



Phylogeny and taxonomy of the genus *Gliocladiopsis*

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Key words

Gliocladiopsis
phylogeny
taxonomy

Abstract Using a global set of isolates and a phylogenetic approach employing DNA sequence data from five genes (β -tubulin, histone H3, internal transcribed spacer region, 28S large subunit region and translation elongation factor 1- α), the taxonomic status of the genus *Gliocladiopsis* (*Glionectria*) (*Hypocreales*, *Nectriaceae*) was re-evaluated. *Gliocladiopsis sagariensis* is reinstated as type species for the genus, which proved to be distinct from its former synonym, *G. tenuis*. The purported teleomorph state of *G. tenuis*, *Glionectria tenuis*, is shown to be distinct based on morphological comparisons supported by phylogenetic inference, and is provided with a new name, *Gliocladiopsis pseudotenuis*. A further four species, mostly isolated from soil, are newly described, namely *G. curvata* (New Zealand, Ecuador and Indonesia), *G. elghollii* (USA), *G. indonesiensis* (Indonesia) and *G. mexicana* (Mexico). Although species of *Gliocladiopsis* are frequently isolated from roots of diseased plants or plant litter in soil, little is presently known of their ecology, or potential role as plant pathogens.

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INTRODUCTION

The genus *Gliocladiopsis* was introduced by Saksena (1954) based on *G. sagariensis* to accommodate a fungal isolate from soil that had penicillate conidiophores resembling *Penicillium* and *Gliocladium*, and cylindrical conidia similar to that of *Calonectria* (as *Cylindrocladium*). Saksena (1954) distinguished *G. sagariensis* from *Penicillium* and *Gliocladium* based on morphological differences in conidium and conidiogenous apparatus morphology, and the apparent lack of chlamyospore formation in culture. Agnihothru (1959), however, was able to observe chlamyospore formation in culture, and based on this as well as morphological similarity, synonymised *G. sagariensis* under *Cylindrocarpon tenue* (Bugnicourt 1939). In contrast, Barron (1968) considered *Gliocladiopsis* as a later synonym of *Calonectria* (as *Cylindrocladium*).

Crous & Wingfield (1993) resurrected the genus *Gliocladiopsis* to accommodate species characterised by dense, penicillate conidiophores, which unlike *Cylindrocladiella* and *Calonectria*, lacked sterile stipe extensions. Based on the characteristic conidiophores, the genus *Cylindrocarpon* was also found to be unsuitable to accommodate these species. These observations led Crous & Wingfield (1993) to place *C. tenue* in *Gliocladiopsis*, retaining *G. sagariensis* as synonym. Watanabe (1994) transferred *G. tenuis* to *Cylindrocladium* based on observations that isolates of *Cylindrocladium* and *Cylindrocladiella* generally lose their ability to produce stipe extensions with continuous subculturing, and therefore he rejected this feature as a stable character to define these genera. Various morphological studies have shown, however, that the presence of a stipe extension and the terminal vesicle shape is an important character to distinguish species of *Calonectria* (Crous & Wingfield 1994, Lombard et al. 2010a–c) and *Cylindrocladiella* (Crous & Wingfield 1993, Victor et al. 1998, van Coller et al. 2005, Lombard et al. 2012).

The first phylogenetic study conducted on this generic complex was that by Schoch et al. (2000), which clearly showed that *Gliocladiopsis* was closely related to *Gliocephalotrichum/Leuconectria*, and removed from *Cylindrocladiella*, *Cylindrocarpon* and *Calonectria* (Fig. 1). Furthermore, the genus *Glionectria* was proposed as teleomorph of *Gliocladiopsis* in this study, and defined by perithecia that are obovoid to broadly obpyriform, with warted, red-brown walls and dark red stromatic bases, producing ellipsoidal, 1-septate ascospores.

Presently *Gliocladiopsis* accommodates three species which include *G. irregularis* (Crous & Peeraly 1996), *G. sumatrensis* (Crous et al. 1997) and *G. tenuis* (Crous & Wingfield 1993), and is defined by densely penicillate conidiophores lacking a stipe extension and terminal vesicle, and produce small, narrow, cylindrical, (0–)1-septate conidia held in yellow droplets, and chains of globose, brown chlamyospores (Crous 2002).

Over the course of several years a collection of *Gliocladiopsis* isolates have been accumulated in the culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands. These isolates were identified as *G. tenuis* based on morphological comparisons only. The aim of this study was to reconsider the taxonomic status of the genus *Gliocladiopsis* using multigene phylogeny and morphological comparisons to correctly identify these isolates.

MATERIALS AND METHODS

Isolates

Isolates and ex-type strains of *Gliocladiopsis* spp. were obtained from the CBS-KNAW Fungal Biodiversity Centre (CBS) and other culture collections as indicated in Table 1. These isolates were either isolated from plant material or baited from soil using the methods described by Crous (2002).

Phylogeny

Total genomic DNA was extracted from single-conidial isolates grown on 2 % malt extract agar (MEA) for 7 d, using the Ultra-Clean™ Microbial DNA isolation kits (Mo Bio Laboratories, Inc., California, USA) according to the manufacturer's protocol.

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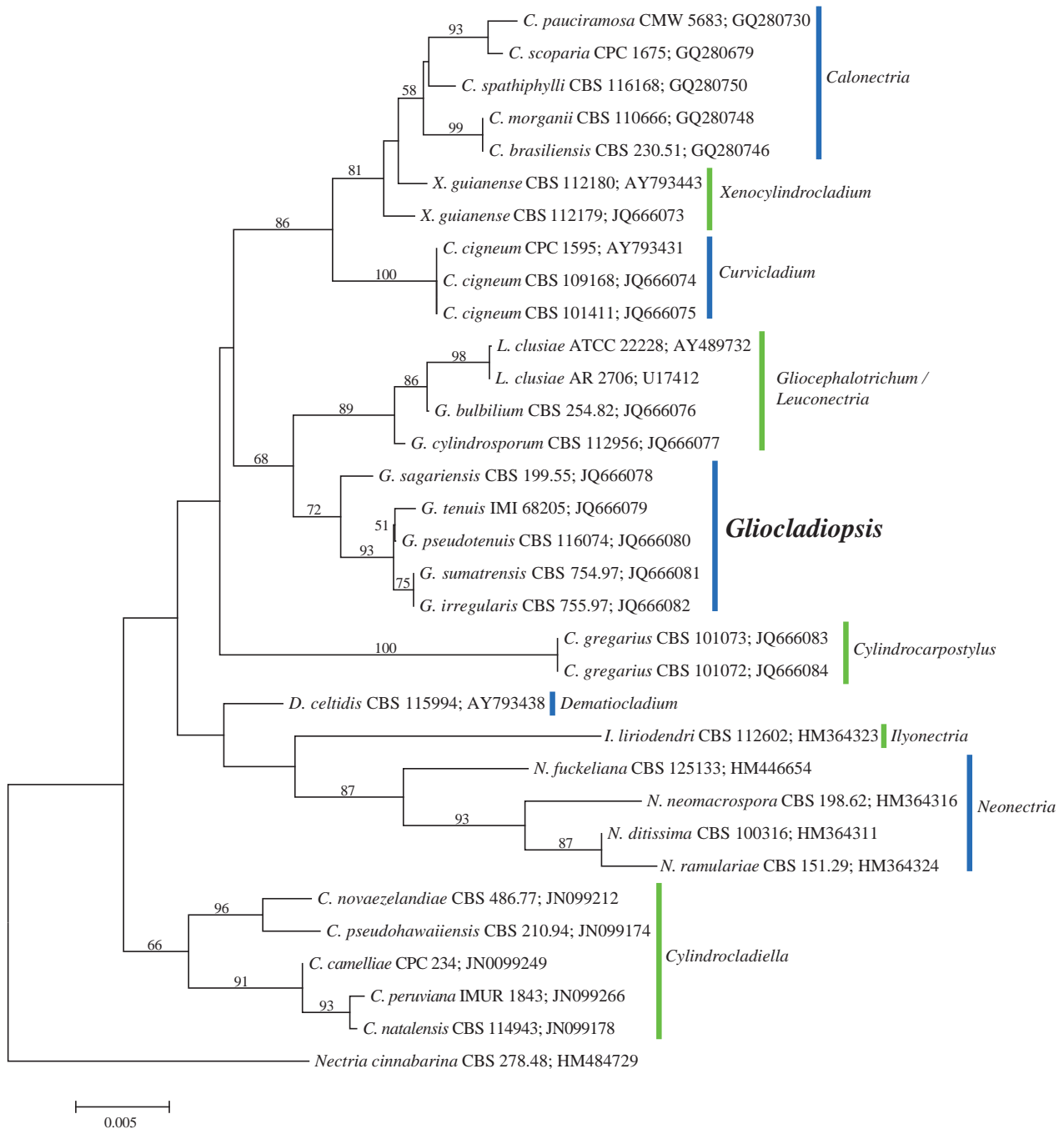


Fig. 1 Neighbour-Joining tree (Kimara-2-parameter) using only the partial LSU sequence alignment with bootstrap values after 1 000 repetitions.

Partial gene sequences were determined for β -tubulin (BT), histone H3 (HIS3), internal transcribed spacer region (ITS), 28S large subunit region (LSU) and translation elongation factor 1- α (TEF 1- α) using the primers and protocols described by Lombard et al. (2010b).

To ensure the integrity of the sequences, the amplicons were sequenced in both directions with the same primer pairs used for amplification, and subsequent alignments were generated using MAFFT v. 6 (Katoh & Toh 2010), and manually corrected where necessary.

Congruency of the sequence datasets for the separate loci, with the exception of LSU, were determined using tree topologies of 70 % reciprocal Neighbour-Joining bootstrap trees with Maximum Likelihood distances that were compared visually to identify conflicts between partitions (Gueidan et al. 2007). Molecular evolution models for the separate gene regions were determined in Modeltest v. 3.7 (Posada & Crandall 1998) and bootstrap analyses were run for 10 000 replicates.

PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10, Swofford 2002) was used to analyse the DNA sequence dataset. Phylogenetic relationships were estimated by heuristic searches with 1 000 random addition sequences and tree bisection-reconnection was used, with the branch swapping option set on 'best trees' only. All characters were weighted equally and alignment gaps were treated as missing data. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC). Bootstrap analysis (Hillis & Bull 1993) was based on 1 000 replications.

A second phylogenetic analysis using a Markov Chain Monte Carlo (MCMC) algorithm was done to generate trees with Bayesian probabilities in MrBayes v. 3.1.1 (Ronquist & Huelsenbeck 2003). Nucleotide substitution models were determined using MrModeltest (Nylander 2004) for each gene region and included in the analyses. Two analyses of four MCMC chains were run from random trees for one million generations and

Table 1 *Gliocladiopsis* isolates included in this study.

Species	Culture accession ¹	GenBank accession ²				Substrate	Country	Collector
		BT	HIS3	ITS	TEF 1- α			
<i>Calonectria brachiatica</i>	CBS 123700	FJ696388	FJ696396	GQ280555	GQ267296	–	–	
<i>C. brassicae</i>	CBS 111869	AF232857	DQ190720	GQ280576	FJ918567	–	–	
<i>G. curvata</i>	CBS 978.73	JQ666119	JQ666009	JQ666043	JQ666085	–	C.S. Hodges	
	CBS 194.80	JQ666120	JQ666010	JQ666044	JQ666086	<i>Persea americana</i>	J.P. Laoh	
	CBS 110840 = MUCL 38873 = CPC 855	JQ666121	JQ666011	JQ666045	JQ666087	Greenhouse	C. Decock	
	CBS 11194 = CPC 1354	JQ666122	JQ666012	JQ666046	JQ666088	Soil	M.J. Wingfield	
	CBS 11195 = CPC 1355	JQ666123	JQ666013	JQ666047	JQ666089	Soil	M.J. Wingfield	
	CBS 11196 = CPC 1356	JQ666124	JQ666014	JQ666048	JQ666090	Soil	M.J. Wingfield	
	CBS 11421 = CPC 1652	JQ666125	JQ666015	JQ666049	JQ666091	Soil	M.J. Wingfield	
	CBS 112365 = CPC 10491 = Lynfield 791-B	JQ666126	JQ666016	JQ666050	JQ666092	<i>Archontophoenix purpurea</i>	F. Klassen	
	CBS 112935 = CPC 4574	JQ666127	JQ666017	JQ666051	JQ666093	<i>Syzygium aromaticum</i>	M.J. Wingfield	
	CBS 114464 = CPC 1656	JQ666128	JQ666018	JQ666052	JQ666094	Soil	M.J. Wingfield	
	CBS 115688 = IFO 9133 = CPC 539 = NBRC 9133	JQ666129	JQ666019	JQ666053	JQ666095	–	M. Kasai	
<i>G. elgholii</i>	CBS 206.94	JQ666130	JQ666020	JQ666054	JQ666096	<i>Chaetochytrium elegans</i>	N.E. El-Gholl	
	CBS 116104 = CPC 636 = P93-2051	JQ666131	JQ666021	JQ666055	JQ666097	<i>C. elegans</i>	N.E. El-Gholl	
<i>G. indonesiensis</i>	CBS 116090 = CPC 715	JQ666132	JQ666022	JQ666056	JQ666098	Soil	A.C. Alfenas	
<i>G. irregularis</i>	CBS 755.97 = CPC 718	JQ666133	JQ666023	AF220977	JQ666099	Soil	A.C. Alfenas	
	CBS 11142 = CPC 1279	JQ666134	JQ666024	JQ666057	JQ666100	<i>Araucaria</i> sp.	M.J. Wingfield	
	CBS 111176 = CPC 1280	JQ666135	JQ666025	JQ666058	JQ666101	<i>Araucaria</i> sp.	M.J. Wingfield	
	CBS 114667 = 1278	JQ666136	JQ666026	JQ666059	JQ666102	<i>Araucaria</i> sp.	M.J. Wingfield	
<i>G. mexicana</i>	CBS 110938 = CPC 964	JQ666137	JQ666027	JQ666060	JQ666103	Soil	M.J. Wingfield	
	CBS 111131 = CPC965	JQ666138	JQ666028	JQ666061	JQ666104	Soil	M.J. Wingfield	
<i>G. pseudotenulis</i>	CBS 114763 = CPC 4575	JQ666139	JQ666029	JQ666062	JQ666105	<i>Vanilla</i> sp.	M.J. Wingfield	
	CBS 116074 = CPC 706	JQ666140	JQ666030	AF220981	JQ666106	Soil	M.J. Wingfield	
<i>G. sagariensis</i>	CBS 199.55	JQ666141	JQ666031	JQ666063	JQ666107	Soil	S.B. Saksena	
<i>G. sumatrensis</i>	CBS 754.97 = CPC 1353	JQ666142	JQ666032	JQ666064	JQ666108	Soil	M.J. Wingfield	
	CBS 11198 = CPC 1352	JQ666143	JQ666033	JQ666065	JQ666109	Soil	M.J. Wingfield	
	CBS 111213	JQ666144	JQ666034	JQ666066	JQ666110	Soil	M.J. Wingfield	
	CBS 111368 = CPC 1351	JQ666145	JQ666035	AF220978	JQ666111	Soil	M.J. Wingfield	
<i>G. tenulis</i>	CBS 111961 = CPC 2910	JQ666146	JQ666036	JQ666067	JQ666112	<i>Coffea</i> sp.	P.W. Crous	
	CBS 111964 = CPC 2909	JQ666147	JQ666037	JQ666068	JQ666113	<i>Coffea</i> sp.	P.W. Crous	
	CBS 114147 = CPC 2912	JQ666148	JQ666038	JQ666069	JQ666114	Soil	P.W. Crous	
	CBS 114148 = CPC 2911	JQ666149	JQ666039	JQ666070	JQ666115	Soil	P.W. Crous	
	IMI 68205 = CPC 2403	JQ666150	JQ666040	AF220979	JQ666116	<i>Indigofera</i> sp.	F. Bugnicourt	
<i>Gliocladiopsis</i> sp.1	CBS 111038 = CPC 1157	JQ666151	JQ666041	JQ666071	JQ666117	Soil	M.J. Wingfield	
<i>Gliocladiopsis</i> sp.2	CBS 116086 = CPC 716	JQ666152	JQ666042	JQ666072	JQ666118	Soil	A.C. Alfenas	

¹ CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: working collection of Pedro Crous housed at CBS; IFO: Institute for Fermentation, 17-85, Jusohonmachi, 2-chome, Yodogawa-ku, Osaka 532, Japan; IMI: International Mycological Institute, CAB International, Egham, Basingstoke Lane, UK; Lynfield: Private collection Frank Hill; MUCL: Mycothèque, Laboratoire de Mycologie Systématique et Appliquée, Université, Louvain-la-Neuve, Belgium; NBRC: National Institute of Technology and Evaluation, NITE Biological Resource Center, 2-49-10 Nishihara, Shibuya-ku, Tokyo, 151-0066, Japan.

² BT = β -tubulin, HIS3 = histone H3, ITS = internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA, TEF 1- α = translation elongation factor 1- α . Ex-type isolates indicated in **bold**.

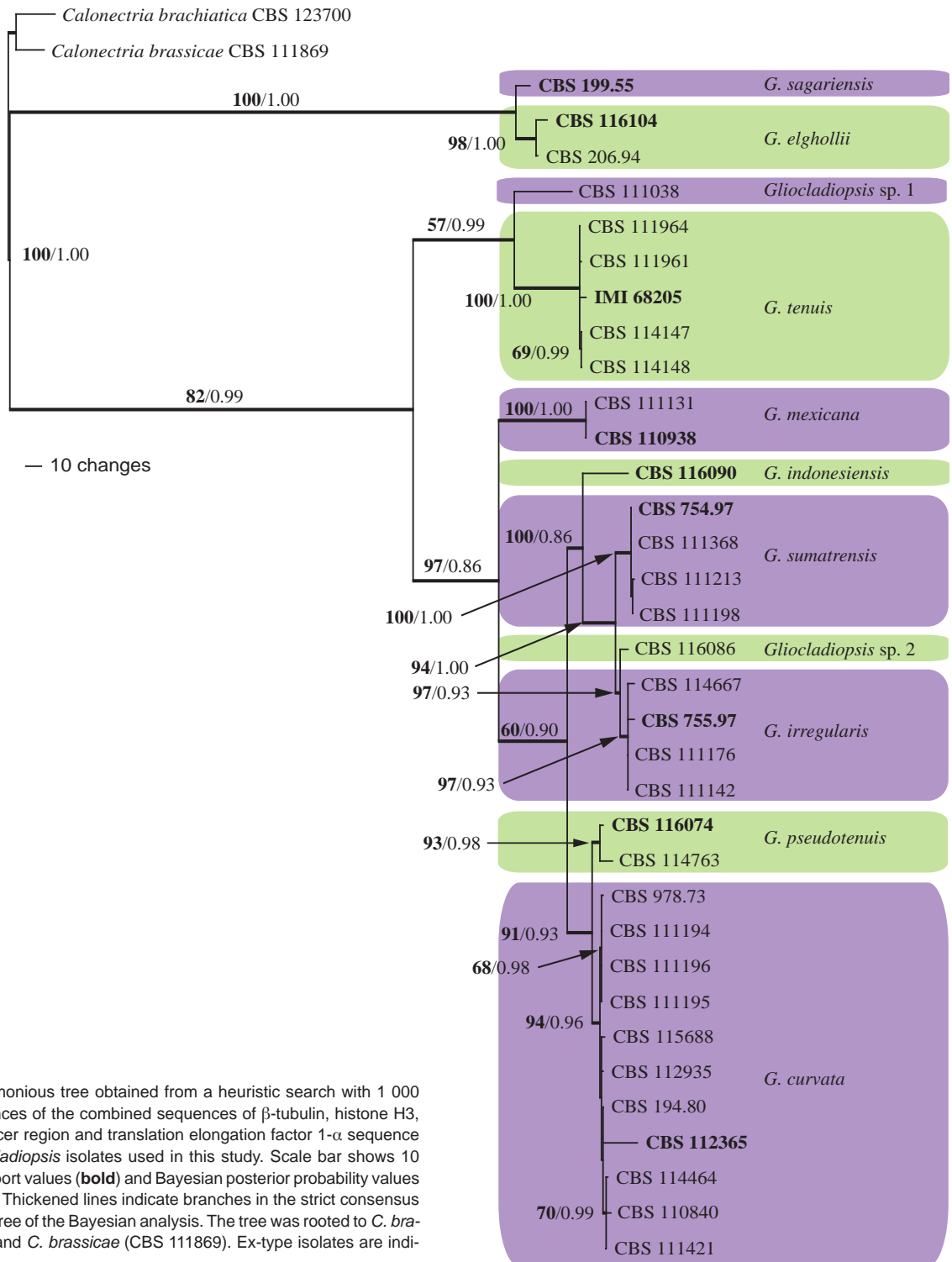


Fig. 2 The most parsimonious tree obtained from a heuristic search with 1 000 random addition sequences of the combined sequences of β -tubulin, histone H3, internal transcribed spacer region and translation elongation factor 1- α sequence alignments of the *Gliocladiopsis* isolates used in this study. Scale bar shows 10 changes. Bootstrap support values (**bold**) and Bayesian posterior probability values are shown at the nodes. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analysis. The tree was rooted to *C. brachiatica* (CBS 123700) and *C. brassicae* (CBS 111869). Ex-type isolates are indicated in **bold**.

sampled every 100 generations. All runs converged on the same likelihood score and tree topology, and therefore the first 1 200 trees were discarded as the burn-in phase of each analysis and posterior probabilities determined from the remaining trees.

The phylogenetic analyses included 36 partial gene sequences for each gene region, with the exception of LSU, representing all known *Gliocladiopsis* spp. (Table 1). *Calonectria brachiatica* (CBS 123700) and *C. brassicae* (CBS 111869) were used as outgroup taxa in both parsimony and Bayesian analyses. All novel sequences were deposited in GenBank and the alignments in TreeBASE (<http://www.treebase.org>).

For the LSU sequence data, distance analyses using neighbour-joining was performed in MEGA v. 5.0 (Tamura et al. 2011)

using the incorrect 'p', Juke-Cantor and Kimura-2-parameter substitution models. The robustness of the trees were evaluated by 1 000 bootstrap replicates. The LSU sequence dataset consisted of 33 partial gene sequences representing 10 genera of which *Nectria cinnabarina* (CBS 278.48) was used as outgroup.

Taxonomy

Morphological characterisation of the *Gliocladiopsis* isolates was done using single conidial cultures prepared on MEA and synthetic nutrient-poor agar (SNA; Nirenburg 1981). Inoculated plates were incubated at room temperature and examined after 7 d. Gross morphological characteristics were determined by mounting fungal structures in clear lactic acid and 30 measure-

ments at $\times 1\ 000$ magnification were made for each isolate using a Zeiss Axioscope 2 microscope with interference contrast (DIC) illumination. The 95 % confidence levels were determined and extremes of conidial measurements are given in parentheses. For other structures, only extremes are presented. Colony characteristics were noted after 7 d of growth on MEA at 24 °C and colony colours determined using the colour charts of Rayner (1970). Descriptions, nomenclature and illustrations were deposited in MycoBank (Crous et al. 2004).

RESULTS

Phylogeny

Amplicons of approximately 500–550 bases were determined for BTUB, HIS3, ITS and TEF 1- α , and 850 for LSU. The phylogenetic analysis included 34 ingroup taxa, with *C. brachiatica* (CBS 123700) and *C. brassicae* (CBS 111869) as outgroup taxa. Comparisons of the 70 % reciprocal bootstrap NJ tree topologies of the individual gene regions showed no conflict and therefore the sequence datasets were combined. The resulting dataset of 2 242 characters, including alignment gaps, consisted of 1 713 constant and 93 parsimony-uninformative characters. Analysis of the 436 parsimony-informative characters yielded one tree (TL = 748; CI = 0.826; RI = 0.914; RC = 0.755), which is presented in Fig. 2. For the Bayesian analysis, a HKY+I+G model was selected for BT and TEF 1- α , GTR+I+G for HIS3, and SYM+I+G for ITS which was incorporated into the analysis. The Bayesian consensus tree confirmed both the tree topology and bootstrap support of the strict consensus tree obtained with maximum-parsimony.

In the phylogenetic tree (Fig. 2) the *Gliocladiopsis* isolates are divided into two main clades. The first main clade (bootstrap support (BS) = 100; posterior probability (PP) = 1.00) contains the ex-type strain of *G. sagariensis* (CBS 199.55) as well as two isolates (CBS 206.94 and CBS 116104) forming a terminal clade (BS = 98; PP = 1.00) representing a unique phylogenetic species.

The second main clade (BS = 82; PP = 0.99) is further divided into two clades. The first of these clades (BS = 57; PP = 0.99) represents the anamorph state of *G. tenuis* (ex-type IMI 68205; BS = 100; PP = 1.00) as well as a unique single lineage (CBS 111038). The second of these clades (BS = 97; PP = 0.86) is also further divided into five well-supported terminal clades and two unique single lineages. Two of these terminal clades represent *G. irregularis* (ex-type CBS 755.97; BS = 97; PP = 0.93) and *G. sumatrensis* (ex-type CBS 754.97; BS = 100; PP = 1.00), respectively. The ex-type strain of the purported teleomorph state of *G. tenuis* (CBS 116074) is also represented by one of these terminal clades (BS = 93; PP = 0.98), indicating that this ex-type strain represents a distinct species from the ex-type strain of the anamorph state. The remaining two terminal clades and two unique single lineages represent possible new species.

The LSU sequence dataset consisted of 892 characters, representing 32 ingroup taxa from nine genera and *N. cinnabarina* (CBS 278.48) as outgroup. Distance analyses using the three substitution models resulted in the same tree topology and bootstrap support values and therefore the tree obtained using the Kimura-2-parameter substitution model is presented in Fig. 1. In the tree, all the *Gliocladiopsis* isolates included, clustered together in one clade (BS = 72) showing a close relationship with *Gliocephalotrichum/Leuconectria* (BS = 89), and showing a more distant relationship with *Calonectria* and *Cylindrocladiella*.

Taxonomy

Based on the phylogenetic inference and morphological observations, numerous *Gliocladiopsis* isolates included in this study

represent novel species. Following the approach of Lombard et al. (2010a–c, 2012) and Crous et al. (2006, 2008, 2009) for other fungal groups, all new species are described in *Gliocladiopsis*, as this represents the older generic, and best established name for this group of fungi (Saksena 1954).

Gliocladiopsis S.B. Saksena, Mycologia 46: 663. 1954.

= *Glionectria* Crous & C.L. Schoch, Stud. Mycol. 45: 58. 2000.

Type species. Gliocladiopsis sagariensis S.B. Saksena, Mycologia 46: 663. 1954.

Perithecia superficial, in dense groups, obovoid to broadly obpyriform; perithecial wall warted, turning red-brown in 3 % KOH+ with a dark red stromatic base, collapsing laterally when dry, consisting of two layers: outer region of thick-walled *textura globulosa*, inner layer of compressed cells of *textura angularis*; ostiolar periphyses tubular with rounded ends. *Asci* unitunicate, 8-spored, cylindrical, sessile, with flattened apex and a refractive apical apparatus. *Ascospores* uniseriate, hyaline, ellipsoidal, smooth, 1-septate, becoming brown and verruculose with age. *Conidiophores* penicillate and/or subverticillate, consisting of a septate, hyaline stipe and penicillate and/or subverticillate arrangement of fertile branches, lacking a stipe extension and terminal vesicle. *Conidiogenous apparatus* with several series of aseptate to 1-septate branches, each terminating in 2–7 phialides; phialides doliform to cymbiform to cylindrical, hyaline, aseptate with obvious collarettes, central phialide sometimes extending above the rest. *Conidia* cylindrical, straight to curved, aseptate to 1-septate, borne in a yellow mass on conidiophores.

Specimen examined. INDIA, Madhya Pradesh, Ghatera Forest c. 100 km east of Sagar, from soil, Mar. 1955, S.B. Saksena, culture ex-type CBS 199.55.

Notes — *Gliocladiopsis sagariensis* was synonymised under *G. tenuis* by Crous & Wingfield (1993) based on their close morphological resemblance. Phylogenetic inference in this study shows that CBS 199.55, ex-type of *G. sagariensis*, is distinct from IMI 69205, ex-type of *G. tenuis*. Based on the description and illustration of Saksena (1954), *G. sagariensis* can be distinguished from *G. tenuis* by having shorter phialides (10–15 μm vs 10–25 μm) and secondary branches (8–12 μm vs 10–18 μm) (Crous & Wingfield 1993).

Gliocladiopsis curvata L. Lombard & Crous, *sp. nov.* — MycoBank MB564399; Fig. 3

Etymology. Name refers to the curved conidia produced by this fungus.

Teleomorph unknown. *Conidiophores* penicillate without stipe extensions and terminal vesicles. *Conidiogenous apparatus* with several series of hyaline branches: primary branches aseptate or 1-septate, 17–37 \times 2–5 μm ; secondary branches aseptate, 12–21 \times 2–4 μm , tertiary branches aseptate, 8–14 \times 2–3 μm ; quaternary branches rare to absent, aseptate, 9–10 \times 3 μm ; phialides cymbiform, 12–21 \times 2–3 μm , arranged in terminal whorls of 2–6 per branch, with minute collarettes. *Subverticillate conidiophores* absent. *Conidia* cylindrical, hyaline, smooth with rounded ends, straight to slightly curved, aseptate to 1-septate, (16–)17–21(–23) \times 3–5 μm (av. = 19 \times 3 μm), lacking a visible abscission scar, but frequently with a flattened base, held in a pale yellow, asymmetrical cluster by colourless slime.

Culture characteristics — Colonies sayal brown to sepia (reverse); chlamydospores extensive, in non-delimited chains. Aerial mycelium dense, off-white to pale luteous.

Specimens examined. ECUADOR, Sabal, soil, 20 June 1997, coll. M.J. Wingfield, isol. P.W. Crous, culture CBS 114464 = CPC 1656. — INDONESIA, Tondano, from soil adjacent to *Syzygium aromaticum*, coll. M.J. Wingfield,

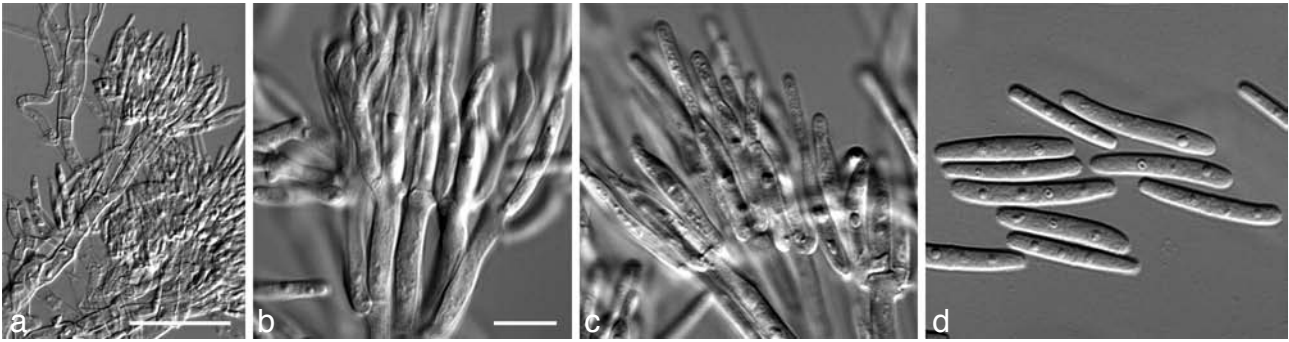


Fig. 3 *Gliocladiopsis curvata* (CBS 112365, ex-type culture). a–c. Conidiophores; d. conidia. — Scale bars: a = 50 μm ; b = 10 μm (applies to c and d).

isol. P.W. Crous, culture CBS 112935 = CPC 4574. — NEW ZEALAND, Auckland, from *Archontophoenix purpurea*, 30 Jan. 2003, F. Klassen, (CBS H-20907, holotype of *G. curvata*) culture ex-type CBS 112365 = CPC 10491 = Lynfield 791-B.

Notes — Although isolates of *G. curvata* were previously treated as *G. tenuis*, they more closely resemble *G. irregularis*, by having slightly curved conidia (Crous & Peerally 1996). The conidia of *G. curvata* (av. = $19 \times 3 \mu\text{m}$) are longer than those of *G. irregularis* (av. = $13 \times 2.5 \mu\text{m}$) and slightly longer than those of *G. tenuis* (av. = $18 \times 2 \mu\text{m}$) (Crous & Wingfield 1993, Crous 2002). *Gliocladiopsis curvata* produces quaternary branches on the conidiogenous apparatus, not reported for *G. irregularis* (Crous & Peerally 1996), and has no central phialide extending above the others as reported for *G. tenuis* (Crous & Wingfield 1993, Crous 2002).

Gliocladiopsis elghollii L. Lombard & Crous, *sp. nov.* — MycoBank MB564400; Fig. 4

Etymology. Named after Prof. N.E. El-Gholl, who isolated this fungus, and contributed greatly to our knowledge of this generic complex.

Teleomorph unknown. *Conidiophores* penicillate without stipe extensions and terminal vesicles. *Conidiogenous apparatus* with several series of hyaline branches: primary branches aseptate or 1-septate, $17\text{--}35 \times 3\text{--}5 \mu\text{m}$; secondary branches aseptate, $12\text{--}20 \times 2\text{--}4 \mu\text{m}$, tertiary branches aseptate, $8\text{--}16 \times 2\text{--}3 \mu\text{m}$; quaternary branches abundant, aseptate, $6\text{--}12 \times 2\text{--}4 \mu\text{m}$; phialides doliiform to cymbiform to cylindrical, $9\text{--}49 \times 2\text{--}4 \mu\text{m}$, arranged in terminal whorls of 2–6 per branch, with minute collarettes, central phialide frequently extending above the rest. *Subverticillate conidiophores* absent. *Conidia* cylindrical, hyaline, smooth with rounded ends, straight, aseptate to 1-septate, $(18\text{--})19\text{--}23\text{--}(29) \times 2\text{--}4 \mu\text{m}$ (av. = $21 \times 3 \mu\text{m}$), lacking a visible

abscission scar, but frequently with a flattened base, held in a pale yellow, asymmetrical cluster by colourless slime.

Culture characteristics — Colonies sayal brown (reverse); chlamydospores extensive, in non-delimited chains. Aerial mycelium dense and off-white.

Specimen examined. USA, Florida, from *Chamaedorea elegans*, June 1993, N.E. El-Gholl, (CBS H-20905, holotype of *G. elghollii*) culture ex-type CBS 116104 = CPC 636 = P93-2051.

Notes — The phylogenetic inference used in this study reveal *G. elghollii* to be closely related to CBS 199.55 (ex-type of *G. sagariensis*). Based on the description by Saksena (1954), *G. elghollii* can be distinguished from *G. sagariensis* by its larger primary (up to $35 \mu\text{m}$ vs up to $22 \mu\text{m}$) and secondary (up to $20 \mu\text{m}$ vs up to $12 \mu\text{m}$) branches formed on the conidiogenous apparatus. The presence of abundant quaternary branches on the conidiogenous apparatus also distinguishes *G. elghollii* from other species in the genus.

Gliocladiopsis indonesiensis L. Lombard & Crous, *sp. nov.* — MycoBank MB564401; Fig. 5

Etymology. Name refers to Indonesia, the country from which the fungus was collected.

Teleomorph unknown. *Conidiophores* penicillate and subverticillate without stipe extensions and terminal vesicles. *Conidiogenous apparatus* with several series of hyaline branches: primary branches aseptate or 1-septate, $17\text{--}24 \times 3\text{--}4 \mu\text{m}$; secondary branches aseptate, $13\text{--}20 \times 2\text{--}3 \mu\text{m}$, tertiary branches aseptate, $8\text{--}15 \times 2\text{--}3 \mu\text{m}$; quaternary branches rare to absent, aseptate, $9\text{--}13 \times 2\text{--}3 \mu\text{m}$; phialides cymbiform to cylindrical, $13\text{--}21 \times 2\text{--}4 \mu\text{m}$, arranged in terminal whorls of 2–6 per branch, with minute collarettes. *Subverticillate conidiophores* moderate, mostly formed in aerial mycelium, with series of hya-

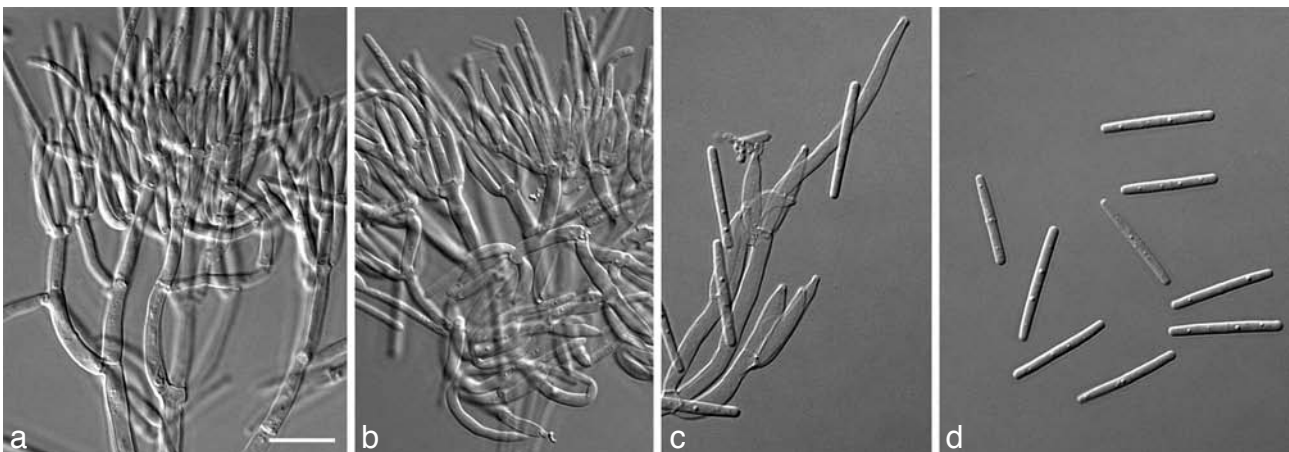


Fig. 4 *Gliocladiopsis elghollii* (CBS 116104, ex-type culture). a–c. Conidiophores; c. conidiophore with extended central phialide; d. conidia. — Scale bar: a = 10 μm (applies to all).

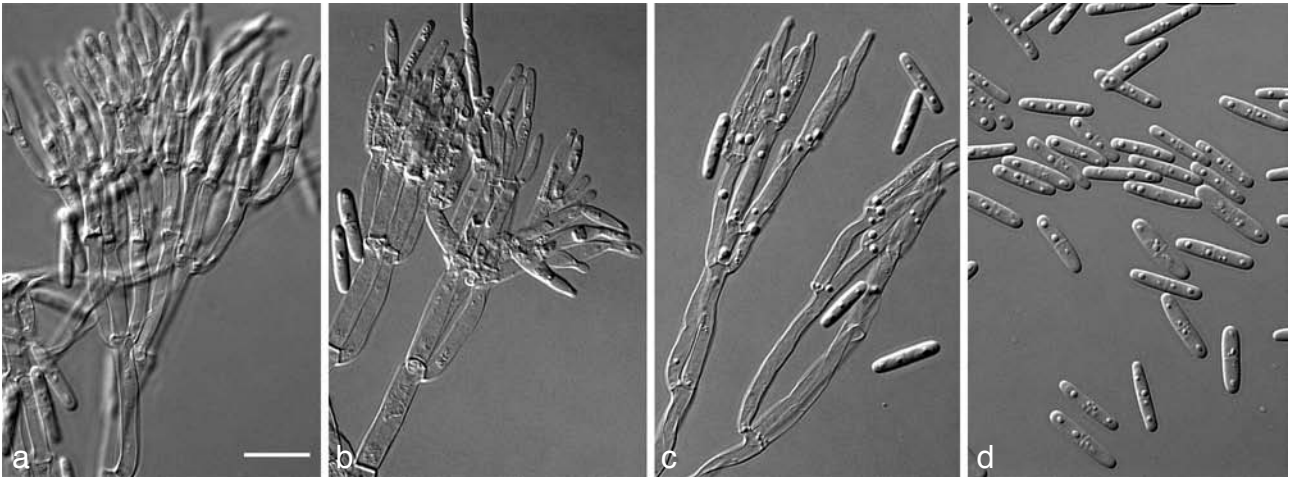


Fig. 5 *Gliocladiopsis indonesiensis* (CBS 116090, ex-type culture). a, b. Penicillate conidiophores; c. subverticillate conidiophore; d. conidia. — Scale bar: a = 10 μ m (applies to all).

line branches: primary branches aseptate or 1-septate, 18–27 \times 3–4 μ m; secondary branches aseptate, 16–24 \times 2–3 μ m; phialides cymbiform, 17–24 \times 2–4 μ m, arranged in terminal whorls of 1–3 per branch, with minute collarettes. *Conidia* cylindrical, hyaline, smooth with rounded ends, straight, 1-septate, (11–)13–15(–17) \times 2–4 μ m (av. = 14 \times 3 μ m), lacking a visible abscission scar, but frequently with a flattened base, held in a pale yellow, asymmetrical cluster by colourless slime.

Culture characteristics — Colonies luteous to cinnamon (reverse); chlamydo spores sparse, forming microsclerotia. Aerial mycelium dense and off-white.

Specimen examined. INDONESIA, from soil, Jan. 1994, coll. A.C. Alfenas, isol. P.W. Crous, (CBS H-20906, holotype of *G. indonesiensis*), culture ex-type CBS 116090 = CPC 715.

Notes — *Gliocladiopsis indonesiensis* is morphologically similar to *G. irregularis* but can be distinguished by the quaternary branches formed on the conidiogenous apparatus, which is not reported for *G. irregularis* (Crous & Peerally 1996).

Gliocladiopsis mexicana L. Lombard & Crous, *sp. nov.* — MycoBank MB564402; Fig. 6

Etymology. Name refers to Mexico, the country from which the fungus was collected.

Teleomorph unknown. *Conidiophores* penicillate without stipe extensions and terminal vesicles. *Conidiogenous apparatus* with several series of hyaline branches: primary branches aseptate or 1-septate, 12–22 \times 3–6 μ m; secondary branches aseptate, 9–15 \times 2–4 μ m, tertiary branches rare to absent, aseptate, 7–14 \times 2–4 μ m; phialides doliiiform to cymbiform, 9–15 \times 3–4 μ m, arranged in terminal whorls of 2–4 per branch,

with minute collarettes. *Subverticillate conidiophores* absent. *Conidia* cylindrical, hyaline, smooth with rounded ends, straight, 1-septate, (15–)17–19(–21) \times 2–4 μ m (av. = 18 \times 3 μ m), lacking a visible abscission scar, but frequently with a flattened base, held in a pale yellow, asymmetrical cluster by colourless slime.

Culture characteristics — Colonies sayal brown to sepia (reverse); chlamydo spores extensive, in non-delimited chains. Aerial mycelium dense, off-white to pale luteous.

Specimens examined. MEXICO, Campeche, Holpechén, from soil, Apr. 1994, coll. M.J. Wingfield, isol. P.W. Crous (CBS H-20908, holotype of *G. mexicana*), culture ex-type CBS 110938 = CPC 964; Campeche, Holpechén, from soil, Apr. 1994, coll. M.J. Wingfield, isol. P.W. Crous, culture CBS 111131 = CPC 965.

Notes — *Gliocladiopsis mexicana* is morphologically similar to *G. tenuis* but can be distinguished based on the number of branches formed on the conidiogenous apparatus. *Gliocladiopsis tenuis* has quaternary branches (Crous & Wingfield 1993), whereas these were not observed for *G. mexicana*, which only rarely formed tertiary branches.

Gliocladiopsis pseudotenuis L. Lombard & Crous, *nom. nov.* — MycoBank MB564403; Fig. 7

Basionym. *Glionectria tenuis* Crous & C.L. Schoch, Stud. Mycol. 45: 58. 2000.

Etymology. Name reflects the fact that this species resembles *G. tenuis*.

Specimens examined. CHINA, Hong Kong, from soil, Nov. 1993, coll. M.J. Wingfield, isol. P.W. Crous, (PREM 56381, holotype of teleomorph state) culture ex-type CBS 116074 = CPC 706. — INDONESIA, Warambungan, from soil next to *Vanilla* sp., ?, coll. M.J. Wingfield, isol. P.W. Crous (CBS H-20904, anamorph state), culture CBS 114763 = CPC 4575.

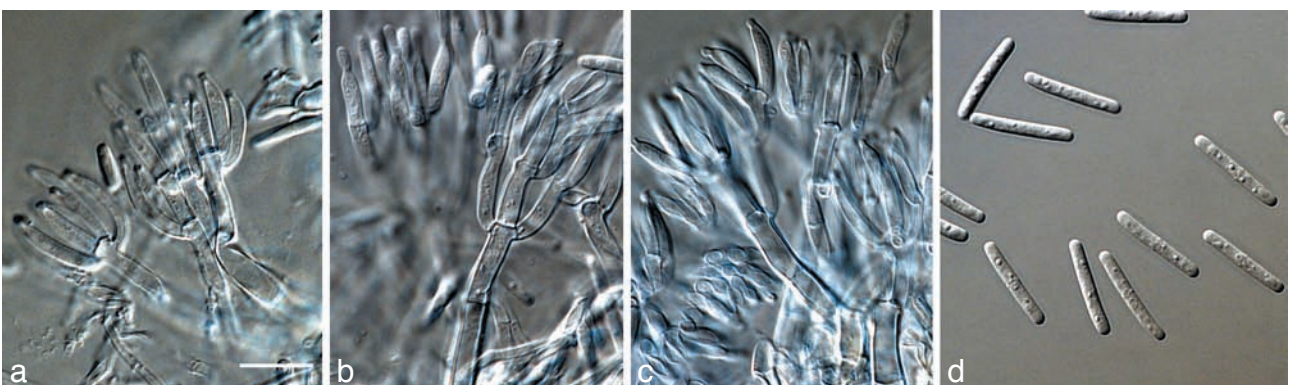


Fig. 6 *Gliocladiopsis mexicana* (CBS 110938, ex-type culture). a–c. Conidiophores; d. conidia. — Scale bars: a = 10 μ m (applies to all).



Fig. 7 *Gliocladiopsis pseudotenuis* (CBS 116074, ex-type culture). a–d. Teleomorph state: a. vertical section through a perithecium; b. ostiolar region of a perithecium; c. vertical section through the wall of a perithecium; d. ascospores. — e–g. Anamorph state: e. conidia; f, g. conidiophores. — Scale bars: a = 100 μ m; b = 10 μ m (applies to c–g).

Notes — *Gliocladiopsis pseudotenuis* is introduced as a new name for *Glionectria tenuis* in the genus *Gliocladiopsis*, which was incorrectly linked to its purported anamorph *G. tenuis* (Schoch et al. 2000). *Gliocladiopsis pseudotenuis* is morphologically similar to *G. tenuis*, but can be distinguished based on the slightly smaller conidia of *G. pseudotenuis* (14–)15–19(–21) \times 2–4 μ m; av. = 17 \times 2 μ m) compared to *G. tenuis* (av. = 18 \times 2 μ m). No quaternary branches were observed on the conidigenous apparatus of *G. pseudotenuis*, and it does not have an elongated central phialide as reported for *G. tenuis* (Crous & Wingfield 1993). However, phylogenetic inference is required to accurately distinguish between these two species.

DISCUSSION

The taxonomy of *Gliocladiopsis* isolates collected from various substrates and countries were investigated in this study using phylogenetic inference and morphological comparisons. This resulted in the identification of seven novel taxa. Following the 'strict priority' option as applied by Gräfenhan et al. (2011) and Lombard et al. (2010c, 2012), these novel taxa were named in the anamorph genus *Gliocladiopsis* (Saksena 1954) and not the teleomorph genus *Glionectria* (Schoch et al. 2000). Two unique phylogenetic lineages could not be provided with names in this study as the isolates were sterile.

Based on the multigene phylogeny used here, *G. sagariensis* is reinstated as the type species for the genus. Phylogenetic inference revealed that the ex-type culture of *G. sagariensis* (CBS 199.55) represents a unique lineage separate from the *G. tenuis* s.str. clade. Morphological comparisons, however, were not possible as the ex-type isolate of *G. sagariensis* is

sterile and therefore comparisons relied on the description and illustration provided by Saksena (1954). *Gliocladiopsis elghollii*, a novel taxon described here, is closely related to *G. sagariensis*, but could be distinguished morphologically, supported by the multigene sequence data.

Glionectria tenuis was described by Schoch et al. (2000) as the teleomorph state of *Gliocladiopsis tenuis* from a soil isolate collected from China that produced perithecia in culture. With additional sequence data supporting morphological observations *Glionectria tenuis* has been provided with a new name, *Gliocladiopsis pseudotenuis*.

The description of *G. curvata*, *G. elghollii*, *G. indonesiensis*, *G. mexicana* and *G. pseudotenuis* adds five more species to this genus, which only included three taxa prior to this study (Crous & Wingfield 1993, Crous & Peerally 1996, Crous et al. 1997). Previously, the isolates representing these new taxa were treated as *G. tenuis* based on morphological identification only. However, closer investigation of the morphology revealed differences distinguishing them from *G. tenuis*, a decision that was strongly supported by phylogenetic inference.

The first phylogenetic study to include *Gliocladiopsis* isolates by Schoch et al. (2000) used only ITS sequence data to distinguish between *G. irregularis*, *G. sumatrensis* and *G. tenuis*. Based on the phylogeny in that study, *G. irregularis* could not be distinguished from *G. sumatrensis*, whereas variation was seen within the *G. tenuis* clade. In this study, the ITS and BT sequence data could also not distinguish between *G. irregularis* and *G. sumatrensis*, and only 5 of the 11 lineages were recovered. The HIS3 and TEF 1- α sequence data, however, resolved all 11 lineages when the various gene regions were analysed separately (results not shown). The LSU sequence

data showed that the *Gliocladiopsis* isolates included formed a monophyletic clade, supporting the generic status of the genus.

Although a large number of the *Gliocladiopsis* isolates included in this study were isolated from symptomatic plant material, their relevance as plant pathogens has never been tested. In general, this group of soil-borne fungi has been regarded as secondary pathogens or saprobes (Crous 2002). Pathogenicity trials conducted by Dann et al. (2012), which included *Gliocladiopsis* isolates, revealed that these isolates were non-pathogenic to avocado plant roots. Inoculation with these isolates, however, improved the overall condition of the plants compared to the controls included. The possibility of using these fungi to improve plant growth in the future, therefore, requires further investigation.

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