Phylogeny and taxonomy of the genus Gliocephalotrichum

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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. nepethi (rambutan fruit from Guatemala), G. queenslandicum (Australia, endophytic isolations) and G. simmonsi (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.

INTRODUCTION

The asexual genus Gliocephalotrichum, with G. bulbilium as type, was introduced by Ellis & Heseltine (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 ascospores. A second species, G. ohiense (Wiley & Simmons 1971), is characterised by having superficial, uniloculate perithecia becoming purple in KOH+ and producing waivered if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

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Table 1  Glíocephalotrichum isolates included in this study.

| Species                  | Culture accession | GenBank accession | Substrate | Country     | Collector            |
|--------------------------|-------------------|-------------------|-----------|-------------|----------------------|
| **Gliocephalotrichum bacillisporum** | CBS 290.91       | KF513182          | plant root | Brazil      | L. Pfennig          |
|                          | CBS 126572 = MUCL 46554 | DQ374413          | leaf litter | French Guiana | C. Decock & V. Robert |
|                          | CBS 132042 = MUCL 46732 | DQ374414          | leaf litter | French Guiana | C. Decock & V. Robert |
|                          | CBS 242.62 = ATCC 22228 = IFO 9325 = IMI 096357 = NRRL 2899 = QM 9007 | DQ377831          | soil       | USA         | L.J. Wickerham       |
| **G. bulbilium**         | CBS 118.68        | KF513183          | air        | Central African Republic | J. Nicot          |
|                          | CBS 562.75        | KF513184          | soil       | Brazil      | L. Pfennig          |
|                          | CBS 451.92 = QS 92-7 = ATCC 90145 = BP1 1113065 | KF513185          | soil       | Puerto Rico | W.R. Buck           |
| **G. cylindrosporum**    | CBS 902.70 = ATCC 22229 = IFO 9326 = IMI 155704 = MUCL 18576 = QM 9009 | KF513208          | soil       | Thailand    | S. Chomchalo         |
|                          | CBS 903.70 = QM 9146 | DQ377842          | leaf litter | Taiwan      | P.W. Crous          |
|                          | CBS 904.70 = MUCL 18580 = QM 9147 | DQ374411          | soil       | South Africa | –                    |
| **G. grandis**           | HMAS 99302        | E9U80472          | leaf litter | China       | W.Y. Zhuang & Y. Nong |
| **G. humicola**          | CBS 139345        | KF513209          | soil       | Taiwan      | P.W. Crous          |
|                          | CPC 2334          | KF513210          | soil       | Taiwan      | P.W. Crous          |
|                          | CPC 23344         | KF513211          | soil       | Taiwan      | P.W. Crous          |
|                          | CPC 23345         | KF513212          | soil       | Taiwan      | P.W. Crous          |
|                          | CPC 23346         | KF513213          | soil       | Taiwan      | P.W. Crous          |
|                          | CPC 23347         | KF513214          | soil       | Taiwan      | P.W. Crous          |
|                          | CPC 23348         | KF513215          | soil       | Taiwan      | P.W. Crous          |
|                          | CPC 23349         | KF513216          | soil       | Taiwan      | P.W. Crous          |
| **G. longibrachium**     | CBS 126571 = MUCL 46694 | DQ377835          | leaf litter | French Guiana | C. Decock & V. Robert |
|                          | CBS 132043 = MUCL 46694 | DQ377836          | leaf litter | French Guiana | C. Decock & V. Robert |
| **G. maxicanum**         | CBS 139347        | KF513220          | leaf litter | Mexico      | L.M. Serrato-Diaz   |
|                          | CBS 139348        | KF513221          | leaf litter | Mexico      | L.M. Serrato-Diaz   |
|                          | CBS 139349        | KF513222          | leaf litter | Mexico      | L.M. Serrato-Diaz   |
| **G. microchaetosporum** | CBS 345.64 = ATCC 22230 = IFO 9329 = IMI 155706 = MUCL 4085 = QM 9042 | KF513226          | leaf litter | Guatemala    | L.M. Serrato-Diaz   |
|                          | CBS 21862 = MUCL 8137 | DQ374411          | soil       | Zaire       | J.A. Meyer          |
|                          | CBS 21863 = MUCL 18049 | DQ374412          | soil       | South Africa | –                    |
| **G. nepetelli**         | CBS 139349        | KF513222          | leaf litter | Guatemala    | L.M. Serrato-Diaz   |
| **G. ohinense**          | CBS 139560        | KF513223          | leaf litter | Guatemala    | L.M. Serrato-Diaz   |
| **G. orbiculatum**       | CBS 765.73 = ATCC 24879 = IMI 176508 = MUCL 39340 | DQ374415          | soil       | USA         | L.H. Huang          |
| **G. queenslandicum**    | CBS 112956 = CPC 4713 | KF513224          | leaf litter | Australia    | I. Steer & B. Paulus |
| **G. simpsonii**         | CBS 139561        | KF513225          | leaf litter | Australia    | I. Steer & B. Paulus |
|                          | CBS 139562        | KF513226          | leaf litter | Guatemala    | L.M. Serrato-Diaz   |
| **G. simplex**           | CBS 267.65 = ATCC 22231 = IFO 9330 = IMI 155705 = MUCL 18577 = QM 9041 | DQ377838          | soil       | South Africa | H.J. Swart          |
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). Mycotechère de l'Université catholique de Louvain (BCCM/MUCL) and the working collection of Pedro Crous (CPC) housed at CBS.

**Phylogen**

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 (TBUT), 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 all 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 × 1 000 magnification using
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

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**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 Glioccephalotrichum 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**.
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. 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. bulbiloid 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).
not collapsing when dry, consisting of two layers: outer region of thick-walled cells of \textit{textura angularis}, inner layer of elliptic to elongate cells. \textit{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. \textit{Conidiophores} consisting of a septate, hyaline, pale luteous to pale brown stipe and a penicillate arrangement of fertile branches subtended by septate stipe extensions. \textit{Conidiogenous apparatus} with a series of aseptate branches, each terminating in 2–8 phialides; \textit{phialides} clavate to cylindrical, hyaline, aseptate, constricted at the apex, with minute periclinal thickening. \textit{Conidia} cylindrical to ellipsoid, straight to slightly curved, aseptate, accumulating in a white to luteous mucoid mass above the phialides.

\textit{Gliocephalotrichum bacillisporum} Decock & Huret, Mycologia 98: 493. — MycoBank MB501190; Fig. 2

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

Specimens examined. \textbf{Brazil}, Pará, Belem, near Capitão Poço, root of unknown plant, May 1991, L. Pfennig, CBS 250.91 = L.P. 504. — \textbf{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.

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

= \textit{Leuconectria clusiae} (Samuels & Rogerson) Rossman, Samuels & Lowen, Mycologia 85: 686. 1993.

= \textit{Pseudonectria clusiae} Samuels & Rogerson, Mem. New York Bot. Gard. 64: 173. 1990.

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

Specimens examined. \textbf{Brazil}, from soil, Jan. 1995, L. Pfennig, CCI 4267 = CBS 104.95. — \textbf{Central African Republic}, La Maboké, from air sample, Feb. 1968, J. Nicot, CBS 118.68. — \textbf{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. — \textbf{Indonesia}, Java, Jakarta, from fruit of \textit{Flacourtia sp.}, Nov. 1975, I. Gandjar, CBS 562.75. — \textbf{Mexico}, from rotten fruit of \textit{Nephelium lappaceum}, 13 Sept. 2013, L.M. Serrato-Diaz, CPC 23334–23339. — \textbf{Puerto Rico}, Bosque Estatal de Guajataca, N18°24’, W66°58’, on decaying fruit of \textit{Clusia sp.}, 17 Jan. 1992, W.R. Buck, specimen BPI 1130065, culture ATCC 30145 = GJS92-7 = CBS 451.92; Mayaguez, USDA-ARS Tropical Agriculture Research Station, from rotten fruit of \textit{N. lappaceum}, 2 Feb. 2011, L.M. Serrato-Diaz, CPC 23321–23333. — \textbf{USA}, Louisiana, Tunica Hills, from a soil sample collected under moss, 24 Aug. 1960, L.J. Wickerham, holotype BPI 414619, culture ex-type NRRL 2898 = ATCC 22228 =IFO 9325 = IMI 096357 = MUCL 18575 = BPI 414619 = QM 9007 = CBS 242.62; North Carolina, Johnston County, from fruit of \textit{Nyssa sylvatica}, 15 Sept. 2006, T. Sutton, culture CPC 13577.
**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 penicilli 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 collar. *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 within 7 days. *Chlamydospores* formed singly, intercalary or terminally, globose to subglobose, hyaline, 8–17 µm diam, not forming bulbiloid 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).
Specimens examined. French Guiana, Cayenne area, Matoury, 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 128571; 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 aspetate, 12–20 × 3–6 µm; secondary branches aspetate, 8–11 × 2–5 µm; tertiary branches aspetate, 6–9 × 2–5 µm, each terminal branch producing 4–6 phialides; phialides cylindrical, slightly ventricose, hyaline, aspetate, 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 bulbiloid 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.
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**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; – Zaire, Yangambi, from soil, Mar. 1960, J.A. Meyer, culture ex-type ATCC 22230 = IFO 9329 = IMI 155706 = MUCL 4085 = OM 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; 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 bulbillloid aggregates on MEA and SNA. Sexual morph not observed.

Culture characteristics — Colonies fast growing (90 mm in 5 d), white with reverse sienna toumber; 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 bulbillloid aggregates. This was not observed for *G. mexicanum,* which in turn, only formed bulbillloid 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
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 terminal, globose to subglobose, hyaline turning brown with age, 43–53 µm diam, forming brown to dark brown, immersed bulbiloid 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 (50 mm in 5 d), white to pale luteous with reverse sienna; aerial mycelium sparse, with abundant conidiophores forming on immersed mycelium.

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 18553 = QM 9368 = CBS 983.69; Pará, Monte Dourado, from soil, Apr. 2011, R.A. 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, Sable 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
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. bacillioporum and G. londriformis 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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