

***Neocordana gen. nov.*, the causal organism of Cordana leaf spot on banana**

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Abstract: *Cordana* leaf spot of banana is shown to be associated with several species of a new genus described here as *Neocordana gen. nov.* Furthermore, *Neocordana* belongs to *Pyriculariaceae (Magnaporthales)* rather than *Cordanaceae* where the type species of *Cordana*, *C. pauciseptata* resides. *Neocordana* is established to accommodate *Cordana musae*, *C. johnstonii*, *C. versicolor*, and a previously undescribed species, *N. musicola*, which is morphologically and phylogenetically distinct. *Neocordana* species are found to be associated with leaves of *Musa* spp. (*Musaceae*) and *Canna denudata* (*Cannaceae*). Based on these results, *Cordanaceae* is best recognized in a separate order, established here as *Cordanales ord. nov.*

Key Words: *Cordanales*, *Magnaporthales*, *Musa*, plant pathogenic fungi, *Pyriculariaceae*, systematics.

Introduction

Cordana leaf spot is a common and widespread disease on banana and plantain. Although it is considered as a minor pathogen of banana, it can cause serious defoliation of plantains in Central America during and following periods of wet weather (Jones 1999, Ploetz *et al.* 2003). Cordana leaf spot is characterised by large, pale brown, oval to fusiform necrotic lesions with pale grey concentric rings, with a dark brown border surrounded by a bright yellow halo, separating lesions from healthy leaf tissue (Jones 1999). Most damage occurs when the pathogen gains entry to leaf tissue weakened because of age, adverse environmental conditions, nutritional deficiencies, wounds or through lesions caused by other pathogens. The leaves ultimately turn brown and dry out (Jones 1999). This is especially apparent when the disease occurs together with Sigatoka leaf spots (Arzanlou *et al.* 2008), in which case the lesions may encompass the entire leaf margin and large portions of the lamina can be affected (Ploetz *et al.* 2003). Leaf infection normally occurs at night during rainy periods or when dew is present. The conidia germinate in a film of moisture on the leaf surface and after a few hours appressoria are formed. The appressoria enable the fungus to penetrate into the host epidermal cells. Species most commonly associated with Cordana leaf spot of banana include *Cordana musae* (Zimm.) Höhn. (1923: 60) and *C. johnstonii* M.B. Ellis (1971: 125). Cordana leaf spot has been reported mainly on *M. sapientum* L. but also can be found on *M. acuminata* ssp. *banksii* N.W.Simmonds, *M. acuminata* ssp. *banksii* × *M. schizozarpa* N.W.Simmonds, *M. balbisiana* Colla, *M. boman* Argent, *M. maclayi* F.Muell., *M. schizozarpa* N.W.Simmonds and *Enset glaucum* (Roxb.) Cheesman (Jones, 1999). Another plant pathogenic species, *Cordana versicolor* D.J. Soares & R.W. Barreto (2005: 18), was described from Brazil causing eye-spot disease on leaves of *Canna denudata* (Soares *et al.* 2005).

The asexual genus *Cordana* was erected by Preuss (1851) with *C. pauciseptata* Preuss (1851: 129) as type species. Species of *Cordana* are characterized by brown, septate conidiophores with swollen conidiogenous zones, terminal and intercalary conidiogenous cells, and pale brown to brown, 0–1-septate conidia. Ecologically, *Cordana* species can be separated into two groups, namely phanerogam leaf-inhabiting species (saprobic or pathogenic) or saprobic on rotten wood or in soil (de Hoog *et al.* 1983). Saprobic species as *C. abramovii* Seman & Davydkina (1983: 20), *C. bisby* (Timonin) M. Hern.-Rest., Gené & Guarro (2014: 106), *C. ellipsoidea* de Hoog (1973: 22), *C. inaequalis* S. Hughes (1983: 246), *C. mercadiana* M. Hern.-

Restr, J. Mena, Gene & Guarro (2014: 106), *C. solitaria* V. Rao & de Hoog (1986: 28), *C. terrestris* (Timonin) M. Hern.-Rest., Gené & Guarro (2014: 106), and *C. verruculosa* M. Hern.-Restr, J. Mena, Gene & Guarro (2014: 106), including the type species, *C. pauciseptata*, cluster in *Cordanaceae* Nann. (1934: 498) (Order *incertae sedis*), in the Sordariomycetes (Hernández-Restrepo *et al.* 2014, Zelski *et al.* 2014). Nevertheless, plant pathogenic species such as *C. musae*, *C. johnstonii*, and *C. versicolor* cluster apart, and are phylogenetically unresolved.

The aims of this work were to designate a suitable neotype specimen for *C. musae* with a living culture and resolve its phylogeny, and to describe a new genus to accommodate *C. musae* and related species.

Materials and methods

Isolates

Fungal isolates used in this study were obtained from culture collection of Pedro Crous (CPC) housed at the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, The Netherlands, and from the CBS culture collection. Isolates were cultured on malt extract agar (MEA), oatmeal agar (OA) and synthetic nutrient deficient agar (SNA) (recipes in Crous *et al.*, 2009b), plus sterilized *Musa* leaves, and incubated at 25 °C under daylight conditions. Reference strains were deposited in CBS, nomenclature and taxonomic information in MycoBank (www.mycobank.org) (Crous *et al.* 2004).

Morphology

Slide preparations were prepared by mounting fungal material in clear lactic acid from sporulating colonies. Observations and photomicrographs were made with a Nikon SMZ1500 stereo-microscope, and with a Nikon eclipse Ni compound microscope, using a Nikon DS-U3 digital camera (Nikon, Tokyo, Japan) and NIS elements imaging software. Colony characters and pigment production were noted after 1 week of growth on MEA and OA incubated at 25 °C. Colony colours (surface and reverse) were treated according to the colour chart of Rayner (1970).

DNA isolation, amplification and analyses

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) and Wizard® Genomic DNA purification kit (Promega, Madison, USA), according to the manufacturer's protocols. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and approximately 900 bp of the 5' end of the 28S rRNA gene. The primers ITS4 (White *et al.* 1990) and LSU1Fd (Crous *et al.* 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The program SeqMan Pro (DNASTAR, Madison, WI, USA) was used to obtain consensus sequences of each isolate. Megablast searches using ITS and LSU sequences were performed in NCBI's GenBank nucleotide sequence database to identify the closest matching sequences, which are added to the sequences alignment. Sequences were aligned with MAFFT v. 7 (Katoh & Standley 2013) using the default settings and adjusted by hand in MEGA v. 6.06 (Tamura *et al.* 2013). To address the phylogenetic relationships among taxa, Bayesian inference (BI) and Maximum Likelihood (ML) were used. MrModel test v. 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model settings prior to the Bayesian analysis in MrBayes v. 3.2.1 (Ronquist *et al.* 2012). ML analysis was performed with RAxML v. 7.0.4 (Stamakis *et al.* 2008). Nodal support was assessed by > 0.95 Posterior probabilities for the Bayesian analysis (PP) determined by calculating 50% majority rule consensus tree from the remaining 2706 trees, and from > 70% maximum likelihood bootstrap values (MLB).

Sequences derived in this study were deposited at GenBank, the alignments and trees in TreeBASE (www.treebase.org/treebase/index.html). The phylogenetic trees were edited using Tree View 1.4.0 and Adobe Illustrator CS5.1.

Results

Phylogeny

Amplicons of approximately 1000 bp were obtained of the partial LSU gene and 550 bp of the ITS region. The LSU alignment was used to resolve the generic placement of the strains and the ITS to determine species identification.

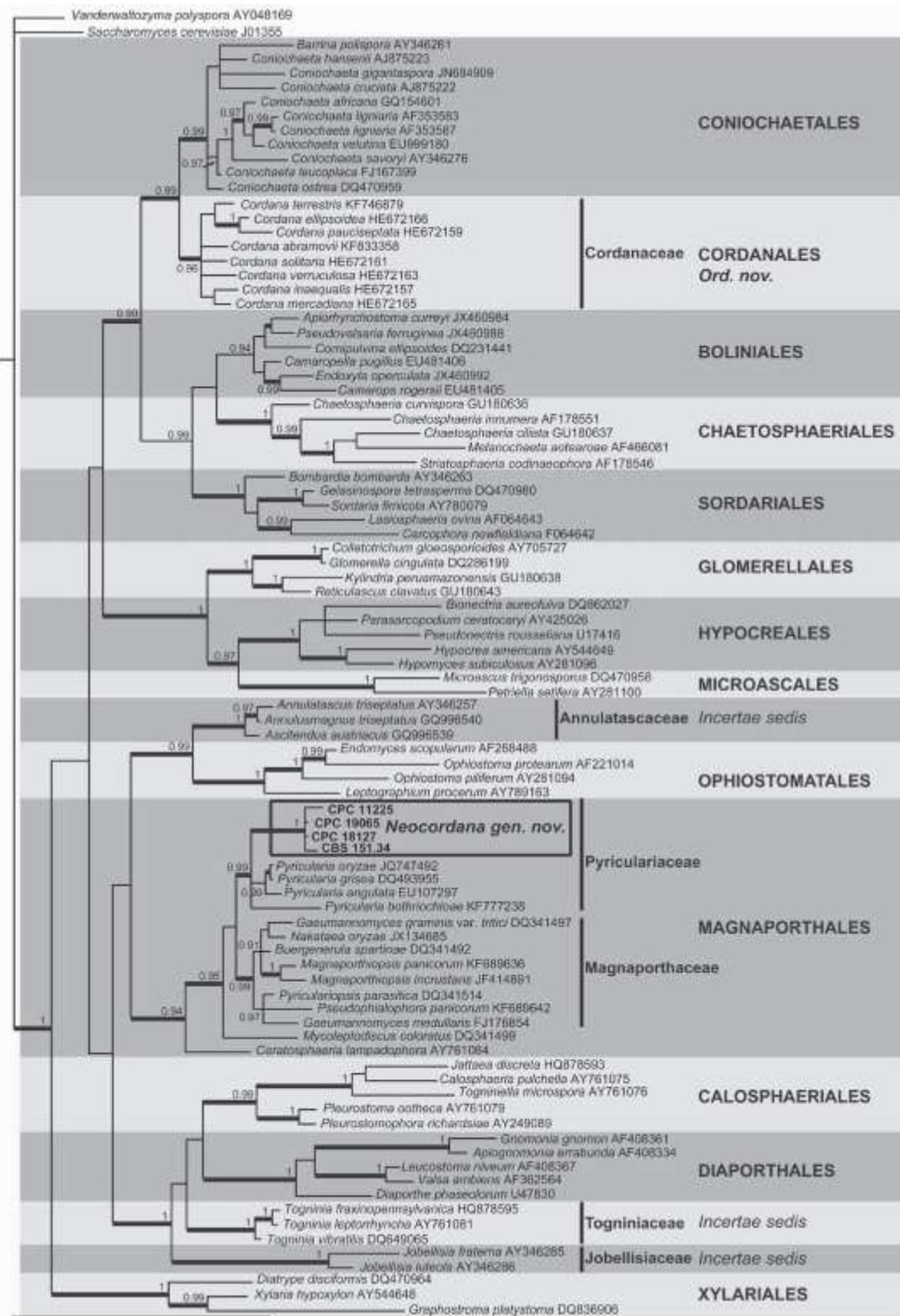


Figure 1. Phylogenetic analyses based on LSU rDNA sequences. Bayesian posterior probabilities above 0.95 are shown at the nodes. Thickened lines indicate a Maximum Likelihood bootstrap supports above 70%. The tree was rooted with *Saccharomyces cerevisiae* J01355 and *Vanderwaltozyma polyspora* AY048169.

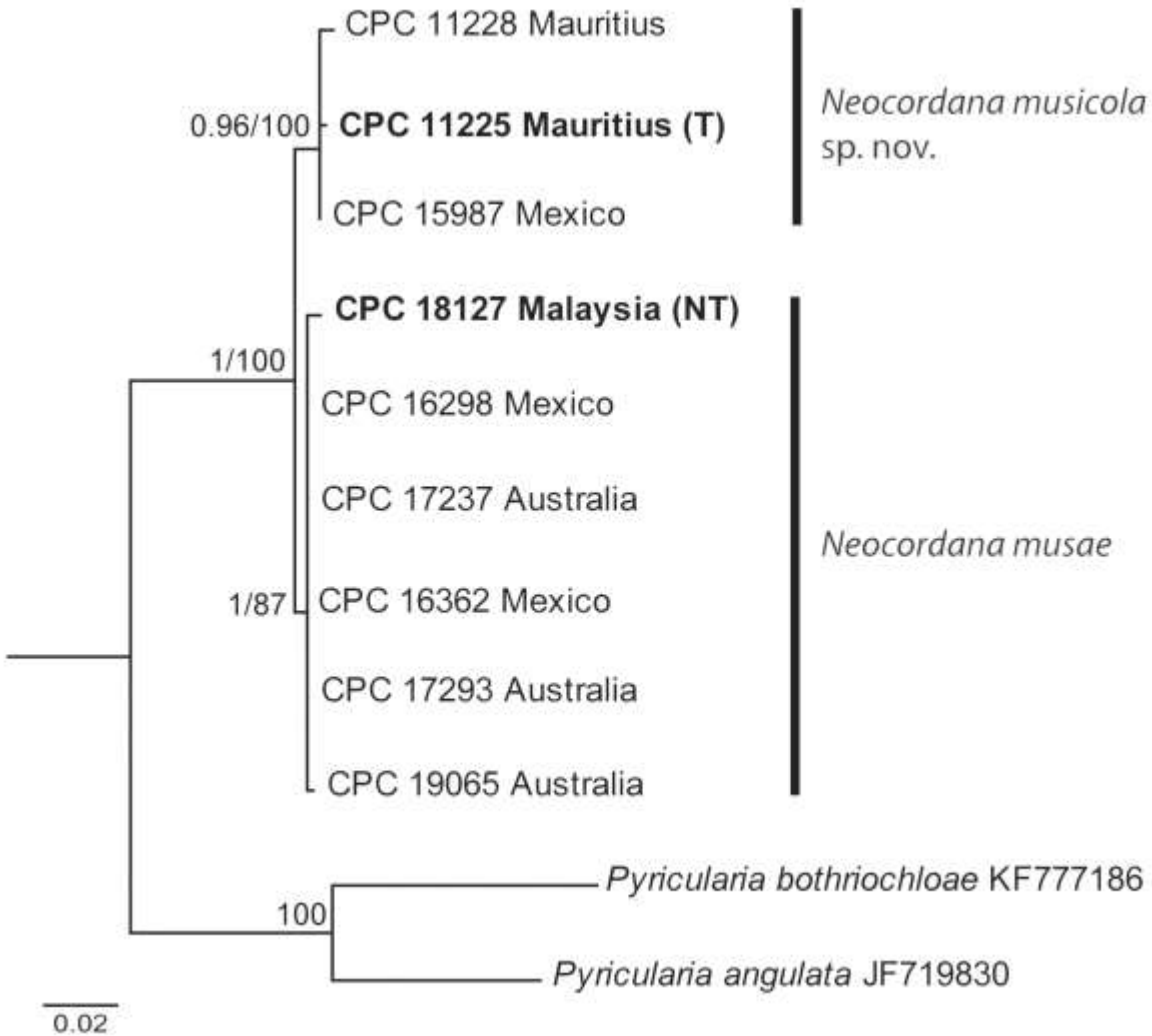


Figure 2. Maximum likelihood (RAxML) phylogenetic tree based on ITS sequences of *Neocordana* isolates. Bayesian posterior probability values and Bootstrap support values are shown at the nodes. The tree was rooted with *Pyricularia bothriochloae* KF777186 and *Pyricularia angulata* JF719830. Ex-type isolates are indicated in bold.

The LSU alignment contained 84 sequences, including two outgroups (*Saccharomyces cerevisiae* J01355 and *Vanderwaltozyma polyspora* AY048169), and 1021 characters including alignment gaps were used in the phylogenetic analysis. Dirichlet base frequencies and the GTR+I+G model with inverse gamma-distributed were used as substitution model. In Bayesian analysis 50% consensus trees and posterior probabilities were calculated from the 5412 tree left after discarding 2706 trees (the first 25% of generations) for burn-in (Figure 1).

The main topology is maintained in the ML tree based on LSU sequences. Clades corresponding with families and orders in *Sordariomycetes* were well-supported

(*Annulastascaceae*, *Boliniales*, *Chaetosphaeriales*, *Coniochaetales*, *Cordanaceae*, *Diaporthales*, *Glomerellales*, *Hypocreales*, *Magnaporthales*, *Microascales*, *Ophiostomatales*, *Sordariales*, and *Xylariales*). Isolates of *Cordana musae* appeared as a distinct lineage among the *Pyriculariaceae* in *Magnaporthales*, rather than *Cordanaceae*, where the type species *C. pauciseptata* is placed. These results indicated that the genus *Cordana* is polyphyletic. A new genus and new combinations are therefore required to accommodate *Cordana musae* and related phytopathogenic species.

The ITS alignment contained 11 sequences (including two outgroups, *Pyricularia bothriochloae* KF777186 and *Pyricularia angulata* JF719830), and 521 characters including alignments gaps were used in the phylogenetic analysis. The ITS phylogenetic tree (Figure 2) resolved two different clades representing isolates from *Musa* spp. collected in different countries.

Taxonomy

Cordanales M. Hern.-Rest. & Crous, *ord. nov.*

MycoBank MB811104

Ascomata superficial or slightly immersed, solitary or gregarious in scattered groups, globose to broadly ovoid, papillate, basal stroma sometimes present, smooth, black to dark brown, periphysate. *Asci* cylindrical, short-stalked, unitunicate, with 8 uniseriate ascospores. *Paraphyses* hyaline, simple, septate. *Ascospores* ellipsoid to fusiform, 1-septate, pale brown or hyaline becoming brown. *Conidiophores* erect, simple or branched, brown, with a few rhizoidal hyphae arising from the base. *Conidiogenous cells* polyblastic, denticulate. *Conidia* 0–1-septate, pale to dark brown, smooth or verruculose.

Type:—*Cordana pauciseptata* Preuss

Family included:—*Cordanaceae* Nann.

Note:—The *Cordanaceae* was established for the genus *Cordana*. Based on the ascomatal morphology that is porosphaerella-like, *Cordana* has been allied with the *Trichosphaeriaceae*

and *Chaetosphaeriaceae* (Müller & Samuels 1982, Réblová *et al.* 1999). Recent molecular studies reported the family to be phylogenetically closely related to the *Coniochaetales* (Réblová & Winka 2000, Huhndorf *et al.* 2004, Réblová & Seifert 2007, Hernández-Restrepo *et al.* 2014, Zelski *et al.* 2014). *Coniochaetales* includes only one family *Coniochaetaceae*, with members characterized by ascomata lacking of stromatic tissue, ascospores with elongated germ slits and phialidic asexual morphs (Huhndorf *et al.* 2004), clearly different from members of *Cordanaceae* that can have a basal stroma, ascospores without germ slits and polyblastic asexual morphs. Our data strongly support the *Cordanaceae* as being monophyletic and a new order, *Cordanales*, is established for the *Cordanaceae*.

Neocordana M. Hern.-Rest. & Crous, *gen. nov.*

MycoBank MB811105

Etymology: *Neo*- new, *Cordana*- asexual genus, in reference to their similarity with the genus *Cordana*.

Colonies effuse, grey to brown, hairy. *Mycelium* superficial and immersed, on the agar and on the plant cuticle, hyphae, smooth, septate, hyaline becoming brown with age. *Conidiophores* differentiated, erect, straight to flexuous, brown at the base becoming paler toward the apex, smooth, swollen basal cell beneath the host epidermis. *Conidiogenous cells* holoblastic, sympodial, denticulate, cylindrical to obclavate, terminal and intercalary, smooth, pale brown to brown. *Conidia* dry, solitary, pale brown, 1-septate, broadly ellipsoidal, oblong, obovoid, obclavate to pyriform, smooth, thick-walled, with a visible darker, thickened hilum. *Appressoria* present.

Type species:—*Neocordana musae* (Zimm.) M. Hern.-Rest. & Crous

Sexual morph: Unknown.

Neocordana johnstonii (M.B. Ellis) M. Hern.-Rest. & Crous, *comb. nov.*

MycoBank MB811113

Basionym: *Cordana johnstonii* M.B. Ellis, *Mycol. Pap.* 125: 8 (1971)

Descriptions and illustrations:—Ellis (1971), Priest (1990).

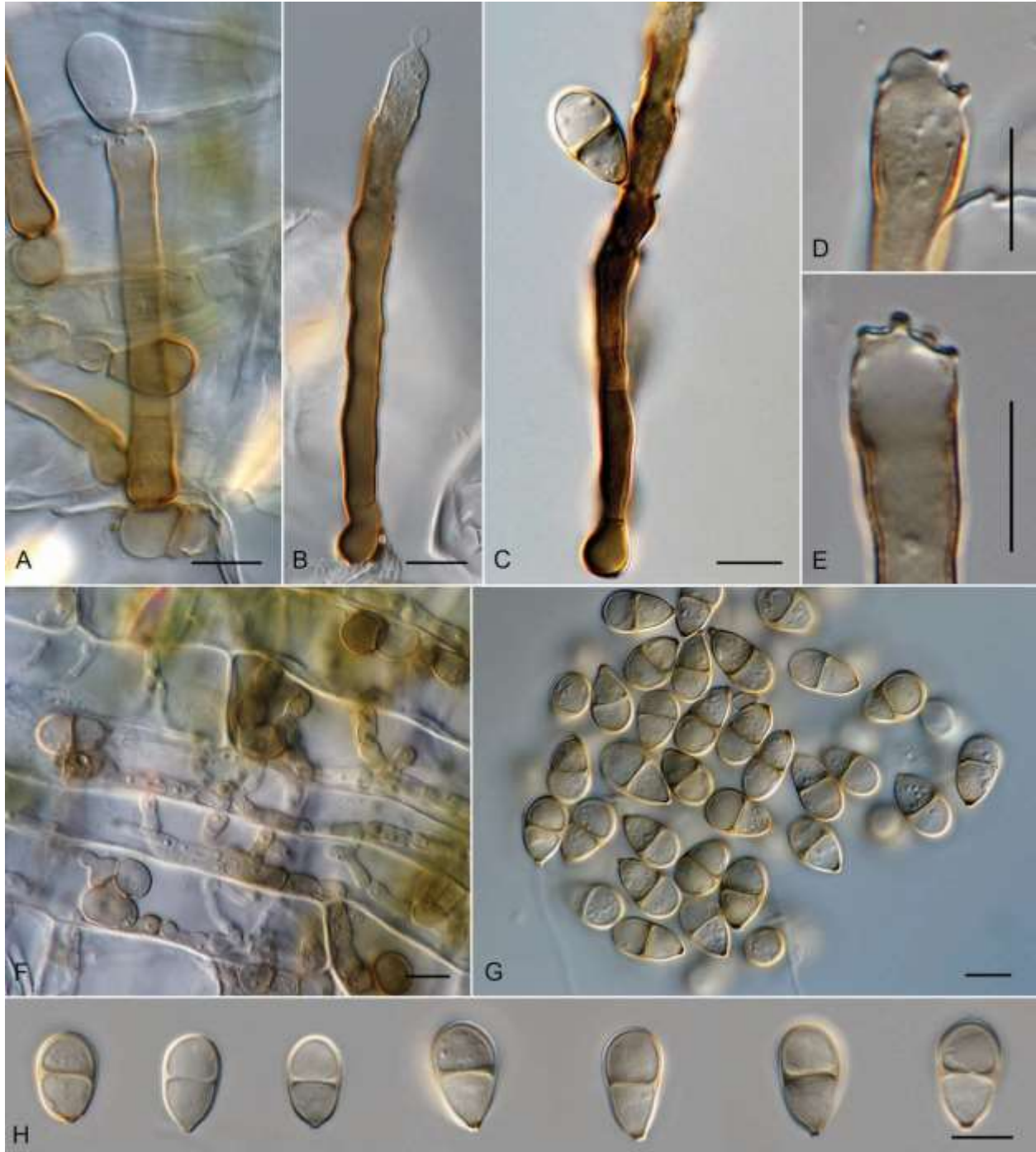


Figure 3. *Neocordana musae* (CPC 18127, ex-neotype culture) on *Musa* leaves on SNA. A–B. Conidiophores. D–E. Conidiogenous cells. f. Intracellular hyphae. G–I. Conidia. Scale bars A–E = 10 μ m.

Type:—INDONESIA. West Irian, Enarotali, 17 May 1960, on leaves of *Musa sapientum*, D. Shaw & A. Johnston, (holotype IMI 81492).

Distribution:—AUSTRALIA. Queensland (Tallebudgera, Mudgeeraba, Currumbin, South coast of Queensland), New South Wales, Norfolk Island, Lord Howe Island. INDONESIA. West Irian. MALAYSIA. Cameron Highlands. PHILIPPINES. Malaya. TONGA.

Neocordana musae (Zimm.) M. Hern.-Rest. & Crous, *comb. nov.*

MycoBank MB811106, Figure 3.

Basionym: *Scolecotrichum musae* Zimm., *Zentbl. Bakt. ParasitKde, Abt. II* 8: 220 (1902) (as *Scolicotrichum*)

= *Cordana musae* (Zimm.) Höhn., *Zentbl. Bakt. ParasitKde, Abt. II* 60: 7 (1923)

Mycelium superficial and immersed, hyphae smooth, hyaline to brown; intracellular hyphae smooth, hyaline to brown. *Conidiophores* brown, smooth, $46\text{--}118.5 \times 5\text{--}6.5 \mu\text{m}$. *Conidiogenous cells* terminal and intercalary, $15\text{--}72.5 \times 5\text{--}9 \mu\text{m}$, denticulate; denticles up to $2 \mu\text{m}$ long, $0.5\text{--}1.5 \mu\text{m}$ wide. *Conidia* obclavate, pyriform, mostly obovoid, $14.5\text{--}19 \times 8\text{--}11.5 \mu\text{m}$, 1-septate, thick-wall, pale brown, base darker, flat, $1\text{--}1.5 \mu\text{m}$ wide.

Culture characteristics:—Colonies on MEA after 1 week attaining 25 mm diam, elevated, cottony, aerial mycelium white with mouse grey zones (sporulation), exudate hyaline to pale luteous, margin diffuse; reverse black in the centre, with concentric rings isabelline to umber or luteous to ochreous. On OA colonies very similar to that observed on MEA.

Type:—INDONESIA. Java, Buitenzorg, 1920, on *Musa sapientum*, Zimmermann A., *Centralbl. F. Bakt. Abt. II. Bd. 8. S220* (holotype not found and presumed missing). MALAYSIA. 1 April 2011, on *Musa* sp., Mee Hua Wong (**neotype designated here** CBS H-22054!, ex-neotype strain CPC 18127 = CBS 139318).

Additional cultures examined:—AUSTRALIA. Queensland, 21 January 2011, on *Musa* sp., unknown collector, CBS 139319 = CPC 19065. MEXICO. Tabasco, Villa Hermosa, 16

December 2008, on *Musa* sp., *Maria de J. Yarez Morales*, CPC 16362; Tapechula, Chiapas, 16 December 2008, on *Musa* sp., *Maria de J. Yarez Morales*, CPC 16298. UNKNOWN COUNTRY, February 1934, on *Musa sapientum*, *G. Stahel*, CBS 151.34.

Distribution:—Widespread in the tropics with the host.

Notes:—In the ITS phylogenetic tree (Figure 2) *Neocordana musae* is represented by six isolates from *Musa* leaves collected from different countries, namely Australia, Malaysia and Mexico. Originally *N. musae* was described from Java (Zimmermann 1902), but the type material is presumably lost. Among the isolates studied here, we chose isolate CPC 18127 as ex-neotype because Malaysia is closest to the location where this fungus was collected for the first time; furthermore this isolate is morphologically similar to the fungus described and illustrated by Zimmermann (1902), and occurs commonly on *Musa* spp. in tropical regions.

Neocordana musicola M. Hern.-Rest. & Crous, *sp. nov.*

MycoBank MB811107, Figure 4.

Etymology: Named after the host from which it was isolated, *Musa*.

Diagnosis: Conidiophores up to $125 \times 4\text{--}5.4 \mu\text{m}$, conidiogenous cells $15\text{--}37.6 \times 4.2\text{--}56 \mu\text{m}$, conidia oblong to obovoid, 1-septate, $14.5\text{--}20 \times 6.5\text{--}9.5 \mu\text{m}$, base $0.9\text{--}1.2 \mu\text{m}$ diam.

Mycelium superficial and immersed, hyphae smooth, hyaline to brown; intracellular hyphae hyaline to brown. *Conidiophores* brown, smooth, $35\text{--}125 \times 4\text{--}5.4 \mu\text{m}$. *Conidiogenous cells* polyblastic, terminal and intercalary, $15\text{--}37.6 \times 4.2\text{--}56 \mu\text{m}$, denticulate; denticles up to $1.5 \mu\text{m}$ long, $0.6\text{--}1 \mu\text{m}$ wide. *Conidia* oblong to obovoid, $14.5\text{--}20 \times 6.5\text{--}9.5 \mu\text{m}$, 1-septate, thick-walled, pale brown, base darker, flat, $0.9\text{--}1.2 \mu\text{m}$ wide.

Culture characteristics:—Colonies on MEA after 1 week attaining 20 mm, cottony, aerial mycelium white, mouse grey towards the edge (sporulation), and margin diffuse; reverse fuscous black in the centre, luteous at the edge. On OA colonies very similar to that observed in MEA.



Figure 4. *Neocordana musicola* (CPC 11225, ex-type culture) on *Musa* leaves on SNA. A–E. F–G. Intracellular hyphae. H. Conidia. Scale bar A–H = 10 μ m.

Type:—MAURITIUS. 15 June 2004, on *Musa* sp., *Yasmina Jaufeerally-Fakim*, (holotype CBS H-22055!, culture ex-type CBS 139316 = CPC 11225 = S 1024).

Additional cultures examined:—MAURITIUS. 15 June 2004, on *Musa* sp., *Yasmina Jaufeerally-Fakim*, CPC 11228. MEXICO. Tecoman, Colima, INIFAB, 30 October 2008, on *Musa* sp., *Maria de J. Yarez Morales*, CPC 15987 = CBS 139317.

Distribution:—MAURITIUS and MEXICO.

Notes:—*Neocordana musicola* is represented by three strains, two of them collected from Mauritius and the third from Mexico. *N. musicola* can be differentiated from the other species in

the genus by their narrower obovoid to oblong conidia. *N. musae* has obovoid to pyriform conidia, $11\text{--}20 \times 8\text{--}11.5 \mu\text{m}$, while those of *N. johnstonii* are broadly ellipsoidal, $20\text{--}30 \times 12\text{--}18 \mu\text{m}$, and conidia of *N. versicolor* (see below) are $15\text{--}25 \times 10\text{--}15 \mu\text{m}$. Molecular data based on the ITS sequences show 2.5% differences between *N. musae* (CPC 18127) and *N. musicola* sp. nov. (CPC 11225).

Neocordana versicolor (D.J. Soares & R.W. Barreto) M. Hern.-Restr. & Crous, *comb. nov.*

MycoBank MB811114

Basionym: *Cordana versicolor* D.J. Soares & R.W. Barreto, Fungal Diver. 18: 148 (2005)

Description and illustration: Soares *et al.* (2005).

Type:—BRAZIL. Minas Gerais, Viçosa, Associacao Atletica Banco do Brasil, 02 May 1999, on living leaves of *Canna denudata*, R.W. Barreto (holotype VIC 22157).

Distribution:—BRAZIL.

Discussion

For many years the causal agents of a distinctive leaf spot disease on banana were considered as members of the genus *Cordana* (von Höhnelt 1923, Ellis 1971, Priest 1990, Jones 1999). The disease was initially attributed to a pathogen described as *Scolecotrichum musae* on *Musa sapientum* from Java (Indonesia) by Zimmermann (1902), and later transferred to *Cordana* by von Höhnelt (1923). Ellis (1971) subsequently introduced a second species isolated on *Musa sapientum* from West Irian (Indonesia) as *C. johnstonii*, which causes a disease very similar in appearance to the one caused by *C. musae*. Morphologically these species can be differentiated based on conidial size and shape. Conidia of *C. musae* are obclavate to pyriform, $14\text{--}18 \times 8\text{--}10 \mu\text{m}$, while those of *C. johnstonii* are broadly ellipsoidal to subglobose, larger and wider, $19\text{--}26 \times 14\text{--}16 \mu\text{m}$. Priest (1990) reported that leaf spots caused by *C. johnstonii* are generally smaller ($3 \times 1 \text{ cm}$) and become fusiform with age, in contrast to the larger ($7 \times 2 \text{ cm}$), oval to elliptical spots caused by *C. musae*.

In our phylogenetic tree based on LSU sequences (Figure 1), cordana-like strains isolated from symptomatic leaves of *Musa* from different countries constituted a distant clade in *Pyriculariaceae* (*Magnaporthales*). Recently phylogenetic studies (Hernández-Restrepo *et al.* 2014) demonstrated that members of *Cordana* occupy a monophyletic clade in the family *Cordanaceae*, as the sister clade of *Coniochaetales* in *Sordariomycetes*. *Cordanales* is here erected for the single family *Cordanaceae*. On the other hand, *Neocordana* is introduced to accommodate phytopathogenic species similar to *Cordana* but closely related to *Pyriculariaceae* (*Magnaporthales*) rather than *Cordanaceae*. The family *Pyriculariaceae* was recently introduced by Klaubauf *et al.* (2014) and includes important plant pathogens along with *Deightoniella* S. Hughes (1952:48) and several pyricularia-like genera. *Deightoniella* can be easily differentiated from *Neocordana* since it produces conidiophores reduced to conidiogenous cells with a flattened scar, and conidia with a central pore in the base (Hughes 1952, Klaubauf *et al.* 2014), while *Neocordana* has septate conidiophores with denticulate conidiogenous cells and conidia with a protruding hilum. On the other hand, pyricularia-like fungi and *Neocordana* are similar in having brown, septate conidiophores with polyblastic, denticulate conidiogenous cells. Nevertheless, pyricularia-like fungi are different from *Neocordana* in having pyriform to obclavate, 2-septate conidia, and grow on grasses and other plants (Seifert *et al.* 2011, Klaubauf *et al.* 2014). *Neocordana* differs by having broadly ellipsoid, obovoid to pyriform, 1-septate conidia and are pathogenic to species of *Musa* or *Canna*.

In the phylogenetic analysis generated here (Figure 1), *Neocordana* is represented by *N. musae* and *N. musicola*. *N. musae* is designated as the type species of the genus, and *N. musicola* is introduced as a new species, similar but different from *N. musae*, based on morphological and molecular data. Unfortunately cultures of *N. johnstonii* and *N. versicolor* were not available to include in the phylogenetic analysis. Nevertheless we propose new combinations for *N. johnstonii*, and *N. versicolor*, based on morphology, pathogenicity and host association. Further studies are needed to confirm their phylogenetic affinities in *Neocordana*.

Acknowledgements

We thank the technical staff, Arien van Iperen (cultures), and Mieke Starink-Willemse (DNA isolation, amplification, and sequencing) for their invaluable assistance.

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