Persoonia 32, 2014: 127–140
www.ingentaconnect.com/content/nhn/pimj

RESEARCH ARTICLE

http://dx.doi.org/10.3767/003158514X680261

Phylogeny and taxonomy of the genus *Gliocephalotrichum*

L. Lombard[1], L.M. Serrato-Diaz[2], R. Cheewangkoon[3], R.D. French-Monar[2], C. Decock[4], P.W. Crous[1,5,6]

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 β-tubulin gene regions. Employing DNA sequence data from four loci (β-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.

Article info Received: 2 August 2013; Accepted: 1 November 2013; Published: 20 March 2014.

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. cylindrosporum*, *G. microchlamydosporum* (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 introduced by Zhuang et al. (2007), although mistakenly connected to the asexual species, *G. cylindrosporum*. 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 & Luna-Ilag 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-

[1] CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; corresponding author e-mail: l.lombard@cbs.knaw.nl.
[2] Department of Plant Pathology and Microbiology, Texas A&M AgriLife Extension Service, 6500 Amarillo Blvd W. Amarillo, Texas 79106, USA.
[3] Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.
[4] Mycothèque de l'Université catholique de Louvain (BCCM/MUCL), Earth and Life Institute, Université catholique de Louvain, Place Croix du Sud 2 bte L07.05.06, 1348 Louvain-la-Neuve, Belgium.
[5] Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.
[6] Laboratory of Phytopathology, Wageningen University and Research Centre (WUR), Doevendaalsesteeg 1, 6708 PD Wageningen, The Netherlands.

Table 1 *Gliocephalotrichum* isolates included in this study.

Species	Culture accession[1*]	GenBank accession[2]				Substrate	Country	Collector
		BTUB	HIS3	ITS	TEF			
Gliocephalotrichum bacillisporum	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
G. bulbilium	**CBS 242.62** = ATCC 22228 = IFO 9325 = IMI 096357 = MUCL 18575 = NRRL 2899 = QM 9007	DQ377831	KF513326	DQ377831	KF513408	soil	USA	L.J. Wickerham
	CBS 118.68	KF513183	KF513327	KF513252	KF513409	air	Central African Republic	J. Nicot
	CBS 562.75	KF513184	KF513328	KF513253	KF513410	*Flacourtia* sp.	Indonesia	I. Gandjar
	CBS 451.92 = GJS 92-7 = ATCC 90145 = BPI 1113065	KF513185	KF513329	KF513254	KF513411	*Clusia* 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	*Nyssa sylvatica*	USA	T. Sutton
	CPC 21866 = MUCL 46552	DQ377830	KF513333	DQ374406	KF513415	leaf litter	French Guiana	C. Decock & V. Robert
	CPC 21867 = MUCL 46553	DQ377829	KF513334	DQ374407	KF513416	leaf litter	French Guiana	C. Decock & V. Robert
	CPC 23321	KF513189	KF513335	KF513258	KF513417	*Nephelium lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23322	KF513190	KF513336	KF513259	KF513418	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23323	KF513191	KF513337	KF513260	KF513419	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23324	KF513192	KF513338	KF513261	KF513420	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23325	KF513193	KF513339	KF513262	KF513421	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23334	KF513194	KF513340	KF513263	KF513422	*N. lappaceum*	Mexico	L.M. Serrato-Diaz
	CPC 23335	KF513195	KF513341	KF513264	KF513423	*N. lappaceum*	Mexico	L.M. Serrato-Diaz
	CPC 23336	KF513196	KF513342	KF513265	KF513424	*N. lappaceum*	Mexico	L.M. Serrato-Diaz
	CPC 23337	KF513197	KF513343	KF513266	KF513425	*N. lappaceum*	Mexico	L.M. Serrato-Diaz
	CPC 23339	KF513198	KF513344	KF513267	KF513426	*N. lappaceum*	Mexico	L.M. Serrato-Diaz
G. cylindrosporum	**CBS 902.70** = ATCC 22229 = IFO 9326 = IMI 155704 = MUCL 18576 = QM 9009	DQ377841	KF513353	DQ366705	KF513435	soil	Thailand	C. Klinsukont
	CBS 903.70 = QM 9146	KF513208	KF513354	KF513277	KF513436	soil	Thailand	S. Chomchalow
	CBS 904.70 = MUCL 18580 = QM 9147	DQ377842	KF513355	DQ366706	KF513437	soil	Thailand	S. Chomchalow
G. grande	**HMAS 98302**	EU984072	–	EF121859	HM054075	leaf litter	China	W.Y. Zhuang & Y. Nong
G. humicola	CBS 135945	KF513209	KF513356	KF513278	KF513438	soil	Taiwan	P.W. Crous
	CBS 135946	KF513210	KF513357	KF513279	KF513439	soil	Taiwan	P.W. Crous
	CPC 23340	KF513211	KF513358	KF513280	KF513440	soil	Taiwan	P.W. Crous
	CPC 23344	KF513212	KF513359	KF513281	KF513441	soil	Taiwan	P.W. Crous
	CPC 23345	KF513213	KF513360	KF513282	KF513442	soil	Taiwan	P.W. Crous
	CPC 23347	KF513214	KF513361	KF513283	KF513443	soil	Taiwan	P.W. Crous
G. longibrachium	**CBS 126571** = MUCL 46693	DQ377835	KF513367	DQ278422	KF513449	leaf litter	French Guiana	C. Decock & V. Robert
	CBS 132043 = MUCL 46694	DQ377836	KF513368	DQ278421	KF513450	leaf litter	French Guiana	C. Decock & V. Robert
G. mexicanum	**CBS 135947**	KF513220	KF513369	KF513289	KF513451	*N. lappaceum*	Mexico	L.M. Serrato-Diaz
	CBS 135948	KF513221	KF513370	KF513290	KF513452	*N. lappaceum*	Mexico	L.M. Serrato-Diaz
G. microchlamydosporum	**CBS 345.64** = ATCC 22230 = IFO 9329 = IMI 155706 = MUCL 4085 = QM 9042	DQ374410	KF513371	DQ366699	KF513453	soil	Zaïre	J.A. Meyer
	CPC 21862 = MUCL 8137	DQ374411	–	DQ366700	KF513454	–	Zaïre	–
	CPC 21863 = MUCL 18349	DQ374412	–	DQ366701	KF513455	–	South Africa	–
G. nephelii	**CBS 135949**	KF513222	KF513372	KF513291	KF513456	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
	CBS 135950	KF513223	KF513373	KF513292	KF513457	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
G. ohiense	**CBS 567.73** = ATCC 24879 = IMI 176508 = MUCL 39340	DQ374415	–	DQ366707	KF513458	soil	USA	L.H. Huang
G. queenslandicum	CBS 112956 = CPC 4713	KF513224	KF513374	KF513293	KF513459	*Eleaeocarpus angustifolius*	Australia	I. Steer & B. Paulus
	CBS 114868 = CPC 4712	KF513225	KF513375	KF513294	KF513460	*E. angustifolius*	Australia	I. Steer & B. Paulus
G. simmonsii	CBS 135951	KF513226	KF513376	KF513295	KF513461	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
	CBS 135952	KF513227	KF513377	KF513296	KF513462	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
	CBS 135953	KF513228	KF513378	KF513297	KF513463	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
G. simplex	**CBS 267.65** = ATCC 22231 = IFO 9330 = IMI 155705 = MUCL 18577 = QM 9041	DQ377838	KF513379	DQ 366702	KF513464	soil	South Africa	H.J. Swart

G. simplex (cont.)	CBS 983.69	KF513229	KF513380	KF513298	KF513465	soil	Brazil	C. Ram
	CBS 511.81	KF513230	KF513381	KF513299	KF513466	*Musa* sp.	New Zealand	H.J. Boesewinkel
	CBS 249.91	KF513231	KF513382	KF513300	KF513467	root of unknown plant	Brazil	L. Pfenning
	CPC 21865 = MUCL 46551 = SING 0061759	DQ377837	KF513383	DQ366704	KF513468	–	Singapore	C. Decock
	CPC 21868 = MUCL 46722	DQ377840	KF513384	KF513301	KF513469	–	Malaysia	–
	CPC 23349	KF513232	KF513385	KF513302	KF513470	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
	CPC 23350	KF513233	KF513386	KF513303	KF513471	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
	CPC 23351	KF513234	KF513387	KF513304	KF513472	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
	CPC 23352	KF513235	KF513388	KF513305	KF513473	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
	CPC 23353	KF513236	KF513389	KF513306	KF513474	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
	CPC 23354	KF513237	KF513390	KF513307	KF513475	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz
	CPC 23355	KF513238	KF513391	KF513308	KF513476	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23356	KF513239	KF513392	KF513309	KF513477	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23357	KF513240	KF513393	KF513310	KF513478	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23358	KF513241	KF513394	KF513311	KF513479	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23359	KF513242	KF513395	KF513312	KF513480	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23360	KF513243	KF513396	KF513313	KF513481	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23361	KF513244	KF513397	KF513314	KF513482	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
	CPC 23362	KF513245	KF513398	KF513315	KF513483	*N. lappaceum*	Puerto Rico	L.M. Serrato-Diaz
Gliocephalotrichum sp. 1	CBS 109446	KF513248	KF513402	KF513320	KF513489	*Miconia* sp.	Venezuela	I. Hernandez
Gliocephalotrichum sp. 2	CBS 254.82	KF513249	KF513403	KF513321	KF513490	*Flacourtia* sp.	Indonesia	J.E. Willemstein-Sytema
Gliocephalotrichum sp. 3	CBS 135954	KF513250	KF513404	KF513322	KF513491	*N. lappaceum*	Guatamala	L.M. Serrato-Diaz

[1] 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-chrome, Yodogawa-ku, Osaka 532, Japan; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; GJS: Gary J. Samuels personal collection; MUCL: Mycothèque, Laboratoire de Mycologie Systématique st Appliquée, l'Université, Louvian-la-Neuve, Belguim; NRRL: Agricultural Research Service, USDA, Washington, USA; QM: Quatermaster 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.

[2] BTUB = β-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 β-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 consistence 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 × 1 000 magnification using

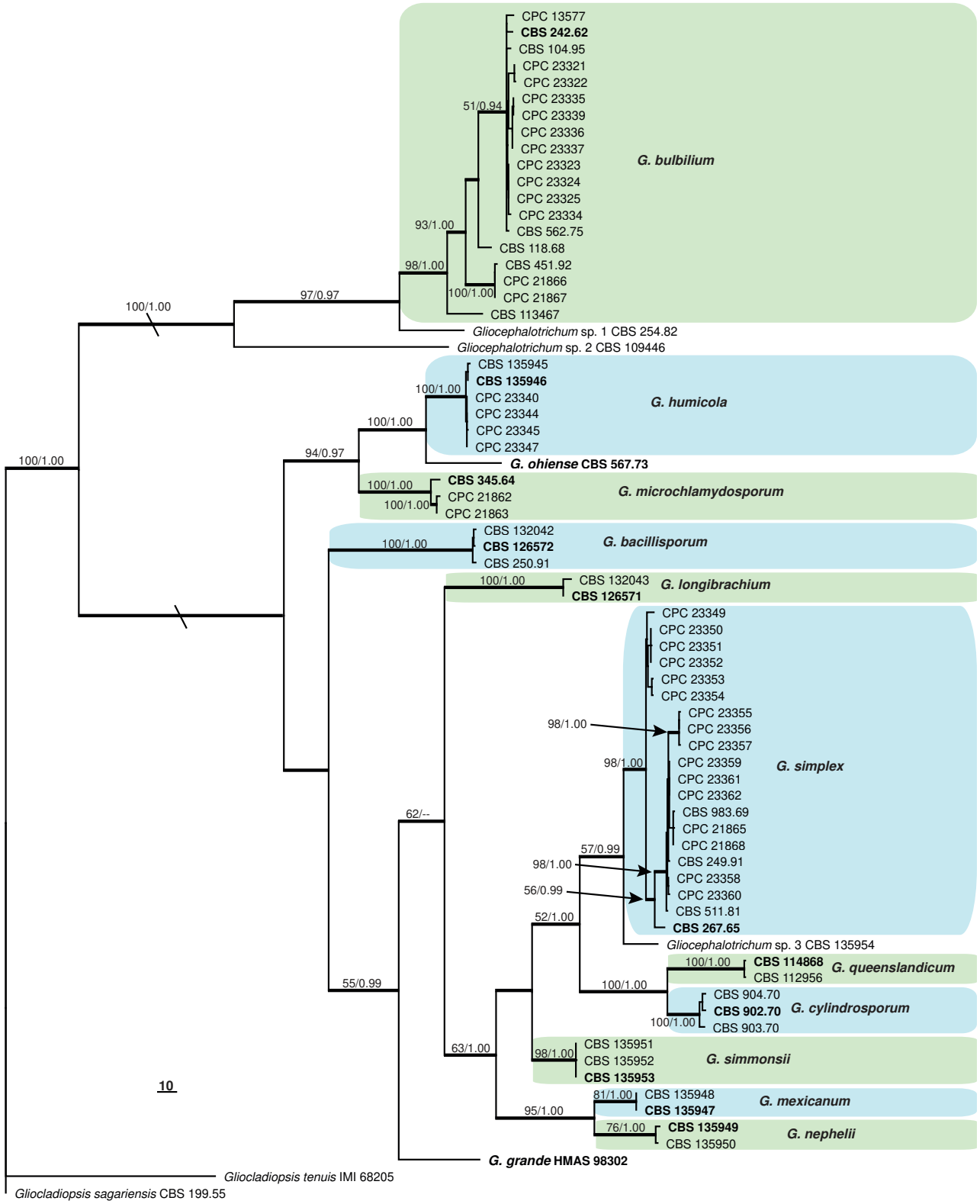

Fig. 1 One of 1 000 most parsimonious trees obtained from a heuristic search with 1 000 random addition sequences of the combined sequences of β-tubulin, histone H3, internal transcribed spacer region and translation elongation factor 1-alpha sequence alignments of the *Gliocephalotrichum* isolates used 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. ohiense* (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,

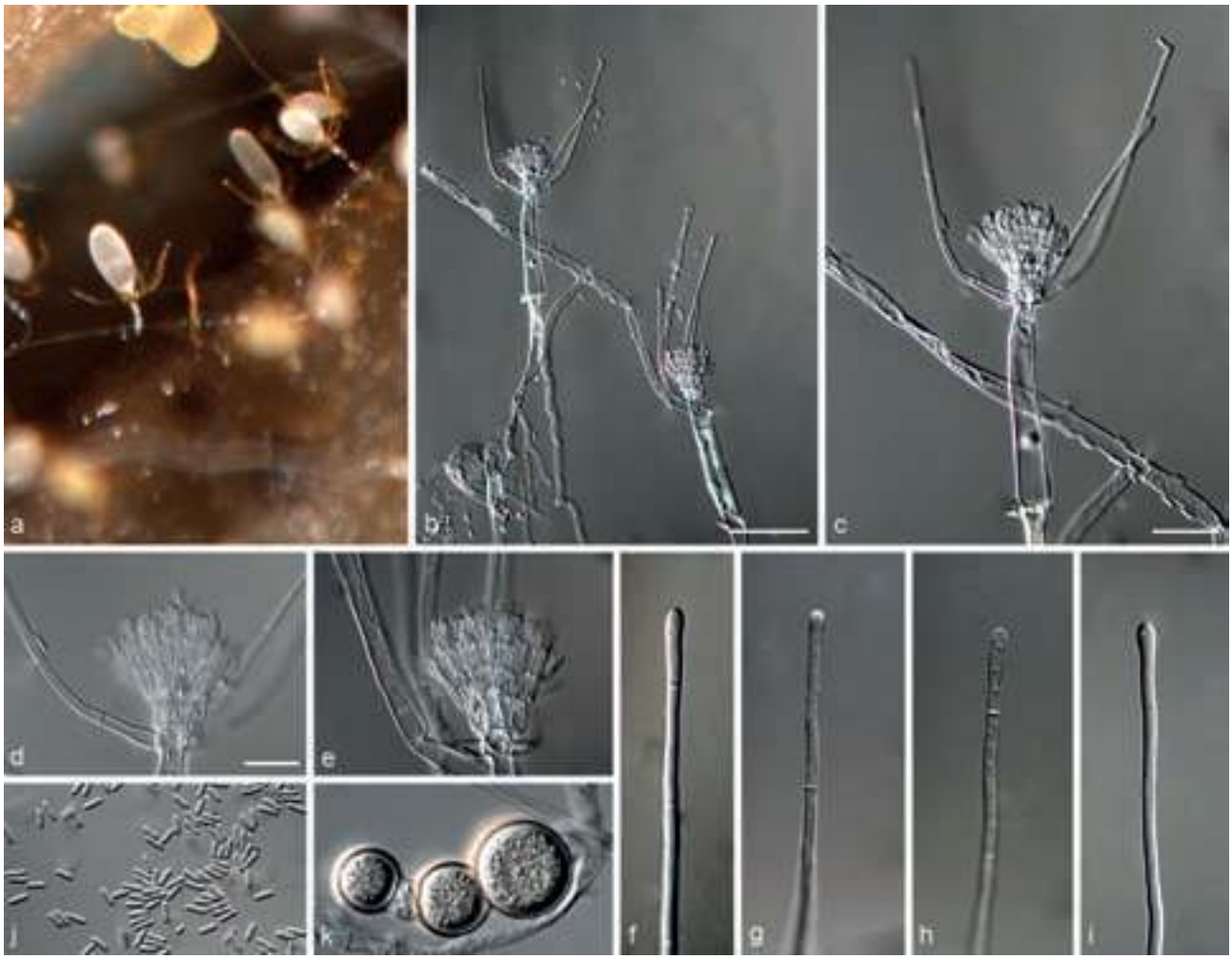

Fig. 2 *Gliocephalotrichum bacillisporum* (CBS 126572, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–i. apex of stipe extensions; j. conidia; k. chlamydospores formed in chains. — Scale bars: b = 50 µm; c = 20 µm; d = 10 µm (applies to e–k).

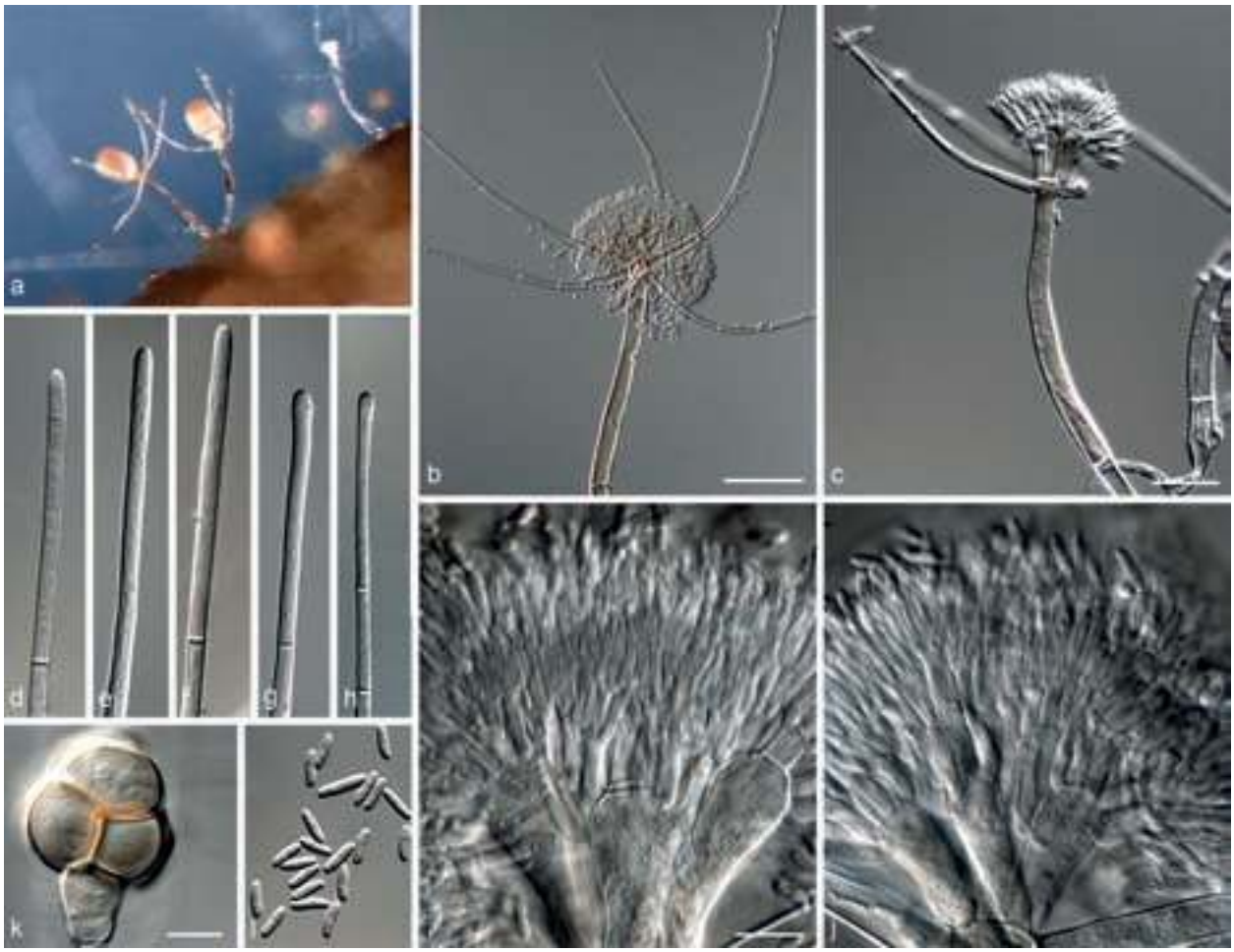

Fig. 3 *Gliocephalotrichum bulbilium* (CBS 242.62, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d–h. apex of stipe extensions; i, j. penicillus; k. bulbilloid aggregate of chlamydospores; l. conidia. — Scale bars: b = 50 µm; c = 20 µm; i = 10 µm (applies to d–j);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

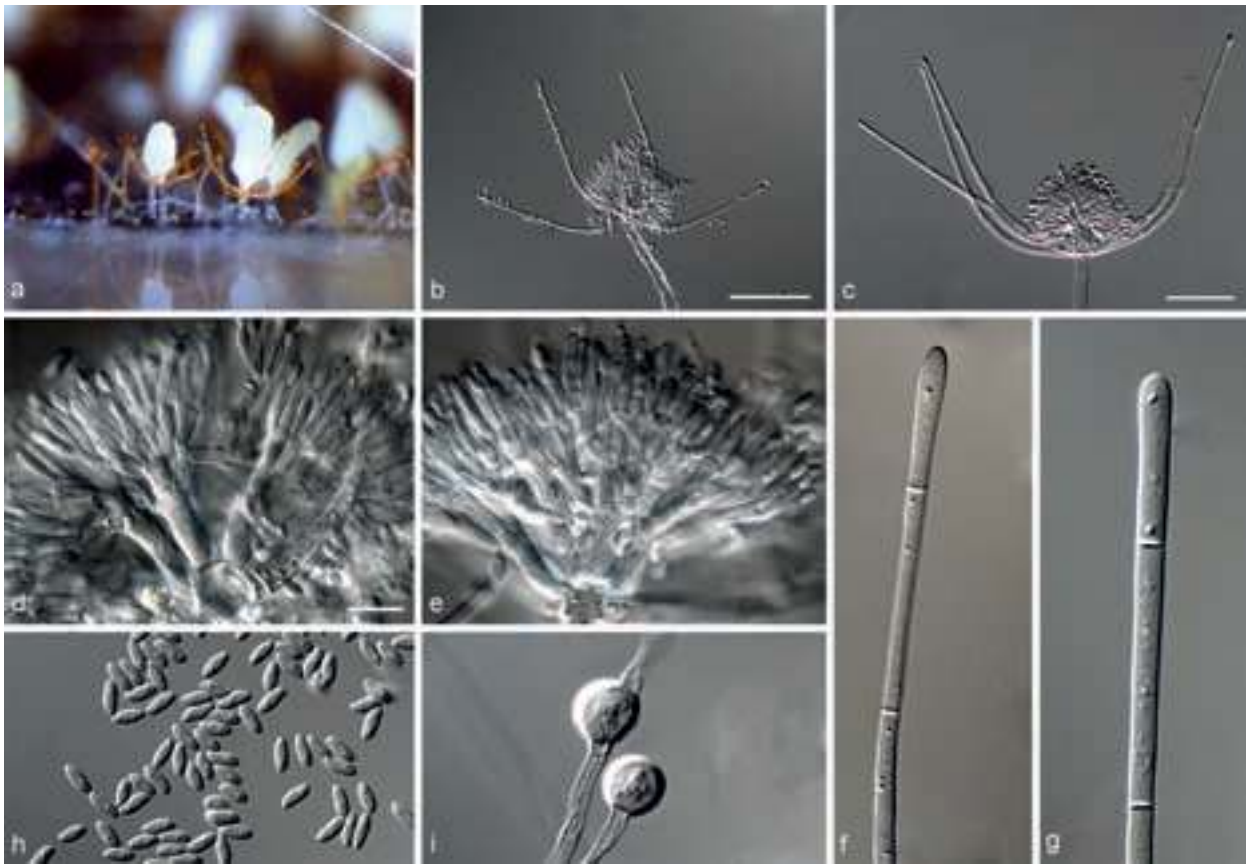

Fig. 4 *Gliocephalotrichum humicola* (CBS 135946, ex-type). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f, g. apex of stipe extensions; h. conidia; i. chlamydospores. — Scale bars: b = 50 µm; c = 20 µm; d = 10 µm (applies to e–i).

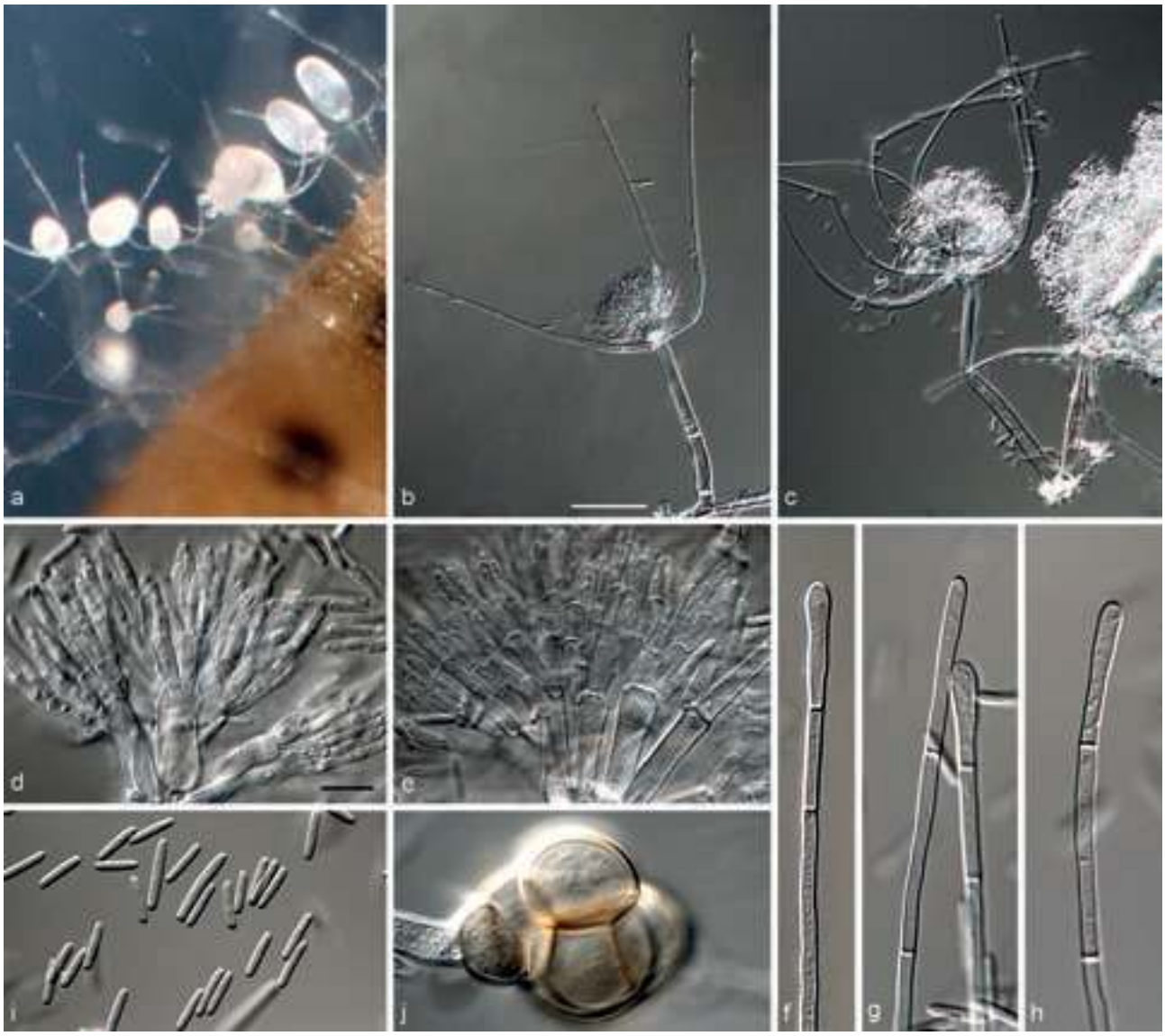

Fig. 5 *Gliocephalotrichum longibrachium* (CBS 126571, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. bulbilloid aggregate of chlamydospores. — Scale bars: b = 50 µm (applies to c); d = 10 µm (applies to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

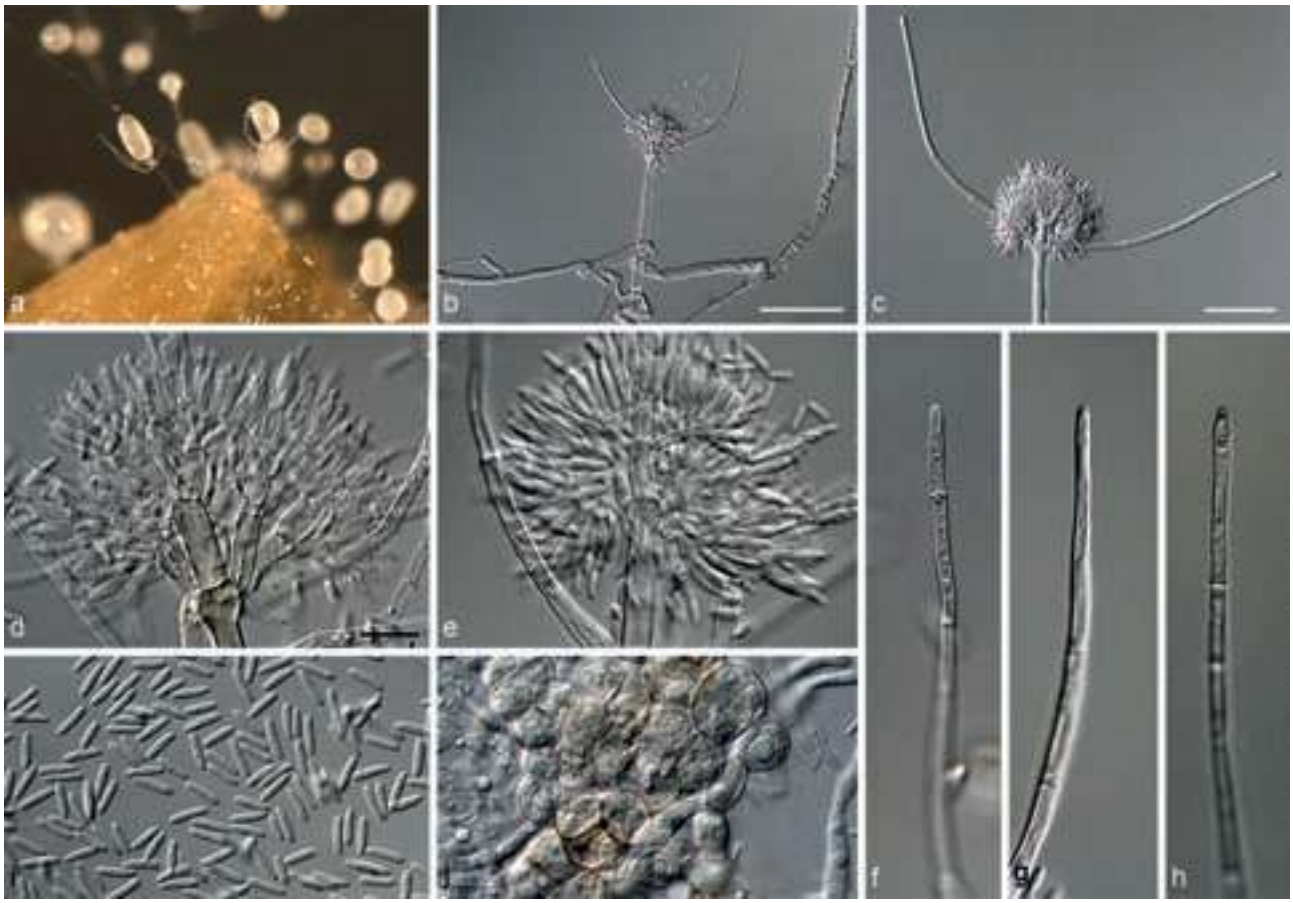

Fig. 6 *Gliocephalotrichum mexicanum* (CBS 135947, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. bulbilloid aggregate of chlamydospores. — Scale bars: b = 50 µm; c = 20 µm; d = 10 µm (applies to e–j).

Gliocephalotrichum 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. Klinsukont*, 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.

Gliocephalotrichum 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).

Gliocephalotrichum 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 × 9–16 µm; stipe extensions 2–5, directly subtending penicillus at right angles, progressively bending upwards, hyaline to pale luteous, septate, 88–199 µm long, 5–10 µm wide at the base, terminating in clavate to broadly clavate vesicle. *Conidiogenous apparatus* densely penicillate, consisting of a whorl of fertile branches, 38–96 µm long, 50–127 µm wide; primary branches aseptate, 13–21 × 4–8 µm; secondary branches aseptate, 8–13 × 2–5 µm; tertiary and additional branches (–4) aseptate, 6–10 × 2–4 µm, each terminal branch producing 4–8 phialides; phialides cylindrical, slightly ventricose, hyaline, aseptate, 8–12 × 1–3 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* hyaline, smooth, ellipsoid, bevelled at one or both ends, 6.5–7.5(–9) × (2–)2.5–3.5(–4) µm (av. 7 × 3 µm), 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 µm 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 — *Gliocephalotrichum humicola* is morphologically similar to *G. ohiense* but can be distinguished by the quaternary branches on the penicillus, which is not reported for *G. ohiense* (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.

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

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

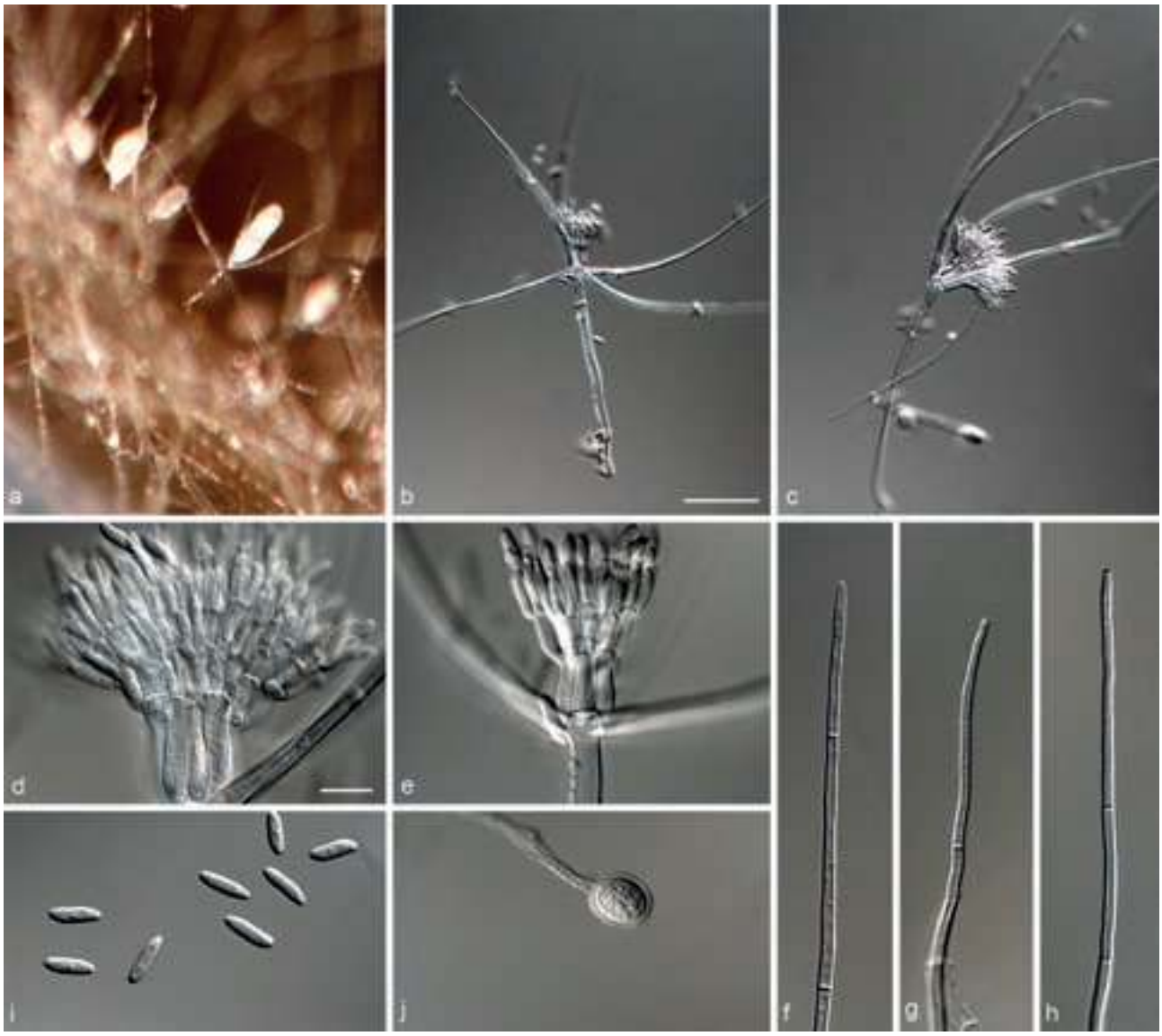

Fig. 7 *Gliocephalotrichum microchlamydosporum* (CBS 345.64, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. chlamydospore. — Scale bars: b = 50 µm (applies to c); d = 10 µm (applies to e–j).

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-Diaz, 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 × 4–15 µm; stipe extensions 2–6, directly subtending penicillus at right angles progressively bending upwards, hyaline, septate, 82–176 µm long, 4–8 µm wide at the base, terminating in narrowly clavate to clavate vesicle. *Conidiogenous apparatus* densely penicillate, consisting of a whorl of fertile branches, 43–135 µm long, 39–60 µm wide; primary branches aseptate, 12–20 × 3–6 µm; secondary branches aseptate, 8–11 × 2–5 µm; tertiary branches aseptate, 6–9 × 2–5 µm, each terminal branch producing 4–6 phialides; phialides cylindrical, slightly ventricose, hyaline, aseptate, 6–10 × 1–3 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* hyaline, smooth, cylindrical, slightly bevelled, rounded at apex, 6–8(–9) × (1–)2(–3) µm (av. 7 × 2 µm), forming a white mucoid droplet at apex of penicillus. *Chlamydospores* form abundant brown to dark brown, immersed bulbilloid aggregates, 55–210 × 50–108 µm, 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-Diaz*, (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.

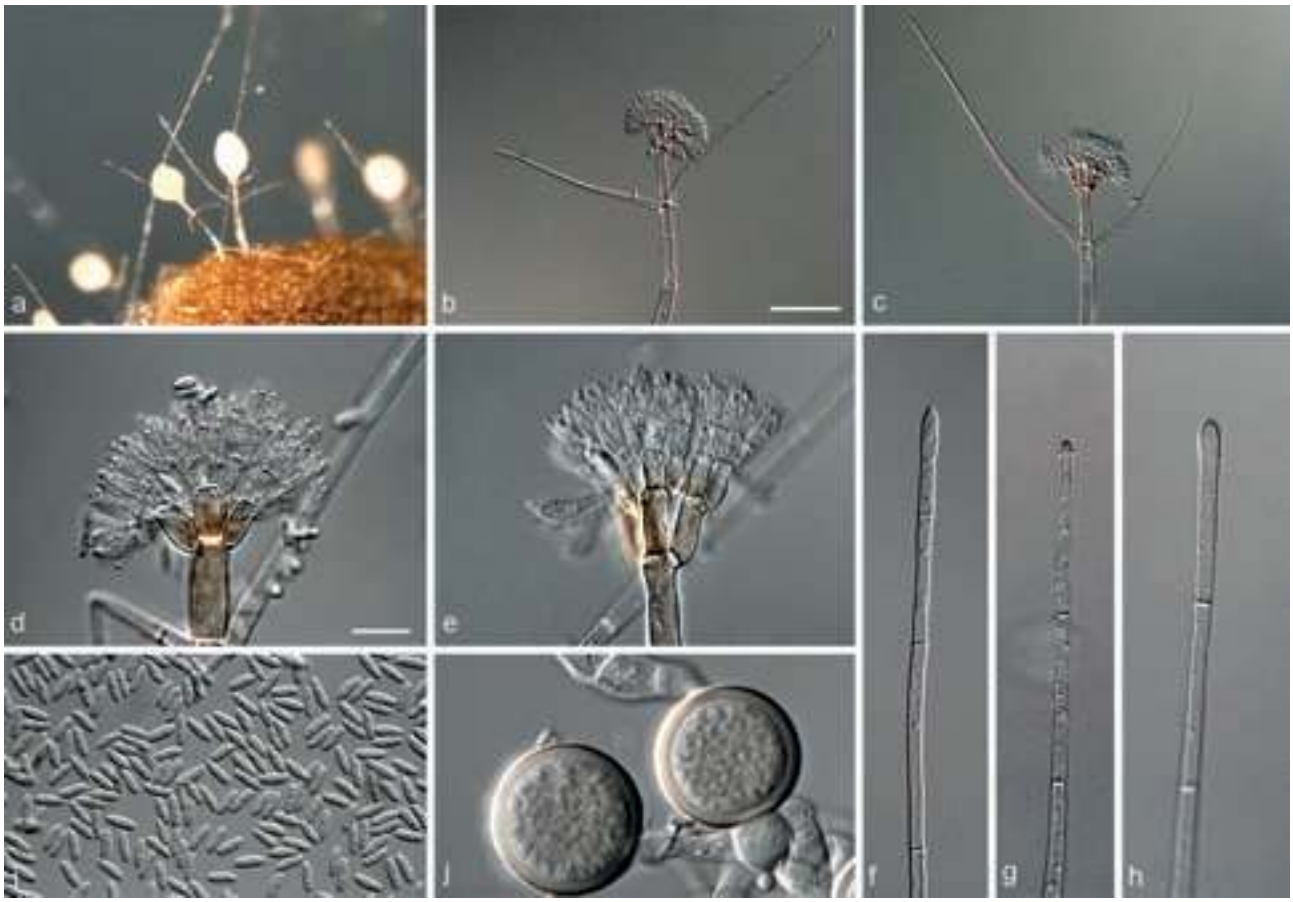

Fig. 8 *Gliocephalotrichum nephelii* (CBS 135949, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. chlamydospores. — Scale bars: b = 50 µm (applies to c); d = 10 µm (applies to e–j).

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

Specimens examined. USA, Illinois, Peoria, MUCL 18349 = NRRL 5212. – Zaïre, 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.

Gliocephalotrichum 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 × 9–14 µm; stipe extensions 2–6, directly subtending penicillus at right angles progressively bending upwards, hyaline, septate, 82–308 µm long, 4–6 µm wide at the base, terminating in narrowly clavate to clavate vesicle. *Conidiogenous apparatus* densely penicillate, consisting of a whorl of fertile branches, 38–75 µm long, 37–45 µm wide; primary branches aseptate, 11–18 × 3–6 µm; secondary branches aseptate, 6–10 × 2–4 µm; tertiary and additional (–4) branches aseptate, 5–9 × 1–4 µm, each terminal branch producing 4–6 phialides; phialides cylindrical, slightly ventricose, hyaline, aseptate, 6–9 × 1–4 µm; 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) × (1–)2(–3) µm (av. 7 × 2 µm), 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 µm diam, not forming bulbilloid 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 — *Gliocephalotrichum 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 bulbilloid aggregates. This was not observed for *G. mexicanum*, which in turn, only formed bulbilloid aggregates of chlamydospores and no solitary chlamydospores.

Gliocephalotrichum ohiense 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. ohiense* is sterile.

Gliocephalotrichum 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 × 6–16 µm; stipe extensions 2–6, directly subtending penicillus at right

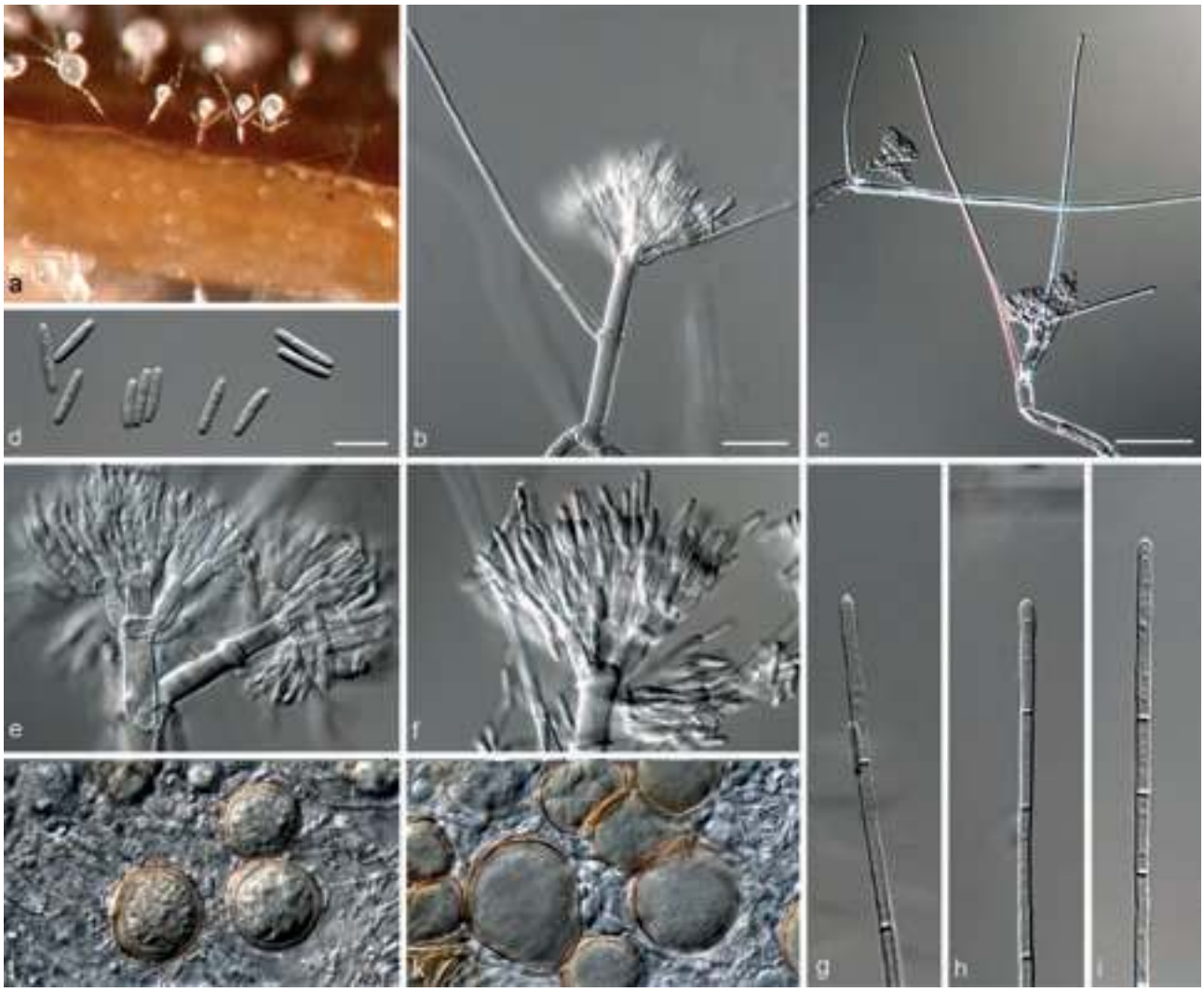

Fig. 9 *Gliocephalotrichum queenslandicum* (CBS 114868, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d. conidia; e, f. penicillus; g–i. apex of stipe extensions; j. chlamydospores; k. bulbilloid aggregate of chlamydospores. — Scale bars: b = 20 µm; c = 50 µm; d =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

angles progressively bending upwards, with a single stipe extension 14–28 µm below the penicillus, hyaline, septate, 45–314 µm long, 4–8 µm wide at the base, terminating in narrowly clavate to clavate vesicle. *Conidiogenous apparatus* densely penicillate, consisting of a whorl of fertile branches, 33–73 µm long, 37–88 µm wide; primary branches aseptate, 17–36 × 5–11 µm; secondary branches aseptate, 7–15 × 2–6 µm; tertiary and additional (–4) branches aseptate, 5–11 × 2–6 µm, each terminal branch producing 4–6 phialides; phialides cylindrical, slightly ventricose, hyaline, aseptate, 6–10 × 2–3 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* hyaline, smooth, cylindrical, slightly bevelled, rounded at apex, 8–9(–11) × (1–)2 µm (av. 8 × 2 µm), forming a white to pale luteous mucoid droplet at apex of penicillus. *Chlamydospores* formed singly or in chains, intercalary or terminally, globose to subglobose, hyaline turning brown with age, 43–53 µm diam, forming brown to dark brown, immersed bulbilloid aggregates, 131–153 × 90–101 µm, 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 *Eleaeocarpus 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 *Eleaeocarpus 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) × (1–)2 µm (av. 8 × 2 µm)) are slightly smaller than those of *G. cylindrosporum* (9.1–13 µm; 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 × 11–18 µm; stipe extensions 1–4, directly subtending penicillus at right angles progressively bending upwards, hyaline, septate, 92–200 µm long, 4–10 µm wide at the base, terminating in clavate to broadly clavate vesicle. *Conidiogenous apparatus* densely penicillate, consisting of a whorl of fertile branches, 41–71 µm long, 25–91 µm wide; primary branches aseptate,

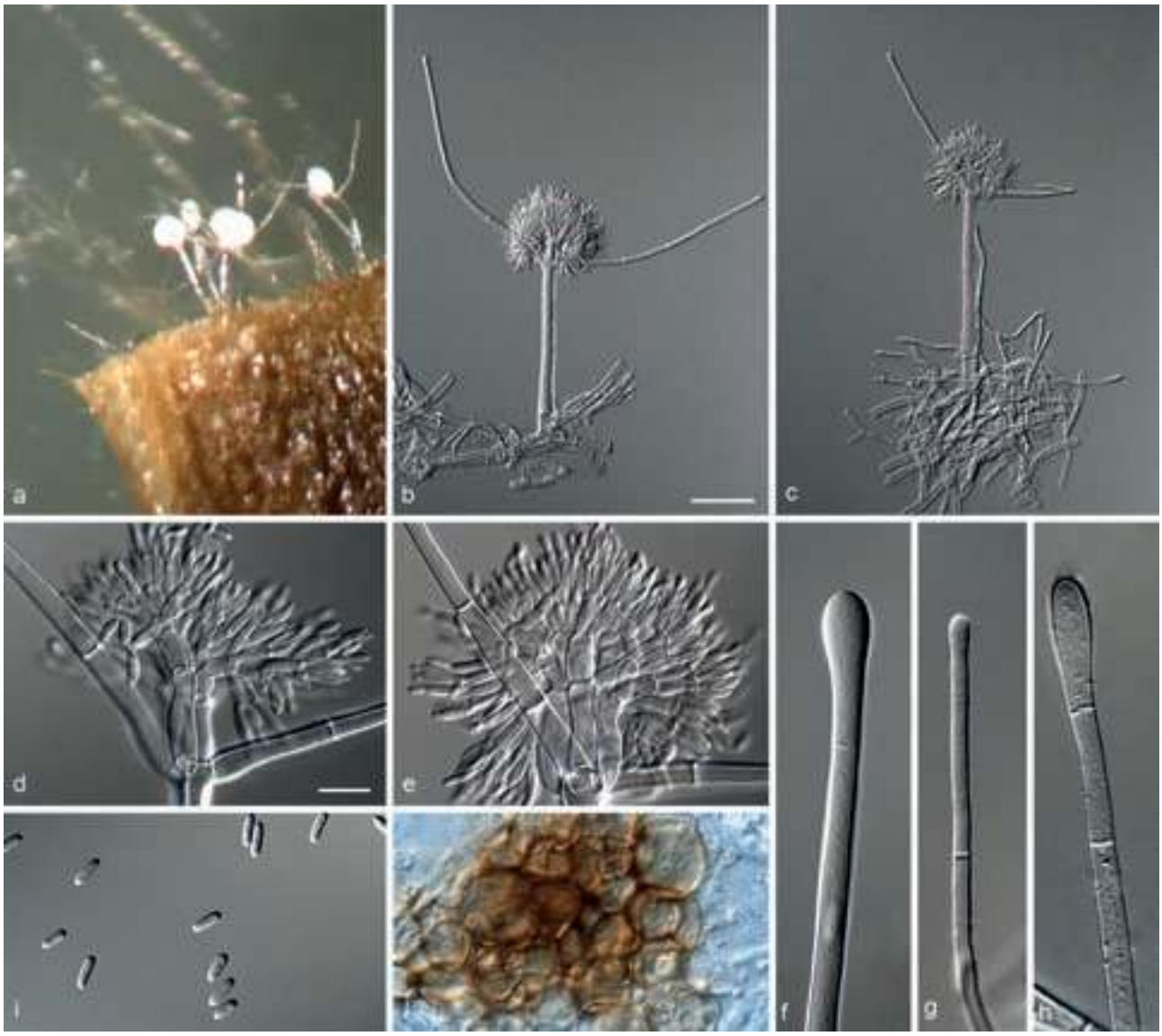

Fig. 10 *Gliocephalotrichum simmonsii* (CBS 135953, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. bulbilloid aggregate of chlamydospores. — Scale bars: b = 50 µm (applies to c); d = 10 µm (applies to e–j).

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

Culture characteristics — Colonies fast growing (90 mm in 5 d), white with reverse sienna turning umber where bulbilloid 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).

Gliocephalotrichum 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. Pfenning*, 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 *Gliocephalotrichum* was investigated in this study using molecular phylogenetic inference and morphological comparisons. The isolates included were

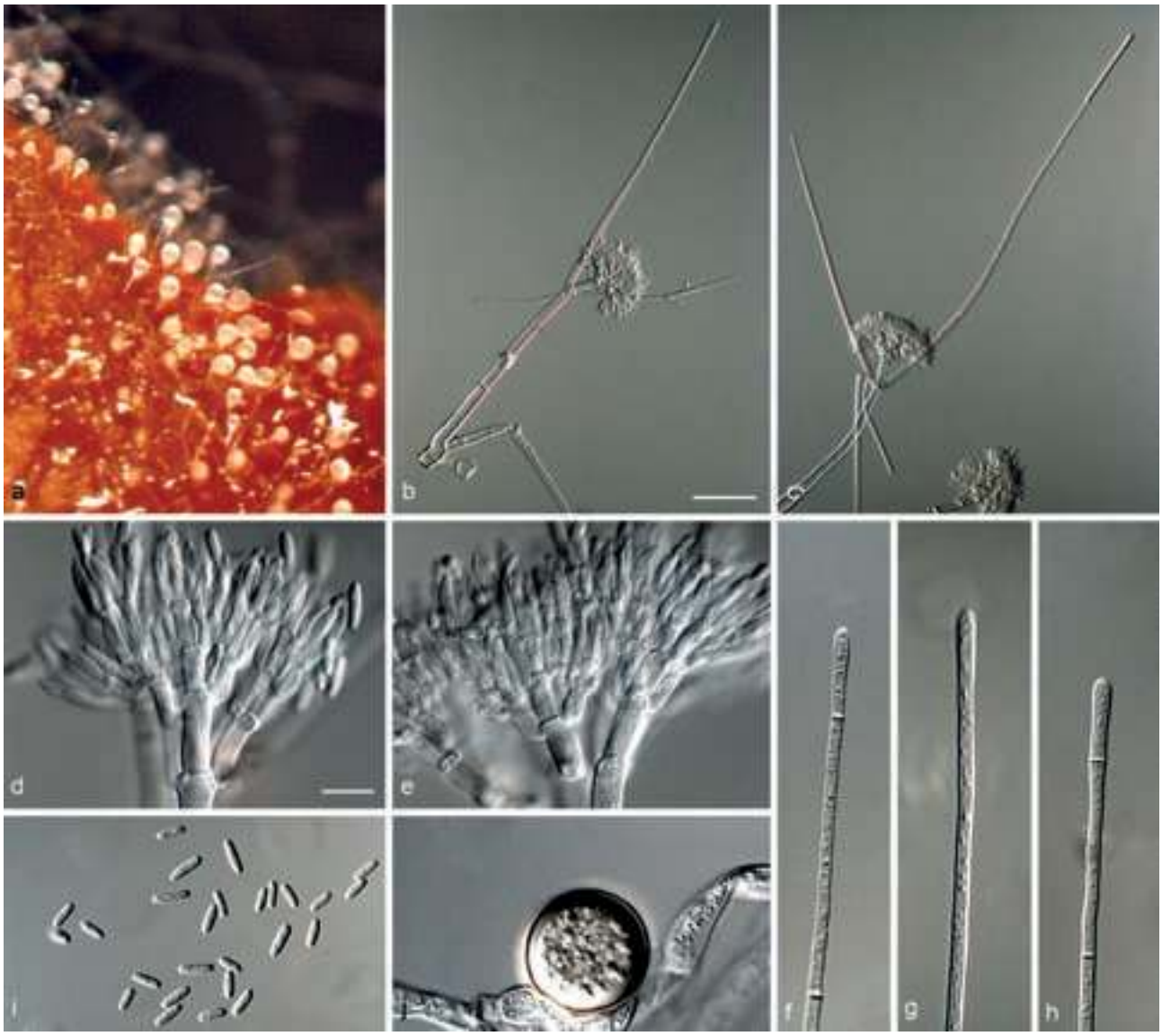

Fig. 11 *Gliocephalotrichum simplex* (CBS267.65, ex-type culture). a. Conidiophores on carnation leaf on SNA; b, c. conidiophores; d, e. penicillus; f–h. apex of stipe extensions; i. conidia; j. chlamydospore. — Scale bars: b = 50 µm (applies to c); d = 10 µm (applies to e–j).

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 *Eleaeocarpus angustifolius* and originally identified as *G. cylindrosporum* 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. cylindrosporum* could be distinguished based on conidial dimensions and the formation of stipe extensions directly below the penicillus for *G. queenslandicum*, not reported for *G. cylindrosporum* (Wiley & Simmons 1971). *Gliocephalotrichum humicola*, baited from soils, is morphologically similar to *G. ohiense*, but could be distinguished by the yellowish stipes and stipe extensions and additional fertile branches not reported for *G. ohiense* (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.

Acknowledgements The authors thank the technical staff, A. van Iperen and Y. Vlug for their invaluable assistance with cultures.

REFERENCES

Constantelos C, Doyle VP, Litt A, Oudemans PV. 2011. First report of Gliocephalotrichum bulbilium causing cranberry fruit rot in New Jersey and Massachusetts. Plant Disease 95: 618.

Crous PW. 2002. Taxonomy and pathology of Cylindrocladium (Calonectria) and allied genera. APS Press, St. Paul, Minnesota USA.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G. 2004. MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50: 19–22.

Decock C, Huret S, Charue P. 2006. Anamorphic fungi from French Guyana: two undescribed Gliocephalotrichum species (Nectriaceae, Hypocreales). Mycologia 98: 488–498.

Ellis JJ, Hesseltine CW. 1962. A new genus of Moniliales having penicillin subtended by sterile arms. Bulletin of the Torrey Botanical Club 89: 21–27.

Farungsang U, Sangchote S, Farungsang N. 1992. Appearance of quiescent fruit rot fungi on rambutan stored at 13 °C and 25 °C. Acta Horticulturae 321: 903–907.

Gueidan C, Roux C, Lutzoni F. 2007. Using multigene phylogeny analysis to assess generic delineation and character evolution in Verrucariaceae (Verrucariales, Ascomycota). Mycological Research 111: 1145–1168.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, et al. 2011. The Amsterdam Declaration on Fungal Nomenclature. IMA Fungus 2: 105–112.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method of assessing confidence in phylogenetic analysis. Systematic Biology 42: 182–192.

Huang LH, Schmitt JA. 1973. Gliocephalotrichum ohiense, a new species from Ohio soils. Mycologia 65: 948–952.

Intan Sakinah MA, Latiffah Z. 2013. First report of Gliocephalotrichum bacillisporum causing fruit rot of rambutan (Nephelium lappaceum) in Malaysia. Plant Disease 97: 1110.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780.

Lombard L, Crous PW. 2012. Phylogeny and taxonomy of the genus Gliocladiopsis. Persoonia 28: 25–33.

Lombard L, Crous PW, Wingfield BD, Wingfield MJ. 2010a. Phylogeny and systematics of the genus Calonectria. Studies in Mycology 66: 31–69.

Lombard L, Crous PW, Wingfield BD, Wingfield MJ. 2010b. Species concepts in Calonectria (Cylindrocladium). Studies in Mycology 66: 1–13.

McNeill J, Barrie FF, Buck WR, Demoulin V, Greuter W, et al. (eds). 2012. International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). [Regnum vegetabile no. 154.] Koeltz Scientific Books, Königstein.

McNeill J, Barrie FF, Burdet HM, Demoulin V, Hawksworth DL, et al. (eds). 2006. International Code of Botanical Nomenclature (Vienna Code). [Regnum vegetabile no. 146.] Gantner Verlag, Ruggell.

Nirenburg HI. 1981. A simplified method to identify Fusarium spp. occurring on wheat. Canadian Journal of Botany 59: 1599–1609.

Nishijima KA, Follett PA, Bushe BC, Nagao MA. 2002. First report of Lasmenia sp. and two species of Gliocephalotrichum on rambutan in Hawaii. Plant Disease 86: 71.

Nylander JAA. 2004. MrModeltest v. 2. Programme distributed by the author. Evolutionary Biology Centre, Uppsala University.

Paulus BC, Kanowski J, Gadek PA, Hyde KD. 2006. Diversity and distribution of saprobic microfungi in leaf litter of an Australian tropical rainforest. Mycological Research 110: 1441–1454.

Pordesimo AN, Luna-Ilag L. 1982. Postharvest diseases of mango and rambutan in the Philippines. Proceedings of the workshop on mango and rambutan, University of the Philippines at los Banos, Philippines: 211–232.

Rayner RW. 1970. A mycological colour chart. Commonwealth Mycological Society, Kew, Surrey. British Mycological Society.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Rossman AY, Samuels GJ, Lowen R. 1993. Leuconectria clusiae gen. nov. and its anamorph Gliocephalotrichum bulbilium with notes on Pseudonectria. Mycologia 85: 685–704.

Rossman AY, Samuels GJ, Rogerson CT, Lowen R. 1999. Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). Studies in Mycology 42: 1–248.

Rossman AY, Seifert KA, Samuels GJ, Minnis AM, Schroers H-J, et al. 2013. Genera in Bionectriaceae, Hypocreaceae, and Nectriaceae (Hypocreales) proposed for acceptance or rejection. IMA Fungus 4: 41–51.

Sangchote S, Farungsang U, Farungsang N. 1998. Pre- and postharvest infection of rambutan by pathogens and effects of post-harvest treatments. In: Coates LM, Hofman PJ, Johnson GI (eds), Disease control and storage life extension in fruit. Proceedings of an international workshop held at Chiang Mai, Thailand, 22–23 May 1997: 87–91. Australian Centre for International Agricultural Research, Canberra, Australia.

Sangchote S, Pongpisutta R. 1998. Fruit rot of mangosteen and their control. In: Coates LM, Hofman PJ, Johnson GI (eds), Disease control and storage life extension in fruit. Proceedings of an international workshop held at Chiang Mai, Thailand, 22–23 May 1997: 81–86. Australian Centre for International Agricultural Research, Canberra, Australia.

Schoch CL, Crous PW, Wingfield MJ, Wingfield BD. 2000. Phylogeny of Calonectria and selected hypocrealean genera with cylindrical macroconidia. Studies in Mycology 45: 45–62.

Serrato-Diaz LM, Latoni-Brailowsky EI, Rivera-Vargas LI, Goenaga R, French-Monar RD. 2012. First report of Gliocephalotrichum bulbilium and G. simplex causing fruit rot of rambutan in Puerto Rico. Plant Disease 96: 1225.

Singh SK, Yadav LS, Singh PN, Sharma R, Mukherjee G. 2012. Additions to Gliocephalotrichum species (anamorphic Hypocreales) from fruit litter of the medicinal plant Terminalia chebula in the Western Ghats, India. Mycoscience 53: 391–395.

Sivakumar D, Wijeratham RSW, Wijesundera RLC, Abeysekera M. 1997. Post-harvest diseases of rambutan (Nephelium lappaceum) in the Western Province. Journal of the National Science Council of Sri Lanka 25: 225–229.

Sivakumar D, Wijeratham RSW, Wijesundera RLC, Abeysekera M. 1999. Field sanitation and the occurrence of brown spot disease of rambutan (Nephelium lappaceum) fruits. Journal of the National Science Council of Sri Lanka 27: 93–97.

Sivakumar D, Wijeratham RSW, Wijesundera RLC, Abeysekera M. 2000. Antagonistic effect of Trichoderma harzianum on post-harvest pathogens of rambutan (Nephelium lappaceum). Phytoparasitica 28: 1–7.

Sivapalan A, Metussin R, Harndan F, Zain RM. 1998. Fungi associated with postharvest fruit rots of Durio graveolens and D. kutejensis in Brunei Darussalam. Australasian Plant Pathology 27: 274–277.

Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods), v. 4.0b10. Computer program. Sinauer Associates, Sunderland, Massachusetts, USA.

Watanabe T, Nakamura K. 2005. Gliocephalotrichum microchlamydosporum and G. simplex in the Ryukyu Island, Japan. Mycoscience 46: 46–48.

Wiley BJ, Simmons EG. 1971. Gliocephalotrichum, new combinations and a new species. Mycologia 63: 575–585.

Zhuang W, Luo J. 2008. Re-identification of the anamorph of Leuconectria grandis. Mycotaxon 106: 409–412.

Zhuang W, Nong Y, Luo J. 2007. New species and new Chinese records of Bionectriaceae and Nectriaceae (Hypocreales, Ascomycetes) from Hubei, China. Fungal Diversity 24: 347–357.