MYCOBIOLOGY
2019, VOL. 47, NO. 2, 173–179
https://doi.org/10.1080/12298093.2019.1611242

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Cladophialophora lanosa sp. nov., a New Species Isolated from Soil

Kallol Das, Seung-Yeol Lee and Hee-Young Jung

School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 41566, Korea

ABSTRACT

Cladophialophora is characterized by simple hyphomycetes with brown hyphae that give rise to branched chains of pale brown conidia and shows affinities with the Herpotrichiellaceae. A fungal strain belonging to the genus *Cladophialophora* was isolated from soil in Daegu, Korea. This strain produces numerous greenish to dark black lanose aerial mycelia with hair like structures. It is morphological similar to *C. chaetospira*, *C. inabaensis*, and *C. multiseptata;* however, the conidiophores and conidia sizes of the newly isolated strain (KNU16-032) are clearly different from them. The novelty of the strain was also confirmed based on phylogenetic analysis using the data sets of the internal transcribed spacer region of and the partial sequence of 28S ribosomal DNA region along with the cultural characteristics. Because morphological observations and phylogenetic analysis indicated that the strain is distinct from previously known *Cladophialophora* species, we propose this species as a new species *Cladophialophora lanosa* sp. nov., and provide the detailed descriptions in this study.

ARTICLE HISTORY
Received 22 November 2018
Revised 18 February 2019
Accepted 8 April 2019

KEYWORDS
Cladophialophora lanosa; herpotrichiellaceae; soil inhabiting fungi

1. Introduction

Cladophialophora Borelli are relatively simple hyphomycetes with brown hyphae that give rise to branched chains of pale brown conidia. *Cladophialophora* is an asexual, morphologically one-celled, and ellipsoidal to fusiform, dry conidia arising through blastic, acropetal conidiogenesis, branched chains and the chains are usually coherent and conidial scars are nearly unpigmented [1]. Currently, *Cladophialophora* comprises fewer than 30 species, which are opportunistic human pathogens, phytopathogens or are isolated from environmental sources and so far 11 species have been shown to cause disease in humans [2–6].

Moreover, the genus was initially erected to accommodate fungal species exhibiting both *Cladosporium* and *Phialophora*-like conidiogenesis. *Cladophialophora* includes species that show affinities with the Herpotrichiellaceae, as revealed by molecular data such as the internal transcribed spacer (ITS) and the large subunit ribosomal DNA region (28S rDNA) [7–9] and by the production of *Cladophialophora*-like anamorphs in some species of *Capronia*, the only known teleomorphic genus in this family [10,11]. A series of molecular phylogenetic studies revealed that *Cladophialophora* is polyphyletic in the order Chaetothyriales and the genus is closely related to members of several anamorphic genera, including *Exophiala*, *Cyphellophora*, *Fonsecaea*, *Knufia*, *Phialophora* and the teleomorphic genus *Capronia*. Particularly, two species of the genus *Cladophialophora* were presented as new species, the ITS and partial 28S rDNA data revealed the relationship with other species [12].

During an extensive investigation on the unreported fungi in Korea, strain KNU16-032 was isolated from soil. Based on morphological characteristics and molecular analysis, the fungus represents an undescribed species belonging to the genus *Cladophialophora*. In this study, the isolated fungus is described and illustrated as a novel species.

2. Materials and methods

2.1. Collection of soil and isolation

During the investigation of unrecorded fungal species in 2016, soil was collected from Daegu, Korea (35°53′41.6″N, 128°35′10.1″E). Afterward, the collected soil (1 g) was diluted with 10 mL of sterile distilled water and vortexed gently to mix with the sterile water. Then, it was diluted serially and spread on the potato dextrose agar (PDA; Difco, Detroit, MI) plates. The plates were incubated at 25 °C for 3 days without any disturbance. After 3 days, numerous single colonies were observed growing on the plates. Then, the single colonies were transferred to new PDA plates and again put into incubation at 25 °C to favor the growth of fungal mycelia. The

CONTACT Hee-Young Jung heeyoung@knu.ac.kr School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 41566, Korea

strain KNU16-032 was selected based on cultural characteristics and molecular phylogenetic analyses.

2.2. Morphological characterization

The morphological characteristics of the strain were determined on PDA, oatmeal agar (OA; Difco), and malt extract agar (MEA; Difco) [13]. All the three media were used to investigate the morphological characteristics of the strain KNU16-032 followed by an incubation period of 21 days at a temperature of 25 °C. After incubation, the diameter of the colonies of each medium was measured, and the colony color of the strain was observed. A light microscope (BX-50, Olympus, Tokyo, Japan) was used to observe the mycological characteristics of the strain.

2.3. DNA extraction, PCR, and sequencing

Genomic DNA was extracted from mycelia using the HiGene Genomic DNA prep kit (Biofact, Daejeon, Korea). Molecular identification of the strain at genus and species levels was conducted using sequences of the ITS and 28S rDNA region. The ITS regions, including 5.8S, were amplified using the ITS primers ITS1/ITS4 [14] and PCR amplification was initiated with 2 min denaturation at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min and 30 s, followed by a final extension at 72 °C for 5 min. In case of 28S ribosomal DNA gene, the PCR amplification was performed using LROR/LR5 primer pair [15]. The amplified PCR products were purified with EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA) and sequenced by Solgent (Daejeon, Korea). The obtained sequences from KNU16-032 were deposited in NCBI GenBank under accession numbers LC387460 and LC387461 for ITS region and the partial 28S ribosomal DNA genes, respectively.

2.4. Phylogenetic analyses

The reference sequences were retrieved from the National Center for Biotechnology Information (NCBI). Evolutionary matrices for the maximum likelihood, neighbor-joining and maximum-parsimony were constructed using Kimura's two-parameter model [16]. Tree topology was inferred by the maximum likelihood, neighbor-joining and maximum-parsimony method using the program MEGA7 with bootstrap values based on 1,000 replications [17].

3. Results

3.1. Taxonomy

The collected *Cladophialophora* species KNU16-032 showed distinct morphological structures compared with other allied species. Therefore, it is described as a new species.

Cladophialophora lanosa K. Das, S.Y. Lee and H.Y. Jung, sp. nov. (Figures 1 and 2).

MycoBank: MB826874

Etymology: The specific name lanosa, derived from Latin *lana*, meaning woolly, refers to the fleece-like appearance of aerial mycelia.

Typus: Daegu, Korea (35°53′41.6″N, 128°35′10.1″E), isolated from soil. The stock culture (NIBRFG0000499862 = KCTC 56424) was deposited in the National Institute of Biological Resources (NIBR) and Korea Collection for Type Cultures (KCTC), metabolically inactive culture.

Habitat: On soil. The soil was yellowish brown, fine gravelly clay loam, lower moisture capacity.

Cultural characteristics: The average diameters of the strain colonies in PDA, OA, and MEA were 24.4, 21.2, and 25.5 mm, respectively. The growth of mycelial colonies was round, and there were also variations in the diameter of the colonies of across the different media (Figure 1). They produced greenish to dark black mycelia with dark brown to black color mycelia appeared in opposite side of the PDA medium (Figure 1(a,b)). The strain was slow growing and developed greenish to dark black lanose aerial mycelia with hair-like structures. The produced numerous lanose aerial mycelia, could be clearly differentiated from aerial mycelia (Figure 2(a,b)).

Morphological characteristics: Hyphae were irregularly septate, straight or bent, smooth, thin-walled, hyaline to brown in color, guttulate, branched, with a size of width 1.4–1.7 µm, with formation of hyphal strands, and differentiated subglobose conidiophores, as well as numerous conidia. Conidiophores were solitary, macronematous, well distinguishable under the light microscope from aerial mycelium, pale to brown, subcylindrical, straight to somewhat curved and erect with the wide 1.2–2.5 µm (Figure 2(f)). Conidiogenous cells were branched, smooth-walled, and round (Figure 2(g–h)). Conidia were one-celled, smooth, acropetal, catenulate, hyaline to pale brown with a size in the range of 2.1–4.4 × 2.0–3.2 µm, ramoconidia subcylindrical, guttulate (Figure 2(c–e)).

Notes: The new species is morphologically similar to *C. chaetospira*, *C. inabaensis*, and *C. multiseptata*. *Cladophialophora lanosa* produces numerous greenish to dark black lanose aerial mycelia with hair like structures, whereas *C. inabaensis* produces

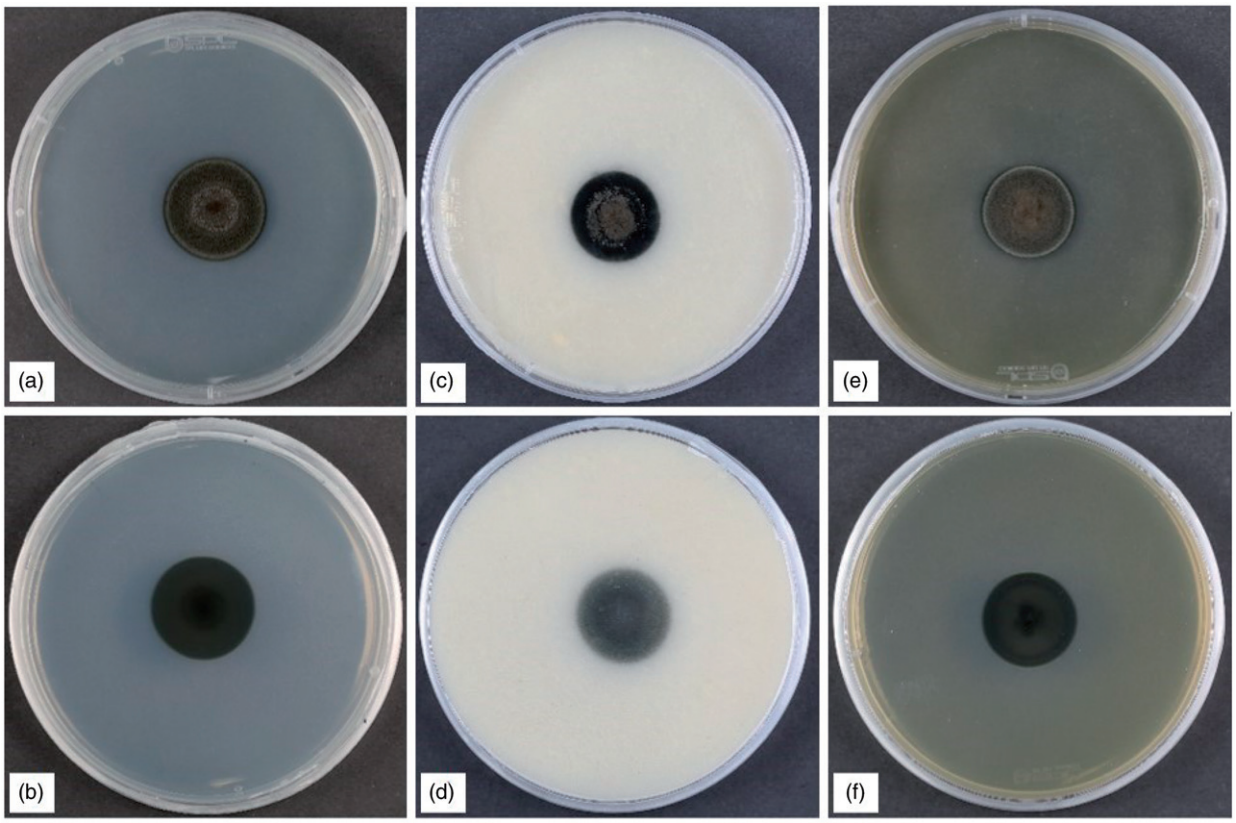

Figure 1. Cultural characteristics of KNU16-032 (a–f), Colony on PDA (a, b), OA (c, d) and MEA (e, f) and in front and reverse, accordingly.

dark gray to dark brown in color, felty, with lanose aerial mycelia on PDA media [18]. The average size of hyphae of *C. lanosa* is 1.4–1.7 µm, while hyphae of *C. inabaensis, C. multiseptata* and *C. chaetospira* are 1.6–3.6 µm, 2.0–4 µm, 2.0–3.5 µm, respectively (Table 1). The average size of conidia of *C. lanosa* (3.4 × 2.7 µm) is shorter than *C. inabaensis* (4.6 × 3.9 µm). In case of *C. multiseptata,* the conidial size ranges 4.5–18 × 3–5 µm [18] and the conidial size of *C. chaetospira* is 25–30 × 3–4 µm (Table 1) [2].

3.2. Phylogenetic analyses

After analyzing the sequences, 581 and 764 bp were obtained from the ITS regions and 28S ribosomal DNA gene, respectively. The BLAST search results of ITS region of *C. lanosa* revealed 92.3% and 92.9% similarities with that of *C. chaetospira* CC 14-28 (KF359558) and *C. inabaensis* EUCL1 (LC128795), respectively. In case of the partial 28S ribosomal DNA gene showed 98.4% and 98.8% similarities with *C. chaetospira* CBS 514.63 (MH869959) and *C. inabaensis* EUCL1 (LC128795), respectively. The lodged sequences of existing *Cladophialophora* species from GenBank were used to compare the ITS region and partial 28S rDNA region to explore this fungal study (Table 2). Maximum-likelihood phylogenetic tree showed the relationship between the strain KNU16-032 and other *Cladophialophora* species based on the ITS and the partial 28S rDNA regions (Figure 3). The neighbor-joining and maximum-parsimony were also constructed to determine the exact taxonomic position of the strain and indicated with the nodes in maximum-likelihood phylogenetic tree. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the neighbor-joining and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the neighbor-joining or maximum-parsimony algorithms (Figure 3). As the results, the phylogenetic tree revealed that the phylogenetic position of KNU16-032 was clearly separated from the *C. inabaensis*. In this reason, the KNU16-032 originating from soil was phylogenetically distinct from the other species of *Cladophialophora*.

4. Discussion

Availability of DNA sequencing technology in the past two-to-three decades has generated an enormous amount of DNA sequence data, allowing fungal taxonomy through phylogenetic revolution. To assess the phylogenetic position of *C. floridana* and *C. tortuosa*, ITS and partial 28S rDNA regions were used previously [12]. The reason for using the gene is to classify the strain KNU16-032 to determine the

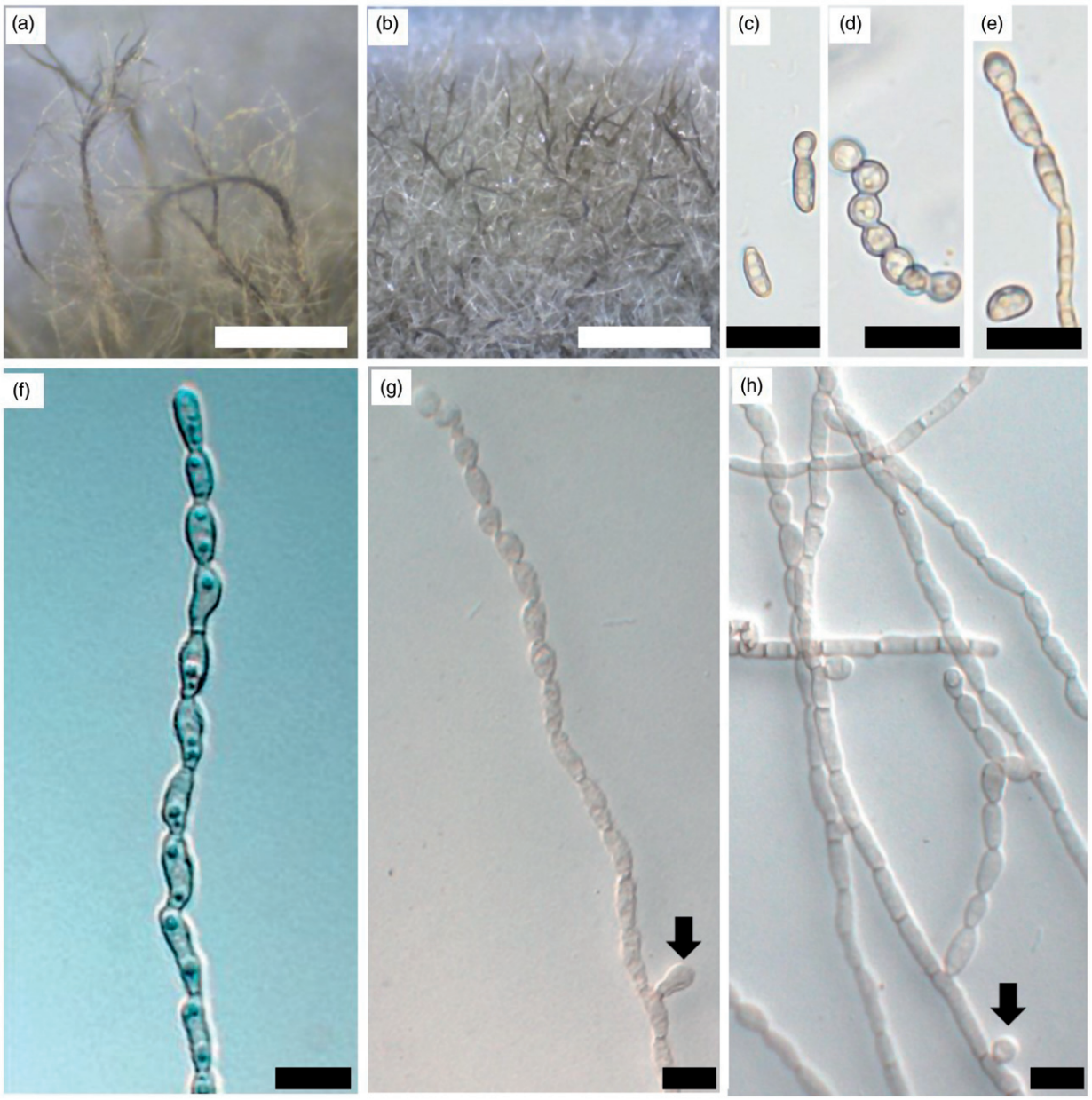

Figure 2. Morphological characteristics of KNU16-032. Lanose aerial mycelia on stereo microscope (a, b); conidia and conidial chains (c, d, e); conidiophores (f), and conidiogenous cells (g, h). Arrows indicated conidiogenous cells. Scale bars: a, b = 1000 μm; c–h = 10 μm.

Table 1. Morphological characteristics of *Cladophialophora lanosa* sp. nov. in this study and comparison with the closest species of the genus *Cladophialophora*.

Characteristics	*Cladophialophora lanosa*[a] (KNU16-032)	*Cladophialophora inabaensis*[b]	*Cladophialophora multiseptata*[c]	*Cladophialophora chaetospira*[d]
Cultural characteristics	Greenish to dark black lanose aerial mycelia with hair like structures and round brown margin at the edge; reverse black on PDA.	Dark gray to dark brown in color, felty, with lanose aerial mycelia on PDA.	Olive-gray with olivaceous-black, slightly lobate margin; reverse black on PDA.	Irony-gray, olivaceous-black; reverse olivaceous-black on PDA
Hyphae wide (μm)	1.4–1.7	1.6–3.6	2.0–4.0	2.0–3.5
Conidiophore wide (μm)	1.2–2.5	N/A	2.0–4.0	3.0–4.0
Conidia (μm)	2.1–4.4 × 2.0–3.2	3.4–7.2	4.5–18 × 3–5	25–30 × 3–4

N/A: not available in previous references.
[a]Fungal strain studied in this paper.
[b]Sources of the descriptions [18].
[c]Sources of the descriptions [23].
[d]Sources of the descriptions [2].

Table 2. List of species used in this study and their GenBank accession numbers for the phylogenetic analysis.

		GenBank Accession Numbers		
Speceis	Strain Numbers	ITS + LSU	ITS	LSU
Cladophialophora arxii	CBS 306.94 ^{T}	–	NR 111280	KX822320
C. australiensis	CBS 112793 ^{T}	EU035402	–	–
C. bantiana	UM 956	–	KU928131	KU928133
C. boppii	CBS 126.86	–	NR 131297	FJ358233
C. chaetospira	CBS 114747	–	KF928450	KF928514
C. devriesii	CBS 147.84 ^{T}	–	NR 111279	KC809989
C. floridana	SR1004	AB986344	–	–
C. immunda	CBS 834.96 ^{T}	–	NR 111283	KC809990
C. inabaensis	EUCL1 ^{T}	LC128795	–	–
C. matsushimae	MFC-1P384	–	FN549916	FN400758
C. minourae	CBS 556.83 ^{T}	–	AY251087	FJ358235
C. multiseptata	FMR 10591	–	HG003668	HG003671
C. mycetomatis	CBS 454.82	–	LC192112	LC192077
C. parmeliae	CBS 129337	–	JQ342180	JQ342182
C. pseudocarrionii	CBS 138591	–	KU705827	KU705844
C. samoensis	CBS 259.83 ^{T}	–	NR 111282	KC809992
C. saturnica	CBS 102230	–	AY857508	KC809993
C. subtilis	CBS 122642 ^{T}	–	NR 111363	KX822322
C. tortuosa	BA4b006 ^{T}	AB986424	–	–
C. yegresii	CBS 114405	–	EU137322	KX822323
C. lanosa	KNU16-032 ^{T}	–	LC387460	LC387461
Phialophora reptans	CBS 113.85 ^{T}	–	NR 121346	JQ766493

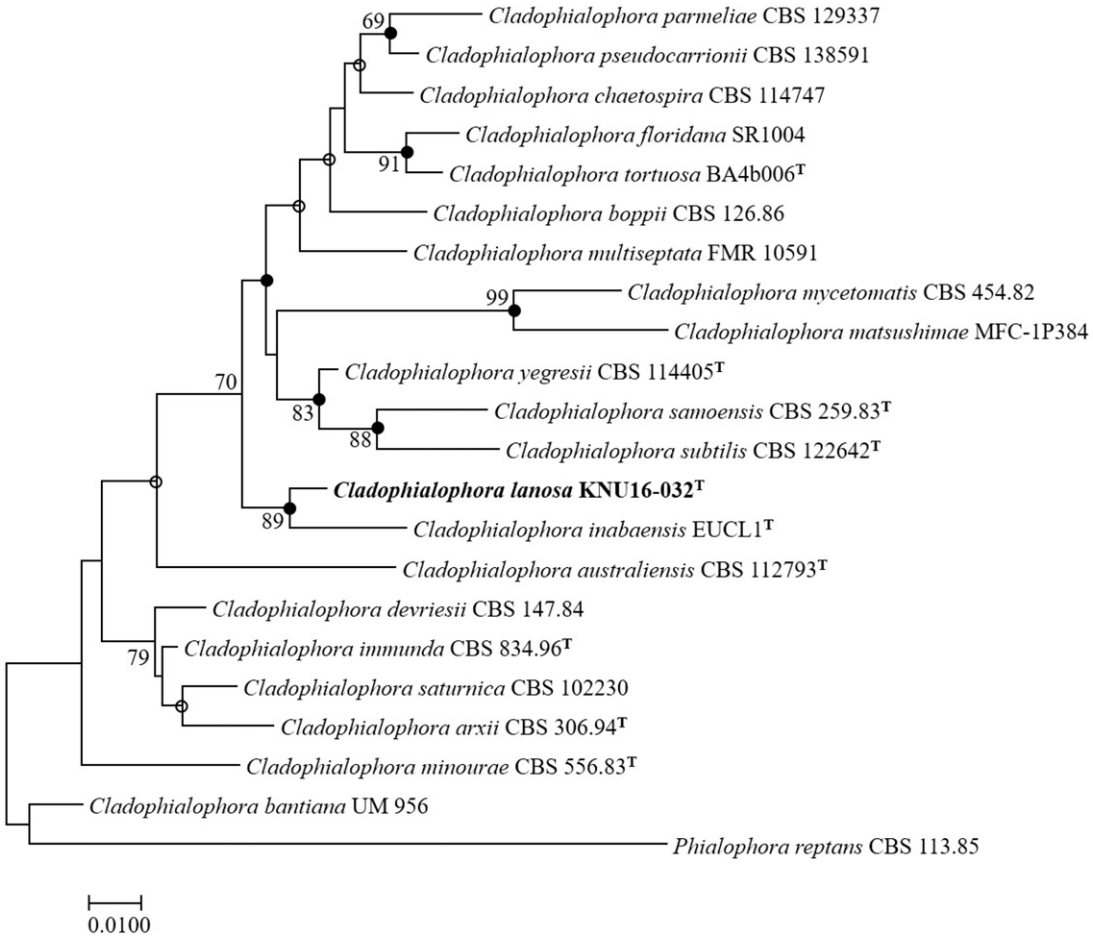

Figure 3. Maximum-likelihood phylogenetic tree based on the combined internal transcribed spacer (ITS) sequences and the partial sequence of 28S ribosomal DNA genes, showing the relationship between *Cladophialophora lanosa* sp. nov. with the closest *Cladophialophora* spp. *Phialophora reptans* CBS 113.85 was used as an outgroup. The numbers above the branches represent the bootstrap values obtained for 1000 replicates (values smaller than 60% were not shown). The isolated strain of this study is indicated in bold. Bar, 0.01 substitutions per nucleotide position.

taxonomic position. The phylogenetic trees revealed that the strain KNU16-032 was distinct from the other known *Cladophialophora* species, confirmed with the results of neighbor-joining, maximum-parsimony and maximum-likelihood phylogenetic trees (Figure 3).

There are numerous fungi from different habitats and diverse geographic regions. *C. inabaensis* isolated from eggplant roots using the baiting method in Japan [18]. Several studies have reported on plant-associated *Cladophialophora* species such as *C. hostae*, *C. scillae*, *C. proteae*, and *C. sylvestris,* whereas, the species *C. australiensis* and *C. potulentorum* were found in soft drinks [4]. *C. chaetospira* found on the roots of Chinese cabbage [19] and also in association with bamboo, on roots of *Picea abies* and from soil collected in a wheat field [2]. Moreover, the genus *Cladophialophora* comprises a number of environmental saprobes such as *C. saturnica* from plant debris in the environment. And the members of the genus can also be isolated from diverse environment conditions [5]. *C. carrionii* was particularly isolated in arid and semiarid climates of e.g. South and Central America and Australia [20–21]. *C. abundans* was isolated from muddy burrows of the mangrove-land crab (*Ucides cordatus*) in Brazilian mangrove habitats [22]. The current study identified a novel species (*C. lanosa* sp. nov.) from soil in Korea. The precise ecological niches of *Cladophialophora* varies from different habitat, hosts and diverse geographic regions. So, this might be a clue to explore their behavior as rare environmental oligotrophs as well as invaders of human tissue containing aromatic neurotransmitters. The understanding of the phylogeny and ecology of *Cladophialophora* is therefore essential.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

We 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).

References

[1] de Hoog GS, Queiroz-Telles F, Haase G, et al. Black fungi: clinical and pathogenic approaches. Med Mycology. 2000;38:243–250.

[2] Crous PW, Schubert K, Braun U, et al. Opportunistic, human-pathogenic species in the Herpotrichiellaceae are phenotypically similar to saprobic or phytopathogenic species in the Venturiaceae. Stud Mycol. 2007;58:185–217.

[3] Horré R, de Hoog GS. Primary cerebral infections by melanized fungi: a review. Stud Mycol. 1999;43:176–193.

[4] Badali H, de Hoog GS, Curfs-Breuker I, et al. Use of amplified fragment length polymorphism to identify 42 *Cladophialophora* strains related to cerebral phaeohyphomycosis with in *vitro* antifungal susceptibility. J Clin Microbiol. 2010;48:2350–2356.

[5] Badali H, Gueidan C, Najafzadeh MJ, et al. Biodiversity of the genus *Cladophialophora*. Stud Mycol. 2008;61:175–191.

[6] Lastoria C, Cascina A, Bini F, et al. Pulmonary *Cladophialophora boppii* infection in a lung transplant recipient: case report and literature review. J Heart Lung Transplant. 2009;28:635–637.

[7] Eriksson OE, Baral H-O, Currah RS, et al. Outline of Ascomycota–2003. Myconet. 2003;9:1–89.

[8] Abliz P, Fukushima K, Takizawa K, et al. Identification of pathogenic dematiaceous fungi and related taxa based on large subunit ribosomal DNA D1/D2 domain sequence analysis. FEMS Immunol Med Microbiol. 2004;40:41–49.

[9] Caligiorne RB, Licinio P, Dupont J, et al. Internal transcribed spacer rRNA gene-based phylogenetic reconstruction using algorithms with local and global sequence alignment for black yeasts and their relatives. J Clin Microbiol. 2005;43:2816–2823.

[10] Muller E, Petrini O, Fisher PJ, et al. Taxonomy and anamorphs of the Herpotrichiellaceae with notes on generic synonymy. Trans Br Mycol Soc. 1987;88:63–74.

[11] Untereiner WA. *Capronia* and its anamorphs: exploring the value of morphological and molecular characters in the systematics of the Herpotrichiellaceae. Stud Mycol. 2000;45:141–149.

[12] Obase K, Douhan GW, Matsuda Y, et al. *Cladophialophora floridana* and *Cladophialophora tortuosa*, new species isolated from sclerotia of *Cenococcum geophilum* in forest soils of Florida, USA. Mycoscience. 2016;57:26–34.

[13] Gams W, Verkley GJM, Crous PW. CBS course of mycology. 5th ed. Utrecht (Netherlands): Centraalbureau voor Schimmelcultures; 2007.

[14] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York (NY): Academic Press, Inc.; 1990. p. 315–322.

[15] Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol. 1990;172:4238–4246.

[16] Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16:111–120.

[17] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–1874.

[18] Usui E, Takashima Y, Narisawa K. *Cladophialophora inabaensis* sp. nov., a new species among the dark septate endophytes from a secondary forest in Tottori, Japan. Microbes Environ. 2016;31:357–360.
[19] Usuki F, Narisawa K. A mutualistic symbiosis between a dark septate endophytic fungus, *Heteroconium chaetospira*, and a nonmycorrhizal plant, Chinese cabbage. Mycologia. 2007;99:175–184.
[20] Trejos A. *Cladosporium carrionii* n. sp. and the problem of *Cladosporia* isolated from chromoblastomycosis. Rev Biol Trop. 1954;2:75–112.
[21] Ridley MF. The natural habitat of *Cladosporium carrionii,* a cause of chromoblastomycosis in man. Aust J Dermatol. 1957;4:23–27.
[22] Feng P-Y, de Hoog GS, Najafzadeh MJ, et al. *Cladophialophora abundans*, a novel species of Chaetothyriales isolated from the natural environment. Mycol Progress. 2014;13: 381–391.
[23] Crous PW, Wingfield MJ, Guarro J, et al. Fungal planet description sheets: 154-213. Persoonia. 2013;31:188–296.