

Comparative Analysis of Conventional Optimization Techniques with RSM and ANN Models for Extracting Oil from Sterculia urens Seeds for Biodiesel Applications

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1 **Novel Hypocrealean Fungi from Rhizophoraceae, *Neocosmospora mangrovei***
2 **sp. nov. and *Neocosmospora ceriopsis* sp. nov., and their Antifungal Activity**
3 **against Anthracnose Pathogen *Colletotrichum* spp.**

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14 **Abstract**

15 *Neocosmospora* is a fungus genus that contains saprobes, endophytes, and pathogens in the family
16 *Nectriaceae*. Prior to this study, 113 morphological species belonging to this genus have been
17 described and published. In this study, we reported two new species of fungus, *Neocosmospora*
18 *mangrovei* (MFLUCC 17-0253; MFLUCC 17-0257) and *N. ceriopsis* (MFLUCC 17-0259) that
19 were isolated from the mangrove habitats in the Southern part of Thailand. Phylogenetic analyses
20 of the combined ITS, EF-1 α , RPB2, and LSU regions showed that the new species clustered within
21 the *Neocosmospora* clade with strong statistical support. Detailed descriptions and illustrations,
22 pairwise homoplasy index (PHI) test, and a phylogenetic tree showing the position of both new
23 species are provided. In addition, both new species showed the potential to be used as biocontrol
24 agents against *Colletotrichum* spp. The *N. mangrovei*, MFLUCC 17-0253 exhibited strong
25 antifungal activity against *C. truncatum*, MFLUCC CG-0064 e.g., 100% of mycelium growth
26 inhibition, 90.83% inhibition of spore germination, and 100% reduction hyphae expansion. The *N.*
27 *ceriopsis* MFLUCC 17-0259 exhibited antifungal activity against *C. acutatum*, MFLUCC CC-0036
28 e.g., 69.39% of mycelium growth inhibition, 79.39% inhibition of spore germination, and 96.42%
29 reduction hyphae expansion. Overall, these findings suggested novel species of *Neocosmospora*
30 that had the capacity to produce various classes of biometabolites including 2,3-hydro-5-hydroxy-
31 8-methoxy-2,4-dimethylnaphthol-[1,2-*b*]-furan-6,9-dione, 2-methoxy-6-methyl-7-acetonyl-8-
32 hydroxy-1,4-naphthalenedione, 5,8-dihydroxy-7-acetonyl-1,4-naphthalenedione,
33 anhydrojavanicin, and fusarnaphthoquinones that can provide an antimicrobial activity that could
34 have the potential in playing a big role in treating anthracnose disease.

35 **Keywords**

36 New species, Mangrove fungi, Multi-locus phylogenetic analyses, Nectriaceae, Sordariomycetes,
37 Biological activity, Anthracnose disease

38 **Introduction**

39 *Neocosmospora*, typified by *N. vasinfecta*, was first introduced by Smith (1899) as the causal agent
40 of wilt disease in cotton, watermelon, and cowpea. Later, the pathogenic disease was identified as
41 *Fusarium neocosporiellum* (Geiser et al. 2013). Both genera, *Fusarium* and *Neocosmospora*, share

similar features including macroconidia, thus there are different opinions as to the taxonomic and phylogenetic distinctions between *Neocosmospora* and the *F. solani* species complex (FSSC) (O'Donnell 2000). Geiser et al. (2013) recognized the *Fusarium* link broadly and considered the *Neocosmospora* as part of the *F. solani* group. The same broad concept of *Fusarium* was adapted in recent works of O'Donnell et al. (2020) and Geiser et al. (2021). Nalim et al. (2011) also treated species in the *F. solani* group as members of *Neocosmospora*. However, later Lombard et al. (2015) justified the FSSC as seven separate genera, of which *Neocosmospora* is included. This distinct genus represents a morphologically aberrant lineage within the other groups in FSSC which was later also justified by reports from Zeng and Zhuang (2017), Sandoval-Denis et al. (2018), and Crous et al. (2021).

Neocosmospora constitutes 113 morphological species, 125 of which have molecular data. In general, this genus is superficial, solitary to gregarious, globose to pyriform, multiseptate, subcylindrical macroconidia slightly curved with the tips cell slightly hooked, thick-walled, and ornamented ascospores lacking germ pores (Pfenning 1995; Rossman et al. 1999; Lombard et al. 2015). Members of *Neocosmospora* are saprobes, endophytes, and pathogens in plant debris, and living plant material (Hirooka et al. 2012; Lombard et al. 2015; Sandoval-Denis et al. 2019). Although *Neocosmospora* is considered to be one of the major groups of plant pathogenic fungi, it is also recognized as a rich species diversity which to a great extent remained unstudied, especially as fungal endophytes.

The members of *Neocosmospora* are mainly documented from woody or herbaceous plants such as *Pistacia vera*, *Citrus sinensis*, *Camellia sinensis*, and *Morus alba* and the genus has a cosmopolitan distribution in tropical or subtropical regions (Guarnaccia et al. 2018 , 2021). More importantly, *Neocosmospora* has been used in several industrial applications, due to its ability to produce several enzymes such as chitosanases, cutinases, hydrolases, laccases, and lyases (Mannesse 1994; Longhi et al. 2000; Liu and Bao 2009; Wu and Nian 2014; Jallouli et al. 2015). These genera are also sources of cytotoxic compounds as well as secondary metabolites with antimicrobial activity (Merlin et al. 2013; Lee et al. 2014; Takemoto et al. 2014; Rathna et al. 2016; Chowdhury et al. 2017; Klomchit et al. 2021). Thus, one of the important issues that can be addressed is exploring the new endophytic fungi of this genus with antimicrobial activity against plant pathogens.

Anthracnose has been reported to be the most destructive disease in several horticulture crops. This disease is seed-borne, soil-borne, waterborne, and airborne. Many species of *Colletotrichum* cause anthracnose disease in chili, including *C. acutatum*, *C. truncatum*, *C. gloeosporioides*, *C. coccodes* (Simmonds 1965; Johnston and Jones 1997; Oh et al. 1999; Nirenberg et al. 2002; Voorips et al. 2004; Sharma et al. 2005; Than et al. 2008). The pathogen infects all chili's aerial parts, including fruit, leaf, and stem (Isaac 1991). This disease caused a major constraint on chili production reducing the marketable value of this fruit dramatically (Manandhar et al. 1995). There were various reports of 10-80% yield loss in several chili production countries, i.e., India, Vietnam, Korea, Malaysia, and Thailand (Alexander and Pernezny 2003; Ramachandran and Rathnamma 2006; Poonpolgul 2007; Kiran et al. 2020). In addition, this disease also affects the quality of seeds wherein the fungi could penetrate the seed coat and endosperm membrane (Manandhar et al. 1995). Since anthracnose causes extensive pre- and post-harvest damage to chili fruits, fungicides have been heavily applied to protect crops from this disease. The extensive use of chemicals can result in increased cost per crop of chili production. More importantly, it has a negative effect on the environment.

In this study, three new species of *Neocosmospora* were isolated from the mangrove habitats in Southern part of Thailand. Sequence analyses of the combined internal transcribed spacer (ITS), translation elongation factor 1- α (EF-1 α), RNA polymerase II second largest subunit (RPB2), and large subunit ribosomal DNA (LSU) regions were carried out. Morphological features and molecular data indicate that the two strains are new species of *Neocosmospora*. The distinctions between the new taxa and closely related taxa are discussed. Furthermore, we also include an evaluation of their antifungal activity against *Colletotrichum* spp. and secondary metabolites produced by *N. ceriopsis* MFLUCC17-0259.

Materials and Methods

Morphological examinations

The fungal specimens were received from Mae Fah Luang University Culture Collection (MFLUCC), Center of Excellence in Fungal Research, Chiang Rai, Thailand. The fungal colonies were characterized using potato dextrose agar (PDA) and Carnation Leaf-Piece Agar (CLA) (Fisher et al. 1982). Strains were incubated at 28 °C with alternating periods of light and darkness (12 hrs/12 hrs). Colony growth rates were measured after one week. Sporulation was observed and induced after two weeks by using a heat loop and incubated for one week. Morphological characteristics were determined from colonies grown on CLA and included size and shape of conidia, conidiogenous cells, and chlamydospores. Micromorphological features were photographed using a Motic SMZ 168 Series dissection microscope for fungal fruiting bodies on the woody substrate while microscopic characters were documented using Nikon Eclipse 80i microscope-camera system. Measurements of microscopic characters were calculated using Tarosoft Image Frame Work program (IFW) version 0.97 and all photographic plates were made using Adobe Photoshop CS6 version 13.12.

DNA extraction, Sequencing, and Phylogenetic analysis

The genomic DNA extraction was performed from fresh mycelium scraped out of the colony surface using the PureDireX Genomic DNA Isolation Kit (Plant), according to the manufacturer's instructions (BIO-HELIX, Keelung City, Taiwan). Four primer pairs were used for PCR amplification, including ITS4/ITS5 primers (internal transcribed spacer region of the ribosomal RNA gene (ITS); White et al. 1990), LROR/LR5 (large subunit ribosomal DNA of the 28S rDNA (LSU); Vilgalys and Hester 1990), EF1-728F/EF1-986 (translation elongation factor 1- α gene (EF-1 α); Carbone and Kohn 1993), and fRPB2-5F/ RPB2-7R (RNA polymerase II gene (RPB2); Liu et al. 1999). PCR purification and sequencing of amplified PCR products were carried out at Biogenomed Co., Ltd. using the primers mentioned above. The phylogenetic relationship between species was studied using a combined data set containing ITS, LSU, EF-1 α , and RPB2 sequences. Sequences were assembled using BioEdit and aligned with MAFFT v.7 on the online server (<https://mafft.cbrc.jp/alignment/server/>) (Katoh and Standley 2013). Aligned sequences were automatically trimmed with the gappyout method using TrimAl v. 1.3 on the web server (<http://phylemon.bioinfo.cipf.es/utilities.html> (accessed on May 20, 2022)). The online tool "ALTER" was used to convert the alignment file to phylip and nexus formats (Glez-Pena et al. 2010). Maximum likelihood analysis was performed with RAXML, and Bayesian inference analyses were done on the CIPRES Science Gateway platform (Miller et al. 2012). Parameters for maximum likelihood were set to rapid bootstrapping, and the analysis was carried out using 1000

replicates. Phylograms were visualized with FigTree v1.4.0 (available at <http://tree.bio.ed.ac.uk/software/figtree/>) and annotated in Microsoft PowerPoint (2010; Table 1).

Assess the antifungal activity against anthracnose disease in chili

Dual culture assay

Two pathogen strains, *C. truncatum*, MFLUCC CG-0064 and *C. acutatum*, MFLUCC CC-0036, which cause anthracnose disease, were kindly provided by Chia Tai Co., Ltd. (Bangkok, Thailand). The dual culture assay was used to screen three new endophytic fungi for antifungal activity against those two pathogenic fungi. Endophytes and pathogenic fungi were incubated on PDA at 28 °C for seven days. The 8-mm mycelium plug of growing endophytic fungi and pathogens was placed 3 cm apart on the PDA plate's surface. The PDA plate inoculated with only one pathogen was used as a control. The dual culture assay was also performed in a completely randomized design with a tri-replicate, and the assay was repeated independently three times. After ten days of incubation, the radial growths of pathogenic fungi were recorded. The radial (r) growths were converted into colony diameter (D) following equation:

$$\text{Diameter of fungal colony} = \pi r^2 \quad (1)$$

The inhibition percent of endophytic fungi to a pathogenic fungus was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{D1-D2}{D1} \times 100 \quad (2)$$

where D1 indicates the colony diameter of the pathogen in the control plate and D2 represents the colony diameter of the pathogen in the antagonistic assay.

Poisoned food assay

Since, two endophyte isolates, *N. mangrovei*, MFLUCC 17-0253 and *N. ceriopsis* MFLUCC 17-0259 exhibited antagonistic effects against *C. truncatum*, MFLUCC CG-0064, and *C. acutatum*, MFLUCC CC-0036, respectively. Thus, these two strains were further analyzed for antagonistic potential with a poisoned food assay. The liquid-liquid chemical extraction method using ethyl acetate was employed to obtain crude extract from the two fungal endophytes following the technique described in Brooks et al. (2022). Briefly, fungal endophytes were cultured in a PDA medium at 28 °C for seven days and mycelium plugs were inoculated into the PDB medium. After four weeks of incubation at 28 °C, the fermentation broth was collected by filtered culture broth through two layers of sterile sheet cloth. Ethyl acetate (equal volume) was added to the fermentation broth, and a separating funnel was used to separate organic layers containing the compounds. The same volume of ethyl acetate was added to the organic layers to obtain a crude extract. The broth was extracted again with ethyl acetate. Fungal crude extracts were dried using rotary evaporator under reduced pressure at 35-40 °C. Dry fungal crude extracts dissolved in methanol were mixed in PDA plates with a final concentration of 0.6 and 0.9 mg/mL.

Mycelial plugs (7 mm diameter) of actively growing pathogenic fungi were placed in the center of prepared PDA plates. After incubating the plate at 28 °C for ten days, a digital caliper was used to measure pathogenic fungi' radial (r) growths. Sterilized water and methanol were used

as negative controls. As Captan showed the highest activity compared with the other fungicide (Carbendazim, Copper hydroxide, Chlorothalonil, and Mancozeb) from pre-screen for antagonistic activity against both pathogenic strains. Captan (0.6 mg/mL) was used as a positive control. Similar to dual culture assay, the radial growths were recorded after incubation for ten days and converted into colony diameter (D). The inhibition percentage was calculated using the equation (2). The experiments were performed in triplicate with three plates for each replicate, and was repeated three times. Statistical analyses were performed using Duncan's multiple range test (DMRT) and the SPSS V16.0 statistical package (SPSS Inc.; Chicago, USA) software program.

Inhibition of spore germination, inhibition of hypha expansion, and disease severity assessment

The spore of pathogenic fungi *C. truncatum*, MFLUCC CG-0064 and *C. acutatum*, MFLUCC CC-0036 were induced by incubation in 1/10 PDA for 25 and 10 days, respectively. Spore solutions were prepared and adjusted to 1×10^6 spores/mL followed the method described in Brooks et al. (2022). The 1 μ L of fungal crude extract (90 mg/mL) was added to 9 μ L of spore solution of pathogenic fungi. While sterile water and Captan were used as a negative and positive control, respectively. The germinated and ungerminated spores were counted under a light microscope after incubating the spore mixture in the dark for 24 hrs. The 20 μ L of the spore mixture with a final concentration of fungal crude extract as 0.9, 2.7, and 5.4 mg/mL was dropped on disinfected chili leaves. Inoculated leaves were placed in plastic containers with moist paper towels for five days. Leaves were cut and stained with Coomassie Brilliant Blue mixture following methods described in Brooks et al. (2022). A light microscope was used to observe hyphal coverage on the plant material. Inhibition of spore germination and inhibition of hypha expansion was calculated using the formula (2).

The experiment was performed in Randomized Completely Block Design (RCBD) with three replications (10 leaves/replication), and the experiments were repeated four times. Statistical analyses were performed using Duncan's multiple range test (DMRT) and the SPSS V16.0 statistical package (SPSS Inc.; Chicago, USA) software program.

Chemical analysis

The fungal extract was subjected to column chromatography (CC) over silica gel (0-20 μ m) eluting with EtOAc/hexane (3:7 v/v) to give two subfractions (A-B). Subfraction A (28.4 mg) was subjected to CC over Sephadex LH-20 (100% MeOH) to give two fractions; compound **1** (11FU-259, 3.2 mg) and compound **2** (12FU-259, 3.7 mg). Subfraction B was also purified by Sephadex LH-20 CC (25-100 μ m, 13 $\times$ 50 cm) using 100% MeOH and then follow by repeated silica gel CC (60-200 μ m, 12 $\times$ 50 cm) using EtOAc/hexane (3:7 v/v) to afford compound **3** (2FU-259, 2.7 mg). The disc diffusion assay was used to determine the antifungal activity of those three compounds. The pathogenic fungus *C. truncatum*, MFLUCC CG-0064 and *C. acutatum*, MFLUCC CC-0036 was cultured in V8 media (200 mL V8 (V8 @, Campbell's), 20 g agar, 3 g CaCO₃) for 22 days to induce sporulation. Spores were washed off from the culture plate using sterile water with 20% (v/v) tween 20. The spore solution (1×10^6 spores/mL) was spread on the dried surface of a PDA plate and air-dried under sterile conditions for 15 min. The mixture of compound: methanol (0.5 mg:5 μ L) was impregnated on sterile filter paper discs (6 mm diameter), then the discs were left to air dry under a laminar hood for 30 mins. Negative and positive controls were sterile water, methanol, and Captan (0.1 mg:5 μ L), respectively. Discs were then placed approximately 2 cm

from the edges of each plate with a total of five discs per plate. All plates were incubated at 28 °C for 7 to 10 days before observing the inhibition zones.

Since only compound 2 (12FU-259) shown antibacterial activity against *C. acutatum*, MFLUCC CC-0036. The NMR spectra of compound 2 were recorded on spectrometer Bruker Avance AV-500 and 600. Chemical shifts were expressed as δ values (ppm), coupling constant (*J*) in Hz, and TMS as internal standard (measured in CDCl₃).

Results

Phylogenetic analyses

The combined LSU, ITS, *tef1- α* , and *rpb2* dataset comprised of 201 taxa from *Nectriaceae*, with *Geejayessia* [*G. atrofusca* (CBS 125482; NRRL 22316), *G. celtidicola* (CBS 125502), *G. cicatricum* (CBS 125550), *G. cicatricum* (CBS 125552)] as outgroup taxa (Table 1). The analyzed dataset, after trimming, comprised a total 3,163 characters including gaps (LSU = 815 bp, ITS = 1066 bp, *TEF1- α* = 602 bp, *RPB2* = 680 bp) with 1,407 distinct alignment patterns and 32.60% proportion of gaps and completely undetermined characters. The ML analysis for the combined dataset provided the best scoring tree (Fig. 1) with a final ML optimization likelihood value of -33795.848267 (ln). Parameters for the GTR+I+G model of the combined LSU, ITS, *tef1- α* , and *rpb2* dataset are as follows: estimated base frequencies; A = 0.241529, C = 0.269280, G = 0.265706, T = 0.223486; substitution rates AC = 1.650028, AG = 3.631450, AT = 1.934861, CG = 0.900810, CT = 7.653185, GT = 1.000000; gamma distribution shape parameter α = 0.276755. The Bayesian analysis indicated the average standard deviation of split frequencies at the end of total MCMC generations is 0.009230. Phylogenetic analyses of the combined data matrix resulted in well-resolved clades (Fig. 1). The tree topologies resulting from maximum likelihood (ML) and Bayesian posterior probabilities (BYPP) analyses were congruent.

In the phylogenetic analyses (Fig. 1), two novel species, *N. mangrovei* (MFLUCC 17-0253, MFLUCC 17-0257) and *N. ceriopsis* (MFLUCC 17-0259), were sister taxa with good bootstrap support (83% ML, 1.00 BYPP), and closely related to *N. macrospora* and *N. ferruginea* (73% MP, 1.00 BYPP). GCSPP result of multi-locus gene of *N. ceriopsis* and *N. mangrovei*, and related species shows no significant recombination happened (Fig. 2).

[Insert Figs.1 & 2]

Taxonomy

Sordariomycetes O.E. Erikss. & Winka, Myconet 1(1): 10 (1997)

Hypocreales Lindau, in Engler & Prantl, Nat. Pflanzenfam., Teil. I (Leipzig) 1(1): 343 (1897)

Nectriaceae Tul. & C. Tul. [as 'Nectriei'], Select. Fung. Carpol. (Paris) 3: 3 (1865)

Neocosmospora E.F. Sm., Bull. U.S. Department of Agriculture 17: 45 (1899)

Type species: *Neocosmospora vasinfecta* E.F. Sm.

***Neocosmospora ceriopsis* Klotzsch, M.S. Calabon, S. Brooks sp. nov.** (Fig. 3)

Index Fungorum number: IF**;
Faces of Fungi number: FoF 12541

Etymology: The epithet reflects the genus name of the host plant *Ceriops decandra*, from which the specimens were collected.

Holotype: MFLU 22-****

Colonies growing on PDA, reaching 40-55 mm diam in 14 d at 24 °C, colony circular, white, flat, entire, velvety to cottony, with or without white to straw concentric rings; reverse white to pale straw.

Conidiophores on aerial mycelium straight, smooth, and thin-walled, simple or branched several times verticillately, bearing terminal or lateral, single monophialides; phialides $35.0\text{--}73 \times 2.2\text{--}4.5 \mu\text{m}$ ($\bar{x} = 52.5 \times 3.0 \mu\text{m}$, $n = 50$) subulate, subcylindrical, to acicular, smooth- and thin-walled, conidiogenous loci with inconspicuous periclinal thickening and non-flared, minute collarettes; aerial conidia of two types: *microconidia* $6.60\text{--}14.5 \times 2.7\text{--}5.5 \mu\text{m}$ ($\bar{x} = 10.4 \times 4.1 \mu\text{m}$, $n = 50$) oval, obovoidal to ellipsoidal, mostly straight to slightly curved, often with a flattened base, aseptate, hyaline, smooth- and thin-walled, clustering in false heads at tip of monophialides; *macroconidia* up to $55 \mu\text{m}$, falcate, rarely straight with moderately curved ends, tapering toward base, septation sometimes indistinct or with thin transverse septa; apical cell of equal length or slightly longer than adjacent cell, blunt to slightly hooked with rounded apex; basal cell inconspicuously to moderately notched, 4-septate, hyaline, smooth- and thick-walled. *Sporodochia* not observed. *Chlamydospores* $9.0\text{--}21.5 \times 5.5\text{--}10 \mu\text{m}$ ($\bar{x} = 13.7 \times 7.7 \mu\text{m}$, $n = 30$) abundantly formed, globose to subglobose, smooth- to verruculose and thick-walled, terminal or intercalary in hyphae or conidia, solitary, in chains or in clusters.

Material examined: THAILAND, Ranong Province, The Ranong Biosphere Reserve, on branches of *Ceriops decandra*, 6 December 2016, Chada Norphanphoun, NG03b (MFLU 22-****, holotype), ex-type living culture MFLUCC 17-0259.

GenBank accession numbers: LSU = ON833276, ITS = MT928794, TEF1- α = **

Notes: In the present study, MFLUCC 17-0253 and MFLUCC 17-0257, showed no morphological distinctions, and pairwise nucleotide comparisons of ITS sequence data showed no nucleotide differences but 8 bp differences (1.29%, 622 bp) in TEF1- α sequence data. This indicates that the two isolates are strains of *N. ceriopsis* (Jeewon and Hyde 2016). The multi-locus phylogenetic analysis shows that *N. ceriopsis* is sister taxon to *N. mangrovei*, with high bootstrap support (83% ML, 1.00 BYPP) (Fig. 1). In pairwise nucleotide comparisons of *N. mangrovei* with the sister taxon *N. ceriopsis*, there is a nucleotide difference of 2.81% (14 bp) and 2.32% (14 bp) in ITS (of 499 nucleotides altogether) and TEF1- α (of 603 nucleotides altogether), respectively. Morphologically, *Neocosmospora ceriopsis* has a larger microconidium ($6.60\text{--}14.5 \times 2.7\text{--}5.5 \mu\text{m}$ vs. $4.5\text{--}8.0 \times 1.5\text{--}3.5 \mu\text{m}$) than *N. mangrovei*. *Neocosmospora ceriopsis* also shared the same subclade with *N. macrospora* and *N. ferruginea* (73% MP, 1.00 BYPP). Morphologically, *N. ceriopsis* is distinct from *N. macrospora* by having longer phialides ($35.0\text{--}73 \times 2.2\text{--}4.5 \mu\text{m}$ vs. $19\text{--}67 \times 2\text{--}5 \mu\text{m}$) and larger chlamydospores ($9.0\text{--}21.5 \times 5.5\text{--}10 \mu\text{m}$ vs. $5\text{--}8.5 \times 4.5\text{--}8 \mu\text{m}$) (Sandoval-Denis et al. 2018). *Neocosmospora ceriopsis* can be distinguished from *N. ferruginea* by the size of phialides [$35.0\text{--}73 \times 2.2\text{--}4.5 \mu\text{m}$ vs. $(29.5\text{--})31\text{--}40\text{--}(45) \times 2.5\text{--}3.5 \mu\text{m}$], conidia [$6.60\text{--}14.5 \times 2.7\text{--}5.5 \mu\text{m}$ vs. $(4\text{--})4.5\text{--}11\text{--}(20) \times (1.5\text{--})2\text{--}4\text{--}(60) \mu\text{m}$], and chlamydospores ($9.0\text{--}21.5 \times 5.5\text{--}10 \mu\text{m}$ vs. $8\text{--}10 \mu\text{m}$ diam) (Sandoval-Denis et al. 2019).

[Insert Fig. 3]

***Neocosmospora mangrovei* Klomchit; M.S. Calabon; S. Brooks sp. nov.** (Fig. 4)

Index Fungorum number: IF**; Faces of Fungi number: FoF 12542

Etymology: Named after the fungal habitat from mangrove habitat, where the fungus found.

Holotype: MFLU 22-****

Colonies growing on PDA, reaching 40-55 mm diam in 14 days at 24 °C, colony circular, white, flat, entire to filiform, velvety; reverse white to pale straw.

Conidiophores on aerial mycelium straight, smooth, and thin-walled, simple, terminal, single monophialides; *phialides* $36.5\text{--}74.0 \times 2.5\text{--}5.5 \mu\text{m}$ ($\bar{x} = 60.8 \times 3.8 \mu\text{m}$, $n = 30$), subulate to acicular, smooth and thin-walled, conidiogenous loci with inconspicuous periclinal thickening and non-flared, minute collarettes; aerial conidia of two types: *microconidia* $4.5\text{--}8.0 \times 1.5\text{--}3.5 \mu\text{m}$ ($\bar{x} = 6.3 \times 2.7 \mu\text{m}$, $n = 50$), subglobose, mostly ellipsoidal, straight to slightly curved, often with a flattened base, aseptate, hyaline, smooth- and thin-walled, clustering in false heads at tip of monophialides; *macroconidia* $39\text{--}45 \times 4.5\text{--}6.0 \mu\text{m}$ ($\bar{x} = 42.8 \times 5.2 \mu\text{m}$, $n = 10$), falcate, straight to slightly dorsiventrally curved, apical cell blunt and slightly curved; basal cell inconspicuously to moderately notched, 4-5-septate, predominantly 4-septate, hyaline, smooth- and thick-walled. *Sporodochia* not observed. *Chlamydospores* $11\text{--}20 \times 5\text{--}10 \mu\text{m}$ ($\bar{x} = 15.6 \times 7.4 \mu\text{m}$, $n = 50$), abundantly formed, globose to subglobose, smooth- to verruculose and thick-walled, terminal or intercalary in hyphae or conidia, solitary or in chains.

Material examined: THAILAND, Ranong Province, The Ranong Biosphere Reserve, on branches of *Rhizophora apiculata* and *R. mucronata*, 7 December 2016, Chada Norphanphoun, NG27b (MFLU 22-****, holotype), ex-type living culture MFLUCC 17-0253; NG37b (MFLU 22-****, paratype), ex-paratype living culture MFLUCC 17-0257.

GenBank accession numbers: MFLUCC 17-0253: LSU = **, ITS = MT928792, TEF1- α = **, MFLUCC 17-0257: LSU = ON833274, ON833275; ITS = MT928793; TEF1- α = **, RPB2 = **

Notes: The combined ITS-LSU-RPB2-TEF1- α sequence data showed that *Neocosmospora mangrovei* is sister taxon to *N. ceriopsis* with strong statistical support. *Neocosmospora mangrovei* also shared the same subclade with *N. macrospora* and *N. ferruginea* (73% MP, 1.00 BYPP). Morphologically, *N. mangrovei* is distinct from *N. macrospora* by having longer phialides ($36.5\text{--}74.0 \times 2.5\text{--}5.5 \mu\text{m}$ vs. $19\text{--}67 \times 2\text{--}5 \mu\text{m}$) and larger chlamydospores ($11\text{--}20 \times 5\text{--}10 \mu\text{m}$ vs. $5\text{--}8.5 \times 4.5\text{--}8 \mu\text{m}$) (Sandoval-Denis et al. 2018). *Neocosmospora mangrovei* can be distinguished from *N. ferruginea* by the size of phialides [$36.5\text{--}74.0 \times 2.5\text{--}5.5 \mu\text{m}$ vs. $(29.5\text{--})31\text{--}40\text{--}(45) \times 2.5\text{--}3.5 \mu\text{m}$], microconidia [$4.5\text{--}8.0 \times 1.5\text{--}3.5 \mu\text{m}$ vs. $(4\text{--})4.5\text{--}11\text{--}(20) \times (1.5\text{--})2\text{--}4\text{--}(6) \mu\text{m}$], and chlamydospores ($11\text{--}20 \times 5\text{--}10 \mu\text{m}$ vs. $8\text{--}10 \mu\text{m}$ diam) (Sandoval-Denis et al. 2019).

[Insert Fig.4]

Antagonistic activity against anthracnose disease

Among the three new strains, *N. mangrovei* MFLUCC 17-0253 and *N. ceriopsis* MFLUCC 17-0259 exhibited antifungal activity against fungi *C. truncatum*, MFLUCC CG-0064 and *C. acutatum*, MFLUCC CC-0036, respectively. Based on the dual culture assay, *N. ceriopsis* MFLUCC 17-0259 presented slightly higher activity against *C. acutatum*, MFLUCC CC-0036 ($46.5 \pm 7.47\%$) compared to *N. mangrovei* MFLUCC 17-0253 with fungi *C. truncatum*, MFLUCC CG-0064 ($21\text{--}25 \pm 6.22\%$).

In contrast, to the dual culture assay, the poisoned food assay, spore germination, and leave inoculation demonstrated that at the same concentration fungal crude extract from *N. mangrovei* MFLUCC 17-0253 is potent toward fungi *C. truncatum*, MFLUCC CG-0064 more than fungal crude extract of *N. ceriopsis* MFLUCC 17-0259 against *C. acutatum*, MFLUCC CC-0036 (Table

2; Fig. 5). For instance, fungal crude extract from *N. mangrovei* MFLUCC 17-0253 at a concentration of 0.9 mg/mL and 2.7 mg/mL was able to completely inhibit the growth of fungi *C. truncatum*, MFLUCC CG-0064 in poisoned food assay and leave inoculation, respectively (Fig. 5). Even though fungal crude extract of *N. ceriopsis* MFLUCC 17-0259 did not present vigorous activity toward pathogen as presented in a fungal crude extract of *N. mangrovei* MFLUCC 17-0253 but with slightly higher concentration, it could effectively inhibit spore germination and hypha growth of *C. acutatum*, MFLUCC CC-0036. For instance, inhibition of hypha expansion on chili leaves after incubation with 2.7 mg/mL of fungal crude extract from *N. ceriopsis* MFLUCC 17-0259 was high as 92.82% (Fig 6; Table 2). Compared to a commercially available fungicide used in this study, fungal crude extract of *N. mangrovei* MFLUCC 17-0253 exhibited more potent antifungal activity throughout the in-vitro assays and in-vivo assay, while the fungal crude extract's antifungal activity of fungal crude extract of *N. ceriopsis* MFLUCC 17-0259 was slightly lower than Captan (Table 2).

[Insert Figs. 5 & 6]

Antifungal compounds

The crude extract of *N. ceriopsis* MFLUCC 17-0259 was further investigated to identify secondary metabolites produced from this fungus and responsible for this antifungal activity. Fungal extraction (2.0 g) from *N. ceriopsis* MFLUCC 17-0259 was subjected to column chromatography (CC) to yield three subfractions (A-C), and Sephadex LH-20 CC purified all subfractions to afford compound 1-3 (2FU-259, 11FU-259, 12FU-259). Three compounds were subject to disc diffusion assay, and only one compound presented an inhibition zone (Fig. 7a), and only one compound exhibited antifungal activity toward anthracnose pathogen. Structure elucidation by NMR revealed the structure of the compound that exhibited antifungal activity toward anthracnose pathogen as 2,3-hydro-5-hydroxy-8-methoxy-2,4-dimethylnaphthol-[1,2-*b*]-furan-6,9-dione (Fig. 7b).

[Insert Fig. 7]

Discussion

The present study dealt with identifying *Neocosmospora* species from the Southern part of Thailand based on morphological and phylogenetic data. The combined sequence analysis of ITS, LSU, EF-1 α , and RPB2 regions and 201 additional taxa of *Neocosmospora* together with morphology confirm the placement of new species in *Neocosmospora*. *N. mangrovei* (MFLUCC 17-0253, MFLUCC 17-0257) and *N. ceriopsis* (MFLUCC 17-0259) are phylogenetically related to *N. Macrospora* (Fig. 1), see *Notes* for morphological differences. These two novel species also add to the increasing number of *Neocosmospora* from mangrove plants wherein Dayarathne et al. (2020) introduced *N. rhizophorae* isolated from *Rhizophora* sp. in Thailand, *N. bostrycoides* reported by Lee and Baker (1973) from *R. mangle*. *Neocosmospora* species are inhabitants of mangrove soil also (Sarma and Vittal 2007; Behera et al. 2012).

Despite the difference in morphological and genetically between *Neocosmospora* and *Fusarium* genus; however, biochemical similarity within both genera was observed. This study revealed the structure of the secondary metabolite, 2,3-hydro-5-hydroxy-8-methoxy-2,4-dimethylnaphthol-[1,2-*b*]-furan-6,9-dione, which is produced by *N. ceriopsis* MFLUCC 17-0259. Interestingly, this compound was the first reported as a secondary metabolized produced by *F.*

solani (Tatum et al. 1989). Similarity, four secondary metabolized produced by *N. mangrovei* MFLUCC 17-0253 were previously reported by Klomchit et al. (2021) which are 2-methoxy-6-methyl-7-acetonyl-8-hydroxy-1,4-naphthalenedione, 5,8-dihydroxy-7-acetonyl-1,4-naphthalenedione, anhydrojavanicin, and fusarnaphthoquinones. Those four compounds were also previously isolated from *Fusarium* spp. (Kimura et al. 1988; Trisuwan et al. 2010). This could be due to, the *Neocosmospora* might still carry the same gene cluster that displays a role in the biosynthesis of secondary metabolites in *Fusarium* genus. Interestingly, the known *Fusarium* produced mycotoxins compounds, Deoxynivalenol and Zearalenone were not produced by *N. mangrovei* MFLUCC 17-0253. These data suggested that in *Fusarium* and *Neocosmospora* genera, the diversity of housekeeping genes is higher than core biosynthetic genes. Contradictory, the inter and intraspecific diversity of the core biosynthetic genes within *Fusarium* genus is consistently higher than that of housekeeping genes (Stępień 2014).

The discovery of *Neocosmospora* sp., MFLUCC 17-0253 was crucial for cucurbit industry because of their antibacterial activity against *Acidovorax citrulli* (Aac), JT-003 which up to date there still has no appropriate method to control this devastating disease (Klomchit et al. 2021). More importantly, Klomchit et al. (2021) reported that the *Neocosmospora* sp., MFLUCC 17-0253 did not cause any disease in the plant host even when the plant host is under stress conditions. This study provided proof that this novel species was lack of mycotoxins that typically produces by *Fusarium* species (Stępień 2014). The mycotoxin such as trichothecenes, fumonisins, and zearalenone play major roles in the biological process, especially in infecting plant hosts (Chelkowski 1998). Thus, lacking important mycotoxin results in loss of pathogenicity toward the plant host. The current study also revealed the strong antifungal activity of *Neocosmospora* sp., MFLUCC 17-0253 toward pathogenetic fungus (*C. truncatum*, MFLUCC CG-0064) causing anthracnose in the chili. This exhibited a broad antimicrobial spectrum of this novel strain and its benefits in sustainability agriculture. The other new species, *N. ceriopsis* MFLUCC 17-0259 also presents antagonistic activity toward the other strain of *Colletotrichum* sp. Previously, the compound 2,3-hydro-5-hydroxy-8-methoxy-2,4-dimethylnaphthol-[1,2-*b*]-furan-6,9-dione was an antifungal compound against the tea pathogenic fungus *Pestalotiopsis theae*. This compound was tested for the downregulation of *P. theae* HMG-CoA synthase gene expression by real-time polymerase chain reaction (RT-PCR). As a result, these compounds showed a significant effect while exhibiting a moderate effect (Alex et al. 2000; Niu et al. 2019).

There are still ongoing studies on the diversity within *Fusarium* population as well as *Neocosmospora* population, our study was able to prove the differentiation between both species in various aspects. More importantly, the new species of *Neocosmospora* in this study did not pose pathogenicity toward the plant host. In contrast, these new species produced secondary metabolites that were potent towards major plant diseases. Thus, it is important to explore new species in this complex genus, so they can be evaluated for their potential as a new product that will help address world sustainable agriculture.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Competing interests: The authors declare no competing interests

Availability of data and materials

All data generated or analyzed in this study are included in this article. All sequences generated here are available from GenBank and given in the “Taxonomy” section.

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by A. Klomchit, MS. Calabon and S. Worabandit. Morphological characterisations, molecular and phylogenetic constructions were performed by A. Klomchit and MS. Calabon. The first draft of the manuscript was written by S. Brooks and all authors commented

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Table 1. The accession numbers of fungal isolations used in this study.

Species name	Strain ¹	GenBank accession number			
		EF-1 α	ITS	LSU	rpb2
<i>Cosmospora cavisperma</i>	CBS 172.31 ^{ET}	–	MW827606	MW827645	MW834000
<i>Cyanonectria buxi</i>	CBS 125551 ^{ET}	KM231939	MH863556	MH875034	MW834001
<i>C. cyanostoma</i>	CBS 101734 ^{ET}	HM626647	FJ474076	MH874353	MW834002
<i>Fusarium agapanthi</i>	NRRL 54463 ^T	KU900630	–	–	KU900625
<i>F. ananatum</i>	CBS 118516 ^T	LT996091	–	–	LT996137
<i>F. buharicum</i>	CBS 796.70	–	U34581	U34552	JX171563
<i>F. bulbicola</i>	CBS 220.76 ^T	KF466415	–	–	KF466404
<i>F. denticulatum</i>	CBS 735.97	AF160269	–	–	LT996143
<i>F. fujikuroi</i>	CBS 221.76 ^T	AF160279	MW827608	MW827648	MW834005

Species name	Strain ¹	GenBank accession number			
		EF-1 α	ITS	LSU	rpb2
<i>F. globosum</i>	CBS 428.97 ^T	KF466417	–	–	KF466406
<i>F. mexicanum</i>	NRRL 47473	GU737416	–	–	LR792615
<i>F. poae</i>	NRRL 13714	–	–	–	JX171572
<i>F. sarcochroum</i>	CBS 745.79	MW834278	MW827611	MW827651	JX171586
<i>F. scirpi</i>	NRRL 13402	GQ505592	GQ505681	–	JX171566
<i>F. tjaetaba</i>	CBS 144400 ^T	KP083263	–	–	KP083275
<i>F. torreyae</i>	CBS 133858 ^T	HM068337	HM068344	MW827652	JX171660
<i>F. tricinctum</i>	CBS 393.93 ^{ET}	AB674263	HM068317	–	JX171629
<i>F. tupiense</i>	NRRL 53984	GU737404	–	–	LR792619
<i>Geejayessia atrofusca</i>	CBS 125482	MW834282	MH863592	MH875066	HQ897775
<i>G. atrofusca</i>	NRRL 22316	AF178361	AF178423	AF178392	EU329502
<i>G. celtidicola</i>	CBS 125502 ^T	HM626638	HM626657	HM626669	MW834013
<i>G. cicatricum</i>	CBS 125550	HM626642	HM626654	HM626666	HQ897697
<i>G. cicatricum</i>	CBS 125552	HM626644	HQ728145	MH875038	HQ728153
<i>Luteonectria albida</i>	CBS 102683	MW834283	MW827615	MH874402	MW834016
<i>L. nematophila</i>	NRRL 54600	–	JABFFA010000104	JABFFA010000104	JX171664
<i>Neocosmospora acutispora</i>	CBS 145461 ^T	LR583593	LR583700	LR583908	LR583814
<i>N. addoensis</i>	CBS 146509	MW248740	MW173041	MW173032	MW446574
<i>N. addoensis</i>	CBS 146510 ^T	MW248741	MW173042	MW173033	MW446575
<i>N. ambrosia</i>	CBS 571.94 ^{ET}	FJ240350	EU329669	–	EU329503
<i>N. ambrosia</i>	NRRL 20438	–	AF178397	AF178366	JX171584
<i>N. ampla</i>	CBS 202.32 ^T	LR583594	LR583701	LR583909	LR583815
<i>N. bataticola</i>	CBS 144397	AF178343	AF178407	AF178376	EU329509
<i>N. bataticola</i>	CBS 144398 ^T	AF178344	AF178408	AF178377	FJ240381
<i>N. borneensis</i>	CBS 145462 ^{ET}	AF178352	AF178415	AF178384	EU329515
<i>N. bostrycoides</i>	CBS 144.25 ^{NT}	LR583597	LR583704	LR583912	LR583818
<i>N. bostrycoides</i>	CBS 392.66	LR583598	LR583705	LR583913	LR583819
<i>N. brevicona</i>	CBS 204.31 ^{ET}	LR583600	LR583707	LR583915	LR583821
<i>N. brevis</i>	CBS 130326	DQ246869	DQ094351	DQ236393	EF470136
<i>N. catenata</i>	CBS 143228	KC808213	KC808255	–	KC808354
<i>N. catenata</i>	CBS 143229 ^T	KC808214	KC808256	–	KC808355
<i>N. ceriopsis</i>	MFLUCC 17–0259	**	MT928794	ON833276	**
<i>N. citricola</i>	CBS 146512	MW248746	MW173047	MW173035	MW446580
<i>N. citricola</i>	CBS 146513 ^T	MW248747	MW173048	MW173036	MW446581
<i>N. crassa</i>	CBS 144386 ^T	LR583604	LR583709	LR583917	LR583823
<i>N. cryptoseptata</i>	CBS 145463 ^T	AF178351	AF178414	AF178383	EU329510
<i>N. cucurbitae</i>	CBS 410.62	DQ247640	LR583710	LR583918	LR583824
<i>N. cucurbitae</i>	CBS 616.66 ^T	DQ247592	LR583711	LR583919	LR583825
<i>N. cyanescens</i>	CBS 518.82 ^T	LR583605	AB190389	LR583920	LR583826
<i>N. cyanescens</i>	CBS 637.82	LR583606	LR583712	LR583921	LR583827
<i>N. diminuta</i>	CBS 144390 ^T	LR583607	LR583713	LR583922	LR583828

Species name	Strain ¹	GenBank accession number			
		EF-1 α	ITS	LSU	rpb2
<i>N. elegans</i>	CBS 144395	AF178328	AF178394	AF178363	EU329496
<i>N. elegans</i>	CBS 144396 ^{ET}	AF178336	AF178401	AF178370	FJ240380
<i>N. epipeda</i>	CBS 146523 ^T	MW834285	MW827624	MW827665	MW834022
<i>N. epipeda</i>	CBS 146524	MW834286	MW827625	MW827666	MW834023
<i>N. euwallaceae</i>	CBS 135854 ^T	JQ038007	JQ038014	–	JQ038028
<i>N. falciformis</i>	CBS 121450	JX435161	JX435211	–	JX435261
<i>N. falciformis</i>	CBS 475.67 ^T	LT906669	MG189935	NG_055724	LT960558
<i>N. falciformis</i>	NRRL 43529	EF452965	EF453117	–	JX171653
<i>N. ferruginea</i>	CBS 109028 ^T	DQ246979	DQ094446	DQ236488	EU329581
<i>N. ferruginea</i>	CPC 28194	LR583602	LT746276	–	LT746341
<i>N. floridana</i>	NRRL 62628 ^T	KC691535	KC691563	–	KC691624
<i>N. gamsii</i>	CBS 143207 ^T	DQ247103	DQ094420	DQ236462	EU329622
<i>N. gamsii</i>	CBS 143211	DQ246951	DQ094563	DQ236605	EU329576
<i>N. gamtoosensis</i>	CBS 146502 ^T	MW248762	MW173063	MW173038	MW446611
<i>N. haematococca</i>	CBS 119600 ^{ET}	DQ247510	KM231797	KM231664	LT960561
<i>N. hengyangensis</i>	HMAS 254518 ^T	KY829448	KY829446	–	–
<i>N. hypothenemi</i>	CBS 145464 ^T	JF740850	LR583715	LR583923	JF741176
<i>N. hypothenemi</i>	CBS 145466	MW834287	MW827626	MW827667	MW834024
<i>N. illudens</i>	CBS 147303	AF178326	AF178393	AF178362	JX171601
<i>N. ipomoeae</i>	CBS 353.87	DQ247639	LR583717	LR583925	LR583831
<i>N. ipomoeae</i>	CBS 833.97	LR583611	LR583719	LR583927	LR583833
<i>N. keleraja</i>	CBS 125720 ^{PT}	LR583612	LR583720	LR583928	LR583834
<i>N. keleraja</i>	CBS 125722 ^{PT}	DQ247515	JF433039		LR583835
<i>N. keratoplastica</i>	CBS 144389	LR583613	LR583722	LR583930	LR583836
<i>N. keratoplastica</i>	CBS 490.63 ^T	LT906670	LR583721	LR583929	LT960562
<i>N. kuroshio</i>	CBS 142642 ^T	KX262216	LR583723	LR583931	LR583837
<i>N. kurunegalensis</i>	CBS 119599 ^T	DQ247511	JF433036	–	LR583838
<i>N. lerouxii</i>	CBS 146514 ^T	MW248768	MW173069	MW173039	MW446617
<i>N. lichenicola</i>	CBS 509.63	LR583618	LR583728	LR583936	LR583843
<i>N. lichenicola</i>	CBS 623.92 ^{ET}	LR583620	LR583730	LR583938	LR583845
<i>N. liriodendri</i>	CBS 117481 ^T	AF178340	AF178404	AF178373	EU329506
<i>N. longissima</i>	CBS 126407 ^T	LR583621	LR583731	LR583939	LR583846
<i>N. macrospora</i>	CBS 142424 ^T	LT746218	LT746266	LT746281	LT746331
<i>N. macrospora</i>	CPC 28193	LT746220	LT746268	LT746283	LT746333
<i>N. magnoliae</i>	MFLUCC 17–2615 ^T	MT212207	NR_171102	NG_073865	MT212200
<i>N. mahasenii</i>	CBS 119594 ^T	DQ247513	JF433045	–	LT960563
<i>N. mangrovei</i>	MFLUCC 17–0253	**	MT928792	ON833274	**
<i>N. mangrovei</i>	MFLUCC 17–0257	**	MT928793	ON8332745	**
<i>N. martii</i>	CBS 115659 ^{ET}	JX435156	JX435206		JX435256
<i>N. merckxiana</i>	CBS 146525 ^T	MW834288	MW827627	MW827668	MW834025

Species name	Strain ¹	GenBank accession number			
		EF-1 α	ITS	LSU	rpb2
<i>N. merckxiana</i>	CBS 146526	MW834289	MW827628	MW827669	MW834026
<i>N. metavorans</i>	CBS 135789 ^T	LR583627	LR583738	LR583946	LR583849
<i>N. metavorans</i>	CBS 143219	LR583629	LR583744	LR583948	LR583851
<i>N. mori</i>	CBS 145467 ^T	AF178358	DQ094305	DQ236347	EU329499
<i>N. mori</i>	CBS 145468	AF178359	DQ094306	DQ236348	EU329493
<i>N. neerlandica</i>	CBS 232.34 ^T	MW847906	MW827629	MW827670	MW847903
<i>N. nelsonii</i>	CBS 309.75 ^T	MW847907	MW827630	MW827671	MW847904
<i>N. nirenbergiana</i>	CBS 145469 ^T	AF178339	AF178403	AF178372	EU329505
<i>N. noneumartii</i>	CBS 115658 ^T	LR583630	LR583745	LR583949	MW446618
<i>N. obliquiseptata</i>	NRRL 62611	KC691548	KC691576	–	KC691637
<i>N. oblonga</i>	CBS 130325 ^T	LR583631	LR583746	LR583950	LR583853
<i>N. oligoseptata</i>	CBS 143241 ^T	KC691538	KC691566	–	LR583854
<i>N. paraeumartii</i>	CBS 487.76 ^T	DQ247549	LR583747	LR583951	LR583855
<i>N. parceramosa</i>	CBS 115695 ^T	JX435149	JX435199	–	JX435249
<i>N. perseae</i>	CBS 144142 ^T	LT991902	LT991940	LT991947	LT991909
<i>N. petroliphila</i>	CBS 203.32	DQ246835	DQ094320	DQ236362	LR583857
<i>N. petroliphila</i>	CBS 224.34	DQ246910	DQ094383	DQ236425	LR583858
<i>N. phaseoli</i>	CBS 265.50	FJ919464	LR583750	LR583954	KJ511278
<i>N. phaseoli</i>	NRRL 22276	AY220186	EU329668	–	JX171608
<i>N. piperis</i>	CBS 145470 ^T	AF178360	AF178422	AF178391	EU329513
<i>N. pisi</i>	CBS 123669 ^{ET}	LR583636	LR583753	LR583957	LR583862
<i>N. pisi</i>	CBS 142372	KY556454	LR583755	LR583959	LR583864
<i>N. plagianthi</i>	NRRL 22632	AF178354	AF178417	AF178386	JX171614
<i>N. protoensiformis</i>	CBS 145471 ^T	AF178334	AF178399	AF178368	EU329498
<i>N. pseudensiformis</i>	CBS 130.78	DQ247635	LR583759	LR583963	LR583868
<i>N. pseudopisi</i>	CBS 266.50	MW834290	MW827631	MW827672	MW834027
<i>N. pseudoradicicola</i>	CBS 145472 ^T	JF740757	JF740899	–	JF741084
<i>N. pseudotonkinensis</i>	CBS 143038 ^{ET}	–	MG189942	MG189927	–
<i>N. quercicola</i>	CBS 141.90 ^T	DQ247634	LR583760	LR583964	LR583869
<i>N. rectiphora</i>	CBS 125726	JF433026	JF433043	–	MW834028
<i>N. rectiphora</i>	CBS 125727 ^T	DQ247509	JF433034	–	LR583871
<i>N. regularis</i>	CBS 190.35	LR583642	LR583762	LR583966	LR583872
<i>N. regularis</i>	CBS 230.34 ^T	LR583643	LR583763	LR583967	MW834029
<i>N. rekana</i>	CMW 52862 ^T	MN249151	MN249094	–	MN249137
<i>N. rhizophorae</i>	MFLUCC 17– 2586 ^T		MN047109	MN017872	–
<i>N. riograndensis</i>	UFMG-CM F12570 ^T	KX534002	KT186366	KX534001	KX534003
<i>N. robusta</i>	CBS 145473 ^T	AF178341	AF178405	LR583968	EU329507
<i>N. samuelsii</i>	CBS 114067 ^T	LR583644	LR583764	LR583969	LR583874
<i>N. silvicola</i>	CBS 119601	LR583645	LR583765	LR583970	LR583875
<i>N. silvicola</i>	CBS 123846 ^T	LR583646	LR583766	LR583971	LR583876
<i>N. solani</i>	CBS 140079 ^{ET}	KT313611	KT313633	–	KT313623

Species name	Strain ¹	GenBank accession number			
		EF-1 α	ITS	LSU	rpb2
<i>Neocosmospora</i> sp.	CBS 143203	DQ246937	DQ094407	DQ236449	EU329571
<i>Neocosmospora</i> sp.	CBS 143206	DQ246945	DQ094414	DQ236456	EU329575
<i>Neocosmospora</i> sp.	CBS 143212	DQ247128	DQ094587	DQ236629	EU329625
<i>Neocosmospora</i> sp.	CBS 143220	JQ743207	JQ743209	–	JQ743211
<i>Neocosmospora</i> sp.	CBS 143222	KC808195	MG189938	–	KC808334
<i>Neocosmospora</i> sp.	NRRL 22642	DQ246844	DQ094329	DQ236371	EU329522
<i>Neocosmospora</i> sp.	NRRL 22834	DQ247663	–	–	FJ240382
<i>Neocosmospora</i> sp.	NRRL 25392	DQ246861	EU329672	–	EU329537
<i>Neocosmospora</i> sp.	NRRL 46704	–	EU329713	–	EU329662
<i>Neocosmospora</i> sp.	NRRL 46705	–	EU329714	–	EU329663
<i>N. spathulata</i>	CBS 145474 ^T	DQ246882	EU329674	–	EU329542
<i>N. stercicola</i>	CBS 142481 ^T	LR583658	LR583779	LR583984	LR583887
<i>N. stercicola</i>	CBS 144388	LR583659	LR583780	LR583985	LR583888
<i>N. suttoniana</i>	CBS 143214 ^T	DQ247163	DQ094617	DQ236659	EU329630
<i>N. suttoniana</i>	CBS 143224	KC808197	MG189940	MG189925	KC808336
<i>N. tenuicristata</i>	NRRL 22155 ^{ET}	JQ265783	NR_169922	–	–
<i>N. tonkinensis</i>	CBS 115.40 ^T	LT906672	MG189941	–	LT960564
<i>N. tonkinensis</i>	CBS 118931	LR583662	LR583784	–	LR583891
<i>N. tuaranensis</i>	NRRL 22231 ^T	KC691542	KC691570	–	KC691631
<i>N. vasinfecta</i>	CBS 325.54	AF178348	AF178412	AF178381	JX171610
<i>N. vasinfecta</i>	CBS 446.93	LR583670	LR583791	LR583996	LR583898
<i>N. vasinfecta</i>	CBS 533.65	LR583671	LR583792	LR583997	LR583899
<i>Nothofusarium devonianum</i>	CBS 147304 ^T	MW834291	MW827632	MW827673	JX171603
<i>Rectifusarium robinianum</i>	CBS 430.91 ^T	KM231923	KM231794	NG_058096	JX171633
<i>R. ventricosum</i>	CBS 748.79 ^T	KM231924	HQ897816	KM231658	JX171597
<i>Scolecofusarium ciliatum</i>	CBS 144385	MW834297	KJ125591	KJ126479	KP835472
<i>S. ciliatum</i>	CBS 155.86	MW834295	MW827635	MW827676	MW834034
<i>S. ciliatum</i>	CBS 191.65 ^{NT}	MW834296	MW827636	MW827677	MW834035
<i>Setofusarium setosum</i>	CBS 574.94	MW834293	MW827633	MW827674	MW834033
<i>S. setosum</i>	CBS 635.92 ^{ET}	MW834294	MW827634	MW827675	JX171651
<i>gularis</i>	CBS 190.35	LR583642	LR583762	LR583966	LR583872
<i>N. regularis</i>	CBS 230.34 ^T	LR583643	LR583763	LR583967	MW834029
<i>N. rekana</i>	CMW 52862 ^T	MN249151	MN249094	–	MN249137
<i>N. rhizophorae</i>	MFLUCC 17–2586 ^T		MN047109	MN017872	–
<i>N. riograndensis</i>	UFMG-CM F12570 ^T	KX534002	KT186366	KX534001	KX534003
<i>N. robusta</i>	CBS 145473 ^T	AF178341	AF178405	LR583968	EU329507
<i>N. samuelsii</i>	CBS 114067 ^T	LR583644	LR583764	LR583969	LR583874
<i>N. silvicola</i>	CBS 119601	LR583645	LR583765	LR583970	LR583875
<i>N. silvicola</i>	CBS 123846 ^T	LR583646	LR583766	LR583971	LR583876
<i>N. solani</i>	CBS 140079 ^{ET}	KT313611	KT313633	–	KT313623

Species name	Strain ¹	GenBank accession number			
		EF-1 α	ITS	LSU	rpb2
<i>Neocosmospora</i> sp.	CBS 143203	DQ246937	DQ094407	DQ236449	EU329571
<i>Neocosmospora</i> sp.	CBS 143206	DQ246945	DQ094414	DQ236456	EU329575
<i>Neocosmospora</i> sp.	CBS 143212	DQ247128	DQ094587	DQ236629	EU329625
<i>Neocosmospora</i> sp.	CBS 143220	JQ743207	JQ743209	–	JQ743211
<i>Neocosmospora</i> sp.	CBS 143222	KC808195	MG189938	–	KC808334
<i>Neocosmospora</i> sp.	NRRL 22642	DQ246844	DQ094329	DQ236371	EU329522
<i>Neocosmospora</i> sp.	NRRL 22834	DQ247663	–	–	FJ240382
<i>Neocosmospora</i> sp.	NRRL 25392	DQ246861	EU329672	–	EU329537
<i>Neocosmospora</i> sp.	NRRL 46704	–	EU329713	–	EU329662
<i>Neocosmospora</i> sp.	NRRL 46705	–	EU329714	–	EU329663
<i>N. spathulata</i>	CBS 145474 ^T	DQ246882	EU329674	–	EU329542
<i>N. stercicola</i>	CBS 142481 ^T	LR583658	LR583779	LR583984	LR583887
<i>N. stercicola</i>	CBS 144388	LR583659	LR583780	LR583985	LR583888
<i>N. suttoniana</i>	CBS 143214 ^T	DQ247163	DQ094617	DQ236659	EU329630
<i>N. suttoniana</i>	CBS 143224	KC808197	MG189940	MG189925	KC808336
<i>N. tenuicristata</i>	NRRL 22155 ^{ET}	JQ265783	NR_169922	–	–
<i>N. tonkinensis</i>	CBS 115.40 ^T	LT906672	MG189941	–	LT960564
<i>N. tonkinensis</i>	CBS 118931	LR583662	LR583784	–	LR583891
<i>N. tuaranensis</i>	NRRL 22231 ^T	KC691542	KC691570	–	KC691631
<i>N. vasinfecta</i>	CBS 325.54	AF178348	AF178412	AF178381	JX171610
<i>N. vasinfecta</i>	CBS 446.93	LR583670	LR583791	LR583996	LR583898
<i>N. vasinfecta</i>	CBS 533.65	LR583671	LR583792	LR583997	LR583899
<i>Nothofusarium devonianum</i>	CBS 147304 ^T	MW834291	MW827632	MW827673	JX171603
<i>Rectifusarium robinianum</i>	CBS 430.91 ^T	KM231923	KM231794	NG_058096	JX171633
<i>R. ventricosum</i>	CBS 748.79 ^T	KM231924	HQ897816	KM231658	JX171597
<i>Scolecofusarium ciliatum</i>	CBS 144385	MW834297	KJ125591	KJ126479	KP835472
<i>S. ciliatum</i>	CBS 155.86	MW834295	MW827635	MW827676	MW834034
<i>S. ciliatum</i>	CBS 191.65 ^{NT}	MW834296	MW827636	MW827677	MW834035
<i>Setofusarium setosum</i>	CBS 574.94	MW834293	MW827633	MW827674	MW834033
<i>S. setosum</i>	CBS 635.92 ^{ET}	MW834294	MW827634	MW827675	JX171651

Abbreviations: **CBS**: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, **NRRL**: collections of the Agricultural Research Service, Peoria, IL, USA, **CPC**: Personal collection of Pedro W. Crous, held at CBS, **MFLUCC**: Mae Fah Luang University Culture Collection at the Center of Excellence in Fungal Research, Thailand, **EF-1 α** : partial fragment of the translation elongation factor 1-alpha, **ITS**: Internal transcribed spacer region of the rDNA, **LSU**: partial fragment of the large ribosomal subunit gene, **RPB2**: partial fragment of the DNA-directed RNA
Type: ¹ ^T Ex-type, ^{ET} Ex-epitype, ^{NT} Ex-neotype. “–” Sequences not available at GenBank.

Table 2. Inhibition percentage presented at the in-vitro assays

Pathogenic fungi/Fungal crude extract	<i>C. acutatum</i> , MFLUCC CC-0036	<i>C. truncatum</i> , MFLUCC CG-0064
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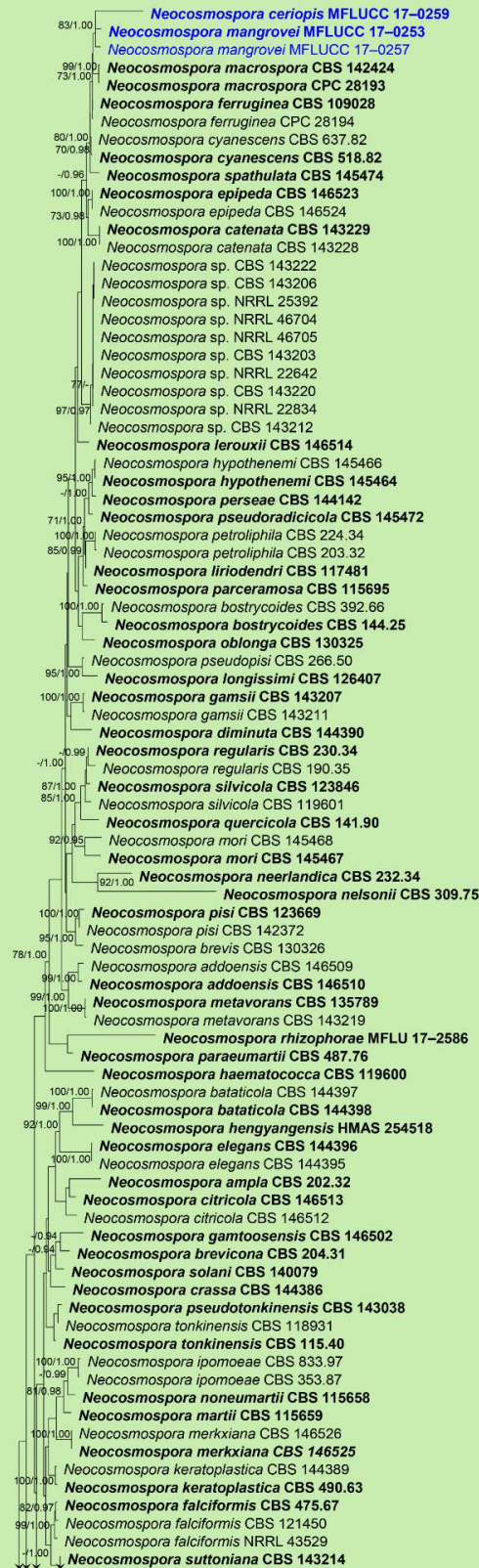
	<i>N. ceriopsis</i> MFLUCC 17-0259	<i>N. mangrovei</i> MFLUCC 17-0253
Inhibition of mycelium growth (%) ^{*1}		
0.6 mg/mL	53.67 ± 3.09b	82.64 ± 0.74b
0.9 mg/mL	69.39 ± 2.42a	100.00 ± 0.00a
Captan (0.6 mg/mL)	21.66 ± 3.00c	78.56 ± 20.79c
Inhibition of spore germination (%) ¹		
0.9 mg/mL	79.39 ± 2.14	90.83 ± 2.07
Captan (0.6 mg/mL)	100.00 ± 00	86.34 ± 1.81
Inhibition of hyphae expansion (%) ¹		
0.9 mg/mL	74.99 ± 11.66	68.75 ± 8.33
2.7 mg/mL	78.57 ± 10.00	100.00 ± 00
5.4 mg/mL	96.42 ± 4.65	100.00 ± 00
Captan (0.6 mg/mL)	100.00 ± 00	25 ± 1.09

* Significant at $p = 0.05$

¹ Data are presented as mean ± S.D. values of three independent experiments.

646

647



Neocosmospora

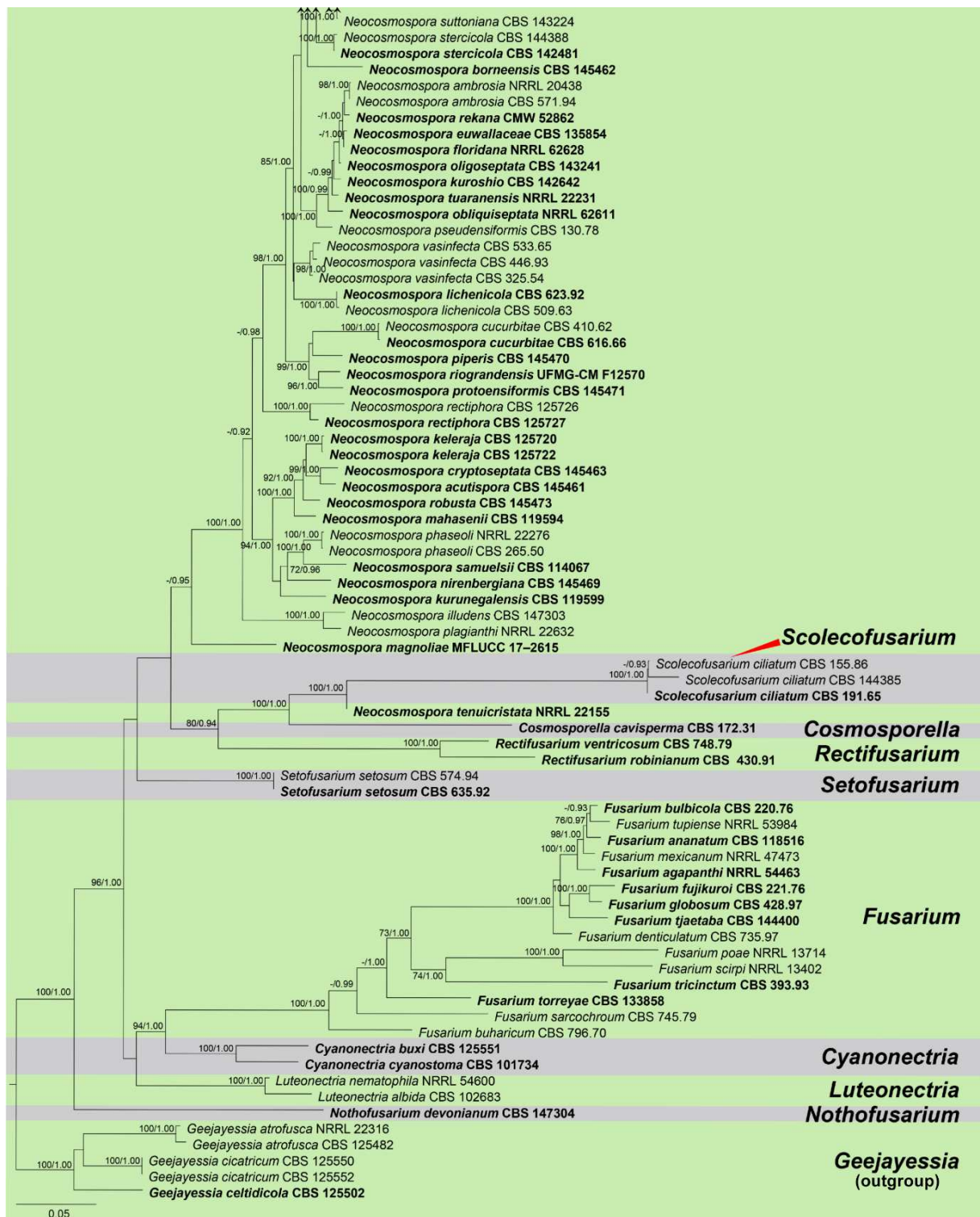


Fig. 1 RAxML analysis of combined EF-1 α , ITS, LSU and RPB2 sequence datasets comprised 125 strains of *Neocosmospora* with *Geejayessia atrofusca* (NRRL 22316) and *G. cicatricum* (CBS 125552) as the outgroup taxa. Bootstrap support values for ML equal to or greater than 70% and BYPP equal to or greater than 0.95 are given above the nodes. Novel taxa generated from this study are in bold.

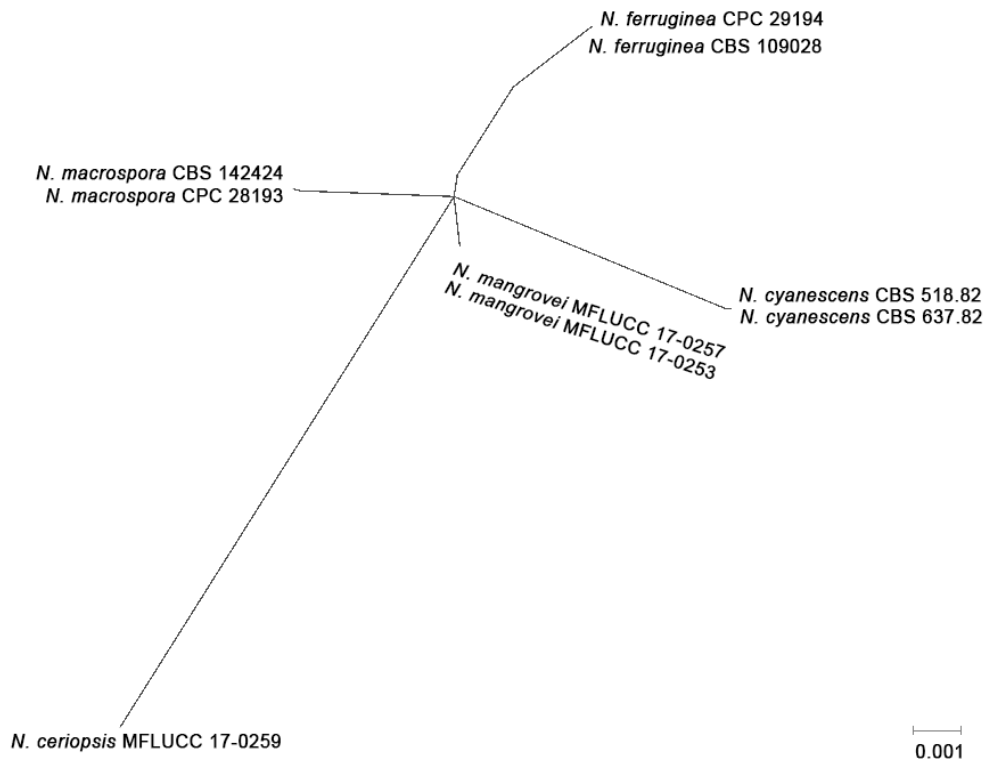


Fig. 2 Results of the pairwise homoplasy index (PHI) test of five species of *Neocosmospora* species using both LogDet transformation and splits decomposition.

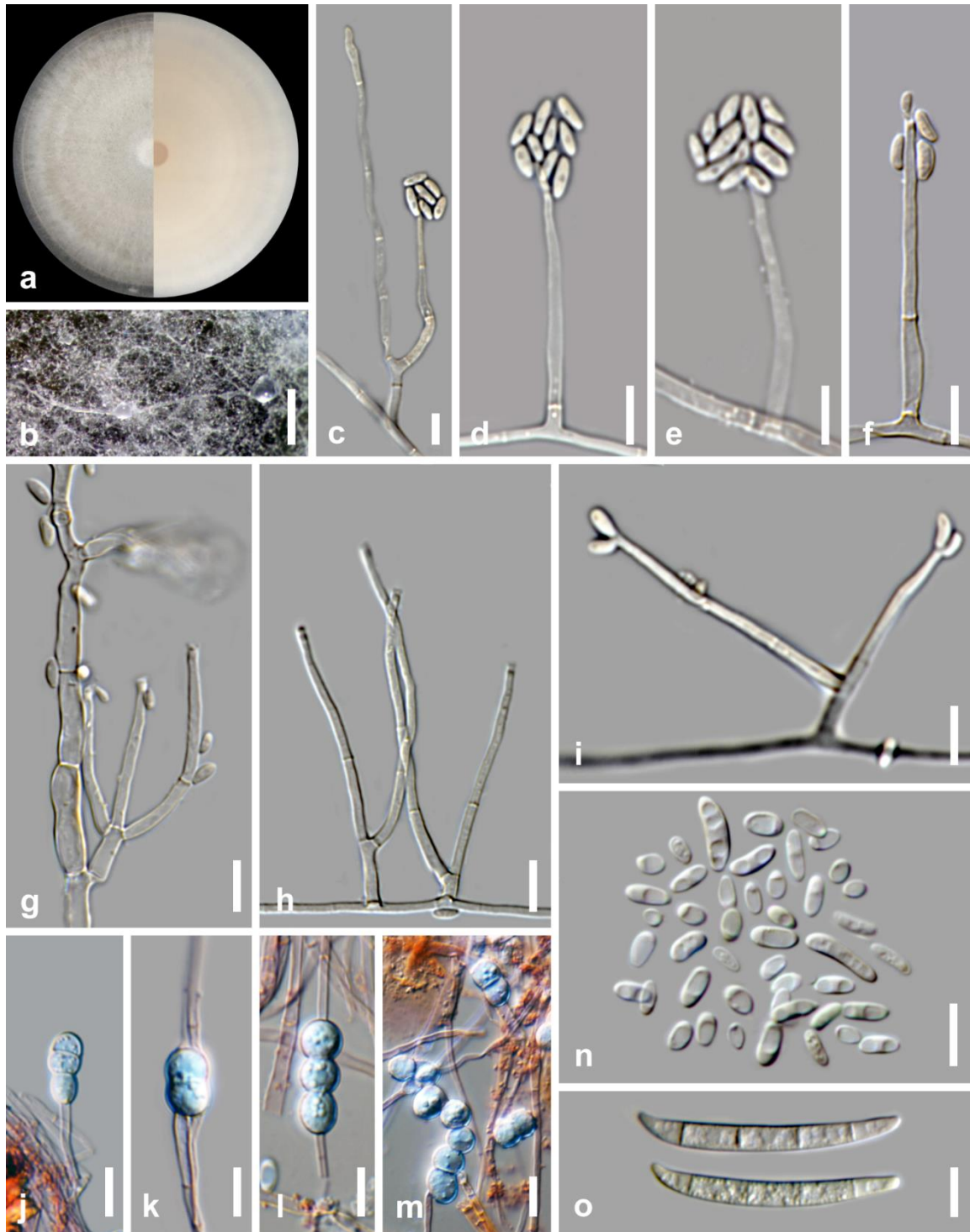


Fig. 3 *Neocosmospora ceriopis* (MFLUCC 17-0259, ex-type culture) (a) Colonies on PDA (obverse and reverse); (b) Mycelia on PDA with aerial conidiophores and mass of conidia; (c, d, e, f, g, h, i) Aerial conidiophores; (j, k, l, m) Chlamydospores; (n) Aerial microconidia; (o) Aerial macroconidia. Scale bars, (b) 500 µm; (c-o) 20 µm.

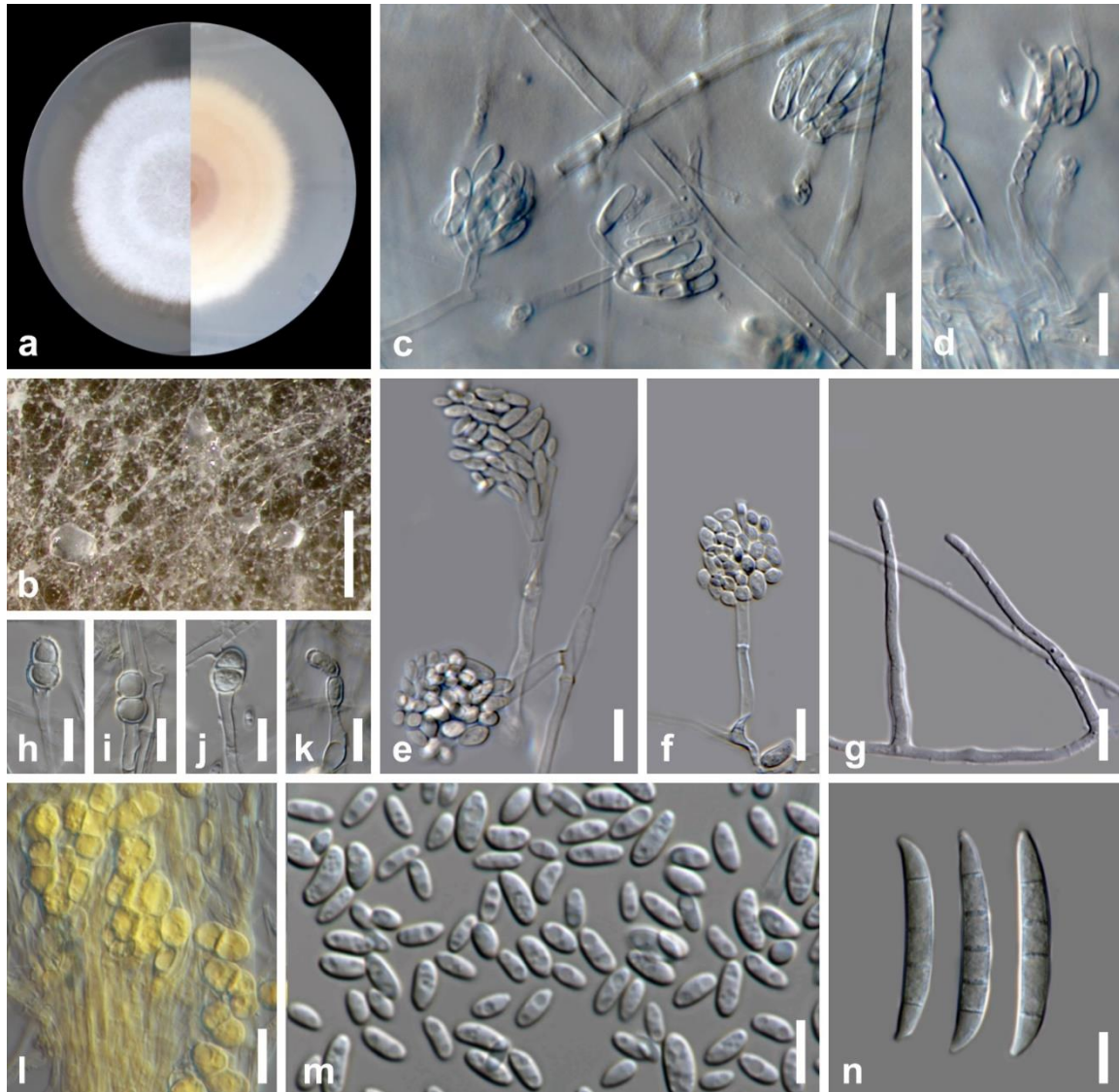


Fig. 4 *Neocosmospora mangrovei* (MFLUCC 17-0253, ex-type culture). (a) Colonies on PDA (obverse and reverse); (b) Mycelia on PDA with aerial conidiophores and mass of conidia; (c, d, e, f, g) Aerial conidiophores; (h, i, j, k, l) Chlamydospores; (m) Aerial microconidia; (n) Aerial macroconidia. Scale bars, (b) 500 µm; (c-n) 10 µm.

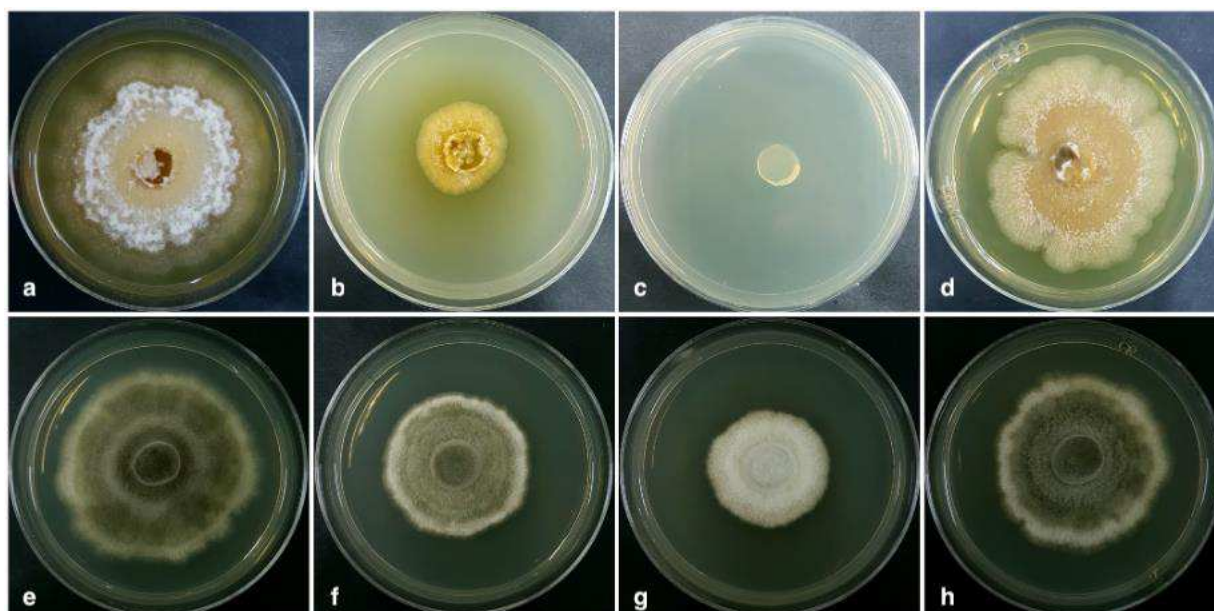


Fig. 5 Growth of *C. truncatum*, MFLUCC CG-0064 in poisoned food assay (a-d) in PDA mixed with sterile water (a), crude extract of *N. mangrovei* MFLUCC 17-0253 (0.6 mg/mL; b), crude extract of *N. mangrovei* MFLUCC 17-0253 (0.9 mg/mL; c), Captan (d) at 7 days after incubation. Growth of *C. acutatum*, MFLUCC CC-0036 in poisoned food assay (e-h) in PDA mixed with sterile water (e) crude extract of *N. ceriopsis* MFLUCC 17-0259 (0.6 mg/mL; f), crude extract of *N. ceriopsis* MFLUCC 17-0259 (0.9 mg/mL; g), Captan (h) at 7 days after incubation.

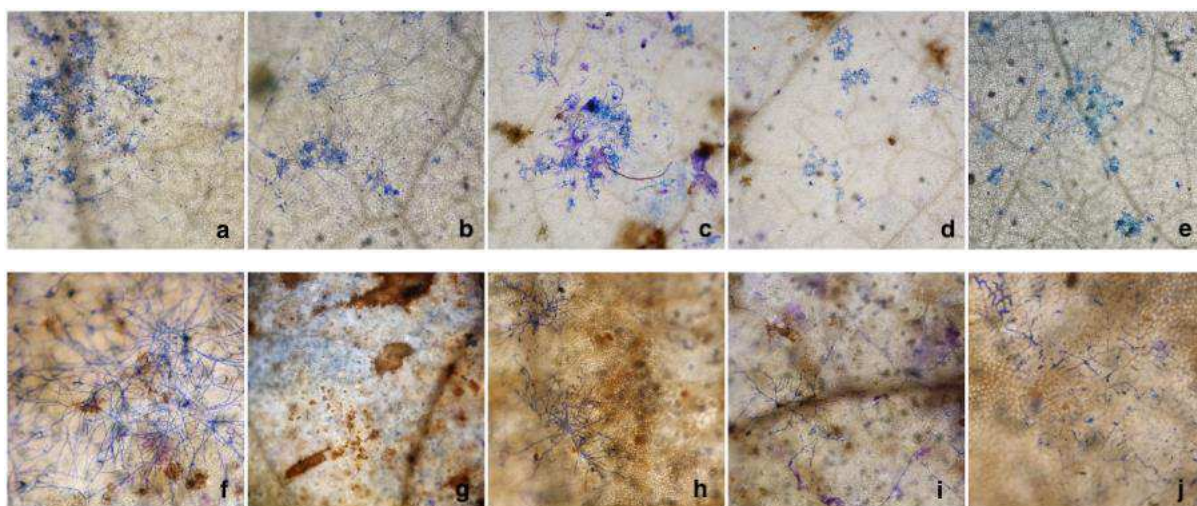


Fig. 6 Hypha growth of *C. truncatum*, MFLUCC CG-0064 (a-e) on chili leaf, spore solution mixed with sterile water (a), Captan (b), crude extract of *N. mangrovei* MFLUCC 17-0253 (0.9 mg/mL; c), crude extract of *N. mangrovei* MFLUCC 17-0253 (2.7 mg/mL; d), crude extract of *N. mangrovei* MFLUCC 17-0253 (5.4 mg/mL; e) at 5 days after incubation. Hypha growth of *C. acutatum*, MFLUCC CC-0036 (f-j) on chili leaf, spore solution mixed with sterile water (f), Captan (g), crude extract of *N. ceriopsis* MFLUCC-0259 (0.9 mg/mL; h), crude extract of *N. ceriopsis* MFLUCC-0259 (2.7 mg/mL; i), crude extract of *N. ceriopsis* MFLUCC-0259 (2.7 mg/mL; j) at 5 days after incubation.

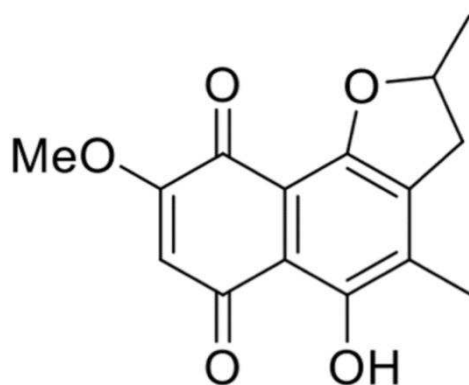
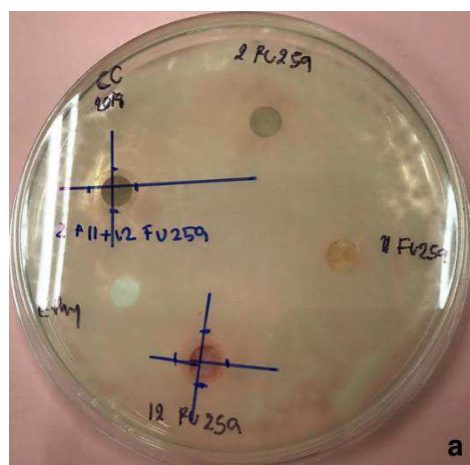


Fig. 7 Disc diffusion assay with negative control (ethyl acetate), 2FU-259, 11FU-259, 12FU-259, and mixture of 11FU-259 and 12FU-259 (a); structure of 2,3-hydro-5-hydroxy-8-methoxy-2,4-dimethylnaphthol-[1,2-*b*]-furan-6,9-dione (b).