Cryptogamie, Mycologie, 2017, 38 (4): 409-434
© 2017 Adac. Tous droits réservés

Cylindrocarpon-like (Ascomycota, Hypocreales) species from the Amazonian rain forests in Ecuador: additions to *Campylocarpon* and *Dactylonectria*

Ana GORDILLO[a, b] & Cony DECOCK[a]*

[a]Mycothèque de l'Université catholique de Louvain (BCCM/MUCL), Earth and Life Institute, Microbiology (ELIM), Croix du Sud 2 bte L7/05.06, 1348 Louvain-la-Neuve, Belgium

[b]Laboratorio de Micología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

Abstract – *Dactylonectria* and *Campylocarpon* are two related genera of Hypocreales sharing a cylindrocarpon-like asexual morph, mostly known as soil-borne pathogens. During a study of the fungal communities of roots (endophyte) and rhizoplanes of plants growing in a layer of compost-like vegetal materials covering crude oil ponds in rain forest areas of the Amazonian Ecuador, a set of isolates with a cylindrocarpon-like asexual morph were studied. Multilocus phylogenetic inferences (based on partial DNA sequences from nuclear ribosomal DNA genes (ITS, 28S) and the housekeeping genes b-tubulin, translation elongation factor 1-α and Histone 3) and morphological studies revealed the occurrence of five undescribed species, of which four belong to *Dactylonectria* and one to *Campylocarpon*. They are described as *Dactylonectria amazonica*, *D. ecuadoriense*, *D. polyphaga*, *D. palmicola* and *Campylocarpon amazonense*.

Hypocreales / phylogeny / systematics / South America

INTRODUCTION

Cylindrocarpon Wollenw [1] and *Cylindrocarpon*-like species are cosmopolitan fungi. They are mostly known as soil-borne pathogens causing plant diseases such as black foot or root rots, but also tree cankers (Cabral *et al.* 2012a, b, Castlebury *et al.* 2006, Chaverri *et al.* 2011, Halleen *et al.*, 2004, 2006, Lombard *et al.* 2014, Samuels & Braford 1994, Hirooka et *et al.* 2005, Kobayashi *et al.* 2005).

Cylindrocarpon was first introduced by Wollenweber (1913) with *C. cylindroides* Wollenw. as type, a species with an asexual morph characterized by long, sausage-shaped, 3–5-septate macroconidia and smaller, ellipsoid, aseptate microconidia. Later on, Wollenweber (1917) associated *Cylindrocarpon* with the sexual form *Neonectria* Wollenw. (*C. cylindroides* was associated with *N. ramulariae* Wollenw.). Subsequently, more than 140 names have been published in *Cylindrocarpon*, at species or subspecies level (*index fungorum* www.indexfungorum.org).

* Corresponding author: cony.decock@uclouvain.be

1. As a rule, authorship of scientific names listed in the table 1 are not repeated in the text.

doi/10.7872/crym/v38.iss4.2017.409

The first revision of *Neonectria* – *Cylindrocarpon* dates back from Booth (1966), who recognized four informal groups on the basis of morphological features of both the sexual and asexual morph, including the anatomy of the perithecial outer layer and the presence or absence of microconidia and chlamydospores. Chaverri *et al.* (2011) proposed the first comprehensive treatment of *Cylindrocarpon* – *Neonectria* of the molecular era, implementing multi-locus phylogenetic inferences combined with morphological analyses. In addition to *Neonectria* (gathering mostly species of Booth's *Cylindrocarpon* group 1) and *Campylocarpon* [previously segregated by Halleen *et al.* (2004)], they acknowledged at generic level three additional lineages, two of which were overlapping with Booth's informal groups: *Thelonectria* P. Chaverri & C. Salgado, which corresponds to Booth's *Cylindrocarpon* group 2, *Ilyonectria* which corresponds to Booth's *Cylindrocarpon* group 3, and *Rugonectria* P. Chaverri & Samuels. Further studies then revealed that *Ilyonectria sensu* Chaverri *et al.* (2011) also was polyphyletic (Cabral *et al.* 2012 a, b; Lombard *et al.* 2013). *Dactylonectria* was therefore introduced to accommodate a bunch of species, the majority of which were isolated from diseased grapevines (Lombard *et al.* 2014). More recently, Aiello *et al.* (2017) introduced *Pleiocarpon* L. Lombard & D. Aiello for a single species, *P. strelitziae* L. Lombard & D. Aiello, associated with basal rot of *Strelitzia reginae* (Strelitziaceae) in Southern Italy.

In this taxonomic frame, we re-evaluated the generic placement, identity and phylogenetic affinities of a set of cylindrocarpon-like strains, isolated from asymptomatic roots of herbaceous plants growing in a layer of compost-like vegetal materials covering weathered crude oil ponds in the Eastern Amazonian Ecuador. A combination of morphological and multilocus DNA-based phylogenetic approaches show these strains to be distributed into five new terminal clades or branches, that represent as much new species. Four species belongs to *Dactylonectria* and one to *Campylocarpon*. These taxa are described and commented.

MATERIAL AND METHODS

Site and Sample procedure

The strains from Ecuador were isolated from internal tissues and rhizoplanes of roots of herbaceous plants growing in a superficial layer of compost-like vegetal debris covering two weathered oil ponds (for detailed methodologies, cf. Gordillo and Decock 2016). Both ponds are located at Charapa camp, Province of Sucumbíos, approx. W 76°48'57" – S 00°11' 49" and W 76°48'54" – S 00°11'46", elevation approx. 300 m asl.

Morphological characterization

Inoculums (mycelial plugs/germinating conidia) were placed in the centre of a 9 cm Petri dish, on Potato Dextrose Agar (PDA) and on a piece of sterile banana leaf on water agar (Banana Leaf Agar, BLA) (Untereiner *et al.* 1998). Cultures were incubated at 25°C with a 12/12 hrs incident near UV light/dark cycle. Cultural characteristics were determined at 7 days after inoculation on PDA. Colours of the colonies are described according to Kornerup and Wanscher (1978). As a rule, the reproductive structures (e.g. conidiophores, conidia, perithecia, ascospores) measured are those produced on BLA. Measurements for length and width of conidia and

ascospores are given as (Minimum-) Lower Limit of a 95% Confidence Interval – Upper Limit of a 95% Confidence Interval (-Maximum). For the other measurements, only the extreme values are given (Cabral *et al.* 2012).

Taxon sampling, DNA isolation, PCR amplification and sequencing

The taxa included in the phylogenetic analysis are listed in Table 1. DNA was extracted from mycelium grown in malt extract broth (2%) at 25°C in the dark, using the innuPREP Plant DNA kit (Analytik Jena, Germany) following the manufacturer's recommendations.

DNA sequences were determined for parts of the genes encoding the nuclear ribosomal internal transcribed spacers region, including the ITS1, ITS2 and 5.8S subunit (ITS), the nuclear ribosomal large subunit (28S, region comprising the D1-D3 domains), β-tubulin (*tub2*, region between exons 1 and 4), translation elongation factor 1-α (*tef1a*, region between exons 1 and 4) and histone H3 (*his3*, region between exons 1 and 3). Amplification and sequencing of the ITS, 28S, *tef1a*, *tub2* and *his3* were performed, respectively, with the primer pairs ITS5/ITS4 (White *et al.* 1990), LR0R (Rehner & Samuels 1995)/LR6 (Vilgalys & Hester 1990), ef1/ef2 (O'Donnell *et al.* 1998), T1 (O'Donnell & Cigelnik 1997)/Bt-2b (Glass and Donaldson 1995) and H3-1a/H3-1b (Glass and Donaldson 1995). The PCR conditions are as described in Lombard *et al.* 2010. Sequencing was performed by Macrogen Ltd. (Seoul, Korea) using the same primers as for amplification. The amplicons were sequenced in both directions. Raw sequences were edited with Sequencher®software version 5.1 (Gene Codes Corporation Ann Arbor n.d.).

Phylogenetic analysis

The affinities of our Amazonian cylindrocarpon-like strains were first searched for using the Blast search engine at GenBank (Altschul *et al.*1990). Subsequently, based on the blast search results, sequence data sets were set up to conduct phylogenetic inferences. The nucleotide alignments were performed with MAFFT v7.213 (Katoh & Standley 2013) and manually corrected in PhyDE-1 (Müller *et al.* 2006) when necessary.

Each data set was partitioned into ITS1, ITS2, 5.8S, 28S and exons/introns for the protein coding genes. The best-fit evolutionary model for each defined partition was estimated using Partition Finder (Lanfear, 2012), following the Akaike information criterion (AIC). Phylogenetic analyses were performed under probabilistic hypothesis, using Bayesian inferences (BI) as implemented in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) and Maximum likelihood (ML) using RAxML 7.0.4 (Stamatakis 2006). For the Bayesian inferences, the best-fit models for each partition were implemented as partition-specific model. All the parameters were linked across partitions.

Bayesian analyses were implemented with two independent runs, each with four MCMC simultaneous, independent chains, for ten million generations for the first data set and six million generations for the second data set, 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 v1.4 (Rambaut & Drummond 2007) were used to reconstruct a 50% majority-rule consensus tree (BC) and to estimate posterior probabilities (PP). Clades with PP above > 0.95 were considered significant supported by the data.

Table 1. List of *Cylindrocarpon-like* isolates used for the various phylogenetic inferences

Genus & species names / *Voucher specimens/cultures reference*	*Substrate*	*Country*	*GenBank accession numbers*				
			ITS	*28S*	his3	tub2	tef1a
Campylocarpon Halleen *et al.*							
Campylocarpon amazonense Gordillo & Decock							
MUCL55434 (T)	Rhizoplane, *Cordia alliodora*	Ecuador	MF683709	MF683729	MF683688	MF683646	MF683667
Campylocarpon fasciculare Schroers *et al.*							
CBS 112613 (T)	Trunk, *Vitis vinifera,*	South Africa	AY677301	HM364313	JF735502	AY677221	JF735691
CBS 112611	Rootstock, *Vitis vinifera*	South Africa	AY677299	–	–	AY677225	–
CBS 112612	Root, *Vitis vinifera*	South Africa	AY677300	–	–	AY677216	–
CBS 113560	Root, *Vitis vinifera*	South Africa	AY677304	–	–	AY677217	–
CBS 112614	Trunk, *Vitis vinifera*	South Africa	AY677302	–	–	AY677220	–
CBS 112600	Root, *Vitis vinifera*	South Africa	AY677298	–	–	AY677219	–
CBS 113554	Rootstock, *Vitis vinifera*	South Africa	–	–	–	AY677223	–
CBS 113559	Root, *Vitis vinifera*	South Africa	AY677303	–	–	AY677218	–
BV2	Rootstock, *Vitis vinifera*	Brazil	JX521864	–	–	JX521835	–
BV3	*Vitis vinifera*	Brazil	JX521865	–	–	JX521836	–
BV4	Rootstock, *Vitis vinifera*	Brazil	JX521866	–	–	JX521837	–
BV5	Rootstock, *Vitis vinifera*	Brazil	JX521867	–	–	JX521838	–
BV6	Rootstock, *Vitis vinifera*	Brazil	JX521868	–	–	JX521839	–
Campylocarpon pseudofasciculare Halleen *et al.*							
CBS 112679 (T)	Root, *Vitis vinifera*	South Africa	AY677306	–	JF735503	AY677214	JF735692.1
CBS 112592	Root, *Vitis vinifera*	South Africa	AY677305	–	–	AY677215	–
BV7	Rootstock, *Vitis vinifera*	Brazil	JX521869	–	–	JX521840	–
Cy1UFSM	*Vitis labrusca*	Brazil	–	–	KF633164	KF633144	–
Cy2UFSM	*Vitis rotundifolia x vinifera*	Brazil	KF447564	–	KF633173	KF633145	–
Cy3UFSM	*Vitis rupestris*	Brazil	KF447565	–	KF633166	KF633146	–
Cy6UFSM	*Vitis labrusca*	Brazil	KF447566	–	KF633169	KF633147	–
Cy14UFSM	*Vitis labrusca*	Brazil	KF447567	–	KF633158	KF633148	–
Cy17UFSM	*Vitis rupestris* x *riparia*	Brazil	KF656730	–	KF633161	KF633149	–
Cy18UFSM	*Vitis berlandieri x rupestris*	Brazil	KF447568	–	KF633162	KF633150	–

Cy19UFSM	*Vitis berlandieri x rupestris*	Brazil	KF447569	–	KF633163	KF633151	–
Cy20UFSM	*Vitis labrusca*	Brazil	KF447570	–	KF633165	KF633152	–
Dactylonectria L. Lombard & Crous							
Dactylonectria alcacerensis (A. Cabral *et al.*) L. Lombard & Crous							
CBS 129087 (T)	*Vitis vinifera*	Portugal	JF735333	KM231629	JF735630	AM419111	JF735819
Cy134	*Vitis vinifera*	Spain	JF735332	–	JF735629	AM419104	JF735818
Dactylonectria anthuriicola (A. Cabral & Crous) L. Lombard & Crous							
CBS 564.95 (T)	Root, *Anthurium sp.*	Netherlands	JF735302	KM515897	JF735579	JF735430	JF735768
Dactylonectria amazonica Gordillo & Decock							
MUCL55430 (T)	Rhizoplane, *Piper sp.*	Ecuador	MF683706	MF683726	MF683685	MF683643	MF683664
MUCL55433	Root, *Piper sp.*	Ecuador	MF683707	MF683727	MF683686	MF683644	MF683665
Dactylonectria ecuadoriense Gordillo & Decock							
MUCL55424 (T)	Rhizosplane, *Piper sp.*	Ecuador	MF683704	MF683724	MF683683	MF683641	MF683662
MUCL55432	Rhizosplane, *Socratea exorrhiza*	Ecuador	MF683702	MF683722	MF683681	MF683639	MF683660
MUCL55226	Root, *Cyathea lasiosora*	Ecuador	MF683703	MF683723	MF683682	MF683640	MF683661
MUCL55431	Rhizosplane, *Carludovica palmata*	Ecuador	MF683701	MF683721	MF683680	MF683638	MF683659
MUCL55425	Rhizoplane, *Piper sp.*	Ecuador	MF683705	MF683725	MF683684	MF683642	MF683663
MUCL55205	Root, *Piper sp.*	Ecuador	MF683700	MF683720	MF683679	MF683637	MF683658
Dactylonectria estremocencis (A. Cabral *et al.*) L. Lombard & Crous							
CBS 129085 (T)	*Vitis vinifera*	Portugal	JF735320	KM231630	JF735617	JF735448	JF735806
CPC 13539	*Picea glauca*	Canada	JF735330	–	JF735627	JF735458	JF735816
Dactylonectria hordeicola L. Lombard & Crous,							
CBS 162.89 (T)	*Hordeum vulgare*	Netherlands	AM419060	KM515898	JF735610	AM419084	JF735799
Dactylonectria macrodidyma (Halleen *et al.*) L. Lombard & Crous							
CBS 112601	Root, *Vitis vinifera*	South Africa	AY677284	KM515899	JF735644	AY677229	JF735833
CBS 112615 (T)	Root, *Vitis vinifera*	South Africa	AY677290	KM515900	JF735647	AY677233	JF735836
Dactylonectria novozelandica (A. Cabral & Crous) L. Lombard & Crous							
CBS 112608	Root, *Vitis vinifera*	South Africa	AY677288	KM515901	JF735632	AY677235	JF735821
Dactylonectria palmicola Gordillo & Decock, sp. nov.							
MUCL55426 (T)	Rhizoplane, *Euterpe precatoria* (Arecaceae)	Ecuador	MF683708	MF683728	MF683687	MF683645	MF683666
Dactylonectria pauciseptata (Schroers & Crous) L. Lombard & Crous							
CBS 120171 (T)	Root, *Vitis sp.*	Slovenia	EF607089	KM515903	JF735587	EF607066	JF735776

…/…

Table 1. List of *Cylindrocarpon-like* isolates used for the various phylogenetic inferences *(continued)*

Genus & species names / *Voucher specimens/cultures reference*	*Substrate*	*Country*	*GenBank accession numbers*				
			ITS	*28S*	his3	tub2	tef1a
CBS 100819, LYN 16202/2	Root, *Erica melanthera*	New Zealand	EF607090	KM515902	JF735582	EF607067	JF735771
CBS 113550	base of trunk, *Vitis sp.*	New Zealand	EF607080	–	JF735583	EF607069	JF735772
CBS 120173, KIS10468	Root, *Vitis sp.*	Slovenia	EF607088	–	JF735589	EF607068	JF735778
CBS Cy196	*Vitis sp*	Portugal	JF735305	–	JF735590	JF735433	JF735779
Dactylonectria pinicola L. Lombard & Crous,							
CBS 159.34	No data	Germany	JF735318	KM515904	JF735613	JF735446	JF735802
CBS 173.37 (T)	*Pinus laricio*	UK: England	JF735319	KM515905	–	JF735447	JF735803
Dactylonectria polyphaga Gordillo & Decock							
MUCL55209 (T)	Root, *Costus sp.* (Costaceae)	Ecuador	MF683689	MF683710	MF683668	MF683626	MF683647
MUCL55208	Root, *Costus sp.* (Costaceae)	Ecuador	MF683699	MF683719	MF683678	MF683636	MF683657
MUCL55238	Root, Costus sp. (Costaceae)	Ecuador	MF683696	MF683717	MF683675	MF683633	MF683654
MUCL55428	Root, *Euterpe precatoria* (Arecaceae)	Ecuador	MF683692	MF683713	MF683671	MF683629	MF683650
MUCL55429	Root, *Euterpe precatoria* (Arecaceae)	Ecuador	MF683691	MF683712	MF683670	MF683628	MF683649
MUCL55206	Root, *Piper sp.* (Piperaceae)	Ecuador	MF683693	MF683714	MF683672	MF683630	MF683651
MUCL55435	Root, *Miconia* sp. (Melastomataceae)	Ecuador	MF683694	MF683715	MF683673	MF683631	MF683652
MUCL55427	Rhizosphera, *Acalypha (Euphorbiaceae)*	Ecuador	MF683695	MF683716	MF683674	MF683632	MF683653
MUCL54780	Root, *Anthurium sp.* (Araceae)	Ecuador	MF683690	MF683711	MF683669	MF683627	MF683648
MUCL54802	Root, *Asplenium* sp. (Apleniaceae)	Ecuador	MF683698	MF683718	MF683677	MF683635	MF683656
MUCL54771	root, *Costus scaber* (Costaceae)	Ecuador	MF683697	–	MF683676	MF683634	MF683655
Dactylonectria torresensis (A. Cabral *et al.*) L. Lombard & Crous							
CBS 119.41	*Fragaria sp.*	Netherlands	JF735349	KM515906	JF735657	JF735478	JF735846
CBS 129086 (T)	*Vitis vinifera*	Portugal	JF735362	KM231631	JF735681	JF735492	JF735870
Dactylonectria vitis (A. Cabral *et al.*) L. Lombard & Crous							
CBS 129082 (T)	*Vitis vinifera*	Portugal	JF735303	KM515907	JF735580	JF735431	JF735769

T = Ex-type isolates

Maximum Likelihood trees were obtained using RAxML v.7.2.8 (Stamatakis, 2006). The analysis first involved 1000 ML searches, under a GTRGAMMA model and all other parameters estimated by the software. ML Bootstrap support values (BS) were obtained running 1000 multi-parametric bootstrapping replicates, under the same model. A node was considered to be strongly supported if it showed a BPP ≥ 0.95 and/or ML BS ≥ 80%.

Phylogenetic congruency between the loci was tested using a 70% reciprocal bootstrap criterion in ML analysis of each individual locus (Mason-Gamer *et al.* 1996, Lombard *et al.* 2014).

RESULTS

Phylogenetic analyses

The amplicons of the ITS, 28S, *tub2*, *tef1a* and *his3* of our Amazonian strains ranged ~ 500-750 bases each. The BLAST search at GenBank (Altschul *et al.*1990) of the ITS, 28S, *tub2*, *tef1a* and *his3* DNA sequences demonstrated each homology mostly with members of *Dactylonectria*. A single strain showed affinities with *Campylocarpon*. Subsequently, both a *Dactylonectria* and a *Campylocarpon* sequence data set were built.

The first data set includes the sequences of the five loci cited above for 40 *Dactylonectria* strains, representing the 10 known species and our related Amazonian strains (Table 1). Several 28S sequences, however, are missing (Table 1). This data set was subdivided into 23 partitions *viz.* ITS1, 5.8S, ITS2, 28S, *tef1a* Introns 1, 2, 3 and 4, *tef1a* Exons 1, 2, 3 and 4, *tub2* Introns 1, 2 and 3, *tub2* Exons 1, 2, and 3, *his3* Exons 1, 2 and 3, *his3* Introns 1, 2 and 3. The models estimated as the best-fit likelihood model of evolution for each partition, subsequently used for the BI, are summarized in Table 2.

Table 2. Summary of data sets *Dactylonectria* of ITS, 28S, *tef1*, *Btub* and *h3*

Partitions	*Data set*								
	ITS1, ITS2, *tub* E1, *tub* I1, *tef1a* I1	5.8S, nrLSU, *tef1a* E3	*tub* E3, *his3* E1, *his3* E2, *his3* E3, *tef1a* E1	*tub* I3, *tef1a* I2, *tef1a* I3	*tef1a* E2, *tef1a* I4	*his3* I1, *tef1a* I4	*tub* I2	*tub* E2	*his3* I2
Model selected	GTR + G	GTR + I	GTR + I + G	HKY + G	JC	GTR + G	SYM + G	SYM + G	GTR + I + G
Freq. A	0.237	0.246	0.169	0.217	0.263	0.226	0.258	0.225	0.392
Freq. C	0.321	0.217	0.381	0.324	0.253	0.367	0.279	0.291	0.387
Freq. G	0.22	0.291	0.243	0.178	0.263	0.164	0.226	0.253	0.094
Freq. T	0.222	0.246	0.207	0.282	0.221	0.243	0.236	0.23	0.126
Proportion of invariable sites		0.916	0.61						0.148
Gamma shape	1.068		0.717	1.007		1.626	1.835	0.448	2.77

I = Intron; E = Exon

The 70% reciprocal bootstrap tree topologies showed no conflicts between phylogenies resulting from the *tub2*, *tef1a* and *his3* gene regions. These individual phylogenies resolved each the same lineages, of which, of interest for our studies, the *D. vitis*, *D. pauciseptata*, and *D. anthuriicola* lineages. They also resolved each the same terminal clades within these lineages. This was already the case for a closely related cylindrocarpon-like data set used by Lombard *et al.* (2014) or Cabral *et al.* (2012). The polymorphism of the *tub2*, *tef1a* and *his3* sequences make them suitable for species discrimination within each lineages.

However, the ITS and 28S genes region revealed conflicting as far as the terminal clades are concerned. The ITS and 28S resolved equally the *D. vitis*, *D. pauciseptata*, and *D. anthuriicola* lineages. However, within each of these lineages, the ITS and 28S sequences did not allow differentiating the terminal species clades shown by the house-keeping genes; their phylogenetic signals, at that level, are null or very weak. This was also reported for the related cylindrocarpon-like data set used by Lombard *et al.* (2014) for the 28S, which has very little phylogenetic signals.

Nonetheless, as emphasized by Cunningham (1997) combining incongruent partitions could increase phylogenetic accuracy. This was the case in previous phylogenetic studies of cylindrocarpon-like species (Lombard *et al.* 2014) but also in other genera of Hypocreales (e.g. Gehesquière *et al.* 2016). Therefore, the five gene regions also were combined in the present study.

The concatenated *Dactylonectria* data set resulted in 3077 positions (including gaps). *Dactylonectria hordeicola* CBS 16289 was used as outgroup, following Lombard *et al.* (2014)). The two Bayesian runs converged to stable likelihood values after 45000 generations. The first 25% of saved trees were discarded as the "burnin" phase. In the ML the searches with RAxML, the combined data set had 406 distinct patterns with a proportions of gaps and undetermined characters of 8.47%. The best scoring ML tree is shown at Fig. 1 (-lnL -8071.916899).

The topologies obtained for the two data sets were overall highly concordant between Bayesian and Maximum likelihood inferences.

The analysis of this data set (Fig. 1) resolve the lineages corresponding to the known *Dactylonectria* species, confirming previous results (Lombard *et al.* 2014). Our Amazonian *Dactylonectria* strains are distributed into four terminal clades/branches (Fig. 1, PS1, PS2, PS3 and PS4), distinct from all the other known species clades.

The clade PS1, formed by 6 strains [MUCL 55431, MUCL 55432, MUCL 55424, MUCL 55425, MUCL55205 & MUCL 55226] and PS2, represented by 2 strains [MUCL 55430 & MUCL 55433] are both closely related to the *D. vitis* branch and the unnamed branch represented by the strain Cy228 (Cabral *et al.* 2012). The clade PS3, represented by 11 strains [MUCL 54780, MUCL 54802, MUCL 54771, MUCL 55206, MUCL 55208, MUCL 55209, MUCL 55238, MUCL 55427, MUCL 55428, MUCL 55429, MUCL55435] is closely related to the *D. anthuriicola* branch (BSML = 100, PP = 1). The clade PS4, represented by a single strain [MUCL 55426], belongs to the *D. pauciseptata* lineage (Fig. 1).

The second data set includes sequences of three loci (ITS, *tub2* and *his3*) from 26 *Campylocarpon* strains, including the type strains of *C. fasciculare* and *C. pseudofasciculare* (Halleen *et al.* 2004), two sets of Brazilian strains identified as *C. fasciculare* (Correia *et al.* 2013) and *C. pseudofasciculare* (Dos Santos *et al.* 2014), and our related single Amazonian strain (Table 1). The ITS sequences are missing for the strains Cy1UFM and CBS 113560. As well, the *his3* sequences are missing for one set of Brazilian strains (Correia *et al.* 2013) and some strains from

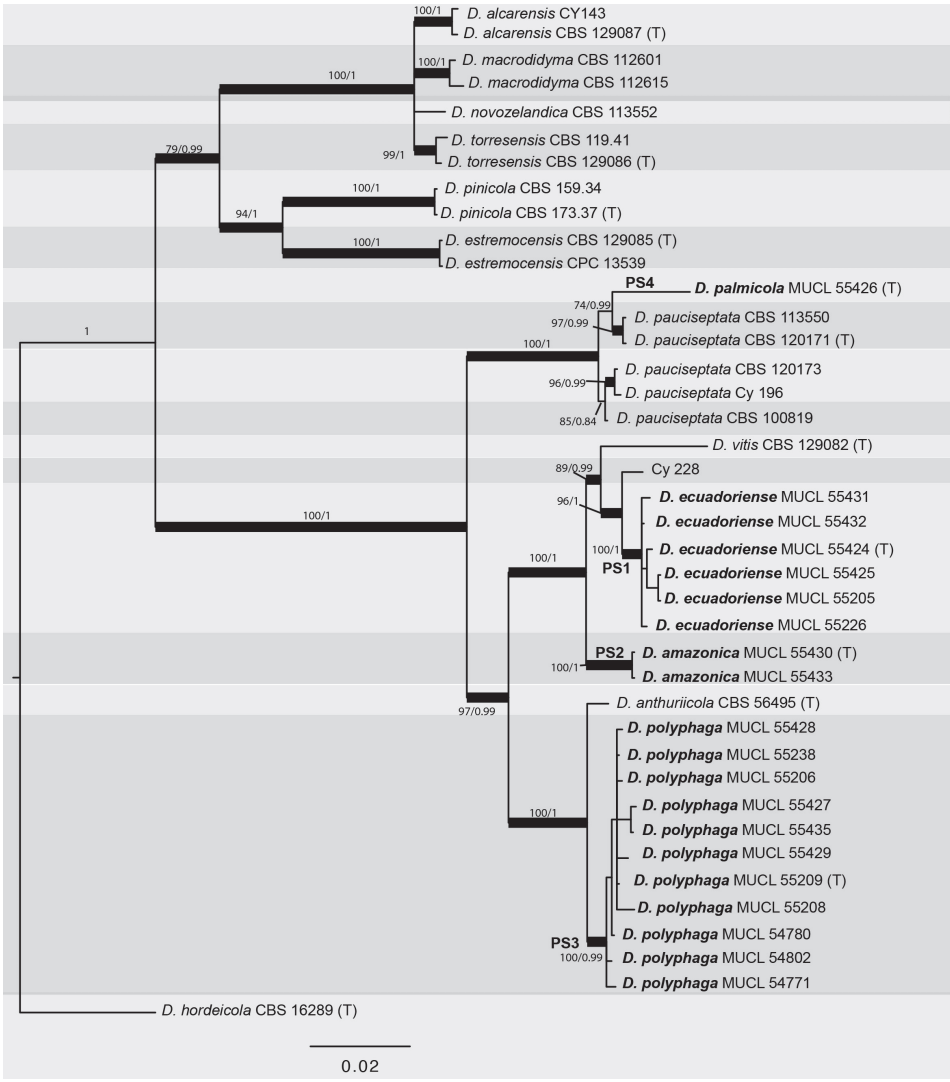

Fig. 1. The ML best tree (-lnL -8071.916899) inferred from the combined five-gene (ITS, 28S, *tub2*, *tef1a* and *his3*) DNA sequence alignment. ML Bootstrap support (BS) values and posterior probability (PP) are indicated and highlighted in bold lines (≥ 80% BS and ≥ 0.95 Bayesian PP.) (T) = Type.

South Africa (Halleen *et al.* 2004) (Table 1). This data set was subdivided into 14 partitions, *viz*. ITS1, 5.8S, ITS2, Intron 1 *tub,* Exon1 *tub*, Intron 2 *tub,* Exon 2 *tub,* Intron 3 *tub,* Exon 3 *tub,* Exon 1 *his3*, Intron 1 *his3,* Exon 2 *his3,* Intron 2 *his3,* Exon 3 *his3.* The models estimated as the best-fit likelihood model of evolution for BI for each partition are summarized in Table 3.

The 70% reciprocal bootstrap tree topologies showed no conflicts between ITS, *tub2* and *his3* gene regions for the *Campylocarpon* data set. The concatenated *Campylocarpon* data set comprises 1588 positions (including gaps). *Ilyonectria*

Table 3. Summary of data sets *Campylocarpon* of ITS, *Btub* and *h3*

Parttions	*Datasets*					
	ITS1	5.8S	ITS2	*tub* I1, *tub* I2, *tub* I3	*tub* E1, *tub* E2, *tub* E3, *his3* E1, *his3* E2, *his3* E3	*his3* I1, *his3* I2
Model selected	SYM + G	JC	HKY + I	GTR	GTR + I	HKY + I
			Base frequencies			
Freq. A	0.234	0.306	0.142	0.278	0.187	0.271
Freq. C	0.323	0.224	0.39	0.32	0.367	0.376
Freq. G	0.212	0.216	0.291	0.177	0.245	0.148
Freq. T	0.231	0.254	0.178	0.226	0.2	0.205
Proportion of invariable sites			0.567		0.752	0.193
Gamma shape	1.108					

I = Intron; E = Exon

radicicola CBS 264.65 was used as outgroup (Halleen *et al.* 2004). The two Bayesian runs converged to stable likelihood values after 30000 generations. The first 25% of saved trees were discarded as the "burnin" phase. In the ML searches with RAxML the combined data s*et ali*gnment had 327 distinct patterns with a proportions of gaps and undetermined characters of 31.45%. The best scoring ML tree is shown at Fig. 2 (-lnL -4375.971595).

The *Campylocarpon* lineage (Lombard *et al.* 2014) (Fig. 2) is divided into two clades, of which only one is well supported. The well-supported clade (BSML = 100, PP = 0.99) is itself divided into two well-supported subclades. The first subclade (BSML = 96, PP = 1) includes the type strain of *C. fasciculare* (CBS 112613) together with several strains originating from grapevine in South Africa (Fig. 2); it represents *C. fasciculare* s.s. The second clade (BSML = 94, PP = 0.66) is composed of several grapevine associated strains, originating from Northeaster Brazil (Correia *et al.*, 2013).

The second clade is not supported (Fig. 2). It includes the type strain of *C. pseudofasciculare*, the strains CBS 112592 and BV7, a set nine strains originating from southern Brazil (Dos Santos *et al.* 2014). Our Amazonian strain MUCL 55434 (branch PS5) forms an isolated branch, which relationships with other *Campylocarpon* are unresolved (Fig. 2).

Morphological analysis

Morphological studies of our Amazonian isolates reveal that the strains of the new clades/branches PS1-5 also present each phenotypic singularities allowing morphological distinction from each other (Tables 4, 5) but also morphological distinction from their closest phylogenetic relatives. The main morphological differences are in the conidial shape and size (Tables 4, 5). Amongst our set of strains, those forming PS4 has the longest 3-septate conidia, averaging 42 × 7.3 µm

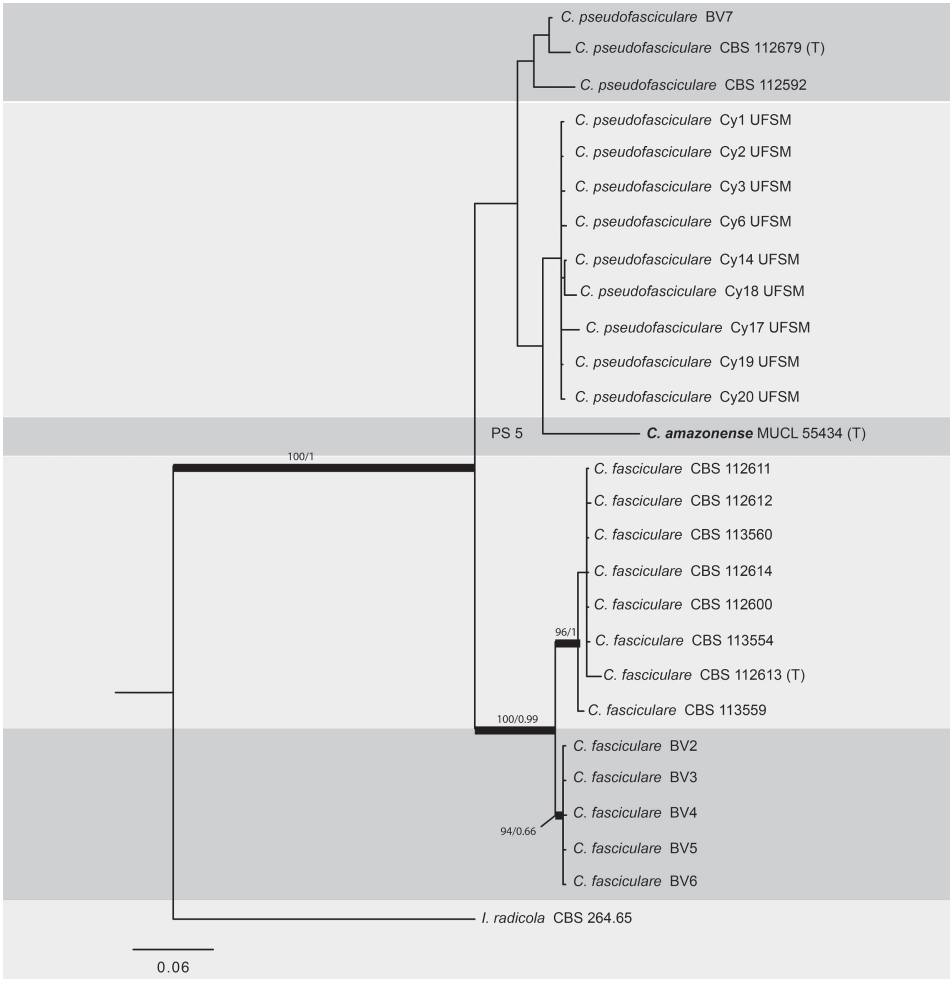

Fig. 2. The MB best tree (-lnL -4375.971595) inferred from the combined tree-gene (ITS, *tub2* and *his3*) sequence alignment. ML Bootstrap support (BS) values and posterior probability (PP) are indicated and highlighted in bold lines (≥ 80% BS and ≥ 0.95 Bayesian PP.) (T) = Type.

whereas those forming PS3 has the smallest conidia, averaging 34.5 × 7.7 µm. The strains forming PS1 and PS2 have relatively similar conidia, differing by their width, respectively 7-8 and 5.5-6.5 µm. Noteworthy, our single isolate MUCL 55426 (PS4) also forms *in vitro* a sexual form, absent in our other Amazonian strains, and, so far, not reported in other strains of the *D. pauciseptata* lineage. Our *Campylocarpon* strain (PS5) has typical, curved conidia, with up to 5 septa.

Taxonomic conclusions

Phylogenetic studies of a set of Amazonian cylindrocarpon-like isolates revealed the occurrence of five clades or branches, which are equated to as

Table 4. Comparison of the conidial size of *Campylocarpon* species

Species	*Substrate*	*# of septa*	*Conidial features* *Conidial size (µm)*	*Conidial av. size (µm)*
C. amazonense Gordillo & Decock	Rhizoplane of *Cordia alliodora*	2-septate	(23.5-) 24-32 (-33.5) × (5.5-) 5.3-6.0 (-6.2)	28 × 5.8
		3-septate	(28-) 33-44 (-47) × (5.5-) 5.9-7 (-7)	39 × 6.5
		4-septate	(31-) 33-47 (-49) × (6.2-) 6.0-7.0 (-7.8)	40 × 6.5
		5-septate	(39-) 46.2-54.2 (54.6-) × (6.2-) 6.7-8 (-7.8)	50 × 7.3
C. fasciculare Schroers *et al.*	Roots, rootstock and trunk of *Vitis vinifera*, causing black foot disease	2-septate	(28.5-) 35-43.5 (-47) × (6-) 6.5-7.5 (-9)	38 × 7
		3-septate	(29-) 38-44.5 (-53) × (5.5-) 6.5-8 (-9)	41.5 × 7.5
		4-septate	(39-) 47-51.5 (-58)×(6.5-) 7.5-8.5 (-9) 44.5-54 × 7.5-9	49 × 8
		5-septate		–
C. pseudofasciculare Halleen *et al.*	Roots of asymptomatic *Vitis vinifera* in nursery	2-septate	24-36 × 6-7	–
		3-septate	(29-) 37-48 (-68.5) × (6.0) 6.5-7.5 (-9.5)	44 × 7
		4-septate	(40.5-) 46.5-53.5 (-62) × (6.5-) 7-8.5 (-9.5)	51 × 8
		5-septate	(35.6) 51-59 (-68) × (6.5-) 7.5-8.9 (-10)	55 × 8

Table 5. Comparison of the conidial features of the new *Dactylonectria* species from the Ecuadorian Amazonia

Species	*Substrate*	*# of septa*	*Conidial size (µm)*	*Conidial av. size (µm)*
Dactylonectria amazonica	Rhizoplane	3-septate conidia	(31-) 31-41 (-43) × (5.5-) 5.7-6.5 (-7.0)	37 × 6.2
Dactylonectria ecuadoriense	Root (endophyte) and rhizoplane	3-septate conidia	(31-) 34.0-43.0 (-43.0) × (7-) 7 -8 (-8.5)	38 × 8
Dactylonectria palmicola	Rhizoplane	3-septate conidia	(37-) 39-47 (-47.0) × (6-) 7.0-7.5 (-8)	42 × 7.3
Dactylonectria polyphaga	Root (endophyte)	3-septate conidia	(31-) 31-37 (-39) × (7.0-) 7 - 8 (-7.8)	34.5 × 7.7

many phylogenetic species. Four species belong to *Dactylonectria* and one to *Campylocarpon*. Each of these phylogenetic species also presents a phenotype allowing to distinguish them (Table 5) but also allowing to differentiate them from their closest phylogenetic relatives (Table 4). These species, therefore, are considered as five undescribed taxa, proposed below as *Campylocarpon amazonense* (PS5), *Dactylonectria amazonica* (PS2), *D. ecuadoriense* (PS1), *D. palmicola* (PS4), and *D. polyphaga* (PS3).

TAXONOMY

Campylocarpon amazonense Gordillo & Decock, **sp. nov.** **Fig. 3 a-c**

MycoBank: MB 822796

Holotype: ECUADOR, Prov. Sucumbíos, Nueva Loja, Lago Agrío canton, Charapa camp, secondary rain forest, rhizoplane, *Cordia alliodora* (Ruiz & Pav.) Oken (Boraginaceae), Jul. 2014, A. Gordillo & C. Decock, MUCL 55434, as a two-week-old dried culture on BLA (living culture ex-holotype MUCL 55434 = PUCE PHPE4-18-1014).

Etymology. "*amazonense*" (Latin): from the area of origin, the Amazonian rain forest.

Culture characteristics: **colonies** on PDA reaching 35 mm diam. in 7 days, with abundant aerial mycelium over the whole colony or in sectors, cottony to felty or forming hyphal strands, white to light brown first (6D5) then yellowish brown, sometimes partly covered by off-white slime, the reverse brown (6E8).

Conidiophores first simple, arising laterally from aerial hyphae, consisting of a short cell from which emerge 1-3 phialides, later gathered in fascicles 80 µm high, 100 µm diam., with a basal stipe 7.5-14.5 × 3-4.0 µm, supporting 1-3 branches, 11.5-31 × 3-4.0 µm, each with a single phialide; **phialides** narrowly flask-shaped, the widest point near the middle, (14-) 15-20 (-27) µm long, 2.5-3.5 (-4.0) µm wide at the base, 3.0-4.0 µm at the widest point, and 1.5-2.3 µm near the aperture (av. = 17.3 × 3.6); **macroconidia** (2-) 3-5 septate, cylindrical, faintly to moderately curved, with obtuse ends, sometimes tapering at the base; 2-septate conidia (23-) 24-33 (-33.5) × (5.5-) 5.3-6.0 (-6.2) µm (av. = 27.8 × 5.8 µm), 3-septate conidia (28-) 31-46 (-47) × (5.5-) 5.9-7 (-7.8) µm (av. = 39 × 6.5 µm), 4-septate conidia (31-) 32-49 (-49) × (6.2-) 6.0-7.0 (-7.8) µm (av. = 40 × 6.5 µm), 5-septate conidia (39-) 40-54 (-54.6) × (6.2-) 6.7-8 (-7.8) µm (av. = 50 × 7.3 µm); **conidial masses** off-white, first hemispherical, then sliding over the mycelium; **microconidia** not observed; **chlamydospore**s not observed.

Sexual morph: not observed.

Substratum, host and habitat: rhizoplane of *Cordia alliodora* (Ruiz & Pav.) Oken (Boraginaceae).

Remarks: *Campylocarpon amazonense* shares with *C. fasciculare* and *C. pseudofasciculare* the 3-5-septate, slightly curved macroconidia. It differs from *C. pseudofasciculare* in having smaller macroconidia and from *C. fasciculare* in having narrower macroconidia (cf. Table 4).

Campylocarpon amazonense is the third species described in the genus, and the first that is not associated with grapevine.

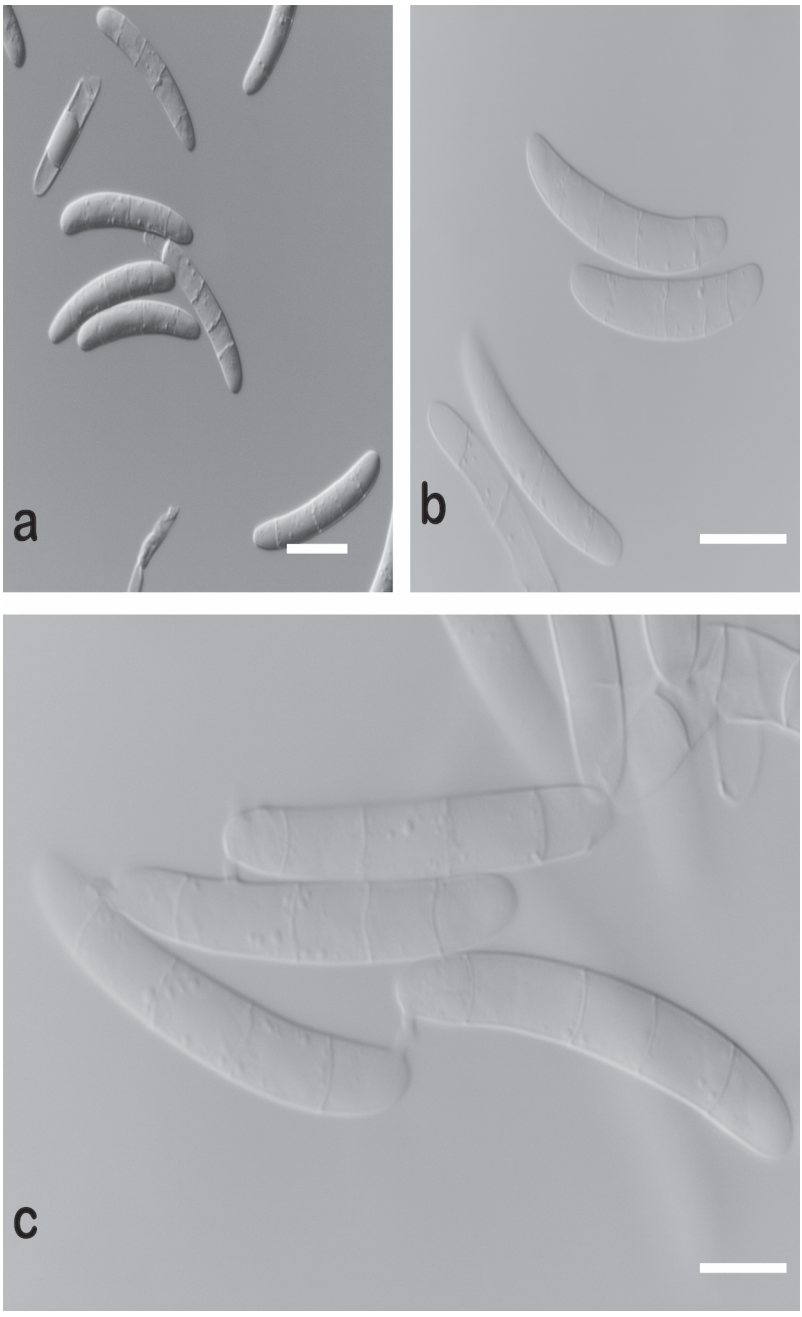

Fig. 3. **a-c.** *Campylocarpon amazonense*, from MUCL 55434 (type). **a-c.** Macroconidia (Figs a, b scale bars = 20 mm; c, scale bars = 10 mm). All from cultures on BLA.

Dactylonectria amazonica Gordillo & Decock, **sp. nov.** **Fig. 4 a-f**

MycoBank: MB 822799

Holotype: ECUADOR, Prov. Sucumbíos, Nueva Loja, Lago Agrío canton, Charapa camp, secondary rain forest, from the rhizoplane of *Piper sp.* (Piperaceae), Jun. 2014, A. Gordillo & C. Decock, MUCL 55430, as two-week-old dried culture on BLA (living culture ex-holotype MUCL 55430, PUCE PHPE4-34-768).

Etymology. "*amazonica*" (Latin): from the area of origin, the Amazonian rain forest.

Culture characteristics: **colonies** on PDA 15 mm diam. in 7 days, with sparse aerial mycelium, the margin light brown (6D6), light brown (6D4) towards the centre, the reverse brown (6F8).

Conidiophores forming macroconidia solitary to loosely aggregated, arising laterally or terminally from aerial mycelium, unbranched or sparsely branched, stipe 30 × 2.5 µm, bearing 1-3 phialides; **phialides** subcylindrical, slightly tapering towards the apical conidiogenous loci, 11.0-15.5 µm long, 2.5-3.0 µm wide at the base, 2.3-4.0 µm at the widest point, approx. in the middle, 1.5-2.3 µm near the aperture; **macroconidia** cylindrical, straight or faintly curved, with both ends rounded, mostly without a visible hilum, predominantly 3-septate, rarely 4-septate; 3-septate conidia (31-) 31-41 (-43) × (5.5-) 5.7-6.5 (-7.0) µm (av. = 37 × 6.2 µm), with a length:width ratio of 5.7-6.1, accumulating in flat, slimy domes; **conidiophores** forming microconidia formed on surface mycelium, mono- or bi-verticillate; **phialides** narrowly flask-shaped, the widest point near the middle, 7-11 × 2.3 µm; **microconidia** formed in heads, aseptate, subglobose to ovoid, rarely ellipsoid, mostly with a visible, centrally located or slightly laterally displaced hilum, 6-10 × 4 µm (av. = 8.4 × 4 µm), with a length:width ratio of 2.1 µm; **chlamydospores** globose to subglobose to ellipsoid, 8-12 × 6-10 µm, smooth, thick-walled, formed intercalary in chains or in clumps, becoming golden-brown.

Sexual morph: not observed.

Substratum, host and habitat: rhizoplane of *Piper* sp (Piperaceae), Neotropical rain forest.

Additional specimens examined: ECUADOR, Prov. Sucumbíos, Nueva Loja, Lago Agrío canton, Charapa camp, secondary rain forest, root, *Piper sp.* (Piperaceae), Jun. 2014, A. Gordillo & C. Decock, living culture as MUCL 55433 = PUCE PHPE4-34-950.

Dactylonectria ecuadoriense Gordillo & Decock, **sp. nov.** **Fig. 5 a-f**

MycoBank: MB 822801

Holotype: ECUADOR, Prov. Sucumbíos, Nueva Loja, Lago Agrío canton, Charapa camp, secondary rain forest, rhizoplane, *Piper sp.* (Piperaceae), Nov. 2013, A. Gordillo & C. Decock, MUCL 55424, as a two-week-old dried culture on BLA (living culture ex-holotype MUCL 55424 = PUCE PHPE3-1-621).

Etymology. "*ecuadoriense*" (Latin): from the country of origin, Ecuador.

Culture characteristics: **colonies** on PDA reaching 30 mm diam. in 7 days, with sparse aerial mycelium, the marginal area light brown (6D6) to brownish orange (5C7), light brown toward the centre (5D6), the reverse brown (6D8).

Conidiophores forming macroconidia solitary to loosely aggregated, arising laterally or terminally from aerial hyphae, unbranched or sparsely branched, stipe 30 × 3.0 µm, bearing 1-3 phialides; **phialides** subcylindrical, slightly tapering

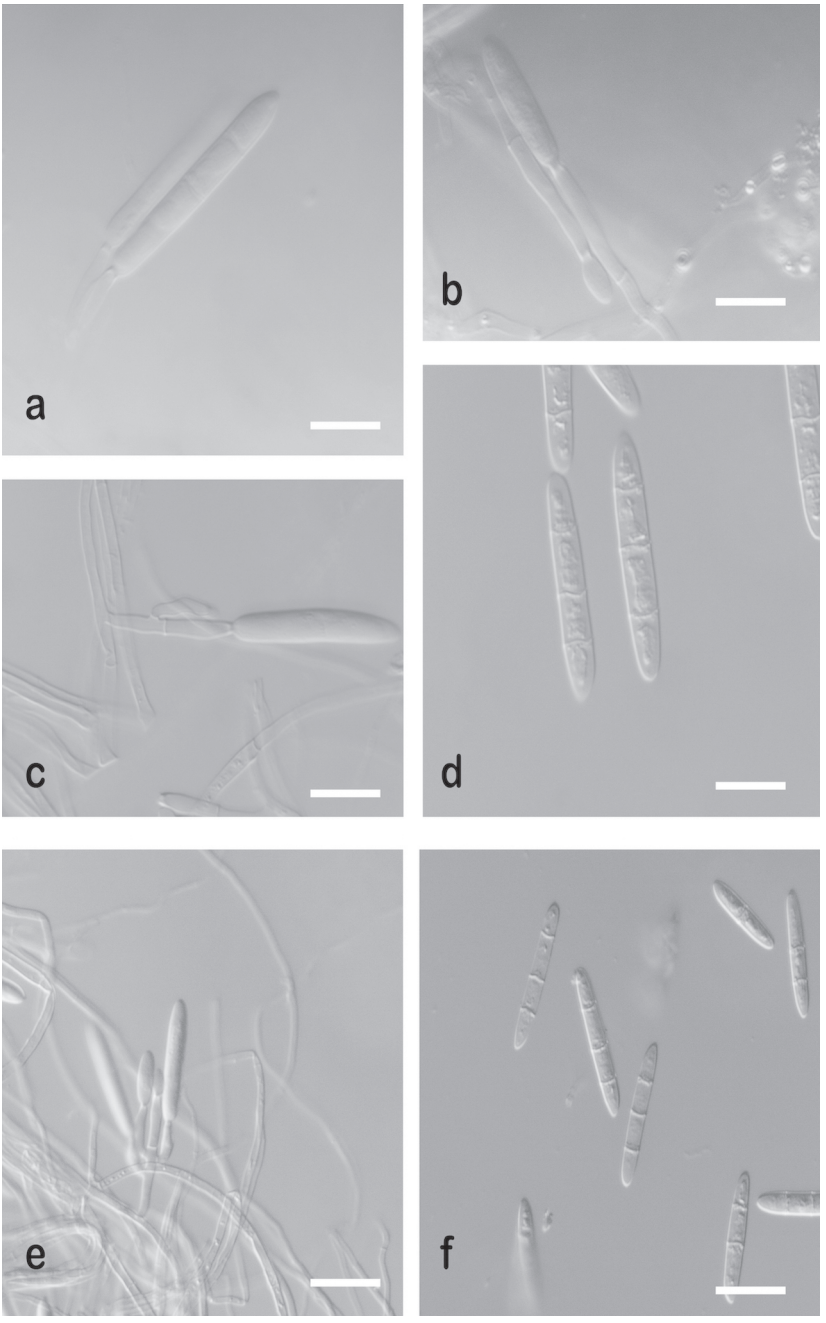

Fig. 4. **a-f.** *Dactylonectria amazonica* from MUCL 55430 (type). **a, d, f.** 3-septate macroconidia; **b, c, e.** Conidiophores (Figs a, c, d scale bars = 20 mm; b, e, f scale bars = 40 mm). All from cultures on BLA.

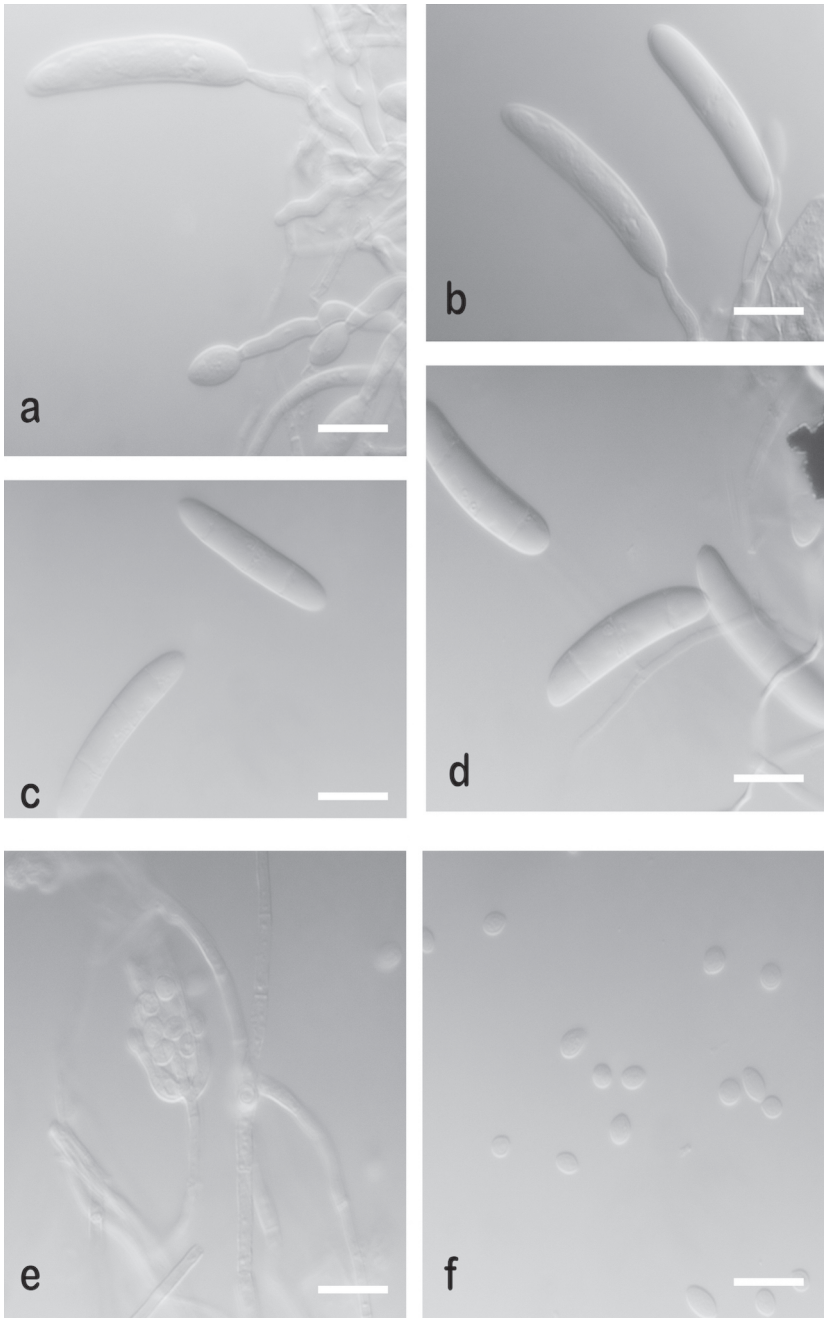

Fig. 5. **a-f.** *Dactylonectria ecuadoriense* from MUCL 55424 (type). **a-b.** Conidiophore; **c-d.** 3-septate macroconidia; **e.** Conidiophores microconidia; **f.** Microconidia (Figs a-f, scale bars: = 15 mm). All from cultures on BLA.

towards the apical conidiogenous loci, 8.0-20 µm long, 2-3 µm wide at the base, 2.5-4.0 µm at the widest point, near the middle, 1.5-2 µm near the aperture; **macroconidia** cylindrical, straight to faintly curved, with both ends rounded, without visible hilum, predominantly 3-, rarely 4-septate, (31-) 34-43 (-43) × (7.0-) 7.0-8.0 (-8.5) µm (av. = 38 × 8 µm), length:width ratio = 4.4-5.4, accumulating in slimy drops, forming flat domes; **conidiophore**s forming microconidia on aerial mycelium, mono- or biverticillate; **phialides** narrowly flask-shaped, the widest point near the middle, 7.8-12 × 2 µm wide; **microconidia** formed in slimy heads, aseptate, subglobose to ovoid, rarely ellipsoid, 3.0-4.5 × 3.0-4.5 (-5.5) µm (av. = 3.8 × 3.7 µm), with a length:width ratio of 0.9-1; **chlamydospore**s intercalary, formed in short chains or in cluster, individually globose, subglobose to ellipsoid, 7.8-14 × 4-11.7 µm, smooth, thick-walled, becoming golden-brown.

Sexual morph: not observed.

Substratum, host and habitat: rhizoplane of several angiosperms and one pteridophyte, including *Socratea exorrhiza* (Mart.) H Wendl (Arecaceae), *Carludovica palmate* Ruiz & Pav. (Cyclanthaceae), *Piper* sp. (Piperaceae) and *Cyathea lasiosora* (Mett. Ex Kuhn) Domin (Cyatheaceae, Pteridophyta), in Neotropical, Amazonian rain forest.

Additional specimens examined: ECUADOR, Prov. Sucumbíos, Nueva Loja, Lago Agrío canton, Charapa camp, secondary rain forest, rhizoplane, *Carludovica palmate* (Cyclanthaceae), Jun. 2014, A. Gordillo & C. Decock, living culture MUCL 55431 = PUCE PHPE4-23-912; ibid., rhizoplane, *Socratea exorrhiza* (Arecaceae), Jun. 2014, A. Gordillo & C. Decock, living culture MUCL 55432 = PUCE PHPE4-37-925; ibid., root, *Cyathea lasiosora* (Cyatheaceae, Pteridophyta), Nov. 2013, A. Gordillo & C. Decock, living culture MUCL 55226 = PUCE PHPE3-26-671; ibid., rhizoplane, *Piper* sp. (Piperaceae), Nov. 2013, A. Gordillo & C. Decock, living culture MUCL 55205 (= MUCL 55425) = PUCE PHPE3-1-622.

Remarks: *Dactylonetria amazonica* (PS2) and *D. ecuadoriense* (PS1) are morphologically similar and share some ecological parameters; both species share the same microhabitat and, partly, their host ranges. Morphologically (Table 5), they mainly differ in the width of their 3-septate macroconidia, respectively 5.7-6.5 µm (av. = 6.2 µm) and 7.5-8 µm (av. = 7.7 µm). *Dactylonectria palmicola* has longer macroconidia whereas *D. polyphaga* has smaller macroconidia (Table 5).

In a phylogenetic perspective, *D. amazonica* and *D. ecuadoriense* are related to *D. vitis*, which is known so far only from *Vitis vinifera* in southern Europe (Cabral *et al.* 2012). They differ from *D. vitis* in having smaller 3-septate macroconidia; the conidial size ranges in *D. amazonica* and *D. ecuadoriense* are, respectively, mostly 34-39 × 5.7-6.5 µm (av. = 37 × 6.2 µm) and 34-40 × 7.5-8 µm (av. = 37.9 × 7.7 µm), reaching up to 43 µm in both. The size range is 41.5-43.5 × 7.9-8.2 µm (av. = 42.5 × 8.0 µm), reaching up to 51.5 µm in *D. vitis* (Cabral *et al.* 2012).

In addition, *D. amazonica* and *D. ecuadoriense* on one side and *D. vitis* on the other side are known from drastically different environments with very different humidity regime and vegetation, *viz*. a hyper humid area of the Amazonian rain forest and a spot of the Mediterranean area of Southern Europe.

Dactylonectria palmicola Gordillo & Decock, **sp. nov.** **Fig. 6 a-f**

MycoBank: MB 822804

Holotype: ECUADOR, Prov. Sucumbíos, Nueva Loja, Lago Agrío canton, Charapa camp, secondary rain forest, rhizoplane, *Euterpe precatoria* (Arecaceae),

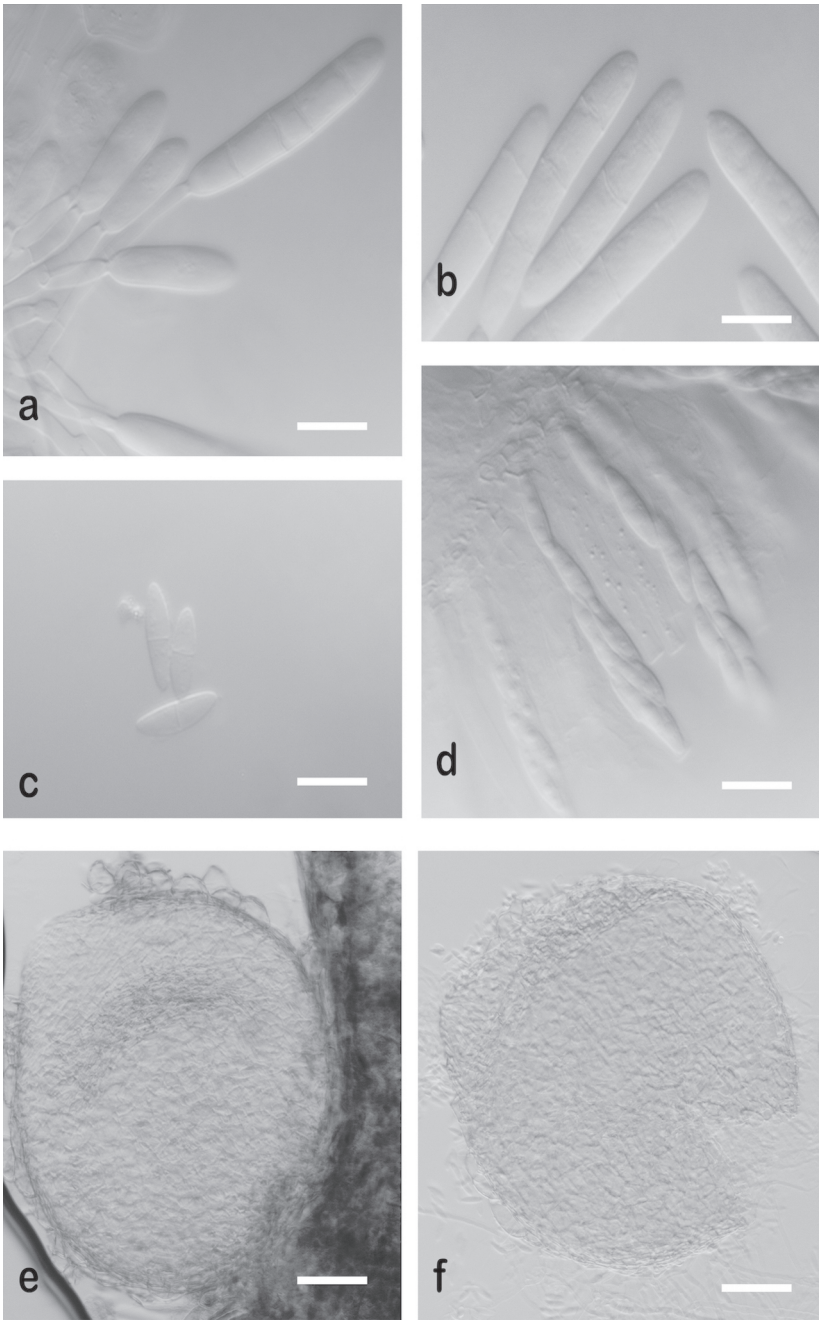

Fig. 6. **a-f.** *Dactylonectria palmicola* from MUCL 55426 (type). **a.** Conidiophores and macroconidia; **b.** 3-septate macroconidia; **c.** Ascospores; **d.** Asci; **e-f.** Perithecia (Figs a-d. scale bars = 15 mm; e-f. scale bars = 35 mm). All from cultures on BLA.

Nov. 2013, A. Gordillo & C. Decock, MUCL 55426, as a two-week-old dried culture on BLA (living culture ex-holotype MUCL 55426 = PUCE PHPE3-11-641).

Etymology. "*palmicola*" (Latin): refers to the common name of the host plant, "palm".

Culture characteristics: **colonies** on PDA reaching 41 mm diam. in 7 days, with sparse or no aerial mycelium, margin white to greyish orange (5B3), orange grey (5B2) in sectors towards the centre, the reverse brownish yellow (5C7) to greyish orange (5B5).

Perithecia formed (homothallically) *in vitro*, developing directly on the agar surface or on sterile pieces of banana leaf, solitarily or aggregated, ovoid to obpyriform, with a flattened apex, smooth to finely warted, dark red, darkening in 3% KOH, 260-310 µm high × 160-280 µm diam.; **perithecial** wall made of two little distinguishable layers; **outer layer** composed of 1-3 rows of angular or subglobose cells; **inner layer** composed of angular to oval cells in subsurface face view; **asci** narrowly clavate to cylindrical, 72-60 × 6-8 µm, 8-spored; **ascospores** ellipsoid to oblong- ellipsoid, somewhat tapering towards both ends, centrally 1-septate, smooth to finely warted, frequently guttulate, hyaline, (12.5-) 13-16 (-17) × (3.5-) 3.5-4.2 (-4.7) µm (av. = 14.7 × 3.9 µm).

Conidiophores forming macroconidia simple or loosely aggregated, arising laterally or terminally from aerial or surface hyphae, unbranched or sparsely branched, stipe 6.2-23.5 × 2.3-4.0 µm, bearing 1-3 phialides; **phialides** subcylindrical, slightly tapering towards the apical conidiogenous loci, 10-15 × 2.3-4.0 µm wide; **macroconidia** cylindrical, straight or faintly curved, with both ends more or less broadly rounded, mostly without a visible hilum, predominantly 3-septate, (37.5-) 39-47 (-47.5) × (6.0-) 7.0-7.5 (-8.0) µm (av. = 42 × 7.3 µm), with a length:width ratio of 6, accumulating in slimy, flat domes; **conidiophores** forming microconidia formed on hyphae at agar surface, mono- or bi-verticillate; **phialides** narrowly flask-shaped, the widest point near the middle, 8.0-11 × 2 µm; **microconidia** formed in heads, aseptate, subglobose to ovoid, rarely ellipsoid, mostly with a visible, centrally located or slightly laterally displaced hilum, (4.0-) 4-6 (-6.0) × (3.0-) 2.5-4.2 (-4.0) µm (av. = 5 × 3.5 µm), with a length:width ratio of 0.78-1.6; **chlamydospores** globose to subglobose to ellipsoid, 10-18× 12-20 µm, smooth, thick-walled, formed intercalary in chains or in clumps, becoming golden-brown.

Substratum, host and habitat: rhizoplane of *Euterpe precatoria* (Arecaceae), Neotropical, Amazonian rain forest.

Remarks: *Dactylonectria palmicola* differs from the other Amazonian species in having a sexual morph and the longest macroconidia (Table 5).

This species is phylogenetically related to *D. pauciseptata*, from which it differs in having narrower macroconidia, mostly 7.0-7.5 µm (av. = 7.3 µm) *vs* 8.5-9.5 (av. = 9 µm, Schroers *et al.* 2008). *Dactylonectria palmicola* also produces a sexual form *in vitro*, which is so far unknown in *D. pauciseptata.* These two species also differ by their habitat; *D. pausiceptata* is known from *Vitis* sp., in vineyard in Portugal and Slovenia (Cabral *et al.* 2012) whereas *D. palmicola* originates from a hyper humid area of the Amazonian rain forest.

Dactylonectria polyphaga Gordillo & Decock, **sp. nov.** **Fig. 7 a-f**

MycoBank: MB 822805

Holotype: ECUADOR, Prov. Sucumbíos, Nueva Loja, Lago Agrío canton, Charapa camp, secondary rain forest, root *Costus sp.* (*Costaceae*), Nov. 2013,

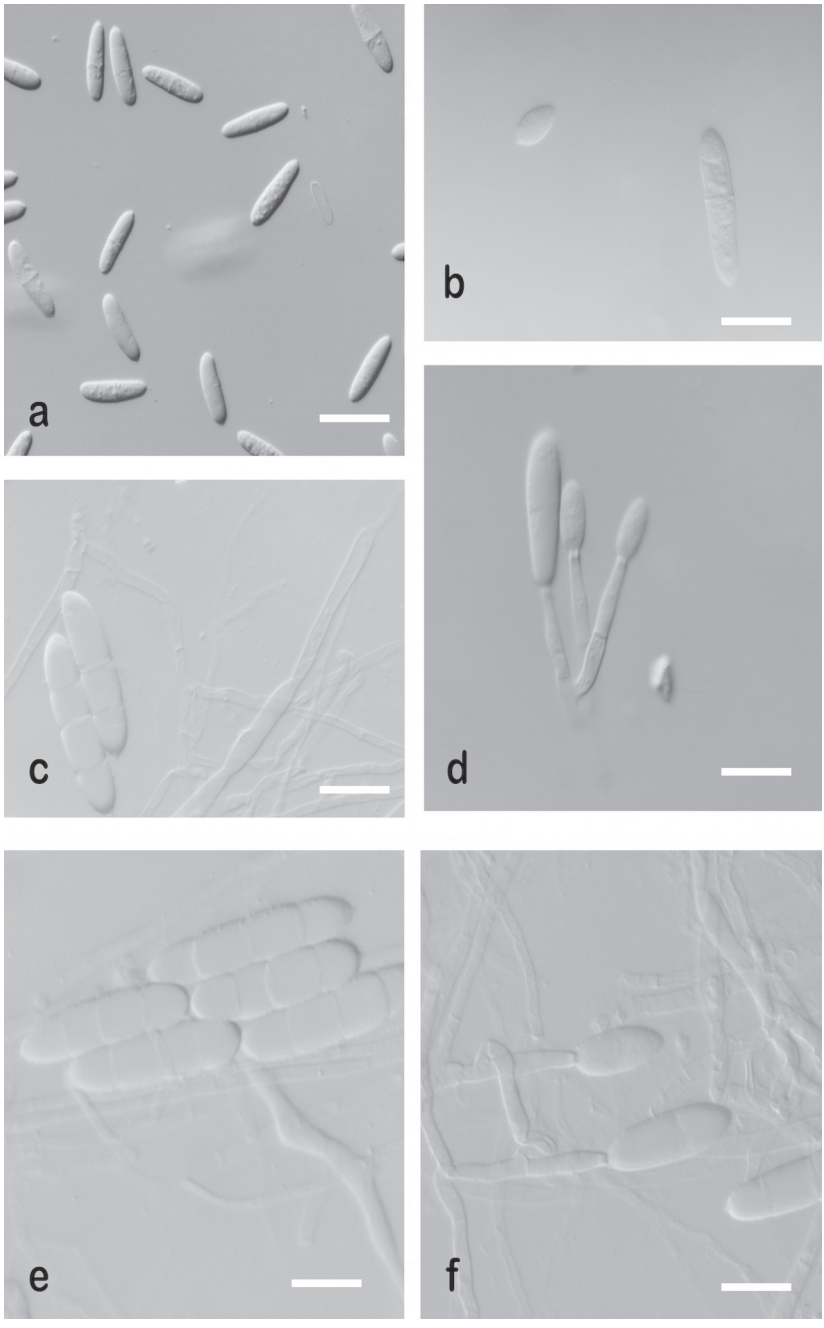

Fig. 7. **a-f.** *Dactylonectria polyphaga* from MUCL 55209 (type). **a, c, e.** 3-septate macroconidia ; **b.** Microconidia; **d, f.** Conidiophores (Figs a, scale bars = 40 mm; b-f scale bars = 20 mm). All from cultures on BLA.

A. Gordillo & C. Decock, MUCL 55209, as a two-week-old dried culture on BLA (living culture ex-holotype MUCL 55209 = PUCE PHPE3-4-628).

Etymology. "*poly-"* (Greek): meaning "many", and *phagein* (Greek): meaning "to eat".

Culture characteristics: **colonies** on PDA reaching 26 mm diam. in 7 days, with sparse aerial mycelium, the margin brownish yellow (5C7), golden brown toward the centre (5D7), the reverse brown (6E7).

Conidiophores forming macroconidia solitary to loosely aggregated, arising laterally or terminally from aerial mycelium, unbranched or sparsely branched, stipe 19 × 3.2 µm, bearing 1-3 phialides; **phialides** subcylindrical, slightly tapering towards the tip, 7.8-22 µm long, 2-3 µm wide at the base, 2.3-4.0 at the widest point, 1.5-2.5 µm near the aperture; **macroconidia** cylindrical, straight or minutely curved, with both ends more or less broadly rounded, mostly without a visible hilum, predominantly 3-septate, rarely 1-2- or 4-septate; 3-septate conidia (31-) 31-37 (-39) × (7.0-) 7.0-8.0 (-7.8) µm (av. = 34.5 × 7.7 µm), with a length:width ratio of 4.4-5, accumulating in slimy, flat domes; **conidiophores** giving rise to microconidia formed on mycelium at agar surface, mono- or bi-verticillate; **phialides** narrowly flask-shaped, typically with widest point near the middle, 7-11 µm long, 2 µm wide; **microconidia** formed in heads, aseptate, subglobose to ovoid, rarely ellipsoid, mostly with a visible, centrally located or slightly laterally displaced hilum, 4.0-4.7 × 3.0-4.0 µm, with a length:width ratio of 1.2; **chlamydospore**s globose to subglobose to ellipsoid, 8-10 × 8-12 µm, smooth, thick-walled, formed intercalary in chains or in clumps, becoming golden-brown.

Sexual morph: not observed.

Substratum, host and habitat: rhizoplanes and roots of *Piper* sp. (Piperaceae), *Asplenium sp.* (Aspleniaceae), *Costus sp., C. scaber* (Costaceae), *Anthurium sp.* (Araceae), *Miconia sp.* (Melastomataceae), *Euterpe precatoria* (Arecaceae) Neotropical rain forest.

Additional specimens examined: ECUADOR, Prov. Sucumbíos, Nueva Loja, Lago Agrío canton, Charapa camp, secondary rain forest, root, *Costus sp.* (*Costaceae*), Nov. 2013, A. Gordillo & C. Decock, living culture MUCL 55208; *ibid*., root, *Asplenium sp.* (Aspleniaceae, Pteridophyta), Jan. 2013, A. Gordillo & C. Decock, living culture MUCL 54802 = PUCE PHPE2-19-240; *ibid*., root, *Costus scaber* Ruiz & Pav (Costaceae), living culture MUCL 54771 = PUCE PHPE2-35-299; *ibid*., root, *Anthurium* (Araceae), living culture MUCL 54780 = PUCE PHPE2-04-399); ibid., root, *Costus* (Costaceae), living culture MUCL 55238 = PUCE PHPE3-4-626; *ibid*., Nov. 2013, root, *Piper* sp. (Piperaceae), living culture MUCL 55206 = PUCE PHPE3-1-624; *ibid*., rhizoplane, *Acalypha* (Euphorbiaceae), living culture MUCL 55427 = PUCE PHPE3-20-657; *ibid*., Jul. 2014, root, *Miconia sp.* (Melastomataceae), MUCL 55435 = PUCE PHPE4-22-1016; *ibid*., Jun. 2014, root, *Euterpe precatoria* (Arecaceae), living cultures MUCL 55429 = PUCE PHPE4-26-750, MUCL 55428 = PUCE PHPE4-40-745.

Remarks: *Dactylonectria polyphaga* differs from the other Amazonian species in having the smallest macroconidia (Table 5).

This species is phylogenetically related to *D. anthuriicola* (Fig 1); they differ in the size range of their 3-septate macroconidia, respectively 32-36 × 7.3-7.9 (av. = 34.5× 7.7 µm) *versus* 29.5-32 × 7.5-8.1 µm (av. = 30.8 × 7.8 µm, Cabral *et al.* 2012).

DISCUSSION

Dactylonectria was introduced to accommodate a bunch of species mostly associated with black foot symptoms of grapevine in Australia, Europe, New Zealand, South Africa and USA (Cabral *et al.* 2012a, b, Lombard *et al.* 2014). Ten species are currently known. *Dactylonectria alcacerensis*, *D. macrodidyma*, *D. novozelandica* and *D. vitis* are only known from grapevines (Cabral *et al.*, 2012a, b; Lombard *et al.* 2014). *Dactylonectria estremocensis*, *D. pauciseptata*, *D. pinicola* and *D. torrecensis* are known from grapevines but also from other hosts including conifers (Lombard *et al.* 2014). So far, only two species are known exclusively from plants other than grapevine, *viz*. *D. anthuriicola* on *Anthurium* sp. (Araceae) and *D. hordeicola* on *Hordeum vulgare* (Poaceae). Furthermore, as far as we have been able to ascertain, there is no record of *Dactylonectria* from from South America and more specifically from the Amazonian rain forest.

Campylocarpon, originally, also was described in association with grapevine, causing black foot symptoms, in South Africa (Halleen *et al.* 2004). *Campylocarpon* species, however, were recorded in several areas of South America, also associated to disease symptoms of grapevine: *C. fasciculare* was reported from Brazil (Correia *et al.* 2013) whereas *C. pseudofasciculare* was reported from Uruguay (Abreo *et al.* 2010), Peru (Álvarez *et al.*, 2012), North-eastern (Correia *et al.*, 2013) and southern Brazil (Dos Santos *et al.*, 2014).

Four *Dactylonectria* and one *Campylocarpon* species are here described, all originating from the Amazonian rain forest in Ecuador. These species were found directly (endophytic) or indirectly (rhizoplane) associated with several herbaceous angiosperms and one pteridophyte (Table 1). They were isolated from externally sane, asymptomatic roots, either from their internal tissues as endophytes or from their rhizoplanes. They are therefore not associated with specific disease symptoms such as root rots or black foot.

Their hosts were growing in a very disturbed, heavily polluted microhabitat in the Amazonian rain forest, which consists of a floating layer of decomposing organic debris, mostly vegetal, forming a compost-like substrate, 10-20 cm thick, and accumulating through the years over crude oil pools. These *Dactylonectria* and *Campylocarpon* species should be searched for in the neighbouring, undisturbed ecosystem to circumscribe their host range and ecology.

The phylogenetic inferences presented above also show some diversities within the *D. pauciseptata* lineage as defined by Lombard *et al.* (2014). In our analyses, the *D. pauciseptata* lineage is divided into four clades or branches. The first clade (Fig. 1, BSML = 100, PP = 1) include the type strain of *D. pauciseptata* (CBS 120171, Slovenia) and the strain CBS 113550 (New Zealand); it corresponds to *D. pauciseptata* s.s. A second clade includes two strains, *viz*. CBS 120173 (Portugal) and Cy 196 (Slovenia) (Cabral *et al.* 2012). *Dactylonectria palmicola* (MUCL 55426, Ecuador) and the strain CBS 100819 (New Zealand) form each an isolated branch. *Dactylonectria pauciseptata* sensu Lombard *et al.* (2014) might be polyphyletic and could encompass four phylogenetic species. In addition to *D. pauciseptata* and *D. palmicola*, two potential species are worth being studied more carefully (Fig. 1).

The South American records of *C. fasciculare* and *C. pseudofasciculare* also are worth to be studied more carefully. The phylogenetic analyses show that the *Campylocarpon* lineage is divided into a well-supported *C. fasciculare* lineage and a poorly supported *C. pseudofasciculare* lineage (Fig. 2). The *C. fasciculare* lineage

(BSML = 100, PP = 0.99) is subdivided into two clades. The first clade (BSML = 96, PP = 1) includes the type strain of *C. fasciculare* (CBS 112613) together with several isolates from South African grapevine (Fig 2); it represents *C. fasciculare* s.s. The second clade (BSML = 94, PP = 0.66) is composed of several grapevine associated strains originating from Northeaster Brazil (Correia *et al.*, 2013); it may represent also an unnamed phylogenetic species. The *C. pseudofasciculare* strains are distributed into two poorly supported clades and the branch represented by the single *C. amazonense* strain (Fig. 2). The first (although not well supported) clade includes the type strain of *C. pseudofasciculare* (CBS 112679, *C. pseudofasciculare* s.s) and the strains CBS 112592 and BV7, both of uncertain identity. A second (also not well supported) clade is composed of nine isolates, originating in southern Brazil (Dos Santos *et al.* 2014) that may represent an unnamed phylogenetic species.

Acknowledgements. The authors gratefully acknowledge the financial support received from the CIUF-CUD (currently ARES, *Académie de Recherche et d'Enseignement Supérieur Wallonie-Bruxelles*) through the PIC Project "Reinforcement of the fungal expertise in Ecuador via case studies of fungal plants interactions in selected ecosystems and the development of biotechnology-oriented fungal resource centres". The authors also thank Petro Amazonas for facilitating access to the oil-polluted ponds and laboratory facilities in the Research Centre for Environmental Technologies located in Sacha camp. The authors also warmly thank Stéphanie Huret for her help with the sequencing program. Ana Gordillo also acknowledges the financial support received from PUCE when working in Ecuador.

REFERENCES

ABREO E., MARTINEZ S., BETTUCCI L. & LUPO S., 2010 — Morphological and molecular characterisation of *Campylocarpon* and *Cylindrocarpon* spp. Associated with black foot disease of grapevines in Uruguay. *Australasian Plant Pathology* 39: 446-452, doi:10.1071/AP10021.

AHLICH K. & SIEBER T. N., 1996 — The profusion of dark septate endophytic fungi in non-ectomycorrhizal fine roots of forest trees and shrubs. *New Phytologist* 132: 259-270, doi: 10.1111/j.1469-8137.1996.tb01845.x.

AIELLO D., POLIZZI G., CROUS P. & LOMBARD L., 2017 — *Pleiocarpon* gen. nov. and a new species of *Ilyonectria* causing basal rot of *Strelitzia reginae* in Italy. *IMA Fungus* 8: 65-76, doi: 10.5598/imafungus.2017.08.01.05.

ALVAREZ L.A., TAMAYO D., CASTILLA C., MUNIVE C., AGUSTÍ-BRISACH C., GRAMAJE D. & ARMENGOL J., 2012 — Occurrence of grapevine trunk pathogens in nurseries and vineyards in the northern and southern coast of Peru. *Phytopathologia Mediterranea* 51: 425.

ALTSCHUL S. F., GISH W., MILLER W., MYERS E. W., & LIPMAN D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology 215(3): 403-10. https://doi.org/10.1016/S0022-2836(05)80360-2

BOOTH C., 1966 — The genus *Cylindrocarpon*. *Mycological Papers* 104: 1-56.

CABRAL A., GROENEWALD J. Z., REGO C., OLIVEIRA H. & CROUS, P. W., 2012a — *Cylindrocarpon* root rot: multigene analysis reveals novel species within the *Ilyonectria radicicola* species complex. *Mycological Progress* 11: 655-688, doi:10.1007/s11557-011-0777-7.

CABRAL A., REGO C., NASCIMENTO T., OLIVEIRA H., GROENEWALD J. Z. & CROUS P. W., 2012b — Multi-gene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines. *Fungal Biology* 116: 62-80, doi:10.1016/j.funbio.2011.09.010.

CASTLEBURY L. A., ROSSMAN A. Y. & HYTEN A. S., 2006 — Phylogenetic relationships of *Neonectria*/*Cylindrocarpon* on *Fagus* in North America. *Canadian Journal of Botany* 84: 1417-1433, doi:10.1139/b06-105.

CHAVERRI P., SALGADO C., HIROOKA Y., ROSSMAN A. Y. & SAMUELS G. J., 2011 — Delimitation of *Neonectria* and *Cylindrocarpon* (Nectriaceae, Hypocreales, Ascomycota) and

related genera with *Cylindrocarpon-like* anamorphs. *Studies in Mycology* 68: 57-78, doi:10.3114/sim.2011.68.03.

CORREIA K. C., CÂMARA M. P. S., BARBOSA M. A. G., SALES R., AGUSTÍ-BRISACH C., GRAMAJE D. & MICHEREFF S. J., 2013 — Fungal trunk pathogens associated with table grape decline in Northeastern Brazil. *Phytopathologia Mediterranea* 52: 380-387, URL: http://hdl.handle.net/10261/95005.

CUNNINGHAM C. W., 1997 — Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14: 733-740, doi:10.1093/oxfordjournals.molbev.a025813.

DOS SANTOS R., BLUME E., MUNIZ M., HARAKAWA R., GARRIDO R. & REGO C., 2014 — Characterization of *Campylocarpon pseudofasciculare* associated with black foot of grapevine in southern Brazil. *Phytopathologia Mediterranea* 53: 06-415, doi:10.14601/Phytopathol.

HALLEEN F., SCHROERS H. J., GROENEWALD J. Z., & CROUS P. W., 2004 — Novel species of *Cylindrocarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.). *Studies in Mycology* 50: 431-455.

HIROOKA Y., KOBAYASHI T. & NATSUAKI K. T., 2005 — *Neonectria castaneicola* and *Neo. rugulosa* in Japan. *Mycologia*, 97(5), 1058-1066. http://doi.org/10.1080/15572536.2006.11832755.

GENE CODES CORPORATION — Ann Arbor, M. U. (n.d.). Sequencher® version 5.4 sequence analysis software. Retrieved from http://www.genecodes.com.

GEHESQUIERE B., CROUCH J.A., MARRA R.E., VAN POUCKE K., RYS F., MAES M., GOBIN B., HOFTE M. & HEUNGENS K., 2016 — Characterization and taxonomic reassessment of the box blight pathogen *Calonectria pseudonaviculata*, introducing *Calonectria henricotae* sp. nov. *Plant Pathology* 65:37-52, doi:10.1111/ppa.12401.

GLASS N. L. & DONALDSON G., 1995 — Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323e1330.

KATOH K. & STANDLEY D. M., 2013 — MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30: 772-780, doi: 10.1093/molbev/mst010.

KOBAYASHI T., HIROOKA Y., NATSUAKI K. T., KAWASHIMA Y & USHIYAMA K., 2005 — New canker diseases of *Abies veitchii* and *Acer crataegifolium* caused by *Neonectria castaneicola*. *Journal of General Plant Pathology*,71(2): 124-126. http://doi.org/10.1007/s10327-004-0172-1.

KORNERUP A. & WANSCHER J. H., 1981 — Methuen handbook of colours. In: E. Methuen (ed.), (3rd ed.). London.

LANFEAR R., 2012 — PartitionFinder v.1.1.0 and PartitionFinderProtein v1.1.0. doi: 10.1093/bioinformatics/btu033.

LOMBARD L., BEZUIDENHOUT C. M. & CROUS P. W., 2013 — *Ilyonectria* black foot rot associated with *Proteaceae*. *Australasian Plant Pathology* 42: 337-349, doi:10.1007/s13313-012-0188-5.

LOMBARD L., VAN DER MERWE N. A., GROENEWALD J. Z. & CROUS P. W., 2014 — Lineages in *Nectriaceae*: re-evaluating the generic status of *Ilyonectria* and allied genera. *Phytopathologia Mediterranea* 53: 515-532, doi:10.14601/Phytopathol.

LOMBARD L., VAN DER MERWE N. A., GROENEWALD J. Z. & CROUS P. W., 2015 — Generic concepts in *Nectriaceae*. *Studies in Mycology* 80: 189-245. http://doi.org/10.1016/j.simyco.2014.12.002.

MANTIRI F. R., SAMUELS G. J., RAHE J. E. & HONDA B. M., 2001 — Phylogenetic relationships in *Neonectria* species having *Cylindrocarpon* anamorphs inferred from mitochondrial ribosomal DNA sequences. *Canadian Journal of Botany* 79: 334-340, doi:10.1139/cjb-79-3-334.

MASON-GAMER R. J., KELLOG E. A. & KELLOG E. A., 1996 — Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Systematic Biology*, 45(4): 524-545. http://doi.org/10.2307/2413529.

MÜLLER K., MÜLLER J., NEINHUIS C. & QUANDT D., 2006 — PhyDE – Phylogenetic Data Editor, v0.995. Program distributed by the authors http://www.phyde.de.

O'DONNELL K. & CIGELNIK E., 1997 — Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7: 103-116.

O'DONNELL K., KISTLER H.C., CIGELNIK E. & PLOETZ R.C., 1998 — Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the USA* 95: 2044e2049.

PEREZ A., 2014 — Diversity, composition and floristic structure of Charapa 1 well pad, Sucumbíos – Ecuador. Quito.
RAMBAUT A. & DRUMMOND A., 2007 — Tracer 1.4. Retrieved from http://beast.bio.ed.ac.uk/Tracer.
REHNER S.A. & SAMUELS G.J., 1995 — Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. *Canadian Journal of Botany* 73: S816-S823.
RONQUIST F. & HUELSENBECK J. P., 2003 — MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574, doi:10.1093/bioinformatics/btg180.
SAMUELS G.J. & BRAYFORD D., 1994 — Species of *Nectria* (sensu lato) with red perithecia and striate ascospores. *Sydowia* 46: 75-161.
SIMMONS M. P., PICKETT K. M. & MIYA M., 2004 — How Meaningful Are Bayesian Support Values? *Molecular Biology and Evolution* 21: 188-199, doi: 10.1093/molbev/msh014.
STAMATAKIS A., 2006 — RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22(21): 2688-2690. http://doi.org/10.1093/bioinformatics/btl446
SWOFFORD D. L., 1996 — Phylogenetic Analysis Using Parsimony. *Options* 42:294-307, doi: 10.1007/BF02198856.
UNTEREINER W.A., BONJEAN B., DECOCK C., EVRARD P., DE FRAHAN M.H., JAMIN N., MASSART L., NÉLISSEN L., ROBERT V., BOSSCHAERTS M., GUISSART F. & DE BRABANDERE J., 1998 — MUCL Catalogue of Strains (Fungi-Yeast). 3e ed. Belgian Office for Scientific, Technical and Cultural Affairs, eds.: Brussels, Belgium.
VILGALYS R. & HESTER M., 1990 — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238-4246.
WHITE T.J., BRUNS T., LEE S. & TAYLOR J., 1990 — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand D, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*. Academic Press, San Diego, pp. 315e322.
WOLLENWEBER H.W., 1913 — *Ramularia*, *Mycosphaerella*, *Nectria*, *Calonectria*. Eine morphologisch pathologische Studie zur Abgren- zung von Pilzgruppen mit cylindrischen und sichelfo ¨rmigen Konidienformen. *Phytopathology* 3: 197-242.
WOLLENWEBER H.W., 1917 — Fusaria autographice delineata. Published by the author, Berlin.