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Article

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New *Cylindrocladiella* spp. from Thailand soils

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Abstract

Species of *Cylindrocladiella* (*Nectriaceae*) are generally regarded as saprobes or weak plant pathogens, typically causing problems in nurseries under high humidity conditions. Although the genus *Cylindrocladiella* is a common soil inhabitant in tropical and subtropical countries, only seven species have thus far been reported from Thailand. The aim of this study was thus to investigate various soil samples from northern Thailand for the presence of *Cylindrocladiella*, and to identify isolates to species level using a combination of cultural studies, morphology and DNA phylogeny (ITS, *tef1* and *tub2* gene regions). From the various isolates obtained, six new *Cylindrocladiella* species could be identified, described here as *C. brevistipitata*, *C. horticola*, *C. humicola*, *C. lateralis*, *C. reginae*, and *C. terrestris*. Further studies are required to establish the ecology of the new species, and also to determine which potential species occur in other as yet unsampled regions in Thailand.

Key words – cryptic species – phylogeny – taxonomy

Introduction

Boesewinkel (1982) established the asexual genus *Cylindrocladiella*, based on *C. parva*, to accommodate several cylindrocladium-like (= *Calonectria*) species characterised by small (< 20 µm long), 1-septate, cylindrical conidia and aseptate, thick-walled stipe extensions. Initially, *Cylindrocladiella* spp. were linked to the sexual genus *Nectricladiella* (Crous & Wingfield 1993, Schoch et al. 2000), however, following the implementation of the International Code of Nomenclature for algae, fungi, and plants (ICN, McNeill et al. 2012), Rossman et al. (2013) proposed that the genus name *Cylindrocladiella* be retained over *Nectricladiella*. Recently, Lombard et al. (2015) showed that the genus *Cylindrocladiella* formed a monophyletic group in the *Nectriaceae*, being closely related to genera such as *Aquanectria* and *Gliocladiopsis*.

To date, 29 species of *Cylindrocladiella* are recognized (Crous & Wingfield 1993, Coller et al. 2005, Inderbitzin et al. 2012, Lombard et al. 2012, 2015, Crous et al. 2017), of which two are linked to nectricladiella-like sexual morphs (Schoch et al. 2000, Crous 2002, Lombard et al. 2012, 2015). These fungi are generally soil-borne, and regarded as saprobes or weak plant pathogens with wide host ranges (Crous 2002, van Coller et al. 2005, Scattolin & Montecchio 2007, Lombard et al. 2012). Disease symptoms associated with *Cylindrocladiella* infection include leaf spots and root,

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stem and cutting rots (Crous et al. 1991, Peerally 1991, Crous & Wingfield 1993, Crous 2002, van Coller et al. 2005, Scattolin & Montecchio 2007, Lombard et al. 2012).

The aim of this study was to consider the identity of several *Cylindrocladiella* isolates isolated from soils collected in Thailand. To achieve this goal, combined morphological characteristics and phylogenetic inference was employed to identify these *Cylindrocladiella* isolates to species level.

Materials & Methods

Isolates

Soils were collected from various regions in Thailand, and baited as described by Crous (2002) using sterilised alfalfa seeds (Table 1). Representative strains are maintained in the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS).

Phylogeny

Total genomic DNA was extracted from axenic cultures grown on 2% malt extract agar (MEA) for 7 days, using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI) according to the manufacturer's protocol. Partial gene sequences were determined for β-tubulin (*tub2*), the internal transcribed spacer region (ITS) of the nuclear rDNA and translation elongation factor 1-alpha (*tef1*) using the primers and protocols described previously (Lombard et al. 2012, 2015). Subsequent alignments were generated using MAFFT v. 7 (Katoh & Standley 2013), and manually corrected where necessary.

The sequence datasets were tested for congruency using the reciprocal 70% bootstrap (BS) threshold method as described by Gueidan et al. (2007) to determine if the three partitions could be combined. Phylogenetic analyses were based on Bayesian inference (BI), maximum parsimony (MP) and maximum likelihood (ML). For BI and ML, the best evolutionary models for each partition were determined using MrModeltest (Nylander 2004) and incorporated into the analysis. MrBayes v. 3.2.1. (Ronquist & Huelsenbeck 2003) was used to generate phylogenetic trees under optimal criteria per partition. A Markov Chain Monte Carlo (MCMC) algorithm of four chains was started in parallel from a random tree topology with the heating parameter set at 0.3. The MCMC analysis lasted until the average standard deviation of split frequencies came below 0.01 with trees saved each 1 000 generations. The first 25% of saved trees were discarded as the "burn-in" phase and posterior probabilities (PP) determined from the remaining trees.

The MP analysis was done using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10, Swofford 2002). Phylogenetic relationships were estimated by heuristic searches with 1 000 random addition sequences. Tree bisection-reconnection was used, with the branch swapping option set on 'best trees' only with all characters weighted equally and alignment gaps treated as fifth state. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC) were calculated for parsimony and bootstrap analysis (Hillis & Bull 1993) was based on 1 000 replications.

The ML analysis was made with RAxML (randomized axelerated [*sic*] maximum likelihood for high performance computing; Stamatakis 2014) through the CIPRES website (http://www.phylo.org) to obtain a second measure of branch support. The robustness of the analysis was evaluated by bootstrap support (BS) analysis with the bootstrap replicates automatically determined by the software. Novel sequences were deposited in GenBank (Table 1) and all alignments in TreeBase as S21153.

Taxonomy

Axenic cultures were grown on synthetic nutrient-poor agar (SNA; Nirenburg 1981) amended with sterile carnation leaf pieces, and on MEA as described by Lombard et al. (2012). Gross morphological characteristics were studied by mounting the fungal structures in 85% lactic acid and 30 measurements were made for all taxonomically informative characters at × 1000 magnification using a Plan-Apochromat × 100/1.4 oil immersion lens (Carl Zeiss, Germany) mounted on a Zeiss

Axioscope 2 microscope, with differential interference contrast (DIC) illumination. The 95 % confidence levels were determined for the conidial measurements with extremes given in parentheses. For all other fungal structures measured, only the extremes are provided. Colony colour was assessed using 7-day-old cultures on MEA incubated at room temperature and the colour charts of Rayner (1970). All descriptions, illustrations and nomenclatural data were deposited in MycoBank (Crous et al. 2004).

Results

Phylogeny

Approximately 500−550 bases were determined for the three loci used in this study. The 70 % reciprocal bootstrap tree topologies for the three loci revealed a conflict between the coding gene regions (*tef1* & *tub2*) and the non-coding ITS gene region. The ITS gene region was unable to resolve majority of the established *Cylindrocladiella* spp. However, the three loci were still combined into a single dataset based on the argument of Cunningham (1997) that combining incongruent partitions could increase phylogenetic accuracy.

The combined alignment of the ITS, *tef1* and *tub2* contained 1579 characters from 68 taxa, including *Gliocladiopsis sagariensis* (CBS 199.55; Lombard et al. 2015) as the outgroup. The number of unique site patterns per data partition, including alignment gaps, was 61 from 503 characters for ITS, 203 from 547 characters for *tef1*, and 176 from 528 characters for *tub2*. MrModeltest determined that all three partitions had dirichlet base frequencies. A GTR+I+G model with inverse gamma-distributed rates was used for ITS and *tef1*, while HKY+I+G with inverse gamma-distributed rates was implemented for *tub2*.

The Bayesian analysis lasted 15 152 generations and the consensus tree, with posterior probabilities, was calculated from 7 576 trees left after 2 525 trees were discarded as burn-in. For the MP analysis 1 058 characters were constant, 175 parsimony-uninformative, and 346 parsimony-informative, yielding 22 equally most parsimonious trees (TL = 1 260; CI = 0.571; RI = 0.886; RC = 0.505). ML analysis resulted in a single best ML tree with –InL = -7042.460298. The best ML tree confirmed the consensus tree topologies obtained from the BI and MP analyses, and therefore only the best ML tree is presented (Fig. 1).

In the phylogenetic tree (Fig. 1), the majority of the *Cylindrocladiella* isolates obtained from soils collected in Thailand clustered into five highly-supported clades, with isolate CBS 142786 forming a distinct single lineage closely related to *C. infestans* and isolates CBS 142787 & CBS 142788. Both later isolates formed a distinct clade (ML-BS & MP-BS > 75 %; PP = 1.0) from the *C. infestans* clade (CBS 191.50 & CBS 111795), representing a novel phylogenetic species. Isolates CBS 142777, CBS 142778 and CBS 142779 formed a distinct clade (ML-BS & MP-BS > 75 %; PP = 1.0) closely related to *C. clavata* (CBS 129563 & CBS 129564), whereas CBS 142780, CBS 142781, CBS 142782 and CBS 142783 formed a unique clade (ML-BS & MP-BS > 75 %; PP = 1.0), distantly related to *C. parva* (CBS 114524), *C. pseudoparva* (CBS 113624 & CBS 129560) and *C. stellenboschensis* (CBS 110688 & CBS 115611). Isolates CBS 142784 and CBS 142785 also formed a unique clade (ML-BS & MP-BS = 100 %; PP = 1.0), distinct from *C. kurandica* (CBS 129576 & CBS 129577), *C. pseudoinfestans* (CBS 114530 & CBS 114531) and *C. queenslandica* (CBS 129574 & CBS 129575), representing a novel phylogenetic species. Isolates CBS 142789 and CBS 142790 formed a distinct clade (ML-BS & MP-BS = 100 %; PP = 1.0), sister to *C. peruviana* (CBS 114697 & IMUR 1843).

Taxonomy

Based on phylogenetic inference and morphological observations, six novel *Cylindrocladiella* species could be identified, isolated from the soils collected in Thailand, and are, therefore, described below.

Table 1. Isolates of *Cylindrocladiella* included in the study.

Species	Isolates	GenBank Accessions			Substrate	Country	Collector
		ITS	tef1	tub2			
C. australiensis	CBS 129567 = CPC 17507^{T}	JN100624	JN099060	JN098747	Soil	Australia	P.W. Crous
	CBS 129568 = CPC 17562	JN100623	JN099059	JN098748	Soil	Australia	P.W. Crous
C. camelliae	CPC234 = PPRI 3990	AF220952	JN099087	AY793471	*E. grandis*	South Africa	P.W. Crous
	CBS 114891 = CPC 277	AF220953	JN099086	AY793472	*E. grandis*	South Africa	P.W. Crous
C. brevistipitata	CBS 142786^{T}	–	MF444940	MF444926	Soil	Thailand	P.W. Crous
C. clavata	CBS 129563 = CPC 17591	JN099096	JN098975	JN098751	Soil	Australia	P.W. Crous
	CBS 129564 = CPC 17592^{T}	JN099095	JN098974	JN098752	Soil	Australia	P.W. Crous
C. cymbiformis	CBS 129553 = CPC 17393^{T}	JN099103	JN098988	JN098753	Soil	Australia	P.W. Crous
C. elegans	CBS 338.92 = IMI 346847^{T}	AY793444	JN099039	AY793474	Leaf litter	South Africa	I. Rong
	CBS 110801 = CPC 525	JN100609	JN099044	JN098755	Leaf litter	South Africa	P.W. Crous
C. ellipsoidea	CBS 129572 = CPC 17558	JN100636	JN099073	JN098756	Soil	Australia	P.W. Crous
	CBS 129573 = CPC 17560^{T}	JN099094	JN098973	JN098757	Soil	Australia	P.W. Crous
C. hahajimaensis	PD684	JN687561	JN687562	–	Soil	Japan	T. Watanabe
C. hawaiiensis	CBS 118704	JN099115	JN098996	JN098760	Soil	Hawaii	Y. Degawa
	CBS 129569 = CPC 12272^{T}	JN100621	JN099057	JN098761	Soil	Hawaii	Y. Degawa
C. horticola	CBS 142784^{T}	MF444911	MF444938	MF444924	Soil	Thailand	P.W. Crous
	CBS 142785	MF444912	MF444939	MF444925	Soil	Thailand	P.W. Crous
C. humicola	CBS 142777	MF444904	MF444931	MF444917	Soil	Thailand	P.W. Crous
	CBS 142778	MF444905	MF444932	MF444918	Soil	Thailand	P.W. Crous
	CBS 142779^{T}	MF444906	MF444933	MF444919	Soil	Thailand	P.W. Crous
C. infestans	CBS 111795 = ATCC 44816 ^{T}	AF220955	JN099037	AF320190	*Pinus pinea*	New Zealand	H.J. Boesewinkel
	CBS 191.50 = IMI 299376	AF220956	JN099036	AY793475	*Arenga pinnata*	Indonesia	K.B. Boedijn & J. Reitsma
C. kurandica	CBS 129576 = CPC 17547	JN100634	JN099071	JN098764	Soil	Australia	P.W. Crous
	CBS 129577 = CPC 17551^{T}	JN100646	JN099083	JN098765	Soil	Australia	P.W. Crous

C. lageniformis	CBS 340.92 = UFV 115^{T}	AF220959	JN099003	AY793481	*Eucalyptus* sp.	Brazil	A.C. Alfenas
	CBS 111060 = CPC 1240	JN100611	JN099046	JN098770	*Eucalyptus* sp.	South Africa	P.W. Crous
C. lanceolata	CBS 129565 = CPC 17566	JN100632	JN099069	JN098788	Soil	Australia	P.W. Crous
	CBS 129566 = CPC 17567^{T}	JN099099	JN098978	JN098789	Soil	Australia	P.W. Crous
C. lateralis	CBS 142787	MF444913	MF444941	MF444927	Soil	Thailand	P.W. Crous
	CBS 142788^{T}	MF444914	MF444942	MF444928	Soil	Thailand	P.W. Crous
C. longiphialidica	CBS 129557 = CPC 18839^{T}	JN100585	JN098966	JN098790	Soil	Thailand	P.W. Crous
	CBS 129558 = CPC 18841	JN100586	JN098967	JN098791	Soil	Thailand	P.W. Crous
C. longistipitata	CBS 112953 = CPC 4720	JN100595	JN099025	JN098792	*O. heterophylla*	Australia	C. Pearce & B. Paulus
	CBS 116075 = CPC 708^{T}	AF220958	JN098993	AY793506	Soil	China	M.J. Wingfield
C. microcylindrica	CBS 111794 = ATCC 38571 ^{T}	AY793452	JN099041	AY793483	*E. elegans*	Indonesia	C.F. Hill
C. natalensis	CBS 114943 = CPC 456^{T}	JN100588	JN099016	JN098794	*A. hypogaea*	South Africa	M.J. Wingfield
	CBS 114944 = CPC 457	JN100589	JN099017	JN098795	*A. hypogaea*	South Africa	M.J. Wingfield
C. nederlandica	CBS 146.94 = PD39/1776	JN099127	JN099011	JN098799	*Rhododendron* sp.	The Netherlands	
	CBS 152.91 = PD90/2015^{T}	JN100603	JN099033	JN098800	*Pelargonium* sp.	The Netherlands	J.W. Veenbaas-Rijks
C. novaezelandica	CBS 486.77 = ATCC 44815 ^{T}	AF220963	JN099050	AY793485	*Rhododendron indicum*	New Zealand	H.J. Boesewinkel
C. parva	CBS 114524 = ATCC 28272 ^{T}	AF220964	JN099009	AY793486	*Telopea speciosissima*	New Zealand	H.J. Boesewinkel
C. peruviana	CBS 114697 = CPC 2573	JN099124	JN099007	JN098802	*Vitis vinifera*	South Africa	S. Lambrecht
	IMUR 1843 = CPC 2404^{T}	AF220966	JN098968	AY793500	ants	Peru	M.P. Herrera
C. pseudocamelliae	CBS 129555 = CPC 18825^{T}	JN100577	JN098958	JN098814	Soil	Thailand	P.W. Crous
	CBS 129556 = CPC 18832	JN100580	JN098961	JN098815	Soil	Thailand	P.W. Crous
C. pseudohawaiiensis	CBS 210.94 = PPRI 4450 ^{T}	JN099128	JN099012	JN098819	*Eucalyptus* sp.	Brazil	A.C. Alfenas
	CBS 115610 = CPC 909	JN100594	JN099024	JN098820		Madagascar	P.W. Crous
C. pseudoinfestans	CBS 114530 = CPC 2320	JN099126	JN099010	JN098821	Soil	Madagascar	J.E. Taylor

	CBS 114531 = CPC 2319^{T}	AF220957	JN099004	AY793508	Soil	Madagascar	J.E. Taylor
C. pseudoparva	CBS 113624 = CPC 752	JN099121	JN099002	JN098822	*Quercus* sp.	Switzerland	L. Petrini
	CBS 129560 = CPC 18149^{T}	JN100620	JN099056	JN098824	Soil	The Netherlands	P.W. Crous
C. queenslandica	CBS 129574 = CPC 17568^{T}	JN099098	JN098977	JN098826	Soil	Australia	P.W. Crous
	CBS 129575 = CPC 17569	JN099097	JN098976	JN098827	Soil	Australia	P.W. Crous
C. reginae	CBS 142780	MF444907	MF444934	MF444920	Soil	Thailand	P.W. Crous
	CBS 142781	MF444908	MF444935	MF444921	Soil	Thailand	P.W. Crous
	CBS 142782^{T}	MF444909	MF444936	MF444922	Soil	Thailand	P.W. Crous
	CBS 142783	MF444910	MF444937	MF444923	Soil	Thailand	P.W. Crous
C. queenslandica	CBS 129574 = CPC 17568^{T}	JN099098	JN098977	JN098826	soil	Australia	P.W. Crous
	CBS 129575 = CPC 17569	JN099097	JN098976	JN098827	soil	Australia	P.W. Crous
C. stellenboschensis	CBS 110668 = CPC 517^{T}	JN100615	JN099051	JN098829	soil	South Africa	P.W. Crous
	CBS 115611 = CPC 4074	JN100593	JN099023	JN098830	*Geum* sp.	New Zealand	P.W. Crous
C. terrestris	CBS 142789	MF444915	MF444943	MF444929	Soil	Thailand	P.W. Crous
	CBS 142790	MF444916	MF444944	MF444930	Soil	Thailand	P.W. Crous
C. thailandica	CBS 129570 = CPC 18834	JN100581	JN098962	JN098833	soil	Thailand	P.W. Crous
	CBS 129571 = CPC 18835^{T}	JN100582	JN098963	JN098834	soil	Thailand	P.W. Crous
C. variabilis	CBS 375.93 = IMI 317057	JN099119	JN099000	JN098836	*Mangifera indica*	India	P.N. Chowdhry
	CBS 129561 = CPC 17505^{T}	JN100643	JN099080	JN098719	soil	Australia	P.W. Crous
C. viticola	CBS 112897 = CPC 5606^{T}	AY793468	JN099064	AY793504	*Vitis vinifera*	South Africa	G.J. van Coller
	CBS 114682 = IMI 297470	JN100612	JN099047	JN098723	*Amorphophallus* sp.	Thailand	R. Stevenson

CBS: Westerdijk Fungal Biodiversity Institute (WI), Utrecht, The Netherlands; CPC: working collection of Pedro Crous housed at WI; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; ATCC: American Type Culture Collection, Virginia, U.S.A.; PPRI: Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa.; UFV: Univeridade Federal de Vicosa, Brazil. ITS = Internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA; *tef1* = Translation elongation factor 1-alpha; *tub2* = β-tubulin. ^{T} Ex-type cultures

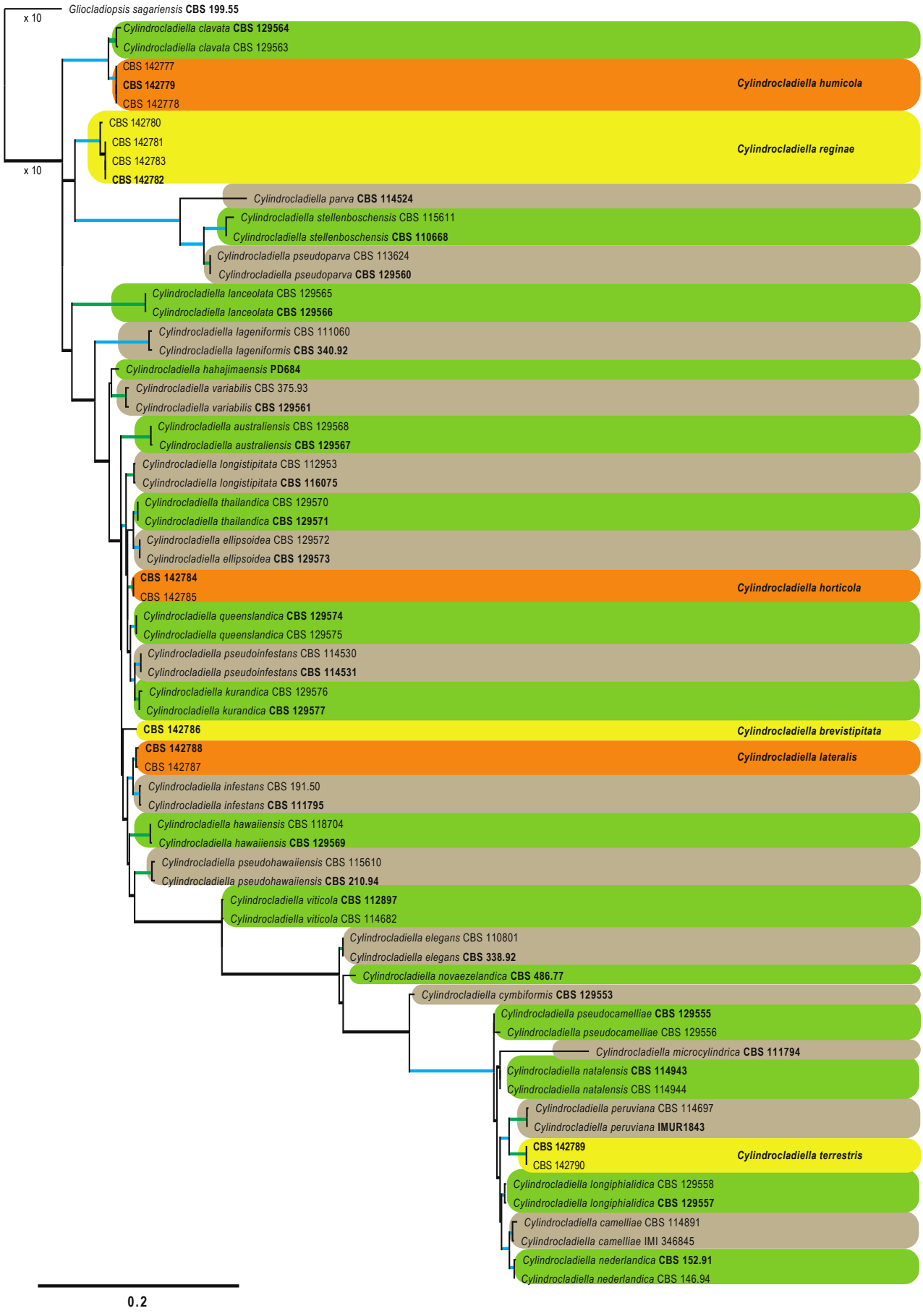
Gliocladiopsis sagariensis CBS 199.55
x 10
Cylindrocladiella clavata CBS 129564
Cylindrocladiella clavata CBS 129563
CBS 142777
CBS 142779
CBS 142778
Cylindrocladiella humicola
CBS 142780
CBS 142781
CBS 142783
CBS 142782
Cylindrocladiella reginae
x 10
Cylindrocladiella parva CBS 114524
Cylindrocladiella stellenboschensis CBS 115611
Cylindrocladiella stellenboschensis CBS 110668
Cylindrocladiella pseudoparva CBS 113624
Cylindrocladiella pseudoparva CBS 129560
Cylindrocladiella lanceolata CBS 129565
Cylindrocladiella lanceolata CBS 129566
Cylindrocladiella lageniformis CBS 111060
Cylindrocladiella lageniformis CBS 340.92
Cylindrocladiella hahajimaensis PD684
Cylindrocladiella variabilis CBS 375.93
Cylindrocladiella variabilis CBS 129561
Cylindrocladiella australiensis CBS 129568
Cylindrocladiella australiensis CBS 129567
Cylindrocladiella longistipitata CBS 112953
Cylindrocladiella longistipitata CBS 116075
Cylindrocladiella thailandica CBS 129570
Cylindrocladiella thailandica CBS 129571
Cylindrocladiella ellipsoidea CBS 129572
Cylindrocladiella ellipsoidea CBS 129573
CBS 142784
CBS 142785
Cylindrocladiella horticola
Cylindrocladiella queenslandica CBS 129574
Cylindrocladiella queenslandica CBS 129575
Cylindrocladiella pseudoinfestans CBS 114530
Cylindrocladiella pseudoinfestans CBS 114531
Cylindrocladiella kurandica CBS 129576
Cylindrocladiella kurandica CBS 129577
CBS 142786
Cylindrocladiella brevistipitata
CBS 142788
CBS 142787
Cylindrocladiella lateralis
Cylindrocladiella infestans CBS 191.50
Cylindrocladiella infestans CBS 111795
Cylindrocladiella hawaiiensis CBS 118704
Cylindrocladiella hawaiiensis CBS 129569
Cylindrocladiella pseudohawaiiensis CBS 115610
Cylindrocladiella pseudohawaiiensis CBS 210.94
Cylindrocladiella viticola CBS 112897
Cylindrocladiella viticola CBS 114682
Cylindrocladiella elegans CBS 110801
Cylindrocladiella elegans CBS 338.92
Cylindrocladiella novaezelandica CBS 486.77
Cylindrocladiella cymbiformis CBS 129553
Cylindrocladiella pseudocamelliae CBS 129555
Cylindrocladiella pseudocamelliae CBS 129556
Cylindrocladiella microcylindrica CBS 111794
Cylindrocladiella natalensis CBS 114943
Cylindrocladiella natalensis CBS 114944
Cylindrocladiella peruviana CBS 114697
Cylindrocladiella peruviana IMUR1843
CBS 142789
CBS 142790
Cylindrocladiella terrestris
Cylindrocladiella longiphialidica CBS 129558
Cylindrocladiella longiphialidica CBS 129557
Cylindrocladiella camelliae CBS 114891
Cylindrocladiella camelliae IMI 346845
Cylindrocladiella nederlandica CBS 152.91
Cylindrocladiella nederlandica CBS 146.94
0.2

Figure 1 – The ML consensus tree inferred from the combined ITS, tef1 and tub2 sequence alignments. Thickened branches indicate branches present in the ML, MP and Bayesian consensus trees. Branches with ML-BS & MP-BS = 100 % and PP = 1.0 are in green. Branches with ML-BS & MP-BS ≥ 75 % and PP ≥ 0.95 are in blue. The scale bar indicates 0.2 expected changes per site. The tree is rooted to Gliocladiopsis sagariensis (CBS 199.55). Epi- and ex-type strains are indicated in bold.

Cylindrocladiella brevistipitata L. Lombard & Crous, **sp. nov.** Fig. 2

MycoBank MB821579; Facesoffungi number: 03582

Etymology – Name refers to the short stipe extension of the conidiophores.

Sexual morph unknown. *Conidiophores* penicillate, mononematous and hyaline, comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 75–150 × 4–6 µm; stipe extension aseptate, straight, 60–95 µm long, thick-walled with one basal septum, terminating in thin-walled, ellipsoidal to lanceolate vesicles, 4–5 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 10–26 × 2–4 µm, secondary branches aseptate, 8–15 × 2–4 µm, tertiary branches aseptate, 6–11 × 2–3 µm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical, hyaline, aseptate, 8–14 × 2–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* not observed. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, (9–)10–12 × 2–3 µm (av. = 11 × 2 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies convex, cottony, with sinuate margins, honey with umber centre; reverse sienna to umber; chlamydospores extensive throughout medium arranged in chains; reaching 65–75 mm after 7 d on MEA at 24 °C in ambient light.

Material examined – Thailand, Mae Rim, Queen Sirikit Botanical Garden, from soil, October 2010, P.W. Crous (holotype CBS H-23173, culture ex-type CBS 142786).

Notes – *Cylindrocladiella brevistipitata* can be distinguished from other species in the genus by its relatively short stipe extensions, and tertiary branches formed on its penicillate conidiogenous apparatus.

Cylindrocladiella horticola L. Lombard & Crous, **sp. nov.** Fig. 3

MycoBank MB821580; Facesoffungi number: FoF03583

Etymology – Named refers to the locality, Queen Sirikit Botanical Garden, from where this fungus was collected.

Sexual morph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline, comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 50–100 × 4–7 µm; stipe extension aseptate, straight, 55–150 µm long, thick-walled with one basal septum, terminating in thin-walled, ellipsoidal to lanceolate vesicles, 3–6 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 10–20 × 2–4 µm, secondary branches aseptate, 7–12 × 2–4 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform to cymbiform, hyaline, aseptate, 7–14 × 2–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* abundant, comprising of a septate stipe and primary branches terminating in 2–4 phialides; primary branches straight, hyaline, 0–1-septate, 15–40 × 2–4 µm; phialides cymbiform to cylindrical, hyaline, aseptate, 11–18 × 2–3 µm, apex with minute periclinal thickening and collarette. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, (11–)11.5–12.5(–14) × (1–)1.5–2.5(–3) µm (av. = 12 × 2 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies convex, cottony, with smooth margins, cream with pale luteous centre; reverse pale luteous to honey with sepia centre; chlamydospores extensive throughout medium arranged in chains; reaching 50–75 mm after 7 days on MEA at 24 °C in ambient light.

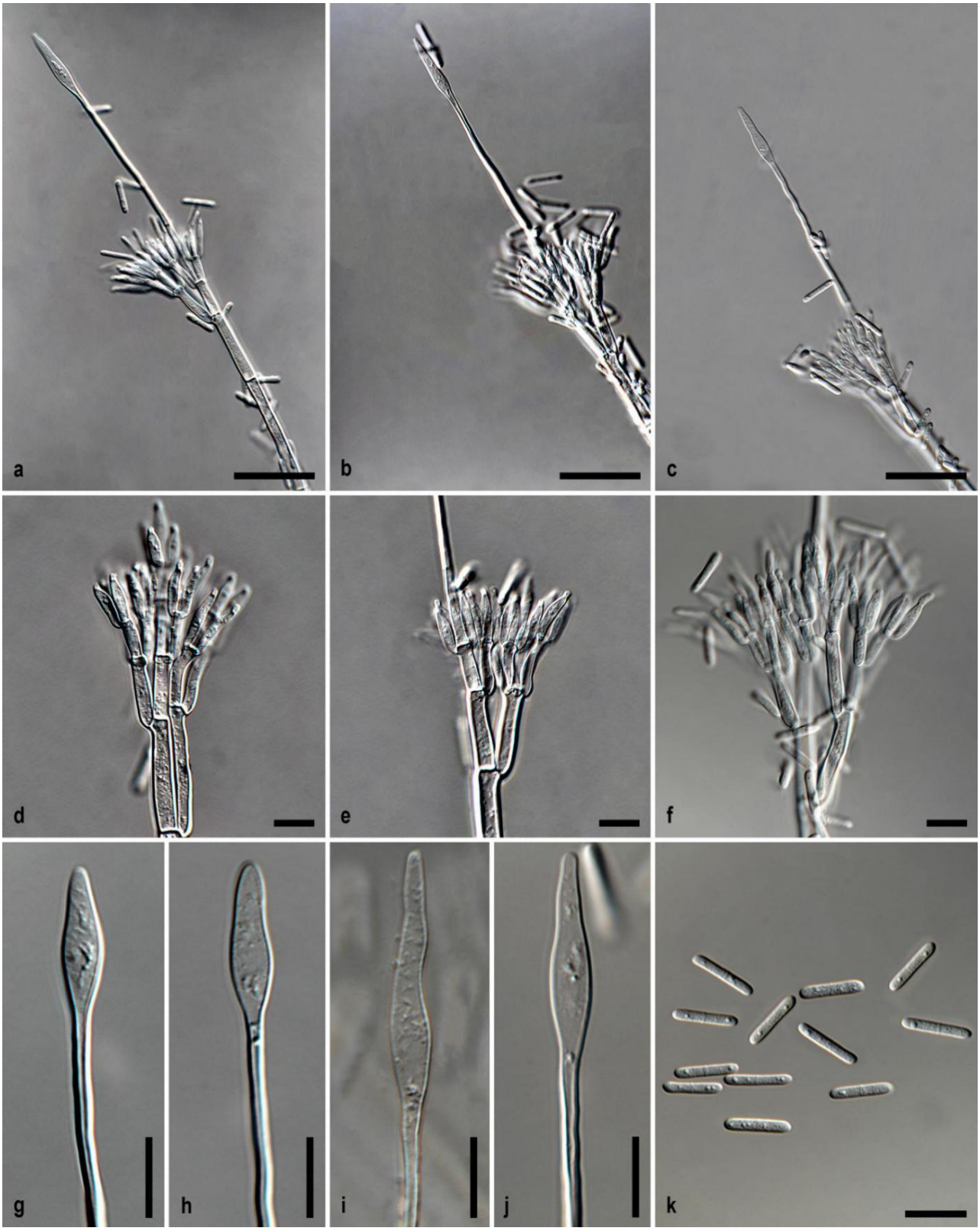

Figure 2 – *Cylindrocladiella brevistipitata* (ex-type CBS 142786). **a–c**. Penicillate conidiophores. **d–f**. Penicillate conidiogenous apparatus. **g–j**. Thin-walled, ellipsoidal to lanceolate vesicles. **k**. Conidia. Scale bars a–c = 20 µm; d–k = 10 µm.

Material examined – Thailand, Mae Rim, Queen Sirikit Botanical Garden, from soil, October 2010, P.W. Crous (holotype CBS H-23174, culture ex-type CBS 142784); ibid., culture CBS 142785.

Notes – *Cylindrocladiella horticola* displays some morphological overlap with *C. kurandica*, *C. pseudoinfestans* and *C. queenslandica* (Schoch et al. 2000, Crous 2002, Lombard et al. 2012). The conidia of *C. horticola* [(11–)11.5–12.5(–14) × (1–)1.5–2.5(–3) µm (av. = 12 × 2 µm)] are slightly smaller than those of *C. kurandica* [(10–)12–14(–16) × 2–4) µm (av. = 13 × 3 µm); Lombard et al. 2012] and *C. pseudoinfestans* [(10–)14–16(–20) × 2(–3) µm (av. = 15 × 2 µm); Schoch et al. 2000] but overlap with those of *C. queenslandica* [(9–)10.5–13.5(–15) × 2–4 µm (av. = 12 × 3 µm); Lombard et al. 2012]. However, the stipe extension of *C. queenslandica* (up to 180 µm; Lombard et al. 2012) is longer than that of *C. horticola* (up to 150 µm).

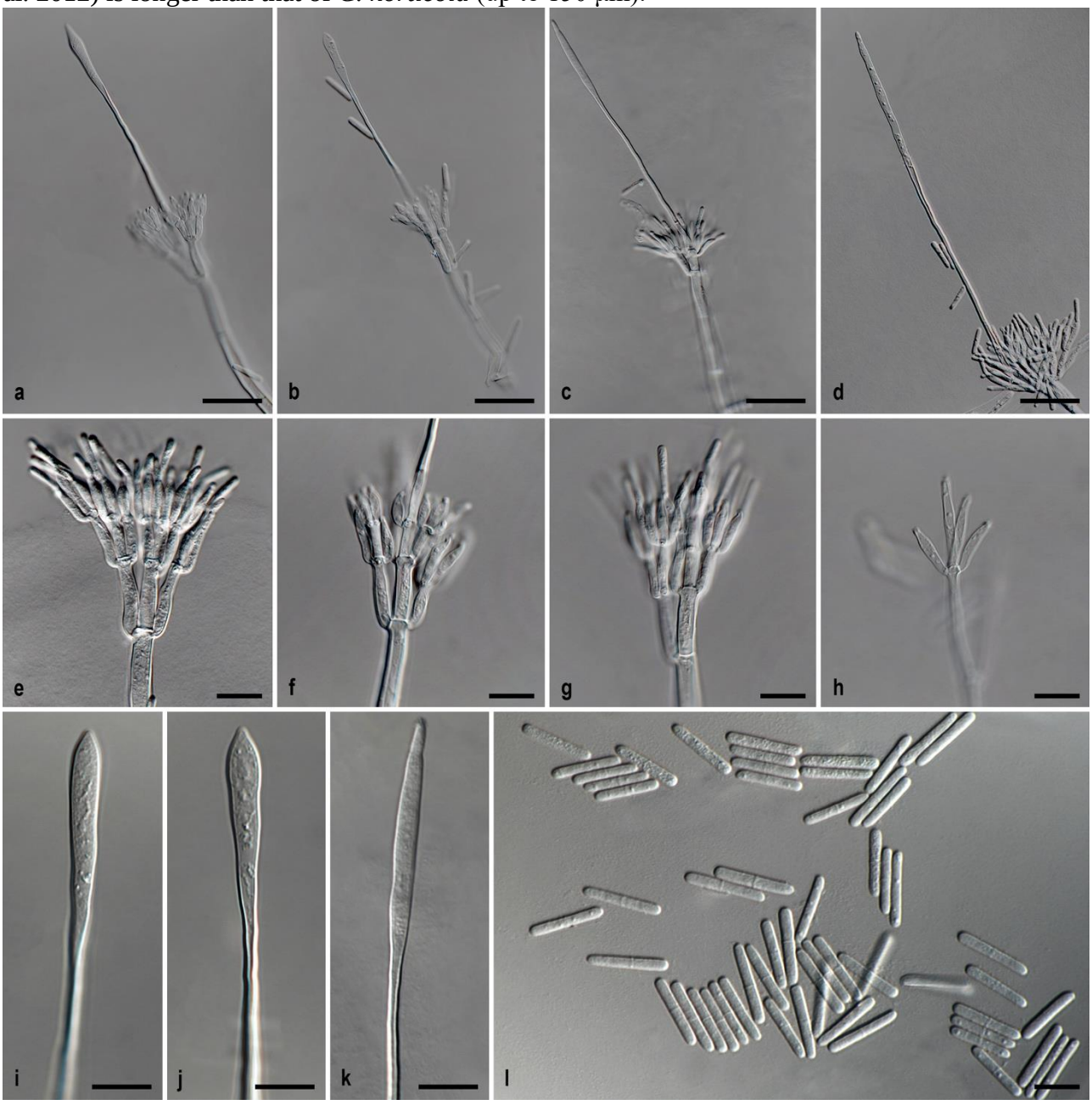

Figure 3 – *Cylindrocladiella horticola* (ex-type CBS 142784). **a–d**. Penicillate conidiophores. **e–g**. Penicillate conidiogenous apparatus. **h**. Subverticillate conidiophores. **i–k**. Thin-walled, ellipsoidal to lanceolate vesicles. **l**. Conidia. Scale bars a–d = 20 µm; e–l = 10 µm.

Cylindrocladiella humicola L. Lombard & Crous, **sp. nov.** Fig. 4

MycoBank MB821581; Facesoffungi number: FoF03584

Etymology – Named after the substrate, soil, from which the fungus was isolated.

Sexual morph unknown. *Conidiophores* penicillate, mononematous and hyaline, comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 30–60 × 5 µm; stipe extension aseptate, straight, 85–110 µm long, thick-walled with one basal septum, terminating in thin-walled, elongate clavate vesicles, 2–4 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 10–20 × 2–4 µm, secondary branches aseptate, 7–10 × 2–3 µm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical to ventricose, hyaline, aseptate, 7–12 × 2–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* not observed. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, (10–)11–13(–14) × 1–3 µm (av. = 12 × 2 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies convex, cottony, with sinuate margins, cream with pale luteous centre; reverse cinnamon to honey with sepia centre; chlamydospores extensive throughout medium, arranged in chains; reaching 45–70 mm after 7 days on MEA at 24 °C in ambient light.

Material examined – Thailand, Chiang Rai, from soil, October 2010, P.W. Crous (holotype CBS H-23175, culture ex-type CBS 142779); ibid., cultures CBS 142777 & CBS 142778.

Notes – *Cylindrocladiella humicola* is closely related to *C. clavata* (Fig. 1). The conidia of *C. humicola* [(10–)11–13(–14) × 1–3 µm (av. = 12 × 2 µm)] are slightly smaller than those of *C. clavata* [(10–)13–15(–16) × 2–3 µm (av. = 14 × 2 µm); Lombard et al. 2012] and the stipe extensions of *C. humicola* are shorter (110 µm) compared to *C. clavata* (up to 170 µm; Lombard et al. 2012).

Cylindrocladiella lateralis L. Lombard & Crous, **sp. nov.** Fig. 5

MycoBank MB821582; Facesoffungi number: FoF03585

Etymology – Named refers to the lateral stipe extensions on the penicillate conidiogenous apparatus.

Sexual morph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline, comprising a stipe, a penicillate arrangement of fertile branches, stipe extensions terminating in vesicles; stipe septate, hyaline, smooth, 30–90 × 3–7 µm; stipe extension aseptate, straight, 100–130 µm long, thick-walled with one basal septum, terminating in thin-walled, elongate clavate to cylindrical vesicles, 3–4 µm wide; lateral stipe extensions sparse, 55–95 µm long, thick-walled with one basal septum, terminating in thin-walled, elongate clavate to cylindrical vesicles, 2–3 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 12–22 × 2–4 µm, secondary branches aseptate, 9–16 × 2–3 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform to cymbiform, hyaline, aseptate, 7–13 × 2–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* abundant, comprising of a septate stipe and primary branches terminating in 2–4 phialides; primary branches straight, hyaline, 0–1-septate, 12–45 × 2–4 µm; phialides cymbiform to cylindrical, hyaline, aseptate, 15–20 × 2–3 µm, apex with minute periclinal thickening and collarette. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, 12–14(–15) × 1–2 µm (av. = 13 × 2 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies convex, cottony, with sinuate margins, cream with pale luteous centre; reverse pale luteous to honey with sepia centre; chlamydospores moderate throughout medium arranged in chains; reaching 45–65 mm after 7 days on MEA at 24 °C in ambient light.

Material examined – Thailand, Mae Rim, Queen Sirikit Botanical Garden, from soil, October 2010, P.W. Crous (holotype CBS H-23176, culture ex-type CBS 142788); ibid., culture CBS 142787.

Notes – *Cylindrocladiella lateralis* is the only species known thus far to produce lateral stipe extensions on its penicillate conidiogenous apparatus, thereby distinguishing it from other species in the genus.

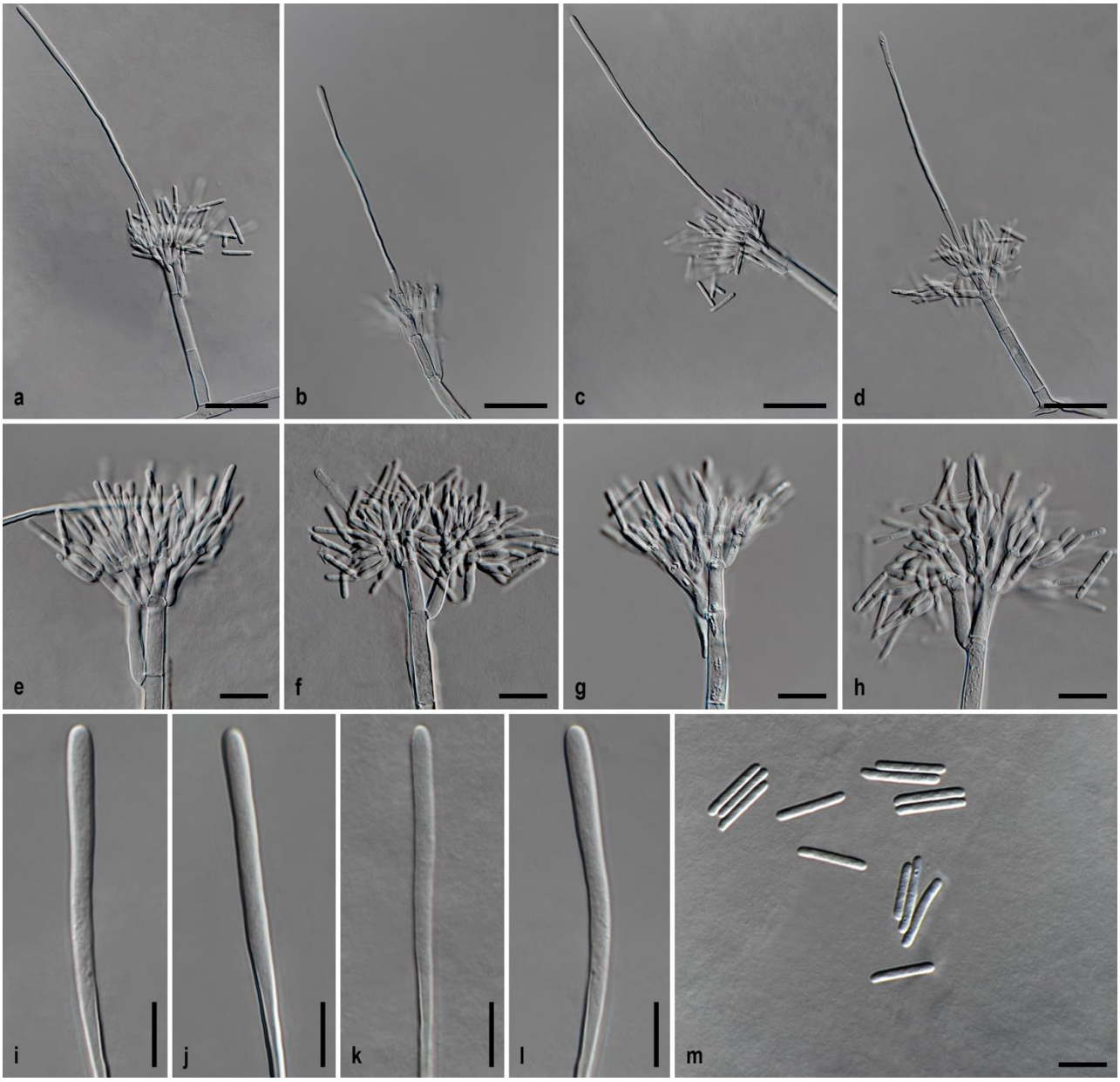

Figure 4 – *Cylindrocladiella humicola* (ex-type CBS 142779). **a–d**. Penicillate conidiophores. **e–h**. Penicillate conidiogenous apparatus. **i–l**. Thin-walled, elongate clavate vesicles. **m**. Conidia. Scale bars a–d = 20 µm; e–m = 10 µm.

Cylindrocladiella reginae L. Lombard & Crous, **sp. nov.** Fig. 6

MycoBank MB821583; Facesoffungi number: FoF03586

Etymology – Named refers to the locality, Queen Sirikit Botanical Garden, from where this fungus was collected.

Sexual morph unknown. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline, comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 40–65 × 4–7 µm; stipe extension aseptate, straight, 85–120 µm long, thick-walled with one basal septum, terminating in thin-walled, elongate clavate vesicles, 3–4 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 8–20 × 2–5 µm, secondary branches aseptate, 8–13 × 2–4 µm, tertiary branches rare, aseptate, 8–10 × 2–3 µm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical to ventricose, hyaline, aseptate, 7–12 × 1–4 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* abundant, comprising of a septate stipe and primary

branches terminating in 1–3 phialides; primary branches straight, hyaline, 0–1-septate, 20–45 × 2–4 µm; phialides cymbiform to cylindrical, hyaline, aseptate, 13–27 × 2–3 µm, apex with minute periclinal thickening and collarette. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, (10–)11.5–12.5(–13) × 2–3 µm (av. = 12 × 2 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

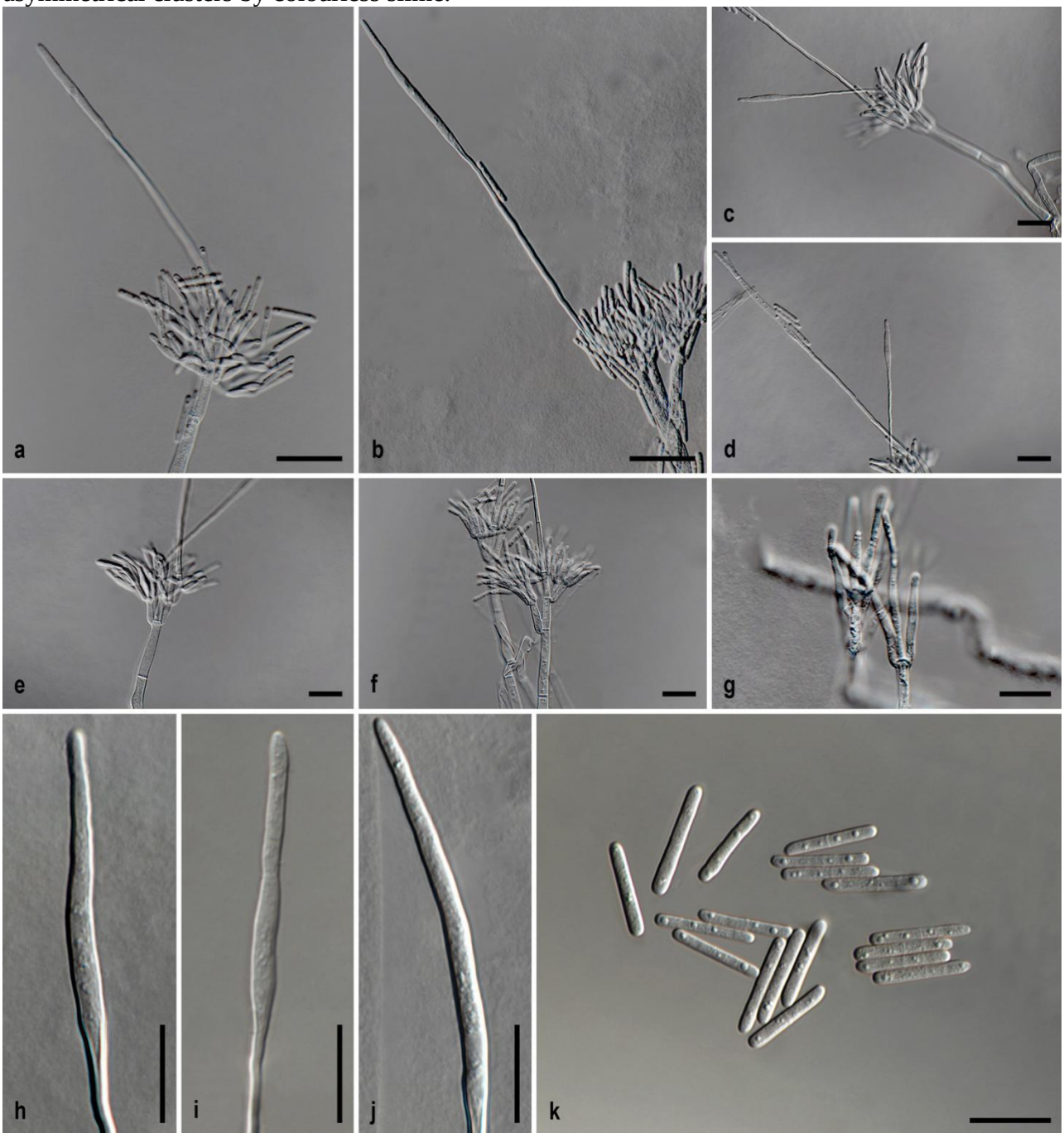

Figure 5 – *Cylindrocladiella lateralis* (ex-type CBS 142788). **a–b**. Penicillate conidiophores. **c–d**. Penicillate conidiophores with lateral stipe extensions. **e–f**. Penicillate conidiogenous apparatus. **g**. Subverticillate conidiophores. **h–j**. Thin-walled, elongate clavate to cylindrical vesicles. **k**. Conidia. Scale bars a–d = 20 µm; e–k = 10 µm.

Culture characteristics – Colonies convex, cottony, with sinuate margins, cream to pale luteous with honey centre; reverse cinnamon to honey with sepia centre and pale luteous margin; chlamydospores extensive throughout medium arranged in chains; reaching 60–65 mm after 7 days on MEA at 24 °C in ambient light.

Materials examined – Thailand, Mae Rim, Queen Sirikit Botanical Garden, from soil, October 2010, P.W. Crous (holotype CBS H-23177, culture ex-type CBS 142782); ibid., cultures CBS 142780, CBS 142781 & CBS 142783.

Notes – *Cylindrocladiella reginae* can be distinguished from its closest phylogenetic neighbours (*C. parva*, *C. pseudoparva* and *C. stellenboschensis*; Fig. 1) by the presence of tertiary branches on its penicillate conidiogenous apparatus, a characteristic not shared by either *C. parva*, *C. pseudoparva* and *C. stellenboschensis* (Boesewinkel 1982, Crous 2002, Lombard et al. 2012).

Figure 6 – *Cylindrocladiella reginae* (ex-type CBS 142782). **a–d**. Penicillate conidiophores. **e–f**. Penicillate conidiogenous apparatus. **g**. Subverticillate conidiophores. **h–j**. Thin-walled, elongate clavate vesicles. **k**. Conidia. Scale bars a–d = 20 µm; e–k = 10 µm.

Cylindrocladiella terrestris L. Lombard & Crous, **sp. nov.** Fig. 7

MycoBank MB821584; Facesoffungi number: FoF03587

Etymology – Named after the substrate, soil, from which the fungus was isolated.

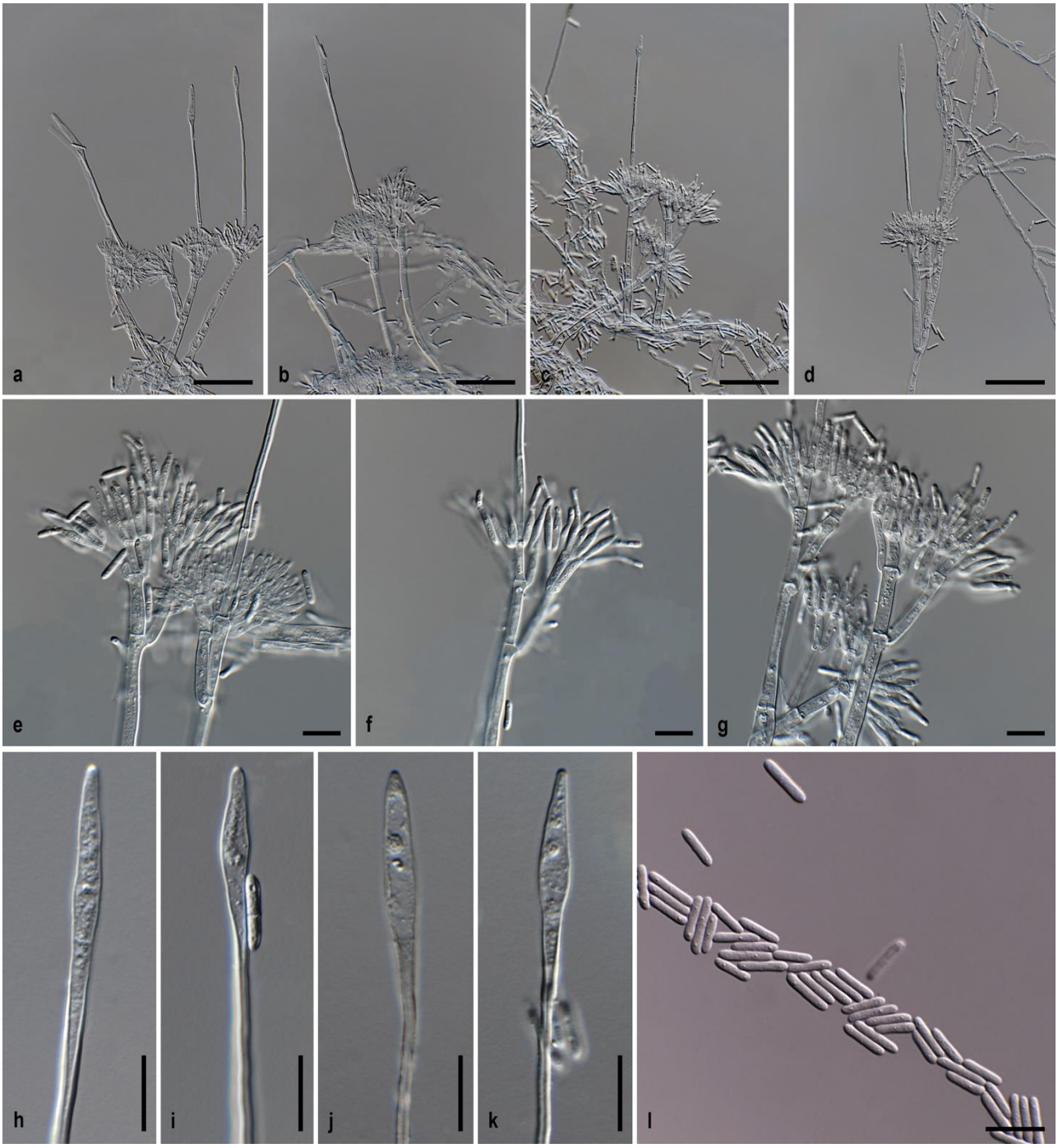

Figure 7 – *Cylindrocladiella terrestris* (ex-type CBS 142789). **a–d**. Penicillate conidiophores. **e–g**. Penicillate conidiogenous apparatus. **h–k**. Thin-walled, ellipsoidal to lanceolate vesicles. **l**. Conidia. Scale bars a–d = 20 µm; e–l = 10 µm.

Sexual morph unknown. *Conidiophores* penicillate, mononematous and hyaline, comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 50–100 × 6–7 µm; stipe extension aseptate, straight, 80–120 µm long, thick-walled with one basal septum, terminating in thin-walled, ellipsoidal to lanceolate vesicles, 4(–5) µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 16–27 × 3–4 µm, secondary branches aseptate, 10–15 × 3–4 µm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical, hyaline, aseptate, 10–15 × 2.5–3.5 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* not observed. *Conidia* cylindrical,

rounded at both ends, straight, 1-septate, (8–)10–12(–14) × 2(–2.5) µm (av. = 11 × 2 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics – Colonies convex, cottony, with sinuate margins, pale luteous with sepia centre; reverse sienna to umber with sepia centre; chlamydospores extensive throughout medium arranged in chains; reaching 60–65 mm after 7 days on MEA at 24 °C in ambient light.

Material examined – Thailand, Mae Rim, Queen Sirikit Botanical Garden, from soil, October 2010, P.W. Crous (holotype CBS H-23178, culture ex-type CBS 142789); ibid., culture CBS 142790.

Notes – *Cylindrocladiella terrestris* is closely related to *C. peruviana* (Fig. 1). The stipe extensions of *C. terrestris* are shorter (up to 120 µm long) compared to those of *C. peruviana* (up to 140 µm long; Boesewinkel 1982, Crous 2002). Additionally, *C. terrestris* did not form subverticillate conidiophores *in vitro*, whereas they are present in *C. peruviana* (Crous 2002).

Discussion

The present study introduces six new *Cylindrocladiella* species, which were all isolated from soil samples collected in northern Thailand. This brings the number of known *Cylindrocladiella* species from Thailand to 13, and includes *C. camelliae, C. longiphialidica, C. microcylindrica, C. peruviana, C. pseudocamelliae, C. thailandica* and *C. viticola* (Crous 2002, Lombard et al. 2012). Of these, only *C. viticola* has been isolated from a plant host (*Vitis vinifera* in South Africa, and *Amorphophallus* sp. in Thailand; Crous 2002), with the remaining 12 originating from soil (Crous 2002, Lombard et al. 2012). However, although their ecology is presently unknown, their relevance as plant pathogens still needs to be determined as *Cylindrocladiella* species are generally regarded as weak plant pathogens, especially in nurseries under tropical conditions (Lombard et al. 2012).

Interestingly, the majority of the *Cylindrocladiella* species presently known from Thailand have thus far been collected only from northern Thailand (Crous 2002, Lombard et al. 2012). The only exceptions are *C. viticola* and *C. microcylindrica*, for which the collection localities within Thailand remains unknown. The apparent high level of species diversity therefore suggests that a more comprehensive soil survey from other regions of Thailand would probably provide a better understanding of the diversity of *Cylindrocladiella* spp. present in this country. It is hoped that future surveys would be able to elucidate this aspect in more detail.

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