CHARACTERISATION OF *NEOFUSICOCCUM* SPECIES CAUSING MANGO DIEBACK IN ITALY

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SUMMARY

Species of Botryosphaeriaceae are important fungal pathogens of mango worldwide. A survey of 11 mango orchards located in the provinces of Catania, Messina, Palermo and Ragusa (Sicily, southern Italy), resulted in the isolation of a large number (76) of *Neofusicoccum* isolates associated with decline and dieback symptoms. Isolates were identified based on morphology and DNA sequence data analyses of the internal transcribed spacer region of the nrDNA and partial translation of the elongation factor 1-alpha gene regions. Two species of *Neofusicoccum* were identified, which included *N. parvum* and *N. australe*, the former of which was the dominant species. The high incidence in local orchards and the pathogenicity results indicate that *N. parvum* and *N. australe* are important pathogens of mango in Sicily where they may significantly limit mango production.

*Key words***:** Botryosphaeriaceae, dieback, ITS, EF-1α, *Mangifera indica*, *Neofusicoccum*,

INTRODUCTION

According to the ancient accounts of travelers and written historical records, the cultivation mango (*Mangifera indica*; Anacardiaceae), a species native to India, began at least 4000 years ago (De Candolle, 1884). Mango trees are able to adapt to various environmental conditions that are normally not conducive to growth of other fruit trees (Wolstenholme and Whiley, 1995). The mango cv. Kensington Pride was first introduced into Sicily (southern Italy) during 1980-1990 by growers in the Catania province. Thereafter, its cultivation expanded to the other provinces of Sicily (Messina, Ragusa, Palermo) and to the nighbouring Calabria region. In these regions, cvs Kensington Pride, R_2E_2, Maya, Kent, Irwin, Keitt and Tommy Atkins are the most commonly grown cultivars (Anonymous, 2010).

During all stages of their life cycle, mango trees can be attacked by over 140 different plant pathogens inciting diverse diseases (Prakash, 2004; Haggag, 2010), some of which have become a limiting factor for mango production (Ploetz, 2004; Prakash, 2004; Javier-Alva *et al.*, 2009; de Olivera Costa *et al.*, 2010). Tip dieback or decline, which is a complex disease, is

considered a serious problem in various mango-producing countries (Ramos *et al*., 1991; Johnson, 1992; Jacobs, 2002; Khanzada *et al.*, 2004a, 2004b). The etiology of this disease remained unclear for several years due to the different causal agents associated with it (Ploetz *et al*., 2003).

Smith and Scudder (1951) found a *Diplodia* sp. associated with dieback of mango but did not confirm its pathogenicity. Ramos *et al*. (1991) isolated *Neofusicoccum ribis* and a *Diplodia* sp. from mango trees showing tip dieback in Florida (USA). *Botryosphaeria dothidea*, diplodia- and fusicoccum-like asexual morphs, were reported as causal agents of fruit rot and decline of mango (Ploetz, 2004). In Florida, *Lasiodiplodia theobromae* (as *D. theobromae*) and *Fusicoccum aesculi* were found responsible for symptoms associated with decline on cvs Keit and Tommy Atkins (Ploetz *et al*., 1996). The latter fungal species have also been reported from Brazil as associated with mango dieback and stem-end rot (de Oliveira Costa *et al.*, 2010). In western Australia, *Neoscytalidium dimidiatum* and *Ne. novaehollandiae* have recently been reported as causal agents of canker and dieback (Ray *et al.*, 2010) whereas, in a subsequent survey, Sakalidis *et al*. (2011) found that *Pseudofusicoccum adansoniae*, *P ardesiacum*, *P. kimberleyense* and *Lasiodiplodia pseudotheobromae* were associated with canker and dieback. Other fungal species in the family Botryosphaeriaceae, including *L. theobromae*, *B. dothidea*, *Neofusicoccum parvum* and *N. mangiferae* have also been reported to cause stem-end rot (SER) of mango (Slippers *et al*., 2005; Johnson, 2008; de Oliveira Costa *et al*., 2010). Recent phylogenetic studies revealed the association of new members of Botryosphaeriaceae e.g., *L. hormozganensis* and *L. iraniensis* with mango diseases in Iran and in Australia (Abdollahzadeh *et al.*, 2010, Sakalidis *et al*., 2011) and *L. egyptiacae* with mango dieback in Egypt (Ismail *et al*., 2012b).

Recent surveys conducted in mango orchards in Italy led to the discovery of several diseases (Ismail *et al.*, 2012a). During these surveys, plants with decline and tip dieback symptoms were frequently observed, from which a large number of Botryosphaeriaceae isolates were recovered that, as reported in this paper, were identified and assayed for pathogenicity assessment.

MATERIALS AND METHODS

Isolations. Isolations were made from 60 symptomatic plant samples showing dieback symptoms on young twigs and branches, dark brown lesions on mature fruits, and necrosis and brown discolouration under cambium tissues, resembling Botryosphaeriaceae infection (Fig. 1). The collected plant materials were surface disinfected by sequential washing in 70% ethanol for 30 sec, a bleach solution (5% sodium hypochlorite) for 1 min followed by rinsing with sterile water, and dried with a sterile filter paper. Small pieces, between the healthy and infected tissues were excised and plated onto potato dextrose agar (PDA) amended with streptomycin sulfate (0.1 g/l). Plates were incubated at 25±2°C in the dark for 2–4 days. The putative isolates resembling the colony morphology of botryosphaeriaceous taxa growing out from the tissues were sub-cultured onto fresh PDA and incubated at 25±2°C for 5 days, and pure cultures were obtained by excising and transferring a hyphal tip to fresh PDA plates.

DNA isolation and amplification. Genomic DNA was extracted from 10-day-old cultures using the UltraClean™ Microbial DNA Isolation Kit (MO-BIO Laboratories, Inc, Carlsbad, USA) following the manufacturer's protocol. The internal transcribed spacer region (ITS) of the nrDNA including the 3' end of 18S small subunit rRNA gene region, the first internal transcribed spacer (ITS1), the complete 5.8S rRNA gene, the second internal transcribed spacer (ITS2) and 5' end of the 28S large subunit rRNA gene region were amplified using primer pair V9G (de Hoog and Gerrits van den Ende, 1998) and ITS4 (White *et al*., 1990). A part of the translation elongation factor 1-alpha (EF-1α) gene was amplified using primers EF1-728F (Carbone and Kohn, 1999) and EF2 (O'Donnell *et al*., 1998). PCR conditions included final concentrations of 0.5 U/μL of *Taq* polymerase (BIOLINE, San Diego, USA), 1× reaction buffer (BIOLINE), 2–2.5 mM $MgCl_2$ (BIOLINE), 0.4–0.6 mM of each dNTP and 0.12–0.2 μM of each primer made up to a final volume of 12.5 μl with sterile deionized water. PCR conditions included the following steps: an initial step of denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 52°C for 30 sec and 72°C for 1 min, with a final elongation step at 72°C for 7 min.

DNA phylogeny. Amplicons of the ITS region were sequenced in both directions using ITS4 and the internal forward primer ITS5 (White *et al.,* 1990). The PCR products of the EF–1α

gene region were sequenced in both directions using the same primer pairs as for amplification. Sequencing reactions were performed using BigDye Terminator v. 3.1 Cycle Sequencing Kit (Perkin-Elmer Applied Bio Systems, Foster City, CA, USA) as recommended by the manufacturer and run on an ABI PRISM^{TM} 3100 DNA automated sequencer (Perkin-Elmer Applied BioSystems, Foster City, CA, USA).

The obtained sequences were compared with those previously identified in GenBank using the Basic Local Alignment Search Tool (BLAST) (Table 1). Sequences were aligned together with those retrieved from GenBank using MAFFT v. 6.0 (Katoh and Toh, 2010) and manually adjusted and corrected where necessary. The nucleotide substitution models were determined individually for each gene region using MrModelTest v. 2.2 (Nylander, 2004). The best fit model, HKY+I+G with dirichlet base frequencies, was selected for both ITS and EF–1α sequence datasets. The 70% reciprocal NJ (Neighbour-Joining) bootstrap analysis was performed for each gene region to determine congruency (Mason-Gamer and Kellogg, 1996; Gueidan *et al.*, 2007).

Bayesian analyses were performed using MrBayes v. 3.1.1 (Ronquist and Huelsenbeck, 2003). For the Bayesian analyses, a Markov Chain Monte Carlo (MCMC) (Larget and Simon, 1999) method was performed to confirm the topology of the tree, by running four chains simultaneously starting from a random tree topology and ending at 1,000,000 generations with trees saved every 100th generation. The burn-in value was graphically estimated from the likelihood scores. The first 1000 trees were discarded from the analysis and the final consensus tree constructed from the remaining trees. Trees were rooted to *Phyllosticta capitalensis* (CBS 115051) and *P. citricarpa* (CBS 102374) as out-group taxa.

The phylogenetic relationship among taxa was determined using PAUP* (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2003). Maximum parsimony (MP) was performed using the heuristic research option with random stepwise addition in 1,000 replicates, tree bisection and reconnection (TBR) as branch swapping algorithms, and random taxon addition sequences for the construction of maximum parsimony trees. Branches of zero length were collapsed and all multiple equally parsimonious trees were saved. MAXTREES was set to 10,000. In the analysis all characters were unordered and had equal weight; gaps were treated as missing data. Parameters measured for parsimony included tree length (TL), consistency index

(CI), rescaled consistency index (RC) and retention index (RI). Bootstrap support values were evaluated using 1,000 bootstrap replicates (Hillis and Bull, 1993). Sequences generated in this study were deposited in GenBank (Table 1).

Morphological characterisation. To induce sporulation, a 6 mm diameter plug of mycelium from each isolate was placed on 2% water agar medium (WA: 20 g agar/l) together with autoclaved pine needles (Smith *et al*., 1996). The plates were incubated at 25±2°C under near-ultraviolet (UV) light for 2–3 weeks. Dimensions of 50 conidia from each of 37 randomly selected isolates were determined at 1.000× magnification mounted in 85% lactic acid. Vertical sections were made through pycnidia with a Leica CM1100 microtome and mounted in lactic acid. The 95% confidence intervals were determined for the conidia with extremes given in parentheses. Cardinal growth temperatures were determined for each isolate on PDA at 10–35°C in 5°C intervals in the dark. Colony colours were determined after 7 days on PDA at 25°C in the dark using the colour charts of Rayner (1970) for comparison.

Pathogenicity tests. Three- to four-month-old mango cv. "Kensington Pride" seedlings ranging in length from 40–60 cm, were used to determine the pathogenicity of nine isolates representing two *Neofusicoccum* spp. (Table 1). The plants were maintained in a growth chamber under artificial light (10/14 h light-and-dark cycles) at 25±2ºC and 70–80% relative humidity (RH). Four plants for each isolate and the controls were used and arranged in a completely randomised block design. Data were subjected to the analysis of variance one-way ANOVA and the mean values of the lesions were compared using the Least Significant Difference (LSD) test ($P < 0.05$) (Stat Soft, Inc. 2004). Isolates were cultured on PDA for 7 days at 25ºC in the dark. Inoculations were performed after the outer tissues were disinfected with 70% ethanol, washed with sterile distilled water and left to dry. Using a cork-borer, a 5 mm incision was made into the epidermis, between two nodes and below the apex of the stem. A 5 mm diameter mycelial PDA plug was removed from the edge of the actively growing test culture and placed in the wounds, with the mycelium facing the cambium. The inoculated wounds were wrapped with Parafilm®, (Laboratory Film, Chicago, IL, USA) to prevent desiccation and contamination. Control plants were inoculated with a sterile PDA plug. Six weeks after inoculation the bark lesion lengths as

well as the length of cambium discolouration were measured and data were log-transformed prior to analysis. Re-isolation of the tested isolates was done from the margins of the necrotic lesions on PDA to confirm Koch's Postulates.

RESULTS

Isolations. In total, 76 isolates of Botryosphaeriaceae were recovered from four sites located in southern-Italy (Sicily). Of these 41 isolates originated from Catania, 27 isolates from Messina, six isolates from Palermo and two isolates from Ragusa. The majority of isolates were recovered from twigs, branches, leaves, fruit and wood (26, 19, 18, 9 and 4, respectively). Of these isolates, 37 were randomly selected and included in the phylogenetic analyses and morphological studies. Isolates obtained in this study were deposited in the culture collection of the Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Sez. Patologia Vegetale, University of Catania, Italy. Representative isolates were also deposited in the culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands (Table 1).

Phylogeny. Amplicons of approximately 550 bp were obtained for ITS using primers ITS4 and ITS5 and approximately 500 bp were obtained for EF–1α using the EF1-728F and EF2 primers. The combined dataset of ITS and EF–1α consisted of 72 taxa, which contained the subset of 37 strains obtained during the survey. A further 35 sequences, including the out-group species, were retrieved from GenBank. The combined dataset contained 909 characters after the uneven ends were truncated. Of these characters, 536 were constant, 95 were uninformative and 278 were parsimony informative. A heuristic search revealed the most parsimonious tree (Fig. 2; tree length = 670 steps, CI = 0.743, RI = 0.917 and RC = 0.682). No conflict between the two gene partitions was detected by 70% reciprocal NJ bootstrap analysis (results not shown). The obtained trees differed only in the arrangement of isolates within the terminal clades while their overall topology was the same and were topologically identical to the 50% majority-rule consensus tree illustrated in Fig. 2. Isolates obtained in this study were accommodated in two distinct clades; of which the first clade contained the majority of strains, which grouped with *Neofusicoccum parvum* strain CMW 9081 (culture ex-type) supported by a bootstrap value (BS)

of 79%. Some strains formed two sub-clades where the first sub-clade contained isolates NF-17, NF-38, NF-37 and NF-67 supported only by a Bayesian posterior probability (BPP) value of 0.95. The second sub-clade contained two isolates (NF-62 and NF-82) supported by a BS/BPP of 63/0.77. Four isolates (NF-70, NF-73, NF-76 and NF-77) clustered in a sub-clade in *N. australe*, supported by a low BS of 64 and highly supported by a BPP of 1.0. The remaining two isolates (NF-2 and NF-22) clustered together in a sub-clade (BS/BPP=58/1.0) with *N. australe* (CMW6837, culture ex-type) (BS/BPP = 72/1.0).

Morphological characterisation. Isolates obtained in this study were separated into two groups based on the phylogenetic inference as well as their conidia and culture morphology. In the first group, conidiomata (Fig. 3a) were formed on pine needles within 10–15 days (Fig. 3a). Pycnidia were solitary, mostly aggregated, globose to subglobose, the outer layers composed of 5–10 dark brown thick-walled *textura angularis* cell layers (Fig. 3b). Conidiogenous cells (Fig. 3c,d) were holoblastic, hyaline, cylindrical, 4.5–19.3 µm long, 1.5–2.7 µm wide. Conidia were hyaline, (14.3–) 15.4–17.6 (–19.3) × (5.0–) 5.4–6.2 (–6.6), mean of 50 conidia ± SD = 16.5 ± 1.1 µm long, 5.8 ± 0.4 µm wide, L/W ratio = 2.8 (Fig. 3e,f). Colonies were initially white, becoming glaucous grey to greenish grey on the upper surface, greenish grey in reverse (Fig. 3g,h). In the second group, conidiomata were formed on pine needles within 10–20 days (Fig. 4a). Pycnidia were solitary, subglobose to ellipsoidal, the outer layers composed of 4–6 dark brown, thick-walled *textura angularis* cell layers (Fig. 4b). Conidiogenous cells (Fig. 4c,d) were holoblastic, hyaline, cylindrical to subcylindrical and phialidic, 8.7–16.2 µm long, 1.8–3.7 µm wide. Conidia were hyaline, (17.3–) 19.7–22.9 (–24.5) × (4.5–) 5.6–6.2 (–6.8), mean of 50 conidia ± SD = 21.3 ± 1.6 µm long, 5.9 ± 0.3 µm wide, L/W ratio = 3.6 (Fig. 4e,f). Colonies were initially white, becoming glaucous grey to greenish grey on the upper surface, and dark slate blue in reverse (Fig. 4g,h).

The cardinal temperatures requirements for the growth of all isolates were: minimum 10°C, maximum 35°C, and optimum 25°C.

Pathogenicity tests. Six weeks after inoculation all seedlings showed bark lesions and cambium discolouration. There was no significant variation observed in bark and cambium

lesions produced among isolates of the same species. In general, *N. parvum* and *N. australe* isolates were equally virulent and produced similar bark and cambium lesions, longer than that of the controls (av. = 25.84 mm) (Fig. 5). However, the longest bark and cambium lesions were developed by isolates NF-69 and NF-5 (av. 45.6 mm and 45 mm; av. 51.2 mm, 49.7 mm) respectively. Although the remaining isolates developed smaller lesions, they also proved to be pathogenic. Koch's postulates were confirmed and the tested isolates were successfully recovered from the inoculated tissues.

DISCUSSION

The present study represents the first attempt to identify botryosphaeriacous fungal pathogens associated with *M. indica* in Italy. The first group consisted of isolates exhibited culture and conidial morphology similar to those of *N. parvum* (Slippers *et al.*, 2004, 2005). The second group contained six isolates revealing culture and conidial characteristics similar to those of *N. australe* (Slippers *et al*., 2004). Combined DNA sequence data and morphological features confirmed the identity of the two groups of isolates as *N. parvum* and *N. australe*. These species have been reported as plant pathogens under different climatic conditions and in very different hosts (Slippers *et al.*, 2004, 2005; van Niekerk *et al.*, 2004; Damm *et al.*, 2007; Begoude *et al*., 2009; Sakalidis *et al.*, 2011). *N. parvum* has been reported from mango in Australia (Johnson, 1992), South Africa (Jacobs, 2002), Peru (Javier-Alva *et al*., 2009) and Brazil (de Oliveira Costa *et al*., 2010). In the present study, it was the most frequently isolated species from branches, twigs, leaves, internal wood and fruits exhibiting various symptoms associated with dieback. This fungus was recovered from almost all monitored areas (Catania, Messina and Palermo), but its relative prevalence differed in each. *N. parvum* was identified in this study based on morphology and phylogenetic inference. The differences among strains might be attributed to genetic variation influenced by environmental conditions in different geographical areas where theses strains were isolated. The conidial morphology did not differ greatly and resembled those reported in previous studies (Slippers *et al*., 2004, 2005; de Oliveira Costa *et al*., 2010). However, no septate conidia were observed as previously reported by Slippers *et al*. (2005) and Oliveira Costa *et al.* (2010). *N. australe* was firstly described by Slippers *et al*. (2004) along with

its sexual morph as occurring on several native Australian plant hosts, which include *Banksia* and *Eucalyptus*, and also from a *Protea* sp. in South Africa, and on *Pistachio* in Italy. The fungus has been reported to cause disease on other hosts such as *Prunus* spp. in South Africa (Damm *et al*., 2007), olive in Italy (Lazzizera *et al*., 2008), grapevine in South Africa (van Niekerk *et al*., 2004), Australia (Taylor *et al.*, 2005), and more recently in Chile (Besoain *et al.*, 2013). *N. australe* and *N. parvum* were also reported as canker-causing agents on blueberry (*Vaccinium* spp.) in Chile (Espinoza *et al*., 2009). *N. australe* was the second most dominant species isolated from twigs and branches of mango showing typical dieback symptoms and found only in three sites, Ragusa, Palermo and Catania.

The origin of the *Neofusicoccum* spp. obtained during this survey is unknown. Some of the cultivated mango varieties in Sicily have been imported either as seeds or as plantlets from Australia (Anonymous, 2010). This might suggest that *N. parvum* and *N. australe* have been introduced into Italy with the importation of exotic mango plant materials (seeds or plantlets) from Australia. Alternatively, most of the mango orchards were neglected and in close proximity to various fruit trees which could have served as source of inoculum or as alternative hosts to these fungi. Therefore, the epidemiology of these fungi needs to be studied further to understand the ecology and the movement of these pathogens, in order to establish integrated control strategies.

To our knowledge, this is the first report of these species causing dieback disease on mango in Italy. It was not surprising to isolate these species from mango orchards, since they have previously been reported on olive in southern Italy (Lazzizera *et al*., 2008). Dieback disease by Botryosphaeriaceae could significantly limit future mango production in Sicily.

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CAPTION TO FIGURES

Fig. 1. a, b. Mango dieback symptoms on the young twigs and branches starting from the tip extending downwards; c**.** dark brown lesions on mature fruit lead to soft rot of the internal pulp and sometimes water drops release from the necrotic area; d**.** with severe infection most of the apical parts die and dry, leading to death of the whole tree; e, f. cross sections through trunks of infected trees showing necrosis and brown vascular discolouration of the cambium tissues.

Fig. 2. The most parsimonious tree obtained from a heuristic research with 1,000 random addition sequences of the combined sequences of the ITS and EF-1α sequence alignments of the *Neofusicoccum* isolates recovered during the survey and other closely related species. Scale bar shows 10 changes. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. Isolates in bold were obtained during the survey.

Fig. 3. *Neofusicoccum parvum* NF-5 = CBS 130995. a.Pycnidia formed on pine needles on WA; b. Longitudinal section through a pycnidium; c, d. Hyaline conidiogenous cells; e, f. Hyaline conidia with granular contents. Colony morphology: g, front side; h, reverse side. - Scale bars: b = 20 μm; c, d, e, f = 10 μm.

Fig. 4. *Neofusicoccum australe* NF-76 = CBS 130997. a. Pycnidia formed on pine needles on WA; b. longitudinal section through a pycnidium; c, d. Hyaline conidiogenous cells; e. Hyaline conidia with granular contents; f. Pale brown 1-3 septate conidia before germination. Colony morphology: g. front side; h. reverse side. Scale bars: b = 20 μm; c, d, e, f = 10 μm.

Fig. 5. Pathogenicity test of *Neofusicoccum* species on mango seedlings cv. Kensington Pride. *Neofusicoccum parvum* is represented by NF-69, NF-5, NF-4, NF-85, NF-20 and NF-50; *N. australe* is represented by NF-70, NF-76 and NF-22. Columns represent the mean value of bark and cambium lesions ± standard deviation (bars), which are significantly different $p<0.05$ according to LSD test.

Table 1. List of isolates used in this study. Isolate numbers marked in bold arethose selected for pathogenicity tests. GenBank accession Nos. in italics were generated in this study.

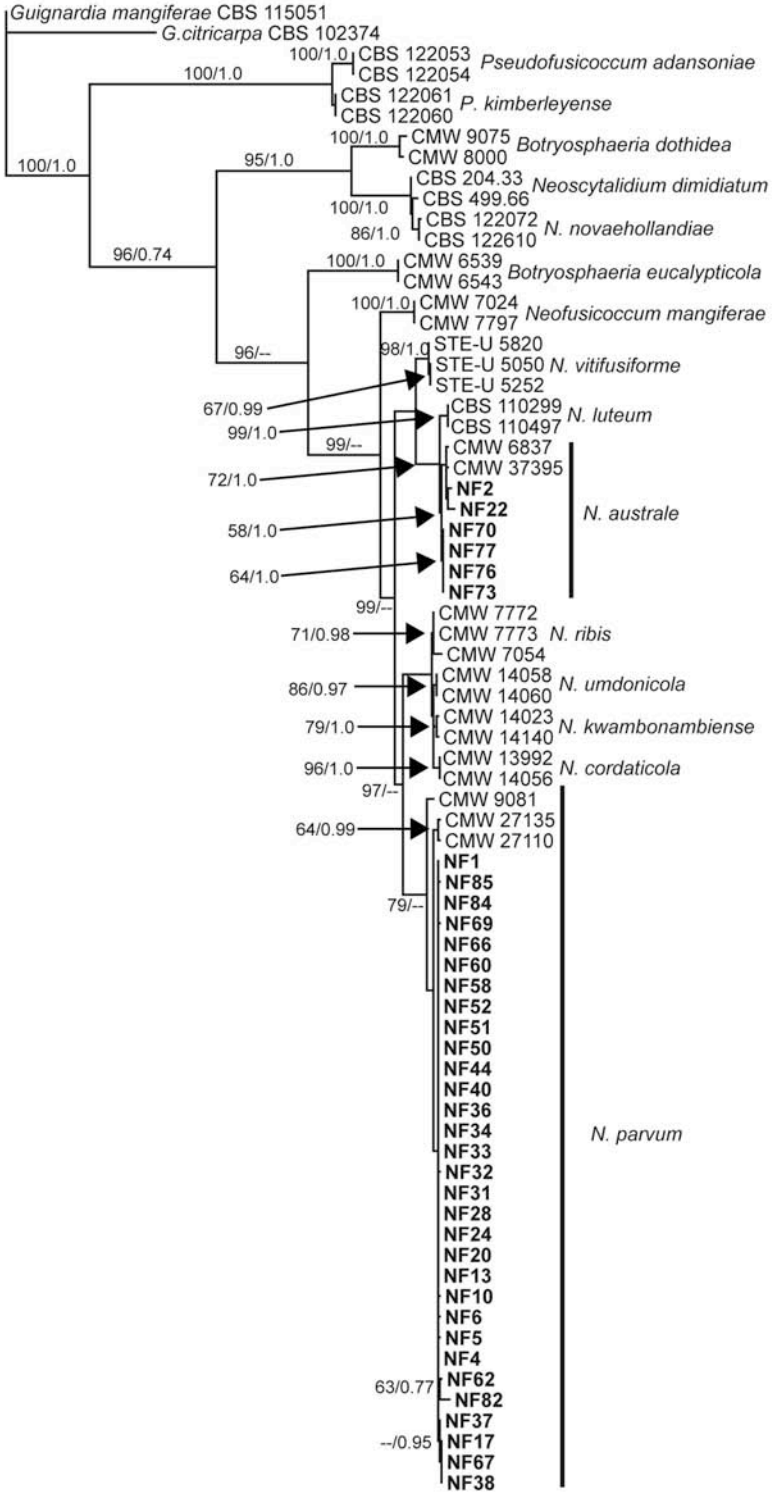
Species Identity	Culture No. [1]	Location	Host	Collector	GenBank accession No.	
					ITS	EF-1
Botryosphaeria dothidea	CMW9075	New Zealand	*Populus* sp.	G.J. Samuels	AY236950	AY236899
	CMW8000	Switzerland	*Prunus* sp.	B. Slippers	AY236949	AY236898
Neofusicoccum australe	CMW6837^{T}	Australia	*Acacia* sp.	B. Slippers	AY339262	AY339270
	CMW 37395	Unknown	*Eucalyptus grandis*	Unknown	JQ744577	JQ744598
	NF-2	Sicily, Italy	*Mangifera indica*	A.M.Ismail	*JN814464*	*JN814507*
	NF-22= CBS 130996	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814465*	*JN814508*
	NF-70	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814466*	*JN814509*
	NF-76= CBS 130997	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814467*	*JN814510*
	NF-73	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814468*	*JN814511*
	NF-77	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814469*	*JN814512*
N. cordaticola	CMW13992^{T}	South Africa	*Syzygium cordatum*	D. Pavlic	EU821898	EU821868
	CMW14056	South Africa	*S. cordatum*	D. Pavlic	EU821903	EU821873
N. eucalypticola	CMW6539	Australia	*E. grandis*	Unknown	AY615141	AY615133
	CMW6543	Australia	*Eucalyptus* sp.	Unknown	AY615140	AY615132
N. kwambonambiense	CMW14023^{T}	South Africa	*S. cordatum*	D. Pavlic	EU821900	EU821870
	CMW14140	South Africa	*S. cordatum*	D. Pavlic	EU821919	EU821889
N. luteum	CBS110299^{T}	Portugal	*Vitis vinifera*	A.J.L Phillips	AY259091	AY573217
	CBS110497	Portugal	*V. vinifera*	A.J.L Phillips	EU673311	EU673277
N. mangiferae	CMW7024^{T}	Australia	*M. indica*	G.I. Johnson	AY615186	AY615173
	CMW7797	Australia	*M. indica*	G.I. Johnson	AY615188	AY615175
N. parvum	CMW27135	China	*Eucalyptus* sp.	M.J. Wingfield	HQ332205	HQ332221
	CMW27110	China	*Eucalyptus* sp.	M.J. Wingfield	HQ332202	HQ332218
	CMW9081 ^{T}	New Zealand	*Populus nigra*	Unknown	AY236943	AY236888
	NF-1	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814429*	*JN814472*
	NF-5= CBS 130995	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814430*	*JN814473*
	NF-13	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814431*	*JN814474*
	NF-24	Sicily,I taly	*M. indica*	A.M.Ismail	*JN814432*	*JN814475*

	NF-36	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814433*	*JN814476*
	NF-38	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814434*	*JN814477*
	NF-85	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814435*	*JN814478*
	NF-33	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814436*	*JN814479*
	NF-34	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814437*	*JN814480*
	NF-40	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814438*	*JN814481*
	NF-60	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814439*	*JN814482*
	NF-44	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814440*	*JN814483*
	NF-66	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814441*	*JN814484*
	NF-37	Sicily, Italy	*M. indica*	A.M.Ismail	*N814442*	*JN814485*
	NF-84	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814443*	*JN814486*
	NF-58	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814444*	*JN814487*
	NF-52	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814445*	*JN814488*
	NF-51	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814446*	*JN814489*
	NF-67	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814447*	*JN814490*
	NF-6	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814448*	*JN814491*
	NF-28	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814449*	*JN814492*
	NF-62	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814450*	*JN814493*
	NF-4	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814451*	*JN814494*
	NF-69	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814452*	*JN814495*
	NF-17	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814453*	*JN814496*
	NF-10	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814454*	*JN814497*
	NF-50	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814455*	*JN814498*
	NF-82	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814456*	*JN814499*
	NF-32	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814458*	*JN814501*
	NF-31	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814459*	*JN814502*
	NF-20	Sicily, Italy	*M. indica*	A.M.Ismail	*JN814460*	*JN814503*
N. ribis	CMW7772^{T}	USA	*Ribes* sp.	B. Slippers	AY236935	AY236877
	CMW7054	USA	*Ribes* sp.	N.E. Stevens	AF241177	AY236879
	CMW7773	USA	*Ribes* sp.	B. Slippers/G. Hdler	AY236936	AY236907
N. umdonicola	CMW14058^{T}	South Africa	*S. cordatum*	D. Pavlic	EU821904	EU821874
	CMW14060	South Africa	*S. cordatum*	D. Pavlic	EU821905	EU821875
N. vitifusiforme	STE-U5050	South Africa	*V. vinifera*	J.M van Niekerk	AY343382	AY343344
	STE-U5252^{T}	South Africa	*V. vinifera*	J.M van Niekerk	AY343383	AY343343
	STE-U5820	South Africa	*Prunus salicina*	U. Damm	EF445347	EF445389
Neoscytalidium dimidiatum	CBS 499.66	Mali	*M. indica*	Unknown	FM211432	EU144063

	CBS 204.33	Egypt	*Prunus* sp.	Unknown	FM211429	EU144064
Ne. novaehollandiae	CBS 122072	Australia	*Adansonia gibbosa*	Unknown	EF585535	EF585581
	CBS 122610	Australia	*Acacia synchronicia*	Unknown	EF585536	EF585578
Phyllosticta capitalensis	CBS 115051	Brazil	*Spondias mombin*	K.F. Rodriques	FJ538325	FJ538383
P. citricarpa	CBS 102374	Brazil	*Citrus aurantium*	Unknown	FJ538313	FJ538371
Pseudofusicoccum adansoniae	CBS 122053	Australia	*A. synchronicia*	Unknown	EF585525	EF585569
	CBS 122054	Australia	*Eucalyptus* sp.	Unknown	EF585532	EF585570
Ps. kimberleyense	CBS 122060	Australia	*A. gibbosa*	Unknown	EU144058	E U144073
	CBS 122061	Australia	*Ficus opposita*	Unknown	EU144059	EU144074

[1]CMW = culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; CBS = CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; NF = culture collection of Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Catania, Italy; STE-U= Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa.
[T]Ex-type cultures.

Fig 1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CBS 122060
P. kimberleyense
100/1.0
CMW 9075
CMW 8000
Botryosphaeria dothidea
100/1.0
95/1.0
CBS 204.33
CBS 499.66
Neoscytalidium dimidiatum
100/1.0
CBS 122072
CBS 122610
N. novaehollandiae
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96/0.74
100/1.0
CMW 6539
CMW 6543
Botryosphaeria eucalypticola
100/1.0
CMW 7024
CMW 7797
Neofusicoccum mangiferae
96/--
98/1.0
STE-U 5820
STE-U 5050
N. vitifusiforme
STE-U 5252
67/0.99
CBS 110299
CBS 110497
N. luteum
99/1.0
99/--
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NF2
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N. australe
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NF76
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99/--
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CMW 7773
N. ribis
CMW 7054
CMW 14058
86/0.97
CMW 14060
N. umdonicola
CMW 14023
79/1.0
CMW 14140
N. kwambonambiense
CMW 13992
96/1.0
CMW 14056
N. cordaticola
97/--
CMW 9081
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CMW 27135
CMW 27110
NF1
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79/--
NF84
NF69
NF66
NF60
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NF51
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NF44
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Fig. 2.

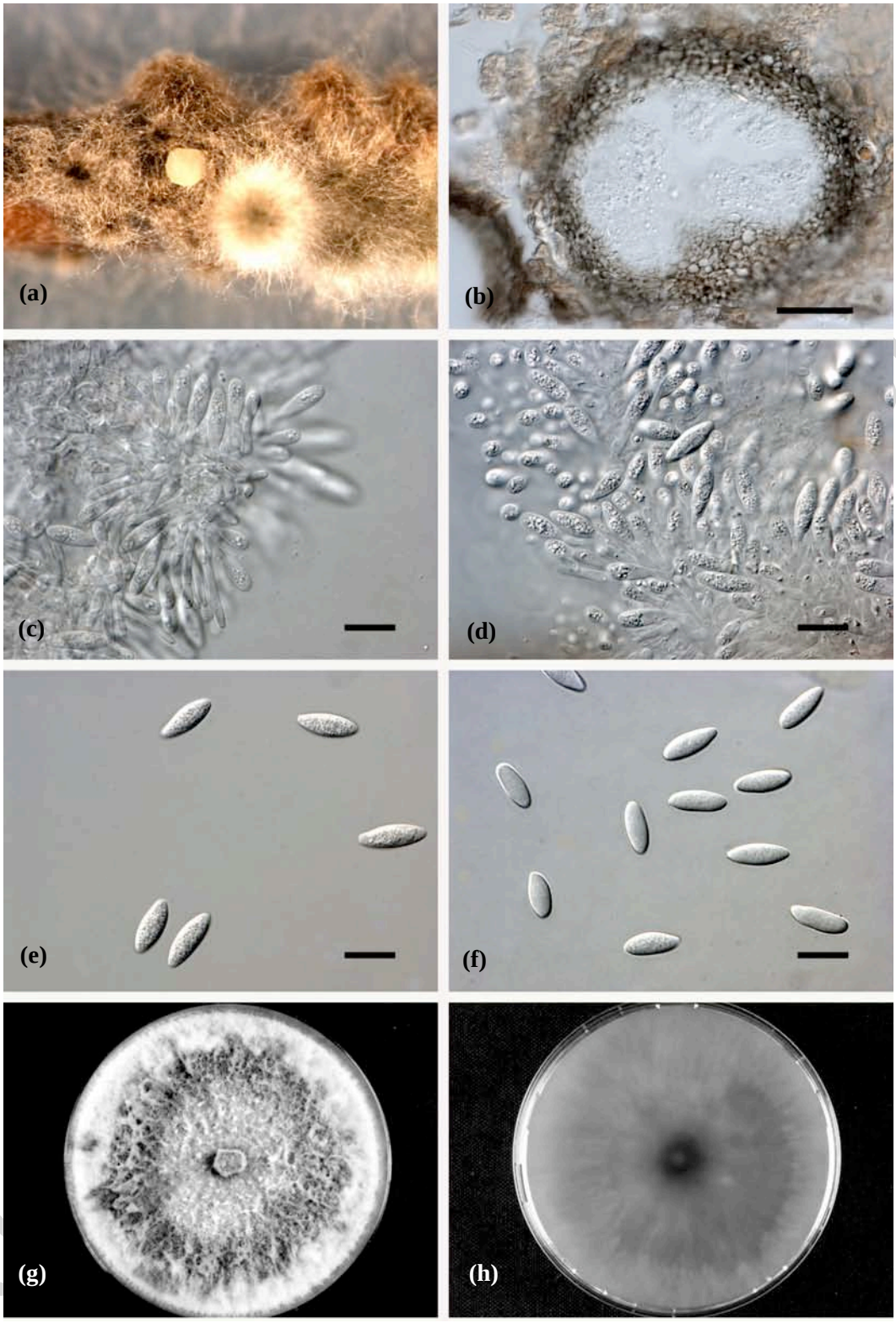

Fig. 3.

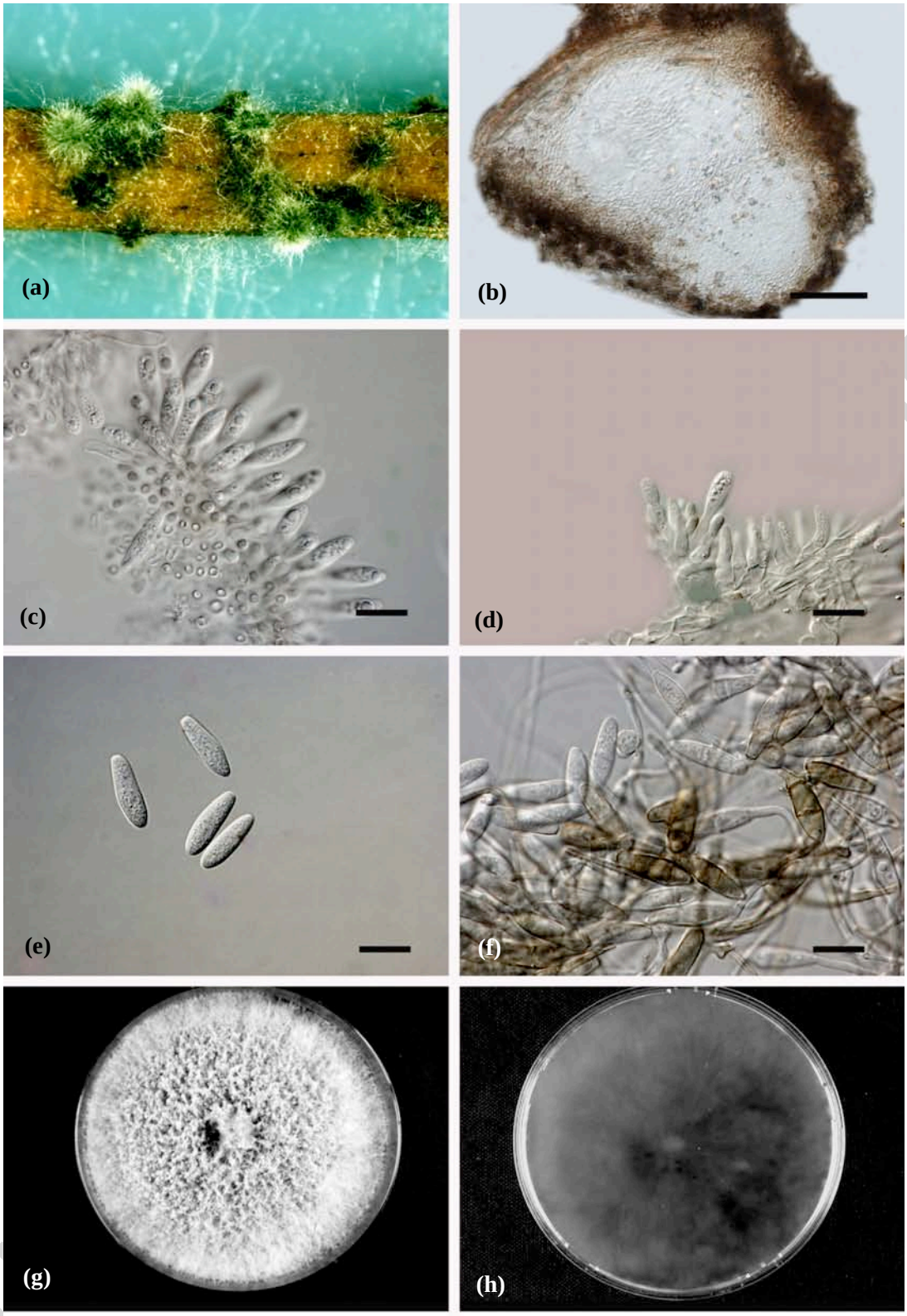

Fig. 4

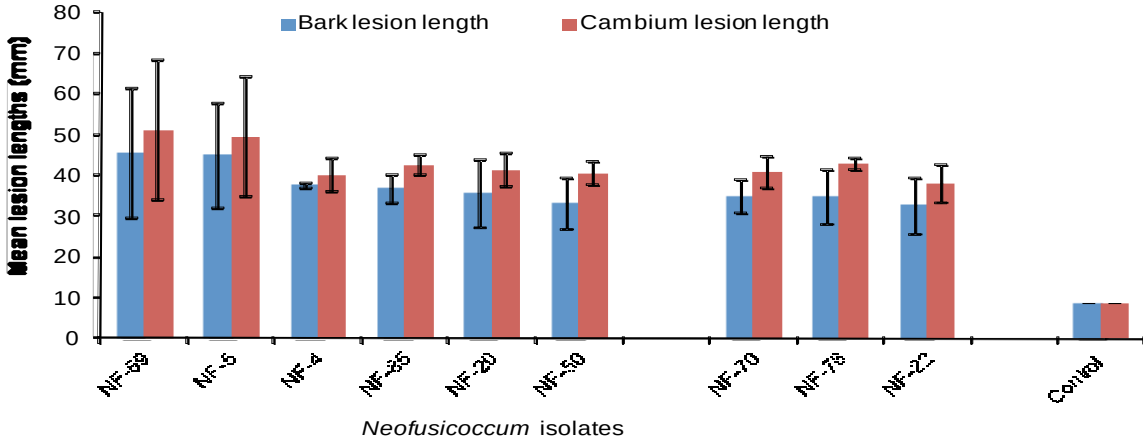

Fig. 5