



# Phylogeny and taxonomy of the genus *Gliocephalotrichum*

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## Key words

*Gliocephalotrichum*  
*Leuconectria*  
phylogeny  
taxonomy

**Abstract** Species in the genus *Gliocephalotrichum* (= *Leuconectria*) (*Hypocreales*, *Nectriaceae*) are soilborne fungi, associated with post-harvest fruit spoilage of several important tropical fruit crops. Contemporary taxonomic studies of these fungi have relied on morphology and DNA sequence comparisons of the internal transcribed spacer region of the nuclear rDNA (ITS) and the  $\beta$ -tubulin gene regions. Employing DNA sequence data from four loci ( $\beta$ -tubulin, histone H3, ITS, and translation elongation factor 1-alpha) and morphological comparisons, the taxonomic status of the genus *Gliocephalotrichum* was re-evaluated. As a result five species are newly described, namely *G. humicola* (Taiwan, soil), *G. mexicanum* (rambutan fruit from Mexico), *G. nephelii* (rambutan fruit from Guatemala), *G. queenslandicum* (Australia, endophytic isolations) and *G. simmonsii* (rambutan fruit from Guatemala). Although species of *Gliocephalotrichum* are generally not regarded as important plant pathogens, their ability to cause post-harvest fruit rot could have an impact on fruit export and storage.

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## INTRODUCTION

The asexual genus *Gliocephalotrichum*, with *G. bulbilium* as type, was introduced by Ellis & Hesseltine (1962) to accommodate a species isolated from soil. The genus was defined as having conidiophores consisting of a penicillate conidiogenous apparatus terminating in phialides producing ellipsoidal, aseptate conidia, and subtended by sterile stipe extensions. Morphologically, this genus closely resembles the asexual morph of *Calonectria* (= *Cylindrocladium*) but is distinguished by the point of origin of the sterile stipe extension (Rossman et al. 1993, 1999, Schoch et al. 2000). In *Gliocephalotrichum*, the stipe extension develops directly below (Ellis & Hesseltine 1962) or some distance below (Wiley & Simmons 1971) the penicillus, whereas the stipe extension originates from within the conidiogenous apparatus of *Calonectria* (Rossman et al. 1993, Lombard et al. 2010b).

Seven species that are recognised within the genus include *G. bacillisporum* (Decock et al. 2006), *G. bulbilium* (Ellis & Hesseltine 1962), *G. cylindrosporium*, *G. microchlamydosporium* (Wiley & Simmons 1971), *G. longibrachium* (Decock et al. 2006), *G. ohiense* (Huang & Schmitt 1973) and *G. simplex* (Wiley & Simmons 1971). The genus *Leuconectria*, with *L. clusiae* as type, was introduced by Rossman et al. (1993) as the sexual morph of *G. bulbilium*. It is characterised by having superficial, uniloculate perithecia becoming purple in KOH+ and producing aseptate ascospores. A second species, *L. grandis*, was intro-

duced by Zhuang et al. (2007), although mistakenly connected to the asexual species, *G. cylindrosporium*. This was later corrected by Zhuang & Luo (2008), although they refrained from providing a name for the asexual morph based on the version of the International Code of Botanical Nomenclature applied at that time (McNeill et al. 2006). Following the abolishment of Art. 59 (Hawksworth et al. 2011), and based on the current International Code of Nomenclature for algae, fungi and plants (ICN; McNeill et al. 2012), Rossman et al. (2013) proposed that the genus name *Gliocephalotrichum* be retained over *Leuconectria*, and therefore provided the name *G. grande* to accommodate *L. grandis* in *Gliocephalotrichum*.

Although limited information is available on the etiology of these fungi, they are generally regarded as saprobes, as they are readily isolated from soils and decaying plant material, such as leaf litter and rotting fruits (Ellis & Hesseltine 1962, Wiley & Simmons 1971, Huang & Schmitt 1973, Rossman et al. 1993, Watanabe & Nakamura 2005, Decock et al. 2006, Zhuang et al. 2007). With the exception of *G. ohiense* (Huang & Schmitt 1973), all species are regarded as tropical or subtropical fungi (Rossman et al. 1993, 1999, Decock et al. 2006). Recently, several new reports have appeared of fruit rots associated with species of *Gliocephalotrichum*, namely of rambutan (*Nephelium lappaceum*) in Hawaii (Nishijima et al. 2002), Malaysia (Intan Sakinah & Latiffah 2013), The Philippines (Pordesimo & Lunallag 1982), Puerto Rico (Serrato-Diaz et al. 2012), Sri Lanka (Sivakumar et al. 1997, 1999, 2000) and Thailand (Farungsang et al. 1992, Sangchote et al. 1998), guava (*Psidium guajava*) in Hawaii (Constantelos et al. 2011), durian (*Durio graveolens* and *D. kutejensis*) in Brunei Darussalam (Sivapalan et al. 1998), *Terminalia chebula* in India (Singh et al. 2012), mangosteen (*Garcinia mangostana*) in Thailand (Sangchote & Pongpisutta 1998) and cranberry (*Vaccinium macrocarpon*) in the USA (Constantelos et al. 2011).

A recent survey of rambutan fruit originating from Guatemala, Mexico and Puerto Rico resulted in the accumulation of several isolates of *Gliocephalotrichum*. Furthermore, baiting from soils collected in Thailand revealed several additional isolates of *Gliocephalotrichum*. A number of isolates have also accumu-

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Table 1 *Gliosphaerotrichum* isolates included in this study.

Species	Culture accession* <sup>†</sup>	GenBank accession <sup>2</sup>			ITS	TEF	Substrate	Country	Collector
		BTUB	HIS3	ITS					
<i>Gliosphaerotrichum bacillisporum</i>	CBS 250.91	KF513182	KF513323	KF513251	KF513405	plant root	Brazil	L. Pfenning	
	CBS 126572 = MUCL 46554	DQ374413	KF513324	DQ374408	KF513406	leaf litter	French Guiana	C. Decock & V. Robert	
	CBS 132042 = MUCL 46732	DQ374414	KF513325	DQ374409	KF513407	leaf litter	French Guiana	C. Decock & V. Robert	
	CBS 242.62 = ATCC 22228 = IFO 9325 = IMI 096357 = MUCL 18575 = NRRL 2899 = QM 9007	DQ377831	KF513326	DQ377831	KF513408	soil	USA	L.J. Wickham	
	CBS 118.68	KF513183	KF513327	KF513252	KF513409	air	Central African Republic	J. Nicot	
	CBS 562.75	KF513184	KF513328	KF513253	KF513410	<i>Flacourtia</i> sp.	Indonesia	I. Gandjar	
	CBS 451.92 = GJS 92-7 = ATCC 90145 = BPI 1113065	KF513185	KF513329	KF513254	KF513411	<i>Clusia</i> sp.	Puerto Rico	W.R. Buck	
	CBS 104.95	KF513186	KF513330	KF513255	KF513412	soil	Brazil	L. Pfenning	
	CBS 113467	KF513187	KF513331	KF513256	KF513413	soil	Thailand	M. Reblova	
	CPC 13577	KF513188	KF513332	KF513257	KF513414	<i>Nyssa sylvatica</i>	USA	T. Suttou	
CPC 21866 = MUCL 46552	DQ377829	KF513333	DQ374407	KF513415	leaf litter	French Guiana	C. Decock & V. Robert		
CPC 21867 = MUCL 46553	KF513189	KF513334	KF513258	KF513416	leaf litter	French Guiana	C. Decock & V. Robert		
CPC 23321	KF513190	KF513335	KF513259	KF513417	<i>Nephelium lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz		
CPC 23322	KF513191	KF513336	KF513260	KF513418	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz		
CPC 23323	KF513192	KF513337	KF513261	KF513419	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz		
CPC 23324	KF513193	KF513338	KF513262	KF513420	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz		
CPC 23325	KF513194	KF513339	KF513263	KF513421	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz		
CPC 23334	KF513195	KF513341	KF513264	KF513422	<i>N. lappaceum</i>	Mexico	L.M. Serrato-Diaz		
CPC 23335	KF513196	KF513342	KF513265	KF513423	<i>N. lappaceum</i>	Mexico	L.M. Serrato-Diaz		
CPC 23336	KF513197	KF513343	KF513266	KF513424	<i>N. lappaceum</i>	Mexico	L.M. Serrato-Diaz		
CPC 23337	KF513198	KF513344	KF513267	KF513425	<i>N. lappaceum</i>	Mexico	L.M. Serrato-Diaz		
CPC 23339	DQ377841	KF513344	KF513267	KF513426	<i>N. lappaceum</i>	Mexico	L.M. Serrato-Diaz		
CBS 902.70 = ATCC 22229 = IFO 9326 = IMI 155704 = MUCL 18576 = QM 9009	DQ377841	KF513353	DQ366705	KF513267	KF513426	soil	Thailand	C. Klinsukont	
CBS 903.70 = QM 9146	KF513208	KF513354	KF513277	KF513436	KF513436	soil	Thailand	S. Chomchalow	
CBS 904.70 = MUCL 18580 = QM 9147	DQ377842	KF513355	DQ366706	KF513437	KF513437	soil	Thailand	S. Chomchalow	
HMAS 98302	EU984072	-	EF 121859	HIM054075	HIM054075	leaf litter	China	W.Y. Zhuang & Y. Nong	
CBS 135945	KF513209	KF513356	KF513278	KF513438	KF513438	soil	Taiwan	P.W. Crous	
CBS 135946	KF513210	KF513357	KF513279	KF513439	KF513439	soil	Taiwan	P.W. Crous	
CPC 23340	KF513211	KF513358	KF513280	KF513440	KF513440	soil	Taiwan	P.W. Crous	
CPC 23344	KF513212	KF513359	KF513281	KF513441	KF513441	soil	Taiwan	P.W. Crous	
CPC 23345	KF513213	KF513360	KF513282	KF513442	KF513442	soil	Taiwan	P.W. Crous	
CPC 23347	KF513214	KF513361	KF513283	KF513443	KF513443	soil	Taiwan	P.W. Crous	
CBS 126571 = MUCL 46693	DQ377835	KF513367	DQ278422	KF513449	KF513449	leaf litter	French Guiana	C. Decock & V. Robert	
CBS 132043 = MUCL 46694	DQ377836	KF513368	DQ278421	KF513450	KF513450	leaf litter	French Guiana	C. Decock & V. Robert	
CBS 135947	KF513220	KF513369	KF513289	KF513451	KF513451	soil	Mexico	L.M. Serrato-Diaz	
CBS 135948	KF513221	KF513370	KF513290	KF513452	KF513452	<i>N. lappaceum</i>	Mexico	L.M. Serrato-Diaz	
CBS 345.64 = ATCC 22230 = IFO 9329 = IMI 155706 = MUCL 4085 = QM 9042	DQ374410	KF513371	DQ366699	DQ366699	KF513453	soil	Zaire	J.A. Meyer	
CPC 21862 = MUCL 8137	DQ374411	-	DQ366700	DQ366700	KF513454	-	Zaire	-	
CPC 21863 = MUCL 18349	DQ374412	-	DQ366701	DQ366701	KF513455	-	Zaire	-	
CBS 135949	KF513222	KF513372	KF513291	KF513456	KF513456	<i>N. lappaceum</i>	South Africa	L.M. Serrato-Diaz	
CBS 135950	KF513223	KF513373	KF513292	KF513457	KF513457	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CBS 567.73 = ATCC 24879 = IMI 176508 = MUCL 39340	DQ374415	-	DQ366707	DQ366707	KF513458	soil	USA	L.H. Huang	
CBS 114868 = CPC 4712	KF513224	KF513374	KF513293	KF513459	KF513459	<i>Eleoacarpus angustifolius</i>	Australia	I. Steer & B. Paulus	
CBS 135951	KF513225	KF513375	KF513294	KF513460	KF513460	<i>E. angustifolius</i>	Australia	I. Steer & B. Paulus	
CBS 135952	KF513226	KF513376	KF513295	KF513461	KF513461	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CBS 135953	KF513227	KF513377	KF513296	KF513462	KF513462	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CBS 135953	KF513228	KF513378	KF513297	KF513463	KF513463	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CBS 267.65 = ATCC 22231 = IFO 9330 = IMI 155705 = MUCL 18577 = QM 9041	DQ377838	KF513379	DQ 366702	DQ 366702	KF513464	soil	South Africa	H.J. Swart	

<i>G. simplex</i> (cont.)	CBS 983.69	KF513229	KF513380	KF513298	KF513465	soil	Brazil	C. Ram
CBS 511.81	KF513230	KF513381	KF513299	KF513466	<i>Musa</i> sp.	New Zealand	H.J. Boesewinkel	
CBS 249.91	KF513231	KF513382	KF513300	KF513467	root of unknown plant	Brazil	L. Pfening	
CPC 21865 = MUC1.46551 = SING 0061759	DO377837	KF513383	DO366704	KF513468	-	Singapore	C. Decock	
CPC 21868 = MUC1.46722	DO377840	KF513384	KF513301	KF513469	-	Malaysia	-	
CPC 23349	KF513232	KF513385	KF513302	KF513470	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CPC 23350	KF513233	KF513386	KF513303	KF513471	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CPC 23351	KF513234	KF513387	KF513304	KF513472	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CPC 23352	KF513235	KF513388	KF513305	KF513473	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CPC 23353	KF513236	KF513389	KF513306	KF513474	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CPC 23354	KF513237	KF513390	KF513307	KF513475	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	
CPC 23355	KF513238	KF513391	KF513308	KF513476	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz	
CPC 23356	KF513239	KF513392	KF513309	KF513477	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz	
CPC 23357	KF513240	KF513393	KF513310	KF513478	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz	
CPC 23358	KF513241	KF513394	KF513311	KF513479	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz	
CPC 23359	KF513242	KF513395	KF513312	KF513480	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz	
CPC 23360	KF513243	KF513396	KF513313	KF513481	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz	
CPC 23361	KF513244	KF513397	KF513314	KF513482	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz	
CPC 23362	KF513245	KF513398	KF513315	KF513483	<i>N. lappaceum</i>	Puerto Rico	L.M. Serrato-Diaz	
CBS 109446	KF513248	KF513402	KF513320	KF513489	<i>Miconia</i> sp.	Venezuela	I. Hernandez	
CBS 254.82	KF513249	KF513403	KF513321	KF513490	<i>Flacourtia</i> sp.	Indonesia	J.E. Willemstein-Sytema	
CBS 135954	KF513250	KF513404	KF513322	KF513491	<i>N. lappaceum</i>	Guatemala	L.M. Serrato-Diaz	

*Gliocephalotrichum* sp. 1  
*Gliocephalotrichum* sp. 2  
*Gliocephalotrichum* sp. 3

<sup>1</sup> ATCC: American Type Culture Collection, Virginia, USA; BPI: U.S. National Fungus Collections, USDA, ARS, Beltsville, Maryland, USA; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: working collection of Pedro Crous housed at CBS; HMAS: Key Laboratory of Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, Chinese Academy of Science, Beijing 100101, P.R. China; IFO: Institute for Fermentation, 17-85, Juso-honmachi, 2-chome, Yodogawa-ku, Osaka 532, Japan; IMI: International Mycological Institute, CAB International, Egham, Basingstoke, UK; GJS: Gary J. Samuels personal collection; MUC1: Mycothèque, Laboratoire de Mycologie Systématique et Appliquée, Université, Louvain-la-Neuve, Belgium; NRR1: Agricultural Research Service, USDA, Washington, USA; QM: Quaternary Culture Collection, Material Protection and Biotechnology Division, Science and Advanced Technology Directorate, U.S. Army Natick Research and Development Center, Natick, Massachusetts 01760-5020, USA; SING: Parks and Recreation Department, Botanical Gardens, Cluny Road, Singapore 1025.

<sup>2</sup> BTUB =  $\beta$ -tubulin, HIS3 = histone H3, ITS = internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal DNA, TEF = translation elongation factor 1- $\alpha$ .

\* Ex-type isolates indicated in bold.

lated over the years in the culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, which were formerly identified based on morphology only. The aim of the present study was to characterise all these diverse isolates using a polyphasic approach incorporating morphology, culture characteristics and multigene DNA phylogenetic data.

## MATERIALS AND METHODS

### Isolates

Isolates were obtained from rambutan fruit, originating from Guatemala, Mexico and Puerto Rico, displaying symptoms of fruit rot as described by Serrato-Diaz et al. (2012). Soils, collected in Thailand, were baited as described by Crous (2002) and indicated in Table 1. Representative strains are maintained in the culture collections of the CBS-KNAW Fungal Biodiversity Centre (CBS), Mycothèque de l'Université catholique de Louvain (BCCM/MUCL) and the working collection of Pedro Crous (CPC) housed at CBS.

### Phylogeny

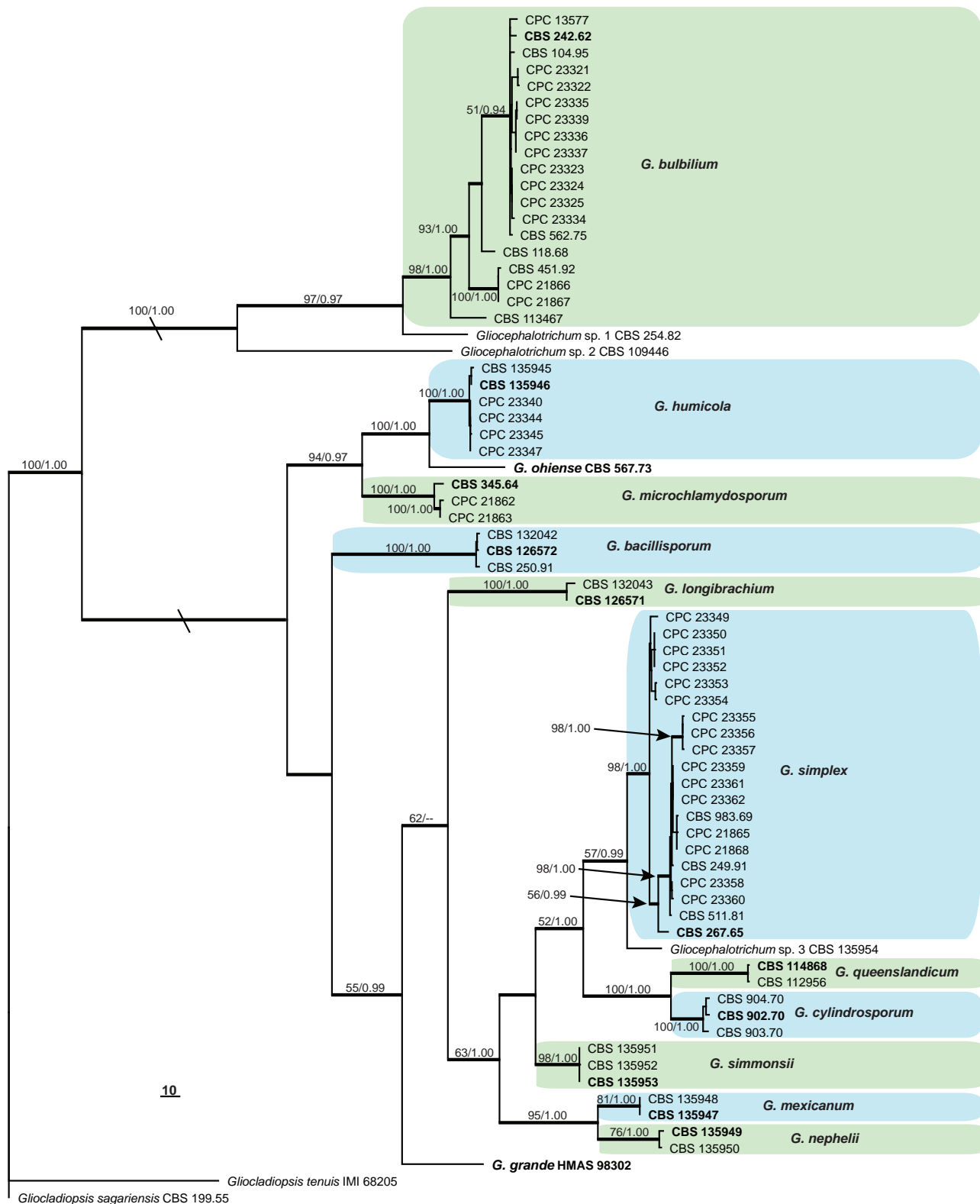
Total genomic DNA was extracted from cultures grown on 2 % malt extract agar (MEA) for 7 d, using the UltraClean™ Microbial DNA isolation kit (Mo Bio Laboratories, Inc., California, USA) according to the manufacturer's protocol. Partial gene sequences were determined for  $\beta$ -tubulin (BTUB), histone H3 (HIS3), the internal transcribed spacer region (ITS) of the nuclear rDNA and translation elongation factor 1- $\alpha$  (TEF) using the primers and protocols described previously (Lombard et al. 2010a, Lombard & Crous 2012). 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 four partitions could be combined. Phylogenetic analyses were based on both Bayesian inference (BI) and Maximum Parsimony (MP). For BI, 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 consistency index (RC) were calculated for parsimony and bootstrap analysis (Hillis & Bull 1993) was based on 1 000 replications.

### Taxonomy

Morphological characterisation of the *Gliocephalotrichum* isolates was done using single conidial cultures prepared on MEA and synthetic nutrient-poor agar (SNA; Nirenburg 1981) amended with sterile carnation leaves, maintained at room temperature. Gross morphological characters were examined after 7 d by mounting fungal structures in clear lactic acid and 30 measurements were made at  $\times$  1 000 magnification using



**Fig. 1** One of 1 000 most parsimonious trees obtained from a heuristic search with 1 000 random addition sequences of the combined sequences of  $\beta$ -tubulin, histone H3, internal transcribed spacer region and translation elongation factor 1-alpha sequence alignments of the *Gliosphaerium* isolates used in this study. Scale bar shows 10 changes. Bootstrap support values 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 *Gliocladiopsis tenuis* (IMI 68205) and *Gliocladiopsis sagariensis* (CBS 199.55). Ex-type isolates are indicated in **bold**.

a Zeiss Axioscope 2 microscope with differential interference contrast (DIC) illumination. The 95 % confidence levels were determined for the conidial measurements and extremes given in parentheses and extremes provided for other structures. Colony characters were noted after 7 d of growth on MEA at 24 °C and 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 around 500–550 bp were determined for the four genes used in this study. The phylogenetic analyses included 70 ingroup taxa, with *Gliocladiopsis sagariensis* (CBS 199.55) and *G. tenuis* (IMI 68205) as outgroup taxa (Lombard & Crous 2012). No topological conflicts were found between the four

partitions based on the reciprocal 70 % BS threshold and therefore the sequence datasets were combined. The combined sequence dataset consisted of 2 491 characters, including alignment gaps. Of these, 1 427 were constant, 159 parsimony-uninformative and 905 parsimony-informative. The MP analysis yielded 1 000 trees (TL = 2716; CI = 0.640; RI = 0.921; RC = 0.590), of which the first is presented (Fig. 1). For the Bayesian inference, a HKY+I+G model was selected for BTUB and TEF, GTR+I+G for HIS3, and SYM+I+G for ITS which was incorporated into the analyses. The Bayesian consensus tree confirmed the tree topology and bootstrap support of the strict consensus tree obtained with MP.

In the phylogenetic tree (Fig. 1), the isolates of *Gliocephalotrichum* included in this study divided into two main clades, one of which was well-supported. The first clade (BS = 100; PP = 1.00) contains *G. bulbilium* (ex-type strain CBS 242.62) and included some of the isolates obtained from rambutan fruits originating from Puerto Rico (CPC 23321–23333) and Mexico (CPC 23334–23339). Isolates CBS 254.82 and CBS 109446 formed basal, sister lineages to the clade representing *G. bulbilium*. The second clade (BS < 50; PP < 0.95) includes the remaining well-established *Gliocephalotrichum* spp. and several unique phylogenetic species. Isolates baited from soils collected in Thailand (CBS 135945, CBS 135946 and CPC 23340–23347), formed a unique terminal clade (BS = 100; PP = 1.00), closely related but separate from the ex-type strain of *G. ohense* (CBS 567.73). Several isolates from the rambutan fruits collected in Guatemala (CPC 23350–23354) and Puerto Rico (CPC 23355–23362), clustered in the clade (BS = 98;

PP = 1.00) representing *G. simplex* (ex-type strain CBS 267.75), with a single isolate (CBS 135954) from Guatemala, forming a basal sister lineage to this clade. The remaining strains isolated from rambutan fruits originating from Guatemala (CBS 135949, CBS 135950 and CBS 135951–135953, respectively) and Mexico (CBS 135947 and CBS 135948), clustered in three separate well-supported clades, each representing a possible new species. Two isolates from Australia (CBS 112956 and CBS 114868) also formed a unique clade (BS = 100; PP = 1.00), closely related but separate from the clade (BS = 100; PP = 1.00) representing *G. cylindrosporum* (ex-type strain CBS 902.70).

### Taxonomy

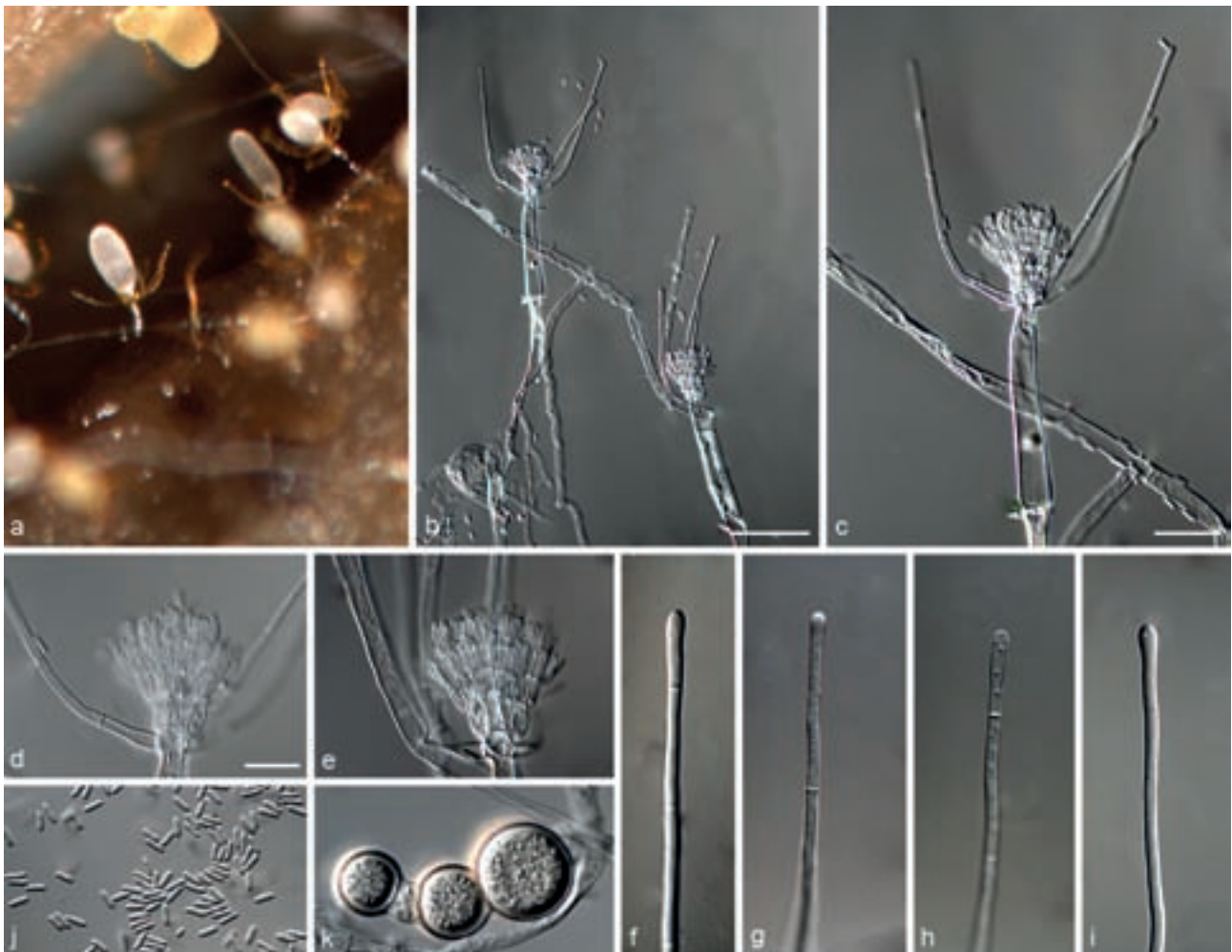
Phylogenetic inference and morphological observations indicate that several strains included in this study represent novel species. Following the proposal by Rossman et al. (2013) these taxa are placed in the genus *Gliocephalotrichum*.

***Gliocephalotrichum* J.J. Ellis & Hesselt., Bull. Torrey Bot. Club 89: 21. 1962.**

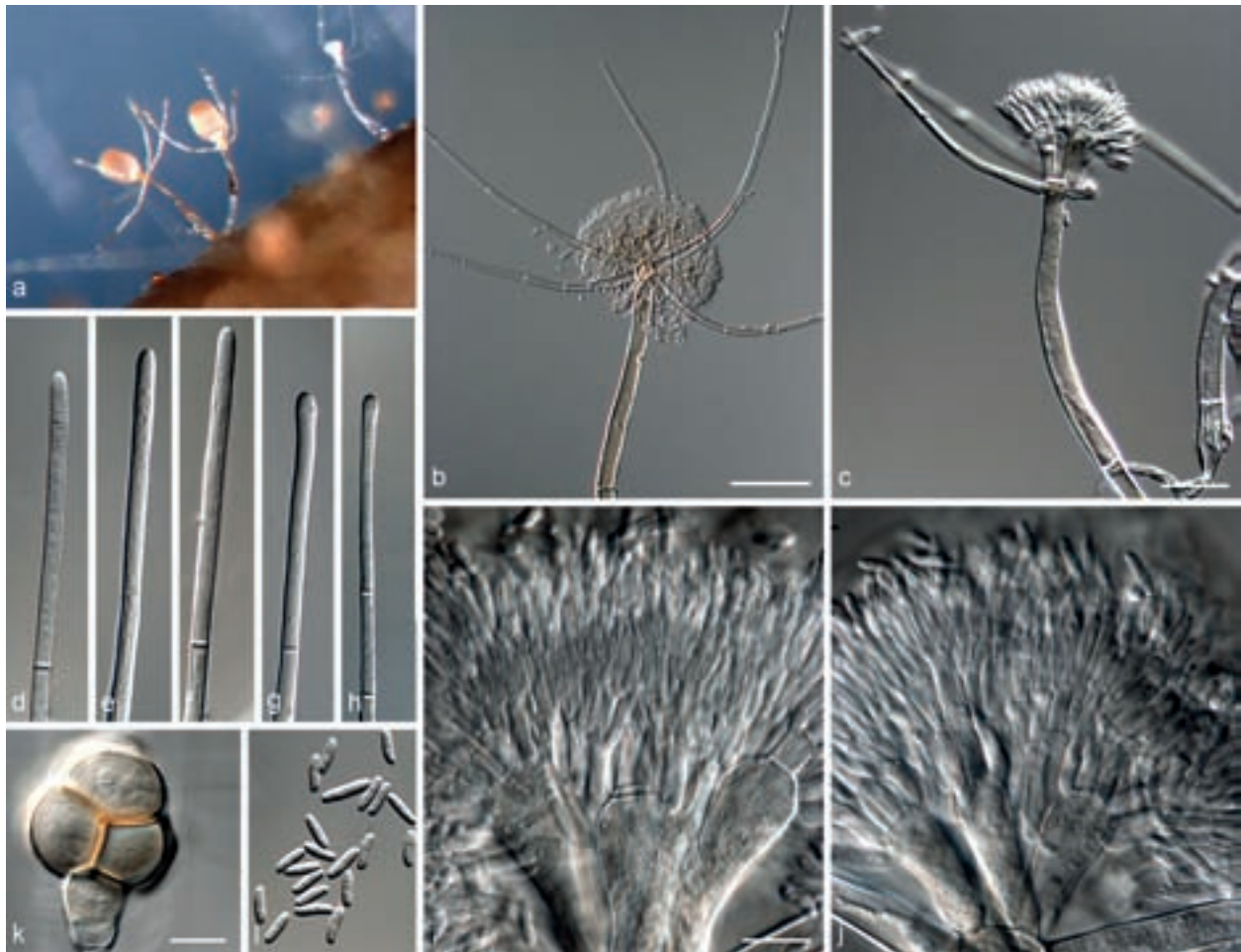
= *Leuconectria* Rossman, Samuels & Lowen, Mycologia 85: 686. 1993.

*Type species. Gliocephalotrichum bulbilium* J.J. Ellis & Hesselt., Bull. Torrey Bot. Club 89: 22. 1962.

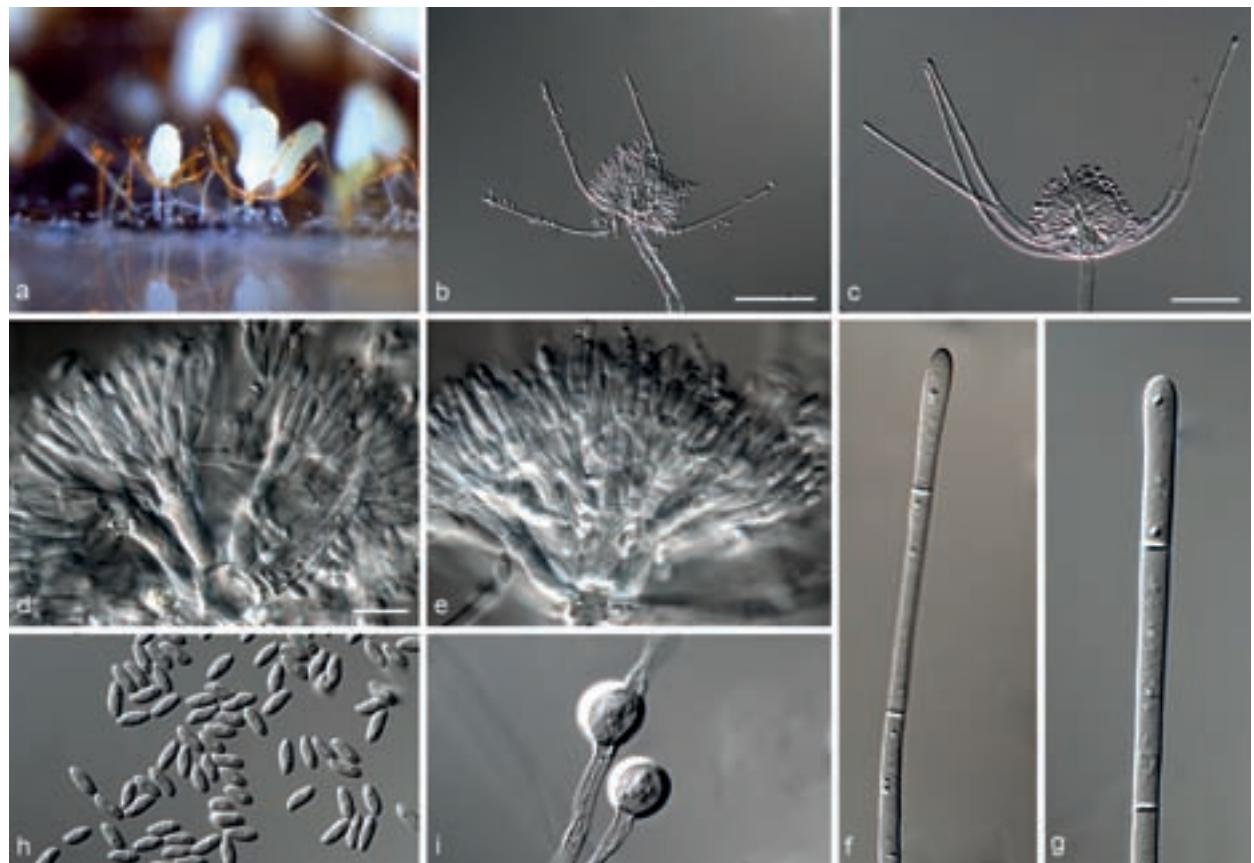
*Perithecia* superficial, solitary, globose to subglobose; perithecial wall scarlet, turning purple in 3 % KOH+, with a white to pale luteous amorphous coating and hyphal stromatic base,



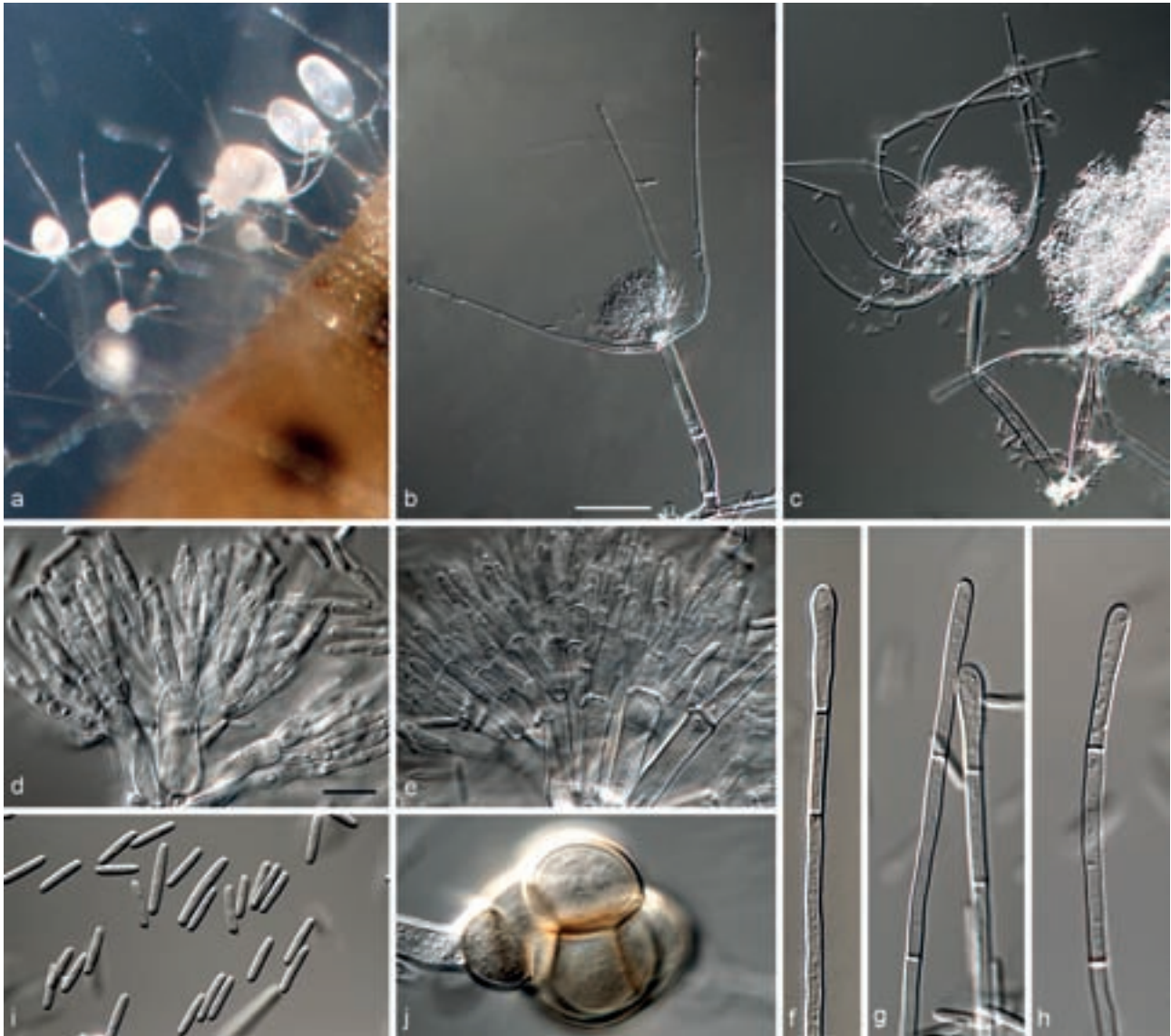
**Fig. 2** *Gliocephalotrichum bacillisporum* (CBS 126572, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–i. apex of stipe extensions; j. conidia; k. chlamydospores formed in chains. — Scale bars: b = 50  $\mu$ m; c = 20  $\mu$ m; d = 10  $\mu$ m (applies to e–k).



**Fig. 3** *Glioscephalotrichum bulbilium* (CBS 242.62, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d–h. apex of stipe extensions; i, j. penicillus; k. bulbiloid aggregate of chlamydospores; l. conidia. — Scale bars: b = 50  $\mu$ m; c = 20  $\mu$ m; i = 10  $\mu$ m (applies to d–j); k = 10  $\mu$ m (applies to l).



**Fig. 4** *Glioscephalotrichum humicola* (CBS 135946, ex-type). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f, g. apex of stipe extensions; h. conidia; i. chlamydospores. — Scale bars: b = 50  $\mu$ m; c = 20  $\mu$ m; d = 10  $\mu$ m (applies to e–i).



**Fig. 5** *Gliocephalotrichum longibrachium* (CBS 126571, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. bulbiloid aggregate of chlamydospores. — Scale bars: b = 50 µm (applies to c); d = 10 µm (applies to e–j).

not collapsing when dry, consisting of two layers: outer region of thick-walled cells of *textura angularis*, inner layer of elliptic to elongate cells. *Asci* unitunicate, 8-spored, narrowly clavate, with flattened apex and a minute refractive apical apparatus. *Ascospores* biserial in the upper part of the ascus, hyaline, ellipsoidal, smooth, aseptate. *Conidiophores* consisting of a septate, hyaline, pale luteous to pale brown stipe and a penicillate arrangement of fertile branches subtended by septate stipe extensions. *Conidiogenous apparatus* with a series of aseptate branches, each terminating in 2–8 phialides; *phialides* clavate to cylindrical, hyaline, aseptate, constricted at the apex, with minute periclinal thickening. *Conidia* cylindrical to ellipsoid, straight to slightly curved, aseptate, accumulating in a white to luteous mucoid mass above the phialides.

***Gliocephalotrichum bacillisporum*** Decock & Huret, *Mycologia* 98: 493. — MycoBank MB501190; Fig. 2

Description and illustration: See Decock et al. (2006).

*Specimens examined.* BRAZIL, Pará, Belem, near Capitão Poço, root of unknown plant, May 1991, *L. Pfennig*, CBS 250.91 = L.P. 504. — FRENCH GUIANA, Cayenne area, Matouri, Sentier d'Interprétation de la Nature 'Lamirande', from dead, decaying leaf of unknown angiosperm in leaf litter, Feb. 1994, *C. Decock & V. Robert*, holotype MUCL 46554, culture ex-type MUCL 46554 = FG 1215 = CBS 126572, MUCL 46732 = FG 2157 = CBS 132042.

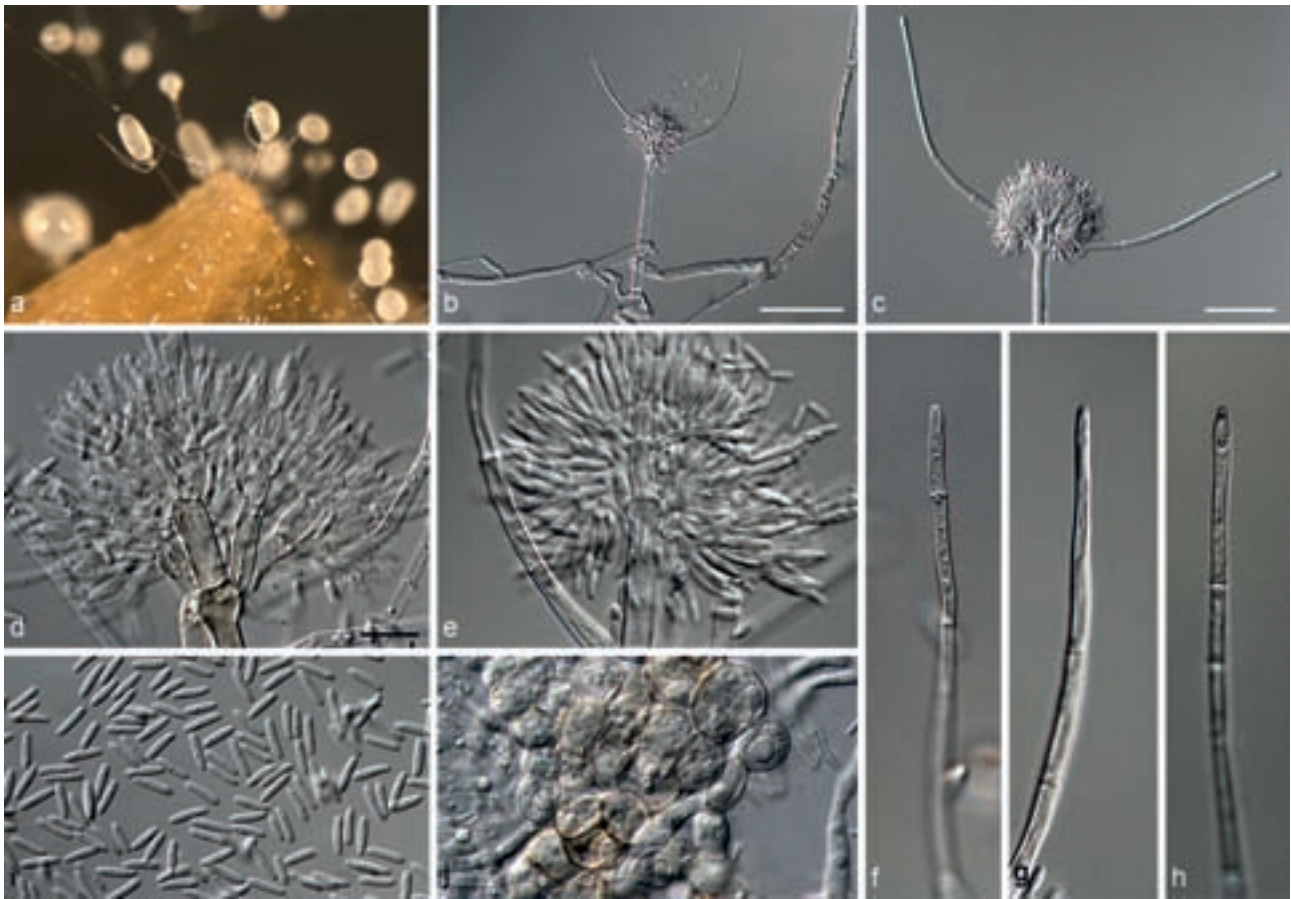
***Gliocephalotrichum bulbilium*** J.J. Ellis & Hesselt., *Bull. Torrey Bot. Club* 89: 21. 1962. — MycoBank MB331344; Fig. 3

= *Leuconectria clusiae* (Samuels & Rogerson) Rossman, Samuels & Lowen, *Mycologia* 85: 686. 1993.

= *Pseudonectria clusiae* Samuels & Rogerson, *Mem. New York Bot. Gard.* 64: 173. 1990.

Description and illustration: See Ellis & Hesseltine (1962).

*Specimens examined.* BRAZIL, from soil, Jan. 1995, *L. Pfennig*, CCT 4267 = CBS 104.95. — CENTRAL AFRICAN REPUBLIC, La Maboké, from air sample, Feb. 1968, *J. Nicot*, CBS 118.68. — FRENCH GUIANA, Cayenne area, Sentier d'Interprétation de la Nature 'Lamirande', from dead, decaying leaf of unknown angiosperm in leaf litter, Feb. 1994, *C. Decock & V. Robert*, MUCL 46552 = CPC 21866, MUCL 46553 = CPC 21867. — INDONESIA, Java, Jakarta, from fruit of *Flacourtia* sp., Nov. 1975, *I. Gandjar*, CBS 562.75. — MEXICO, from rotten fruit of *Nephelium lappaceum*, 13 Sept. 2011, *L.M. Serrato-Diaz*, CPC 23334–23339. — PUERTO RICO, Bosque Estatal de Guajataca, N18°24', W66°58', on decaying fruit of *Clusia* sp., 17 Jan. 1992, *W.R. Buck*, specimen BPI 1113065, culture ATCC 90145 = GJS92-7 = CBS 451.92; Mayaquez, USDA-ARS Tropical Agriculture Research Station, from rotten fruit of *N. lappaceum*, 2 Feb. 2011, *L.M. Serrato-Diaz*, CPC 23321–23333. — USA, Louisiana, Tunica Hills, from a soil sample collected under moss, 24 Aug. 1960, *L.J. Wickerham*, holotype BPI 414619, culture ex-type NRRL 2899 = ATCC 22228 = IFO 9325 = IMI 096357 = MUCL 18575 = BPI 414619 = QM 9007 = CBS 242.62; North Carolina, Johnston County, from fruit of *Nyssa sylvatica*, 15 Sept. 2006, *T. Sutton*, culture CPC 13577.



**Fig. 6** *Glioscephalotrichum mexicanum* (CBS 135947, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. bulbilloid aggregate of chlamydospores. — Scale bars: b = 50  $\mu$ m; c = 20  $\mu$ m; d = 10  $\mu$ m (applies to e–j).

***Glioscephalotrichum cylindrosporum*** B.J. Wiley & E.G. Simmons, *Mycologia* 63: 582. 1971. — MycoBank MB314499

Description and illustration: See Wiley & Simmons (1971).

*Specimens examined.* THAILAND, Pak Thong Chai area, from forest soil, Dec. 1967, C. Klinasukont, culture ex-type QM 9009 = ATCC 22229 = IFO 9326 = IMI 155704 = MUCL 18576 = CBS 902.70; from forest soil, 1968, S. Chomchalow, QM 9146 = CBS 903.70; from root of tree, 1968, S. Chomchalow, QM 9147 = MUCL 18580 = CBS 904.70.

*Notes* — All three isolates representing *G. cylindrosporum* are sterile.

***Glioscephalotrichum grande*** (Y. Nong & W.Y. Zhuang) Rossman & L. Lombard, *IMA Fungus* 4: 47. 2013. — MycoBank MB802537

*Basionym.* *Leuconectria grandis* Y. Nong & W.Y. Zhuang, *Fung. Diversity* 24: 349. 2007.

Description and illustration: See Zhuang et al. (2007).

***Glioscephalotrichum humicola*** L. Lombard, Cheew. & Crous, *sp. nov.* — MycoBank MB805189; Fig. 4

*Etymology.* Name refers to the fact that this fungus was isolated from soil.

*Conidiophores* formed abundantly, scattered, solitary, erect, hyaline, arising from submerged hyphae, consisting of a stipe and stipe extensions subtending a penicillate conidiogenous apparatus; stipe septate, hyaline, pale luteous to luteous, smooth, 60–136  $\times$  9–16  $\mu$ m; stipe extensions 2–5, directly subtending penicillus at right angles, progressively bending upwards, hyaline to pale luteous, septate, 88–199  $\mu$ m long, 5–10  $\mu$ m wide at the base, terminating in clavate to broadly clavate vesicle. *Conidiogenous apparatus* densely penicil-

late, consisting of a whorl of fertile branches, 38–96  $\mu$ m long, 50–127  $\mu$ m wide; primary branches aseptate, 13–21  $\times$  4–8  $\mu$ m; secondary branches aseptate, 8–13  $\times$  2–5  $\mu$ m; tertiary and additional branches (–4) aseptate, 6–10  $\times$  2–4  $\mu$ m, each terminal branch producing 4–8 phialides; phialides cylindrical, slightly ventricose, hyaline, aseptate, 8–12  $\times$  1–3  $\mu$ m; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* hyaline, smooth, ellipsoid, bevelled at one or both ends, 6.5–7.5(–9)  $\times$  (2–)2.5–3.5(–4)  $\mu$ m (av. 7  $\times$  3  $\mu$ m), forming a mucoid droplet at apex of penicillus, turning pale luteous to luteous within 7 days. *Chlamydospores* formed singly, intercalary or terminally, globose to subglobose, hyaline, 8–17  $\mu$ m diam, not forming bulbilloid aggregates on MEA and SNA. *Sexual morph* not observed.

*Culture characteristics* — Colonies fast growing (90 mm in 5 d), pale luteous to luteous with reverse pale luteous to sienna; no aerial mycelium formed, but abundant conidiophores covering the whole surface.

*Specimens examined.* TAIWAN, Taichung, Daikin walking trail, N24° 13' 35.2" E120° 58' 18.7", from soil, Oct. 2012, coll. P.W. Crous, isol. L. Lombard, (holotype CBS H-21385) culture ex-type CBS 135946; CBS 135945; CPC 23340–23348.

*Notes* — *Glioscephalotrichum humicola* is morphologically similar to *G. ohiiense* but can be distinguished by the quaternary branches on the penicillus, which is not reported for *G. ohiiense* (Huang & Schmitt 1973). Furthermore, Huang & Schmitt (1973) indicated that the conidiophores are pale brown to brown, whereas those of *G. humicola* are pale luteous to luteous.

***Glioscephalotrichum longibrachium*** Decock & Charue, *Mycologia* 98: 489. 2006. — MycoBank MB501189; Fig. 5

Description and illustration: See Decock et al. (2006).





**Fig. 7** *Gliocephalotrichum microchlamydosporum* (CBS 345.64, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. chlamydospore. — Scale bars: b = 50  $\mu\text{m}$  (applies to c); d = 10  $\mu\text{m}$  (applies to e–j).

*Specimens examined.* FRENCH GUIANA, Cayenne area, Matouri, Sentier d'Interprétation de la Nature 'Lamirande', from dead, decaying leaf of unknown angiosperm in leaf litter, Feb. 1994, C. Decock & V. Robert, holotype MUCL 46693, culture ex-type MUCL 46693 = FG 1143 = CBS 126571; MUCL 46694 = FG 1149 = CBS 132043.

***Gliocephalotrichum mexicanum*** L. Lombard, L.M. Serrato-Díaz, R.D. French-Monar & Crous, *sp. nov.* — MycoBank MB805190; Fig. 6

*Etymology.* Name refers to Mexico, the country from where the fruit was imported into the USA.

*Conidiophores* formed abundantly, scattered, solitary, erect, hyaline, arising from submerged hyphae, consisting of a stipe and stipe extensions subtending a penicillate conidiogenous apparatus; stipe septate, hyaline, smooth, 81–158  $\times$  4–15  $\mu\text{m}$ ; stipe extensions 2–6, directly subtending penicillus at right angles progressively bending upwards, hyaline, septate, 82–176  $\mu\text{m}$  long, 4–8  $\mu\text{m}$  wide at the base, terminating in narrowly clavate to clavate vesicle. *Conidiogenous apparatus* densely penicillate, consisting of a whorl of fertile branches, 43–135  $\mu\text{m}$  long, 39–60  $\mu\text{m}$  wide; primary branches aseptate, 12–20  $\times$  3–6  $\mu\text{m}$ ; secondary branches aseptate, 8–11  $\times$  2–5  $\mu\text{m}$ ; tertiary branches aseptate, 6–9  $\times$  2–5  $\mu\text{m}$ , each terminal branch producing 4–6 phialides; phialides cylindrical, slightly

ventricose, hyaline, aseptate, 6–10  $\times$  1–3  $\mu\text{m}$ ; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* hyaline, smooth, cylindrical, slightly bevelled, rounded at apex, 6–8(–9)  $\times$  (1–)2(–3)  $\mu\text{m}$  (av. 7  $\times$  2  $\mu\text{m}$ ), forming a white mucoid droplet at apex of penicillus. *Chlamydospores* form abundant brown to dark brown, immersed bulbiloid aggregates, 55–210  $\times$  50–108  $\mu\text{m}$ , made of globose to ellipsoid cells; solitary chlamydospores absent. *Sexual morph* not observed.

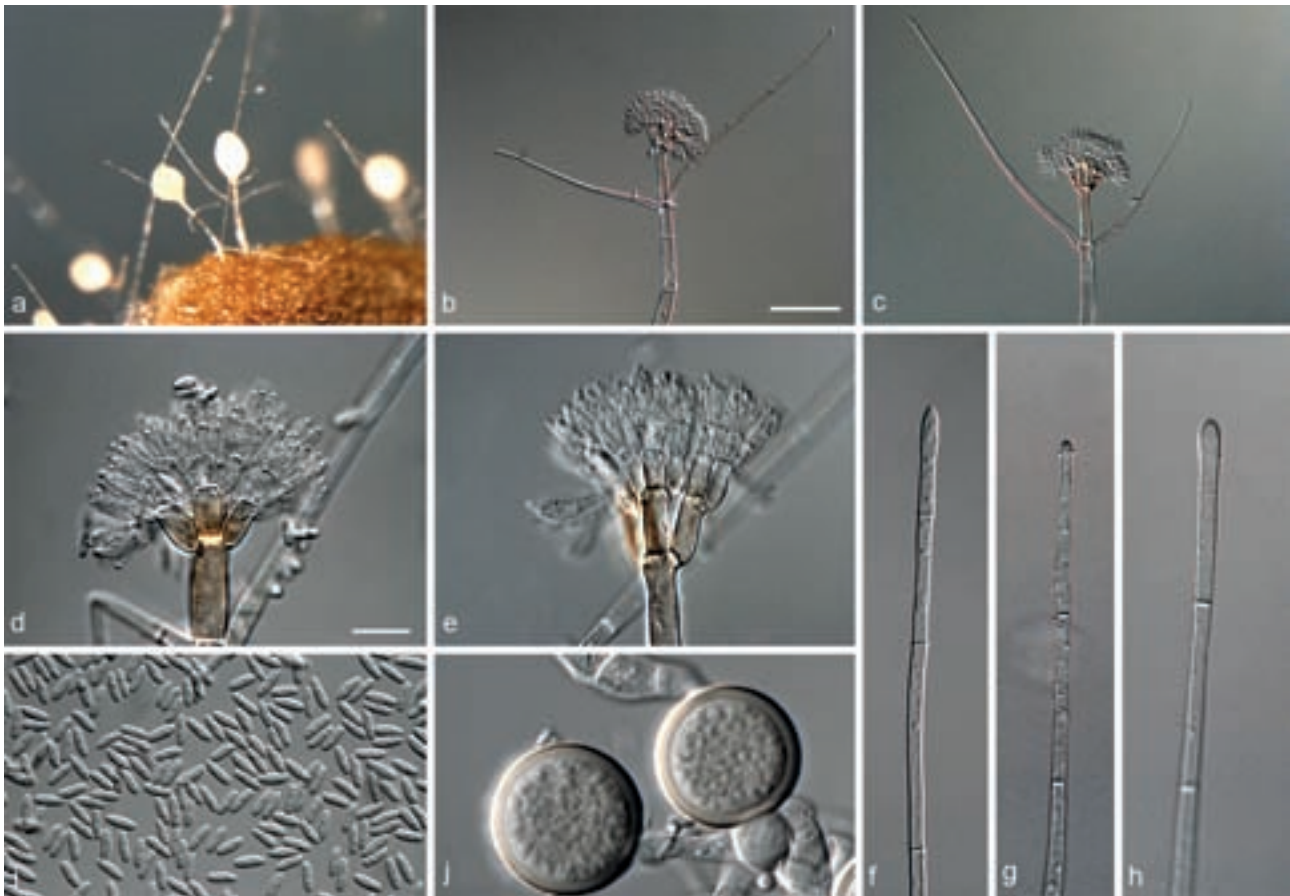
*Culture characteristics* — Colonies fast growing (90 mm in 5 d), white to sienna with reverse sienna to umber; aerial mycelium sparse, with abundant conidiophores forming on immersed mycelium.

*Specimens examined.* MEXICO, from fruit of *Nephelium lappaceum* imported into the USA, 9 July 2011, L.M. Serrato-Díaz, (holotype CBS H-21386) culture ex-type CBS 135947; CBS 135948.

*Notes* — *Gliocephalotrichum mexicanum* formed a unique phylogenetic lineage, sister to *G. nephelii* (see below), which was well-supported by both BI and MP analyses.

***Gliocephalotrichum microchlamydosporum*** (J.A. Mey.) B.J. Wiley & E.G. Simmons, *Mycologia* 63: 580. 1971. — MycoBank MB314500; Fig. 7

*Basionym.* *Cylindrocladium simplex* var. *microchlamydosporum* J. Mey., *Publ. Inst. Natl. Etude Agron. Congo Belge* 75: 148. 1959.



**Fig. 8** *Gliosphaerotrichum nephelii* (CBS 135949, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. chlamydospores. — Scale bars: b = 50  $\mu\text{m}$  (applies to c); d = 10  $\mu\text{m}$  (applies to e–j).

Description and illustration: See Wiley & Simmons (1971).

*Specimens examined.* USA, Illinois, Peoria, MUCL 18349 = NRRL 5212. — ZAIRE, Yangambi, from soil, Mar. 1960, J.A. Meyer, culture ex-type ATCC 22230 = IFO 9329 = IMI 155706 = MUCL 4085 = QM 9042 = CBS 345.64; MUCL 8137.

***Gliosphaerotrichum nephelii*** L. Lombard, L.M. Serrato-Diaz, R.D. French-Monar & Crous, *sp. nov.* — MycoBank MB805191; Fig. 8

*Etymology.* Name refers to *Nephelium lappaceum*, from which the fungus was isolated.

*Conidiophores* formed abundantly, scattered, solitary, erect, hyaline, arising from submerged hyphae, consisting of a stipe and stipe extensions subtending a penicillate conidiogenous apparatus; stipe septate, hyaline, smooth, 98–143  $\times$  9–14  $\mu\text{m}$ ; stipe extensions 2–6, directly subtending penicillus at right angles progressively bending upwards, hyaline, septate, 82–308  $\mu\text{m}$  long, 4–6  $\mu\text{m}$  wide at the base, terminating in narrowly clavate to clavate vesicle. *Conidiogenous apparatus* densely penicillate, consisting of a whorl of fertile branches, 38–75  $\mu\text{m}$  long, 37–45  $\mu\text{m}$  wide; primary branches aseptate, 11–18  $\times$  3–6  $\mu\text{m}$ ; secondary branches aseptate, 6–10  $\times$  2–4  $\mu\text{m}$ ; tertiary and additional (–4) branches aseptate, 5–9  $\times$  1–4  $\mu\text{m}$ , each terminal branch producing 4–6 phialides; phialides cylindrical, slightly ventricose, hyaline, aseptate, 6–9  $\times$  1–4  $\mu\text{m}$ ; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* hyaline, smooth, cylindrical to ellipsoid, slightly bevelled, rounded at apex, (6–)6.5–7.5(–8)  $\times$  (1–)2(–3)  $\mu\text{m}$  (av. 7  $\times$  2  $\mu\text{m}$ ), forming a white mucoid droplet at apex of penicillus. *Chlamydospores* formed singly or in chains, intercalary or terminally, globose to subglobose, hyaline turning brown with age, 19–33  $\mu\text{m}$  diam, not forming bulbiloid aggregates on MEA and SNA. *Sexual morph* not observed.

*Culture characteristics* — Colonies fast growing (90 mm in 5 d), white with reverse sienna to umber; aerial mycelium sparse, with abundant conidiophores forming on immersed mycelium.

*Specimen examined.* GUATEMALA, from fruit of *Nephelium lappaceum* imported into the USA, 3 Sept. 2011, L.M. Serrato-Diaz, (holotype CBS H-21387) culture ex-type CBS 135949; CBS 135950.

*Notes* — *Gliosphaerotrichum nephelii* forms a sister lineage to *G. mexicanum* and can be morphologically distinguished by its large chlamydospores which develop singly or in chains, and do not form bulbiloid aggregates. This was not observed for *G. mexicanum*, which in turn, only formed bulbiloid aggregates of chlamydospores and no solitary chlamydospores.

***Gliosphaerotrichum ohienne*** L.H. Huang & J.A. Schmitt, *Mycologia* 65: 949. 1973. — MycoBank MB314501

Description and illustration: See Huang & Schmitt (1973).

*Specimen examined.* USA, Ohio, Belmont County, Dysart Woods, from soil, Aug. 1972, L.H. Huang, culture ex-type ATCC 24879 = IMI 176508 = MUCL 39340 = CBS 567.73.

*Notes* — This isolate of *G. ohienne* is sterile.

***Gliosphaerotrichum queenslandicum*** L. Lombard & Crous, *sp. nov.* — MycoBank MB805192; Fig. 9

*Etymology.* Name refers to Queensland, Australia, where this fungus was collected.

*Conidiophores* formed abundantly, scattered, solitary, erect, hyaline, arising from submerged hyphae, consisting of a stipe and stipe extensions subtending a penicillate conidiogenous apparatus; stipe septate, hyaline, smooth, 54–184  $\times$  6–16  $\mu\text{m}$ ; stipe extensions 2–6, directly subtending penicillus at right



**Fig. 9** *Gliocephalotrichum queenslandicum* (CBS 114868, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d. conidia; e, f. penicillus; g–i. apex of stipe extensions; j. chlamydozoospores; k. bulbilloid aggregate of chlamydozoospores. — Scale bars: b = 20  $\mu\text{m}$ ; c = 50  $\mu\text{m}$ ; d = 10  $\mu\text{m}$  (applies to e–k).

angles progressively bending upwards, with a single stipe extension 14–28  $\mu\text{m}$  below the penicillus, hyaline, septate, 45–314  $\mu\text{m}$  long, 4–8  $\mu\text{m}$  wide at the base, terminating in narrowly clavate to clavate vesicle. *Conidiogenous apparatus* densely penicillate, consisting of a whorl of fertile branches, 33–73  $\mu\text{m}$  long, 37–88  $\mu\text{m}$  wide; primary branches aseptate, 17–36  $\times$  5–11  $\mu\text{m}$ ; secondary branches aseptate, 7–15  $\times$  2–6  $\mu\text{m}$ ; tertiary and additional (–4) branches aseptate, 5–11  $\times$  2–6  $\mu\text{m}$ , each terminal branch producing 4–6 phialides; phialides cylindrical, slightly ventricose, hyaline, aseptate, 6–10  $\times$  2–3  $\mu\text{m}$ ; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* hyaline, smooth, cylindrical, slightly bevelled, rounded at apex, 8–9(–11)  $\times$  (1–)2  $\mu\text{m}$  (av. 8  $\times$  2  $\mu\text{m}$ ), forming a white to pale luteous mucoid droplet at apex of penicillus. *Chlamydozoospores* formed singly or in chains, intercalary or terminally, globose to subglobose, hyaline turning brown with age, 43–53  $\mu\text{m}$  diam, forming brown to dark brown, immersed bulbilloid aggregates, 131–153  $\times$  90–101  $\mu\text{m}$ , consisting of globose to ellipsoid cells on MEA and SNA. *Sexual morph* not observed.

**Culture characteristics** — Colonies fast growing (90 mm in 5 d), white to pale luteous with reverse sienna; aerial mycelium sparse, with abundant conidiophores forming on immersed mycelium.

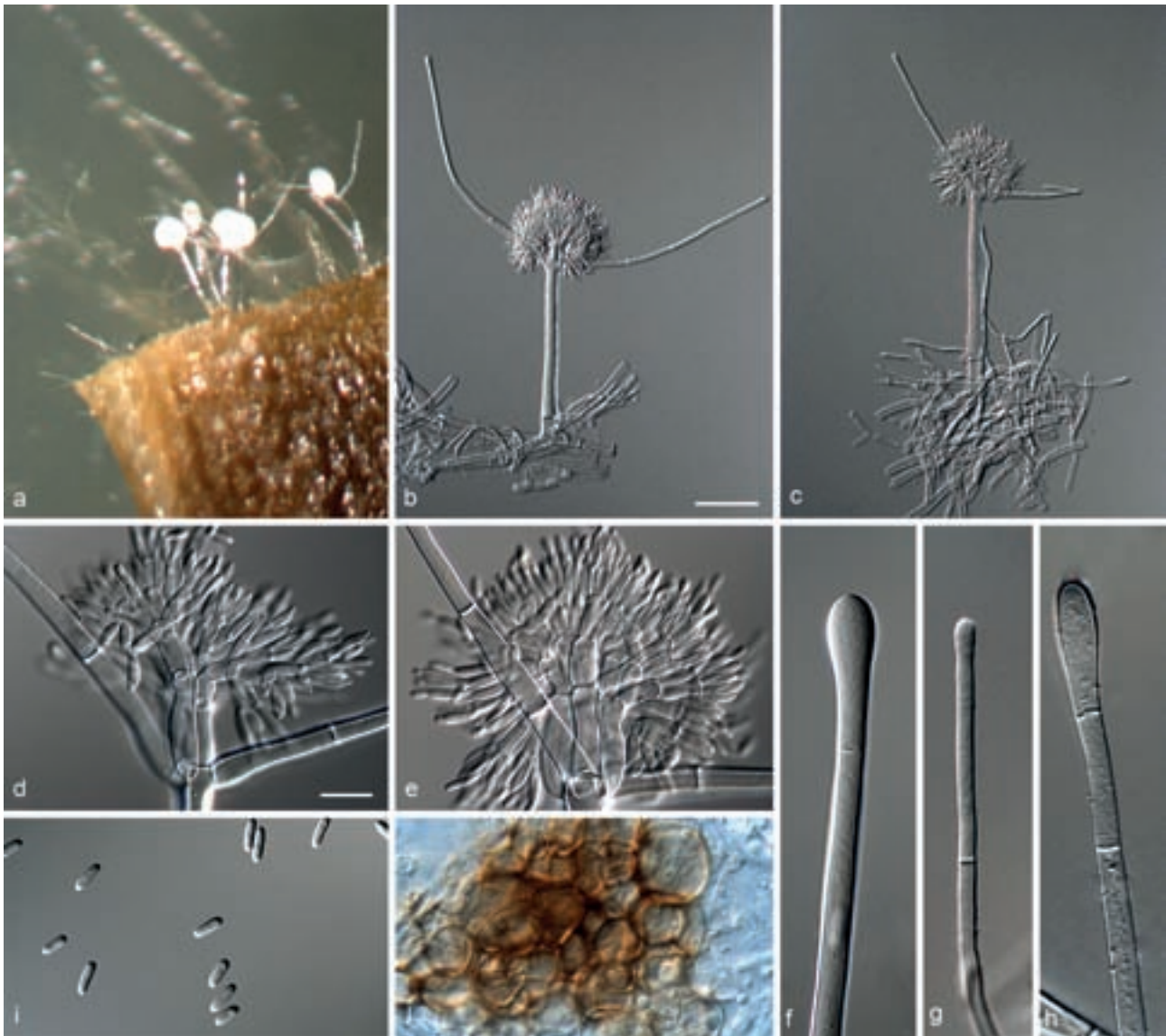
**Specimens examined.** AUSTRALIA, Queensland, Atherton Tablelands, Millaa Millaa, from roots of *Eleaocarpus angustifolius*, 27 Feb. 2001, I. Steer & B. Paulus, (holotype CBS H-21384) culture ex-type CBS 114868 = B. Paulus # 3096 = CPC 4712; Topaz, from roots of *Eleaocarpus angustifolius*, 27 Feb. 2001, I. Steer & B. Paulus, CBS 112956 = B. Paulus # 3223 = CPC 4713.

**Notes** — *Gliocephalotrichum queenslandicum* is closely related to *G. cylindrosporum* but can be distinguished based on their conidial morphology. Conidia of *G. queenslandicum* (8–9(–11)  $\times$  (1–)2  $\mu\text{m}$  (av. 8  $\times$  2  $\mu\text{m}$ )) are slightly smaller than those of *G. cylindrosporum* (9.1–13  $\mu\text{m}$ ; Wiley & Simmons 1971). Furthermore, *G. queenslandicum* produces a single stipe extension some distance below the penicillus, with the remaining stipe extensions positioned directly beneath the penicillus, a characteristic not reported for *G. cylindrosporum* (Wiley & Simmons 1971).

***Gliocephalotrichum simmonsii*** L. Lombard, L.M. Serrato-Diaz, R.D. French-Monar & Crous, *sp. nov.* — MycoBank MB805193; Fig. 10

**Etymology.** This species is named in honour of Dr Emory G. Simmons (deceased), recognising his contribution to the taxonomy of *Gliocephalotrichum*.

**Conidiophores** formed abundantly, scattered, solitary, erect, hyaline, arising from submerged hyphae, consisting of a stipe and stipe extensions subtending a penicillate conidiogenous apparatus; stipe septate, hyaline, smooth, 136–322  $\times$  11–18  $\mu\text{m}$ ; stipe extensions 1–4, directly subtending penicillus at right angles progressively bending upwards, hyaline, septate, 92–200  $\mu\text{m}$  long, 4–10  $\mu\text{m}$  wide at the base, terminating in clavate to broadly clavate vesicle. *Conidiogenous apparatus* densely penicillate, consisting of a whorl of fertile branches, 41–71  $\mu\text{m}$  long, 25–91  $\mu\text{m}$  wide; primary branches aseptate,



**Fig. 10** *Glioscephalotrichum simmonsii* (CBS 135953, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. bulbiloid aggregate of chlamydozoospores. — Scale bars: b = 50  $\mu$ m (applies to c); d = 10  $\mu$ m (applies to e–j).

10–26  $\times$  3–6  $\mu$ m; secondary branches aseptate, 6–13  $\times$  2–4  $\mu$ m; tertiary branches aseptate, 6–11  $\times$  2–3  $\mu$ m, each terminal branch producing 4–6 phialides; phialides cylindrical, slightly ventricose, hyaline, aseptate, 7–13  $\times$  1–3  $\mu$ m; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* hyaline, smooth, cylindrical to ellipsoid, slightly bevelled, rounded at apex, (5–)7–8  $\times$  (1–)2  $\mu$ m (av. 7  $\times$  2  $\mu$ m), forming a white mucoid droplet at apex of penicillus. *Chlamydozoospores* form abundant brown to dark brown, superficial and immersed bulbiloid aggregates, 75–225  $\times$  70–176  $\mu$ m, consisting of globose to ellipsoid cells; solitary chlamydozoospores absent. *Sexual morph* not observed.

**Culture characteristics** — Colonies fast growing (90 mm in 5 d), white with reverse sienna turning umber where bulbiloid aggregates are formed; aerial mycelium sparse, with abundant conidiophores forming on immersed mycelium.

**Specimen examined.** GUATEMALA, from fruit of *Nephelium lappaceum* imported into the USA, 3 Sept. 2011, *L.M. Serrato-Diaz*, (holotype CBS H-21388) cultures ex-type CBS 135953; CBS 135951; CBS 135952.

**Notes** — Isolates representing *G. simmonsii* formed a unique, distinct lineage (Fig. 1).

***Glioscephalotrichum simplex*** (J.A. Mey.) B.J. Wiley & E.G. Simmons, *Mycologia* 63: 578. 1971. — MycoBank MB314502; Fig. 11

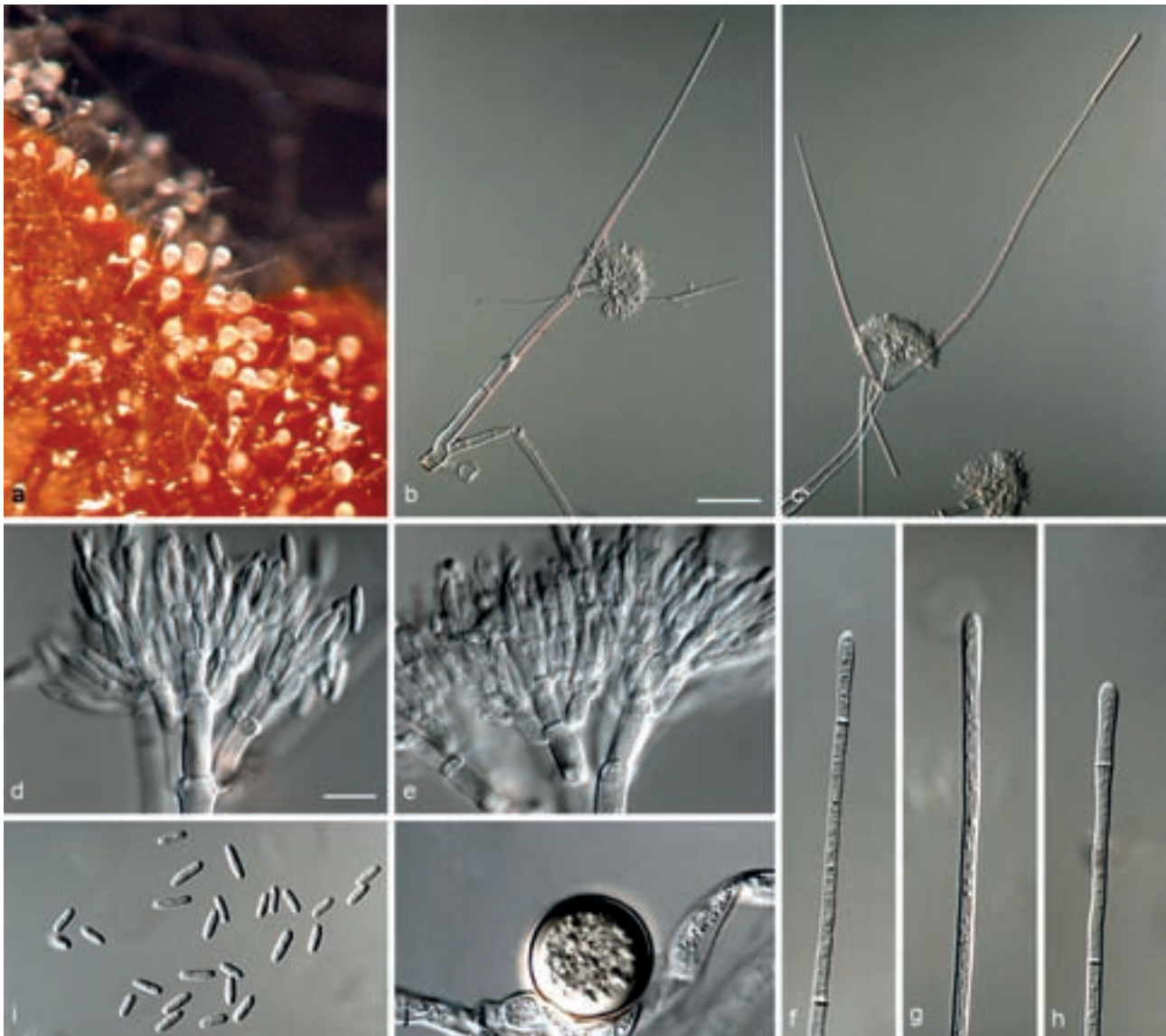
**Basionym.** *Cylindrocladium simplex* J.A. Mey., *Publ. Inst. Natl. Etude Agron. Congo Belge* 75: 148. 1959.

**Description and illustration:** See Wiley & Simmons (1971).

**Specimens examined.** BRAZIL, Pará, Belem, near Capitão Poço, from root of unknown plant, May 1991, *L. Pfennig*, CBS 249.91; Salvador, from soil, Nov. 1969, *C. Ram*, MUCL 18583 = QM 9366 = CBS 983.69; Pará, Monte Dourado, from soil, Apr. 2011, *R.F. Alfenas*, LPF317 = CPC 23349. — GUATEMALA, from fruit of *Nephelium lappaceum*, 3 Sept. 2011, *L.M. Serrato-Diaz*, CPC 23350–23354. — MALAYSIA, MUCL 46722 = CPC 21868. — NEW ZEALAND, Niue Island, from *Musa*, Nov. 1981, *H.J. Boesewinkel*, CBS 511.81. — PUERTO RICO, from fruit of *Nephelium lappaceum*, 2 Feb. 2011, *L.M. Serrato-Diaz*, CPC 23355–23367. — SINGAPORE, Lower Pierce Reservoir, from submerged leaf litter, 2003, *C. Decock*, MUCL 46551 = SING 0061759 = CPC 21865. — SOUTH AFRICA, Sabie River area, from soil, May 1954, *H.J. Swart*, culture ex-type ATCC 22231 = IFO 9330 = IMI 155705 = MUCL 18577 = QM 9041 = CBS 267.65.

## DISCUSSION

The taxonomy of the genus *Glioscephalotrichum* was investigated in this study using molecular phylogenetic inference and morphological comparisons. The isolates included were



**Fig. 11** *Gliocephalotrichum simplex* (CBS267.65, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. chlamydospore. — Scale bars: b = 50  $\mu$ m (applies to c); d = 10  $\mu$ m (applies to e–j).

collected from various substrates and countries. Following the proposal of Rossman et al. (2013) and the ICN (McNeill et al. 2012), five novel taxa are introduced in the asexual genus *Gliocephalotrichum* and not in the sexual genus *Leuconectria*. The taxonomic status of three unique phylogenetic lineages (CBS 254.82, CBS 109446 and CBS 135954) remain unresolved as they are represented by only a single isolate and isolates CBS 254.782 and CBS 109446 are sterile.

The description of *G. humicola*, *G. mexicanum*, *G. nephelii*, *G. queenslandicum* and *G. simmonsii* adds five more species to this genus, which included seven taxa prior to this study (Ellis & Hesseltine 1962, Wiley & Simmons 1971, Huang & Schmitt 1973, Decock et al. 2006). Of these seven taxa, only *G. bulbilium* (= *Leuconectria clusiae*; Rossman et al. 1993) and *G. grande* (= *Leuconectria grandis*; Zhuang et al. 2007) have been found to produce a sexual morph. No sexual morph could be induced for any of the new taxa described in this study.

*Gliocephalotrichum mexicanum*, *G. nephelii* and *G. simmonsii* were isolated from rambutan fruits displaying symptoms of post-harvest fruit rot, with *G. mexicanum* isolated from fruit from Mexico, and the latter two species from Guatemala. The remaining isolates from Mexico were identified as *G. bulbilium*, and those from Guatemala as *G. simplex*. Both *G. bulbilium* and *G. simplex* have previously been reported on rambutan fruit,

in Puerto Rico and Hawaii (Nishijima et al. 2002, Serrato-Diaz et al. 2012).

The phylogenetic inference done in this study revealed some variation within the clades representing *G. bulbilium* and *G. simplex*, respectively, either indicating possible cryptic speciation within both these *Gliocephalotrichum* species, or geographical variation. Morphological studies of the isolates representing both these taxa in this study, revealed no differences when compared to each other and the ex-type strains. Therefore, a larger sampling of taxa and the addition of more gene regions is required to investigate this further.

Isolates representing *G. queenslandicum* (CBS 114868, CBS 112956) were isolated as endophytes from the roots of *Eleaocarpus angustifolius* and originally identified as *G. cylindrosporium* based on morphology (Paulus et al. 2006). Closer investigation of the morphology, supported by phylogenetic inference in this study, revealed that *G. queenslandicum* and *G. cylindrosporium* could be distinguished based on conidial dimensions and the formation of stipe extensions directly below the penicillus for *G. queenslandicum*, not reported for *G. cylindrosporium* (Wiley & Simmons 1971). *Gliocephalotrichum humicola*, baited from soils, is morphologically similar to *G. ohiiense*, but could be distinguished by the yellowish stipes and stipe extensions and additional fertile branches not reported for *G. ohiiense* (Huang & Schmitt 1973).

The first comprehensive phylogenetic study on the genus *Gliocephalotrichum* by Decock et al. (2006) employed both ITS and BTUB sequence data, resulting in the introduction of *G. bacillisporum* and *G. longibrachium* isolated from leaf litter collected in French Guiana. Based on the phylogenies in that study, all *Gliocephalotrichum* species treated could be resolved, with BTUB providing the best resolution for all species treated. Furthermore, the phylogenies supported the segregation of the species into two informal groups (Wiley & Simmons 1971) based on the position of the stipe extensions in relation to the penicillus. In our studies, BTUB sequence data still provided the best resolution for all species treated, followed by TEF and HIS3 sequence data when the various gene regions were analysed separately (results not shown). However, our multilocus phylogenetic analysis did not resolve the informal segregation suggested by Wiley & Simmons (1971).

Identification of several new species within the genus *Gliocephalotrichum*, of which three were associated with fruit rot of rambutan, highlights the limited information available for this genus of fungi. Although fungi in the genus *Gliocephalotrichum* are not regarded as important plant pathogens, their ability to cause post-harvest fruit rot of tropical fruits could have an impact on fruit exports and imports. Therefore, further surveys from different geographical regions and additional etiological studies are required to determine the potential threat of *Gliocephalotrichum* species as causal agents of post-harvest diseases of tropical fruits globally.

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