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RESEARCH ARTICLE

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Gliocladiopsis koreensis sp. nov., *Ilyonectria koreensis* sp. nov., and *Mariannaea koreensis* sp. nov. (Nectriaceae), Novel Fungi Isolated from Soil in Jeju Island and Upo Wetland in the Republic of Korea

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ABSTRACT

In this study, three novel fungal species belonging to the Nectriaceae family, *Gliocladiopsis koreensis* sp. nov., *Ilyonectria koreensis* sp. nov., and *Mariannaea koreensis* sp. nov., were discovered from soil samples collected at Iseung-ak Oreum on Jeju Island and the Upo Wetland in Changnyeong, Republic of Korea. They were confirmed as distinct species through molecular phylogenetic analyses using the ITS, *TUB*, *Tef1*, HIS3, and LSU sequences. Maximum-likelihood and Bayesian inference trees show that *G. koreensis* forms a sister clade with *G. curvata*, *G. singaporiensis*, and *G. peggii*, *I. koreensis* clusters closely with *I. qitaiheensis* and *I. changbaiensis*, and *M. koreensis* is phylogenetically related to *M. atlantica*, *M. fusiformis*, *M. elegans* var. *punicea*, and *M. terricola*. While all three new species exhibit unique morphological characteristics such as colony growth patterns, pigmentation, and microstructures that differentiate them from their closest relatives. The findings of these novel species contribute to the understanding of fungal diversity in these ecologically significant regions and highlight their potential applications in agriculture, nutrient cycling, and environmental restoration.

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1. Introduction

The Nectriaceae family, circumscribed by brothers Charles and Louis René Tulasne in 1865 [1], refers to fungi which form uniloculate perithecia that are generally orange red to purple under KOH or yellow under 100% lactic acid conditions [2]. The characteristic feature of the asexual morph of Nectriaceae is that it forms amerosporous to phragmosporous conidia [3]. Nectriaceae has high species diversity, with generally higher species diversity in warm temperate and tropical regions [2, 4–6]. Wijayawardene et al. reported that by 2022, 70 genera, including *Gliocladiopsis*, *Ilyonectria*, and *Mariannaea* belong to Nectriaceae [7].

The genus *Gliocladiopsis* was introduced by Saksena with *G. sagariensis* as the representative species, and includes fungi isolated from soil with penicillate conidiophores similar to *Penicillium* and *Gliocladium* and cylindrical conidia similar to *Calonectria* [8]. *Glionectria*, a teleomorph of *Gliocladiopsis*, has been reported to have the following morphological characteristics: perithecia that are obovoid to broadly obpyriform, with warted, red-brown walls and dark red stromatic bases, producing ellipsoidal, 1-septate ascospores [9]. The first molecular genetic discussion of *Gliocladiopsis* was made by Schoch et al., which showed that *Gliocladiopsis* is a closely related genus to *Gliocephalotrichum* and *Leuconectria* [10]. *Gliocladiopsis* includes 16 species, including *G. curvata*, *G. elghollii*, *G. indonesiensis*, *G. mexicana*, and *G. pseudotenuis*, as reported by Gordillo and Decock [11]. Four species: *G. wuhanensis* [12], *G. aquatica* [13], *G. swieteniae* [14], and *G. siamensis* [15] have been additionally reported, and a total of 20 species are currently included in the genus *Gliocladiopsis*. *Gliocladiopsis* is also known as a pathogen that causes black rot in avocado roots [16].

The genus *Ilyonectria* was introduced by Booth to accommodate species of *Neonectria* belonging to the *Neonectria radicicola* group that has asexual forms and produces chlamydospores and microconidia, with *I. radicicola* as the type species [17]. *I. radicicola* was later renamed as *I. destructans* [6]. Species belonging to the genus *Ilyonectria* are mainly isolated from host roots or stems such as *Quercus suber*, *Vitis vinifera*, *Malus domestica*, *Olea europaea*, and *Pinus laricio* [18]. A variety of *Ilyonectria* species, including

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I. mors-panacis [19], *I. destructans* [20], and *I. robusta* [21], cause root rot in ginseng (*Panax ginseng*). Species of *Ilyonectria* have red globose to subglobose perithecia and oval ascospores with one septum, which are characteristics of the sexual form. The characteristic of their asexual form is that they have oval or ovoid microconidia with 0–1 septa and macroconidia with almost straight oval shape with 1–3 septa [22]. Although there have been several morphological and phylogenetic studies of species in the genera *Neonectria* and *Cylindrocarpon*, there have been no taxonomic proposals to formally separate groups of species that do not clearly belong to these two groups. [23–26]. In 2011, a multi-locus sequence phylogenetic tree analysis using DNA sequences of partial large subunit (LSU), internal transcribed spacers (ITSs) 1 and 2, including 5.8S of the nuclear ribosomal DNA (ITS), partial β-tubulin (*tub*), α-actin (*act*), RNA polymerase II subunit 1 (*rpb1*), and translation elongation factor 1α (*tef1*) sequences was performed by Chaverri et al. [4]. This analysis led Chaverri et al. to propose a new group, *Ilyonectria radicicola* comb. nov., which is part of the genera *Neonectria* and *Cylindrocarpon* but forms a different clade [4]. There are 37 species of the genus *Ilyonectria* registered in MycoBank, of which 27 species have the current name *Ilyonectria*.

The genus *Mariannaea*, reestablished by Samson to accommodate *Mariannaea camptospora* Samson, *M. elegans* (Corda) Samson [27], was placed in Nectriaceae by Samuels and Seifertet [28]. *Mariannaea* was first reported to be characterized by forming one-celled conidia with sticky heads connected with flask-shaped phialides [27]. However, later, some species with straight conidial chains such as *M. nipponica* [29] and *M. clavispora* [30] and *M. superimposita* [31] with two-celled conidia with one septum were reported. The morphological concept was broadened with the addition of species such as *Mariannaea*. Species belonging to the genus *Mariannaea* include *Nectria* (Fr.) [32] or *Cosmospora* Rabenh [33]. Genera of several teleomorph types of the same Nectriaceae were mixed in the phylogenetic tree [28, 34]. Through various phylogenetic evaluations, *Mariannaea* was reevaluated as a monophyletic group [6, 35, 36]. There are currently 28 species reported in the genus *Mariannaea* on MycoBank (https://www.mycobank.org). Species of the genus *Mariannaea* have mostly been reported from terrestrial habitats [6, 30, 31, 34, 37, 38], but *M. aquaticola* was first reported from freshwater by Cai et al. [35]. Additionally, Hu et al. reported *M. chlamydospora*, *M. cinerea*, and *M. fusiformis* from trees submerged in water [36].

Soil is one of the most studied fungal habitats, where the discovery of unrecorded and new species is actively taking place [39–41]. However, fungi in the soil of Jeju Island and Upo Wetland, ecologically important regions in the Republic of Korea, have not been studied much. In the process of isolating fungi from soils in these regions, three new species belonging to Nectriaceae were discovered. In this paper, we described their morphological characteristics and phylogenetic position.

2. Materials and methods

2.1. Collection of soil samples

On August 28 2023, the hyphosphere soil around the fruiting bodies of mushroom, *Russula nigricans* and *Calostoma japonicum*, was collected at Iseung-ak Oreum, Jeju Island, Republic of Korea. On September 18 2023, swamp soil was collected at the Upo Wetland, Changnyeong-gun, Republic of Korea. The collected soil samples were placed in an icebox and transported to the laboratory. The transported soils were stored in a refrigerator at 4 °C until used for analysis.

2.2. Isolation of fungi

The collected soil (1 g) was mixed with 10 mL of sterilized distilled water and vortexed for 30 min. After vortex, the soil mixture was diluted from 10^{-1} to 10^{-3}. The diluted soil mixture (100 µL) was plated in triplicate on Dichloran glycerol 18% (DG18) agar, which inhibits excessive growth of the fast-growing fungus due to its low water activity and promotes the isolation of single colonies. The spread DG18 medium was cultured in a 25 °C incubator for 1 week to 1 month. After culturing for seven days, the medium was checked every day, and the growing fungal colonies were selected one by one and transferred to the PDA. Single spore isolates were obtained from 2-week-old PDA cultures, and one isolate was selected and used for identification. Representative isolates with NIBR numbers are deposited at the National Institute of Biological Resources in Incheon, South Korea.

2.3. Morphology analysis

For the observation of colony morphology of fungal isolates in different mediums, PDA, Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA), and Oatmeal Agar (OA) were used. Each medium was inoculated with agar plugs of each single spore isolate grown on PDA, and the morphology of the

colony was observed after culturing for 14 days in an incubator under dark conditions at 25 °C. The microstructure of the fungal isolates cultured for certain period was observed with an optical microscope (BX53, OLYMPUS, Tokyo, Japan). The length and thickness of microstructures such as chlamydospores, conidiophores, phialides, and conidia were measured with 50 replicates.

Table 1. Information about the sequences used to construct the phylogenetic tree of species belonging to the genus *Gliocladiopsis*.

				GenBank accession number			
Scientific name	Strain	Source	Location	ITS	*tub*	*tef1*	*his3*
Gliocladiopsis forsbergii	BRIP 61349 (T)	Mycelium of *Grevillea* sp.	Australia	KX274071	KX274037	–	KX274054
Gliocladiopsis whileyi	BRIP 61430 (T)	Mycelium of *Grevillea* sp.	Australia	KX274086	KX274052	–	KX274069
Gliocladiopsis curvata	CBS 114464	–	Ecuador	JQ666052	JQ666128	JQ666094	JQ666018
Gliocladiopsis curvata	CBS 110840	–	–	JQ666045	JQ666121	JQ666087	JQ666011
Gliocladiopsis curvata	CBS 978.73	Soil	Brazil	JQ666043	JQ666119	JQ666085	JQ666009
Gliocladiopsis curvata	CBS 194.80	*Persea americana*	Ecuador	JQ666044	JQ666120	JQ666086	JQ666010
Gliocladiopsis curvata	CBS 112365 (T)	*Archontophoenix purpurea*	New Zealand	JQ666050	JQ666126	JQ666092	JQ666016
Gliocladiopsis wuhanensis	HEAC17307 (T)	Soil	China	MH024520	MH169602	–	MH255786
Gliocladiopsis guangdongensis	LC1349	Submerged wood	China	KC776123	KC776125	KC776119	KC776121
Gliocladiopsis guangdongensis	LC1340 (T)	Submerged wood	China	KC776122	KC776124	KC776118	KC776120
Gliocladiopsis pseudotenuis	CBS 114763	*Vanilla* sp.	Indonesia	JQ666062	JQ666139	JQ666105	JQ666029
Gliocladiopsis pseudotenuis	CBS 116074 (T)	Soil	China	AF220981	JQ666140	JQ666106	JQ666030
Gliocladiopsis irregularis	CBS 755.97 (T)	Soil	Indonesia	AF220977	JQ666133	JQ666099	JQ666023
Gliocladiopsis irregularis	CBS 111176	*Araucaria* sp.	Malaysia	JQ666058	JQ666135	JQ666101	JQ666025
Gliocladiopsis irregularis	CBS 111142	*Araucaria* sp.	Malaysia	JQ666057	JQ666134	JQ666100	JQ666024
Gliocladiopsis sp. 2	CBS 116086	Soil	Indonesia	JQ666072	JQ666152	JQ666118	JQ666042
Gliocladiopsis indonesiensis	CBS 116090 (T)	Soil	Indonesia	JQ666056	JQ666132	JQ666098	JQ666022
Gliocladiopsis sumatrensis	CBS 754.97 (T)	Soil	Indonesia	JQ666064	JQ666142	JQ666108	JQ666032
Gliocladiopsis sumatrensis	CBS 111198	Soil	Indonesia	JQ666065	JQ666143	JQ666109	JQ666033
Gliocladiopsis peggii	BRIP 60983 (T)	Mycelium of *Persea americana*	Australia	NR_147649	KX274038	–	KX274065
Gliocladiopsis peggii	BRIP 63709a	Mycelium	Australia	KX274085	KX274041	–	KX274057
Gliocladiopsis peggii	BRIP 63710a	Mycelium	Australia	KX274084	KX274051	–	KX274068
Gliocladiopsis mexicana	CBS 110938 (T)	Soil	Mexico	JQ666060	JQ666137	JQ666103	JQ666027
Gliocladiopsis singaporiensis	MUCL 48728 (T)	Submerged leaf litter in freshwater	Singapore	KX671138	KX611500	KX671130	–
Gliocladiopsis koreensis	**NIBRFGC000512620 (T)**	**Soil**	**The Republic of Korea**	**OR742029**	**PP111909**	**PP111911**	**PP111892**
Gliocladiopsis koreensis	**DUCC15782**	**Soil**	**The Republic of Korea**	**PQ479937**	**PP111910**	**PP111912**	**PP111893**
Gliocladiopsis hennebertii	MUCL 54818 (T)	Rhizosphere, *Costus scaber*	Ecuador	KX671140	KX611502	KX671132	–
Gliocladiopsis elghollii	CBS 116104 (T)	*Chamaedorea elegans*	USA	JQ666055	JQ666131	JQ666097	JQ666021
Gliocladiopsis sagariensis	CBS 199.55 (T)	Soil	India	JQ666063	JQ666141	JQ666107	JQ666031
Gliocladiopsis swieteniae	MFLUCC 18-2767 (T)	Decaying fruits of *Swietenia mahagoni* (Meliaceae)	Thailand	MT215501	MT212214	–	MT212194
Gliocladiopsis siamensis	MFLUCC 18-2743 (T)	Stem of woody plant	Thailand	NR_189389	ON364481	–	ON364457
Gliocladiopsis aquaticus	MFLU 17-1976 (T)	Decaying wood	Thailand	MG543924	MG574421	–	MG734182
Gliocladiopsis aquaticus	MFLUCC 17-2028	Decaying wood	Thailand	MG543925	MG574422	–	MG734183
Gliocladiopsis sp. 1	CBS 111038	Soil	Colombia	JQ666071	JQ666151	JQ666117	JQ666041
Gliocladiopsis tenuis	IMI68205 (T)	*Indigofera* sp.	Indo-China	AF220979	–	–	–
Gliocladiopsis tenuis	CBS 111961	*Coffee* sp.	Vietnam	JQ666067	JQ666146	JQ666112	JQ666036
Gliocladiopsis ecuadoriensis	MUCL 54740 (T)	Rhizosphere of *Polybotrya* sp.	Ecuador	KX671139	KX611501	KX671131	KX671146
Penicillifer pulcher	CBS 560.67 (T)	Soil	Netherlands	KM231742	KM231998	KM231862	KM231456

(T): type strain.
The strains isolated in this paper are indicated in bold.

Table 2. Information about the sequences used to construct the phylogenetic tree of species belonging to the genus *Ilyonectria*.

Scientific name	Strain	Source	Location	GenBank accession number			
				ITS	*tub*	*tef1*	*his3*
Ilyonectria zarorii	CPC 37837	Rhizosphere	Chile	MW114894	MW119264	MW119262	MW119260
Ilyonectria zarorii	CPC 37835 (T)	Rhizosphere	Chile	MW114893	MW119263	MW119261	MW119259
Ilyonectria crassa	NSAC SH 1	–	Canada	AY295311	JF735395	JF735725	JF735536
Ilyonectria crassa	CBS 158.31	Root	Netherlands	JF735276	JF735394	JF735724	JF735535
Ilyonectria crassa	CBS 139.30 (T)	Root	Netherlands	JF735275	JF735393	JF735723	JF735534
Ilyonectria pseudodestructans	CBS 117824	Root	Austria	JF735292	JF735419	JF735751	JF735562
Ilyonectria pseudodestructans	CBS 129081 (T)	*Vitis vinifera*	Portugal	AJ875330	AM419091	JF735752	JF735563
Ilyonectria pseudodestructans	PR20-11	–	–	MT678562	MT810735	MT800963	MT800946
Ilyonectria panacis	CBS 129079 (T)	*Panax quinquefolium*	Canada	MH865176	JF735424	JF735761	JF735572
Ilyonectria rufa	CBS 153.37 (T)	Dune sand	France	MH855863	AY677251	JF735729	JF735540
Ilyonectria rufa	CBS 640.77	*Abies alba*	France	JF735277	JF735399	JF735731	JF735542
Ilyonectria communis	CGMCC 3.18788 (T)		China	MF350456	MF350402	MF350483	MF350429
Ilyonectria communis	J410	*Panax ginseng*	China	MF350457	MF350403	MF350484	MF350430
Ilyonectria communis	CB4-2	*Cyclamen* sp.	Netherlands	JF735304	JF735432	JF735770	JF735581
Ilyonectria cyclaminicola	CBS 302.93 (T)	*Cyclamen* sp.	Netherlands	JF735304	JF735432	JF735770	JF735581
Ilyonectria cyclaminicola	EFA 444	–	–	MF440369	MF797792	MH070096	MF471472
Ilyonectria leucospermi	CBS 132809 (T)	*Leucospermum* sp.	South Africa	JX231161	JX231113	JX231129	JX231145
Ilyonectria leucospermi	CBS 132810	*Protea* sp.	South Africa	JX231161	JX231113	JX231129	JX231145
Ilyonectria protearum	CBS 132812	*Protea* sp.	South Africa	JX231165	JX231117	JX231133	JX231149
Ilyonectria protearum	CBS 132811 (T)	*Protea* sp.	South Africa	JX231157	JX231109	JX231125	JX231141
Ilyonectria ilicicola	Cy-FO-224	*Ilex* sp. roots	Spain	KY676883	KY676877	KY676871	KY676865
Ilyonectria ilicicola	Cy-FO-225 (T)	*Ilex* sp. roots	Spain	KY676884	KY676878	KY676872	KY676866
Ilyonectria ilicicola	Cy-FO-226	*Ilex* sp. roots	Spain	KY676885	KY676879	KY676873	KY676867
Ilyonectria coprosmae	CBS 119606	*Metrosideros* sp.	Canada	JF735260	JF735373	JF735694	JF735505
Ilyonectria lusitanica	CBS 129080 (T)	*Vitis vinifera*	Portugal	JF735296	JF735423	JF735759	JF735570
Ilyonectria vredehoekensis	CBS 132807 (T)	*Protea* sp.	South Africa	JX231155	JX231107	JX231123	JX231139
Ilyonectria vredehoekensis	CBS 132808	*Protea* sp.	South Africa	JX231159	JX231111	JX231127	JX231143
Ilyonectria capensis	CBS 132815 (T)	*Protea* sp.	South Africa	JX231151	JX231103	JX231119	JX231135
Ilyonectria venezuelensis	CBS 102032 (T)	Bark	Venezuela	AM419059	AY677255	JF735760	JF735571
Ilyonectria europaea	CBS 102892	Stem	Germany	JF735295	JF735422	JF735758	JF735569
Ilyonectria europaea	CBS 129078 (T)	*Vitis vinifera*	Portugal	JF735294	JF735421	JF735756	JF735570
Ilyonectria robusta	CBS 129084	*Vitis vinifera*	Portugal	JF735273	JF735391	JF735721	JF735532
Ilyonectria robusta	CBS 117818	Root	Austria	JF735267	JF735382	JF735712	JF735523
Ilyonectria robusta	CBS 308.35 (T)	*Panax quinquefolius*	Canada	MH855684	JF735377	JF735707	JF735518
Ilyonectria liliigena	CBS 732.74	*Lilium* sp.	Netherlands	JF735298	JF735426	JF735763	JF735574
Ilyonectria liliigena	CBS 189.49 (T)	*Lilium regale*	Netherlands	JF735297	JF735425	JF735762	JF735573
Ilyonectria gamsii	CBS 940.97 (T)	Soil	Netherlands	AM419065	AM419089	JF735766	JF735577
Ilyonectria koreensis	**NIBRFGC000512618 (T)**	**Soil**	**The Republic of Korea**	**OR742035**	**PP111901**	**PP111903**	**PP111894**
Ilyonectria koreensis	**DUCC15749**	**Soil**	**The Republic of Korea**	**PQ533830**	**PP111902**	**PP111904**	**PP111895**
Ilyonectria qitaiheensis	CGMCC 3.18787 (T)	*Panax ginseng*	China	MF350472	MF350418	MF350499	MF350445
Ilyonectria qitaiheensis	R3-2	Root	China	MT678569	MT810742	MT800970	MT800953
Ilyonectria qitaiheensis	J919	*Panax ginseng*	China	MF350473	MF350419	MF350500	MF350446
Ilyonectria changbaiensis	Q24-5	Root	China	MT678568	MT810741	MT800969	MT800952
Ilyonectria changbaiensis	CB4-7	Root	China	MT678567	MT810740	MT800968	MT800951
Ilyonectria changbaiensis	CGMCC 3.18789 (T)	*Panax ginseng*	China	MF350464	MF350410	MF350491	MF350437
Ilyonectria changbaiensis	72R2	*Panax ginseng*	China	MF350465	MF350411	MF350492	MF350438
Ilyonectria mors-panacis	CBS 306.35 (T)	*Panax quinquefolium*	Canada	JF735288	JF735414	JF735746	JF735557
Ilyonectria mors-panacis	H6-1	Root	China	MT678563	MT810736	MT800964	MT800947
Ilyonectria mors-panacis	XFC1	Root	China	MT678564	MT810737	MT800965	MT800948
Ilyonectria liriodendri	CBS 117526	*Vitis vinifera*	Portugal	DQ178164	DQ178171	JF735697	JF735508
Ilyonectria liriodendri	CBS 110.81 (T)	*Liriodendron tulipifera*	USA	DQ178163	DQ178170	JF735696	JF735507
Ilyonectria destructans	CBS 264.65(T)	*Cyclamen persicum*	Sweden	AY677273	AY677256	JF735695	JF735506
Ilyonectria palmarum	DiGeSA-HF7	Basal stem rot	Italy	HF937432	HF922609	HF922615	HF922621
Ilyonectria palmarum	DiGeSA-HF3 (T)	Basal stem rot	Italy	HF937431	HF922608	HF922614	HF922620
Ilyonectria strelitziae	CBS 142253 (T)	Dry basal rot of *Strelitzia reginae*	Italy	KY304649	KY304755	KY304727	KY304621
Ilyonectria strelitziae	ST8	Dry basal rot of *Strelitzia reginae*	Italy	KY304651	KY304757	KY304729	KY304623
Ilyonectria vivaria	CBS 145414 (T)	Grapevine	Spain	MK602795	MK602810	MK602825	MK602830
Neonectria lugdunensis	CBS 125485 (T)	*Populus fremontii*	USA	MH863594	KM232019	KM231887	KM231482

(T): type strain.
The strains isolated in this paper are indicated in bold.

Table 3. Information about the sequences used to construct the phylogenetic tree of species belonging to the genus *Mariannaea*.

Scientific name	Strain	Source	Location	GenBank accession number		
				ITS	LSU	*tub*
Mariannaea chlamydospora	LC1715 (T)	Submerged wood	China	KX986134	KX986141	KX986147
Mariannaea elegans	CBS 217.73A (T)	*Pinus sylvestris*, decayed bark	Netherlands	KX986132	KX986139	KX986145
Mariannaea camptospora	CBS 209.73 (T)	Forest soil	Netherlands	MH860663	MH872365	AY624245
Mariannaea macrochlamydospora	FKI-4735 (T)	Soil	Japan	AB855777	AB855782	–
Mariannaea lignicola	LC1791 (T)	Submerged wood	China	KX986136	KX986143	KX986149
Mariannaea lignicola	LC1792	Submerged wood	China	KX986137	KX986144	KX986150
Mariannaea submersa	MFLU 19-0549	Submerged wood	Thailand	MT496744	MT496752	–
Mariannaea submersa	MFLU 19-0542 (T)	Submerged wood	Thailand	MT496743	MT496751	–
Mariannaea pinicola	CBS 745.88 (T)	*Pinus* sp.	Venezuela	MH862152	MH873845	KM232011
Mariannaea samuelsii	CBS 125515 (T)	–	Guatemala	MH863675	MH875139	KM232015
Mariannaea samuelsii	CBS 746.88	Bark	Jamaica	MH862153	MH873846	KM232014
Mariannaea humicola	CBS 740.95 (T)	Soil	Brazil	KM231755	KM231619	KM232012
Mariannaea aquaticola	MFLU090223 (T)	Submerged wood	Thailand	GQ153834	GQ153833	–
Mariannaea aquaticola	MFLU090224	Submerged wood	Thailand	GQ153836	GQ153835	–
Mariannaea cinerea	LC1766 (T)	Submerged wood	China	KX986135	KX986142	KX986148
Mariannaea superimposita	CBS 124559	Soil	Japan	AB855781	AB855786	–
Mariannaea superimposita	CBS 113472	Soil	Japan	AB855780	AB855785	–
Mariannaea catenulatae	CBS 491.62 (T)	Wood	Venezuela	KM231752	KM231617	KM232009
Mariannaea dimorpha	HMAS 266564 (T)	Rotten bark	China	KF767353	KJ002443	–
Mariannaea camelliae	CMU 329 (T)	–	Thailand	NR_175622	NG_088070	–
Mariannaea koreensis	**NIBRFGC000512616 (T)**	**Soil**	**The Republic of Korea**	**OR835254**	**OR835270**	**OR841123**
Mariannaea koreensis	**DUCC15750**	**Soil**	**The Republic of Korea**	**PQ533829**	**PQ533831**	**PP111899**
Mariannaea atlantica	URM 8146 (T)	Soil	Brazil	MN151372	MN151398	–
Mariannaea fusiformis	LC1701 (T)	Submerged wood	China	KX986133	KX986140	KX986146
Mariannaea elegans var. *punicea*	CBS 239.56 (T)	–	Zaire	AY624201	AY526489	AY624244
Mariannaea terricola	URM 92163 (T)	Soil	Brazil	MK101011	MK101012	–
Nectria balansae	A.R. 4446	*Coronilla* sp.	France	HM484552	GQ505996	HM484607

(T): type strain.
The strains isolated in this paper are indicated in bold.

2.4. DNA extraction, sequencing, and phylogenetic analysis

About 100 mg of fungal isolate hyphae were collected and genomic DNA was extracted as described by Kim et al. [42]. Using the extracted DNA as a template, each target DNA region was amplified using PCR with primer sets in Supplementary Table 1. Amplificated PCR products were confirmed by electrophoresis on a 1% (w/v) agarose gel. Purification of the PCR product was performed using a silica gel column and 80% ethanol. Sequence analysis was entrusted to BIONICS Co., Ltd. (Seoul, South Korea). After editing using Chromas, the determined sequences were identified through BLAST (Basic Local Alignment Search Tool) search in NCBI database (https://www.ncbi.nlm.nih.gov) and deposited in GenBank with accession numbers in Tables 1–3. Fungal species that were closely related to the isolates of this study were included in the phylogenetic analysis. Reference sequences were obtained from GenBank and are listed in Tables 1–3. Individual sequence datasets of the ITS region, the LSU rDNA region, β-tubulin gene (*tub*), translation elongation factor 1 alpha gene (*tef1*), histone H3 gene (*HIS3*) were aligned using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) alignment tool of MEGA X [43]. The phylogenetic tree was constructed by maximum-likelihood (ML) algorithms using the General Time Reversible (GTR) model of nucleotide substitution with a gamma distribution and statistical support for the node value was calculated from 1000 bootstrap replicate trees. The Bayesian inference (BI) analysis was conducted using MrBayes v 3.2.7 with the following parameters. The model was set to assume the GTR model with a gamma-distributed rate variation across sites. The

Markov chain Monte Carlo (MCMC) analysis was run for 500,000 generations. For post-analysis, a burn-in of 20,000 generations was applied for both parameter estimation and tree summarization. The reliability of each node was represented by posterior probability (PP) values.

3. Results and discussion

3.1. Phylogenetic analyses

The sequences of ITS, *tub*, *tef1*, and *HIS3* of *G. koreensis* were analyzed. The size of ITS was 522 bp, *tub* was 330 bp, *tef1* was 493 bp, and *his3* was 477 bp. The four sequences, ITS (446 bp), *tub* (247 bp), *his3* (445 bp), and *tef1* (219 bp), were concatenated. The meaningful sequences among the aligned series were ITS (50/446), *tub* (52/247), *his3* (182/445), and *tef1* (92/219). As a result of aligning the concatenated sequence with the sequences of other *Gliocladiopsis* species, a final sequence of 1398 bp including gaps was obtained. A total of 25 species and 37 strains were used for phylogenetic analysis to distinguish *G. koreensis* from other *Gliocladiopsis* species (Table 1). Based on a concatenated 1398 bp sequence with gaps of ITS + *tub* + *his3* + *tef1*, the results of MLBS and BI phylogenetic analyses showed that *G. koreensis* formed a sister clade with the clade comprising *G. curvata*, *G. singaporiensis*, and *G. peggii*. However, it was identified as a monophyletic species with moderate statistical support (MLBS/PP = 78/88; Figure 1).

The sequences of the ITS, *tub*, *tef1*, and *HIS3* of *I. koreensis* were analyzed. The size of the ITS was 516 bp, *tub* was 323 bp, *tef1* was 561 bp, and *his3* was 495 bp. The four sequences, the ITS (415 bp), *tub* (263 bp), *his3* (371 bp), and *tef1* (393 bp), were concatenated. The meaningful sequences among the aligned series were the ITS (83/415), *tub* (63/263), *his3* (153/371), and *tef1* (116/393). As a result of aligning the linked sequence with the reference sequences of other *Ilyonectria* species, a final sequence of 1539 bp including gaps was obtained. A total of 29 species and 57 strains were used for phylogenetic analysis to distinguish *I. koreensis* from

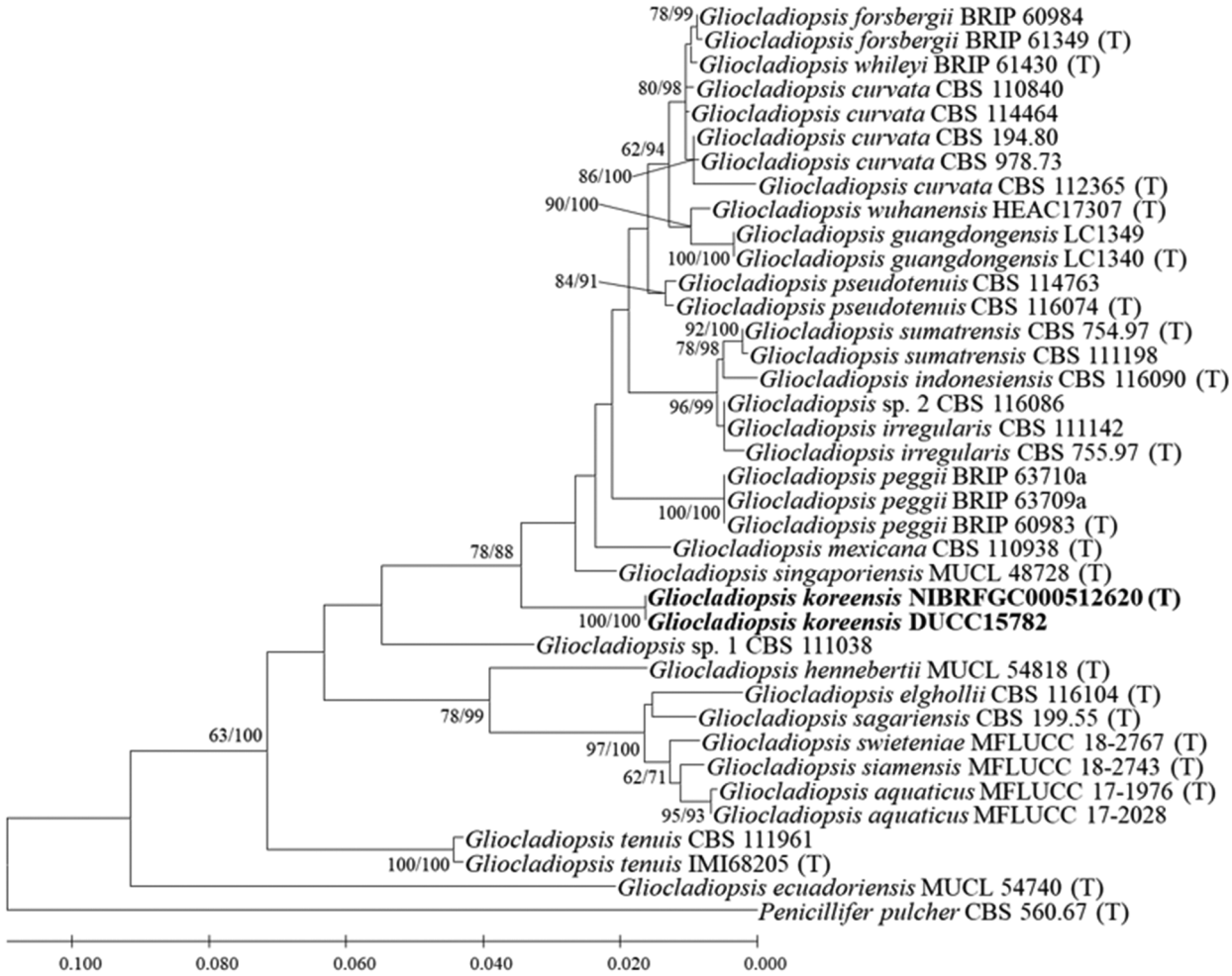

Figure 1. Mximum-likelihood phylogenetic tree based on the concatenated ITS, *tub*, *tef1*, *his3* nucleotide sequences of *Gliocladiopsis* species. New species are indicated in bold. The reliability value of each node was represented in the order of MLBS/PP. Nodes with reliability values less than MLBS <60 or PP <70 were removed. *Penicillifer pulcher* CBS 560.67 was used as an outgroup.

other *Ilyonectria* species (Table 2). Based on a concatenated 1539 bp sequence with gaps of the ITS + *tub* + *his3* + *tef1*, the results of MLBS and BI phylogenetic analyses revealed that *I. koreensis* formed a sister clade with *I. qitaiheensis* and *I. changbaiensis*. However, it was identified as a monophyletic species with strong statistical support (MLBS/PP = 89/100; Figure 2).

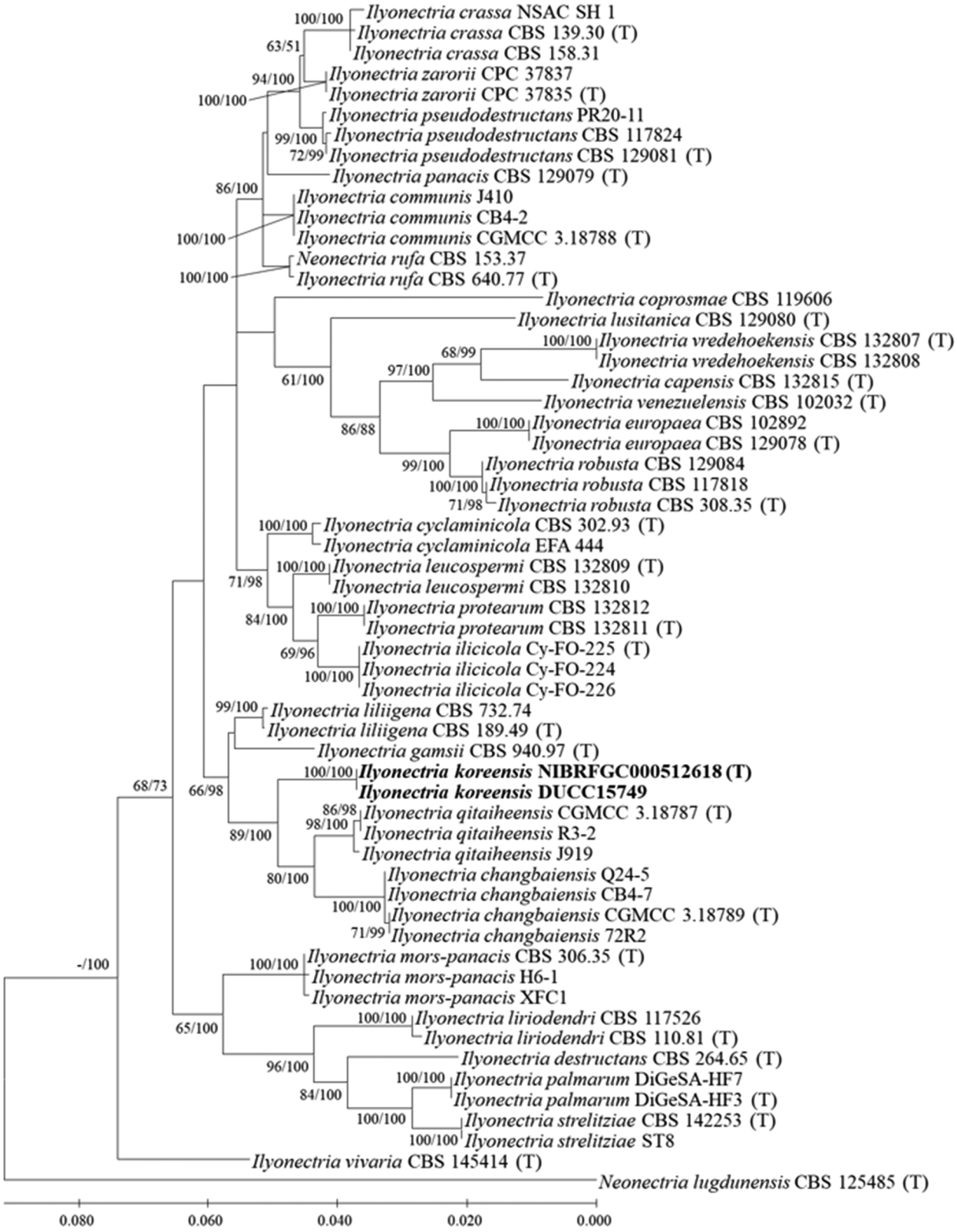

Figure 2. Mximum-likelihood phylogenetic tree based on the concatenated ITS, *tub*, *tef1*, *his3* nucleotide sequences of *Ilyonectria* species. New species are indicated in bold. The reliability value of each node was represented in the order of MLBS/PP. Nodes with reliability values less than MLBS <60 or PP <70 were removed. *Neonectria lugdunensis* CBS 125485 was used as an outgroup.

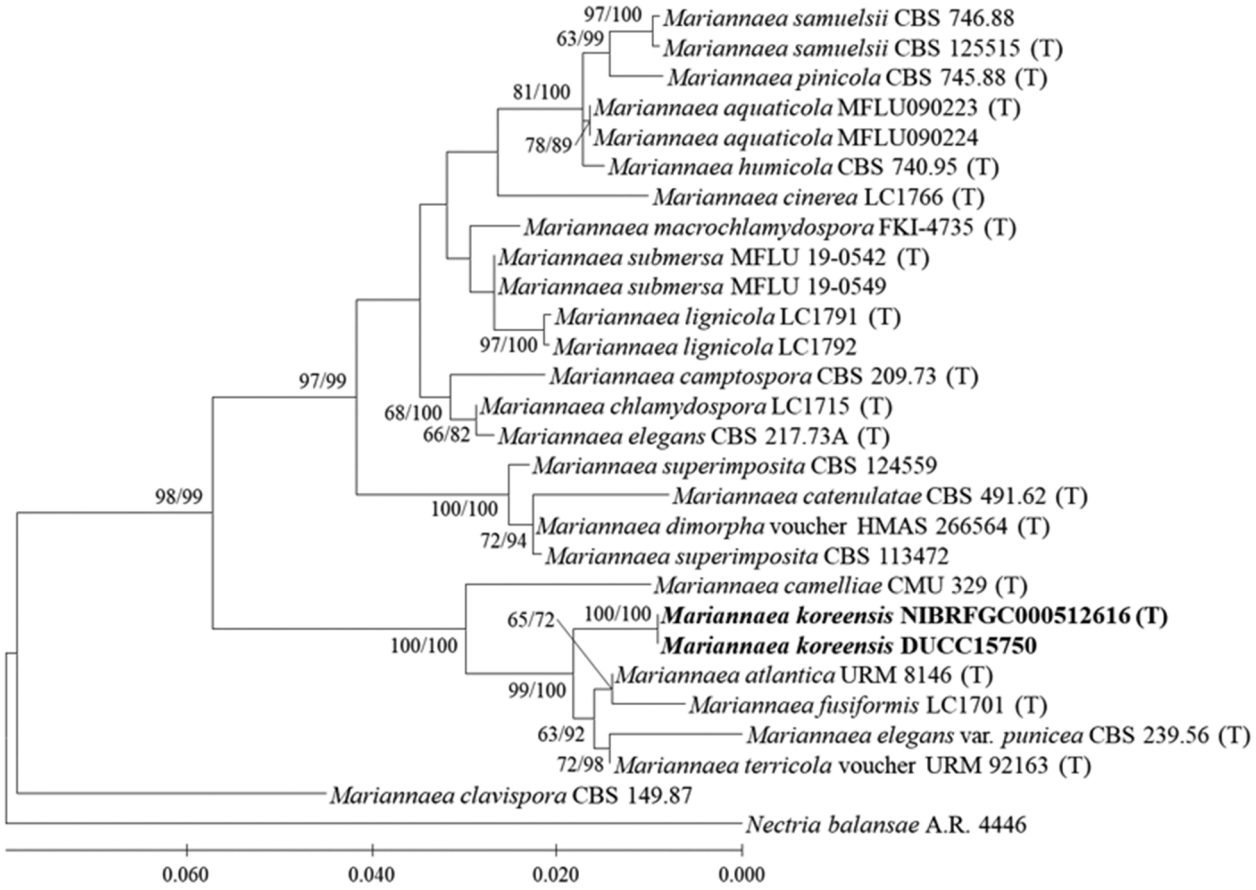

Figure 3. Mximum-likelihood phylogenetic tree based on the concatenated ITS, LSU, *tub* nucleotide sequences of *Mariannaea* species. New species are indicated in bold. The reliability value of each node was represented in the order of MLBS/PP. Nodes with reliability values less than MLBS <60 or PP <70 were removed. *Nectria balansae* A.R. 4446 was used as outgroup.

The sequences of the ITS, LSU, and *tub* of *M. koreensis* were analyzed. The size of the ITS was 456 bp, LSU was 845 bp, and *tub* was 281 bp. The three sequences, the ITS (410 bp), LSU (650 bp), and *tub* (223 bp), were concatenated. The meaningful sequences among the aligned series were the ITS (106/410), LSU (296/650), and *tub* (76/223). As a result of aligning the concatenated sequence with the sequences of other *Mariannaea* species, a final sequence of 1381 bp including gaps was obtained. A total of 20 species and 26 strains were used for phylogenetic analysis to distinguish *M. koreensis* from other *Mariannaea* species (Table 3). Based on a concatenated 1381 bp sequence with gaps of the ITS + LSU + *tub*, the results of MLBS and BI phylogenetic analyses revealed that *M. koreensis* formed a sister clade with the clade comprising *M. atlantica*, *M. fusiformis*, *M. elegans* var. *punicea*, and *M. terricola*. However, it was identified as a monophyletic species with high statistical support (MLBS/PP = 99/100; Figure 3).

3.2. Taxonomy

Gliocladiopsis koreensis Noh., H. and Kim., S.H., sp. nov. (Figure 4(A–Q)) [MycoBank No. 856848].

Typification: Jeju Island, The Republic of Korea: Soil around fruiting bodies of *Calostoma japonicum* from Iseung-ak Oreum (33°20′13.3″N, 126°37′24.2″E) collected on August 28 2023. NIBRFGC000512620.

Etymology: Latin, koreensis, referring to the Republic of Korea, origin of this species.

Description:

Macro morphological characteristics. Colonies were grown on PDA 51–51.8 mm, on MEA 50–50.5 mm, on CYA 48.9–49.3 mm, and on OA 51.3–51.6 mm (Figure 4(A-H)). The morphology of the colonies on PDA was submerged circular, with a dark yellow center and beige and dark brown edges. The aerial hyphae developed in the central part were 17–26 mm long. Clumps of white or yellow powdery spores were present throughout the colony. The back of the colony was dark brown and yellow pigment released extracellularly was observed. On MEA, the colonies were apricot-colored circular with beige borders and dark yellow center with aerial hyphae 10–18 mm long. The aerial hyphae outside the center were 3–5 mm long. Although no extracellular pigment was observed, the underside of the colony was greenish gray. On CYA, colonies were circular, radial, with white borders and dark orange or

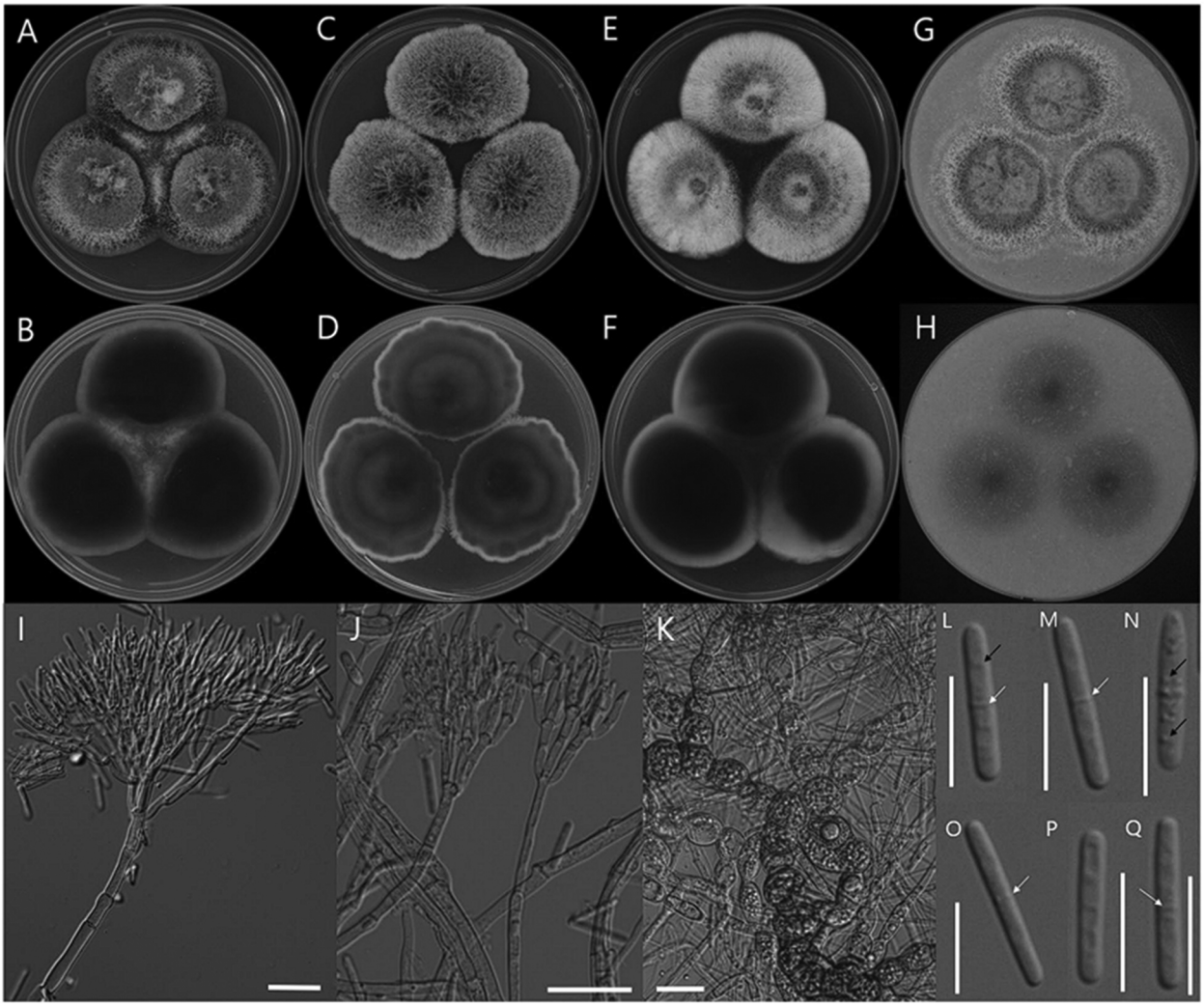

Figure 4. Colony morphology on different media (A–H) and light microscopic images (I–Q) of *Gliocladiopsis koreensis* Noh., H. and Kim., S.H., sp. nov. grown at 25 °C for 14 d. (A, B) PDA; (C, D) MEA; (E, F) CYA; (G, H) OA; (I, J) conidiophores and branches; (K) chlamydospores; (L–Q) conidium. Scale bar, I–K = 20 µm, L–Q = 10 µm. White narrows: septate of conidia. Black narrows: oily droplets.

yellow centers. Aerial hyphae of 14–26 mm were developed in the center and 5–6 mm at the edges. The back of the colony was dark brown, and light brown pigment was observed to be secreted extracellularly. Colonies in OA were yellow, radial, and circular with submerged white borders. Aerial hyphae measuring 8–11 mm were observed in the center of the colony and 5–7 mm just inside the border. The underside of the colony was greenish gray with light yellow border.

Microscopic morphological characteristics. The conidiophores were penicillate and subverticillate, hyaline, with the tips differentiated into three primary branches (Figure 4(I,J)). Primary branches were aseptate, thin-walled, measuring 15.4–34.9 × 2.8–6.4 µm (average = 22.99 × 4.15 µm, n = 50). Secondary branches were aseptate, thin-walled, and measured 10.6–24.7 × 2.3–4.1 µm (average = 19.62 × 3.12 µm, n = 50). Tertiary branches were aseptate, thin-walled, and measured 8.1–20.5 × 1.9–4.0 µm (average = 14.41 × 3.31 µm, n = 50). Quaternary branches were absent. Each branch contains 2–4 phialides, which were cymbiform to cylindrical, hyaline, and measured 8.2–24.5 × 1.9–3.5 µm (average = 14.86 × 2.73 µm, n = 50). Conidia were elongated, cylindrical, hyaline, guttulate, smooth, 0–1 septate, and measured 17.8–23.1 × 2.3–3.4 µm (average = 19.84 × 2.88 µm, n = 53) (Figure 4(L–Q)). Chlamydospores were frequently observed in older cultures, forming long chains of ovoid to cylindrical cells, with individual cells measuring 16.3–29.5 × 12.5–23.7 µm (average = 21.64 × 17.47 µm, n = 53) (Figure 4(K)). Teleomorph stage was not observed.

Notes. *G. koreensis* was closely related to *G. singaporiensis* and *G. mexicana* on the phylogenetic tree. However, it has primary branches of 15.4–34.9 × 2.8–6.4 µm, which are slightly thicker than the 20–31 × 3–4 µm of *G. singaporiensis*. Quaternary

branches are present in *G. singaporiensis*, but not in *G. koreensis*. Additionally, *G. koreensis* is morphologically distinct from *G. singaporiensis* in that it has larger and thicker phialides (8.2–24.5 × 1.9–3.5 µm vs. 8–14 × 2 µm), conidia (17.8–23.1 × 2.3–3.4 µm vs. 14–19 × 1.5–2 µm), and chlamydospores (16.3–29.5 × 12.5–23.7 µm vs. 8–14 × 9–13 µm) than *G. singaporiensis*. *G. mexicana* has aseptate or 1-septate primary branches, but *G. koreensis* only has aseptate primary branches. *G. koreensis* showed morphological differences in having longer secondary and tertiary branches (8.1–20.5 × 1.9–4 µm vs. 7–14 × 2–4 µm) than *G. mexicana* and having aseptate conidia. *G. peggii* shows significant morphological differences in that it does not have tertiary branches, chlamydospores, and aseptate conidia. *G. sumatrensis* has morphological differences in that it lacks chlamydospores and aseptate conidia and has 1-septate primary branches. *G. indonesiensis* and *G. irregularis* have morphological differences in that they do not have aseptate primary branches, chlamydospores, and aseptate conidia (Table 4). Based on the morphological differences with these closely related species and the phylogenetic tree results, we propose *G. koreensis* as a new species of the genus *Gliocladiopsis*.

Ilyonectria koreensis Noh., H. and Kim., S.H., sp. nov. (Figure 5(A–δ)) [MycoBank No. 856849].

Typification: Jeju Island, The Republic of Korea: Soil around fruiting bodies of *Russula nigricans* from Iseung-ak Oreum (33°20′13.3″N 126°37′24.2″E) collected on August 28 2023. NIBRFGC000512618.

Etymology: Latin, koreensis, referring to Korea, origin of this species.

Description:

Macro morphological characteristics. Colonies were grown on PDA 69.1–72.8 mm, on MEA 73.8–74.5 mm, on CYA 89–90 mm, and on OA 89–90 mm (Figure 5(A-H)). On PDA, colonies were irregular, light brown to brown, submerged with 2–4 mm aerial hyphae. Clumps of powdery conidia were present on the aerial hyphae. The back of the colony was light brown with a dark brown center. Colonies on MEA were light brown, circular, radial, and had aerial hyphae measuring 3–7 mm. Irregular-shaped mass made of hyphae was observed in the aerial hyphae. The back was brown with a light beige border. On CYA, colonies were light beige color, circular, radial, and had 2–4 mm of aerial hyphae. Irregular-shaped mass made of hyphae was observed in the aerial hyphae like MEA. The back of the CYA was yellow with clumps of brown mycelium. On OA, colonies were white with yellowish center and brown border, circular, radial, and had 4–7 mm of aerial hyphae. As the culture period became longer, the aerial hyphae in the central part disappeared. Clumps of powdery conidia were present on the aerial hyphae like PDA. The back of OA was gray purple.

Microscopic morphological characteristics. Conidiophores were hyaline, thin walled, 1–4 septate, 40–230(–350) × 2.3–3.5 (average = 107 × 2.82, *n* = 50) µm (Figure 5(I,J)). Conidiophores branched out from the hyphae and formed spores at the end or branched once more in the middle and formed spores at the end. Macroconidia were hyaline, 1–3 septate, straight or minutely curved, cylindrical with rounded ends; 1-septate, (19.8–)20.6–31.6 (–33.2) × 4.4–5.9(–6.5) µm (average = 25.45 × 5.18 µm, *n* = 50), with a length:width ratio of 4.0–5.9 (Figure 5(K–N)); 2-septate, (24.1–)26.1–33.5(–36.1) × 5.4–6.2 µm (average = 29.81 × 5.85 µm, *n* = 50), with a length:width ratio of 4.5–5.8 (Figure 5(S–V)); 3-septate, 31.4–39.6 × 5.5–6.5(–6.7) µm (average = 35.14 × 6.03 µm, *n* = 50), with a length:width ratio of 5.2–6.4 (Figure 5(O–R)). Microconidia were hyaline, 0–1 septate, ovoid to ellipsoid; aseptate, (3.5–)7.2–12.5(–13.8) × (3.2–)3.5–4.5(–4.7), with a length:width ratio of 1.7–3.6 (Figure 5(W–Y)); 1-septate, (11.2–)12.5–15.5(–16.6) × (3.3–)4.1–4.7(–5.1), with a length:width ratio of 2.8–3.8: 1-septate microconidia (Figure 5(Z–β)). Chlamydospores were formed by the swelling of the ends of browned hyphae, initially colorless and hyaline, when matured dark brown and translucent, contain large oil droplet, 7.6–18.2 × 7.3–16.6 µm (Figure 5(γ–δ)).

Notes. *I. koreensis* formed the same clade with *I. qitaiheensis*, *I. changbaiensis*, *I. gamsii*, and *I. liliigena*, and was most closely related to *I. qitaiheensis* and *I. changbaiensis*. *I. liliigena* has slightly smaller 2-septate (26.1–33.5 × 5.4–6.2 µm vs. 26.1–27.7 × 4.7–5 µm) and 3-septate (31.4–39.6 × 5.5–6.5 µm vs. 27.9–29.8 × 4.7–5.1 µm) macroconidia and a slightly thinner 1-septate microconidia (12.5–15.5 × 4.1–4.7 µm vs. 12.9–14.3 × 3.3–3.6 µm) than *I. koreensis*. *I. gamsii* showed morphological differences by having a maximum of three septate, unlike *I. koreensis*, which have a maximum of four septate, smaller chlamydospores (7.6–18.2 × 7.3–16.6 µm vs. 8–14 × 7–12 µm), and shorter aseptate microconidia (7.2–12.5 × 3.5–4.5 µm vs. 6.9–8.0 × 4.0–4.5 µm). *I. qitaiheensis* had morphological differences compared to *I. koreensis* in that it had conidiophores with 0–3 septate (*I. koreensis* have conidiophores with 1–4 septate) and slightly shorter aseptate microconidia (7.2–12.5 × 3.5–4.5 µm vs. 7.9–8.8 × 3.0–3.8 µm). *I. changbaiensis* had morphological differences

Table 4. Representative morphological characteristics between *Gliocladiopsis* species that are closely related to *G. koreensis* on the phylogenetic tree.

Scientific name Strain no.	Primary branches	Secondary branches	Tertiary branches	Quaternary branches	Phialides	Conidia	Chlamydospores	Reference
G. koreensis NIBRFGC000512620 (T)	Aseptate, 15.4–34.9 × 2.8–6.4 µm	Aseptate, 10.6–24.7 × 2.3–4.1 µm	Aseptate, 8.1–20.5 × 1.9–4 µm	Absent	Aseptate, 2–4 per branch, aseptate, 8.2–24.5 × 1.9–3.5 µm	0–1 septate, 17.8–23.1 × 2.3–3.4 µm	Common, long chains, 16.3–29.5 × 12.5–23.7 µm	In this study
G. singaporiensis MUCL 48728 (T)	Aseptate, 20–31 × 3–4 µm	Aseptate, 16–22 × 3–4 µm	Aseptate, 10–20 × 2 µm	Aseptate, 9–16 × 2–3 µm	Aseptate, 2–4 per branch, 8–14 × 2 µm	0(–1) septate, (13–)14–19 × 1.5–2 µm	Sparse, in short chains, 8–14 × 9–13 µm	[11]
G. Mexicana CBS 110938 (T)	Aseptate or 1-septate, 12–22 × 3–6 µm	Aseptate, 9–15 × 2–4 µm	Aseptate, 7–14 × 2–4 µm	Absent	Aseptate, 2–4 per branch, 9–15 × 3–4 µm	1-septate, (15–)17–19(–21) × 2–4 µm	Extensive, in non-delimited chains	[9]
G. peggii BRIP 60983 (T)	Aseptate, 20–25 × 3–4.5 µm	Aseptate, 8–15 × 2.5–4 µm	Absent	Absent	Aseptate, 3–4 per branch, 10–15 × 2–3 µm	1-septate, (10.5–)14–18(–19) × (1.5–)2–3 µm	Absent	[44]
G. sumatrensis CBS 754.97 (T)	1-septate, 17–40 × 3–5 µm	Aseptate, 12–17 × 3–5 µm	Aseptate, 7–10 × 3.5–4 µm	Absent	Aseptate, up to 6 per branch, 8–20 × 2.5–3 µm	1-septate, (10–)14–17(–18) × 2–2.5(–3) µm	Absent	[45]
G. indonesiensis CBS 116090 (T)	1-septate, 17–24 × 3–4 µm	Aseptate, 13–20 × 2–3 µm	Aseptate, 8–15 × 2–3 µm	Rare to absent, aseptate, 9–13 × 2–3 µm	Aseptate, 2–6 per branch, 13–21 × 2–4 µm	1-septate, (11–)13–15(–17) × 2–4 µm	Absent	[9]
G. irregularis CBS 755.97 (T)	1-septate, (15–)17(–25) × (3–)3.5 µm	Aseptate, (15–)18(–20) × 2.5(–3) µm	Aseptate, (9–)11(–14) × 2(–2.5)	Absent	Aseptate, (10–)13(–16) × (2–)3 µm	1-septate, (11–)13(–14) × 2.5(–3) µm	Absent	[46]

(T): type strain.

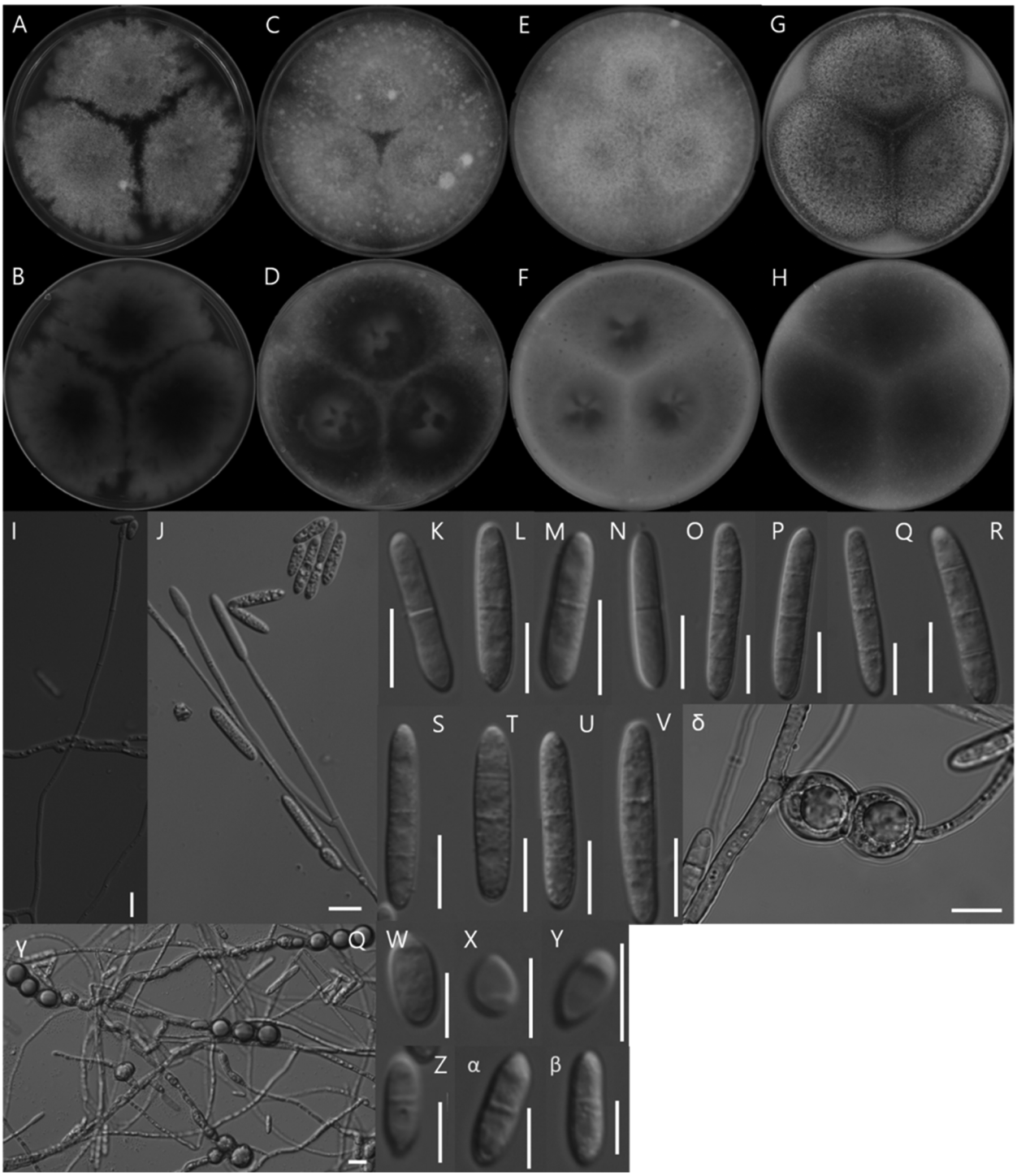

Figure 5. Colony morphology on different media (A-H) and light microscopic images (I–δ) of *Ilyonectria koreensis* Noh., H. and Kim, S.H., sp. nov. grown at 25°C for 14 d. (A, B) PDA; (C, D) MEA; (E, F) CYA; (G, H) OA; (I, J) conidiophores; (K–N) 1-septate macroconidia; (O–R) 2-septate macroconidia; (S-V) 3-septate macroconidia; (W–Y) aseptate microconidia; (Z–β) 1-septate microconidia; (γ, δ) chlamydospores. Scale bar, I–V, γ, δ = 10 µm, W–β = 5 µm.

compared to *I. koreensis*, with shorter aseptate microconidia (7.2–12.5 × 3.5–4.5 µm vs. 7.4–8.1 × 3.8–4.0 µm) and shorter but thicker 1-septate macroconidia (20.6–31.6 × 4.4–5.9 µm vs. 22.8–23.9 × 6.2–6.5 µm), and a conidiophore with up to three septate (Table 5). Based on the morphological differences with these closely related species and the phylogenetic tree results, we propose *I. koreensis* as a new species of genus *Ilyonectria*.

Mariannaea koreensis Noh., H. and Kim., S.H., sp. nov. (Figure 6(A–P)) [MycoBank No. 856850].

Typification: Changnyeong-gun, The Republic of Korea: Swamp soil from Upo Wetland (35°32′55.4″N 128°24′53.0″E) collected on September 18 2023. NIBRFGC000512616.

Etymology: Latin, koreensis, referring to Korea, origin of this species.

Description:

Macro morphological characteristics. On PDA, colonies were white and purple with rich wavy pattern, circular, with diameters of 36.5–39.5 mm and aerial hyphae 2–3 mm high (Figure 6(A-B)). A wavy pattern could be seen on the back, and light brown color pigment was observed. On MEA, colonies were white with pale wavy pattern, circular, radial with diameter of 38.5–42.7 mm (Figure 6(C-D)). The back of the colony was light ivory. On CYA, colonies were white, circular, no wavy pattern, radial with 37.2–39.4 mm and aerial hyphae 3–5 mm high (Figure 6(E-F)). The underside of the colony was ivory with a white outline. On OA, colonies were diameters of 41.8–43.2 mm, reddish brown, circular, pale wavy pattern, white powdery conidia and chlamydospore (Figure 6(G-H)).

Microscopic morphological characteristics. Hyphae 2–7 µm wide, smooth, septate, hyaline, thin-walled, branched (Figure 6(I–K)). Conidiophores were up to 450 µm long, 3.0–4.8 µm wide, straight, the main axis can primarily exhibit dichotomous or single branching at the base or middle part, smooth, thin-walled, septate, hyaline, each branch or main axis bears 3–7 phialides arranged in a whorled pattern, with the phialides at the tip of the main axis being generally longer. Phialides were 9.5–23.1 × 2.5–5.3 µm, flask-like, hyaline, smooth-walled, arranged in a whorled pattern on the branches or the main axis of the conidiophore (Figure 6(L)). Conidia were 4.7–6.7 × 3.2–4.1 µm, hyaline, smooth, fusoid, thin-walled, connected in an imbricate chain (Figure 6(O,P)). Chlamydospores had two forms: single and doliiform; single, terminal and intercalary, globose with 4.8–10.3 µm diameter and doliiform with 9.1–14.8 × 7.9–10.7 µm, hyaline, thick-walled (Figure 6(M,N)).

Notes. *M. koreensis* was closely related to *M. atlantica*, *M. fusiformis*, *M. elegans* var. *punicea*, *M. terricola*. However, *M. koreensis* was distinguished from other species in that it had 3–7 phialides per branch (*M. atlantica*: 2–7; *M. camelliae*: 1–4; *M. fusiformis*: 3–6; *M. elegans* var. *punicea*: 3–6; *M. terricola*: 3 per branch). *M. camelliae*, *M. fusiformis*, *M. elegans* var. *punicea* had only single chlamydospore, but *M. koreensis*, *M. atlantica*, and *M. terricola* had the characteristic of having doliiform chlamydospores. Additionally, *M. koreensis* had morphological difference in that it has slightly shorter doliiform chlamydospores than *M. atlantica* and *M. terricola* (9.1–14.8 × 7.9–10.7 µm vs. 8–20 × 5–10 µm and 7.5–20 × 4–10 µm). The conidia of *M. koreensis* were like those of *M. camelliae* and *M. elegans* var. *punicea* (4.7–6.7 × 3.2–4.1 µm vs. 4–7 × 3–5 µm and 4–7 × 2–3.5 µm), but smaller than those of *M. atlantica* (5–10 × 2–4 µm) and *M. fusiformis* (5–10 × 3–4 µm) (Table 6). The most notable characteristic of *M. koreensis* is that, unlike other *Mariannaea* species, it forms purple and white wavy colonies with a white border on the PDA. Although *M. fusiformis* forms purple colonies, the difference is that *M. fusiformis* had no wavy pattern.

Jeju Island, designated as a UNESCO World Natural Heritage site, boasts unique volcanic topography and diverse ecosystems [52]. Iseung-ak Oreum in this Island is a parasitic volcano with an elevation of 539 m and a height of 114 m. And it is a popular tourist destination for its lush cedar trees, cherry blossoms in spring, and silver grass in fall. Since soil-derived mushrooms play a role in recycling materials in the soil, saprotrophic mushroom such as *Russula nigricans* or *Calostoma japonicum* from Lee Seungak Oreum is also expected to play that role. The presence of diverse bacteria in the hyphosphere of mushroom is known, but the presence of diverse fungi has not been much studied. Thus, the discovery of two new species, *Gliocladiopsis koreensis* and *Ilyonectria koreensis*, in the hyphosphere soil of these basidiomycetes is significant in itself. It is expected that the fungi in the hyphosphere soil of the mushroom would have interactions with diverse bacteria and the mushroom's hyphae within soil environment.

Upo Wetland, an internationally ecologically protected Ramsar site, is renowned for its ecological diversity and serves as a habitat for rare species [53]. The outstanding value of Upo Wetland is evident, especially in its scale and biodiversity. As fungal diversity has been poorly studied, the discovery of a new species, *Mariannaea koreensis*, in the area highlights Upo Wetlands as worthy of protection. Members of the Nectriaceae family are known to produce potent degradative enzymes capable of breaking down complex organic compounds such as lignin and cellulose [54, 55]. This enzymatic activity contributes to organic matter decomposition and nutrient cycling in forest ecosystems, thereby improving soil health [56–58]. Some members of the Nectriaceae family have been reported to remediate soils contaminated with heavy metals or chemicals [59, 60]. This makes these fungi valuable candidates for sustainable bioremediation approaches in ecologically sensitive regions like Jeju Island and the Upo Wetland. Furthermore, the secondary metabolites produced by these fungi have great potential for various industrial and pharmaceutical applications. Members of the Nectriaceae family are well-documented producers of diverse bioactive compounds, including antibiotics, antifungal agents,

Table 5. Representative morphological characteristics between *Ilyonectria* species that are closely related to *I. koreensis* on the phylogenetic tree.

Scientific name Strain no.	Conidiophores	Chlamydospores	Macroconidia	Microconidia	Reference
I. koreensis NIBRFGC000512618 (T)	1–4 septate, 40–230(–350) × 2.3–3.5	7.6–18.2 × 7.3–16.6 µm	1-septate, (19.8–)20.6–31.6(–33.2) × 4.4–5.9(–6.5) µm, with a length:width ratio of 4.0–5.9; 2-septate, (24.1–)26.1–33.5(–36.1) × 5.4–6.2 µm, with a length:width ratio of 4.5–5.8; 3-septate, 31.4–39.6 × 5.5–6.5(–6.7) µm, with a length:width ratio of 5.2–6.4	Aseptate, (3.5–)7.2–12.5(–13.8) × (3.2–)3.5–4.5(–4.7), with a length:width ratio of 1.7–3.6; 1-septate, (11.2–)12.5–15.5(–16.6) × (3.3–)4.1–4.7(–5.1), with a length:width ratio of 2.8–3.8	In this study
I. liliigena CBS 189.49 (T)	1–4 septate, 50–170 µm long	6–14 × 5–12 µm	1-Septate, (19.0–)22.9–24.6(–30.0) × (3.3)4.2–4.5(5.2) µm, with a length:width ratio of 4.0–7.0; 2-septate, (21.0–)26.1–27.7(–32.1) × (4.0)4.7–5(5.7) µm, with a length:width ratio of 3.8–7.0; 3-septate, (23.9–)27.9–29.8(–35.0) × (3.9)4.7–5.1(6.0) µm, with a length:width ratio of 4.0–8.3	Aseptate, (5.9–)8.9–10.3(–17.0) × (2.5–)3.0–3.2(–4.4) µm, with a length:width ratio of 2.0–4.6; 1-septate, (10.0–)12.9–14.3(–18.0) × (2.5–)3.3–3.6(–4.5) µm, with a length:width ratio 2.8–5.6	[47]
I. gamsii CBS 940.97 (T)	1–3 septate, 50–150 µm long	8–14 × 7–12 µm	1-Septate, (22.0–)25.7–27.9(–33.0) × (4.0)5.1–5.5(6.0) µm, with a length:width ratio of 4.3–6.2; 2-septate, (25.0–)28.2–31.7(–39.0) × (5.0)5.5–5.9(6.5) µm, with a length:width ratio of 4.2–7.1; 3-septate, (24.0–)34.3–38.5(–44.0) × (5.0)5.9–6.3(7.0) µm, with a length:width ratio of 4.3–7.3	Aseptate, (4.0–)6.9–8.0(–10.0) × (3.0–)4.0–4.5(–5.0) µm, with a length:width ratio of 1.3–2.9; 1-septate, (8.0–)12.9–15.7(–18.0) × (4.0–)4.2–4.7(–5.5) µm, with a length:width ratio 1.8–4.0	
I. qitaiheensis CGMCC 3.18787 (T)	0–3 septate, 46- to 132 µm long	8–14 × 7–20 µm	1-Septate, (15.0–)21.8–23.9(–34.0) × (4.0–)5.1–5.5(–7.0) µm, with a length:width ratio of 3.6–4.9; 2-septate, (21.0–)27.9–29.9(–37.0) × (4.0–)5.6–6.0(–8.0) µm, with a length:width ratio of 4.3–5.7; 3-septate, (22.0–)29.3–32.0(–44.0) × (5.0–)5.7–6.1(–8.0) µm, with a length:width ratio of 4.4–5.8	Aseptate, (3.0–)7.9–8.8(–12.0) × (3.0–)3.4–3.8(–6.0) µm, with a length:width ratio of 1.0:3.7; 1-septate microconidia, (9.0–) 10.5–11.6(–14.0) × (3.0–)3.7–4.2(–6.0) µm, with a length:width ratio of 2.5:3.3	[48]
I. changbaiensis CGMCC 3.18789 (T)	0–3 septate, 46- to 72 µm long	7–16 × 7–14 µm	1-Septate, (16.0–)22.8–23.9(–33.0) × (4.0–)6.2–6.5(–8.0) µm, with a length:width ratio of 2.4–5.2; 2-septate, (22.0–)27.7–28.9(–36.0) × (5.0–)6.6–6.9(–8.0) µm, with a length:width ratio of 3.1–5.0; 3-septate, (25.0–)30.0–31.5(–38.0) × (6.0–)6.7–7.0(–8.0) µm, with a length:width ratio of 3.3–5.4	Aseptate, (4.0–)7.4–8.1(–12.0) × (3.0–)3.8–4.0(–5.0) µm, with a length:width ratio of 1.3–3.3; 1-septate, (9.0–)11.7–12.4(–16.0) × (3.0–)4.14.3(–5.0) µm, with a length:width ratio of 2.0–4.0	

(T): type strain.

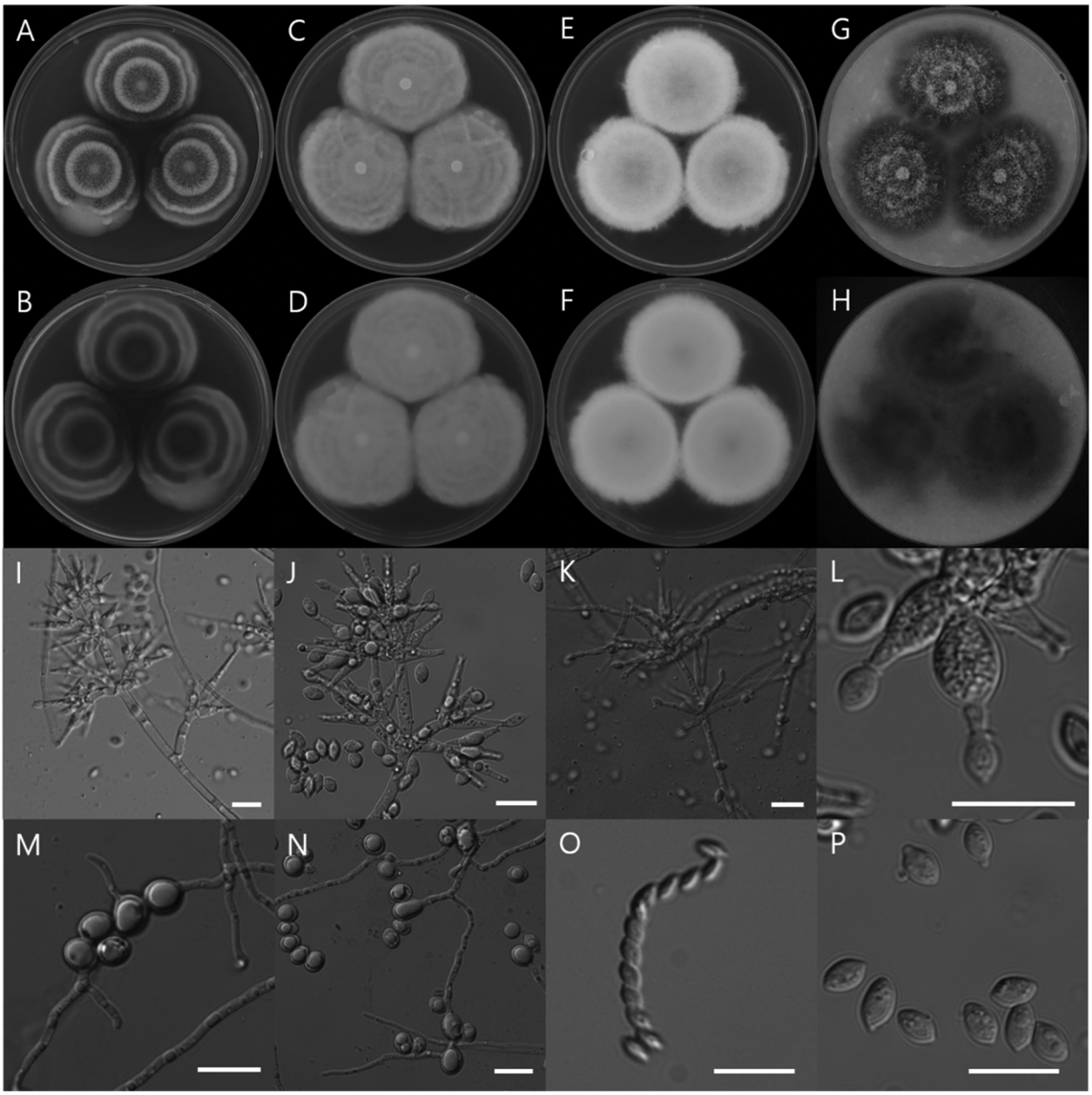

Figure 6. Colony morphology on different media and light microscopic images of *Mariannaea koreensis* Noh., H. and Kim., S.H., sp. nov. grown at 25 °C for 14 d. (A, B) PDA; (C, D) MEA; (E, F) CYA; (G, H) OA; (I–K) conidiophores and phialides; (L) phialides; (M, N) chlamydospores; (O) chained conidia; (P) conidia. Scale bar = 10 µm.

Table 6. Representative morphological characteristics between *Mariannaea* species that are closely related to *M. koreensis* on the phylogenetic tree.

Scientific name Strain no.	Conidiophores	Phialides	Chlamydospores	Conidia	Reference
M. koreensis NIBRFGC000512616 (T)	Up to 450 µm long, 3.0–4.8 µm wide	3–7 per branch, 9.5–23.1 × 2.5–5.3 µm	Single, 4.8–10.3 µm diam., doliiform, 9.1–14.8 × 7.9–10.7 µm	4.7–6.7 × 3.2–4.1 µm	In this study
M. camelliae CMU329 (T)	105–225 × 3.5–7.5 µm	1–4 per branch, 13–17.5 × 2–4.5 µm	6–11 × 4–9 µm	4–7 × 3–5 µm	[49]
M. atlantica URM 8146 (T)	Up to 327.5 µm long, 3–5 µm wide	2–7 per branch, 12–20 × 2–3 µm	Single, 4–15 µm; doliiform, 8–20 × 5–10 µm	5–10 × 2–4 µm	[50]
M. fusiformis CGMCC 3.17272 (T)	Up to 800 µm long, 4–6 µm wide	3–6 per branch, 14–38 × 4–5 µm	8–10 × 5–7 µm	5–10 × 3–4 µm	[36]
M. elegans var. *punicea* CBS 239.56 (T)	160–300 µm long, 6–9 µm wide	3–6 per branch, 9–15 × 2–6 µm	6–10 µm diam.	4–7 × 2–3.5 µm	
M. terricola UMR 92163 (T)	Up to 575 µm long, 5–12 µm wide	3 per branch, 14–22 × 2–5 µm	Single, 7.5–8 µm; doliiform, 7.5–20 × 4–10 µm	3–9 × 2–4 µm	[51]

(T): type strain.

and bioactive polyketides [61, 62]. Thus, the discovery of these novel species not only expands our understanding of fungal biodiversity but also highlights their potent roles in decomposition, bioremediation, and secondary metabolite production, providing a strong foundation for future research.

Disclosure statement

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

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