MYCOBIOLOGY
2023, VOL. 51, NO. 2, 87–93
https://doi.org/10.1080/12298093.2023.2195735

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Morphological and Phylogenetic Analyses Reveal a New Species of Genus *Monochaetia* Belonging to the Family Sporocadaceae in Korea

Seong-Keun Lim[a]*, Kallol Das[a]*, Soo-Min Hong[a], Sang Jae Suh[a,b], Seung-Yeol Lee[a,b] and Hee-Young Jung[a,b]

[a]College of Agriculture and Life Sciences, Kyungpook National University, Daegu, South Korea; [b]Institute of Plant Medicine, Kyungpook National University, Daegu, South Korea

ABSTRACT

The fungal strain belonging to the genus *Monochaetia* of the family Sporocadaceae was isolated from hairy long-horned toad beetle (*Moechotypa diphysis*) during the screening of microfungi associated with insects from Gangwon Province, Korea. The strain KNUF-6L2F produced white, light brown to dirty black surface, and olivaceous green colonies with the higher growth, while the closest strain *M. ilicis* KUMCC 15–0520^{T} were light brown to brown, and *M. schimae* SAUCC 212201^{T} light brown to brown toward center. The strain KNUF-6L2F produced shorter (5.7–14.0 μm) apical appendages than *M. ilicis* (6.0–24.0 μm), but similar to *M. schimae* (7.0–12.5 μm). Three median cells of KNUF-6L2F were light brown to olivaceous green, whereas brown and olivaceous cells were observed from *M. ilicis* and *M. schimae*, respectively. And the strain KNUF-6L2F produced larger conidiogenous cells than *M. ilicis* and *M. schimae*. Additionally, phylogenetic analyses based on molecular datasets of internal transcribed spacer (ITS) regions, translation elongation factor 1-alpha (*TEF1α*), and β-tubulin (*TUB2*) genes corroborated the strain's originality. Thus, the strain is different from other known *Monochaetia* species, according to molecular phylogeny and morophology, hence we suggested the new species *Monochaetia mediana* sp. nov. and provided a descriptive illustration.

ARTICLE HISTORY
Received 8 March 2023
Revised 22 March 2023
Accepted 22 March 2023

KEYWORDS
Moechotypa diphysis; *Monochaetia*; morphology; phylogeny; Sporocadaceae

1. Introduction

The family Sporocadaceae was introduced by Corda in 1842 with the type genus *Sporocadus* and the species of this family are endophytic, plant pathogenic or saprobic, and associated with a wide range of host plants [1–3]. The family included genus *Bartalinia*, *Monochaetia*, *Neopestalotiopsis*, *Pestalotiopsis*, *Pseudopestalotiopsis*, *Seiridium*, and many other species of asexual morph bearing conidial appendages [5–6]. Most genera have conidia that are multi-septate, essentially fusiform, have appendages at one or both ends, and frequently have some melanized cells. Also referred to as pestalotioid fungus since they resemble taxa that have affinities to genus *Pestalotia* [3]. The genus *Monochaetia* was introduced considering the type species as *M. monochaeta* including 23 species [7]. Numerous *Monochaetia* species were reassigned to *Pestalotiopsis* or *Truncatella* and the monograph of Guba recognized more than 40 species of *Monochaetia* [8,9]. There are 136 *Monochaetia* epithets in MycoBank (https://www.mycobank.org- accessed on March 8 2023). Moreover, *Monochaetia* is a pestalotiopsis-like genus [4] and introduced the family Pestalotiopsidaceae to accommodate with other pestalotiopsis-like genera [10]. Additionally, the similar conidia with an apical appendage can be seen in *Monochaetia*, *Pestalotia*, and *Pestalotiopsis*. Conidia that are septate and have a single apical appendage distinguish *Monochaetia* from the latter two taxa. The species in this genus are typically saprophytes, plant parasites, and carriers of leaf spot diseases on a variety of hosts [11]. Although it can be a rare occurrence and distribution, the majority of *Monochaetia* species lack molecular data [12]. It has been also noted that several *Monochaetia* species produce bioactive substances such taxol, ambuic acid, and chaetiacandin [13–15].

The purpose of continuing study on the native fungal species in Korea is to improve our understanding of their diversity, distribution, and ecology, as well as the possible applications of these findings in a variety of industries, including Korean agricultural environments.

2. Material and methods

2.1. Sample collection and fungal isolation

The insect samples were collected from Gangwon Province (37°14′13.2″N, 129°04′04.4″E), Korea,

CONTACT Seung-Yeol Lee leesy1123@knu.ac.kr
*Seong-Keun Lim and Kallol Das contributed equally to this manuscript.

transferred immediately to the laboratory and stored at 4 °C until use. The insect was then rinsed three times: twice with sterile distilled water, once with 70% ethanol, and finally ground with a hand grinder. The isolation was performed using the dilution plating technique, as described previously [16]. After transferring the single colonies from the plates to fresh PDA plates, they were kept in an incubator at 25 °C for five to seven days. Subsequently, the strain was selected for further molecular analyses and fungal strain was maintained in 20% glycerol at −80 °C for further study.

2.2. Cultural and morphological characterization

Cultural characteristics and morphological observations were studied using the cultural media according to genus by following previous studies and the strain was transferred to PDA incubated at 25 °C for 7 and 15 days [12,17]. The characteristics of the colonies were then recorded, and the mycological features were observed by examining the fungal structures under a light microscope (BX-50; Olympus, Tokyo, Japan).

2.3. Genomic DNA extraction, PCR amplification, sequencing

Total genomic DNA of strain KNUF-6L2F was extracted from the fungal mycelia grown on the PDA plate using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) following the manufacturer's protocol. The internal transcribed spacer regions with intervening 5.8S nrRNA gene (ITS), 28S ribosomal DNA for the large subunit (LSU), partial β-tubulin (*TUB2*), and translation elongation factor 1-alpha (*TEF1α*) genes were amplified and sequenced by using primers pairs ITS1F/ITS4 [18,19], LROR/LR5 [20,21], T1/Bt2b [22,23], and EF1-728F/EF-2 [24,25]. The quality of PCR products was analyzed by 1.2% agarose gel electrophoresis and stained with ethidium bromide. The purified product was purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and then sent to SolGent for sequencing (Daejeon, Korea).

2.4. Molecular phylogenetic analyses

Sequences from NCBI, the National Center for Biotechnology Information, were used to construct the phylogenetic trees (Table 1). Ambiguous regions were deleted from alignments and evolutionary distance matrices for the neighbor-joining (NJ) algorithm were calculated using Kimura's two-parameter model [26]. This analysis also identified nodes with filled circles in the NJ phylogenetic tree [27]. Open circles showed corresponding nodes from maximum likelihood or maximum parsimony algorithms [28,29]. The NJ method was inferred by tree topology using MEGA7.0 software program with bootstrap values based on 1000 replications [30].

3. Results

3.1. Taxonomical analysis of Monochaetia mediana sp. nov.

The strain KNUF-6L2F exhibited distinct morphology from those of allied species of *Monochaetia* and was therefore described as a new species.

Monochaetia mediana **S.Y. Lee and H.Y. Jung, sp. nov.** (Figure 1).

MycoBank: MB 847592

Etymology: The specific name is derived from the Latin adjective "*medianus*", -a, -um, meaning "median", referring to the median cells of conidia.

Typus: Gangwon Province (37°14′13.2″N, 129°04′04.4″E), isolated from hairy long-horned toad beetle (*Moechotypa diphysis*). The stock culture (NIBRFGC000509947) was deposited in the National Institute of Biological Resources (NIBR) as a metabolically inactive culture.

Habitat and known distribution: Samcheok-si, Gangwon Province, Korea, from hairy long-horned toad beetle (*Moechotypa diphysis*).

Cultural characteristics: Colonies on PDA 50.0–54.0 and 60.0–63.0 mm in diameter after 7 and 15 days at 25 °C, respectively. Colonies were circular, zonate, undulate, canaliculate, whitish margin, light brown, dirty black surface at the center, olivaceous green colonies irregularly shown; reverse white to light brown toward center and dark brown at the center (Figure 1(A,B)).

Morphological characteristics: Conidiomata 150–805 µm diam ($n = 20$), pycnidial, mostly solitary, scattered, immersed, brown to black, glabrous, releasing conidia by breaking the surface grown on PDA (Figure 1(C)). Conidiophores indistinct. Conidiogenous cells 6.0–23.8 × 3.0–6.2 µm, holoblastic, phialidic, discrete, cylindrical, hyaline, smooth, thin-walled (Figure 1(D,E)). Conidia 17.8–28.1 × 4.4–5.5 µm diam. ($\bar{x} = 22.1 \times 4.8$ µm), fusiform, tapering at both ends, 4-septate, erect or sometimes slightly curved; apical cell 3.1–5.3 µm long ($\bar{x} = 4.0$ µm), conical, hyaline and smooth-walled; three median cells together 11.5–17.0 µm long ($\bar{x} = 13.8$ µm), doliiform, light brown to olivaceous green, rough-walled, upper second cell 3.1–5.1 µm long ($\bar{x} = 4.3$ µm), upper third cell 3.0–5.9 µm long ($\bar{x} = 4.2$ µm), upper fourth cell 3.4–4.8 µm long ($\bar{x} = 4.1$ µm); basal cell 3.1–5.8 µm long ($\bar{x} = 4.3$ µm), conic, hyaline and smooth-walled; apical appendage 5.7–14.1 µm long ($\bar{x} = 9.3$ µm),

Table 1. GenBank accession numbers used for the phylogenetic analyses in this study.

Species	Strain numbers	GenBank accession numbers		
		ITS	*TEF1α*	*TUB2*
Monochaetia mediana	**KNUF-6L2F^T**	**OQ443083**	**OQ454909**	**OQ454910**
Monochaetia castaneae	SM9-2	MW166223	MW199742	MW218516
Monochaetia castaneae	CFCC 54354^T	MW166222	MW199741	MW218515
Monochaetia ilicis	CFCC 55248	–	OK358482	OK358491
Monochaetia ilicis	CFCC 55515	–	OK358481	OK358490
Monochaetia ilicis	CBS 101009	MH553953	MH554371	MH554612
Monochaetia monochaeta	CBS 546.80	MH554056	MH554491	MH554732
Monochaetia monochaeta	CBS 115004	AY853243	MH554398	MH554639
Monochaetia quercus	CBS 144034^T	MH554171	MH554606	MH554844
Monochaetia schimae	SAUCC212203	MZ577567	OK104876	OK104869
Monochaetia schimae	SAUCC212202	MZ577566	OK104875	OK104868
Monochaetia schimae	SAUCC212201^T	MZ577565	OK104874	OK104867
Bartalinia robillardoides	CBS 122705^T	LT853104	LT853202	LT853252

Notes: ITS: Internal transcribed spacer regions of the rDNA; *TEF1α*: partial translation elongation factor gene; *TUB2*: Beta-tubulin. The newly generated sequences were indicated in bold.

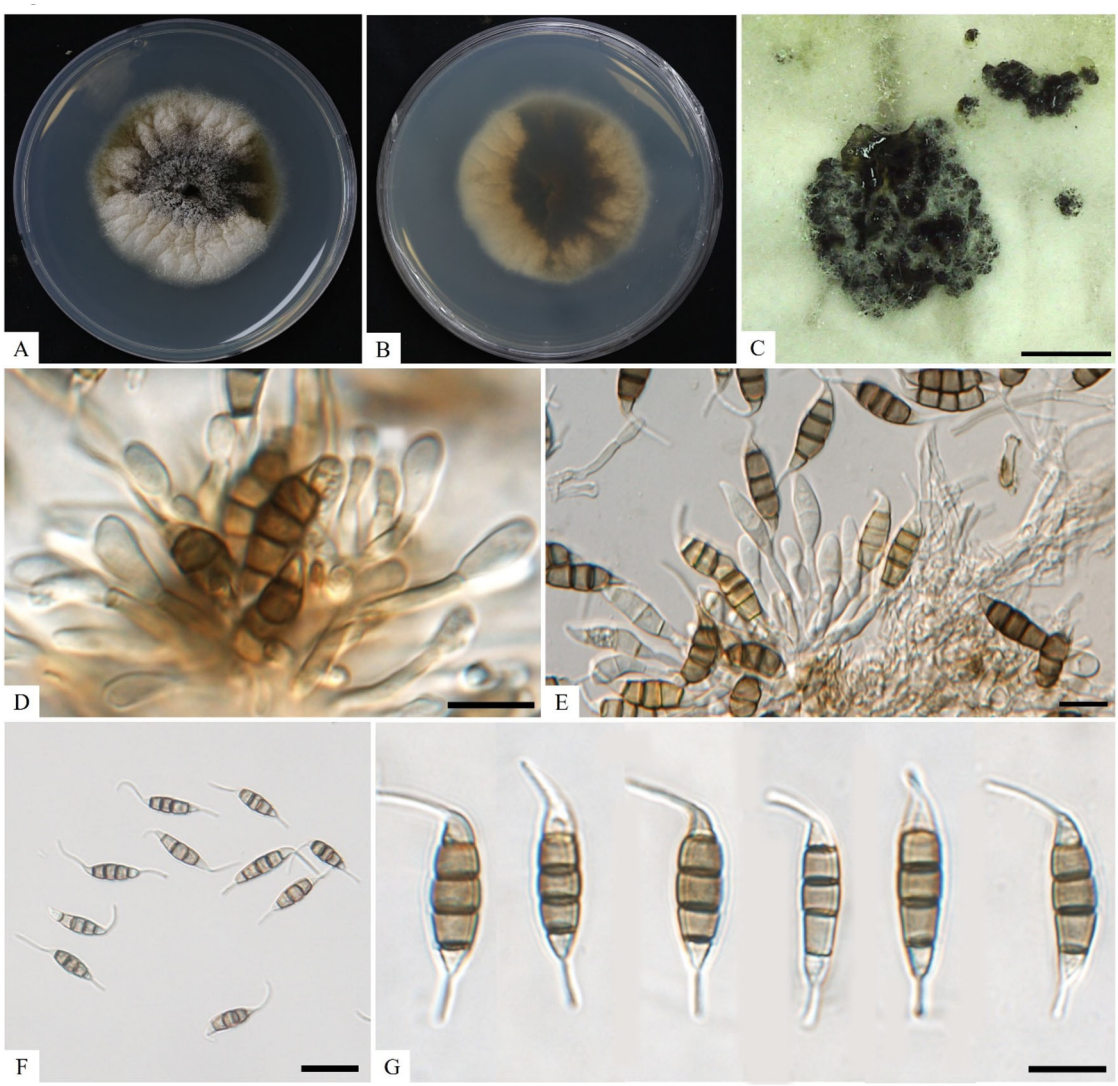

Figure 1. Cultural and morphological characteristics of *Monochaetia mediana* KNUF-6L2F^T. The colony growth on potato dextrose agar at 25 °C after 15 days, front and reverse, respectively (A, B). Conidiomata (C); conidiogenous cells with conidia (D, E); conidia (F, G). Scale bars: C = 1000 μm; D,E,G = 10 μm; F = 20 μm.

single, tubular, filiform; basal appendage 3.9–8.3 μm long ($\bar{x}$ =5.7 μm), single, central, tubular, filiform (Figure 1(G)).

Note: The strain KNUF-6L2F produced white, light brown to dirty black surface, and olivaceous green colonies, with the growth of 50.0–54.0 and 60.0–63.0 mm in diameter on PDA after 7 and 15 days at 25 °C, respectively, while the closest strain *M. ilicis* KUMCC 15–0520^T (syn. *M. ilexae*) were light brown to brown toward center with the growth

Table 2. Morphological characteristics of *Monochaetia mediana* (KNUF-6L2F^T) and comparison with the closest species of *Monochaetia*.

Characteristics	*M. mediana* KNUF-6L2F[a]	*M. ilicis* KUMCC 15–0520^T[b]	*M. schimae* SAUCC 212201^T[c]	*M. castaneae* CFCC 54354^T[d]
Colony color	PDA: White, light brown to dirty black surface, olivaceous green	PDA: Light brown at the margin, brown at the center	PDA: Light brown at the margin, brown at the center	PDA: Spreading, with sparse aerial mycelium and smooth.
Shape	Circular, zonate, undulate, canaliculate	Circular, raised, dense, zonate	Irregularly circular, raised, dense, lobate edge, zonate	Flat, lobate margin, cinnamon
Size (diam.)	50–54 mm after 7 at 25 °C	35 mm diameter after 7 days at 25 °C	39–45 mm after 15 days at 25 °C	40 mm in 15 days
Conidia (μm)	17.8–28.1 × 4.4–5.5	20.0–27.0 × 5.0–8.0	18.0–24.0 × 4.5– 6.0	20.0–24.0 × 5.4–6.2
Conidiogenous cells (μm)	20.0–27.0 × 3.0–5.0	4.0–6.0 × 1.0–2.0	9.0–16.5 × 1.2–2.2	12.0–20.0 × 1.5–2.5
Length of 3 median cells (μm)	11.5–17.0	13.0–18.0	12.5–15.5	10.5–16.5
Length of apical appendage (μm)	5.7–14.0	6.0–24.0	7.0–12.5	17.5–35.0
Length of basal appendage (μm)	3.9–8.3	3.0–12.0	2.5–5.0	10.0–20.0

[a]Fungal strain studied in this research.
[b–d]Sources of descriptions [12,17,31].

of 35 mm after 7 days, and *M. schimae* SAUCC 212201^T light brown to brown toward center with the growth of 39.0–45.0 mm after 15 days at 25 °C (Table 2). The strain KNUF-6L2F produced shorter (5.7–14.0 μm) apical appendages than *M. ilicis* KUMCC 15–0520^T (6.0–24.0 μm), but almost similar to *M. schimae* SAUCC 212201^T (7.0–12.5 μm). The three median cells of KNUF-6L2F were light brown to olivaceous green, whereas brown and olivaceous cells were observed from *M. ilicis* KUMCC 15–0520^T and *M. schimae* SAUCC 212201^T, respectively. The strain KNUF-6L2F produced larger (6.0–23.8 × 3.0–6.2 μm) conidiogenous cells than *M. ilicis* KUMCC 15–0520^T (4.0–6.0 × 1.0–2.0 μm) and *M. schimae* SAUCC 212201^T (9.0–16.5 × 1.2–2.2 μm) (Table 2). The proposed strain KNUF-6L2F differed from the closest strain of *M. ilicis* and *M. schimae* with the colony's growth and color, apical appendages, color of three median cells, and conidiogenous cells. Thus, the morphology of strain KNUF-6L2F was distinct from the previously identified allied species of *Monochaetia*.

3.2. Molecular phylogeny of strain KNUF-6L2F

The ITS regions (553 bp) demonstrated maximum 100% with the different strains of *Monochaetia ilicis* (CBS 101009, BK76, BK75, 464E), and 97.4–98.9% similarities with the strains of *M. sinensis* HKAS 10065^T, *M. junipericola* CBS 143391^T, *M. monochaeta* CBS 118.66, *M. castaneae* CFCC 54354^T. The sequences (803 bp) for the 28S ribosomal DNA (LSU) of KNUF-6L2F revealed a high sequence similarity of 99.5–100% with the strains of *M. ilicis* (CBS 101009, KUMCC 15-0517, HKAS 92492, KUMCC 15-0520), *M. monochaeta* M5-2, *M. kansensis* PSHI2004Endo1030, *M. junipericola* CBS 143391, *M. sinensis* HKAS 10065^T, *M. castaneae* CFCC 54354^T. A partial sequence of *TEF1α* (510 bp), the isolated strain showed maximum 89.96–91.90% similarities with the strains of *M. ilicis* (CBS 101009, CFCC 55515, CFCC 55248). Also, a partial of *TUB2* (724 bp) gene displayed highest 95.68–96.84% identities with the strains of *M. ilicis* (CBS 101009, CFCC 55515), 86.82–89.23% identities with the strains of *M. junipericola* CBS 143391, *M. sinensis* HKAS 10065. The concatenated sequences of the ITS regions, *TUB2* and *TEF1α* genes, as well as the nodes in the NJ phylogenetic tree, filled nodes in maximum likelihood and maximum parsimony trees, were used to determine the taxonomic position of KNUF-6L2F (Figure 2). The taxonomic position of strain KNUF-6L2F was determined by phylogenetic studies based on maximum parsimony using a combination of sequences (tree length = 586, consistency index = 0.74, retention index = 0.78, and composite index = 0.62). Phylogenetic analyses and morphological observations revealed that strain KNUF-6L2F was different from the *Monochaetia* species have previously been described (Figure 2). Thus, it needs to be classified as a novel species in the genus and proposed the name *Monochaetia mediana* sp. nov.

4. Discussion

Monochaeta species are often found as plant pathogens that also cause post-harvest losses and can infect members of the Coniferales, Ericales, Rosales, and Salicales order of plants [6,9,32,33]. In the previous studies, several species of *Monochaetia* were reported from Fagaceae hosts, including *M. dimorphospora* (*Castanea pubinervis*), *M. castaneae* (*C. mollissima*), *M. monochaeta* (*Quercus pubescens*) etc. [3,31]. *M. schimae* was first reported from *Schima superba* and *M. ilicis* was recorded from *Ilex* sp. [12,17]. Also, *M. concentrica* and *M. kansensis* were recovered from *Castanea* leaves, and *M. junipericola* isolated from the twigs of *Juniperus communis* [34,35]. Moreover, some species, namely, *M. karstenii* produces different secondary metabolites such as

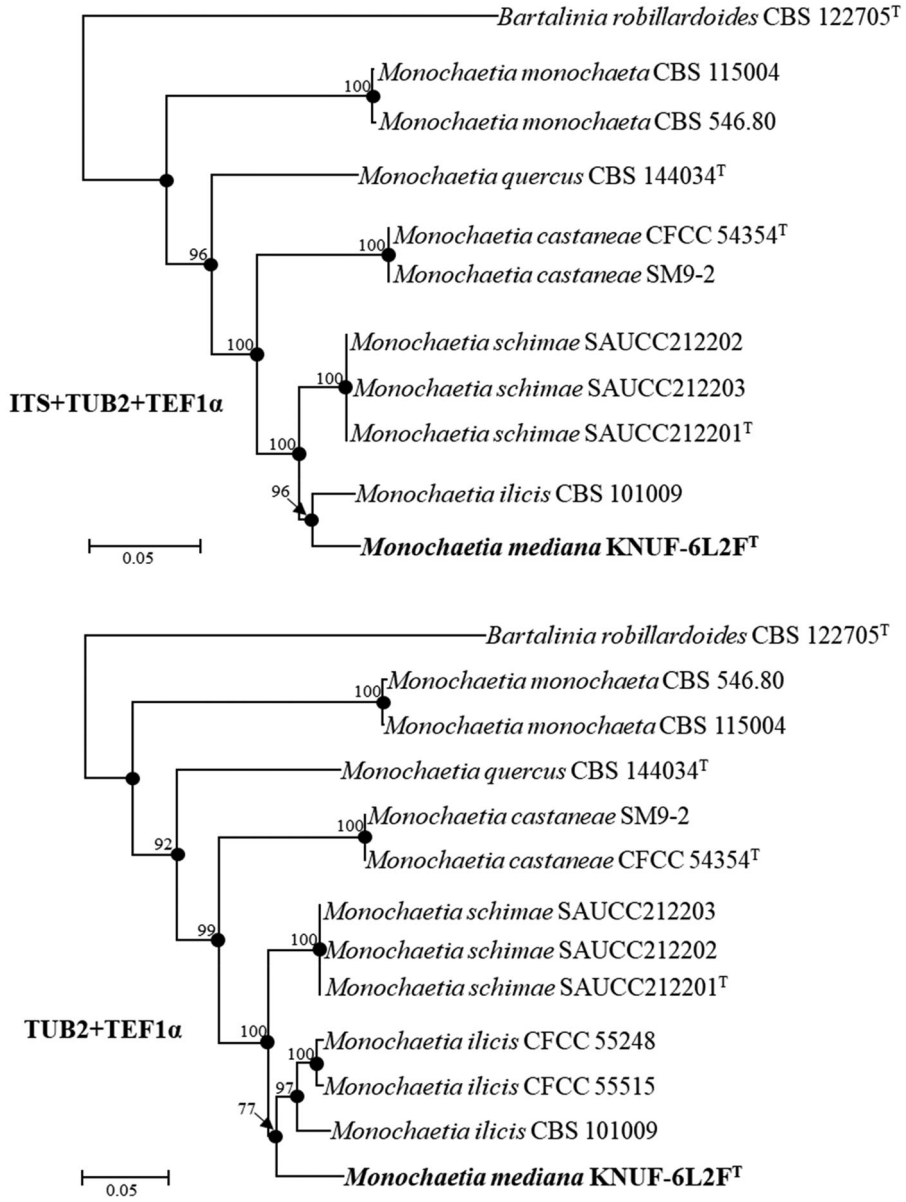

Figure 2. Neighbor-joining phylogenetic tree of KNUF-6L2F^T based on ITS, *TEF1α*, and *TUB2* sequences, showing the phylogenetic position among the related strains in the genus *Monochaetia*. *Bartalinia robillardoides* CBS 122705^T was used as an outgroup. The strain isolated in this study is in bold, and the numbers above the branches represent the bootstrap values are obtained for 1000 replicates. Bar = 0.05 substitutions per nucleotides position.

cyclohexenone derivatives, cinnamic acid, isooxazoline 3-phenyl-benzodiazepine, 2-propenoic acid, 3-phenyl-(E)-dodecene, and 3-undecen-1-yne (E) having antimicrobial and antioxidant activity [36]. Another strain of *Monochaetia* called *Monochaetia* sp. Tbp-2 can produce paclitaxel [37]. In 2019, the species *M. camelliae* was recorded from seawater in Korea [38]. However, there is no other species of *Monochaetia* that has been recorded from any insects in Korea until now. Therefore, there is possibility that the numbers of novel secondary metabolites and habitats remain to be discovered from this genus. Though species of this genus *Monochaetia* occur in many habitats worldwide, the identified strain, KNUF-6L2F, was isolated from the hairy long-horned toad beetle (*Moechotypa diphysis*) in Korea.

In conclusion, the species in this genus are known for their distinctive morphological features and their occurrence on twigs, leaves, and stems of various plant species. Therefore, considering all the aspects of the new species of *Monochaetia*, further investigation is essential to explore the etiology as well as their pathogenicity along with their ecological importance based on Korean soils and environmental conditions.

Disclosure statement

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

Funding

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the

Ministry of Environment (MOE) of the Republic of Korea [NIBR202231206].

ORCID

Kallol Das http://orcid.org/0000-0003-0906-3983
Seung-Yeol Lee http://orcid.org/0000-0003-1676-0330
Hee-Young Jung http://orcid.org/0000-0002-4254-3367

References

[1] Maharachchikumbura SSN, Guo LD, Chukeatirote E, et al. A destructive new disease of *Syzygium samarangense* in Thailand caused by the new species *Pestalotiopsis samarangensis*. Trop Plant Pathol. 2013;38(3):227–235.
[2] Jayawardena RS, Zhang W, Liu M, et al. Identification and characterization of *Pestalotiopsis*-like fungi related to grapevine diseases in China. Fungal Biol. 2015;119(5):348–361.
[3] Liu F, Bonthond G, Groenewald JZ, et al. Sporocadaceae, a family of coelomycetous fungi with appendage-bearing conidia. Stud Mycol. 2019; 92:287–415.
[4] Jaklitsch WM, Gardiennet A, Voglmayr H. Resolution of morphology-based taxonomic delusions: *Acrocordiella*, *Basiseptospora*. *Blogiascospora*, *Clypeosphaeria*, *Hymenopleella*, *Lepteutypa*, *Pseudapiospora*, *Requienella*, *Seiridium* and *Strickeria*. Persoonia. 2016;37:82–105.
[5] Samarakoon M. Evolution of Xylariomycetidae (Ascomycota: Sordariomycetes). Mycosphere. 2016; 7(11):1746–1761.
[6] Wijayawardene NN, Hyde KD, Rajeshkumar KC, et al. Notes for genera: Ascomycota. Fungal Divers. 2017;86(1):1–594.
[7] Allescher A. Fungi Imperfecti: Gefärbt-sporige Sphaerioideen. In: Kummer E. Rabenhorst's Kryptogamen-Flora von Deutschland. Österreich und der Schweiz. 2nd ed. Leipzig: Kummer; 1902. p. 65–128.
[8] Steyaert RL. Contribution à l'étude monographique de Pestalotia De Not. et *Monochaetia* Sacc. (*Truncatella* gen. nov. et *Pestalotiopsis* gen. nov.). Bulletin du Jardin Botanique de l'État à Bruxelles. 1949;19(3):285–354.
[9] Guba EF. Monograph of *Monochaetia* and *Pestalotia*. Cambridge: Harvard University Press; 1961.
[10] Senanayake IC, Maharachchikumbura SSN, Hyde KD, et al. Towards unraveling relationships in Xylariomycetidae (Sordariomycetes). Fungal Divers. 2015;73(1):73–144.
[11] Mehrotra RS, Aneja KR. An introduction to mycology. New Delhi: New Age International (P) limited, Publishers; 1990.
[12] de Silva NI, Phookamsak R, Maharachchikumbura SSN, et al. *Monochaetia ilexae* sp. nov. (Pestalotiopsidaceae) from Yunnan province in China. Phytotaxa. 2017;291(2):123–132.
[13] Strobel GA, Hess WM, Ford F, et al. Taxol from fungal endophytes and the tissue of biodiversity. J Ind Micro Biotech. 1996;17(5-6):417–423.
[14] Li JY, Harper JK, Grant DM, et al. Ambuic acid, a highly functionalized cyclohexenone with antifungal activity from *Pestalotiopsis* spp. and *Monochaetia* sp. Phytochemistry. 2001;56(5):463–468.
[15] Komori T, Yamashita M, Tsurumi Y, et al. Chaeticandin, a novel papulacandin I. Fermentation, isolation and characterization. J Antibiot. 1985;38(4):455–459.
[16] Das K, Kim JH, Choi KS, et al. A new report of *Biscogniauxia petrensis* isolated from mosquitoes in Korea. Kor J Mycol. 2020;48:87–93.
[17] Zhang Z, Liu R, Liu S, et al. Morphological and phylogenetic analyses reveal two new species of Sporocadaceae from Hainan, China. MycoKeys. 2022;88:171–192.
[18] Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol. 1993;2(2):113–118.
[19] 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.
[20] Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol. 1990;172(8):4238–4246.
[21] Rehner SA, Samuels GJ. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycol Res. 1994;98(6): 625–634.
[22] Glass NL, Donaldson G. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol. 1995;61(4):1323–1330.
[23] O'Donnell K, Cigelnik E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* were nonorthologous. Mol Phylogenet Evol. 1997;7(1):103–116.
[24] O'Donnell K, Kistler HC, Cigelnik E, et al. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci USA. 1998;95(5):2044–2049.
[25] Carbone I, Kohn LM. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 1999;91(3):553–556.
[26] Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16(2):111–120.
[27] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–425.
[28] Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol. 1981;17(6):368–376.
[29] Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool. 1971;20(4):406–416.
[30] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–1874.
[31] Jiang N, Fan XL, Tian CM. Identification and characterization of leaf-inhabiting fungi from *Castanea* plantations in China. JoF. 2021;7(1):64.

[32] Gonthier P, Gennaro M, Nicolotti G. Effects of water stress on the endophytic mycota of *Quercus robur*. Fungal Divers. 2006;21:69–80.
[33] Wijayawardene NN, Hyde KD, Wanasinghe DN, et al. Taxonomy and phylogeny of dematiaceous coelomycetes. Fungal Divers. 2016;77(1):1–316.
[34] de Silva N, Maharachchikumbura SSN, Thambugala KM, et al. *Monochaetia sinensis* sp. nov. from Yunnan province in China. Phytotaxa. 2018;375(1):59–69.
[35] Crous PW, Schumacher RK, Wingfield MJ, et al. New and interesting fungi. 1. Fungal Syst Evol. 2018;1(1):169–215.
[36] Subramaniam Y, Subbiah R, Balan L, et al. Bioprospecting of bioactive metabolites from *Monochaetia karsteni*. J Pure Appl Microbiol. 2020;14(2):1557–1566.
[37] Zhao J, Zhou L, Wang J. Endophytic fungi for producing bioactive compounds originally from their host plants. In: Mendez-Vilas DA, editor. Current research, technology and education topics in applied microbiology and microbial biotechnology. Badajoz: Formatex Research Center; 2011. p. 567–576.
[38] Nguyen TTT, Lim HJ, Chu SJ, et al. Two new species and three new records of Ascomycetes in Korea. Mycobiology. 2022;50(1):30–45.