
C. malorum	CBS 165.42^{T}	AY249059		KM497134	
C. melinii	ONC1	KM497033		KM497114	
C. melinii	CBS 268.33^{T}	AY249072		KM497132	
C. meredithiae	BAG2^{T}	MF979574		MF677914	
C. novi-eboraci	NYC1	KM497035		KM497116	
C. novi-eboraci	NYC2	KM497034		KM497115	
C. novi-eboraci	NYC14^{T}	KM497037		KM497118	
C. novi-eboraci	NYC13	KM497036		KM497117	
C. novi-eboraci	CBS 101359	DQ404350		KM497135	
C. novi-eboraci	**CNUFC MSW11-6-2**	**MW020711**		**MW310411**	
C. novi-eboraci	**CNUFC MSW11-6-2-1**	**MW020712**		**MW310412**	
C. orchidicola	UAMH8152	AF214576		MF677921	
C. orientoamericana	NHC1^{T}	KM497018		KM497099	
C. orientoamericana	NHC2	KM497019		KM497100	
C. spadicis	QCC1	KM497031		KM497112	
C. spadicis	CBS 111743	DQ404351		KM497136	
C. viticola	Cme-1	HQ661096			
Gibellulopsis aquatica	CBS 117131^{T}	LR026720	LR025850		
G. catenata	CBS 113951^{T}	LR026721	LR025851		
G. fusca	CBS 560.65^{T}	LR026724	LR025854		
G. nigrescens	CBS 120949^{NT}	LR026738	LR025868		
G. serrae	CBS 290.30^{T}	LR026742	LR025872		
Lectera capsici	CBS 142534^{T}	NR_155338	KY979825		
L. colletotrichoides	CBS 109728	KM231851	KM231731		
L. humicola	IMI 265740^{T}	JQ647449	LR025896		
L. longa	IMI 181698^{T}	JQ647448	LR025897		
L. nordwiniana	CBS 144921^{T}	MK047461	MK047511		
L. nordwiniana	CBS 144922	MK047463	MK047513		
L. nordwiniana	JW231013	MK047462	MK047512		
L. nordwiniana	**CNUFC HRS5-3**	**MW020713**	**MW023397**		
L. nordwiniana	**CNUFC HRS5-3-1**	**MW020714**	**MW023398**		
L. phaseoli	IMI 366179		LR025898		
Monilinia laxa	CBS 298.31	HQ846949			
Monilochaetes melastomae	CBS 145059	MK047430	MK047481		
Musicillium elettariae	CBS 252.80^{T}	LR026765	LR025899		

(*continued*)

Table 1. Continued.

Taxon name	Collection No.	GenBank accession No.			
		ITS	LSU	BenA	CaM
M. theobromae	CBS 968.72^{NT}	LR026773	LR025907		
M. tropicale	CBS 120009^{T}	LR026783	LR025917		
Mycoarthris cf. corallina	UFMGCB 6326	KC485428			
M. corallina	IMI 385400^{T}	AH009124			
M. corallina	**CNUFC MSW242-6**	**MW020715**			
M. corallina	**CNUFC MSW242-8**	**MW020716**			
Mycoarthris sp.	S-8	KJ735002			
Mycoarthris sp.	P2895	KT270092			
Mycoarthris sp.	837	JX982481			
Mycoarthris sp.	11MA09	JX270444			
Paramusicillium asperulatum	CBS 120158	LR026792	LR025930		
Plectosphaerella cucumerina	CBS 137.37	KU140674	KY662256		
Sarocladium bacillisporum	CBS 425.67^{T}	MH859020	HE608658		
S. bactrocephalum	CBS 749.69^{T}	MH859409	HQ231994		
S. kiliense	CBS 122.29^{T}	AJ621775	HQ232052		
S. strictum	CBS 346.70^{T}	MH859705	HQ232141		
S. zeae	CBS 801.69		HQ232152		
Sclerotinia sclerotiorum	CBS 499.50	KF859933			
Simplicillium lanosoniveum	CBS 321.72^{T}	MH860488	HQ232006		
Talaromyces aculeatus	CBS 289.48	KF741995		KF741929	KF741975
T. adpressus	CBS 140620^{T}	KU866657		KU866844	KU866741
T. amestolkiae	CBS 132696^{T}	JX315660		JX315623	KF741937
T. angelicus	KACC 46611^{T}	KF183638		KF183640	KJ885259
T. apiculatus	CBS 312.59^{T}	JN899375		KF741916	KF741950
T. beijingensis	CBS 140617^{T}	KU866649		KU866837	KU866733
T. dendriticus	CBS 660.80^{T}	MH861305		JX091391	KF741965
T. flavovirens	CBS 102801^{T}	JX013916		JX091376	KF741933
T. funiculosus	CBS 272.86^{T}	JN899377		JX091383	KF741945
T. fuscoviridis	CBS 193.69^{T}	KF741979		KF741912	KF741942
T. galapagensis	CBS 751.74^{T}	JN899358		JX091388	KF741966
T. indigoticus	CBS 100534^{T}	JN899331		JX494308	KF741931
T. liani	CBS 225.66^{T}	JN899395		JX091380	KJ885257
T. macrosporus	CBS 317.63^{T}	JN899333		JX091382	KF741952
T. muroii	CBS 756.96^{T}	JN899351		KJ865727	KJ885274
T. mycothecae	URM 7622^{T}	MF278326		LT855561	LT855564
T. neofusisporus	AS3.15415^{T}	KP765385		KP765381	KP765383
T. pinophilus	CBS 631.66^{T}	JN899382		JX091381	KF741964
T. ruber	CBS 132704^{T}	JX315662		JX315629	KF741938
T. rubicundus	CBS 342.59^{T}	JN899384		JX494309	KF741956
T. sayulitensis	CBS 138204^{T}	KJ775713		KJ775206	KJ775422
T. siamensis	CBS 475.88^{T}	JN899385		JX091379	KF741960
T. siamensis	DTO 269I3	KJ775726		KJ775219	KJ775428
T. siamensis	**CNUFC DMW2-2**	**MW020717**		**MW056317**	**MW056319**
T. siamensis	**CNUFC DMW2-2-1**	**MW020718**		**MW056318**	**MW056320**
T. stollii	CBS 408.93^{T}	JX315674		JX315633	JX315646
T. veerkampii	CBS 500.78^{T}	NR_153228		KF741918	KF741961
Tetracladium apiense	CCM F-23299	EU883422			
Te. breve	CCM F-12505	EU883431			
Te. ellipsoideum	MIDUI20^{T}	JX029111			
Te. ellipsoideum	MIDUI30	JX029113			
Te. furcatum	CCM F-06983	FJ000373			
Te. furcatum	CCM F-11883	FJ000375			
Te. globosum	HAILUO215^{T}	JX029109			
Te. globosum	MY25	JX029133			
Te. globosum	MY24	JX029118			
Te. globosum	**CNUFC CPWS-1**	**MW020719**			
Te. globosum	**CNUFC CPWS-2**	**MW020720**			
Te. marchalianum	CCM F-26399	AY204623			
Te. maxilliforme	CCM F-13186	EU883430			
Te. palmatum	CCM F-10001	FJ000372			
Te. psychrophilum	HAILUO380^{T}	JX029119			
Te. psychrophilum	MY376	JX029129			
Te. setigerum	CCM F-10186	EU883427			
Xenoacremonium recifei	CBS 137.35^{T}		KM231713		

Bold letters indicate isolates and accession numbers determined in our study. ITS: internal transcribed spacer region; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, The Netherlands; DTO: Internal culture collection of CBS-KNAW Fungal Biodiversity Center; CNUFC: Chonnam National University Fungal Collection (Gwangju, South Korea); KACC: Korean Agricultural Culture Collection; URM: University Recife Mycologia (URM) Culture Collection (Micoteca URM), Brazil.^{T} and ^{NT} = ex-type and ex-neotype strains.

were salmon in color, sporodochial, punctiform, solitary or gregarious and were abundantly formed on PDA, 2% MEA, and OA. Setae were dark brown, 3–8 septate, flexuous or rigid, tapering to acutely rounded apices, surrounding conidiomata, and measured 57–205 × 4–6.4 µm. Phialides were subcylindrical to subulate, hyaline, smooth–walled, and measured 12.5–29 × 1.8–3.5 µm. Conidia were fusiform to navicular, hyaline, orange in mass, and measured 4.5–9 × 2–4.5 µm.

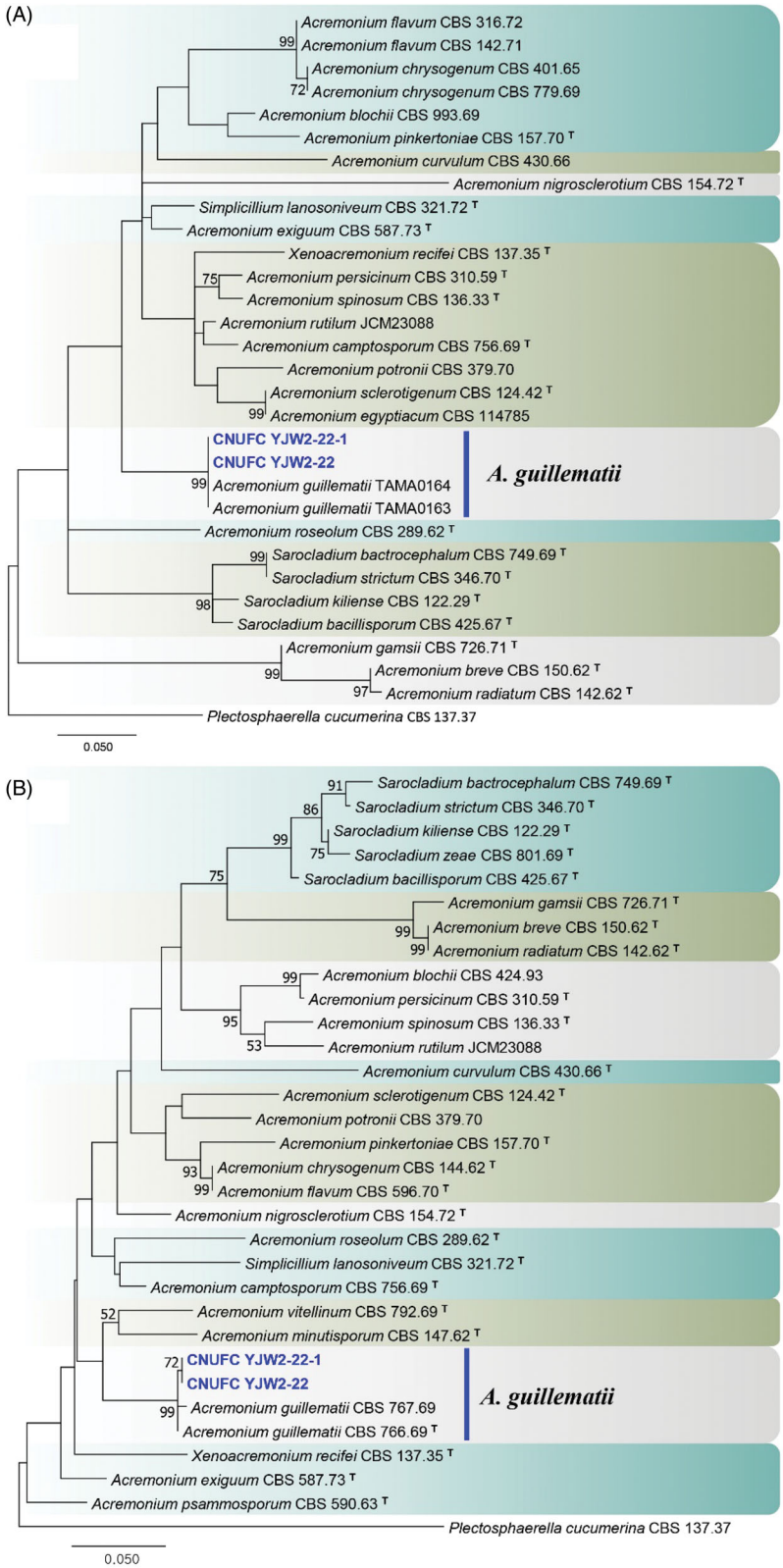

Figure 1. Phylogenetic tree of *Acremonium guillematii* CNUFC YJW2-22 and CNUFC YJW2-22-1, and related species, based on a maximum likelihood analyses of internal transcribed spacer (A) and large subunit (B) sequences. The sequence of *Plectosphaerella cucumerina* was used as an outgroup. Bootstrap support values of ≥50% are indicated at the nodes. Ex-type strains are indicated by T.

3.2.4. Taxonomy of CNUFC MSW242-6

Mycoarthris corallina Marvanová & Fisher, Nova Hedwigia 75: 258 (2002) [24] (Figure 10).

Description: Colonies grew slowly on 2% MEA in the dark at 15 °C, reaching 18 mm in diameter over 13 days, were off–white in color, and were flat. The reverse side of each colony was pale beige. Conidiophores were terminal or lateral, simple or sparsely branched, hyaline, and measured up to 39 × 2.5–3 µm. Conidia were cylindrical or slightly

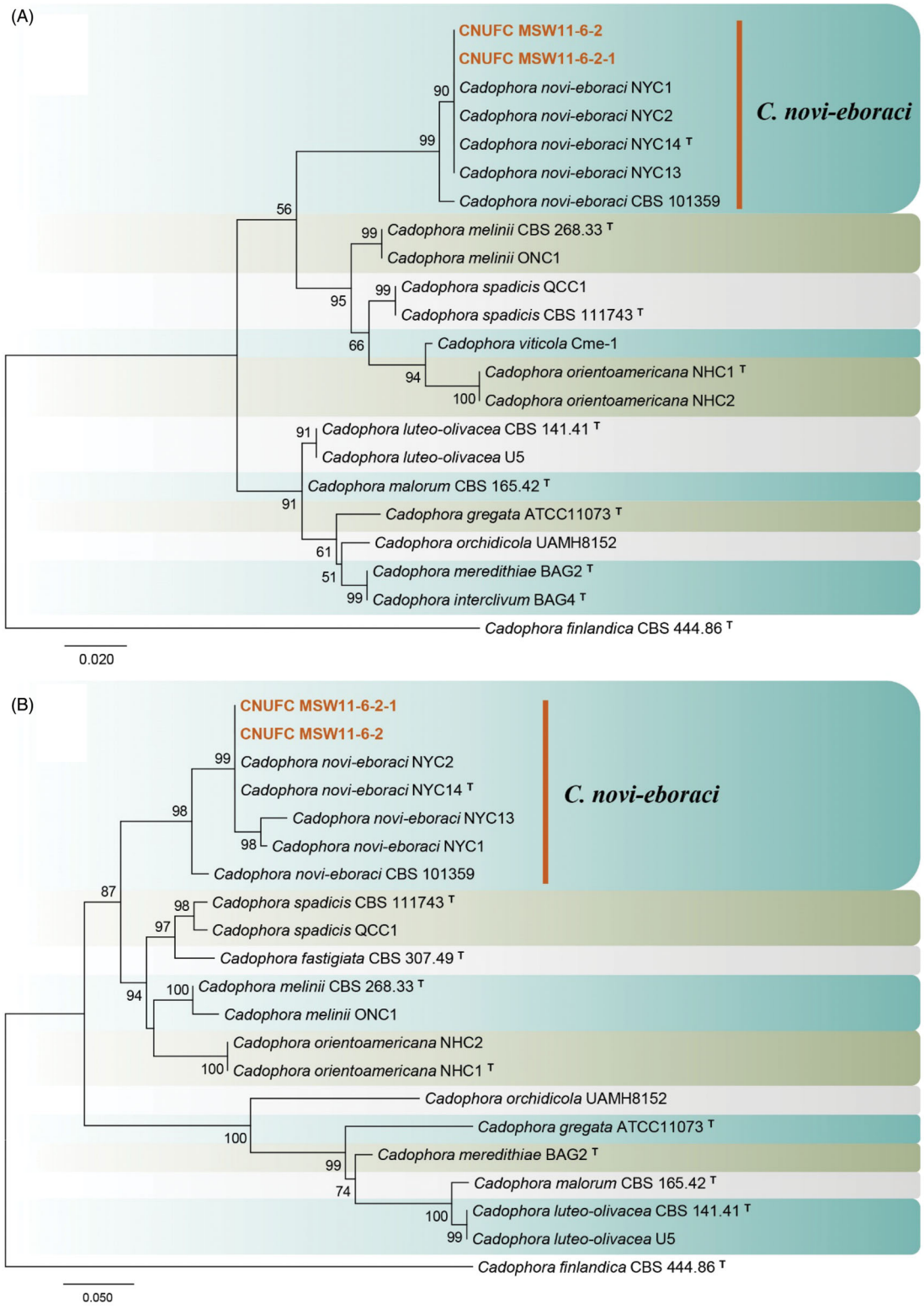

Figure 2. Phylogenetic tree of *Cadophora novi-eboraci* CNUFC MSW11-6-2 and CNUFC MSW11-6-2-1, and related species, based on maximum likelihood analyses of internal transcribed spacer (A) and beta-tubulin (B) sequences. The sequence of *Cadophora finlandica* was used as an outgroup. Bootstrap support values of ≥50% are indicated at the nodes. Ex-type strains are indicated by T.

curved, produced a chain of arthroconidia, and measured 9.5–36 × 1.5–2.5 µm.

3.2.5. Taxonomy of CNUFC DMW2-2

Talaromyces siamensis (Manoch & Ramírez) Samson, Yilmaz & Frisvad, Studies in Mycology 70: 177 (2011) [26] (Figure 11).

Description: Colonies grew slowly on CYA in the dark at 25 °C, reaching 30 mm in diameter over 7 days and were olive green. The reverse side of each colony was reddish at the center and faded into a bright red. Conidiophores were biverticillate, solitary or branched, with smooth walled stipes, and measured 115–475 × 2.8–4 µm. Metulae were three

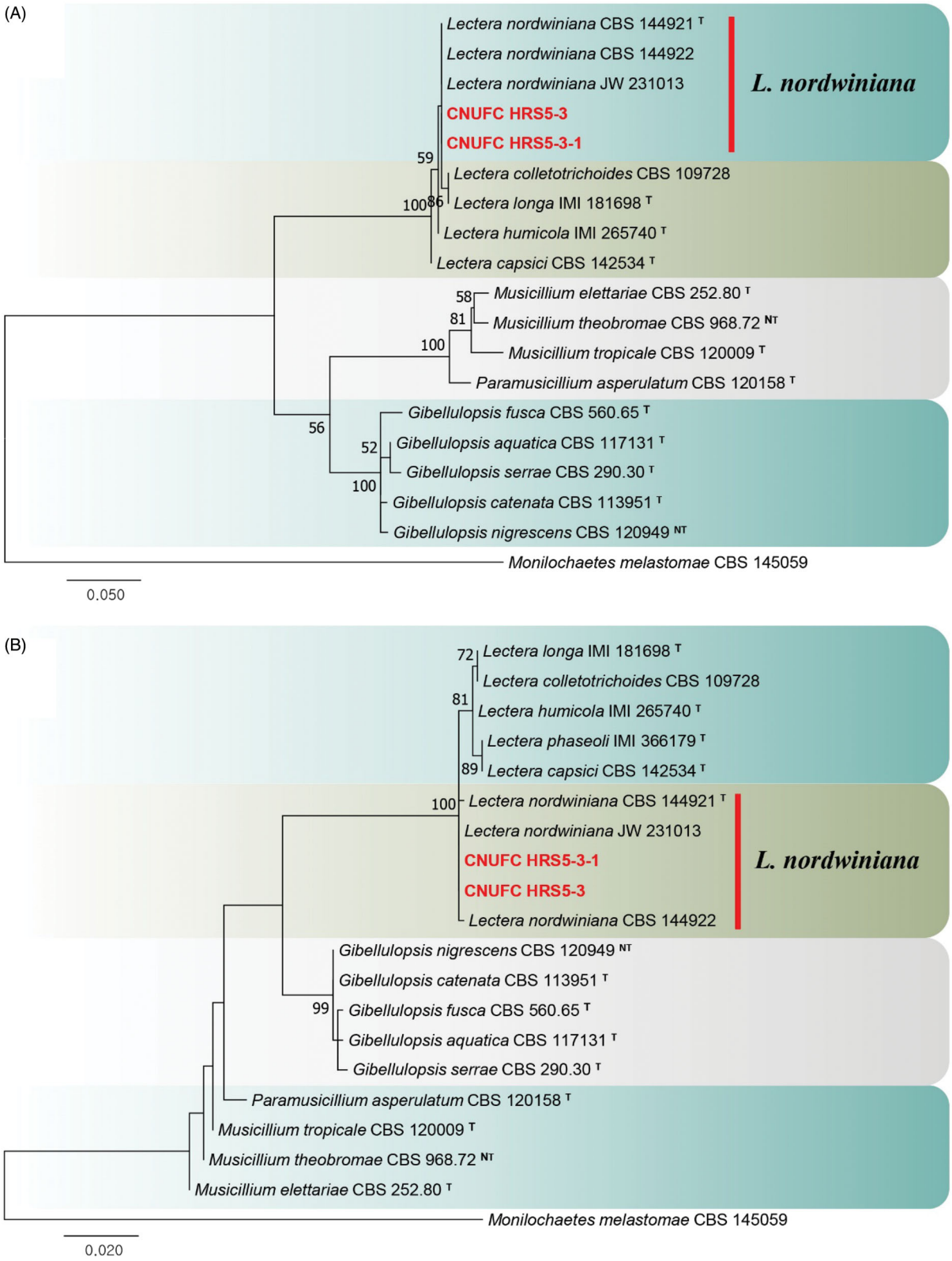

Figure 3. Phylogenetic tree of *Lectera nordwiniana* CNUFC HRS5-3 and CNUFC HRS5-3-1, and related species, based on maximum-likelihood analyses of internal transcribed spacer (A) and large subunit (B) sequences. The sequence of *Monilochaetes melastomae* was used as an outgroup. Bootstrap support values of ≥50% are indicated at the nodes. Ex-type and ex-neotype strains are indicated by T and NT.

to six divergent and measured 9.3–16 × 2.2–3.5 µm. Phialides were acerose, numbered three to five per metulae, and measured 9–14.5 × 2–3 µm. Conidia were ovoid, ellipsoidal to fusiform, and measured 2.8–4 × 2–3 µm.

3.2.6. Taxonomy of CNUFC CPWS-1

Tetracladium globosum M.M. Wang & Xing Z. Liu, Persoonia 34: 108 (2015) [39] (Figure 12).

Description: Colonies grew slowly on PDA, reaching 8 mm in diameter over 7 days at 15 °C. The

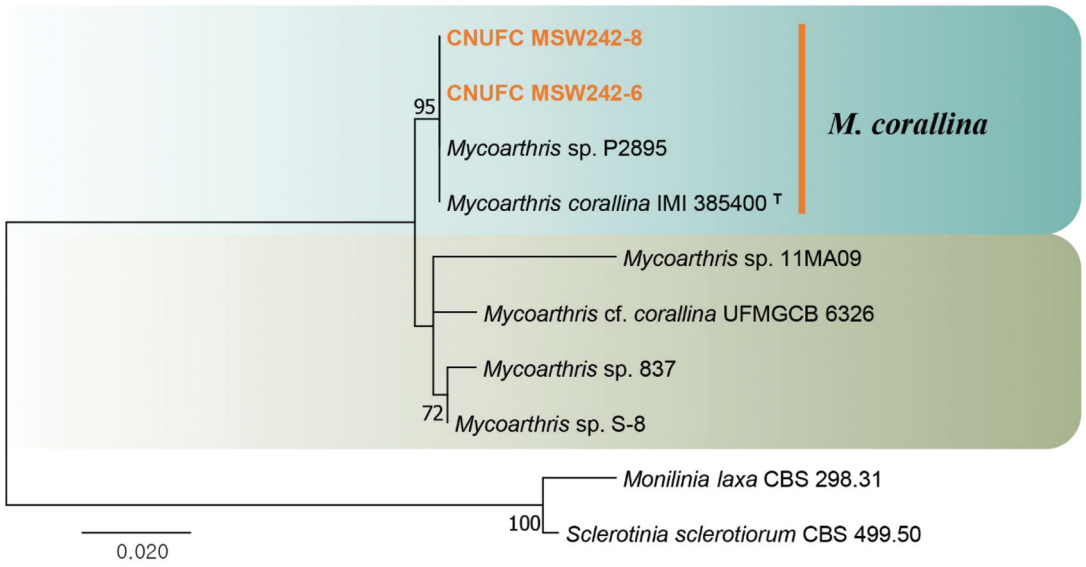

Figure 4. Phylogenetic tree of *Mycoarthris corallina* CNUFC MSW242-6 and CNUFC MSW242-8, and related species, based on maximum-likelihood analysis of internal transcribed spacer sequences. The sequences of *Monilinia laxa* and *Sclerotinia sclerotiorum* were used as outgroups. Bootstrap support values of ≥50% are indicated at the nodes. Ex-type strains are indicated by T.

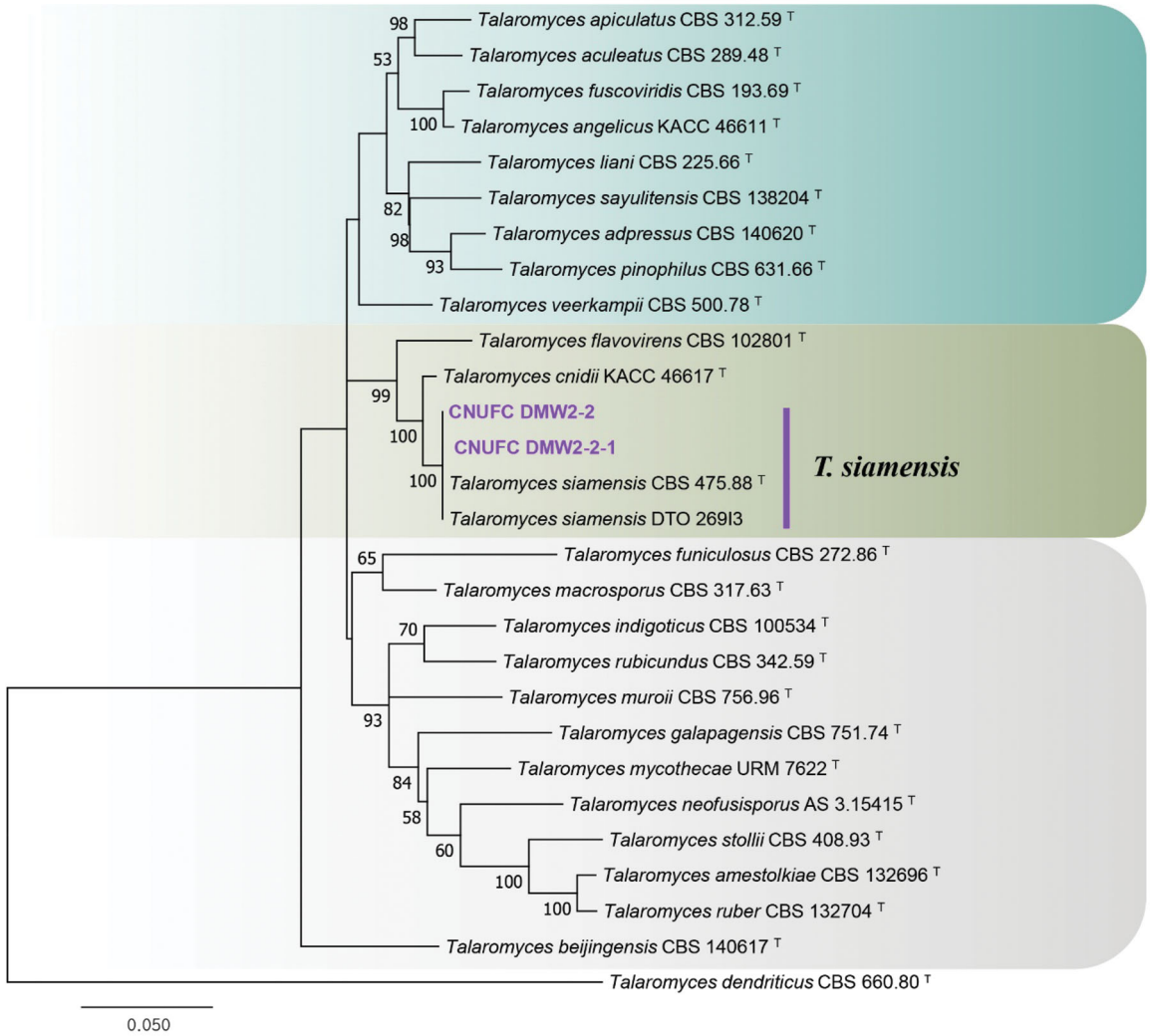

Figure 5. Phylogenetic tree of *Talaromyces siamensis* CNUFC DMW2-2 and CNUFC DMW2-2-1, and related species, based on maximum-likelihood analyses of internal transcribed spacer, beta-tubulin, and calmodulin sequences. The sequence of *Talaromyces dendriticus* was used as an outgroup. Bootstrap support values of ≥50% are indicated at the nodes. Ex-type strains are indicated by T.

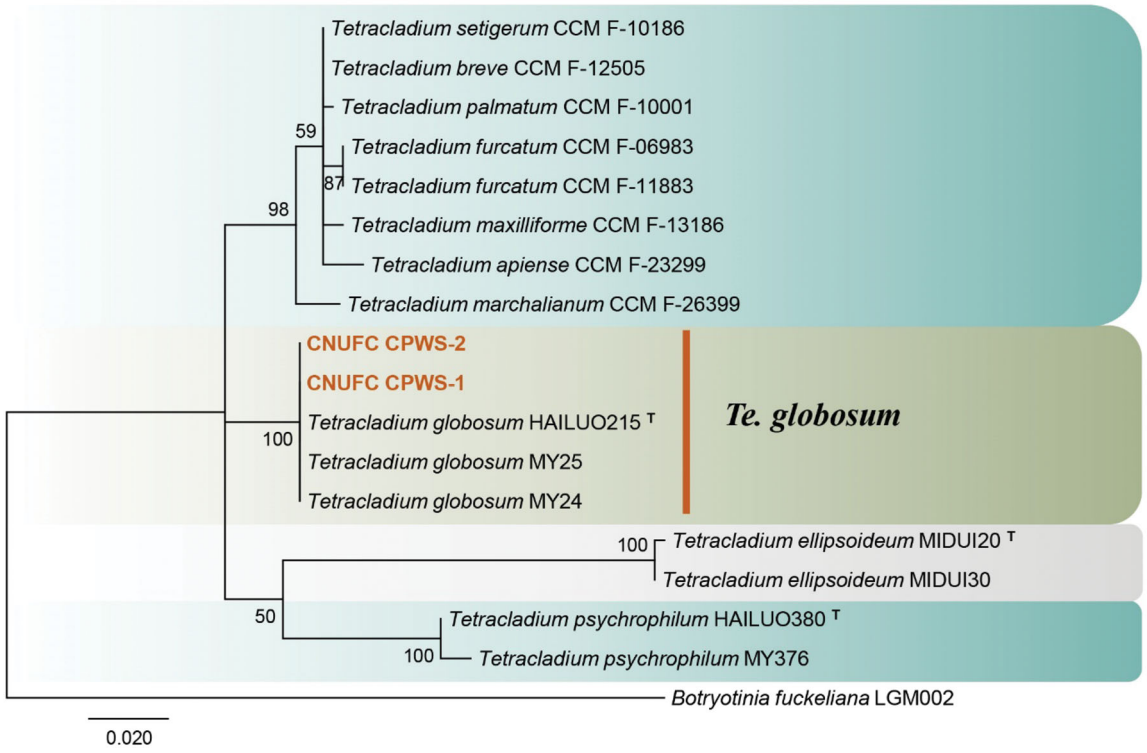

Figure 6. Phylogenetic tree of *Tetracladium globosum* CNUFC CPWS-1 and CNUFC CPWS-2, and related species, based on maximum-likelihood analysis of internal transcribed spacer sequences. The sequence of *Botryotinia fuckeliana* was used as an outgroup. Bootstrap support values of ≥50% are indicated at the nodes. Ex-type strains are indicated by T.

colony color was pale yellow. Vegetative hyphae possessed transverse septa, measured 1–4 µm, and were smooth, thin walled, and hyaline. Conidia were globose, smooth-walled, hyaline, measured 4.7–6.5 µm, and were attached to the hyphae with short conidiophores.

4. Discussion

To date, few studies have reported undescribed species in Korean freshwater environments. Therefore, collecting freshwater samples to identify undescribed fungal species is the best way to enhance our knowledge of Korean filamentous fungi. The discovery of *A. guillematii*, *C. novi-eboraci*, *L. nordwiniana*, *M. corallina*, *T. siamensis*, and *Te. globosum* in the present study improves our knowledge of the occurrence and distribution of freshwater fungi in Korea, especially, rare genera of *Lectera*, *Mycoarthris*, and *Tetracladium*.

The ITS rDNA region, which is regarded as barcode marker, is well-established as a method for identifying fungal taxa [58]. Additionally, LSU and *β*-tubulin have previously been used to accurately identify fungal strains at the species level. In the present study, the CNUFC YJW2-22 and CNUFC YJW2-22-1 isolates were identified to *A. guillematii* based on ITS and LSU sequence data (Figure 1). The morphological features of the CNUFC YJW2-22 isolate were similar to those of *A. guillematii*, as described by Gams [56]. In the current study, the strain produced yellow colonies and octahedral crystals on 2% MEA, which was found to be similar to *A. guillematii* [56,59]. Many *Acremonium* species have been identified as useful fungal resources as they produce bioactive metabolites. Tian et al. [60] reviewed the secondary metabolites of *Acremonium* species and revealed a total of 356 metabolites including steroids, terpenoids, polyketides, meroterpenoids, peptides, and alkaloids.

The analyses of ITS and BenA gene sequences showed that the CNUFC MSW11-6-2 and CNUFC-MSW11-6-2-1 isolates were clustered with *C. novi-eboraci* (type strain), with well-supported branches, in full agreement with the results of Travadon et al. [16] (Figure 2). The CNUFC MSW11-6-2 isolate was most morphologically-similar to *C. novi-eboraci,* as described by Travadon et al. [16]. *Cadophora luteo-olivacea* isolated from huts in Antarctica have been reported to produce cadopherone and colomitide polyketides [61]. In addition, Almeida et al. [62] isolated four types of hydroxylated sclerosporin from the marine-derived *Cadophora malorum* fungus.

Phylogenetic analysis of ITS and LSU sequences showed that the CNUFC HRS5-3 and CNUFC

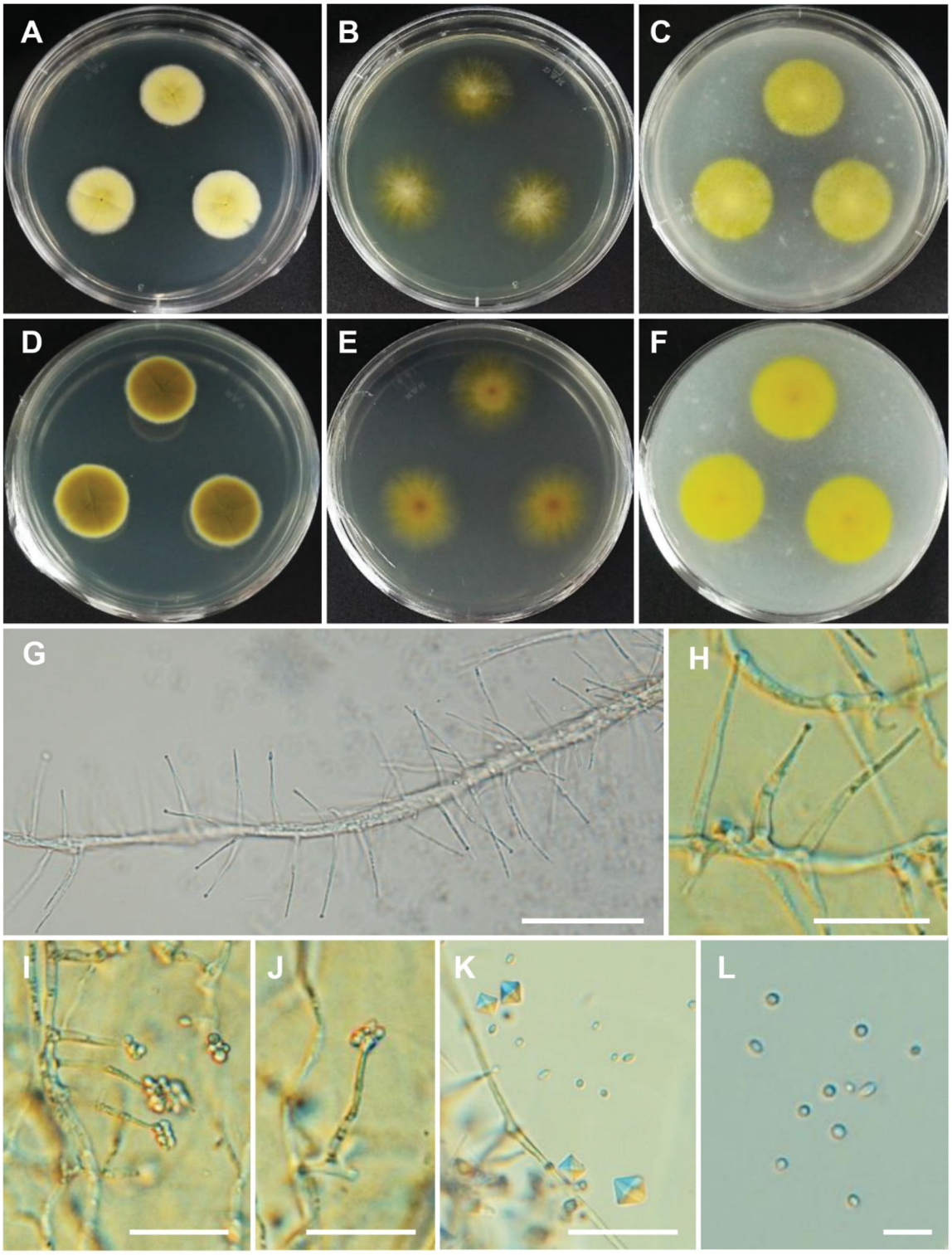

Figure 7. Morphology of *Acremonium guillematii* CNUFC YJW2-22. (A,D) Colonies on potato dextrose agar. (B,E) Colonies on malt extract agar. (C,F) Colonies on oatmeal agar. (A–C) obverse view, (D–F) reverse view. (G,H) Branched conidiophores. (I,J) Conidiophores and clustered conidia in slimy heads. (K) Octahedral crystals in culture. (L) Conidia. Scale bars: *G-K* = 20 μm, *L* = 10 μm.

HRS5-3-1 strains were clustered within the same clade as *L. nordwiniana* (type strain) (Figure 3). Molecular data analysis results were consistent with the phylogeny presented by Giraldo and Crous [63] and Crous et al. [57]. The morphological characteristics of the CNUFC HRS5-3 isolate were generally similar to those previously described by Crous et al. [57] such as long flexuous setae (up to 205 μm).

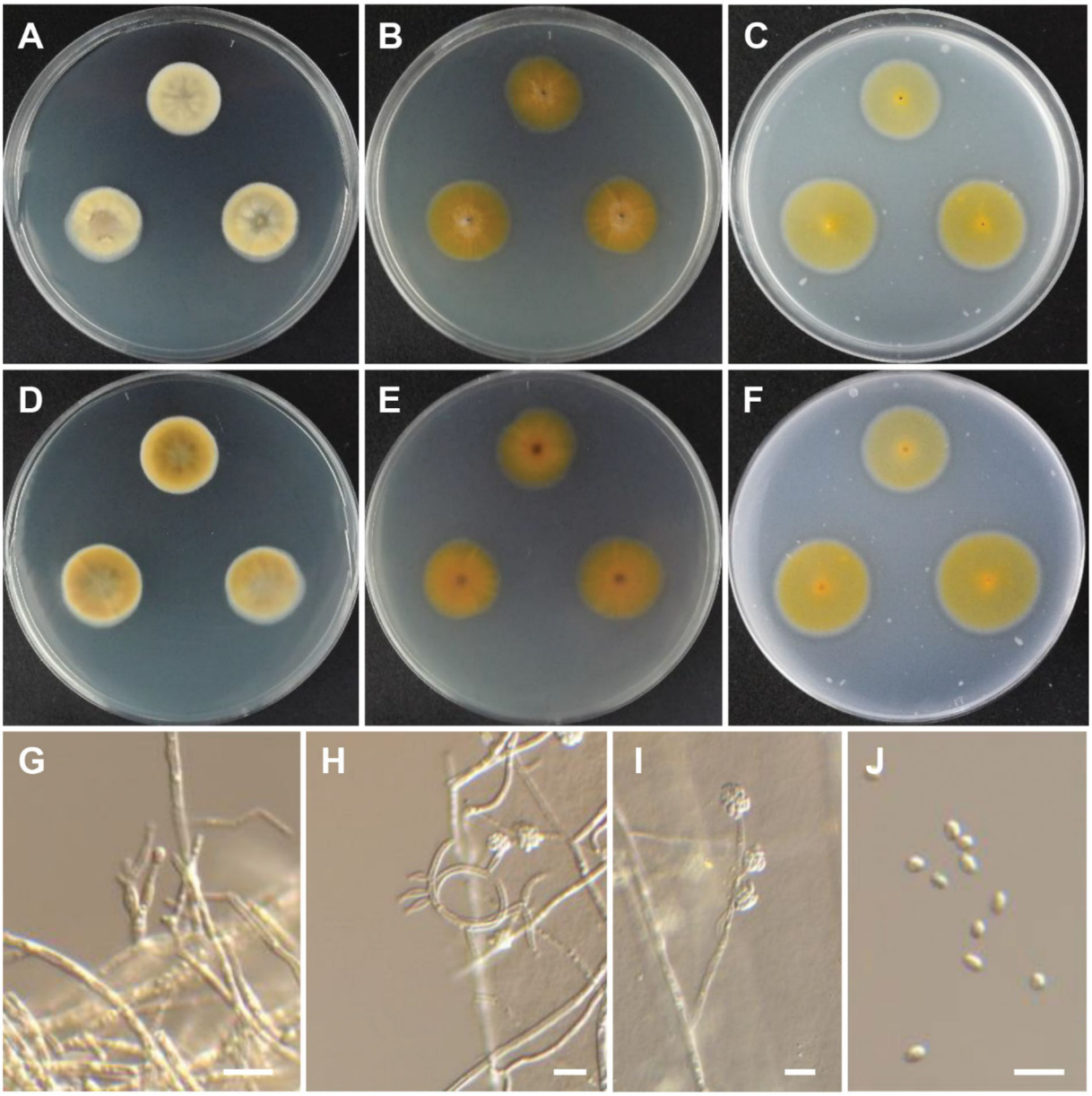

Figure 8. Morphology of *Cadophora novi-eboraci* CNUFC MSW11-6-2. (A,D) Colonies on potato dextrose agar. (B,E) Colonies on malt extract agar. (C,F) Colonies on oatmeal agar. (A–C) obverse view, (D–F) reverse view. (G–I) Conidiogenous cells and conidia in slimy heads. (J) Conidia. Scale bars: *G-J* = 10 μm.

However, the observations of the current study showed that the CNUFC HRS5-3 isolate produced salmon conidiomata, whereas *L. nordwiniana* (type strain) produces brown conidiomata [57] Interestingly, the strain isolated in the present produced extracellular enzymes including protease and lipase (data not shown). Therefore, the *Lectera* species obtained in this study may have useful applications based on this property. *L. nordwiniana* was previously isolated from Dutch soils [57], making the present study the first instance of the species being recovered from freshwater.

Phylogenetic analyses revealed that the CNUFC MSW242-6 and CNUFC MSW242-8 isolates were clustered with *M. corallina* (type strain) (Figure 4). The CNUFC MSW242-6 isolate was morphologically similar to *M. corallina* as it produced arthroconidia on 2% MEA medium [24]. Similar to *M. corallina* isolated by Marvanová et al. [24], the strain was isolated from stream foam in the present study. Therefore, stream foam is a habitat for *M. corallina*.

The phylogenetic tree created using ITS, BenA, and CaM sequences showed that the CNUFC DMWW2-2 and CNUFC DMWW2-2-1 isolates were placed within the *T. siamensis* clade, belonging to the section *Talaromyces* (Figure 5). The morphological characteristics of the *T. siamensis* isolate in this study were similar to those previously described by Yilma et al. [27]. However, differences in colony diameter were observed on CYA (CYA: 20–22 mm

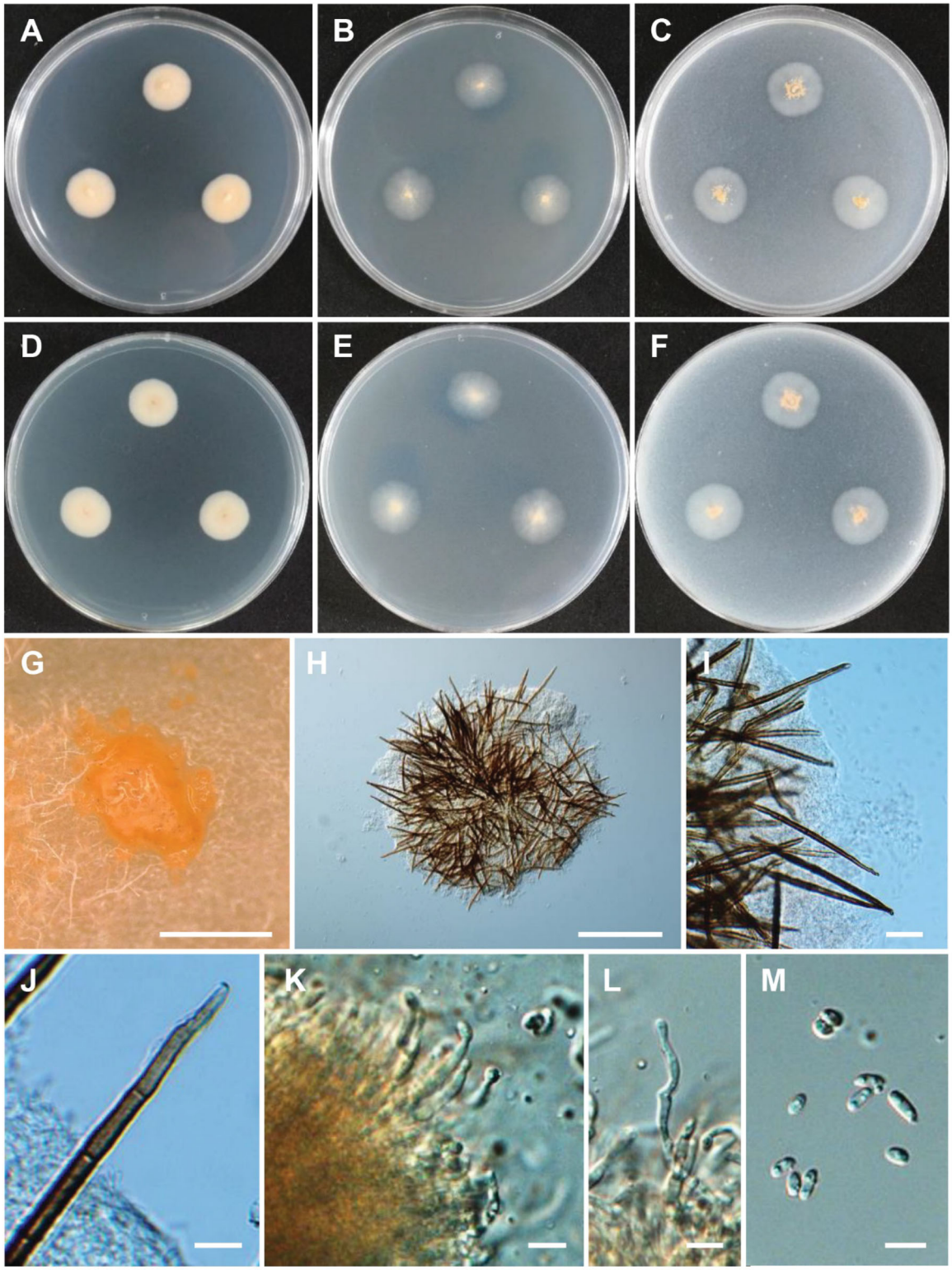

Figure 9. Morphology of *Lectera nordwiniana* CNUFC HRS5-3. (A,D) Colonies on potato dextrose agar. (B,E) Colonies on malt extract agar. (C,F) Colonies on oatmeal agar. (A–C) obverse view, (D–F) reverse view. (G) Young conidiomata on potato dextrose agar. (H,I) Conidiomata with setae. (J) Apex of a seta. (K,L) Phialides with conidia on acervuli. (M) Conidia. Scale bars: *G* = 1 mm, *H* = 200 μm, *I* = 20 μm, *J-M* = 10 μm.

after 7 days) and MEA (MEA: 32–33 mm after 7 days) media. *T. siamensis* is known to produce mitorubrins, penicillides, purpactins, vermixocins, secalonic acids D & F, and vermicellin [27], and the species was previously isolated from forest soil and house dust from Thailand [27]. The present study describes the first instance of *T. siamensis* isolated from freshwater.

Molecular phylogenetic analysis using ITS sequence indicated that CNUFC CPWS-1 and

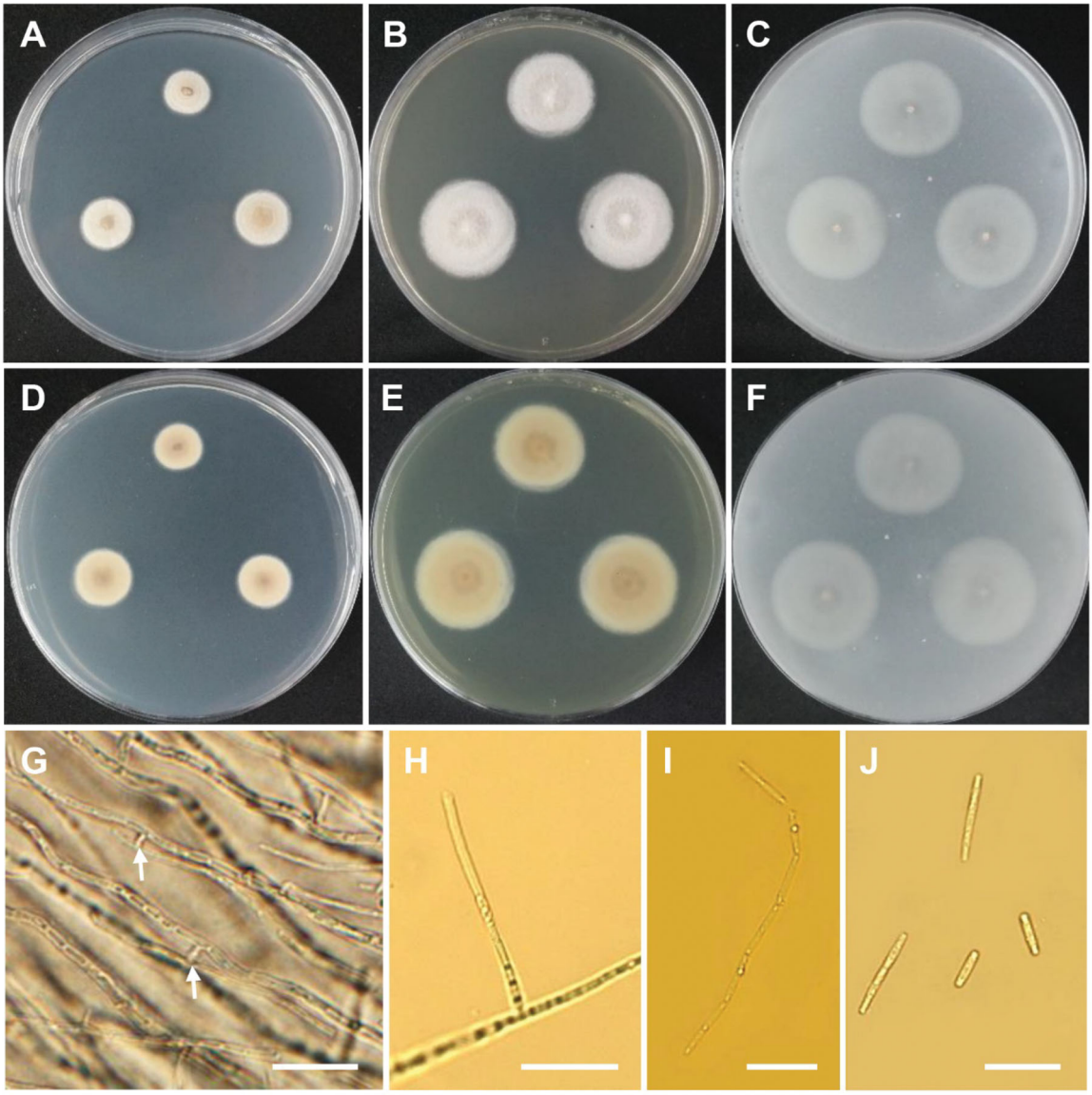

Figure 10. Morphology of *Mycoarthris corallina* CNUFC MSW242-6. (A,D) Colonies on potato dextrose agar. (B,E) Colonies on malt extract agar. (C,F) Colonies on oatmeal agar. (A–C) obverse view, (D–F) reverse view. (G) Anastomosing hyphae (white arrows). (H) Conidiophore. (I) Chain of arthroconidia. (J) Conidia. Scale bars: *G-J* = 20 µm.

CNUFC CPWS-2 were grouped with *Te. globosum* (type strain) (Figure 6), and the molecular data analyses of these species were consistent with the phylogeny presented by Wang et al. [39]. The CNUFC CPWS-1 isolate is morphologically-similar to *Te. globosum* as it produces globose and 1-celled conidia. However, the conidia sizes reported in the literature (3.0–5.5 µm) are slightly smaller than those of the isolates in the present study. *Te. globosom* was first isolated in a glacial soil sample from the Hailuogou Glacier in China, one of the most extreme environments on earth [39]. *Te. globosum* species has been isolated for the first time from freshwater in Korea. The occurrence of the species in such freshwater niche suggests that it plays an important role in aquatic ecosystems.

Recently, novel species in *Penicillium* (*Penicillium acidum* Hyang B. Lee, T.T. Duong & T.T.T. Nguyen, and *Penicillium aquaticum* Hyang B. Lee, T.T. Duong & T.T.T. Nguyen), *Rhizophydium* (*Rhizophydium koreanum* Hyang B. Lee, S.J. Jeon, T.T.T. Nguyen), and *Mucor* (*Mucor fluvii* Hyang B. Lee, S.H. Lee & T.T.T. Nguyen) have been discovered from freshwater niche in Korea [64,65]. A number of undescribed fungal species were also reported from freshwater [33,43–46]. However, only a small number of aquatic hyphomycetes were found in freshwater [47]. Therefore, a large number of undescribed fungal taxa in freshwater still remain undiscovered on planet. Future research focusing on aquatic ascomycetes or aquatic hyphomycetes known as Ingoldian fungi in Korea are needed to

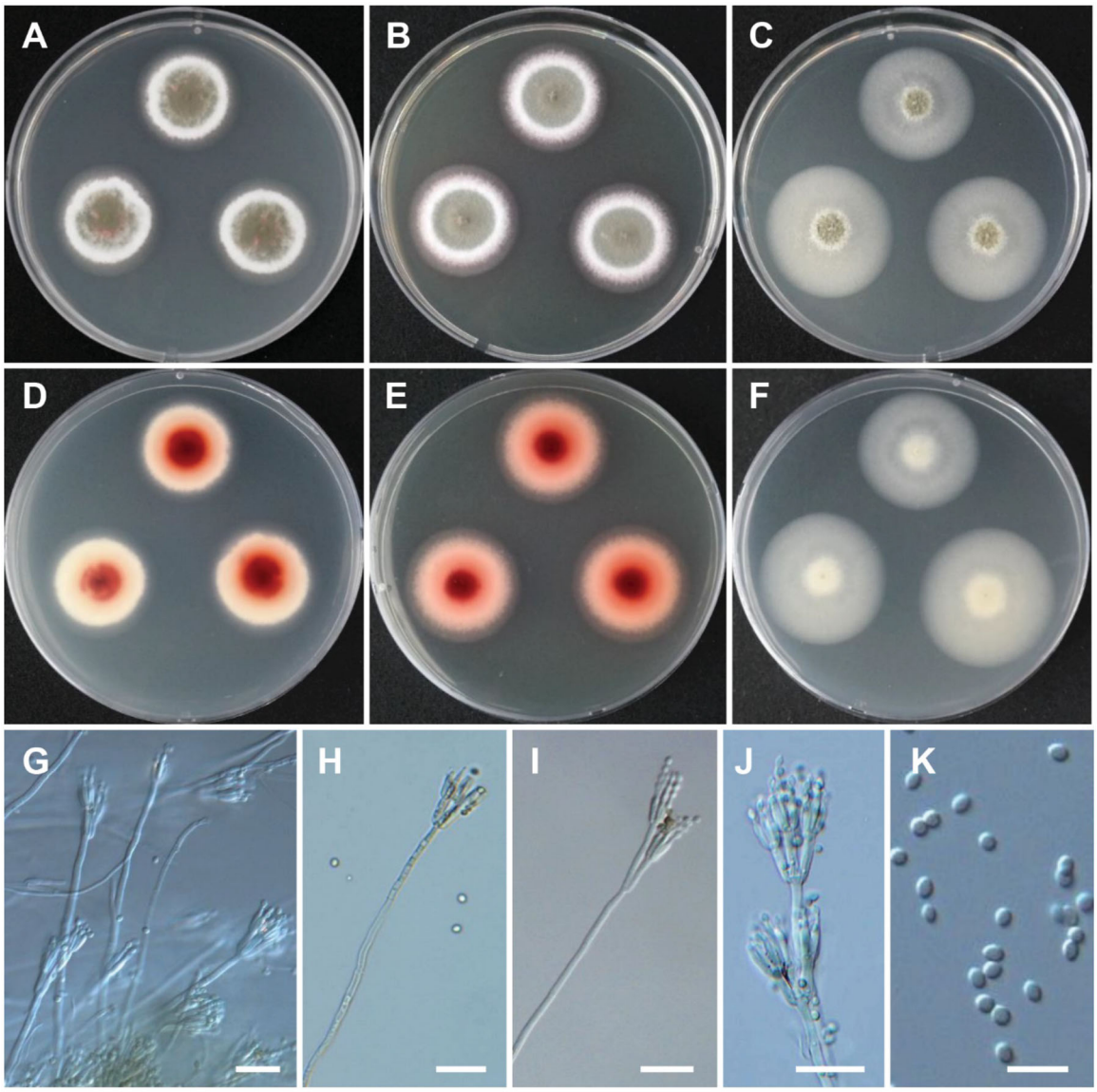

Figure 11. Morphology of *Talaromyces siamensis* CNUFC DMW2-2. (A,D) Colonies on czapek yeast autolysate agar. (B,E) Colonies on yeast extract sucrose agar. (C,F) Colonies on malt extract agar. (A–C) obverse view, (D–F) reverse view. (G–J) Conidiophores. (K) Conidia. Scale bars: *G-J* = 20 μm, *K* = 10 μm.

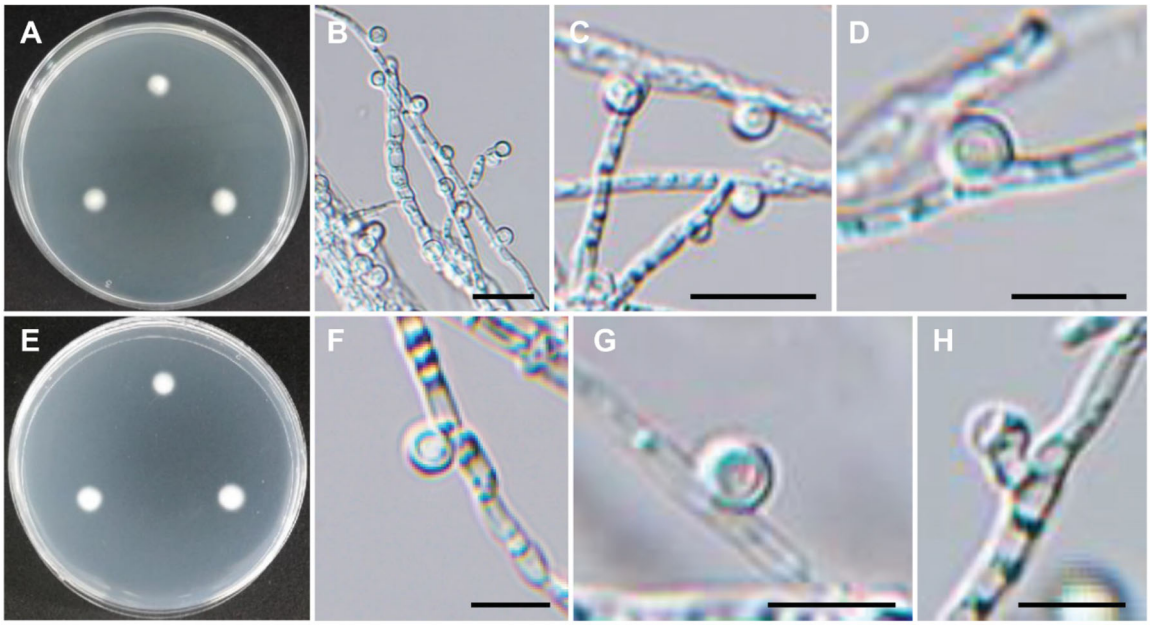

Figure 12. Morphology of *Tetracladium globosum* CNUFC CPWS-1. (A,E) Colonies on potato dextrose agar. (A) obverse view, (E) reverse view. (B–D) Conidia and hyphae. (F–H) Conidia. Scale bars: *B-C* = 20 μm, *D, F-H* = 10 μm.

improve our understanding of the ecology of these groups, especially rare genera.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was financially supported by Chonnam National University [Grant number: 2017-2827]. This work was in part supported by the Graduate Program for the Undiscovered Taxa of Korea funded by NIBR, and in part by the Project on Discovery of Fungi from Freshwater and Collection of Fungarium funded by NNIBR of the Ministry of Environment (MOE).

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