The plant pathogenic genus *Neocordana*

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Abstract

Neocordana species mainly causes leaf spots on *Musa* spp. (banana and plantain) and on *Canna denudata* (an ornamental plant). Leaf spots on *Musa* spp. reduce the quality of the commodity and result in significant economic loss. Based on molecular and morphological data, the genus accommodates seven species at present with a worldwide distribution. Most of the taxonomic work has been conducted on *Neocordana musae*. In this study, updates on the diversity, distribution, and morpho-molecular taxonomy of *Neocordana* are provided. In addition, important aspects such as pathogenicity, disease control, antagonistic activity and association with other pathogenic fungi are discussed. The phylogenetic relationships of *Neocordana* with other genera in the family Pyriculariaceae based on LSU and ITS DNA sequence data are investigated. Our results support the monophyletic nature of *Neocordana* which is sister to *Barretomyces*. This study provides the first comprehensive account on diversity, taxonomy, and pathogenicity of *Neocordana*.

Introduction

Fungi are organisms that play various ecological roles in the environment, including decomposition of organic matter, recycling of nutrients, and exhibit pathogenic and endophytic lifestyles (Tang et al. 2005, Treseder & Lennon 2015, Rampadarath et al. 2018, Wanasinghe et al. 2018b). The asexual genus, *Neocordana* was established by Hernández-Restrepo et al. (2015) and accommodated in the family Pyriculariaceae, order Magnaporthales. *Neocordana musae* (Zimm.) Hern.-Restr. & Crous is the type species (Hernández-Restrepo et al. 2015) and seven plant pathogenic and saprobic taxa (Index Fungorum 2019) are included in the genus from a limited number of hosts (Musaceae and Cannaceae) worldwide (Jones 1999, Hernández-Restrepo et al. 2015, Crous et al. 2016, 2017, 2019) (Table 1). Several studies have reported that fungi can exhibit different lifestyles such as endophytes, saprobes or pathogens (Promputtha et al. 2007, Maharachchikumbura et al. 2011, Jeewon et al. 2013). *Neocordana* is an excellent example for a

fungal genus that represents all three modes of ecological strategies, notably, as a plant pathogen and saprobe (Hernández-Restrepo et al. 2015, Crous et al. 2016, 2017, 2019), as well as an endophyte (Photita et al. 2001). As plant pathogens, the overwhelming majority of species causes leaf spots on *Musa* spp. (banana) (Allen & Dettmann 1990, Jones 1999, Fan et al. 2012). In addition, *Neocordana versicolor* infects the leaves of *Canna denudata*, a common ornamental plant in Brazil (Soares et al. 2005). The damage caused by the pathogens is severe, resulting in major defoliation (Hernández-Restrepo et al. 2015) and minimize the rate of photosynthesis in host plants. Generally, the leaf spots, resulting from the pathogen can affect the productivity, quality and the market value of commodities (Gaikwad et al. 2017) and contribute to considerable economic loss. The objectives of this study are to i) provide an updated account of the diversity, distribution and morpho-molecular taxonomy of the genus *Neocordana*; ii) reinvestigate intergeneric relationships of *Neocordana* based on LSU and ITS sequence data; iii) discuss the pathogenicity aspects, potential of disease control and endophytic nature of *Neocordana*.

Table 1 Recorded species of *Neocordana,* hosts and distribution.

Species	Host	Distribution	Reference
Neocordana johnstonii (M.B. Ellis) Hern.-Restr. & Crous	*Musa sapientum*	Australia, Indonesia Malaysia, Philippines, Tonga	Hernández-Restrepo et al. (2015)
N. malayensis Crous	*Musa* sp.	Malaysia	Crous et al. (2019)
N. musae (Zimm.) Hern.-Restr. & Crous	*Musa sapientum*	Has been recorded around the tropical region	Hernández-Restrepo et al. (2015)
N. musarum Crous	*Musa* sp.	France	Crous et al. (2016)
N. musicola Hern.-Restr. & Crous	*Musa* sp.	Mauritius, Mexico	Hernández-Restrepo et al. (2015)
N. musigena Crous	*Musa* sp.	Morocco	Crous et al. (2017)
N. versicolor (D.J. Soares & R.W. Barreto) Hern.-Restr. & Crous	*Canna denudata*	Brazil	Hernández-Restrepo et al. (2015)

Diversity, distribution, and morphology

The sexual morph of *Neocordana* species is still unknown (Hernández-Restrepo et al. 2015, Crous et al. 2016, 2017, 2019) while the asexual morphs are brown hyphomycetes with differentiated conidiophores which are straight, and erect. Conidiophores bear pale brown conidia that arise from the terminal and intercalary conidiogenous cells (Hernández-Restrepo et al. 2015). Colonies are usually grown on potato dextrose agar (PDA). The mycelia are superficial, hairy and grey to brown. This mycelial growth has also been recorded on the cuticle of host plants. The septate mycelia are hyaline and become brown with age (Hernández-Restrepo et al. 2015). The morphological features of *N. musae* are illustrated in Fig. 1. The main morphological characters (especially conidial size and shape) differentiating species of genus *Neocordana* are outlined in Tables 2, 3, 4 and 5.

Neocordana versicolor can be delineated from *N. musae* in terms of conidial shape, colour and size (Table 3) while *N. johnstonii* and *N. versicolor* also bear some morphological dissimilarities in the conidiophore (Table 4). When comparing *N. musae* and *N. johnstonii*, the conidiophores of both taxa are brown, erect, straight, septate and denticulate. The conidiophores arise as single small groups and paler towards the apex in color. Conidia of both organisms are two-celled, smooth and pale brown to hyaline. A thickened hylum can be seen in conidia. Colonies are hypophyllous, effuse to hairy and dark brown. The size of conidiophores and conidia are different from each other as listed in Table 5 (Jones 1999).
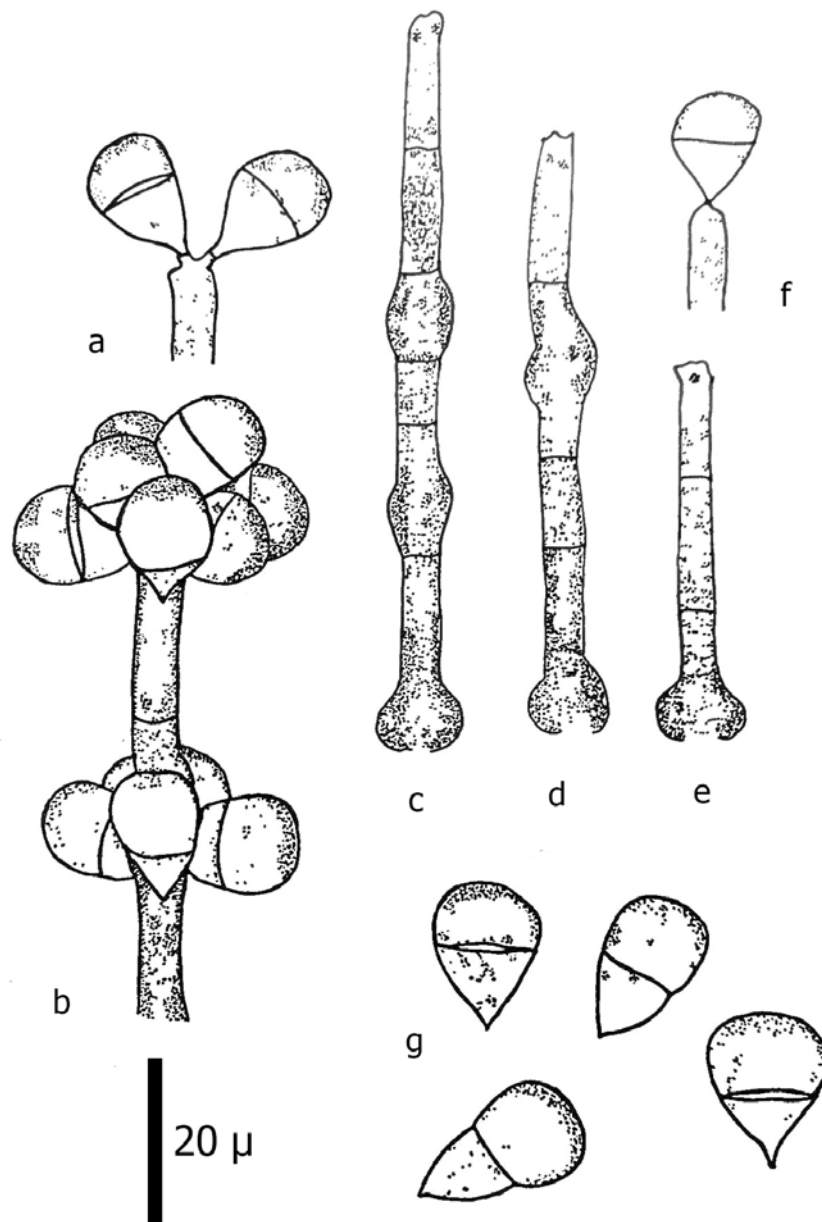
Fig. 1 – *Neocordana musae.* a, b, f Conidiophores with attached conidia. c, d, e Conidiophores from which conidia are detached. g Conidia (redrawn from Meredith (1962b).

Table 2 Comparison of the size and shape of conidia in *Neocordana*.

Taxa	Shape of the conidia	Size of the conidia	Reference
Neocordana johnstonii	Ellipsoidal to subglobose	19–26 ×14–16 μm	Hernández-Restrepo et al. (2015)
N. malayensis	oblong to obovoid	14–18 × 8–10 μm	Crous et al. (2019)
N. musae	Obclavate to pyriform	14–18 × 8–10 μm	Hernández-Restrepo et al. (2015)
N. musarum	Oblong to obovoid	15–20 × 9–13 μm	Crous et al. (2016)
N. musicola	Oblong to obovoid	14.5–20 × 6.5–9.5	Hernández-Restrepo et al. (2015)
N. musigena	Oblong to obovoid	15–18 × 7–9 μm	Crous et al. (2017)
N. versicolor	Ellipsoidal	15–25 × 10–15 μm	Hernández-Restrepo et al. (2015)

Table 3 Morphological differences between *Neocordana musae* and *N. versicolor*.

Character	*Neocordana musae*	*Neocordana versicolor*
Appressoria	Dark grey	Dark brown
Conidia	Broadly ellipsoid	Obovoid to pyriform
Family of the host	Musaceae	Cannaceae
Germ tube	End with amulliform	Shaped a boxing glove

Table 4 Distinct features of the conidiophore between *N. johnstonii* and *N. versicolor*.

Character of the conidiophore	*Neocordana johnstonii*	*Neocordana versicolor*
Apical and intercalary part	Not swollen	Becomes bulbous and swollen
Presence of denticles	More than one denticle at the swollen parts	One denticle per swelling

Table 5 Comparison of conidia and conidiophores of *N. musae* and *N. johnstonii*.

Character	*Neocordana musae*	*Neocordana johnstonii*
Conidiophore	Often nodose	Often twisted in the basal part
	Up to 150 µm long	Up to 300 µm long
	4-6 µm in diameter	5-9 µm in diameter
Conidia	Short	Longer and wider than *N. Musae*

Crous et al. (2016) mentioned that *N. musarum* resembles *N. musae*. Size of the conidia can be used to differentiate between the two species, as *N. musarum* has wider conidia than *N. musae*. According to Crous et al. (2017), *N. musigena* is similar to *N. musicola* but phylogenetically distinct. Crous et al. (2019) mentioned that some of the morphological features of *N. malayensis* (such as conidial dimensions) overlap with *N. musae* and *N. musicola*. *Neocordana malayensis* can be separated from other *Neocordana* species as it has very long, flexuous conidiophores and the phylogenetic distinct.

Fungal colony characters of species in the genus are almost similar and only a few contrasting features were noticed and these are detailed in Table 6.

Table 6 Comparison of culture characteristics in *Neocordana*.

Species	Colonies on Malt Extract Agar (MEA)		Reference
	Upper surface of the plate	Lower surface of the plate	
Neocordana johnstonii	*In vitro* cultures are not available	*In vitro* cultures are not available	Hernández-Restrepo et al. (2015)
N. malayensis	Flat, spreading with moderate, aerial mycelium, having a feathery margin. dirty white surface having patches of pale olivaceous grey or pale luteous in colour	Not mentioned	Crous et al. (2019)
N. musae	Elevated and cottony, aerial mycelium is white, mouse gray zones represent the sporulation, having a defuse margin	Centre is black, have concentric rings, isabelline to umber or luteous to ochreous in colour	Hernández-Restrepo et al. (2015)

Table 6 Continued.

Species	Colonies on Malt Extract Agar (MEA)		Reference
	Upper surface of the plate	Lower surface of the plate	
N. musarum	Colonies are flat and spreading, aerial mycelium is moderate and smooth, having even margins, dirty white in colour	Pale luteous in colour	Crous et al. (2016)
N. musicola	Cottony, aerial mycelium is white and mouse grey towards the edge that shows the sporulation, having a diffuse margin	Fuscous black in the center, luteous at the edge	Hernández-Restrepo et al. (2015)
N. musigena	Colonies flat, spreading, with moderate aerial mycelium, and feathery margins	Luteous in colour	Crous et al. (2017)
N. versicolor	*In vitro* cultures are not available	*In vitro* cultures are not available	Hernández-Restrepo et al. (2015)

History and taxonomy of *Neocordana*

Neocordana species were classified under the asexual dematiaceous hyphomycetes genus *Cordana* Preuss. *Cordana* was established by Preuss (1851) with *C. pauciseptata* Preuss as the type specimen. It was characterized by brown conidiophores which are septate, bearing swollen conidiogenous zones. *Cordana* consisted of saprobic and pathogenic species. Saprobes use soil and rotten wood as substrates whereas pathogens colonize leaves. With DNA sequence data and phylogenetic concepts, pathogens such as *C. musae*, *C. johnstonii* and *C. versicolor* were placed in the order Magnaporthales, family Pyriculariaceae and accommodated in *Neocordana.*

Hernández-Restrepo et al. (2015) provided a phylogenetic tree based on analyses of LSU and ITS sequence data of *N. musae* and *N. musicola* isolates with other taxa of Magnaporthales. The isolates of *N. musae* and *N. musicola* were from different localities such as Australia, Mauritius, Malaysia and Mexico (Hernández-Restrepo et al. 2015). With high bootstrap support, *Neocordana* isolates were clearly separated as a monophyletic clade in Pyriculariaceae. DNA sequence data of *N. versicolor* and *N. johnstoni* are not available and therefore not included in the phylogenetic analysis. Based on the morphological data and host pathogenic interactions, *N. versicolor* and *N. johnstoni* were included in the genus. Crous et al. (2016) have introduced *N. musarum* as a new species with the help of descriptive, morphological and molecular data (Bayesian analysis of the LSU sequences). Next species introduced by Crous et al. (2017) is *N. musigena*. Crous et al. (2019) recently introduced *N. malayensis* based on LSU sequence data. The taxonomic placement of five species (*N. musae, N. musicola, N. musarum, N. musigena* and *N. malayensis*) are supported by molecular data but the taxonomic placement of two species (*N. versicolor* and *N. johnstoni*) are still uncertain due to the lack of molecular data. Currently, LSU, ITS, ACT, and RPB1 gene sequences are available in GenBank for the recently introduced above mentioned four species. The published record for *N. versicolor* was provided by Soares et al. (2005) and its establishment was mainly based on morphology, plant family of the host and pathogenicity.

Taxonomic placement of the genus *Neocordana* in the family Pyriculariaceae

In this study, we present an updated tree of the Pyriculariaceae by using all the available LSU and ITS sequences. Multiple alignments were automatically done by MAFFT v. 7.036 (http://mafft.cbrc.jp/alignment/server/index.html; Katoh & Standley 2013) using BioEdit v. 7.0.5.2 (Hall 1999). Maximum likelihood trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using the GTR+I+G model of evolution. Maximum Likelihood bootstrap values (ML) equal to or greater than 60% are given above each node in blue (Fig. 2). The parameters as set by Wanasinghe et al. (2018a) were used to conduct the Bayesian analysis. Branches with Bayesian posterior probabilities

greater than 0.95 above each node in blue (Fig. 2). Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2012) and reorganized in Microsoft power point (2010). 38 taxa that have been used in the study are shown in Table 7. Alignment data and phylogenetic tree are submitted to TreeBASE (www.treebase.org, S23711).

Table 7 Taxa used in the phylogenetic analysis of Pyriculariaceae and their corresponding GenBank accession numbers. Type species of each genus are in bold.

| Species	Culture accession numbers	Gene bank accession numbers	
		LSU	ITS
Bambusicularia brunnea	**CBS 133599**	**KM484948**	**KM484830**
Barretomyces calatheae	CBS 129274	KM484950	KM484831
Gaeumannomyces radicicola	Z 426 AJ	KM484966	KM484849
G. tritici	CBS 186.65	KM484955	KM484838
Macgarvieomyces borealis	**CBS 461.65**	**DQ341511**	MH858669
M. juncicola	CBS 610.82	KM484970	KM484855
Neocordana malayensis	CBS 144604	MK442533	MK442593
N. musae	**CPC 18127**	**LN713290**	**NR-154265**
N. musae	CPC 16362	LN713289	LN713281
N. musae	CPC 16298	LN713288	LN713282
N. musae	CPC 19065	LN713291	LN713276
N. musarum	CPC 28529	KY173516	KY173425
N. musarum	CPC 28525	KY173515	KY173424
N. musicola	CPC 15987	LN713287	LN713283
N. musicola	CPC 11225	LN713286	NR-154266
N. musigena	CPC 29779	KY979805	KY979750
N. musigena	CPC 29777	KY979804	KY979749
N. musigena	CPC 29140	KY979803	KY979748
Neopyricularia commelinicola	**CBS 128303**	**KM484982**	**KM484868**
Proxipyricularia zingiberis	**CBS 303.39**	**KM484989**	**KM484871**
Pseudopyricularia bothriochloae	CBS 136427	KF777238	KF777186
P. cyperi	CBS 133595	KM484990	KM484872
P. hagahagae	CPC 25635	KT950865	KT950851
P. higginsii	09-2007-1470	KM484991	KM484875
P. hyrcaniana	Ck2	KY457266	NA
P. iraniana	18d	KY457269	KY465726
P. kyllingae	**CBS 133597**	**KM484992**	**KM484876**
P. kyllingae	CB8959	KM484993	KM484877
Pyricularia ctenantheicola	GR0001	KM484994	KM484878
P. grisea	BR0029	KM484995	KM484880
P. oryzae	CBS 365.52	KM485000	KM484890
P. oryzae	CBS 375.54	KM485001	KM484891
P. penniseticola	BF0017	KM485031	KM484925
P. pennisetigena	ML0036	NA	KM484935
P. urashimae	CPC 29421	KY173529	KY173439
P. zingibericola	RN0001	KM485037	KM484941
Xenopyricularia zizaniicola	**CBS 133593**	NA	**KM484947**
X. zizaniicola	KYZL201-1-1	KM485042	KM484946
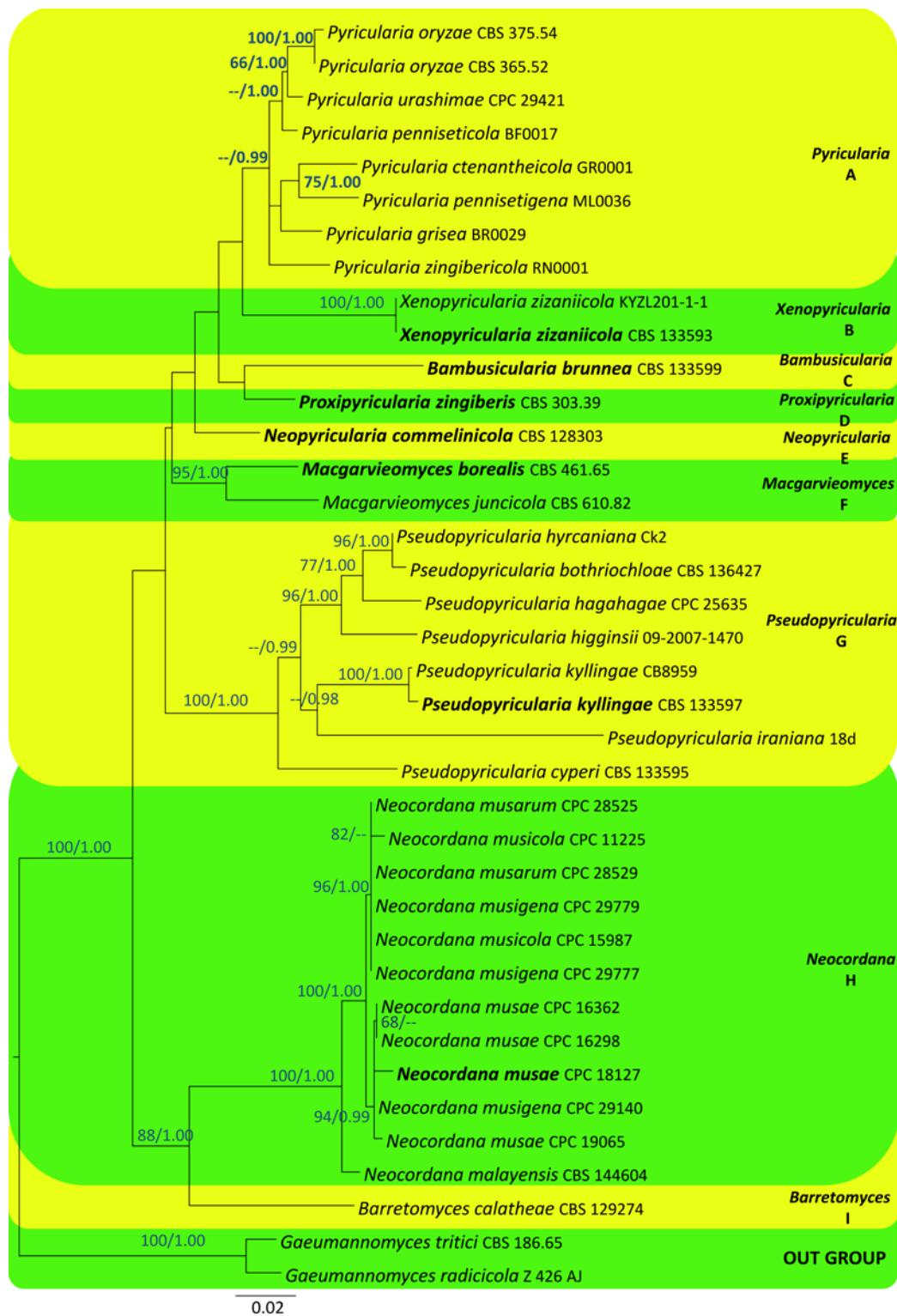
Fig. 2 – Maximum likelihood tree revealed by RAxML from an analysis of combined LSU and ITS partial sequence data of the genera of family Pyriculariaceae, showing the phylogenetic position of the genus *Neocordana*. ML bootstrap supports (≥60%) and Bayesian posterior probabilities (≥0.95PP) are given above the branches respectively. The tree is rooted to *Gaeumannomyces radicicola* and *G. tritici* (Magnaporthaceae). Type strains of genera are in bold. Estimated base frequencies were as follows; A =0.248133, C =0.257797, G =0.288552, T =0.205518; substitution rates AC =2.347792, AG = 1.844686, AT =2.746626, CG =1.125430, CT =5.574905, GT =1.000000; proportion of invariable sites I =0.566499; gamma distribution shape parameter α =0.505155. The best scoring RAxML tree is shown with a final ML optimization likelihood value of -6111.997.

The combined LSU and ITS gene dataset comprised 38 taxa from selected genera of the family Pyriculariaceae and the matrix had 346 distinct alignment patterns, with 13.16% of undetermined characters or gaps. A total of 36 in group taxa corresponding to 9 monophyletic clades (ML = 100 %, PP = 1.00), within the Pyriculariaceae is presented herein (Fig. 2). Nine genera previously recognized, namely *Bambusicularia* (clade C), *Barretomyces* (clade I, ML = 88% / PP = 1.00), *Macgarvieomyces* (clade F, ML = 95% / PP = 1.00), *Neocordana* (clade H, ML = 88% / PP = 1.00), *Neopyricularia* (clade E), *Proxipyricularia* (clade D), *Pseudopyricularia* (clade G)), *Pyricularia* (clade A) and *Xenopyricularia* (clade B) are given here. The genus *Neocordana* constitutes a well-supported monophyletic clade (Clade H), sister to *B. calatheae* (clade I). *Neocordana* taxa were not well separated phylogenetically within the clade, for instance, *N. musae*, *N. musicola* and *N. musigena*.

Biology and pathogenicity of *Neocordana*

Disease symptoms of *Neocordana* leaf spots in *Musa* sp. and *Canna denudata*

Neocordana pathogens only target the leaves of the hosts (Jones 1999, Hernández-Restrepo et al. 2015). No published records regarding disease occurrence in other plant parts such as fruits roots or stem are known. Cross infectivity and alteration of hosts are still not observed. The leaf spots on *Musa* sp. are very common in the field and result in significant crop loss (Jones 1999, Hernández-Restrepo et al. 2015). It was observed that the penetration and the invasion of the *Neocordana* pathogens are very common during humid environmental conditions (Jones 1999). The severity of the damage depends on the age, nutritional deficiency or the occurrence of previous wounds in the plant. Environmental conditions that the plants are being exposed to also significantly affect the severity of the damage. *Neocordana* leaf spots on *Musa* sp. have distinct concentric ring patterns at the gray centers of the necrotic areas (Hernández-Restrepo et al. 2015, Crous et al. 2016). The necrotic lesions are pale brown, large and having an oval to fusiform shape (Jones 1999). Around the brown necrotic area, a bright yellow chlorotic lesion is present and it separates the diseased area of the leaf from the healthy area (Jones 1999). The lesions also can be observed in places where other pathogens had previously invaded. Penetration of the *Neocordana* pathogens occur at leaf margins and nutrient deficiencies trigger the invasion (Jones 1999, Cooke et al. 2009). The disease symptoms in *Canna* caused by *N. versicolor* are also somewhat similar to above (Soares et al. 2005). Eye shaped lesions can be seen in the leaf. They are widely distributed on the surface and finally results in complete necrosis of the leaf (Soares et al. 2005). Leaf spots of *N. johnstonii* were also found to be smaller, more regular in outline and more tapered than *N. musae*. The leaf spots of *N. musae* were recorded as large, elliptical and oval with respect to *N. johnstonii* (Jones 1999).

Ecological factors and the formation of conidia and appressoria

Violent conidial discharge of *Neocordana musae* under decreasing vapor pressure at Jamaican plantations of *Musa* sp. was recorded by Meredith (1962a). In an infected leaf when the relative humidity is lower than 95%, conidial formation has not been reported. During dry climatic conditions, conidia formation was only recorded at night. Dispersal of conidia was high when the leaf surface got wet during raining season. Meredith (1962a) concluded that the rain, humidity and irrigation can increase conidial concentration on the leaf. Meredith (1962b) discussed that the discharge of conidia is independent from light. As a result of drying, water evaporates from conidia and the conidiophores. Negative pressure was developed soon after water evaporation and finally, the walls are drawn inward and tension builds up. As a result of releasing the tension, conidia are dispersed away from the conidiophores. The gaseous phase of the terminal cell might provide the energy for the discharge of conidia (Meredith 1962b).

Allen & Dettmann (1990) observed the production of conidia and appressoria formation of *N. johnstonii in vitro* and was considered as a pathogen causing leaf spots. As recorded in Allen & Dettmann (1990) several *Neocordana* leaf spots were observed in cold locations compared to warm

habitats. Sporulation was rapid on the abaxial surface of the leaf under moist weather conditions. Compared to dead leaves, high sporulation was recorded on living leaves. In young leaf spots, sporulation was highly observed. The spores had a short lifespan on dry leaf surfaces. The sporulation continues at a constant rate at least for five days. The optimum temperature for spore production was 21°C–22°C. The lowest temperature requirement for the production of spores was 10 °C on wet leaves. Rate of conidia production was recorded as 0.04 conidia mm^{-2} of lesion h^{-1} on dead leaves. Appressoria were formed under 22°C–25°C on detached leaves. In addition, the production of spores had a low optimum temperature with respect to *Mycosphaerella musae* and *M. musicola* (Allen & Dettmann 1990). Jones (1999) concluded that *N. johnstonii* was more adapted to cooler environments than *N. musae*.

Disease formation of *Neocordana musae*

Disease formation has been widely studied only on *N. musae*. The pathogen can penetrate on living and dead cells of the host. Penetration of the pathogen depends on the health of the tissue hence *N. musae* was recorded as a weak pathogen. The penetration is wound-induced. Invasion usually occurs at leaf margins and the places where the leaf lamina got tear off. Effect of senescence, nutritional deficiencies, adverse environmental conditions, wounds and the lesions caused by other pathogens can felicitate the invasion of *N. musae* (Jones 1999). Conidia of *N. musae* can be germinated on a moisture film on the leaf surface. Within 8 hours after the deposition, appressoria formation starts. The appressoria next give rise to the infection peg which penetrates the epidermal cell. After that, one or few fungal cells are formed and fill the epidermal cell. The infection peg starts to swell next. Finally, the epidermal cells face necrotic cell death and result in pale brown lesions (Jones 1999).

Isolation, culturing, identification and biological characteristics of *Neocordana*

Few studies were conducted on isolation, culturing and biology and so far, restricted to *N. musae*. In Gaikwad et al. (2017) the fungus was isolated from the diseased leaves of *Musa* sp. and grown in different media. The culture morphology and colony diameter were observed with respect to the media that have been used in that study. Potato Dextrose Agar media (PDA), Malt Extract Agar media (MEA), Czapeks dox agar media, Asthana & Hawkars agar, Richard agar, Kings agar and host leaf extract agar media were used. Among all tested mycelia, growth was not observed in Asthana & Hawkars agar media and Richard agar media. On other media, the colony is gray to white in colour. Distinct concentric to circular rings at the periphery was observed in PDA and King's agar media. A compact growth was observed in Czapeks dox agar and Kings Agar media. When the organism grew on MEA and Czapek's dox agar media, a sparse growth was seen. Sporulation was observed on MEA media from the periphery of the concentric rings. Highest radial growth was recorded on PDA and MEA while the lowest was observed at Czapeks dox agar media. Pure cultures were taken from single spore isolations. Molecular data were not used to identify the organism and pathogenicity tests were not conducted during the study. For the other *Neocordana* members, detailed studies of the cultures are yet to be conducted. Colony characteristics were recorded in Hernández-Restrepo et al. (2015) and Crous et al. (2016, 2017).

Pathogenic nature of *N. musae* was also studied on *Musa* sp. in Yunnan province, China (Fan et al. 2012). Morphological observations were conducted from fungal samples associated with leaf spots and optimum favorable temperature for the growth was recorded as 28 °C and pH 7. Whole light treatment was identified as a good criterion for mycelium growth and the mycelium grows better in sucrose and carbon-rich media. Yeast extract was figured out as better sources for nitrogen in the media. Pathogen showed a profuse growth on PDA and PSA media and the optimum temperature was recorded as 25°C for the sporulation on above media. The lethal temperature for the mycelium of *N. musae* was recorded as 70°C and the time of exposure was 30 minutes (Fan et al. 2012).

Endophytic nature of *Neocordana* members and association with *Mycosphaerella* sp.

Neocordana musae was recorded as an endophyte in Thailand (Photita et al. 2001). In-depth study on the endophyte was again conducted by Photita et al. (2004). Pathogenic nature (leaf spots formation) of the endophyte was checked on detached *Musa* leaves. The isolates used were unable to cause the disease on leaves. Photita et al. (2004) concluded that *N. musae* is latent in the host tissue for a long time before causing the disease. There are no published records on the endophytic nature of other *Neocordana* members.

Neocordana musae was recorded around the lesions as a result of *M. musicola*, *M. fijiensis* and another *Mycosphaerella* sp. which is responsible for Septoria leaf spot disease (Jones 1999). Another study was conducted in northern Thailand by Kaewjan (2012) on *Mycosphaerella* species and fungi associated with diseases of *Musa* sp. which recovered *N. musae* as an endophyte. The authors clearly state that *N. musae* can be a weak pathogen which causes diseases under stressed conditions. The pathogen can stay latent during a long period of time inside the host. Tsigbey (1996) reported that black sigatoka symptoms were associated with a lot of conidia and conidiophores of *N. musae* but unable to collect the spores of *M. fijiensis*. Tsigbey (1996) also observed *N. musae* at the centers and margins of necrotic leaf tissues. Tsigbey (1996) has stated that future studies are warranted on the pathogenic roles of *N. musae* and its link to the severity of black sigatoka disease on *Musa* sp.

Antagonistic activity of *Neocordana musae*

Antagonistic activity of *N. musae* was investigated *in vitro* on the growth of *Colletotrichum musae* (Nuangmek et al. 2008). *Neocordana musae* was recorded as an endophyte from a wild variety of *Musa* sp., which significantly reduces the radial growth of mycelium of *C. musae*. In addition, this inhibition occurred due to the competitive nature and faster growth of *N. musae.* As a result, the growth of the mycelium of *C. musae* is also inhibited. Production of secondary metabolites by *N. musae* and a related antagonistic activity was not recorded in (Nuangmek et al. 2008).

Controlling of leaf spots

Fungicides used for Sigatoka leaf disease and black streak disease in *Musa* sp. can be used to control *Neocordana* leaf spots (Jones 1999). Addition of oil with the systemic fungicides use in controlling Sigatoka leaf disease in *Musa* sp. can enhance the severity of *Neocordana* spots and no specific controlling method was indicated in this study (Ploetz 2003). Biological control was applied on *N. musae* using *Jatropha* plant extracts to reduce leaf spot diseases in *Musa* sp. (Torre & Tangonan 2008). Extracts of several parts of *Jatropha* plant such as seeds, young fruits, roots and leaves are very effective to control *N. musae*. Alvindia (2012) evaluated the impact of *in vitro* application of several biocontrol agents on the mycelium growth of *N. musae*. Selected extracts of plant oils, inorganic salts, *Trichoderma harzianum, Bacillus amyloliquefaciens*, and Serenade ASO (*B. subtilis* 1.34% EC) totally controlled the mycelium development of *N. musae* in 10 weeks (Alvindia 2012). In addition to that, Piper betel oil (60%), potassium bicarbonate (28%), garlic oil and capsicum oil (20%) could limit mycelium growth. Jalander & Gachande (2013) studied the *in vitro* efficacy of two fungicides named Captasul (capton-50) and Ridomil gold on mycelium growth of *N. musae*. Different concentrations (50ppm, 100ppm, 150ppm, 200 ppm, 250 ppm, and 300 ppm) were used against the pathogen and Captasul (300ppm) was reported as highly toxic and very effective to control *N. musae*. Recently Samuelian (2016) investigated antagonistic activities of *T. harzianum* against the growth of *N. musae* as an integrated disease management approach. At present, almost all the biological control methods are applied on *N. musae,* but similar studies are needed on other *Neocordana* pathogens.

Possible future studies

Limited number of studies have been conducted on *Neocordana* when compared to other plant pathogenic genera such as *Colletotrichum, Fusarium* and *Mycosphaerella* which cause diseases in *Musa* sp. Detailed studies were not conducted and few studies could be included in this

paper. Host and pathogenic interactions, natural disease resistance mechanisms such as induced systemic resistance (ISR), Systemic Acquired Resistance (SAR) and the associated chemical and physiological changes in host plants can also be studied. Postharvest physiology of infected commodities by *Neocordana* pathogens can be investigated. The infection process was studied in the past but no recent reports can be found on this aspect. As *Musa* sp. is one of the major fruit crops, it will be useful to investigate more on its pathogenesis, disease cycle and its epidemiology. It is necessary to come up with more biological control strategies such as integrated pest management approaches in the future to cut down treatment costs.

There are only seven species reported at present but very likely that this genus harbors many more species. We have also observed the establishment of new species based on minute morphological differences which are not conclusive to differentiate species. According to Jeewon & Hyde (2016), phenotypic variations such as the size or shape of conidia must be considered with caution as they can vary with several environmental factors. Therefore, more molecular studies are warranted for a better understanding of species relationships and reveal the potential of species complexes in the future. There are possibilities for investigations on whether *Neocordana* endophytes can switch to pathogenic and saprobic lifestyles. We suggest to undertake proteomic studies to characterize proteins associated with pathogenicity and disease formation that will be useful in the future for better taxonomic resolutions and disease control.

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