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Multigene phylogenetic and morphological evidence for seven new species of *Aquanectria* and *Gliocladiopsis* (Ascomycota, Hypocreales) from tropical areas

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ABSTRACT

Aquanectria and *Gliocladiopsis* are two closely related genera of Hypocreales. They are also morphologically similar, forming hyaline, penicillate conidiophores and hyaline, straight to sinuous, 0–1-septate phialoconidia. During a revision of gliocladiopsis-like isolates originating from rain forest areas of South America (Ecuador, French Guiana) and Southeast Asia (Singapore), multilocus phylogenetic inferences, based on DNA sequences encoding partial β-tubulin (*TUB2*), translation elongation factor 1-α (*TEF1- α*), histone H3 (*HIS3*) genes and the nuc rDNA internal transcribed spacer region (ITS1-5.8S-ITS2 = ITS), revealed the occurrence of seven new phylogenetic species. These phylogenetic species also revealed unique combinations of phenotypes, allowing morphological distinction from their closest phylogenetic relatives. Four new species of *Aquanectria* and three new species of *Gliocladiopsis* are described and illustrated. Three of the four *Aquanectria* species deviate from the other species in the genus by having shorter conidia, which are in the size range observed in *Gliocladiopsis* species. They are placed in *Aquanectria* based on the phylogenetic analysis, but this also makes the morphological distinction between these two genera obsolete.

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Ecuador; oil pollution; Singapore; South America; Southeast Asia; systematics; 7 new taxa

INTRODUCTION

Gliocladiopsis (Hypocreales) is characterized by complex, hyaline, penicillate conidiophores, which consist of a simple-septate stipe bearing 2–4 successive whorls of branches subtending whorls of phialides. Conidia are hyaline, cylindrical, 0–1-septate, accumulating in whitish to pale yellowish mucoid drops (Lombard and Crous 2012). So far, connection to a teleomorph is known only for *G. pseudotenuis* (syn. *Glionectria tenuis* Crous & C.L. Schoch; Schoch et al. 2000).

Originally described with a single species, *G. sagariensis* (Saksena 1954), *Gliocladiopsis* was subsequently reduced to synonymy either under *Cylindrocarpon* (Agnihothrudu 1959), with the type species considered a synonym of *Cylindrocarpon tenue* Bugnic., or under *Cylindrocladium* (Barron 1968). Crous and Wingfield (1993) reconsidered the status of *Gliocladiopsis*, based on one species, *G. tenuis* (syn. *G. sagariensis*). Later, Crous and Peerally (1996) and Crous et al. (1997) added *G. irregularis* and *G. sumatrensis*.

Lombard and Crous (2012) provided the most comprehensive revision of *Gliocladiopsis*, combining morphological and phylogenetic species concepts; *G. sagariensis* and *G. tenuis* were accepted as two distinct species (Lombard and Crous 2012). Additionally, five species were added, namely, *G. curvata, G. elghollii, G. indonesiensis, G. mexicana*, and *G. pseudotenuis*, and two sterile strains were recognized as two phylogenetic species and left unnamed, designated as *Gliocladiopsis* sp. 1 and *Gliocladiopsis* sp. 2 (Lombard and Crous 2012). Liu and Cai (2013) proposed that *Gliocladiopsis* sp. 2 could be equated to *G. irregularis*. They also added *G. guangdongensis* based on a single strain isolated from southern China (Liu and Cai 2013). Parkinson et al. (2017) described three new species, *G. peggii, G. whileyi*, and *G. forsbergii*, isolated from avocado roots, in Australia.

Limited information is currently available on the ecology of these fungi. *Gliocladiopsis* species are primarily soil-borne fungi, isolated as soil-borne pathogens (Booth 1966; Crous et al. 1997) and from diseased roots (Dann et al. 2012; Parkinson et al. 2017) but also from asymptomatic rhizomes (Li et al. 2008). Their trophic or nutritional relationships, including their potential relevance as soil-borne pathogens (Booth 1966) or in contrast as growth promotors (Dann et al.

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2012), are poorly understood. Nevertheless, the type strain of *G. guangdongensis* was isolated from wood submerged in freshwater (Liu and Cai 2013), whereas unidentified *Gliocladiopsis* isolates were isolated as endophytes from the flower (*Rafflesia cantleyi*, Rafflesiacae; Refaei et al. 2011) and seeds (*Cecropia insignis*, Cecropiaceae; U'ren et al. 2009), suggesting a broader ecological range.

Gliocladiopsis is morphologically similar to *Aquanectria*, which is also its closest phylogenetic relative (Lombard et al. 2015). The conidiophores, conidiogenous cells, and conidiogenesis and, to some extent, the conidia are similar in both genera. As originally delimited, *Aquanectria* would differ in having longer, filiform to slightly sinuous conidia (Lombard et al. 2015), a morphology that might reflect an adaptation to aquatic habitats (Baschien et al. 2013). *Aquanectria* hitherto includes only two species, *A. penicillioides* (type species) and *A. submersa* (Lombard et al. 2015), which are both aquatic fungi growing on submerged plant debris (Ingold 1942; Hudson 1961; Duarte et al. 2012).

In this context, we reevaluated the identity and phylogenetic affinities of a set of gliocladiopsis-like strains originating from rain forests in South America (Ecuador and French Guiana) and Southeast Asia (Singapore). Using multilocus, DNA-based phylogenetic approaches, these strains were shown to belong to seven terminal clades, which could be equated to new phylogenetic species (Taylor et al. 2000). Each presents a distinct phenotype, allowing morphological distinction from closest phylogenetic relatives.

Three species nest within the *Gliocladiopsis* lineage as defined by Lombard and Crous (2012) or Parkinson et al. (2017). Their morphology agrees with this placement. One species belongs to the *Aquanectria* lineage as defined by Lombard et al. (2015), a relationship that also is concordant with its phenotype. The other three phylogenetic species group together in a single lineage, sister to the *Aquanectria* lineage. However, this is discordant with their phenotypes, because their conidial shapes and sizes point toward *Gliocladiopsis* rather than *Aquanectria*. *Aquanectria* is considered their natural classification based on their phylogenetic affinities; however, this placement and the appropriateness of continuing to recognize a distinction between these two genera can be questioned.

MATERIALS AND METHODS

Site and sampling procedure.—The strains from Ecuador were isolated from the rhizosphere and roots of several Monocotyledons and one Pteridophyte growing in a layer of organic debris forming a mat floating over oil ponds, in the Amazonian forest. Oil ponds are poor-quality oil deposits left in the Amazonian forest during the 1980s. Over time, these ponds were slowly covered by plant debris, the accumulation and decomposition of which resulted in a floating organic mat recolonized by plants, whose roots dive into the oil. The local vegetation is dominated by *Carludovica palmata* (Cyclanthaceae), *Dimerocostus strobilaceus* (Costaceae), *Heliconia* cf. *chartacea* (Heliconiaceae), and several species of Araceae. Weather conditions are characterized by warm temperatures and high humidity (Perez 2014).

Isolation of fungal strains.—In Ecuador, roots and adjacent soils were collected, placed into polyethylene bags, and kept in a refrigerator at 4–7 C until processed within 24 h. Roots were gently washed to remove the rough soil particles then rinsed with sterile water. This rinsing solution was diluted in 9 mL sterilized water (1 vol solution into 9 vol water). Of each dilution, 1 mL was poured on malt extract agar supplemented with 50 ppm of L-chloramphenicol (MEA50; Untereiner et al. 1998 [agar, Rocc AG0238-02, Sart-Eustache, Belgium; malt, Duchefa Biochemie 8002-48-0, Haarlem, The Netherlands; chloramphenicol, Applichem Panreac A6435,0100, Darmstadt, Germany), in duplicate. The plates were incubated in the dark at 25 C and examined daily for fungal growth. Pure cultures were obtained from germinating spores or growing hyphae and transferred to MEA in 9-cm-diam Petri dishes.

To isolate endophytes, roots were washed to remove soil particles, cut into six 5-cm-long segments, and surface-sterilized using successive baths: 1 min in 99% (*v*/*v*) ethanol, 5 min in 35% (*v*/*v*) hydrogen peroxide, 1 min in 99% (*v*/*v*) ethanol, then rinsed with sterile, distilled water for a few minutes. Immediately after rinsing, segments of 2–3 mm length were aseptically excised from the middle of each root piece and plated on MEA50 (see above). The plates were incubated at 25 C in the dark (Ahlich and Sieber 1996) and examined every day for fungal growth. Isolates were obtained by excising hyphal tips emerging from the root pieces.

Strains from Singapore were obtained by C.D. by a single conidium isolation from gliocladiopsis-like colonies emerging from decaying leaves submerged in freshwater. The strain from French Guiana was obtained by C.D. by a single conidium isolation from a gliocladiopsis-like colony emerging from a fragment of a basidiome of a *Phellinus* sp. (Basidiomycota, Hymenochaetaceae), plated on on MEA50 (see above).

All strains obtained were deposited at BCCM/MUCL (Agro-food & Environmental Fungal Collection).

Morphological characterization.—Cultures were grown on malt extract agar (MEA; see above), synthetischer nährstoffarmer agar (SNA [agar, Rocc AG0238-02]) and banana leaf agar (BLA [agar, Rocc AG0238-02]), at 25 C with a 12/12 h near-ultraviolet (UV) light/dark cycle (Untereiner et al. 1998). Cultural characters were determined at 7 d after inoculation. As a rule, the descriptions of the conidiophores and conidia are based on morphologically informative structures developed on BLA. The conidiophore organization is described from the base upward, with a stipe giving rise to an apical series of branches (the primary branches arising from the stipe) subtending the phialides. For conidial measurements, the 95% confidence levels were determined, and the extremes of the conidial measurements are given in parentheses. For other structures, only the extremes are presented. Colors of the colonies are described using capitalized color names and alphanumeric codes derived from Kornerup and Wanscher (1981).

DNA isolation, amplification, sequencing, and data sets.—DNA was extracted from mycelium grown in malt broth at 25 C in the dark, using innuPREP Plant DNA kit (Analytik, Jena, Germany) following the manufacturer's recommendations. Sequences were determined for partial genes encoding β-tubulin (*TUB2*; region between exon 1 and exon 4), translation elongation factor 1-α (*TEF1-α*; region between exon 1 and exon 4), and histone H3 (*HIS3*; region between exon 1 and exon 3) and the nuc rDNA internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) region. The *TUB2* gene fragment was amplified using primer pair T1 (O'Donnell and Cigelnik 1997) and Bt-2b (Glass and Donaldson 1995). The *TEF1-α* gene fragment was amplified using the primers ef1 and ef2 (O'Donnell et al. 1998). The *HIS3* gene fragment was amplified with primer pair H_3-1a and H_3-1b (Glass and Donaldson 1995). The ITS region was amplified with the primer pair ITS5 and ITS4 (White et al. 1990). Polymerase chain reaction (PCR) conditions were as described in Lombard et al. (2010). Amplicons were sequenced in both directions by Macrogen (Seoul, Korea) using the same primers.

Raw sequences were edited with Sequencher 5.1 (Gene Codes, Ann Arbor, Michigan). Reference sequences (Lombard and Crous 2012; Lombard et al. 2015; Parkinson et al. 2017) were downloaded from GenBank. Nucleotide sequence alignments were determined using MAFFT 7.213 (Katoh and Standley 2013) and manually corrected in PhyDE 1 (Müller et al. 2006) when necessary. Species, strains, and sequences used in this study are listed in TABLE 1.

Three data sets were assembled to conduct phylogenetic inferences. The concatenated alignments are deposited at TreeBASE (study nos. S21752, S21531, and S21757).

The first data set comprises 56 strains of *Gliocladiopsis* (13 species), *Aquanectria* (2 species), *Dematiocladium* (1 species), *Penicillifer* (1 species), *Corallonectria* (1 species), *Cylindrocladiella* (1 species), our unidentified gliocladiopsis-like isolates, and *Calonectria* (2 species) selected as outgroup (Lombard et al. 2015). The second data set comprises the 13 species of *Gliocladiopsis* and our unidentified gliocladiopsis-like strains that, as a result of the first set analysis, were found to belong to the *Gliocladiopsis* lineage (Lombard and Crous 2012). *Calonectria brachiatica* and *C. brassicae* were selected as outgroups (Lombard and Crous 2012). The third data set includes the described species of *Aquanectria* (Lombard et al. 2015) and several of our unidentified gliocladiopsis-like strains that, as a result of the first analysis, were found to belong to or to be related to *Aquanectria* (Lombard et al. 2015).

Phylogenetic analysis.—Phylogenetic analyses of the three data sets were performed using maximum parsimony (MP) as implemented in PAUP* 4.0b10 (Swofford 2003), Bayesian inference (BI) as implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), and maximum likelihood (ML) using RAxML 7.0.4 (Stamatakis 2014). The best-fit likelihood model of evolution for the different data sets was estimated using PartitionFinder (Lanfear 2012).

For MP analyses, gaps were treated as missing data. The most parsimonious trees were identified using heuristic searches with 1000 random addition sequences, further evaluated by bootstrap analysis, retaining clades compatible with the 50% majority rule in the bootstrap consensus tree. Analysis conditions were tree bisection addition branch swapping, starting tree obtained via stepwise addition, steepest descent not in effect and MULTREES in effect. Clades with bootstrap support value (BS) above 90% were considered strongly supported by the data.

Bayesian inference (BI) analyses were implemented with two independent runs, each with four Markov chain Monte Carlo (MCMC) simultaneous independent chains, starting from random trees, and keeping one tree every 1000th generation. All trees sampled after convergence (average standard deviation of split frequencies <0.01, confirmed using Tracer 1.4 [Rambaut and

Table 1. List of species, collections, and sequences used in the phylogenetic analyses.

Genus/species name			GenBank accession number			
Voucher specimen/ culture reference	Substrate	Country	*TUB2*	*HIS3*	ITS	*TEF1-a*
Aquanectria L. Lombard & Crous						
Aquanectria penicillioides (Ingold) L. Lombard & Crous						
CBS 257.54; ATCC 16261	Acer sp.	USA	KM232000	—	KM231743	KM231865
Aquanectria submersa (H.J. Huds.) L. Lombard & Crous						
CBS 394.62	Unknown	UK	KM231999	KM231458	HQ897796	—
Aquanectria filiformis Gordillo & Decock						
MUCL 54681	Endophyte root, *Monotagma* sp.	Ecuador	KX611499	KX671145	KX671137	KX671129
Aquanectria devians						
MUCL 48197	Submerged leaf litter in freshwater	Singapore	KX611506	KX671150	KX671144	KX671136
Aquanectria tenuispora						
MUCL 48047	Submerged leaf litter in freshwater	Singapore	KX611505	KX671149	KX671143	KX671135
MUCL 48016	Submerged leaf litter in freshwater	Singapore	KX611503	KX671147	KX671141	KX671133
Aquanectria tenuissima						
MUCL 53250	Unknown	French Guiana	KX611504	KX671148	KX671142	KX671134
Calonectria de Not.						
Calonectria brachiatica L. Lombard et al.						
CBS 123700	*Pinus maximinoi*	Colombia	FJ696388	FJ696396	GQ280555	GQ267296
Calonectria brassicae (Panwar & Bohra) L. Lombard et al.						
CBS 111869	—	—	AF232857	DQ190720	GQ280576	FJ918567
Corallonectria C. Herrera & P. Chaverri 2013						
Corallonectria jatrophae (Möller) C. Herrera & P. Chaverri						
CBS 913.96(T); GJS 96-18	Unknown tree	Puerto Rico	KC479787	KM231457 KC479758	KM231863	
Cylindrocladiella Boesew						
Cylindrocladiella camelliae (Venkataram. & C.S.V. Ram) Boesew						
CPC 234 (T); PPRI 3990; IMI 346845	*Eucalyptus grandis*	South Africa	AY793471	AY793509	AF220952	JN099087
Dematiocladium Allegr., Aramb., Cazau & Crous						
Dematiocladium celtidis Allegr., Aramb., Cazau & Crous						
CBS 115994 (T)	*Celtis tala*	Argentina	—	—	AY793430	KM231864
Gliocladiopsis S.B. Saksena						
Gliocladiopsis curvata L. Lombard & Crous						
CBS 978.73	soil	Brazil	JQ666119	JQ666009	JQ666043	JQ666085
CBS 194.80	*Persea americana*	Ecuador	JQ666120	JQ666010	JQ666044	JQ666086
CBS 110840	Greenhouse	Belgium	JQ666121	JQ666011	JQ666045	JQ666087
CBS 111194	Soil	Mauritius	JQ666122	JQ666012	JQ666046	JQ666088
CBS 111195	Soil	Mauritius	JQ666123	JQ666013	JQ666047	JQ666089
CBS 111196	Soil	Mauritius	JQ666124	JQ666014	JQ666048	JQ666090
CBS 111421	Soil	Ecuador	JQ666125	JQ666015	JQ666049	JQ666091
CBS 112365(T)	*Archontophoenix purpurea*	New Zealand	JQ666126	JQ666016	JQ666050	JQ666092
CBS 112935	*Syzygium aromaticum*	Indonesia	JQ666127	JQ666017	JQ666051	JQ666093
CBS 114464	Soil	Ecuador	JQ666128	JQ666018	JQ666052	JQ666094
CBS 115688	—	Japan	JQ666129	JQ666019	JQ666053	JQ666095
Gliocladiopsis ecuadoriensis Gordillo & Decock						
MUCL 54740	Rhizospher, *Polybotrya* sp.	Ecuador	KX611501	KX671146	KX671139	KX671131
Gliocladiopsis elghollii L. Lombard & Crous						
CBS 206.94	*Chamaedorea elegans*	USA	JQ666130	JQ666020	JQ666054	JQ666096
CBS 116104 (T)	*Chamaedorea elegans*	USA	JQ666131	JQ666021	JQ666055	JQ666097
Gliocladiopsis forsbergii L.E. Parkinson, E.K. Dann & R.G. Shivas						
BRIP 61349 (T)	*Persea americana*	Australia	KX274037	KX274054	KX274071	—
Gliocladiopsis guangdongensis F. Liu & L. Cai						
LC 1340	Submerged wood	China	KC776124	KC776120	KC776122	KC776118
Gliocladiopsis hennebertii Gordillo & Decock						
MUCL 54818	Rhizospher, *Costus scaber*	Ecuador	KX611502	—	KX671140	KX671132
Gliocladiopsis indonesiensis L. Lombard & Crous						
CBS 116090 (T)	Soil	Indonesia	JQ666132	JQ666022	JQ666056	JQ666098
Gliocladiopsis irregularis Crous & Peerally						
CBS 755.97(T)	Soil	Indonesia	JQ666133	JQ666023	AF220977	JQ666099

(*Continued*)

Table 1. (Continued).

Genus/species name			GenBank accession number			
Voucher specimen/ culture reference	Substrate	Country	*TUB2*	*HIS3*	ITS	*TEF1-α*
CBS 111142	*Araucaria* sp.	Malaysia	JQ666134	JQ666024	JQ666057	JQ666100
CBS 111176	*Araucaria* sp.	Malaysia	JQ666135	JQ666025	JQ666058	JQ666101
CBS 114667	*Araucaria* sp.	Malaysia	JQ666136	JQ666026	JQ666059	JQ666102
Gliocladiopsis mexicana L. Lombard & Crous						
CBS 110938 (T)	Soil	Mexico	JQ666137	JQ666027	JQ666060	JQ666103
CBS 111131	Soil	Mexico	JQ666138	JQ666028	JQ666061	JQ666104
Gliocladiopsis peggii L.E. Parkinson, E.K. Dann & R.G. Shivas						
BRIP 55019	*Persea americana*	Australia	JN243766	JN243767	JN243765	—
BRIP 60983 (T)	*Persea americana*	Australia	KX274038	KX274065	KX274083	—
Gliocladiopsis sagariensis S.B. Saksena						
CBS 199.55 (T)	Soil	India	JQ666141	JQ666031	JQ666063	JQ666107
Gliocladiopsis singaporiensis Decock & Gordillo						
MUCL 48728	Submerged leaf litter in freshwater	Singapore	KX611500	—	KX671138	KX671130
MUCL 48412	Submerged leaf litter in freshwater	Singapore	—	—	—	—
Gliocladiopsis sumatrensis Crous & M.J. Wingf.						
CBS 754.97 (T)	Soil	Indonesia	JQ666142	JQ666032	JQ666064	JQ666108
CBS 111198	Soil	Indonesia	JQ666143	JQ666033	JQ666065	JQ666109
CBS 111213	Soil	Indonesia	JQ666144	JQ666034	JQ666066	JQ666110
CBS 111368	Soil	Indonesia	JQ666145	JQ666035	AF220978	JQ666111
Gliocladiopsis pseudotenuis L. Lombard & Crous						
CBS 114763	*Vanilla* sp.	Indonesia	JQ666139	JQ666029	JQ666062	JQ666105
CBS 116074 (T)	Soil	China	JQ666140	JQ666030	AF220981	JQ666106
Gliocladiopsis tenuis (Bugnic.) Crous & M.J. Wingf						
CBS 111961	*Coffea* sp.	Vietnam	JQ666146	JQ666036	JQ666067	JQ666112
CBS 111964	*Coffea* sp.	Vietnam	JQ666147	JQ666037	JQ666068	JQ666113
CBS 114147	Soil	Vietnam	JQ666148	JQ666038	JQ666069	JQ666114
CBS 114148	Soil	Vietnam	JQ666149	JQ666039	JQ666070	JQ666115
IMI 68205 (T)	*Indigofera* sp.	Indonesia	JQ666150	JQ666040	AF220979	JQ666116
Gliocladiopsis whileyi L.E. Parkinson, E.K. Dann & R.G. Shivas						
BRIP 61340 (T)	*Persea americana*	Australia	KX274052	KX274069	KX274086	—
Gliocladiopsis sp.1						
CBS 111038	Soil	Colombia	JQ666151	JQ666041	JQ666071	JQ666117
Gliocladiopsis sp.2						
CBS 116086	Soil	Indonesia	JQ666152	JQ666042	JQ666072	JQ666118
Penicillifer Emden						
Penicillifer pulcher Emden						
CBS 560.67(T); ATCC 18931; MUCL 11607						
BRIP 61340	Soil	The Netherlands	KM231998	KM231456	KM231742	KM231862

Note. T = type. —, missing number.

Drummond 2007]) were used to reconstruct a 50% majority-rule consensus tree (BC) and to estimate posterior probabilities. Clades with posterior probability (PP) above 0.95 were considered strongly supported by the data (Simmons et al. 2004).

ML searches were conducted with RAxML involving 1000 replicates under the GTRGAMMA model. In addition, 1000 bootstrap (BSML) replicates were run with the same model. Nodes with maximum bootstrap values ≥85% were considered to be significantly supported.

To detect topological conflicts among data partitions, the nodes between the trees obtained in the ML analysis from the individual loci were compared. Paired trees were examined for conflicts only involving nodes with bootstrap support values >70%. A conflict was assumed to be significant if two different relationships for the same set of taxa (one being monophyletic and the other nonmonophyletic) were observed on the different trees (Mason-Gamer et al. 1996; Lombard et al. 2015).

RESULTS

Phylogenetic analysis.—The amplification products of *TUB2, TEF1-α, HIS3*, and ITS ranged from ~500 to 550 bases each. The *TUB2* gene fragment is missing for the type strain of *Dematiocladium celtidis*. The *HIS3* gene fragments could not be amplified for the strains MUCL 48728, MUCL 48412, and MUCL 54818; it is also missing for the type strain of *Aquanectria penicillioides* and *D. celtidis*. The *TEF1-α* gene fragment is missing for *A. submersa* CBS 394.62, *Gliocladiopsis forsbergii* BRIP 61349, *G. peggii* BRIP 60983 and BRIP 55019, and *G. whileyi* BRIP 61340.

Individual data set comparisons.—Tree topologies, considering the 70% reciprocal bootstrap criterion, showed some conflicts in the terminal clades among the individual DNA loci (ITS, *TUB2, TEF1-α*, and *HIS3*), in all three data sets used. Each of the individual phylogenies

resolved most but not all of the same terminal clades/branches or phylogenetic species, within *Gliocladiopsis* and *Aquanectria* (SUPPLEMENTARY FILES 1–8). For example, in the *TEF1-α*-based inferences, using the *Gliocladiopsis* data set (second data set), including all the positions, *G. curvata* was not monophyletic (SUPPLEMENTARY FILE 2), whereas in the ITS-based inferences, *G. irregularis, G. sumatrensis, G. indonesiensis*, and *G. mexicana* were unresolved as distinct phylogenetic species (SUPPLEMENTARY FILE 1). Using the *Auqanectria* data set (second data set), including all the positions, the relationships of the stains MUCL 48197, MUCL 48047, and MUCL 48016 also conflicted; they formed either a single clade (ITS, *TEF1-α*; SUPPLEMENTARY FILES 5, 6) or two closely related clades (*TUB2, HIS3*; SUPPLEMENTARY FILES 7, 8).

The concatenated data set resolved all of the phylogenetic species identified to date (Lombard and Crous 2012; Parkinson et al. 2017) and several additional terminal clades/branches formed by our own isolates. The degree of polymorphism of *TUB2* makes it, hitherto, the most suitable marker for species discrimination within *Gliocladiopsis* and our unidentified strains related to *Aquanectria*, followed by *HIS3*. However, *HIS3* could not be amplified for some of our strains (TABLE 1). The ITS was the least discriminant marker.

Concerning the separation of the *Gliocladiopsis* and *Aquanectria* lineages, the results were inconclusive. The *Aquanectria* lineage (sensu Lombard et al. 2015) was present in all the phylogenies but well supported only in the *TEF1-α*- and *TUB2*-based inferences (SUPPLEMENTARY FILES 10, 12). Inversely, the *Gliocladiopsis* lineage sensu Lombard and Crous (2012) or Parkinson et al. (2017) occurred in the ITS-, *TEF1-α*-, and *TUB2*-based inferences but was not a well-supported clade in any of them (SUPPLEMENTARY FILES 9, 10, 12). Only the *TEF1-α*-based phylogenetic inference grouped *Gliocladiopsis* and *Aquanectria* in two well-distinct sister lineages, as shown by Lombard et al. (2015) (SUPPLEMENTARY FILE 10). The inferences based on the other genes remained equivocal about the relationships between these two clades (SUPPLEMENTARY FILES 9, 11, 12) because of the lack of support.

Nevertheless, as emphasized by Cunningham (1997), combining "noncongruent" gene partitions could increase phylogenetic accuracy. This was the case in previous phylogenetic studies dealing with various groups of Hypocreales, including, e.g., cylindrocarpon-like species (Lombard et al. 2014), *Cylindrocladium* (Gehesquière et al. 2016), or *Cylindrocladiella* (Lombard et al. 2017). Therefore, the five gene regions also were combined in our study.

Concatenated data set analysis.—The final first concatenated data set comprised 2447 positions, including gaps. Four segments located in the introns 1 and 2 of *HIS3* (108 positions), in the introns 1 and 3 of *TUB2* (50 positions), in the ITS*2* (110 positions), and in the introns 1 and 2 of *TEF1-α* (19 positions) could not be confidently aligned and were excluded from the analysis. The general time-reversible model (GTR+I+G), using proportion of invariant sites and distribution of rates at variable sites modeled on a discrete gamma distribution with four rate classes, was estimated to be the best-fit likelihood model of evolution for BI and ML. The base frequencies are A = 0.22, C = 0.33, G = 0.22, T = 0.22, the gamma distribution shape parameter = 0.73, and the proportion of invariable sites = 0.41.

For the MP analysis, gaps were excluded, 1368 positions were constant, 237 variable-uninformative, and 612 parsimony-informative. The heuristic search yielded 57 most parsimonious trees (length = 977 steps, consistency index [CI] = 0.648, retention index [RI] = 0.773. The differences in the topologies between the 20 trees result from variable relationships within the main *Gliocladiopsis* lineage, especially within the *G. curvata* species clade. The two Bayesian runs converged to stable likelihood values after 330 100 generations. In the ML searches, the combined data set had 1024 distinct patterns, with a proportion of gaps and undetermined characters of 10.60%. The topologies obtained were overall highly concordant in the resolution of the terminal species clades, regardless of the methodology (cladistic or probabilistic).

The second final concatenated data set comprises 2264 positions, including gaps. This analysis includes the segments that were removed from the first data set (see above). The general time-reversible model (GTR+I+G), using proportion of invariant sites and distribution of rates at variable sites modeled on a discrete gamma distribution with four rate classes, was estimated to be the best-fit likelihood model of evolution for BI and ML. The base frequencies are A = 0.22, C = 0.32, G = 0.22, T = 0.22, the gamma distribution shape = 0.68, and the proportion of invariable sites = 0.48.

For the MP analysis, gaps were excluded, 1606 positions were constant, 77 variable-uninformative, and 356 parsimony-informative. The heuristic search yielded 118 most parsimonious trees (length = 679 steps, CI = 0.772, RI = 0.88. The differences in the topologies between the 118 trees results from variable relationships within the main *Gliocladiopsis* lineage and within the *G. curvata* clade. The two BI runs converged to stable likelihood values after 650 000 generations. In

the ML searches, the combined data set alignment had 558 distinct patterns, with a proportion of gaps and undetermined characters of 8.16%. The topologies obtained were overall highly concordant in the resolution of the terminal species clades, regardless of the method.

The third final concatenated data set comprises 2268 positions, including gaps. The best-estimated model was JC+G with unequal base frequencies for ITS (A = 0.23, C = 0.28, G = 0.24, T = 0.23, gamma distribution shape parameter of 0.49) and HKY+G with unequal base frequencies for *TUB2, HIS3*, and *TEF1-α* (A = 0.23, C = 0.33, G = 0.21, T = 0.21, gamma distribution shape parameter of 0.26).

For the MP analysis, gaps were excluded, 1751 positions were constant, 148 variable-uninformative, and 157 parsimony-informative. The heuristic search yielded one single parsimonious trees (length = 378 steps, CI = 0.93, RI = 0.88). The two BI runs converged to stable likelihood values after 10 000 generations. In the ML searches with RAxML, the combined data set alignment had 343 distinct patterns, with a proportion of gaps and undetermined characters of 13.78%. The topologies obtained were highly concordant in the terminal clades in all cases.

The first concatenated data set phylogenetic analyses resolved 28 species clades (FIG. 1). Twenty-two species clades were distributed into two well-supported related lineages (FIG. 1), viz., an expanded *Aquanectria* lineage (BS = 100, BSML = 100, PP = 1) and the *Gliocladiopsis* lineage (BS = 100, BSML = 90, PP = 1). The *Aquanectria* lineage was divided into *Aquanectria* sensu Lombard et al. (2015) lineage (BS = 100, BSML = 100, PP = 1) and an additional clade (BS = 100, BSML = 100, PP = 1), sister to the former. *Dematiocladium, Penicillifer, Corallonectria*, and *Cylindrocladiella* are basal to the well-supported (BS = 96, BSML = 94, PP = 1) *Gliocladiopsis-Aquanectria* lineage.

The *Gliocladiopsis* lineage sensu Lombard et al. (2012) or Parkinson et al. (2017), whether using the first (FIG. 1) or second (FIG. 2) data set, was strongly supported, regardless of the hypothesis, parsimony or probabilistic. This lineage was divided into three well-supported sublineages, confirming previous results (Lombard and Crous 2012; Parkinson et al. 2017).

The first sublineage (FIG. 1; BS = 95, BSML = 97, PP = 1) corresponded to the *G. sagariensis–G. elghollii* clade, also present in Lombard and Crous (2012). The singleton MUCL 54818 nested within this clade, forming a distinct branch.

The second sublineage (BS = 90, BSML = 94, PP = 1) corresponded to the *G. tenuis–Gliocladiopsis* sp. 1 clade, also detected by Lombard and Crous (2012). The strain MUCL 54740 nested within this clade, forming a well-supported subclade (BS =100, BSML = 99, PP = 1) with *Gliocladiopsis* sp. 1.

The strains MUCL 48412 and MUCL 48728 formed a distinct clade (FIGS. 1, 2; BS = 87, BSML = 93, PP = 1), which is related to the *G. curvata, G. forsbergii*, and *G. whileyi* clades (FIG. 2).

The *Aquanectria* lineage, as originally defined (Lombard et al. 2015), contained *A. penicillioides* and *A. submersa*. MUCL 54681 was closely related to these two species but formed a distinct branch (FIGS. 1, 3).

Four strains (MUCL 48016, MUCL 48047, MUCL 48197, and MUCL 53250) formed a distinct, well-supported lineage (FIGS. 1, 3; BS = 100, BSML = 100, PP = 1), sister to the *Aquanectria* lineage (Lombard et al. 2015). It is further divided into three subclades, corresponding to MUCL 48016 and MUCL 48047 (BS = 100, BSML = 100, PP = 1), MUCL 48197, and MUCL 53250.

Taxonomic conclusions.—Gliocladiopsis-like strains isolated from South America and Southeast Asia are shown to represent seven new lineages (FIGS. 1–3), which we interpret as phylogenetic species. Examination of the strains corresponding to each of these phylogenetic species revealed singular combinations of phenotypic characters, in particular the branching pattern of the conidiophores and the conidial shape and size, allowing morphological distinction from their closest phylogenetic relatives. Hence, they are interpreted as new species and described below.

Three species nest within the *Gliocladiopsis* lineage (Lombard and Crous 2012). Their morphology also agrees with the prevailing morphological circumscription of the genus. They have penicillate conidiophores and cylindrical, 0–1-septate conidia, with a size range of 12–22 µm long. They are described below as *Gliocladiopsis ecuadoriensis, G. hennebertii*, and *G. singaporiensis*.

MUCL 54681 nests together with *A. penicillioides* and *A. submersa* (Lombard et al. 2015). The morphology of this strain also agrees with the current morphological circumscription of the genus (Lombard et al. 2015). *Aquanectria* was distinguished from *Gliocladiopsis* by having much longer, straight to variably sinuous conidia. *Aquanectria penicillioides* and *A. submersa* have sinuous conidia, with size ranges of 45–55 × 2–3 µm (Ingold 1942; Ranzoni 1956; Lombard et al. 2015) and 26–44 × 1.5–2.5 µm (Hudson 1961), respectively. MUCL 54681 produces straight to only occasionally slightly sinuous conidia, with a size range of 25–30 × 2 µm. It is described below as *Aquanectria filiformis*.

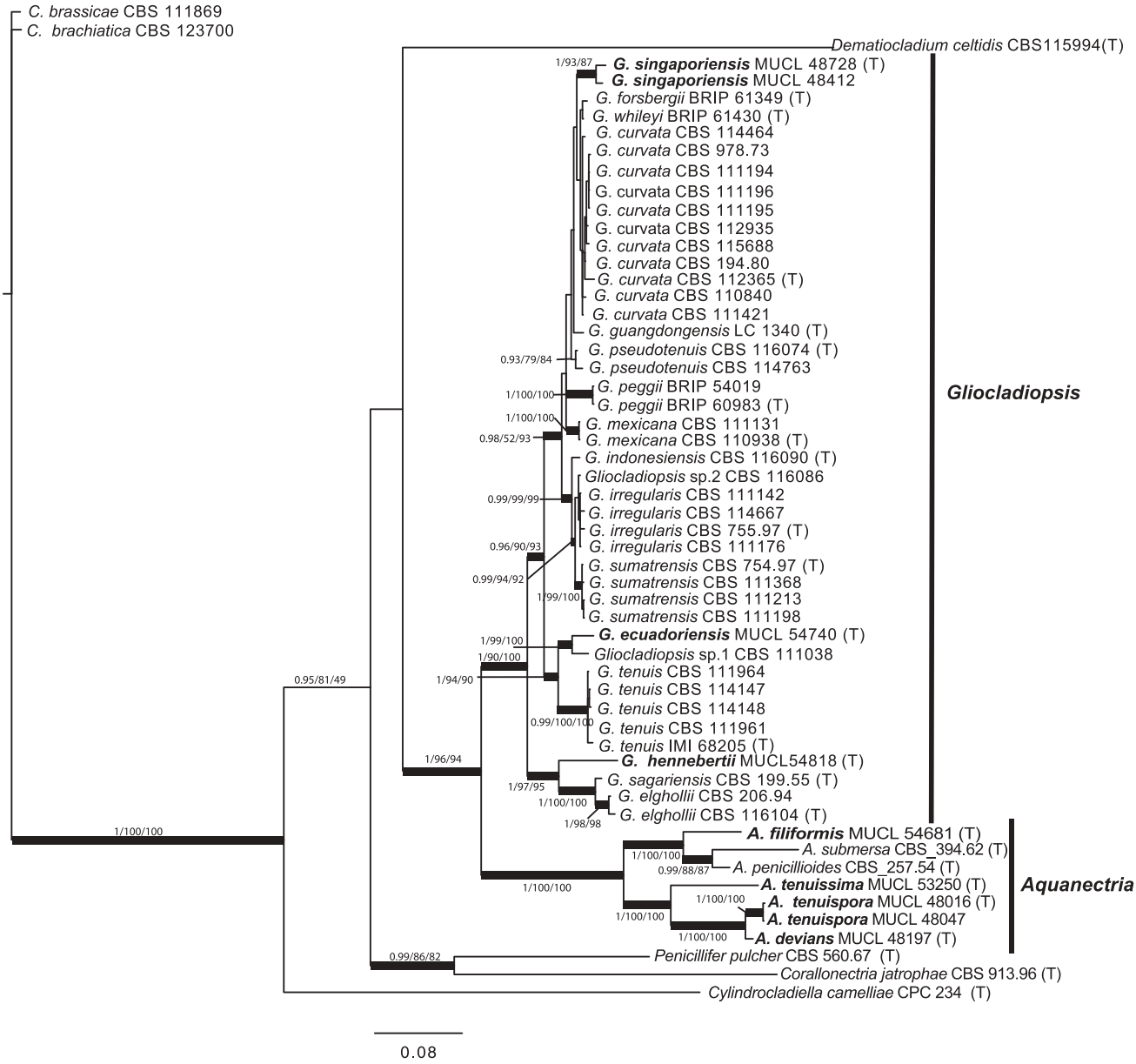

Figure 1. Multilocus phylogenetic tree (Bayesian inference) derived from a combined four-gene sequence alignment from *Aquanectria* and *Gliocladiopsis* data sets. New species are indicated in bold. Bootstrap support values and posterior probability are indicated with bold lines when PP (≥0.95)/ML (≥85%)/MP (≥90%). Thickened lines represent nodes also present in ML and MP trees. The tree was rooted to *Calonectria brachiatica* and *Calonectria brassicae*. Bar = 10 changes.

The three phylogenetic species represented by MUCL 48016 and MUCL 48047, MUCL 53250, and MUCL 48197 formed together a well-supported clade (BS = 100, BSML = 100, PP = 1), sister to the *Aquanectria* lineage (FIG. 1) (Lombard et al. 2015). However, their phenotypes deviate, to some extent, from *Aquanectria* as originally defined (Lombard et al. 2015). They have much shorter conidia, which are in the size range observed in *Gliocladiopsis* species; morphology-based taxonomy would place these strains in *Gliocladiopsis*. However, giving preference to the phylogenetic analyses (e.g., Lombard et al. 2015), they are described below as *A. devians*, *A. tenuispora*, and *A. tenuissima*, a decision more fully considered in Discussion.

TAXONOMY

Aquanectria devians Gordillo & Decock, sp. nov. FIG. 4a–b

MycoBank MB823328

Typification: SINGAPORE. MacRitchie Reservoir Park, approx. 1°20′41″N, 103°49′20″E, a dried, 2-wk-old culture on BLA, isolated from a decaying leaf submerged in freshwater, Dec 2001, *Olivier Laurence, isol.*

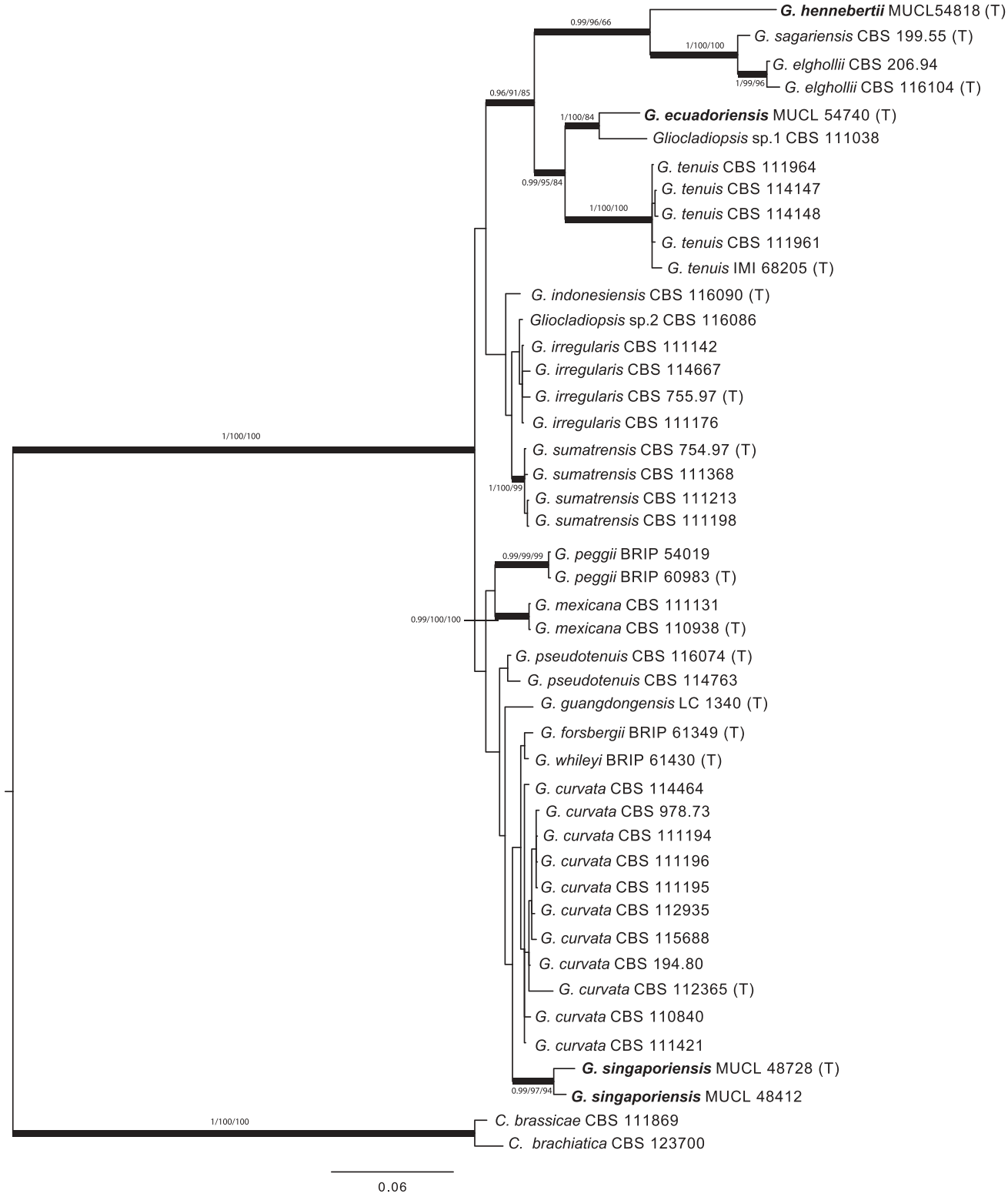

Figure 2. Multilocus phylogenetic tree (Bayesian inference) derived from a combined four-gene sequence alignment from *Gliocladiopsis* data set. New phylogenetic species are indicated in bold. Bootstrap support values and posterior probability are indicated with bold lines PP (≥0.95)/ML (≥85%)/MP (≥90%). Thickened lines represent nodes also present in ML and MP trees. The tree was rooted to *Calonectria brachiatica* and *Calonectria brassicae*. Bar = 10 changes.

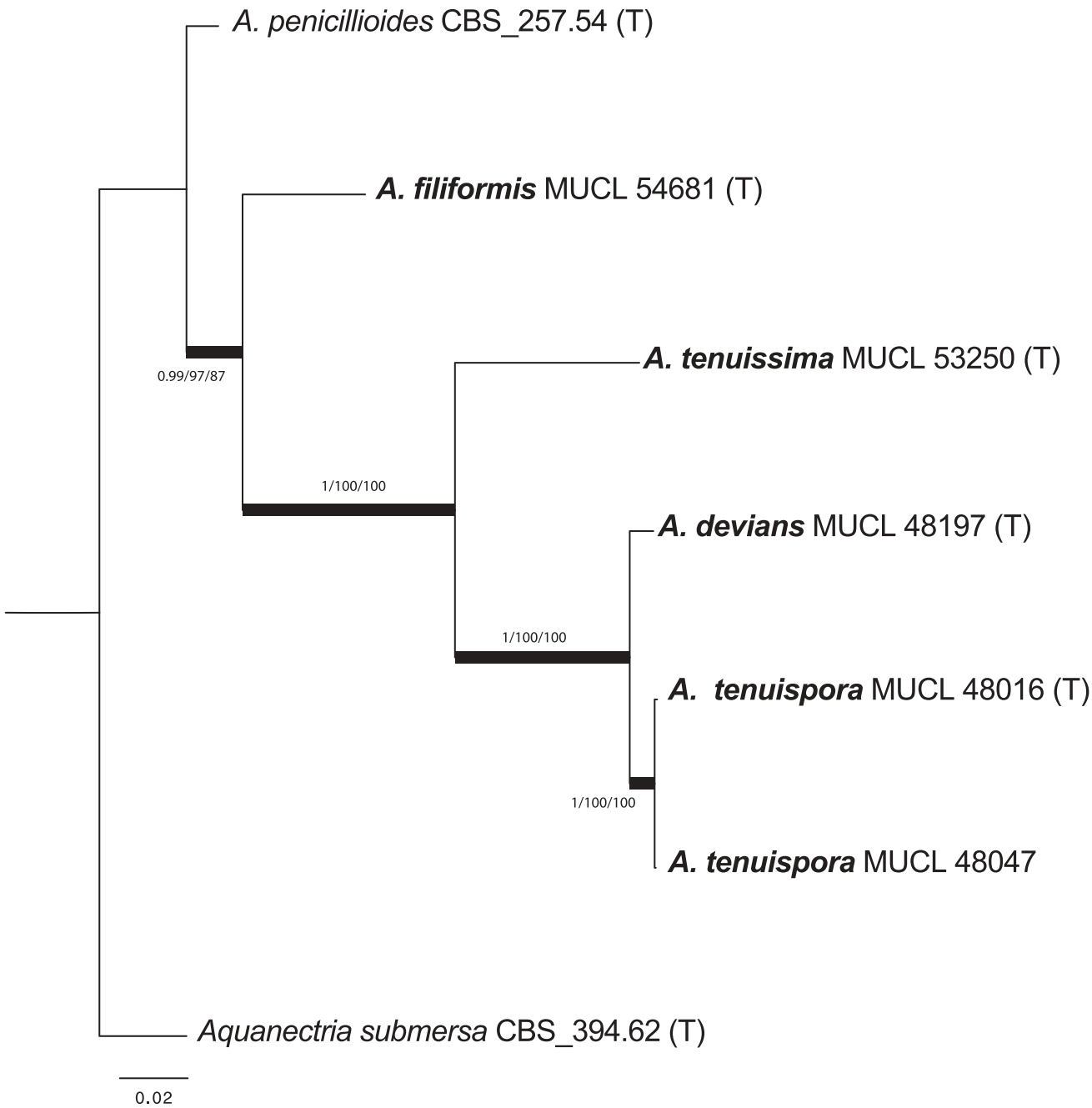

Figure 3. Multilocus phylogenetic tree (Bayesian inference) derived from a combined four-gene sequence alignment from *Aquanectria* data set. New phylogenetic species are indicated in bold. Bootstrap support values and posterior probability are indicated with bold lines PP (≥0.95)/ML (≥85%)/MP (≥90%). Thickened lines represent nodes also present in ML and MP trees. The tree was rooted to *Aquanectria sumersa*. Bar = 10 changes.

Cony Decock, from colonies on natural substrate (**holotype** MUCL 48197). Ex-type culture: MUCL 48197. GenBank: *HIS3* = KX671150; ITS = KX671144; *TEF1-α* = KX671136; *TUB2* = KX611506.

Etymology: *devians* (Latin), referring to small size of the conidia, the smallest conidia hitherto known in the genus.

Cultural characters: Colonies on SNA effuse, reaching 5 mm diam in 7 d, 40 mm diam in 14 d; aerial mycelium hyaline. Colonies on MEA reaching 40 mm diam in 7 d, 65 mm diam in 14 d, with a concentric growth pattern; aerial mycelium finely floccose, white, the reverse Dark Brown (6F5).

Conidiophores hyaline, penicillate, with a stipe and an apical series of branches subtending the phialides; stipe hyaline, thin-walled, 80–160 × 2 μm with 1(–3) levels of hyaline branches; primary branches aseptate,

15.5–17 × 2 µm; secondary branches aseptate, 8–12.5 × 2 µm; tertiary branches absent to rare, 8–10 × 2 µm; phialides cymbiform, 8–12 × 2 µm, in whorls of 2–4; conidia cylindrical, straight with rounded ends, hyaline, smooth, 0(–1)-septate, (10–)11–16(–17) × 1.5–2 µm, av. = 14 × 1.6 µm; chlamydospores not observed. Teleomorph not observed.

Substratum: Submerged leaf in fresh water.

Habitat: Rain forest.

Distribution: Singapore.

Notes: Aquanectria devians has the smallest conidia in the genus, averaging 14 µm long, a character that distinguished it from all other species of the genus. In that sense, it is the most "deviating" species of *Aquanectria* as originally conceived (Lombard et al. 2015).

Gliocladiopsis irregularis has conidia similar in length, averaging 13 × 2.5 µm (Crous and Peerally 1996). However, conidia are 2.5–3 µm wide in *G. irregularis* (Crous and Peerally 1996) but thinner, and 1.5–2 µm wide in *A. devians* (FIG. 4a).

Aquanectria filiformis Gordillo & Decock, sp. nov. FIG. 5a–b

MycoBank MB823329

Typification: ECUADOR. SUCUMBÍOS PROVINCE: Nueva Loja, Lago Agrío canton, Charapa camp, approx. 76°48′W, 00°11′S, secondary rain forest, a dried 2-wk-old culture on BLA isolated from root of *Monotagma* sp. (Marantaceae), Nov 2012, *A. Gordillo & C. Decock, PUCE PHPE1-14-54* (**holotype** MUCL 54681). **Isotype** PUCE PHPE1-14-54. Ex-type culture: MUCL 54681 = PUCE PHPE1-14-54. GenBank: *HIS3* = KX671145; ITS = KX671137; *TEF1-α* = KX671129; *TUB2* = KX611499.

Etymology: filiformis (Latin), referring to the shape of the conidia.

Cultural characters: Colonies on SNA effuse, reaching 4 mm diam in 7 d, 15 mm diam in 14 d; aerial mycelium hyaline. Colonies on MEA reaching 21 mm diam in 7 d, 42 mm diam in 14 d with a concentric growth pattern; aerial mycelium floccose, Greyish Brown (5D3), Yellowish Brown (5E8) toward the center, Pale Orange (5A3) toward the margin, the reverse Dark Brown (5E4).

Conidiophores hyaline, penicillate, with a stipe and an apical series of branches subtending the phialides; stipe hyaline, thin-walled, up 31–47 × 3 µm, with 1(–3) series of hyaline branches; primary branches aseptate, 16–31 × 2 µm; secondary branches aseptate, 9–14 × 2 µm; tertiary branches absent to rare, aseptate, 9–12.5 × 1.5 – 2 µm, with terminal branches bearing 2–3 phialides; phialides cylindrical to cymbiform, 12–17 × 2 µm; conidia filiform, straight to occasionally slightly sigmoid, with rounded ends, hyaline, smooth, guttulate, 0–1-septate, (23–)24–30(–31) × 1.5–2 µm, av. = 27 × 2 µm; chlamydospores brownish, sparse, in chains of 2–3 ovoid to cylindrical cells, individually 16–23 × 10–13 µm. Teleomorph not observed.

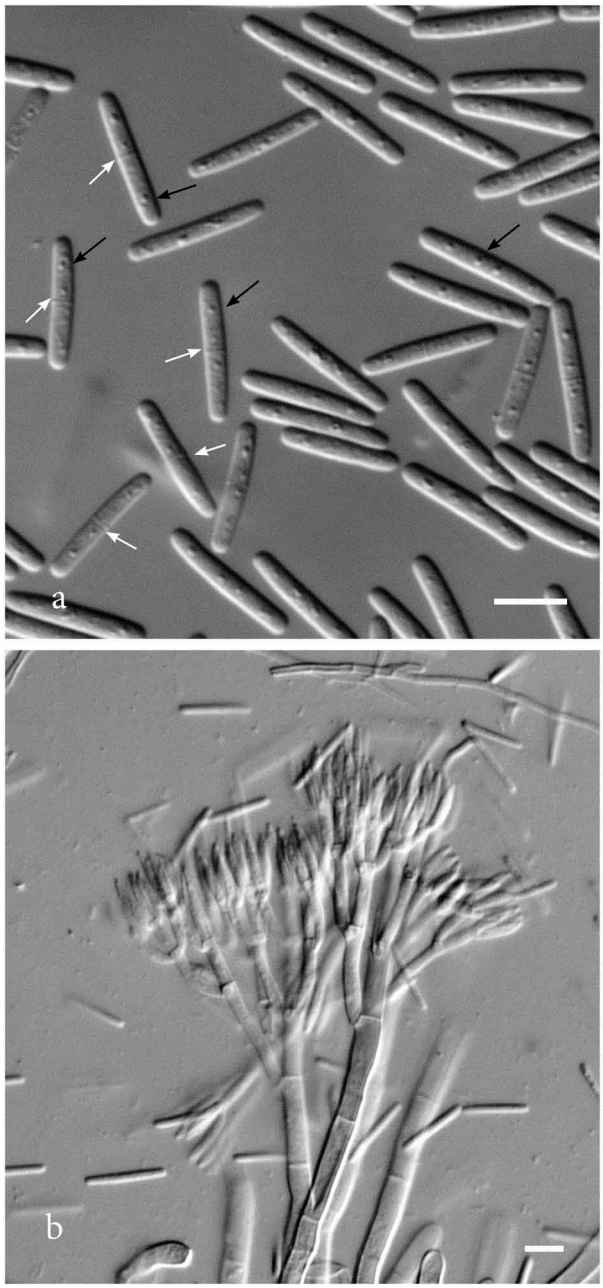

Figure 4. *Aquanectria devians* (ex-type culture). a. Conidia. b. Conidiophores. White arrows: septa; black arrows: guttules. Bars = 10 µm.

Substratum: Root of *Monotagma* sp. (Marantaceae).

Habitat: Secondary Amazonian rain forest.

Distribution: Eastern Ecuador.

Notes: Aquanectria filiformis is related to *A. penicillioides* and *A. submersa* (FIGS. 1, 3), from which it can be distinguished by its conidial size and shape. *Aquanectria penicillioides* has longer (45–55 ×

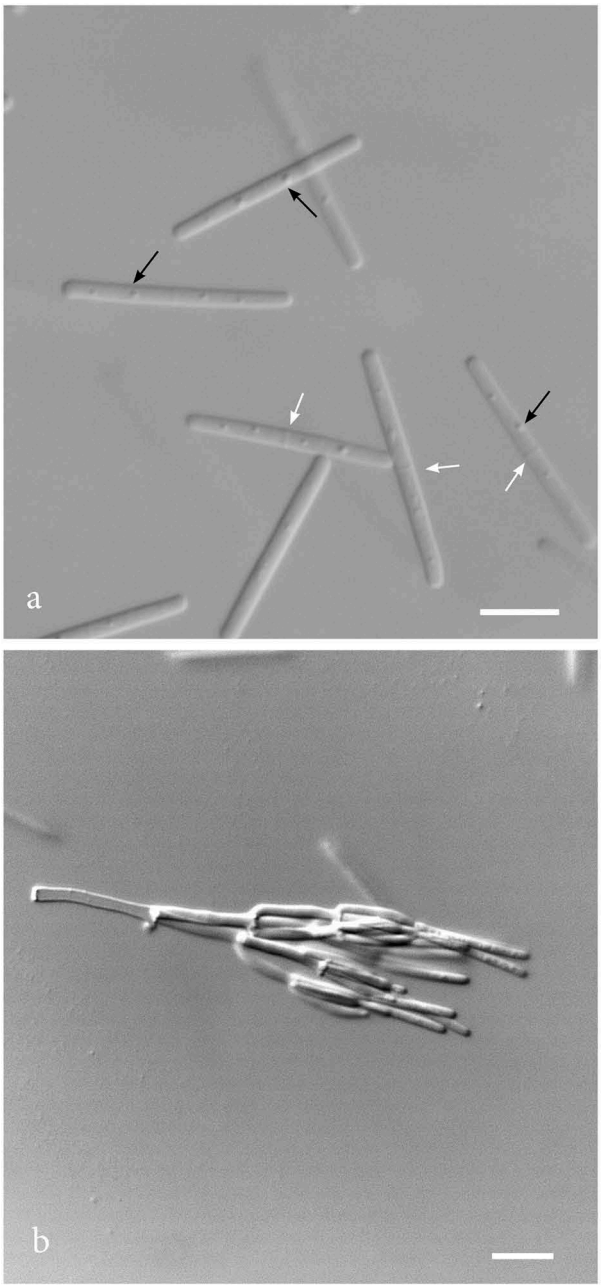

Figure 5. *Aquanectria filiformis* (ex-type culture). a. Conidia. b. Conidiophores. White arrows: septa; black arrows: guttules. Bars = 10 µm.

2–3 µm), curved to sigmoid conidia (Ingold 1942; Lombard et al. 2015). The conidia of *A. submersa* were originally described as 29–51 (or 26–44) × 1.5–2 µm (Hudson 1961). The conidia of *A. filiformis* are mostly 24–30 × 1.5–2 µm (av. = 27 × 2), mostly straight (FIG. 5e), and only unfrequently slightly sigmoid.

Aquanectria penicillioides and *A. submersa* were described as "aquatic" fungi growing on plant material submerged in freshwater (Ingold 1942; Ranzoni 1956; Hudson 1961; Duarte et al. 2012; Lombard et al. 2015). *Aquanectria filiformis* was isolated from roots, not an aquatic habitat. Nevertheless, its macrohabitat, a surface layer of organic debris covering an oil pond, is regularly flooded during frequent heavy rains.

Aquanectria tenuissima Gordillo & Decock, sp. nov. FIG. 6a–b

MycoBank MB823330

Typification: FRENCH GUIANA. Nouragues Nature Reserve, approx. 4°05′N, 52°41′W, a dried 2-wk-old dried culture on BLA, isolated from a single conidium from conidiophores emerging on a fragment of a basidiomata of a *Phellinus* sp. (voucher specimen FG-10-218) plated on malt extract agar, during field work, Aug 2010, *C. Decock FG-10-218c* (**holotype** MUCL 53250). Ex-type culture: MUCL 53250. GenBank: *HIS3* = KX671148; ITS = KX671142; *TEF1-α* = KX671134; *TUB2* = KX611504.

Etymology: tenuissima (Latin), referring to the small conidia.

Cultural characters: Colonies on SNA effuse, reaching 31 mm diam in 14 d; aerial mycelium hyaline. Colonies on MEA reaching 30 mm diam in 7 d, 55 mm diam in 14 d, with a concentric growth pattern; aerial mycelium floccose, Brown (6E7), Greyish Orange toward the margin (6B5), the reverse yellowish brown.

Conidiophores hyaline, penicillate, with a stipe and apical series of branches subtending the phialides; stipe hyaline, thin-walled, 25–100 × 2–4 µm with 1(–3) hyaline branches; primary branches aseptate, 16–31 × 2 µm; secondary branches aseptate, 9–12 × 2 µm; tertiary branches absent to occasional, 9–11.5 × 2 µm; phialides cymbiform, 8–10(–12) × 2 µm, arranged in terminal whorls of 2–4; conidia cylindrical, straight with rounded ends, hyaline, smooth, oil guttule, 0(–1) septate, 16–23(–24) × 2 µm, av. = 20 × 2 µm; chlamydospores brown, in short chains, individually 6–12 × 12 µm. Teleomorph not observed.

Substratum: Unknown, isolated as a culture contaminant, from a fragment of a basidiome of a *Phellinus sp.* (Basidiomycota, Hymenochaetaceae).

Habitat: Rain forest.

Distribution: French Guiana.

Notes: The conidia of *A. tenuispora* and *A. tenuissima* are similar. These two species can be differentiated by the lengths of their conidiophore stipes, respectively 40–55 µm and 25–100 µm, and the lengths of their phialides, respectively 9–13 µm (av. = 12 µm) and 8–10 (–12) µm (av. = 9 µm). *Aquanectria devians* (MUCL 48197) has smaller conidia, (10–)11–16(–17) µm (av. = 14 × 1.6 µm), than *A. tenuispora*, with conidia (16–) 17–20(–21) × 2 µm (av. = 19 × 2 µm) and *A. tenuissima* 16–23(–24) × 2 µm (av. = 20 × 2 µm).

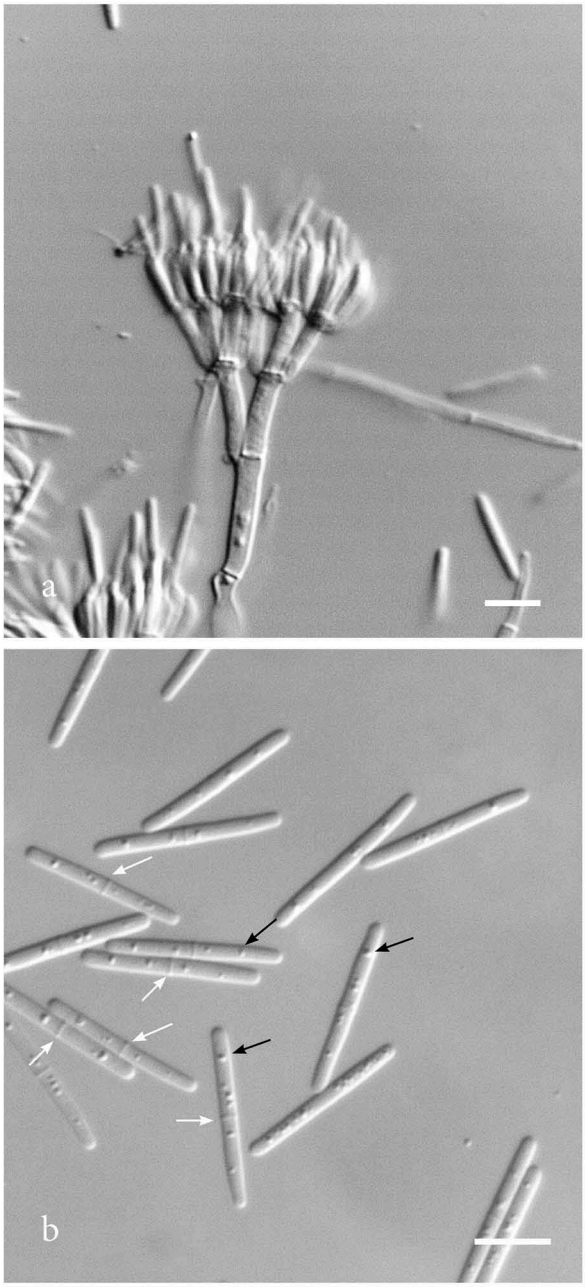

Figure 6. *Aquanectria tenuispora* (ex-type culture). a. Conidiophores. b. Conidia. White arrows: septa; black arrows: guttules. Bars: a = 7 µm; b = 10 µm.

Aquanectria tenuispora Gordillo & Decock, sp. nov. FIG. 7a–d

MycoBank MB823331

Typification: SINGAPORE. Mac Ritchie Reservoir Park, approx. 1°20′41″N, 103°49′20″E, Nov 2001, a dried 2-wk-old dried culture on BLA from conidia picked up from colonies on a decaying leaf of an unidentified angiosperm submerged in freshwater, *Olivier Laurence, isol. Cony Decock* (**holotype** MUCL 48016). Ex-type culture: MUCL 48016. GenBank: *HIS3* = KX671147; ITS = KX671141; *TEF1-α* = KX671133; *TUB2* = KX611503.

Etymology: tenuispora (Latin), referring to the small size of the conidia.

Cultural characters: Colonies on SNA effuse, reaching 35 mm diam in 7 d, 62 mm diam in 14 d; aerial mycelium hyaline. Colonies on MEA reaching 28 mm diam in 7 d, 50 mm diam in 14 d, with concentric growth pattern; aerial mycelium floccose, Brown (6E6), Greyish Brown toward the margin (6E4), the reverse Dark Brown (6F5).

Conidiophores hyaline, penicillate, with a stipe and an apical series of branches subtending the phialides; stipe hyaline, thin-walled, 40–55 × 2–3 µm, with apical 1(–3) series of hyaline branches; primary branches aseptate, 14–20 × 2–3 µm; secondary branches aseptate, 8–16 × 2–3 µm; tertiary branches absent to rare aseptate, 9–12 × 2 µm; phialides in whorl of 2–4, cylindrical to cymbiform, 9–13 × 2 µm; conidia cylindrical, straight with rounded ends, hyaline, smooth, oil guttule, 0(–1)-septate, (16–) 17–20(–21) × 2 µm, av. = 19 × 2 µm; chlamydospores present in short chains of 3–5 cells, individually 9–17 × 8–12 µm. Telemorph not observed.

Substratum: Submerged leaf in freshwater reservoir.

Habitat: Rain forest litter.

Distribution: Singapore.

Other specimens examined: SINGAPORE. MacRitchie Reservoir Park, Nov 2001, decaying, submerged leaf litter in freshwater, unidentified angiosperm, *Olivier Laurence, isol. Cony Decock*, from colonies on natural substrate (living culture MUCL 48047).

Gliocladiopsis ecuadoriensis Gordillo & Decock, sp. nov. FIG. 8a–d

MycoBank MB823333

Typification: ECUADOR. SUCUMBÍOS PROVINCE: Nueva Loja, Lago Agrío Canton, Charapa Camp, approx. 76°48′W, 00°11′S, secondary rain forest, a dried 2-wk-old culture on BLA, isolated from the rhizoplane of *Polybotrya* sp. (Dryopteridaceae), Jan 2013, *A. Gordillo & C. Decock, PUCE PHPE2-20-368* (**holotype** MUCL 54740). **Isotype** PUCE PHPE2-20-368. Ex-type culture: MUCL 54740 = PUCE PHPE2-20-368. GenBank: *HIS3* = KX671146; ITS = KX671139; *TEF1-α* = KX671131; *TUB2* = KX611501.

Etymology: "*ecuadoriensis*" (Latin), referring to the country of origin, Ecuador.

Cultural characters: Colonies on SNA effuse, reaching 44 mm diam in 7 d, 75 mm diam in 14 d; aerial mycelium mostly white. Colonies on MEA reaching 30 mm diam in 7 d, 50 mm diam in 14 d; aerial mycelium floccose, Greyish Orange (5B4), darker, Yellowish Brown toward the center (5E8), Brownish

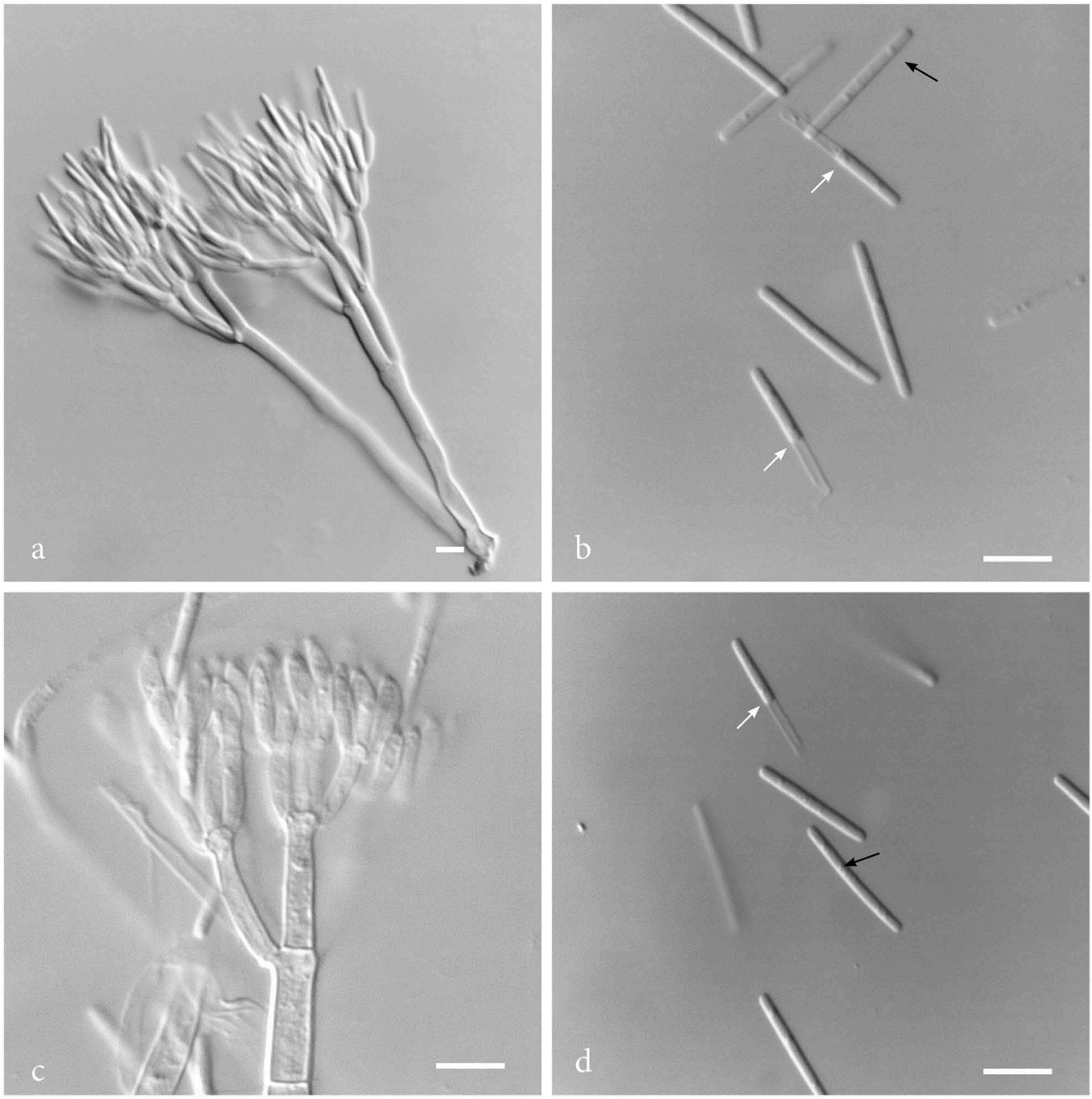

Figure 7. *Aquanectria tenuissima* (ex-type culture). a, b. Conidiophores. c, d. Conidia. White arrows: septa; black arrows: guttules. Bars: a, c = 7 µm; b, d = 10 µm.

Orange toward the margin (5C4), the reverse Olive Brown (4F8).

Conidiophores hyaline, penicillate, with a stipe and an apical series of branches subtending the phialides; stipe hyaline, thin-walled, septate, 20–140 × 2–4 µm bearing 2(–3) series of hyaline branches; primary branches aseptate, 16–21 × 2–3 µm; secondary branches aseptate, 12–20 × 2 µm; tertiary branches aseptate, 9–20 × 2 µm; phialides in whorls of 2–4, cymbiform to cylindrical, 9–16 × 2 µm; conidia cylindrical, straight with rounded ends, hyaline, smooth, 0(–1) septate, (8–)8–16(–16) × 1.5–2 µm, av. = 13 × 2 µm; chlamydospores brown, in short chains of 3–5 ovoid to spherical, smooth-walled cells, individually 14–24 × 8–16 µm. Teleomorph not observed.

Substratum: Rhizoplane of *Polybotrya* sp. (Dryopteridaceae).

Habitat: Secondary Amazonian rain forest.

Distribution: Ecuador, western Amazonia.

Notes: Gliocladiopsis ecuadoriensis (MUCL 54740) is closely related to *Gliocladiopsis* sp. 1 represented by CBS 111038; these two strains could be

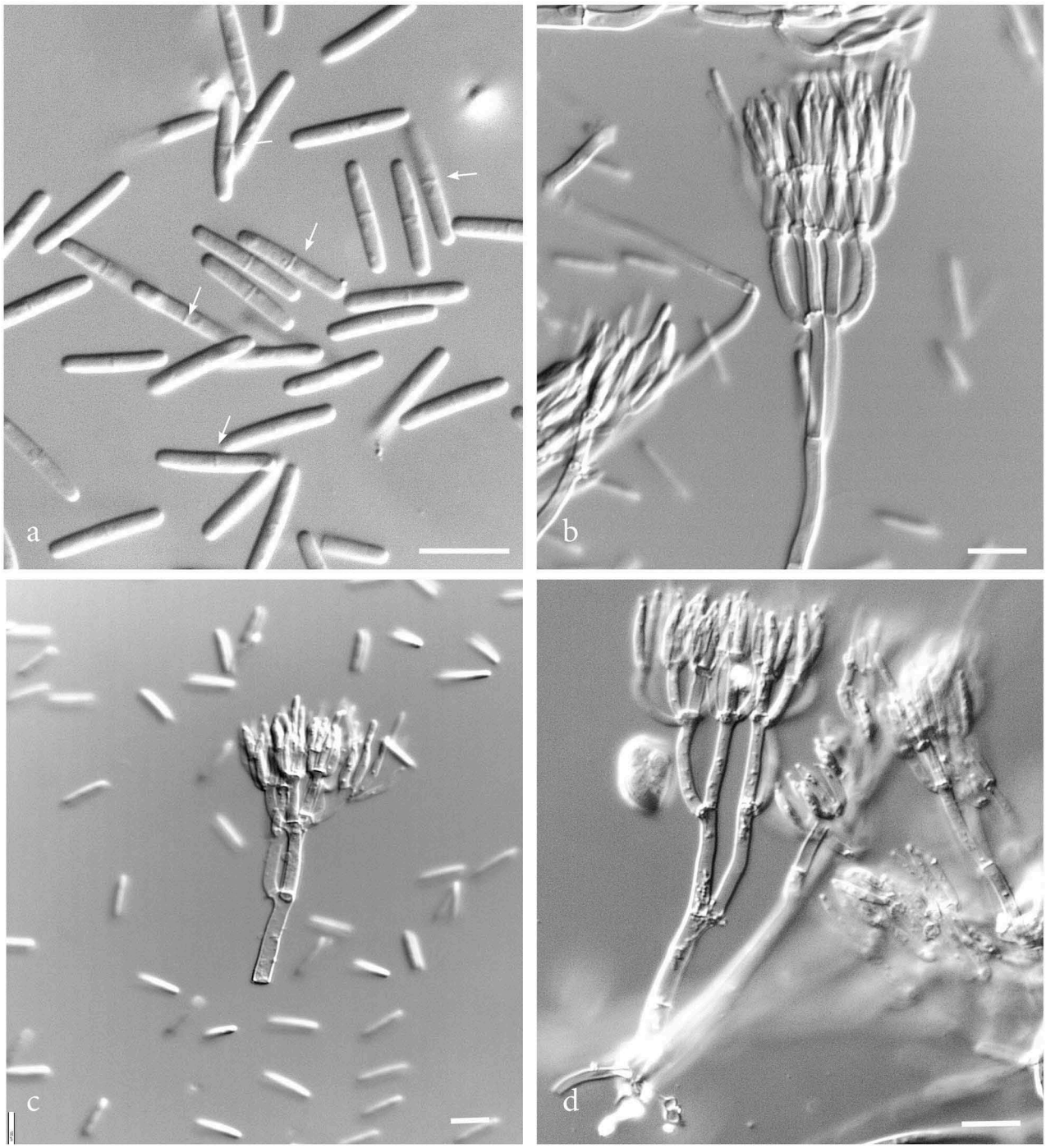

Figure 8. *Gliocladiopsis ecuadoriensis* (ex-type culture). a. Conidia. b–d. Conidiophores. White arrows: septa. Bars = 10 µm.

conspecific, although slightly genetically divergent (FIG. 2). However, the absence of reproductive structure in the strain CBS 111038 (Lombard and Crous 2012) hampers confirmation. It could be of interest to grow this strain on BLA, a medium that could stimulate sporulation (Gordillo and Decock 2017).

To a lesser degree, *G. ecuadoriensis* also is related to *G. tenuis* (FIG. 2); these species are distinguished by their conidial sizes, respectively 8–16 × 1.6–2 µm (av. = 13.2 × 2 µm) and 16.5–20 × 1.5–2 µm (av. = 18 × 2 µm) (Crous and Wingfield 1993; Lombard and Crous 2012). The number of branches of the conidiogenous apparatus also differentiates these two species:

G. ecuadoriensis has predominantly primary and secondary branches, rarely tertiary, whereas *G. tenuis* produces regularly tertiary and quaternary branches (Crous and Wingfield 1993).

Gliocladiopsis hennebertii Gordillo & Decock, sp. nov. FIG. 9a–b

MycoBank MB823334

Typification: ECUADOR. SUCUMBÍOS PROVINCE: Nueva Loja, Lago Agrío canton, Charapa camp, approx. 76°48′W, 00°11′S, secondary rain forest, a dried 2-wk-old culture on BLA, isolated from the rhizoplane of *Costus scaber* (Costaceae), Jan 2013, *A. Gordillo & C. Decock PUCE PHPE*2-33-332 (**holotype** MUCL 54818). **Isotype**: PUCE PHPE2-33-332. Ex-type culture: MUCL 54818 = PUCE PHPE2-33-332. GenBank: ITS = KX671140; *TEF1-α* = KX671132; *TUB2* = KX611502.

Etymology: "*hennebertii*" (Latin), dedicated to Prof G. L. Hennebert, former director of MUCL and initiator of the cooperation with PUCE, Ecuador.

Cultural characters: Colonies on SNA effuse, reaching 23 mm diam in 7 d, 55 mm diam in 14 d; aerial mycelium hyaline. Colonies on MEA reaching 23 mm diam in 7 d, 37 mm diam in 14 d; aerial mycelium floccose, Greyish Brown at center (5D3, nougat), Orange Grey (5B2) toward the margin, the reverse Yellowish Brown (5F8, raw amber).

Conidiophores hyaline, penicillate, with a stipe and an apical series of branches subtending the phialides; stipe, thin-walled, septate, 27–39 × 2–4 µm, with 2–3(–4) series of hyaline branches; primary branches aseptate, 27–39 × 3–4 µm; secondary branches aseptate, 13–25 × 2–3 µm; tertiary branches aseptate, 9–17 × 2–3 µm; quaternary branches absent to occasional, 8–12 × 2–3 µm, phialides in terminal whorls of 2–4 per branch, cylindrical, 12–21(–23) × 2–3 µm; conidia cylindrical, hyaline, smooth, guttulate, straight with rounded ends, 0(–1) septate, (16–)18–21(–22) × 1.5–2 µm, av. = 20 × 2 µm; chlamydospores sparse, in short chains of ovoid to cylindrical cells, individually 16–22 × 8–16 µm. Teleomorph not observed.

Substratum: Rhizoplane of *Costus scaber* Ruiz & Pav. (Costaceae).

Habitat: Secondary Amazonian rain forest.

Distribution: Ecuador, western Amazonia.

Notes: Gliocladiopsis hennebertii is related to *G. elghollii* and *G. sagariensis*; these three species form a well-supported subclade (FIGS. 1, 2). *Gliocladiopsis hennebertii* and *G. sagariensis* can be distinguished by the length of their primary and secondary branches and phialides. The branches are 27–39 µm and 13–25 µm long and the phialides 12–21(–23) µm long in *G. hennebertii* vs., respectively, 14–22 µm and 8–12 µm and 10–15 µm long in *G. sagariensis* (Saksena 1954). Their conidia are generally similar, although marginally smaller in *G. hennebertii* than in *G. sagariensis*, viz., 16–22 × 1.5–2 µm (av. = 20 × 2 µm) and 18–24 × 1.5–2 µm (Saksena 1954), respectively.

The branching pattern of the penicillus in *G. sagariensis* is unknown. The ex-type strain at the CBS is sterile according to Lombard and Crous (2012), and there are some uncertainties about how Saksena (1954) interpreted

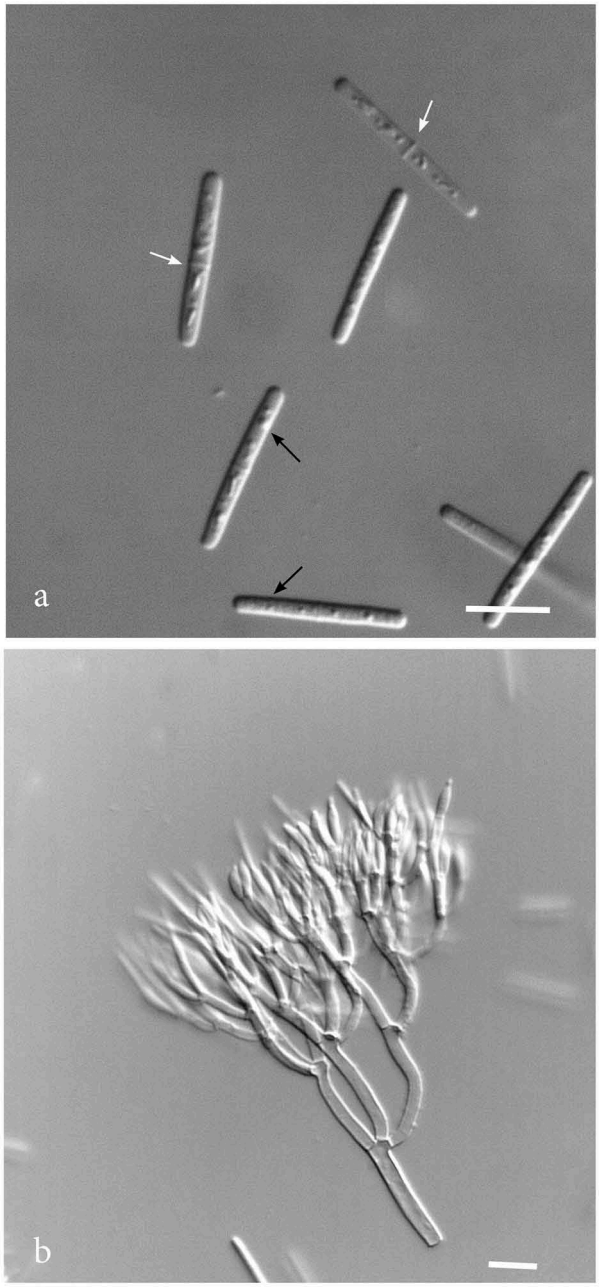

Figure 9. *Gliocladiopsis hennebertii* (ex-type culture). a. Conidia. b. Conidiophores. White arrows: septa; black arrows: guttules. Bars = 10 µm.

the branching of conidiophores in *G. sagariensis*. As noted above for *G. ecuadoriensis*, it would be also of interest to grow this strain on BLA, a medium that could stimulate sporulation (Gordillo and Decock 2017).

Gliocladiopsis hennebertii can be distinguished from *G. elghollii* by the absence or occasional presence of quaternary branches, which are abundantly formed in the latter. Both species also can be distinguished by their conidial widths, respectively 1.5–2 µm and 2–4 µm (Lombard and Crous 2012).

Gliocladiopsis singaporiensis Decock & Gordillo, sp. nov. FIG. 10a–d

MycoBank MB823335

Typification: SINGAPORE. Lower Peirce Reservoir, approx. 1°22′12″N, 103°49′13″E, a dried 2-wk-old culture on BLA, isolated from conidia picked up from colonies on a decaying leaf submerged in freshwater, Apr 2002, *Olivier Laurence* (*Mycosphere S.a.r.l.*), *isol. Cony Decock* (**holotype** MUCL 48728). Ex-type culture: MUCL 48728. GenBank: ITS = KX671138; *TEF1-α* = KX671130; *TUB2* = KX611500.

Etymology: "*singaporiensis*" (Latin), referring to the country of origin, Singapore.

Cultural characters: Colonies on SNA effuse, white, reaching 40 mm diam in 7 d, 70 mm diam in 14 d; aerial mycelium hyaline. Colonies on MEA reaching 35 mm diam in 7 d, 60 mm diam in 14 d, aerial mycelium floccose, initially homogeneously white then Light Brown (5D4), Orange Grey (5B2) toward the margin, the reverse Olive Brown (4D5).

Conidiophores hyaline, penicillate, with a stipe and an apical series of branches subtending phialidic conidiogenous cells; stipe hyaline, thin-walled, septate, 34–140 × 3–4 µm, with 2–3(–4) series of hyaline branches: primary branches aseptate, 20–31 × 3–4 µm; secondary branches aseptate, 16–22 × 3–4 µm; tertiary branches aseptate, 10–20 × 2 µm; quaternary branches 9–16 × 2–3 µm; phialides in whorls of 2–4, cymbiform to cylindrical, 8–14 × 2 µm; conidia cylindrical, hyaline, smooth, straight with rounded ends, 0(–1) septate, (13–) 14–19 × 1.5–2 µm, av. = 16 × 2 µm; chlamydospores sparse, in short chains of ovoid to cylindrical, individually 8–14 × 9–13 µm. Teleomorph not observed.

Substratum: Submerged leaf litter in stream.

Habitat: Leaf litter, Southeast Asian rain forest.

Distribution: Singapore.

Other specimens examined: SINGAPORE: Lower Peirce Reservoir, approx. 1°22′12″N, 103°49′13″E, from conidia picked up from colonies on a decaying leaf submerged in freshwater, unidentified angiosperm, Apr 2002, *Olivier Laurence* (*Mycosphere S.a.r.l.*), *isol. Cony Decock*, culture MUCL 48412.

Note: Gliocladiopsis singaporiensis has an isolated phylogenetic position, closed related to the subclade formed by *G. curvata, G. forsbergii*, and *G. whileyi* (FIG. 2). *Gliocladiopsis singaporiensis* can be differentiated from these species by the conidial shape and/or size and the number of branches in the penicillus. *Gliocladiopsis singaporiensis* has slightly shorter and especially narrower conidia (13–19 × 2 µm, av. = 16 × 2 µm) compared with those of *G. curvata* (17–21(–23) × 3–5 µm, av. = 19 × 3 µm; Lombard and Crous 2012) and those of *G. whileyi* (17–21(–22) × 1.5–3 µm; Parkinson et al. 2017). Conidia are straight in *G. singaporiensis* and slightly curved in the two latter species (Lombard and Crous 2012; Parkinson et al. 2017). Quaternary branches are frequent in *G. singaporiensis* and *G. whileyi* (Parkinson et al. 2017) but absent to rare in *G. curvata* (Lombard and Crous 2012).

Gliocladiopsis forsbergii differs from *G. curvata, G. singaporiensis*, and *G. whileyi* by having penicilli with up to five levels of branches (Parkinson et al. 2017). *Gliocladiopsis irregularis, G. mexicana, G. pseudotenuis*, and *G. sumatrensis* all lack quaternary branches (Lombard and Crous 2012), by which they differ from *G. singaporiensis*.

DISCUSSION

Aquanectria and *Gliocladiopsis* are closely related, sister genera (Lombard et al. 2015) that can hardly be distinguished morphologically, although they tend to occur in different ecological niches. The isolation of *Aquanectria devians, A. tenuispora*, and *A. tenuissima* raises questions about the delimitation and the appropriateness of maintaining the distinction between these two genera. The phenotypes of these three species are strongly reminiscent of *Gliocladiopsis*, as evidenced by their conidia, which have shapes and sizes that overlap with those of *Gliocladiopsis* species (Lombard and Crous 2012). Their conidial sizes are, on average, ≤20 µm long, far smaller than the conidia observed in other species of *Aquanectria* sensu Lombard et al. (2015). In solely morphological perspective, they undoubtedly would have been placed in *Gliocladiopsis*. Nonetheless, these species form a well-supported clade (FIG. 1) that is more closely related (sister) to the *Aquanectria* lineage (sensu Lombard et al. 2015) than to the *Gliocladiopsis* lineage (sensu Lombard and Crous 2012). Their intron 1 of *HIS3*, for instance, is confidently alignable with that of *A. submersa* and *A. filiformis* (sensu Lombard and Crous 2012) but hardly alignable with that of the *Gliocladiopsis* species.

Following the current trend for DNA-based classification (e.g., Lombard et al. 2016), giving preference to

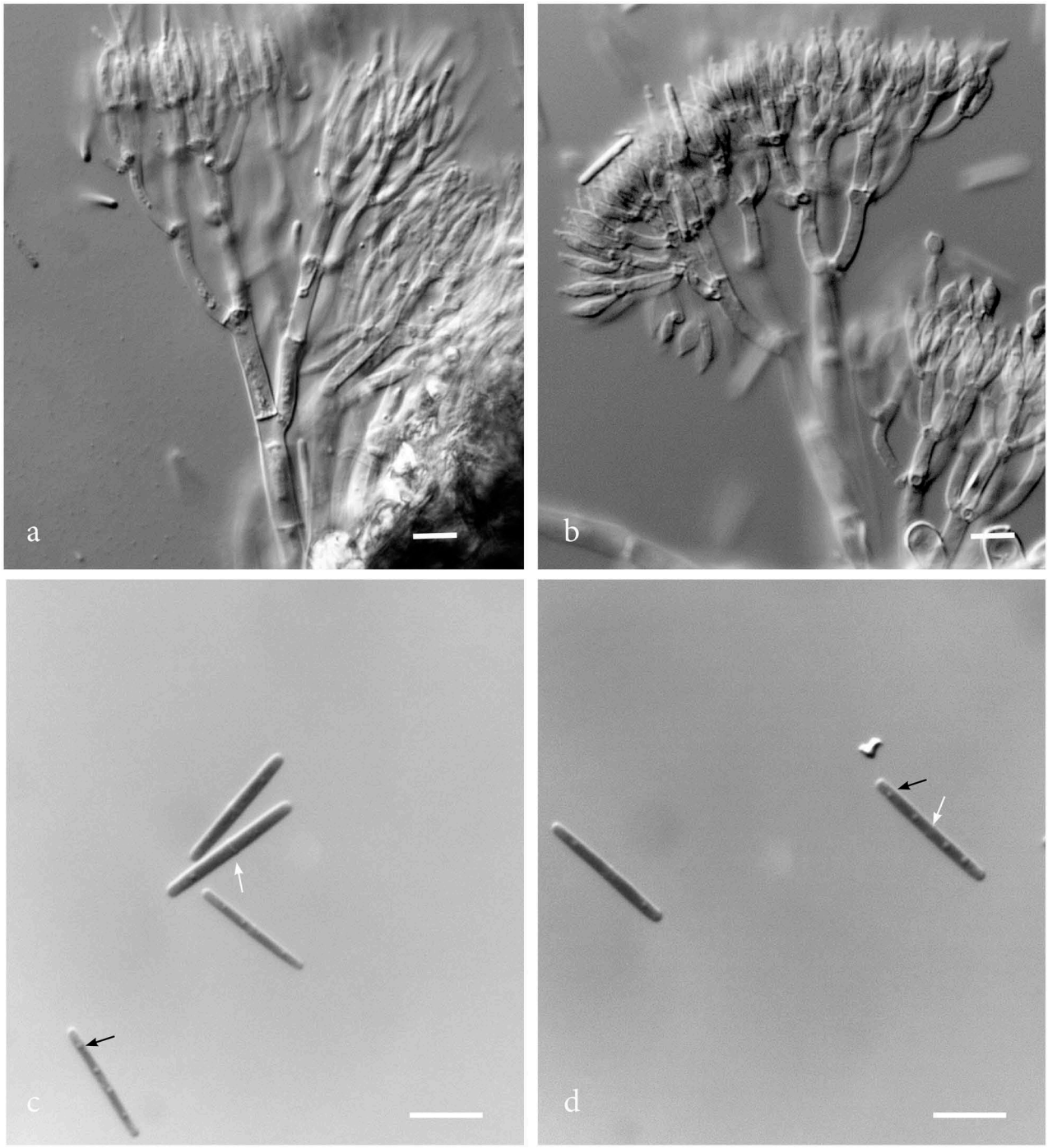

Figure 10. *Gliocladiopsis singaporiensis* (ex-type culture). a–b. Conidiophores. c, d. Conidia. White arrows: septa; black arrows: guttules. Bars = 8 µm.

the phylogenetic signals of the different markers used, *Aquanectria* is here considered as an ad hoc "taxonomic" placement for these strains. This option reduces, de facto, the shared morphological (conidiophores, conidiogenous cells) and ontogenetic (conidiogenesis) criteria to subordinate elements. This classification also renders the hitherto sole apparent (or known) morphological distinction between the closely related *Aquanectria* and *Gliocladiopsis* (Lombard and Crous 2012) obsolete, viz., the shape and size of the asexual spores. The teleomorph of *Gliocladiopsis* and *Aquanectria* species are still very poorly known, preventing the use of those characters to draw conclusions. The classification adopted here maintains the status

quo but results in a morphologically heterogeneous *Aquanectria*, divided into a "typical" *Aquanectria* morphotypes and a deviating gliocladiopsis-like morphotype, each corresponding to a well-supported subclade (FIGS. 1, 2).

Species of this expanded *Aquanectria* lineage may share the autecological feature of inhabiting aquatic or temporarily flooded habitat; the type strains of *A. penicillioides, A. submersa, A. devians*, and *A. tenuispora* originate from aquatic or periodically flooded habitat. The habitat of the type strain of *A. tenuissima* is uncertain.

One alternative would be to merge *Aquanectria* and *Gliocladiopsis*, emphasizing the key morphological and ontogenetic similarities of the hyaline, penicillate conidiogenous apparatus, the conidiogenous cells, and the conidiogenesis, which are identical in both genera. This expanded *Gliocladiopsis* also would comprise a well-supported, well-delimited lineage within the Nectriaceae (sensu Lombard et al. 2015). The resulting concept of *Gliocladiopsis* also would be morphologically more homogeneous, although with an atypical morphotype represented by the *A. penicillioides, A. submersa*, and *A. filiformis* clade, which has species in which conidia are much longer and variably sinuous compared with the typical morphotype. Eventually, whether we place these three latter species in *Gliocladiopsis* or *Aquanectria*, the long and variably sigmoid conidia could represent a derived adaptation to an aquatic habitat (Baschien et al. 2013), apomorphic for the *A. penicillioides, A. submersa*, and *A. filiformis* subclade, but of secondary importance and not relevant for distinguishing closely related genera. A third option would be to describe the well-supported *A. devians, A. tenuispora*, and *A. tenuissima* clade as a new genus. This putative new genus would be morphologically indistinguishable from *Gliocladiopsis*. This option would be defendable only if any associated teleomorph were discovered that presented singular characters justifying it. This was the case, for example, for the hypocrealean *Cylindrocarpon* complex of genera (Chaverri et al. 2011). However, teleomorphs are unknown in these three species and, as noted above, are very poorly known in the *Gliocladiopsis–Aquanectria* lineage, preventing the use of those characters to draw conclusions.

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