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Diversity of soil-borne *Gliocladiopsis* from Indonesia, Malaysia and Vietnam

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Abstract: *Gliocladiopsis* comprises a diverse group of soil-borne fungi predominantly found in tropical and subtropical regions of the world. This study investigated *Gliocladiopsis* strains collected from soil samples across three Southeast Asian countries, namely, Indonesia, Malaysia and Vietnam. Morphological characteristics and multi-gene phylogenetic analyses based on four loci (ITS, *TEF1, TUB2*, and *HIS3*) were used to identify the strains. Eleven *Gliocladiopsis* spp., including a novel taxon, *Gliocladiopsis vietnamensis sp. nov*., were identified. The six species, *G. curvata*, *G. forsbergii*, *G. guangdongensis*, *G. irregularis*, *G. whileyi* and *G. wuhanensis*, are newly reported in Vietnam, *G. curvata* in Malaysia, and *G. mexicana* in Indonesia. This study has significantly expanded the known diversity of *Gliocladiopsis* in Southeast Asia. The results also suggest that Southeast Asia is a biodiversity hotspot for this genus, highlighting the importance of further research to explore fungal diversity in understudied tropical and subtropical ecosystems.

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INTRODUCTION

The genus *Gliocladiopsis* was established by Saksena (1954) based on *G. sagariensis* from a soil sample from Ghatera forests in Sagar, India. The name was chosen to reflect its similarity to *Gliocladium* in having slimy spore droplets. Saksena (1954) also noted a resemblance of the genus to *Cylindrocladium* (*Cm.*) and *Penicillium* in having conidiophores terminating in penicillate branches and, in the case of *Cylindrocladium*, cylindrical conidia. *Gliocladiopsis* spp. are characterised by having penicillate (rarely subverticillate) conidiophores, (0–)1-septate cylindrical conidia resembling the conidial morph of *Calonectria* (*Cylindrocladium*) but without stipe extensions terminating in terminal vesicles, and pale yellow spore droplets at the tips of the conidiogenous apparatuses (Crous & Wingfield 1993, Crous 2002, Lombard & Crous 2012). In some species, globose and brown chlamydospore chains have been observed in culture (Crous 2002).

Gliocladiopsis has undergone a number of taxonomic treatments since its first description. Saksena (1954) initially assigned *Gliocladiopsis* to the *Moniliaceae*, an obsolete family accommodating numerous unrelated hyphomycetes. Later, Agnihothrudu (1959), after studying the ex-types of *G. sagariensis* and *Cylindrocarpon* (*Cp.*) *tenue*, reduced the monotypic *Gliocladiopsis* to synonymy with *Cylindrocarpon*, and treated *G. sagariensis* as a heterotypic synonym of *Cp. tenue* (Bugnicourt 1939). Independently, Barron (1968) considered *Gliocladiopsis* as a synonym of *Cylindrocladium.* Crous & Wingfield (1993) later resurrected *Gliocladiopsis* as a separate genus based on *G. sagariensis* as a type species and established *G. tenuis* for *Cp. tenue.* The placement of *Gliocladiopsis* within the *Nectriaceae* was confirmed using multi-gene phylogenetic analyses (Lombard & Crous 2012, Lombard *et al.* 2015). *Gliocladiopsis* has been linked to the sexual genus *Glionectria* (Schoch *et al.* 2000, Crous 2002), but following the "One Fungus = One Name" convention (Hawksworth 2011), the older asexual genus name *Gliocladiopsis* was chosen over the lesser-known *Glionectria* (Rossman *et al.* 2013).

Lombard & Crous (2012) provided the most comprehensive monograph for *Gliocladiopsis* spp., using the combination of morphological characters and sequence data from the 28S large subunit (LSU) and internal transcribed spacer (ITS) region of the ribosomal DNA, β-tubulin (*TUB2*), histone H3 (*HIS3*), and translation elongation factor 1-α (*TEF1*). They accepted *G. sagariensis* as the type species and confirmed the taxonomic status of nine *Gliocladiopsis* spp. known from culture. To date, 20 *Gliocladiopsis* spp. are recognised (Lombard & Crous 2012, Parkinson *et al.* 2017a, Liu & Cai 2013, Hyde *et al.* 2018, Zhai *et al.* 2019, Gordillo & Decock 2019, Perera *et al.* 2020, 2023).

Gliocladiopsis species are predominantly found in tropical and subtropical regions and are commonly associated with plant litter, soil and rhizosphere (Crous 2002, Lombard & Crous 2012). Although they are generally soil-borne, some species have been found in aquatic habitats (Liu & Cai 2003, Gordillo & Decock 2019, Hyde *et al.* 2019), and others have been isolated as endophytes from asymptomatic plant tissues (Li *et al.* 2008, Refaei *et al.* 2011, Hidayat *et al.* 2016). Most of these species have never been experimentally tested for pathogenicity, and they are generally regarded as saprobes (Crous 2002, Lombard &

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Table 1. Collection details and GenBank accessions of isolates included in the phylogenetic analyses.

Species	Isolate numbers	Substrate	Locality	GenBank accession				Reference
				TUB2	*HIS3*	ITS	*TEF1*	
Gliocladiopsis aquaticus	MFLUCC 17-1811^{T}	submerged wood	Thailand	MG574421	MG734182	MG543924	N/A	Hyde *et al*. (2018)
G. aquaticus	MFLUCC 17-2028	submerged wood	Thailand	MG574422	MG734183	MG543925	N/A	Hyde *et al*. (2018)
G. curvata	CBS 978.73	N/A	Brazil	JQ666119	JQ666009	JQ666043	JQ666085	Lombard & Crous (2012)
	CBS 194.80	*Persea americana*	Ecuador	JQ666120	JQ666010	JQ666044	JQ666086	Lombard & Crous (2012)
	CBS 110840 = MUCL 38873 = CPC 855	green house	Belgium	JQ666121	JQ666011	JQ666045	JQ666087	Lombard & Crous (2012)
	CBS 111194 = CPC 1354	soil	Mauritius	JQ666122	JQ666012	JQ666046	JQ666088	Lombard & Crous (2012)
	CBS 111195 = CPC 1355	soil	Mauritius	JQ666123	JQ666013	JQ666047	JQ666089	Lombard & Crous (2012)
	CBS 111196 = CPC 1356	soil	Mauritius	JQ666124	JQ666014	JQ666048	JQ666090	Lombard & Crous (2012)
	CBS 111421 = CPC 1652	soil	Ecuador	JQ666125	JQ666015	JQ666049	JQ666091	Lombard & Crous (2012)
	CBS 112365^{T} = CPC 10491 = Lynfield 791-B	*Archontophoenix purpurea*	New Zealand	JQ666126	JQ666016	JQ666050	JQ666092	Lombard & Crous (2012)
	CBS 112935 = CPC 4574	*Syzygium aromaticum*	Indonesia	JQ666127	JQ666017	JQ666051	JQ666093	Lombard & Crous (2012)
	CBS 114464 = CPC 1656	soil	Ecuador	JQ666128	JQ666018	JQ666052	JQ666094	Lombard & Crous (2012)
	CBS 115688 = IFO 9133 = CPC 539 = NBRC 9133	N/A	Japan	JQ666129	JQ666019	JQ666053	JQ666095	Lombard & Crous (2012)
	CMW 47217	soil associated with *Acacia* hybrid	Cuc Phuong National Park, Ninh Binh, Vietnam	PV420432	PV420382	PV405799	PV434096	This study
	CMW 47331	soil associated with *Acacia mangium*	Son Duong, Tuyen Quang, Vietnam	PV420433	PV420383	PV405800	PV434097	This study
	CMW 48247	soil associated with *Eucalyptus* sp.	Aek Nauli, North Sumatra, Indonesia	PV420434	PV420384	PV405801	PV434098	This study
	CMW 48275	soil associated with *Eucalyptus urophylla*	Tawau, Sabah, Malaysia	PV420435	PV420385	PV405802	PV434099	This study
G. ecuadoriensis	MUCL 54740^{T}	rhizosphere of *Polybotrya* sp.	Ecuador	KX611501	KX671146	KX671139	KX671131	Gordillo & Decock (2019)
G. elghollii	CBS 206.94	*Chamaedorea elegans*	USA	JQ666130	JQ666020	JQ666054	JQ666096	Lombard & Crous (2012)
	CBS 116104^{T} = CPC 636 = P93-2051	*Chamaedorea elegans*	USA	JQ666131	JQ666021	JQ666055	JQ666097	Lombard & Crous (2012)
G. forsbergii	BRIP 60984	*Grevillea* sp.	Australia	KX274036	KX274053	KX274070	N/A	Parkinson *et al.* (2017a)
	BRIP 61349a^{T}	*Persea americana*	Australia	KX274037	KX274054	KX274071	N/A	Parkinson *et al.* (2017a)

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Table 1. (continued).

Species	Isolate numbers	Substrate	Locality	GenBank accession				Reference
				*TUB*2	*HIS*3	ITS	*TEF*1	
	CMW 47195	soil associated with *Acacia* hybrid	Tuyen Quang, Vietnam	PV420436	PV420386	PV405803	PV434100	This study
	CMW 47287	soil associated with *Acacia* hybrid	Cuc Phuong National Park, Ninh Binh, Vietnam	PV420437	PV420387	PV405804	PV434101	This study
	CMW 47292	soil associated with *Acacia* hybrid	Cuc Phuong National Park, Ninh Binh, Vietnam	PV420438	PV420388	PV405805	PV434102	This study
G. guangdongensis	LC 1340^{T}	submerged wood	China	KC776124	KC776120	KC776122	KC776118	Liu & Cai (2013)
	LC 1349	submerged wood	China	KC776125	KC776121	KC776123	KC776119	Liu & Cai (2013)
	CMW 47386 = CBS 144576	soil associated with *Camellia* sp.	Tam Dao, Vinh Phuc, Vietnam	PV420439	PV420389	PV405806	PV434103	This study
	CMW 47388 = CBS 144577	soil associated with *Camellia* sp.	Tam Dao, Vinh Phuc, Vietnam	PV420440	PV420390	PV405807	PV434104	This study
	CMW 47397 = CBS 144578	soil associated with *Camellia* sp.	Tam Dao, Vinh Phuc, Vietnam	PV420441	PV420391	PV405808	PV434105	This study
	CMW 47398	soil associated with *Camellia* sp.	Tam Dao, Vinh Phuc, Vietnam	PV420442	PV420392	PV405809	PV434106	This study
G. hennebertii	MUCL 54818^{T}	rhirosphere of *Costus scaber*	Ecuador	KX611502	N/A	KX671140	KX671132	Gordillo & Decock (2019)
G. indonesiensis	CBS 116090^{T} = CPC 715	soil	Indonesia	JQ666132	JQ666022	JQ666056	JQ666098	Lombard & Crous (2012)
	CMW 48255	soil associated with *Eucalyptus* sp.	Aek Nauli, North Sumatra, Indonesia	PV420443	PV420393	PV405810	PV434107	This study
	CMW 48258	soil associated with *Eucalyptus* sp.	Aek Nauli, North Sumatra, Indonesia	PV420444	PV420394	PV405811	PV434108	This study
G. irregularis	CBS 755.97^{T} = CPC 718	soil	Indonesia	JQ666133	JQ666023	AF220977	JQ666099	Lombard & Crous (2012)
	CBS 111142 = CPC 1279	*Araucaria* sp.	Malaysia	JQ666134	JQ666024	JQ666057	JQ666100	Lombard & Crous (2012)
	CBS 111176 = CPC 1280	*Araucaria* sp.	Malaysia	JQ666135	JQ666025	JQ666058	JQ666101	Lombard & Crous (2012)
	CBS 114667 = 1278	*Araucaria* sp.	Malaysia	JQ666136	JQ666026	JQ666059	JQ666102	Lombard & Crous (2012)
	CBS 116086 = CPC 716	soil	Indonesia	JQ666152	JQ666042	JQ666072	JQ666118	Lombard & Crous (2012)
	CMW 47170 = CBS 144564	soil associated with *Acacia auriculiformis*	Do Luong, Nghe An, Vietnam	PV420445	PV420395	PV405812	PV434109	This study
	CMW 47177	soil associated with *Acacia auriculiformis*	Do Luong, Nghe An, Vietnam	PV420446	PV420396	PV405813	PV434110	This study
	CMW 47189 = CBS 144570	soil associated with *Acacia* hybrid	Quang Tri, Vietnam	PV420447	PV420397	PV405814	PV434111	This study

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Table 1. (continued).

Species	Isolate numbers	Substrate	Locality	GenBank accession				Reference
				*TUB*2	*HIS*3	ITS	*TEF*1	
	CMW 47328 = CBS 144571	soil associated with *Acacia mangium*	Tan Huong, Yen Bai, Vietnam	PV420448	PV420398	PV405815	PV434112	This study
	CMW 47345	soil associated with *Acacia mangium*	Dai Lai, Vinh Phuc, Vietnam	PV420449	PV420399	PV405816	PV434113	This study
	CMW 47346 = CBS 144565	soil associated with *Camellia* sp.	Thanh Son, Phu Tho, Vietnam	PV420450	PV420400	PV405817	PV434114	This study
	CMW 47383 = CBS 144572	soil associated with *Camellia* sp.	Tam Dao, Vinh Phuc, Vietnam	PV420451	PV420401	PV405818	PV434115	This study
	CMW 47390	soil associated with *Camellia* sp.	Tam Dao, Vinh Phuc, Vietnam	PV420452	PV420402	PV405819	PV434116	This study
	CMW 47415 = CBS 144566	soil associated with *Eucalyptus urophylla*	Nghia Dan, Nghe An, Vietnam	PV420453	PV420403	PV405820	PV434117	This study
	CMW 47431	soil associated with *Eucalyptus* hybrid	Bac Tu Liem, Hanoi, Vietnam	PV420454	PV420404	PV405821	PV434118	This study
	CMW 47518	soil associated with *Pinus* sp.	Do Luong, Nghe An, Vietnam	PV420455	PV420405	PV405822	PV434119	This study
	CMW 47520	soil associated with *Pinus* sp.	Do Luong, Nghe An, Vietnam	PV420456	PV420406	PV405823	PV434120	This study
	CMW 47521	soil associated with *Pinus* sp.	Do Luong, Nghe An, Vietnam	N/A	PV420407	PV405824	PV434121	This study
	CMW 47524	soil associated with *Pinus* sp.	Do Luong, Nghe An, Vietnam	PV420457	PV420408	PV405825	PV434122	This study
	CMW 47528	soil associated with *Cinnamomum cassia*	Mau A, Yen Bai, Vietnam	PV420458	PV420409	PV405826	PV434123	This study
	CMW 47529 = CBS 144567	Soil associated with *Cinnamomum cassia*	Mau A, Yen Bai, Vietnam	PV420459	PV420410	PV405827	PV434124	This study
	CMW 47533	soil associated with *Cinnamomum cassia*	Mau A, Yen Bai, Vietnam	PV420460	PV420411	PV405828	PV434125	This study
	CMW 47535	soil associated with *Cinnamomum cassia*	Mau A, Yen Bai, Vietnam	PV420461	PV420412	PV405829	PV434126	This study
	CMW 47537	Soil in natural forest	Anh Son, Nghe An, Vietnam	PV420462	PV420413	PV405830	PV434127	This study
	CMW 47555	soil associated with *Castanea* sp.	Van Ban, Lao Cai, Vietnam	PV420463	PV420414	PV405831	PV434128	This study
	CMW 49939	soil in natural forest	Bu Gia Map National Park, Binh Phuoc, Vietnam	PV420464	PV420415	PV405832	PV434129	This study

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Table 1. (continued).

Species	Isolate numbers	Substrate	Locality	GenBank accession				Reference
				TUB2	*HIS3*	ITS	*TEF1*	
	CMW 48272	soil associated with *Acacia mangium*	Tawau, Sabah, Malaysia	PV420465	PV420416	PV405833	PV434130	This study
G. mexicana	CBS 110938^{T} = CPC 964	soil	Mexico	JQ666137	JQ666027	JQ666060	JQ666103	Lombard & Crous (2012)
	CBS 111131 = CPC965	soil	Mexico	JQ666138	JQ666028	JQ666061	JQ666104	Lombard & Crous (2012)
	CMW 48270	soil associated with *Eucalyptus* sp.	Aek Nauli, North Sumatra, Indonesia	PV420466	PV420417	PV405834	PV434131	This study
	CMW 48250	soil associated with *Eucalyptus* sp.	Aek Nauli, North Sumatra, Indonesia	PV420467	PV420418	PV405835	PV434132	This study
G. peggii	BRIP 60983^{T}	*Persea americana*	Australia	KX274038	KX274065	KX274083	N/A	Parkinson *et al.* (2017a)
	BRIP 60987	*Persea americana*	Australia	KX274040	KX274062	KX274074	N/A	Parkinson *et al.* (2017a)
G. pseudotenuis	CBS 114763 = CPC 4575	*Vanilla* sp.	Indonesia	JQ666139	JQ666029	JQ666062	JQ666105	Lombard & Crous (2012)
	CBS 116074^{T} = CPC 706	soil	China	JQ666140	JQ666030	AF220981	JQ666106	Lombard & Crous (2012)
G. sagariensis	CBS 199.55^{T}	soil	India	JQ666141	JQ666031	JQ666063	JQ666107	Lombard & Crous (2012)
G. siamensis	MFLUCC 18-0576T	decaying stem	Thailand	ON364481	ON364457	ON361571	N/A	Perera *et al.* (2023)
G. singaporiensis	MUCL 48728T	submerged leaf litter	Singapore	KX611500	N/A	KX671138	KX671130	Gordillo & Decock (2019)
G. sumatrensis	CBS 754.97^{T} = CPC 1353	soil	Indonesia	JQ666142	JQ666032	JQ666064	JQ666108	Lombard & Crous (2012)
	CBS 111198 = CPC 1352	soil	Indonesia	JQ666143	JQ666033	JQ666065	JQ666109	Lombard & Crous (2012)
	CBS 111213	soil	Indonesia	JQ666144	JQ666034	JQ666066	JQ666110	Lombard & Crous (2012)
	CBS 111368 = CPC 1351	soil	Indonesia	JQ666145	JQ666035	AF220978	JQ666111	Lombard & Crous (2012)
	CMW 48262	soil associated with *Eucalyptus* sp.	Aek Nauli, North Sumatra, Indonesia	PV420468	PV420419	PV405836	PV434133	This study
	CMW 48267	soil associated with *Eucalyptus* sp.	Aek Nauli, North Sumatra, Indonesia	PV420469	PV420420	PV405837	PV434134	This study
G. swieteniae	MFLUCC 17-2616^{T}	*Swietenia mahagoni*	Thailand	MT212214	MT212194	MT215501	N/A	Perera *et al.* (2020)
G. tenuis	CBS 111961 = CPC 2910	*Coffee* sp.	Vietnam	JQ666146	JQ666036	JQ666067	JQ666112	Lombard & Crous (2012)
	CBS 111964 = CPC 2909	*Coffee* sp.	Vietnam	JQ666147	JQ666037	JQ666068	JQ666113	Lombard & Crous (2012)
	CBS 114147 = CPC 2912	soil	Vietnam	JQ666148	JQ666038	JQ666069	JQ666114	Lombard & Crous (2012)
	CBS 114148 = CPC 2911	soil	Vietnam	JQ666149	JQ666039	JQ666070	JQ666115	Lombard & Crous (2012)
	IMI 68205^{T} = CPC 2403	*Indigofera* sp.	Indonesia	JQ666150	JQ666040	AF220979	JQ666116	Lombard & Crous (2012)
	CMW 49920	soil associated with *Hevea brasiliensis*	Duong Minh Chau, Tay Ninh, Vietnam	PV420470	PV420421	PV405838	PV434135	This study

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Table 1. (continued).

Species	Isolate numbers	Substrate	Locality	GenBank accession				Reference
				TUB2	*HIS3*	ITS	*TEF1*	
	CMW 49927	soil associated with *Hevea brasiliensis*	Long Thanh, Dong Nai, Vietnam	PV420471	PV420422	PV405839	PV434136	This study
	CMW 49916	soil associated with *Hevea brasiliensis*	Duong Minh Chau, Tay Ninh, Vietnam	PV420472	PV420423	PV405840	PV434137	This study
G. vietnamensis	**CMW 47402 = CBS 144585**	soil associated with *Eucalyptus urophylla*	Son Duong, Tuyen Quang, Vietnam	PV420473	PV420424	PV405841	PV434138	This study
	CMW 49944 = CBS 144586	soil associated with *Alnus nepalensis*	Hoang Lien National Park, Lao Cai, Vietnam	PV420474	PV420425	PV405842	PV434139	This study
	CMW 49945^{T} = CBS 144587	soil associated with *Alnus nepalensis*	Hoang Lien National Park, Lao Cai, Vietnam	PV420475	PV420426	PV405843	PV434140	This study
	CMW 49947	soil associated with *Alnus nepalensis*	Hoang Lien National Park, Lao Cai, Vietnam	PV420476	PV420427	PV405844	PV434141	This study
G. whileyi	BRIP 61430^{T}	*Persea americana*	Australia	KX274052	KX274069	KX274086	N/A	Parkinson *et al.* (2017a)
	CMW 47392	soil associated with *Camellia* sp.	Tam Dao, Vinh Phuc, Vietnam	PV420477	PV420428	PV405845	PV434142	This study
G. wuhanensis	HEAC17307^{T}	soil	China	MH169602	MH255786	MH024520	N/A	Zhai *et al.* (2019)
	CMW 47540 = CBS 144579	soil in natural forest	Nhu Xuan, Thanh Hoa, Vietnam	PV420478	PV420429	PV405846	PV434143	This study
	CMW 47541 = CBS 144580	soil in natural forest	Nhu Xuan, Thanh Hoa, Vietnam	PV420479	PV420430	PV405847	PV434144	This study
	CMW 49936 = CBS 144581	soil in natural forest	Bu Gia Map Natural Park, Binh Phuoc, Vietnam	PV420480	PV420431	PV405848	PV434145	This study
Gliocladiopsis sp. 1	CBS 111038 = CPC 1157	soil	Colombia	JQ666151	JQ666041	JQ666071	JQ666117	Lombard & Crous (2012)
Penicillifer pulcher	CBS 560.67^{T} = ATCC 18931 = MUCL 11607	soil	The Netherlands	KM231998	KM231456	KM231742	KM231862	Lombard *et al.* (2015)

Note: N/A represents information that is not available. Isolates obtained in this study are indicated in **bold**. ^{T} denotes ex-type strain.
ATCC, American Type Culture Collection, VA, USA; **BRIP**, Biosecurity Queensland Plant Pathology Herbarium, Department of Agriculture and Fisheries, Dutton Park, Australia; **CBS**, the culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; **CMW**, the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; **CPC**, Pedro Crous working collection housed at Westerdijk Fungal Biodiversity Institute; **HEAC**, the Herbarium of Henan Agricultural University, Zhengzhou, China; **IMI**, International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; **LC**, Herbarium of Microbiology, Academia Sinica, Taipei, Taiwan; **MFLUCC,** Mae Fah Luang University culture collection, Chiang Rai, Thailand; **MUCL**, Mycotheque, Laboratoire de Mycologie Systematique st Appliqee, l'Universite, Louvain-la-Neuve, Belgium.
ITS, internal transcribed spacer regions 1 and 2 including the 5.8S region of ribosomal RNA; ***HIS3***, histone H3; ***TEF1***, translation elongation factor 1-alpha; ***TUB2***, β-tubulin.

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Crous 2012). In this study, we used the combination of multi-gene phylogenetic inference and morphological observations to identify a collection of isolates resembling *Gliocladiopsis* species from soil samples collected in Indonesia, Malaysia and Vietnam.

MATERIALS AND METHODS

Sampling and fungal isolations

Surveys were conducted in various forestry plantations, nurseries and natural forests across 13 Vietnamese provinces (Binh Phuoc, Dong Nai, Hanoi, Lao Cai, Nghe An, Ninh Binh, Phu Tho, Quang Tri, Tay Ninh, Thanh Hoa, Tuyen Quang, Vinh Phuc and Yen Bai), Indonesia (North Sumatra) and Malaysia (Sabah). Soil samples were placed in plastic bags and transferred to the laboratory for fungal isolations. The collected samples were baited with germinating *Medicago sativa* (alfalfa) seeds following the method described by Crous (2002). Gliocladiopsis-like spore masses were located under a dissecting microscope and transferred using a sterile hypodermic needle directly onto Petri dishes containing 2 % (w/v) malt extract agar (MEA; Biolab, Midrand, South Africa) supplemented with 1 % streptomycin sulphate (Sigma-Aldrich).

Primary isolations were incubated for 3–7 d at 25 °C for fungal growth. Single hyphal tips cut from young fungal colonies were transferred to fresh MEA plates to obtain pure cultures. These isolates were deposited in the research culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and the publicly accessible culture collection (CMW-IA) of the Innovation Africa at the University of Pretoria, and the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. Dried cultures representing the holotype of the novel taxon were deposited in the H.G.W.J. Schweickerdt herbarium (PRU), University of Pretoria, South Africa.

DNA sequencing and phylogenetic analyses

DNA was extracted from 7-d-old pure fungal cultures grown on MEA, using Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA), following the protocols suggested by the manufacturer. Four loci, namely, ITS, *TEF1*, *TUB2* and *HIS3*, were amplified using the set of primers as described by Pham *et al.* (2018). The PCR amplification were conducted using an Applied Biosystems ProFlex PCR System (Thermo Fisher Scientific, Waltham, MA, USA) following the preparation described by Pham *et al.* (2018). Amplified fragments were cleaned using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA). Amplicons were sequenced in both directions using an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems, USA) at the Sequencing Facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria. Geneious Prime v. 2025.0.3 (https://www.geneious.com) was used to assemble and edit the raw sequences. All sequences generated in this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov).

Representative sequences of *Gliocladiopsis* spp. published previously were obtained from GenBank to compare with sequences generated in this study (Table 1). Alignments of all sequences were assembled using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013), then confirmed manually in MEGA v. 7 (Kumar *et al.* 2016) where necessary. Maximum likelihood (ML) and Bayesian inference

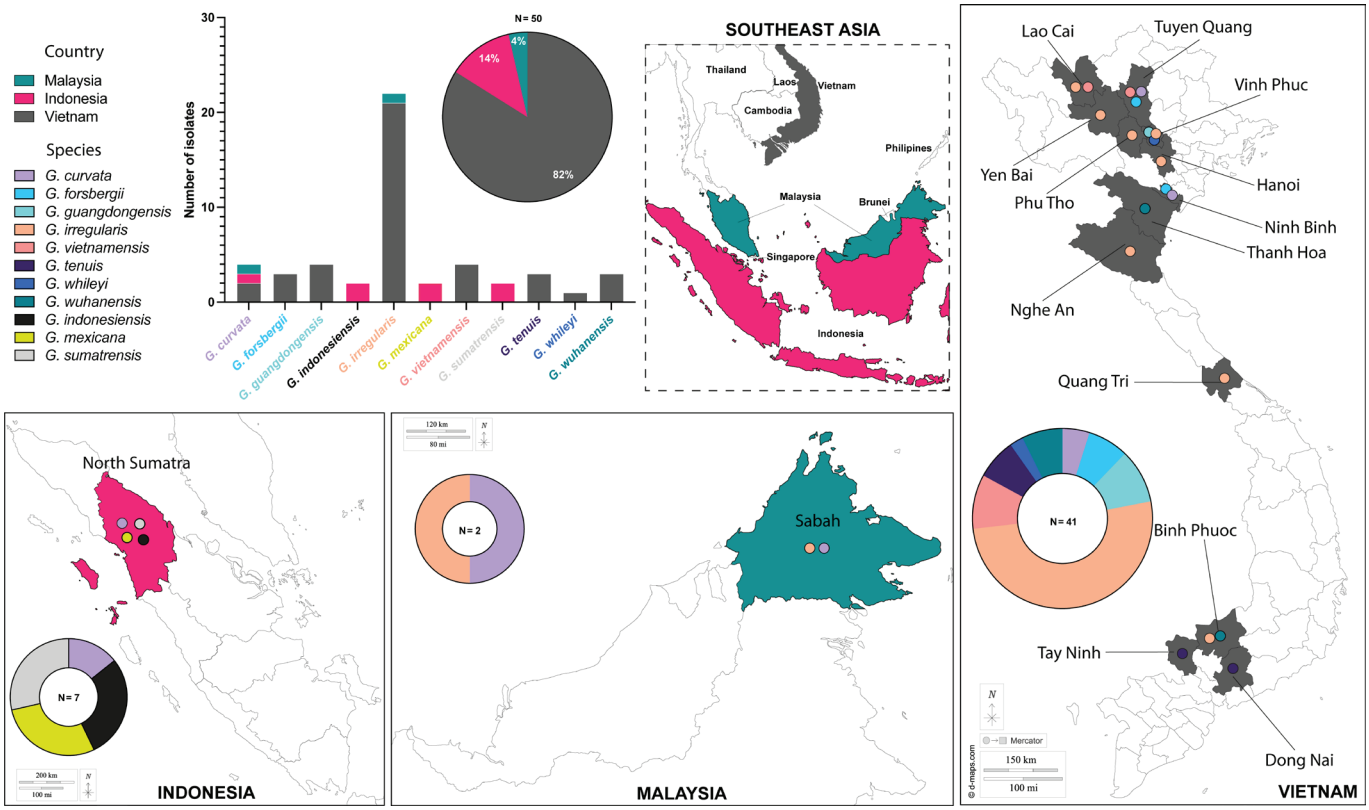

Fig. 1. Geographic location of the sampling sites in Vietnam, Indonesia and Malaysia and the diversity of *Gliocladiopsis* spp. isolated in each region.

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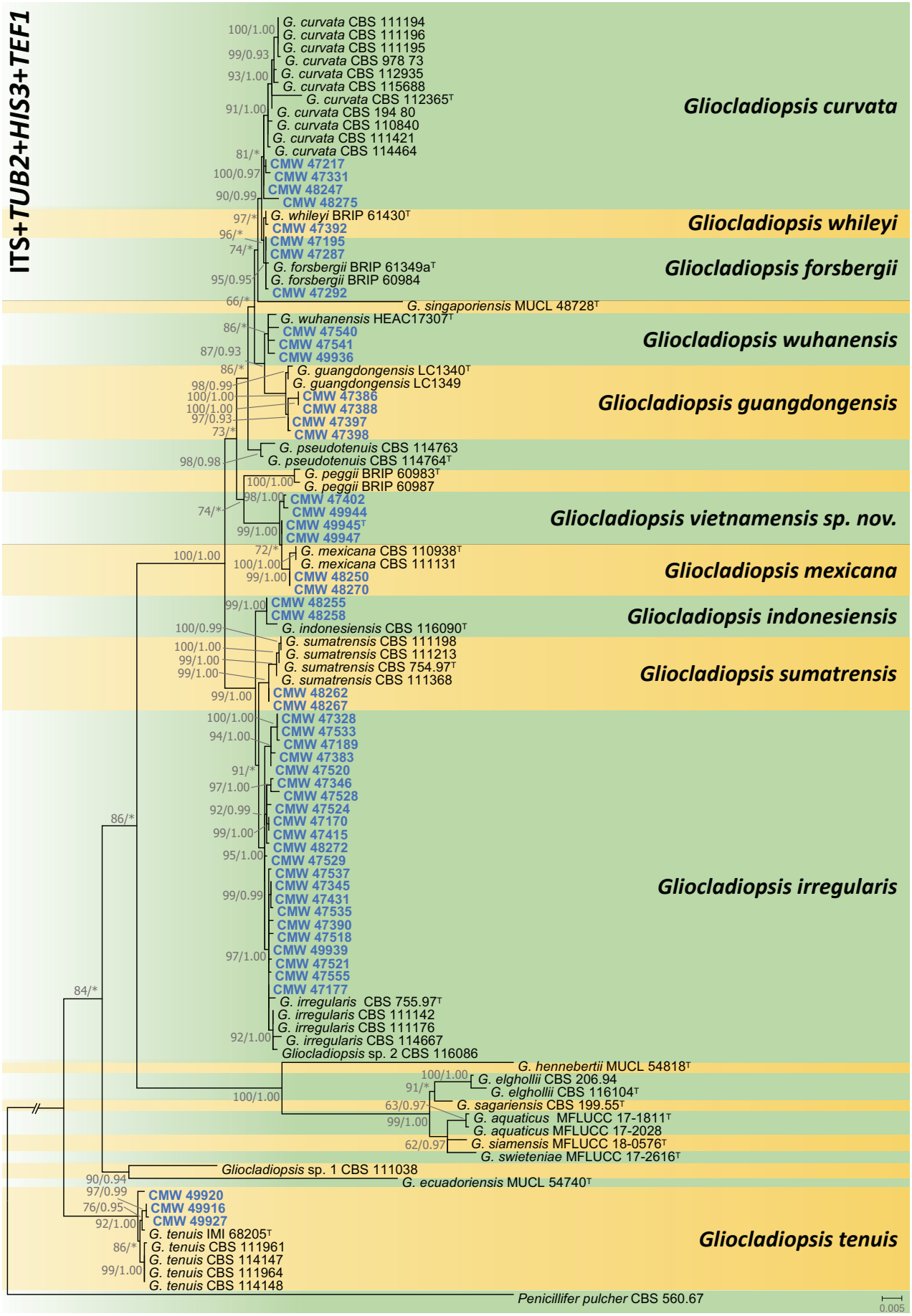

Fig. 2. Phylogenetic tree based on maximum likelihood (ML) analysis of a combined DNA dataset of ITS, *TUB2*, *HIS3*, and *TEF1* sequences for *Gliocladiopsis* spp. Bootstrap support values ≥ 60 % for ML analyses and posterior probabilities values ≥ 0.90 obtained from Bayesian inference (BI) are indicated at the nodes as ML/BI. Bootstrap support values < 60 % or probabilities values < 0.90 are marked with "*". Isolates representing ex-type material are marked with "T" and novel isolates from this study are in blue. *Penicillifer pulcher* (isolate CBS 560.67) represents the outgroup.

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(BI) analyses were performed on the combined dataset of four regions. For ML, analyses were conducted using W-IQ-TREE with an optimal substitution model automatically determined and 1000 ultrafast bootstraps (Trifinopoulos *et al.* 2016). For BI, analyses were performed using MrBayes v. 3.2.6 (Ronquist *et al.* 2012) on the CIPRES Science Gateway v. 3.3. Four Markov chain Monte Carlo (MCMC) chains were run from a random starting tree for five million generations, and trees were sampled every 100th generation. The first 25 % of trees sampled were eliminated as burn-in, and the remaining trees were used to determine the posterior probabilities. *Penicillifer pulcher* (CBS 560.67) was used as the outgroup taxon. The resulting trees were visualised using MEGA v. 7 (Kumar *et al.* 2016).

Microscopy and morphological characteristics

The isolates were grown on synthetic nutrient-poor agar (SNA; Nirenburg 1981) to study morphological characteristics. The conidiophores were initially mounted in water and later replaced with 85 % lactic acid for further observation and preservation. Nikon microscopes (Eclipse Ni, SMZ 18, Nikon, Tokyo, Japan) were used to study microscopic features. Images were captured with a Nikon DS-Ri2 camera mounted on the microscopes, and characteristic structures were measured utilising the NIS-Elements BR program. Twenty-five to 50 measurements of all taxonomically relevant characteristics were made whenever possible. The dimensions of the structures are presented as ranges other than those where an average is included. Colony characteristics were observed for cultures on 2 % MEA incubated in the dark in 7 d and were described using Rayner's colour charts (Rayner 1970). Colony growth was determined on MEA at temperatures ranging from 10–35 °C at 5 ° intervals with five replicate plates for each temperature. Two measurements of colony diameter were taken for cultures grown for 7 d, and averages were computed.

RESULTS

Fungal isolates

Collectively, 50 cultures having a morphology typical of *Gliocladiopsis* spp. were isolated from 84 soil samples collected in Vietnam, eight from Indonesia, and six from Malaysia. Of these, 41 isolates (82 %) were from Vietnam, seven (14 %) from Indonesia and two (4 %) from Malaysia (Fig. 1). Twenty-two isolates (44 %) were of a single species, and of these, 21 were from Vietnam (Fig. 1). The remaining 19 isolates represented a maximum of four isolates of any one species. Only one species occurred in all three countries. Three species were found only in Indonesia, and five were found only in Vietnam (Fig. 1).

Phylogenetics analyses

Amplicons of approximately 520 bp were generated for the ITS region, 450 bp for the *TUB2*, 470 bp for the *HIS3*, and 500 bp for the *TEF1*. The combined sequence dataset used in the phylogenetic analyses included 99 ingroup taxa and 2023 characters, including gaps. The ML and BI analyses generated phylogenetic trees with concordant topologies, revealing similar phylogenetic relationships among taxa. The ML tree with bootstrap support values and the posterior probabilities obtained from BI are presented in Fig. 2. Isolates obtained from this study resided in 11 different clades. Of these, most isolates (22) grouped in a well-supported clade (ML/BI = 95/1.00) with the ex-type and representative isolates of *G. irregularis*. The remaining isolates grouped with *G. curvata* (four isolates), *G. whileyi* (one isolate), *G. forsbergii* (three isolates), *G. wuhanensis* (three isolates), *G. guangdongensis* (four isolates), *G. mexicana* (two isolates), *G. indonesiensis* (two isolates), *G. sumatrensis* (two isolates) and *G. tenuis* (three isolates). Four isolates (CMW 47402, CMW 49944, CMW 49945, CMW 49947) resided in a cluster distinct from the most closely related species *G. mexicana*, and are thus described as a novel taxon.

Taxonomy

Gliocladiopsis vietnamensis N.Q. Pham, Marinc. & M.J. Wingf., ***sp. nov***. MB 858515. Fig. 3.

Etymology: The name refers to Vietnam, where the species was first discovered.

Diagnosis: Morphologically similar to *G. mexicana* but can be differentiated from *G. mexicana* by ITS (2 bp), *TUB2* (1 bp), *HIS*3 (2 bp), and *TEF1* (5 bp) sequences.

Typus: **Vietnam**, Lao Cai Province, Hoang Lien National Park, from soil associated with *Alnus nepalensis*, 1 Sep. 2013, *N.Q. Pham, Q.N. Dang & T.Q. Pham* [**holotype** specimen PRU(M) 4633; ex-holotype culture CBS 144587 = CMW 49945 = CMW-IA 7117]. GenBank: PV420475 (*TUB2*); PV420426 (*HIS3*); PV405843 (ITS); PV434140 (*TEF1*).

Description: *Sexual morph* not observed. *Conidiophores* penicilliate, upright, simple. *Conidiogenous apparatus* often consisting of a layer of conidiogenous cells or branching in 2–3 tiers; 1° branches 6–25.5 × 1–5 µm; 2° branches 5–20.5 × 1–4 µm. *Conidiogenous cells* hyaline, cylindrical, phialidic, tapering towards the apex, 6–22 × 1–3.5 µm. *Conidia* hyaline, cylindrical, straight or curved, medianly 1-septate, 13–23 × 2.5–4 µm (avg. 17 × 3 µm).

Colony characteristics: On MEA after 7 d, colonies buff on the surface, ochreous becoming paler toward the margin in reverse, margin entire, elevation flat. Optimal growth temperature at 25 °C reaching 37.9 mm in 7 d, followed by 20 °C (24.5 mm), 30 °C (22.9 mm), 15 °C (11.2 mm), and no growth at 10 °C and 35 °C.

Distribution: Vietnam (Tuyen Quang and Lao Cai Province)

Additional materials examined: **Vietnam**, Tuyen Quang Province, Son Duong, from soil collected in an *Eucalyptus urophylla* plantation, 1 Nov. 2013, *N.Q. Pham & T.Q. Pham*, culture CBS 144585 = CMW 47402, GenBank: PV420473 (*TUB2*); PV420424 (*HIS3*); PV405841 (ITS); PV434138(*TEF1*); Lao Cai Province, Hoang Lien National Park, from soil associated with *Alnus nepalensis*, 1 Sep. 2013, *N.Q. Pham,*

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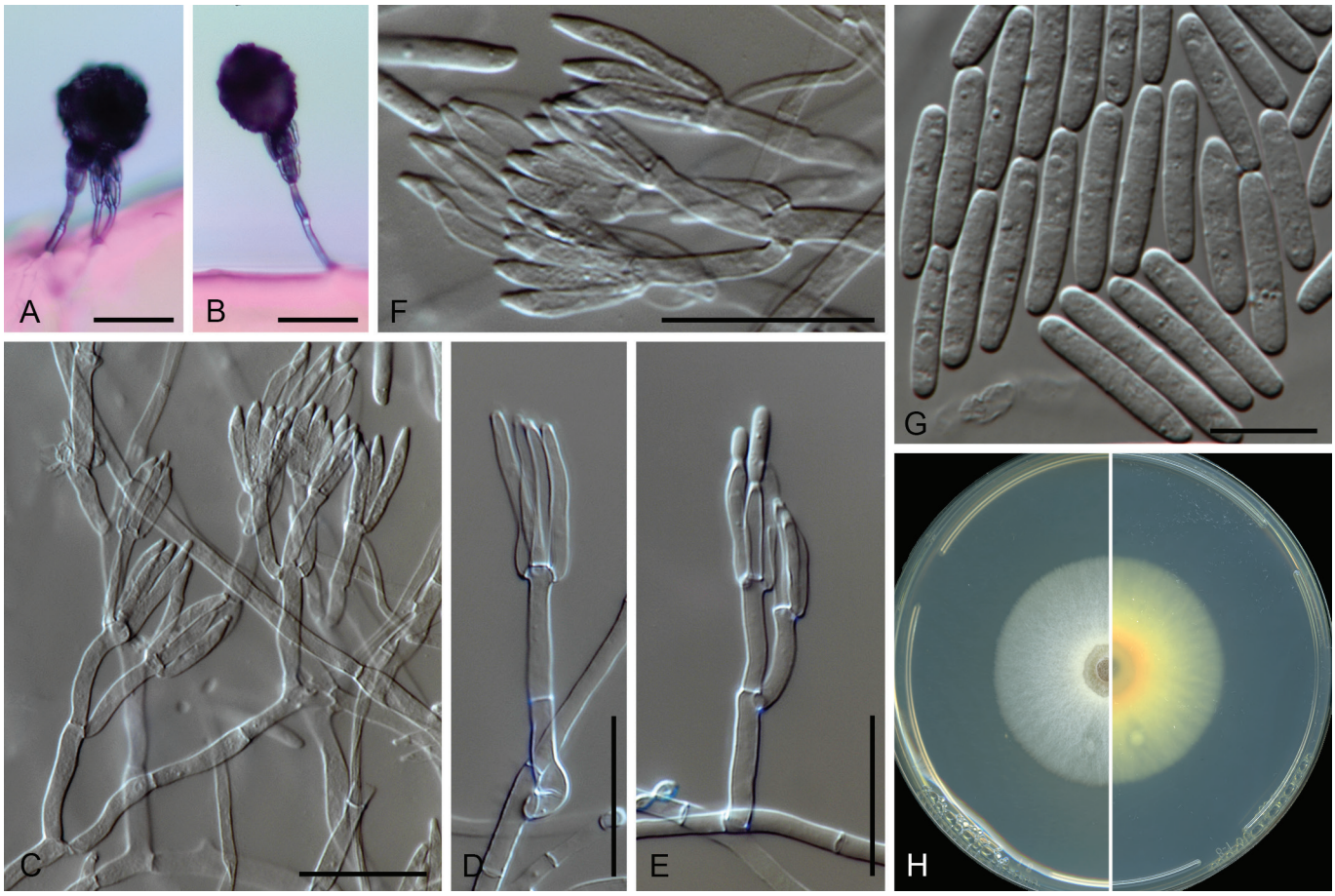

Fig. 3. Microscopic features of *Gliocladiopsis vietnamensis* (ex-holotype CBS 144587 = CMW 49945 = CMW-IA 7117). **A, B.** Conidiophores on SNA. **C–E.** Conidiophores. **F.** Conidiogenous cells. **G.** Conidia. **H.** Culture grown on PDA for 7 d at 25 °C in the dark (left, above; right, reverse). Scale bars: A, B = 50 µm; C–F = 25 µm; G = 10 µm.

N.Q. Dang & T.Q. Pham, PRU(M) 4632, culture CBS 144586 = CMW 49944 = CMW-IA 7116, GenBank: PV420474 (*TUB2*); PV420425 (*HIS3*); PV405842 (ITS); PV434139(*TEF1*).

Notes: *Gliocladiopsis vietnamensis* is phylogenetically closely related to *G. mexicana*. They are morphologically similar in not having tertiary branches (rarely found in *G. mexicana*) and in having similar conidial dimensions (15–21 × 2–4 µm for *G. mexicana*, 13–23 × 2.5–4 µm for *G. vietnamensis*) (Lombard & Crous 2012). The colony colour of *G. mexicana* was described as sayal brown to sepia in reverse, whereas *G. vietnamensis* cultures are buff to ochreous in reverse.

Gliocladiopsis mexicana was first reported from soil samples in Mexico in 1994 (Lombard & Crous 2012). This study added two strains (CMW 48250, 48270) of the species, which were isolated from Indonesian soil samples. *Gliocladiopsis peggii* is a sister taxon to *G. vietnamensis* and *G. mexicana* and is morphologically similar. However, unlike the other two from soil samples, it was isolated from necrotic roots of avocado and necrotic stem lesions on *Grevillea* sp. (Parkinson *et al.* 2017a).

DISCUSSION

This study represents the largest collection of *Gliocladiopsis* isolates from Southeast Asia that has ever been subjected to DNA sequence analyses. Based on morphological characteristics and phylogenetic analyses of four loci (ITS, *TUB2, HIS3,* and *TEF1*), 11 *Gliocladiopsis* spp. were identified from soils collected from different regions of Indonesia, Malaysia and Vietnam. These included 10 of the 20 previously described species in the genus, namely, *G. curvata*, *G. guangdongensis*, *G. indonesiensis*, *G. irregularis*, *G. forsbergii*, *G. mexicana*, *G. sumatraensis*, *G. tenuis*, *G. whileyi*, and *G. wuhanensis.* In addition, a new species was discovered, described in this study as *G. vietnamensis*.

Six species, namely, *G. curvata*, *G. forsbergii*, *G. guangdongensis*, *G. irregularis*, *G. whileyi*, and *G. wuhanensis,* are reported for the first time from Vietnam. *Gliocladiopsis curvata* is reported for the first time in Malaysia. *Gliocladiopsis mexicana*, previously known only in Mexico, was also recovered from soils in Indonesia. This increases the number of known *Gliocladiopsis* spp. in Southeast Asia to 15 (Crous 2002, Hyde *et al.* 2008, Lombard & Crous 2012, Gordillo & Decock 2019, Perera *et al.* 2020, 2023), suggesting that this region represents a biodiverse hotspot for soil-borne *Gliocladiopsis* spp. The identification of a relatively high number of species from a relatively small number of isolates, exclusively from soils collected in areas not previously examined, also suggests that many more *Gliocladiopsis* species await discovery, especially in the tropics and the Southern Hemisphere.

Gliocladiopsis irregularis was one of the most commonly isolated species (44 %) in this study. This species was found to have a broad distribution across Vietnam, encompassing eight provinces, as well as in Sabah, Malaysia. Although *G. irregularis* had previously been reported from Indonesia

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(Crous 2002, Lombard & Crous 2012), we did not isolate this species from North Sumatra. *Gliocladiopsis irregularis* was recovered from soils associated with a wide range of planted tree species, *i.e.*, *Acacia*, *Camellia*, *Eucalyptus*, *Pinus* and *Cinnamomum* spp., as well as from native and largely undisturbed forests. Given its common occurrence and wide distribution, it seems probable that *G. irregularis* is native to this area. However, further studies, including those at a population genetics level, would be required to resolve that question.

All *Gliocladiopsis* species identified in this study were isolated exclusively from baited soils and could not be directly linked to disease symptoms. Their isolation from germinating alfalfa seedlings and thus green tissues used in baiting suggests some level of pathogenicity; however, their role, even as weak plant pathogens, remains to be determined. To date, the only species that has been experimentally tested for pathogenicity is *G. peggii*, which has been found to be non-pathogenic (Parkinson *et al.* 2017b) and, in some cases, even promotes plant growth (Dann *et al.* 2012). Clearly, a much deeper understanding of the biology, particularly their ecological role in the rhizosphere, is needed for species of *Gliocladiopsis*.

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Conflict of Interest: The authors declare no conflicts of interest.

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