

Article



https://doi.org/10.11646/phytotaxa.530.2.1

Neodeightonia arengae sp. nov., Botryosphaeriaceous taxa on Arenga tremula (Arecaceae) from Guangdong, China

YINRU XIONG^{1,2,3,4}, ISHARA S. MANAWASINGHE^{1,5}, CHUNFANG LIAO^{1,2,3,6}, KEVIN D. HYDE^{1,2,7} & ZHANGYONG DONG^{1,8}*

- ¹ Innovative Institute for Plant Health, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, P.R. China
- ² Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand
- ³ School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
- ⁴ richard xyr@163.com; https://orcid.org/0000-0002-4673-606X
- ⁵ ishara.ishara@yahoo.com; https://orcid.org/0000-0001-5730-3596
- ⁶ 371105002@lamduan.mfu.ac.th; https://orcid.org/0000-0002-6309-1101
- 7 \blacksquare kdhyde3@gmail.com; $^{\odot}$ https://orcid.org/0000-0002-2191-0762
- 8 dongzhangyong@hotmail.com; https://orcid.org/0000-0001-7524-0226
- *Corresponding author: Zhang-yong Dong, ■dongzhangyong@hotmail.com

Abstract

Microfungi associated with palm are a significant fungal group with a unique ecological niche and a broad distribution in tropical regions. Even though many fungal species have been reported from palm hosts, studies on fungi from *Arenga tremula* are considerably few. In this study, we isolated a saprobic *Botryosphaeriaceae* species on *A. tremula*, collected from Guangzhou, Guangdong Province, China. Morphological characteristics and phylogenetic analysis of the internal transcribed spacer (ITS), small subunit nuclear rRNA gene (SSU),part of the large subunit nuclear rRNA gene (LSU) and translation elongation factor 1–alpha gene (*tef* 1-α). Based on phylogenetic results and morphology we introduced *Neodeightonia arengae sp. nov.*, with species description and illustrations. In addition, we provide a comparison of morphological characters of currently accepted *Neodeightonia* species. This is the first report of a *Neodeightonia* species associated with *Arenga tremula* and so represents an additional contribution to the knowledge of fungi associated with palm trees.

Keywords: 1 new species, Botryosphaeriaceae, multi-gene, palm fungi, saprobic

Introduction

Palm fungi are one of the most diverse and largest groups of fungi. There are more than 1500 species previously described from palm trees representing almost all major fungal classes (Fröhlich & Hyde 2000, Taylor & Hyde 2003). Up until 2000, a diverse group of fungal species had been described from palm hosts. In total 794 ascomycetes species belonged to 79 families, 278 genera and 79 families had been introduced. (Hyde 1994, Htde & Cannon 1999, Fröhlich & Hyde 2000, Hyde *et al.* 2000, Taylor & Hyde 2003). Thereafter, several new genera and species have been described (Pinruan *et al.* 2010, Ligoxigakis *et al.* 2013). The consistent new discoveries indicate the potential identification of novel fungal taxa from palm trees due to their yet to be fully appreciated diversity.

Guangdong Province is one of the most populated cities with a subtropical climate (Gao *et al.* 2020). During the last decades, more than 70 varieties of palm plants from other provinces and abroad were introduced into the Guangdong Province. Palm trees are mostly used as a greening tree species, ornamental plants and garden plants or natural gardens in Guangdong Province. (Cai *et al.* 2007). According to the data from the National Specimen Information Infrastructure (NSII), 71 palm species in Guangdong Province have been identified. (http://www.nsii. org.cn/2017/query.php?name=\u5e7f\u4e1c data retrieved on 23/5/2021), including *Caryota mitis, C. ochlandra*, and *Rhapis gracilis*. However, microfungi associated with these species have not yet been well studied or documented.

Botryosphaeriaceae (Botryosphaeriales, Dothideomycetes) species are well-known plant pathogens, saprobes, and endophytes on a wide range of hosts worldwide (Phillips et al. 2013). Their ecological role varies with the host and geography (Slippers & Wingfield 2007, Phillips et al. 2013). Currently, there are 24 genera accepted in this

family including *Neodeightonia* (Garcia *et al.* 2021). Punithalingam (1969) described *Neodeightonia subglobosa* as the type species in *Neodeightonia*. However, Arx & Müller (1975) transferred *N. subglobosa* to *Botryosphaeria*, and *Neodeightonia* was synonymized under *Botryosphaeria*. Phillips *et al.* (2008) distinguished *Neodeightonia* from *Botryosphaeria* based on differences in morphology (the dark, one-septate ascospores) and phylogeny, then *Neodeightonia* was redefined as a separate genus and *N. phoenicum* was described as a new species. Currently, there are seven species accepted in the genus with accompanying molecular data (Konta *et al.* 2016, Jayasiri *et al.* 2019, Wu *et al.* 2021). Up until now, four *Neodeightonia* species have been recorded from palm and only two of these species have been reported as pathogens. Ligoxigakis *et al.* (2013) reported *N. phoenicum* causing palm rot in Greece and Liu *et al.* (2010) described *N. palmicola* on the palm *Arenga westerhoutii* from Thailand.

In this study, a saprobic *Neodeightonia* species was isolated from *Arenga tremula*. The isolated taxa were identified as novel species based on both phylogeny and morphology. Morphological examinations showed that this palm fungus is different from the previously described species in *Neodeightonia*.

Materials & methods

Sample collection, Morphological observations, and isolation

A decaying leaf sample of the *Arenga tremula* was collected from the palm garden of the South China Botanical Garden in Guangzhou City, Guangdong Province, China on December 17, 2020. The sample was collected and photographed in the laboratory.

Macro morphological characters of the fungi were observed using a Cnoptec SZ650 (China) series stereo microscope, and photographs were taken using the Nikon Eclipse 80i and the industrial DigitaL Sight DS-Fi1 (Panasonic, Japan) microscope imaging system. Fruiting bodies were cut into 30 μm thin sections by a freezing sliding microtome (Bio-Key science and technology Co., LTD, LEICA CM1860, Germany) for photographing and measuring. Digital images of micromorphological structures were recorded with an Eclipse 80i photographic microscope (Nikon, Japan). Conidial length and width were measured for at least 40 spores and processed by TaroSoft® Image FrameWorks v0.9.7. The mean values were calculated along with standard deviations (SDs). Adobe Photoshop CS6 software (Adobe Systems Inc.) was used to develop images. Following Senanayake *et al.* (2020) single spore isolation was performed to obtain pure cultures. Germinated spores were transferred to potato dextrose agar (PDA) plates after 16 h in water agar (WA) at 25 °C. Cultures were incubated at 25 °C in natural light/dark for up to 7 days. Culture characteristics such as colony shape, and mycelium colour were determined and colony colour was assessed as previously described by Rayner (1970). All pure cultures obtained in this study were deposited in the culture collection of Zhongkai University of Agriculture and Engineering (ZHKUCC) in PDA slants at 4°C. Herbarium materials were deposited in the herbarium of Zhongkai University of Agriculture and Engineering (ZHKUC).

DNA extraction and PCR amplification

Genomic DNA was extracted from fresh mycelium grown on PDA for four days using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, P.R. China). The ITS region was amplified using ITS4 and ITS5 primer pairs (White *et al.* 1990). The LSU region with LR0R and LR5 (Vilgalys & Hester 1990), the SSU with NS1 and NS4 (White *et al.* 1990), and the *tef* 1-α gene with EF1-728F and EF1-986R (Carbone & Kohn 1999). The PCR reaction mixture contained 25 μL of total volume, which consisted of 12.5 μL 2×FastTaq Premix (mixture of FastTaq TM DNA Polymerase, buffer, dNTP Mixture, and stabilizer) (Beijing Qingke Biological Technology Co., Ltd., Beijing, P.R. China), 1 μL of each forward and reverse primers, 9.5 μL ddH₂O and 1 μL DNA. PCR amplification was performed in BIO-RAD. Thermal cycler conditions for each primer pair is given in **Table 1.** The PCR products were checked on 1% agarose electrophoresis gels stained with ethidium bromide under UV light using a Gel DocTM XR Molecular Imager (Bio-Rad, USA). The unpurified PCR products were sequenced by Tianyi Huiyuan Gene Technology Co., Ltd, China.

TABLE 1. Gene regions, primer pairs and respective thermal cycler conditions used in this study.

Locus	Primer Reaction condition (5'-3')	Amplification	References
ITS	ITS5: 5'-DDAAGTAAAAGTCGTAACAAGG-3' (Forward) ITS4: 5'-TCCTCCGCTTATTGATATGC-3' (Reverse)	Initial denaturation at 94°C for 3 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1	White et al. (1990)
LSU	LR0R: 5'-ACCCGCTGAACTTAAGC-3' (Forward)	min, and a final extension at 72°C for 10 min Initial denaturation at 94°C for 3 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s,	Vilgalys, & Hester. (1990)
	LR5: 5'-TCCTGAGGGAAACTTCG-3' (Reverse)	annealing at 55°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 10 min	
tef 1- α	EF1-728F: 5'-CATCGAGAAGTTCGAGAAGG-3' (Forward)	Initial denaturation at 94°C for 3 min, followed by 35 cycles consisting of denaturation at 94°C for 45 s,	Carbone & Kohn (1999)
	EF1-986R: 5'-TACTTGAAGGAACCCTTACC-3' (Reverse)	annealing at 55°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 10 min	
SSU	NS1: 5'-GTAGTCATATGCTTGTCTC-3' (Forward)	Initial denaturation at 94°C for 3 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s,	White <i>et al</i> . (1990)
	NS4: 5'-CTTCCGTCAATTCCTTTAAG-3' (Reverse)	annealing at 55°C for 30 s and extension at 72°C for 1 min, and a final extension at 72°C for 10 min	

Phylogenetic analysis

The sequence quality was assured by checking chromatograms of resulted sequences using BioEdit v.7.0.5.2 (Hall 1999). BioEdit v.7.0.5.2 was used to combine the sequences generated by the forward and reverse primers. Sequences obtained in this study were analyzed using the National Center for Biotechnology Information (NCBI) search engine BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for initial species confirmation. Based on blast results, reference sequences were obtained from GenBank following Konta *et al* (2016). and Jayasiri *et al*. (2019) (**Table 2**). Sequences obtained in this study were aligned with sequences downloaded from GenBank using MAFFT v. 7 at the webserver (http://mafft.cbrc.jp/alignment/server). The sequence sets of ITS, LSU, SSU, and *tef* 1-α were combined by BioEdit v.7.0.5.2 (Hall 1999). Using Alignment Transformation Environment online (https://sing.ei.uvigo.es/ALTER/) files were converted to run phylogenetic trees. Phylogenetic analyses were conducted using maximum likelihood (ML) inferred in RAxML v. 8.2.12 (Stamatakis 2014), maximum parsimony (MP) implied on PAUP v. 4.0b10 (Swofford 2003), and Bayesian analysis on MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001).

Maximum parsimony analysis was performed in PAUP (phylogenetic analysis using parsimony) v.4.0b10 (Swofford 2003) using the heuristic search option with tree bisection-reconnection (TBR) branch swapping and 1000 random sequence additions. Ambiguous regions in the alignment were excluded, and gaps were treated as missing data. The stability of the trees was evaluated by 1000 bootstrap replications. Branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive statistics including tree length (TL), consistency index (CI), retention index (RI), relative consistency index (RC), and homoplasy index (HI) were calculated.

The best evolution model was determined by MrModeltest v. 2.2 for each gene. Maximum likelihood analyses were accomplished using RAxML-HPC2 on XSEDE v. 8.2.8 (Stamatakis *et al.* 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller *et al.* 2010) using the GTR+I+G model of evolution with 1000 non-parametric bootstrapping iterations. MrBayes v.3.0b4 was (Huelsenbeck & Ronqvist 2001) used for the Bayesian analyses. The Markov Chain Monte Carlo sampling (BMCMC) analysis was conducted with four simultaneous Markov chains. They were run for 1,000,000 generations: sampling the trees at every 100th generation. From the 10,000 trees obtained, the first 2,000 representing the burn-in phase were discarded. The remaining 8,000 trees were used for calculating posterior probabilities in the majority rule consensus tree. Taxonomic novelties were submitted to the Faces of Fungi database (Jayasiri *et al.* 2015) and Index Fungorum (http://www.indexfungorum.org). All sequences derived from this study are deposited in GenBank (**Table 2**).

TABLE 2. GenBank accession numbers of the sequences used in phylogenetic analyses. All type strains are bold. The newly generated sequences are indicated in red.

Species Tayon	Culture Collection	GenBank acc	cession number		
Species Taxon	number	ITS	LSU	SSU	tef 1-a
Alanphillipsia aloeicola	CBS 138896	KP004444	KP004472	-	-
Alanphillipsia aloeigena	CBS 136408	KF777137	KF777193	-	-
Alanphillipsia aloes	CBS 136410	KF777138	KF777194	-	-
Alanphillipsia aloetica	CBS 136409	KF777139	KF777195	-	-
Alanphillipsia euphorbiae	CBS 136411	KF777140	KF777196	-	-
Neodeightonia arengae	ZHKUCC 21-0074	MZ647513	MZ647497	MZ647509	MZ669855
Neodeightonia arengae	ZHKUCC 21-0075	MZ647514	MZ647498	MZ647510	MZ669856
Neodeightonia arengae	ZHKUCC 21-0076	MZ647515	MZ647499	MZ647511	MZ669857
Neodeightonia licuriensis	COAD1780	KP165429	-	-	KP165430
Neodeightonia microspora	MFLUCC 11-0483	KU940110	KU863099	-	-
Neodeightonia palmicola	MFLUCC 10-0822	HQ199221	HQ199222	HQ199223	-
Neodeightonia planchoniae	MFLUCC 17-2427	MK347755	MK347972	MK347861	-
Neodeightonia phoenicum	CBS 122528	KF766198	EU673261	KF766285	EU673309
Neodeightonia rattanica	MFLUCC 15-0313	KX646358	KX646353	-	KX646361
Neodeightonia rattanicola	MFLUCC 15-0319	KX646359	KX646354	KX646358	KX646362
Neodeightonia subglobosa	CBS 448.91	KF766199	DQ377866	KF766286	EU673306
Phaeobotryon aplosporum	CFCC 53774	MN215836	MN215871	-	MN205996
Phaeobotryon cupressi	CBS 124700	FJ919672	KX464538	-	FJ919661
Phaeobotryon mamane	CBS 122980	EU673332	EU673248	-	EU673298
Phaeobotryon rhoinum	CFCC 52450	MH133924	MH133941	-	MH133958
Phaeobotryon rhois	CFCC 89662	KM030584	KM030591	-	KM030598
Phaeobotryon ulmi	CBS 123.30	KX464232	DQ377861	-	KX464766
Phaeobotryon negundinis	MFLUCC 15-0436	KU820970	NG_069332.1	-	KU853997
Sphaeropsis chromolaenicola	MFLUCC 17-1499	MT214366	MT214460	MT214412	-
Sphaeropsis citrigena	ICMP 16812	EU673328	EU673246	EU673180	EU673294
Sphaeropsis eucalypticola	CBS 133993	JX646802	JX646819	JX646835	-
Sphaeropsis porosa	CBS 110496	AY343379	DQ377894	EU673179	AY343340
Sphaeropsis visci	CBS 100163	EU673324	EU754215	EU754116	EU673292

Abbreviations of culture and herbarium collections: CBS, Centraalbureau voor Schimmelcultures, The Netherlands; MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; CFCC, China Forestry Culture Collection Centre; ICMP, International Collection of Microorganisms from Plants, Landcare Research, New Zealand; ZHKUCC, Zhongkai University of Agriculture and Engineering Culture Collection.

ITS: the internal transcribed spacer, LSU: large subunit nuclear rRNA gene, SSU: small subunit nuclear rRNA, tef 1- α : translation elongation factor 1-alpha.

Results

Phylogenetic analysis of Neodeightonia species and other related taxa in Botryosphaeriaceae

Phylogenetic trees were generated by ML, MP, and Bayesian analyses of combined ITS (589 bp), LSU (998 bp), SSU (1054 bp), and tef 1- α (319 bp) sequence data. The tree topologies generated by these three methods were similar and the best scoring MP tree is shown in **FIGURE 1**. The sequence alignment comprised 29 taxa of representative strains of *Botryosphaeriaceae*, including three different isolates obtained in this study. *Sphaeropsis* species were used as the outgroup taxa. Maximum parsimony analysis consisted of 1,342 constant characters and 208 informative characters resulting in 545 equally parsimonious trees (**FIGURE 1**) (CI = 0.694, RI= 0.846, RC = 0.587, HI = 0.306). The best scoring ML tree had an optimization likelihood value of -5345.279103. The matrix had 379 distinct alignment patterns with a 13.16% proportion of gaps and completely undetermined characters. Estimated base frequencies were as follows: A = 0.228596, C = 0.259861, G = 0.290292, T = 0.221251; substitution rates: AC = 0.979993, AG = 2.226357, AT = 0.422697, CG = 1.473656, CT = 4.848545, GT = 1.000000; gamma distribution shape parameter α = 0.078161. Incomplete portions at the ends of the sequences were excluded from the analysis. Three single spore isolates from this study clustered within the *Neodeightonia* clade which forms a sister clade to *Alanphillipsia* in *Botryosphaeriaceae*. Our isolates developed a sister clade to *N. palmicola* with high bootstrap support (75% ML, 78% MP, 1.00 BYPP). Based on the phylogenetic evidence and morphology, here we describe a novel *Neodeightonia* species following the recent species delineation guidelines given in Chethana *et al.* (2021) and Pem *et al.* (2021).

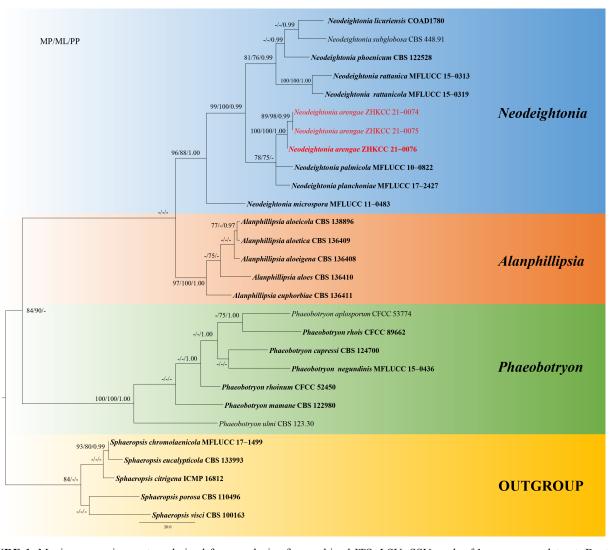


FIGURE 1. Maximum parsimony tree derived from analysis of a combined ITS, LSU, SSU, and tef 1- α sequence dataset. Bootstrap support values for maximum parsimony (MP) greater than 75% and maximum likelihood (ML) greater than 75% and Bayesian posterior probabilities (BYPP) greater than 0.95 are given at the nodes. Five *Sphaeropsis* taxa are used as the outgroup. Strain numbers are given after the species names. The new species identified are highlighted in red. Ex-type strains are in bold.



FIGURE 2. *Neodeightonia arengae* (**ZHKU 21-0095 Holotype**): a. Specimen observed. b–c. Conidiomata. d, e Peridium. f. Conidiogenous cells. g–j. conidia. k. germinating conidia. l. The front of culture on PDA at 25°C. m. The reverse of culture on PDA at 25°C. Scale bars: b, c = 300 μ m, d = 100 μ m, e = 30 μ m, f = 15 μ m, g–j, k = 10 μ m.

Taxonomy

Neodeightonia arengae Y.R. Xiong, Manawas., K.D. Hyde & Z.Y. Dong, sp. nov. (FIGURE 2)

Index Fungorum number: IF558659 Facesoffungi number: FoF 10227

Etymology: Epithet refers to the host genus from which the fungus was isolated

Holotype: ZHKU 21-0095

Saprobic on leaf of Arenga tremula (Arecaceae). Sexual morph: not observed. Asexual morph: Conidiomata 200–330 μ m length \times 140–190 μ m diam. (\overline{x} =250 \times 160 μ m, n=10), pycnidial, semi-immersed, solitary, globose, unilocular, black dots, a crack on the surface of the host where the conidiomata is located. Peridium 20–30 μ m (\overline{x} =21 μ m, n=5), composed of thick-walled, brown-black cells of textura angularis, thin inner wall, almost reduced to conidiogenesis region. Conidiophores reduced to conidogenous cells. Conidiogenous cells hyaline, holoblastic, rough. Conidia 10–30 μ m \times 10–15 μ m (\overline{x} =21 \times 11 μ m, n=40), hyaline, subglobose to ellipsoid, aseptate, granular content, one to two large guttules, broadly rounded at both ends, thin-wall without mucilaginous.

Culture characters: colonies on PDA reach 7 cm diam. at 28°C after five days. Upper view wrinkled, filamentous, entire margin, flat, cloudy, fluffy for aerial hyphae, become gray-black with time, dense for aerial hyphae, reverse becomes black.

Material examined: CHINA. South China Botanical Garden, Guangzhou, Guangdong Province, dead leaves of *Arenga tremula* (Blanco) Becc., 17 December 2020, Yinru Xiong, (holotype ZHKU 21-0095), living cultures ZHKUCC 21-0074 ex-holotype; ZHKUCC 21-0075, ZHKUCC 21-0076 ex-Paratype.

Notes: Three isolates in the present study clustered together with the Neodeightonia species. The taxa from this study form two sister clades to N. palmicola (MFLUCC 10-0822) and N. planchoniae (MFLUCC 17-2427). Neodeightonia planchoniae is a sexual morph and it was characterized by sheaths around the ascospores, while N. arenge is an asexual morph and there was no sheath observed around the conidia. Neodeightonia arengae differs from N. palmicola conidia by the absence of the truncate base, while N. palmicola conidia sometimes appeared truncate at the base (Table 3). Neodeightonia arengae has one or two large guttules in conidia, while the conidia of N. palmicola lack guttules (Liu et al. 2010). The conidia of N. arengae have one morphological stage, hyaline, and aseptate, while the conidia of N. palmicola have two morphological stages initially hyaline. With age, conidia become cinnamon to sepia, forming one septum. Based on these polyphasic approaches we identified our isolates as novel Neodeightonia species.

Discussion

Palms are one of the most widely planted trees worldwide (Sayan 2001). They are monocot plants that comprise over 181 genera and about 2,600 species (Christenhusz & Byng 2016). In a simple search on the USDA plant and fungal database (https://nt.ars-grin.gov/fungaldatabases/), a dazzling array of palm plants and fungal associations can be observed (Farr & Rosaman 2021). However, fungi on the *Arenga tremula* have not been well documented (Farr & Rosaman 2021). Additionally, there are no records on *Botryosphaeriaceae* species on *A. tremula arengae* and so the novel taxon described in this study is the first of such species to be reported on *Arenga tremula*.

Botryosphaeriaceae is a well-established family with opportunistic fungal pathogens (Phillips et al. 2013). Thus, the identification and characterization of species belonging to this family have both ecological and economic importance. The updated taxonomic treatments of this family are given in Hongsanan et al. (2020) and Zhang et al. (2021). The sexual morph of Neodeightonia is characterized by hyaline, aseptate ascospores, with polar apiculi and surrounded by a mucilaginous sheath (Liu et al. 2012). The asexual morph is characterized by conidia that are initially hyaline that may become brown and 1-septate upon maturity, with smooth to finely roughened walls or with fine striations (Phillips et al. 2008, Liu et al. 2012, Phillips et al. 2013, Dai et al. 2016, Konta et al. 2016). Based on these morphological characteristics and combined with phylogenetic analysis, seven Neodeightonia species are currently accepted (Phillips et al. 2008, Liu et al. 2010, Dai et al. 2016, Konta et al. 2016, Jayasiri et al. 2019).

Although the sexual morph of *Neodeightonia subglobosa* (type species) has been reported in cultures (Punithalingam 1969), we could not observe the sexual morph of *Neodeightonia arengae* after two months of incubation on PDA at 25°C. The only pathogenicity record on this genus is palm rot caused by *N. phoenicum* (Ligoxigakis *et al.* 2013). However, in this study, we isolated a species from dead leaf materials. Therefore, whether this novel species is the main pathogen of the diseased host previously described remains to be studied.

 TABLE 3. Comparison of Asexual morph characters of Neodeightonia species.

Neodeightonia arengae Neodeightonia arlamical Neodeightonia arlamical Neodeightonia arlamicola Neodeightonia arlamicola Neodeightonia arengae $245-349$ high \times $208-305$ μm diam. $6.1-11.2 \times 2.1-3.2$ μm MFLUCC 15-0712 $420-510$ high \times $357-443$ μm diam. $4.4-12.5 \times 1.5-4.3$ μm Neodeightonia palmicola N/A N/A 12528 Neodeightonia arengae 122528 Neodeightonia arengae $1223-325$ high $128-189$ μm diam. N/A $123-189$ μm diam.	n. 6.1 –11.2 × 2.1–3.2 µm			
2 2	; ; ; ;	19–22 × 7–9 μm	MEA fast growing, after 2 weeks, 7–8.5 cm diam. at 25°C.	Konta <i>et al.</i> 2016
ı e	m. $4.4 - 12.3 \times 1.3 - 4.5 \mu m$	13–20 × 7–8 μm	MEA fast growing, after 2 weeks, 7–8.5 cm diam. at 25°C.	Konta <i>et al.</i> 2016
2	N/A	$(14.5-)17-21(-24) \times (9-)10-12.5(-14) \mu m \text{ 1-septate}$ N/A	N/A	Phillips <i>et al.</i> 2008, 2013
	$9-20 \times 3-6 \ \mu m$.	$17.5 - 24.5 \times 9.5 - 12.5 \mu m$ 1-septate	PDA fast growing, after 4 days 50 mm. Liu et al. 2010	Liu <i>et al.</i> 2010
Neodejohtonia nlanchoniae	n. N/A	16.4–26.4 × 9.7–13.1µm	PDA fast growing, after 4 days 730 mm.	This study
MFLU 18–2140			Undetermined	Jayasiri SC <i>et al.</i> 2019
Neodeightonia licuriensis N/A COAD1780	8.7–16.2 × 2.3–4.4 µm	14.6-20.5 × 8.3-11.4 µm	grew to 90 mm diam. in 10 days at 25°C on PDA	Adamčík <i>et al.</i> 2015
Neodeightonia microspora N/A MFLUCC 11–0483	N/A	N/A	Undetermined	Dai <i>et al.</i> 2016
Neodeightonia subglobosa CBS 448.91 150–200 µm diam. (type species)	5–12.5 × 2–3μm	11–13.5 × 8–10.5 µm	After I week, 5 cm diam. at 28°C	Phillips <i>et al.</i> 2008, 2013, Lui <i>et al.</i> 2015, Dai <i>et al.</i> 2016

Acknowledgements

This research was funded by the Key Realm R&D Program of Guangdong Province, grant no: 2018B020205003. The work was partly funded by the High-level Talents in Zhongkai University of Agriculture and Engineering, grant no: J2201080102.

References

- Adamčík, S., Cai, L., Chakraborty, D., Chen, X.H., Cotter, H.V.T., Dai, D.Q., Dai, Y.C., Das, K., Deng, C.Y., Masoomeh, G.N., Hyde, K.D., Langer, E., Latha, K.P.D., Liu, F., Liu, S.L., Liu, T.T., Wei, L.V., Shu-Xia, LV, Machado, A.R., Pinho, D.B., Pereira, O.L., Prasher, I.B., Rosado, A.W.C., Qin, J., Qin, W.M., Verma, R.K., Wang, Q., Yang, Z.L., Yu, X.D., Zhou, L.W., Buyck, B. (2015) Fungal Biodiversity Profiles 1–10. *Cryptogamie, Mycologie* 36 (2): 121–166. https://doi.org/10.7872/crym/v36.iss2.2015.121
- Arx, J.A.V. & Müller, E. (1975) A re-evaluation of the bitunicate Ascomycetes with keys to families and genera. *Studies in Mycology* 9: 1–159. [Baarn]
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series* 41: 95–98.
- Cai, W.Q., Wang, Z., Deng, J.Y. (2007) Occurrence status and control strategies of dangerous palm plant diseases and insect pests in Guangdong Province. *Hunan Forestry Science and Technology* 2007 (03): 48–50. [https://kns.cnki.net/kcms/detail/detail.aspx?File Name=HLKJ200703016&DbName=CJFQ2007]
- Carbone, I. & Kohn, L. (1999) A method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. *Mycologia* 91 (3): 553–556.
 - https://doi.org/10.2307/3761358
- Chethana, K.W.T., Manawasinghe, I.S., Hurdeal, V.G., Bhunjun, C.S., Appadoo, M.A., Gentekaki, E., Raspé, O., Promputtha, I. & Hyde, K.D. (2021) What are fungal species and how to delineate them? *Fungal Diversity* 109: 1–25. https://doi.org/10.1007/s13225-021-00483-9
- Christenhusz, M.J.M. & Byng, J.W. (2016) The number of known plants species in the world and its annual increase. *Phytotaxa* 261 (3): 201–207.
 - http://dx.doi.org/10.11646/phytotaxa.261.3.1
- Dai, D.Q., Phookamsak, R., Wijayawardene, N.N., Li, W.J., Bhat, D.J., Xu, J.C., Taylor, J.E., Hyde, K.D. & Chukeatirote, E. (2016) Bambusicolous fungi. *Fungal Diversity* 82 (1): 1–105. https://doi.org/10.1007/s13225-016-0367-8
- Frohlich, J., Taylor, J.E. & Hyde, K.D. (2000) Palm microfungi. Fungal Diversity Press.
- Gao, W., Tu, R.X., Li, H., Fang, Y.L. & Que, Q.M. (2020) In the Subtropical Monsoon Climate High-Density City, What Features of the Neighborhood Environment Matter Most for Public Health? *International Journal of Environmental Research and Public Health* 17: 9566.
 - https://doi.org/10.3390/ijerph17249566
- Garcia, J.F., Lawrence, D.P., Morales-Cruz, A., Travadon, R., Minio, A., Hernandez-Martinez, R., Rolshausen, P.E., Baumgartner, K. & Cantu, D. (2021) Phylogenomics of Plant-Associated *Botryosphaeriaceae* Species. *Frontiers in microbiology* 12: 652802. https://doi.org/10.3389/fmicb.2021.652802
- Hongsanan, S., Hyde, K.D., Phookamsak, R., Wanasinghe, D., Mckenzie, E., Sarma, V., Lücking, R., Boonmee, S., Bhat, D.J., Liu, N.G., Tennakoon, D., Pem, D., Karunarathna, A., Jiang, S.H., Jones, G., Phillips, A., Manawasinghe, I., Tibpromma, S., Jayasiri, S., Sandamali, D., Jayawardena, R.S., Wijayawardene, N.N., Ekanayaka, A.H., Jeewon, R., Lu, Y.Z., Phukhamsakda, C., Dissanayake, A.J., Zeng, X.Y., Luo, Z.L., Tian, Q., Thambugala, K.M., Dai, D.Q., Samarakoon, M.C., Chethana, K.W.T., Ertz, D., Doilom, M., Liu, J.K., Pérez Ortega, S., Suija, A., Senwanna, C., Wijesinghe, S.N., Niranjan, M., Zhang, S.N., Ariyawansa, H.A., Jiang, H.B., Zhang, J.F., Norphanphoun, C., Silva, N.I.D., Thiyagaraja, V., Zhang, H., Bezerra, J.D.P., Miranda González, R., Aptroot, A., Kashiwadani, H., Harishchandra, D., Sérusiaux, E., Abeywickrama, P.D., Bao, D.F., Devadatha, B., Wu, H.X., Moon, K.H., Gueidan, C., Schumm, F., Bundhun, D., Mapook, A., Monkai, J., Bhunjun, C.S., Chomnunti, P., Suetrong, S., Chaiwan, N., Dayarathne, M.C., Yang, J., Rathnayaka, A.R., Xu, J.C., Zheng, J.S., Liu, G., Feng, Y. & Xie, N. (2020) Refined families of Dothideomycetes: orders and families incertae sedis in Dothideomycetes. *Fungal Diversity* 104: 1–302.
 - https://doi.org/10.1007/s1322 5-020-00462-6
- Hyde, K.D. (1994) Fungal from Palms. XIII. The genus Oxydothis, a revision. Sydowia 46: 265-314.

- Hyde, K.D. & Cannon, P.F. (1999) Fungal Causing Tar Spots on Palms. Mycological Papers 175: 1–114.
- Hyde, K.D., Taylor, J.E. & Fröhlich, J. (2000) Genera of Ascomycetes from Palms. Fungal Diversity Press.
- Huelsenbeck, J.P., Ronquist, F. (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754.
- Jayasiri, S.C., Hyde, K.D., Ariyawansa, H.A., Bhat, J., Buyck, B., Cai, L., Dai, Y.C., AbdElsalam, K.A., Ertz, D., Hidayat, I., Jeewon, R., Jones, E.B.G., Bahkali, A.H., Karunarathna, S.C., Liu, J.K., Luangsaard, J.J., Lumbsch, H.T., Maharachchikumbura, S.S.N., McKenzie, E.H.C., Moncalvo, J.M., GhobadNejhad, M., Nilsson, H., Pang, K.L., Pereira, O.L., Phillips, A.J.L., Raspé, O., Rollins, A.W., Romero, A.I, Etayo, J., Selçuk, F., Stephenson, S.L., Suetrong, S., Taylor, J.E., Tsui, C.K.M., Vizzini, A., AbdelWahab, M.A., Wen, T.C., Boonmee, S., Dai, D.Q., Daranagama, D.A., Dissanayake, A.J., Ekanayaka, A.H., Fryar, S.C., Hongsanan, S., Jayawardena, R.S., Li, W.J., Perera, R.H., Phookamsak, R., de Silva, N.I., Thambugala, K.M., Tian, Q., Wijayawardene, N.N., Zhao, R.L., Zhao, Q., Kang, J.C., Promputtha, I. (2015) The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. *Fungal Diversity* 74: 3–18. http://dx.doi.org/10.1007/s13225-015-0351-8
- Jayasiri, S.C., Hyde, K.D., Jones, E.B.G., McKenzie, E.H.C., Jeewon, R., Phillips, A.J.L., Bhat, D.J., Wanasinghe, D.N., Liu, J.K., Lu, Y.Z., Kang, J.C., Xu, J., Karunarathna, S.C. (2019) Diversity, morphology and molecular phylogeny of Dothideomycetes on decaying wild seed pods and fruits. *Mycosphere* 10 (1): 1–186. https://doi.org/10.5943/mycosphere/10/1/1
- Konta, S., Hongsanan, S., Phillips, A.J.L., Jones, E.B.G., Boonmee, S., Hyde, K.D. (2016) Botryosphaeriaceae from palms in Thailand II two new species of Neodeightonia, N. rattanica and N. rattanicola from Calamus (rattan palm). Mycosphere 7 (7): 90–961. https://doi.org/10.5943/mycosphere/si/1b/6
- Ligoxigakis, E.K., Markakis, E.A., Papaioannou, I.A., Typas, M.A. (2013) First Report of Palm Rot of *Phoenix* spp. Caused by *Neodeightonia phoenicum* in Greece. *Plant Disease* 97 (2): 286. https://www.researchgate.net/publication/263040162
- Liu, J.K., Chomnunti, P., Cai, L., Phookamsak, R., Chukeatirote, E., Jones, E.B.G., Moslem, M., Hyde, K.D. (2010) Phylogeny and morphology of *Neodeightonia palmicola sp. nov.* from palms. *Sydowia* 62: 261–276. [https://www.researchgate.net/publication/249968435]
- Liu, J.K., Phookamsak, R., Doilom, M., Wikee, S., Li, Y.M., Ariyawansha, H., Boonmee, S., Chomnunti, P., Dai, D.Q., Bhat, J.D., Romero, A.I., Zhuang, W.Y., Monkai, J., Gareth, J.E.B., Chukeatirote, E., Thida, W.K.K, Zhao, Y.C., Wang, Y. & Hyde, K.D. (2012) Towards a natural classification of *Botryosphaeriales*. *Fungal Diversity* 57 (1): 149–210. https://doi.org/10.1007/s13225-012-0207-4
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. *In: Gateway Computing Environments Workshop (GCE)*. pp. 1–8. https://doi.org/10.1109/GCE.2010.5676129
- NSII (2017) The NSII Specimen search, version 2020, Beijing China. Available from: http://www.nsii.org.cn/2017/query.php?name=\u5e7f\u4e1c (accessed 23 May 2021).
- Pem, D., Jeewon, R., Chethana, K.W.T. Hongsanan, S., Doilom, M., Suwannarach, N. & Hyde, K.D. (2021) Species concepts of Dothideomycetes: classification, phylogenetic inconsistencies and taxonomic standardization. *Fungal Diversity* 109: 283–319. https://doi.org/10.1007/s13225-021-00485-7
- Phillips, A.J.L., Alves, A., Pennycook, S.R., Johnston, P.R., Ramaley, A., Akulov, A. & Crous, P.W. (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the *Botryosphaeriaceae*. *Persoonia* 21: 29–55. https://doi.org/10.3767/003158508X340742
- Phillips, A.J.L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M.J., Groenewald, J.Z. & Crous, P.W. (2013) The *Botryosphaeriaceae*: genera and species known from culture. *Studies in Mycology* 76 (1): 51–167. https://doi.org/10.3114/sim0021
- Pinruan, U., Rungjindamai, N., Sakayaroj, J., Lumyong, S., Hyde, K.D. & Jones, E.B.G. (2010) *Baipadisphaeria gen. nov.*, a freshwater ascomycete *Hypocreales*, *Sordariomycetes* from decaying palm leaves in Thailand. *Mycosphere* 1 (1). [https://www.cabdirect.org/cabdirect/abstract/20198647685]
- Punithalingam, E. (1969) Studies on Sphaeropsidales in culture. Commonwealth Mycological Institute.
- Rayner RW. (1970) A Mycological Colour Chart. Kew, UK: Commonwealth Mycological Institute & British Mycological Society.
- Sayan, M.S. (2001) Landscaping with Palms in the Mediterranean. Palms 45 (4): 171-176
- Senanayake, I.C., Rathnayaka, A.R., Marasinghe, D.S., Calabon, M.S., Gentekaki, E., Lee, H.B., Hurdeal, V.G., Pem, D., Dissanayake, L.S., Wijesinghe, S.N., Bundhun, D., Nguyen, T.T., Goonasekara, I.D., Abeywickrama, P.D., Bhunjun, C.S., Jayawardena, R.S., Wanasinghe, D.N., Jeewon, R., Bhat, D.J., Xiang, M.M. (2020) Morphological approaches in studying fungi: collection, examination, isolation, sporulation and preservation. *Mycosphere* 11: 2678–2754.

- https://doi.org/10.5943/mycosphere/11/1/20
- Slippers, B. & Wingfield, M.J. (2007) *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* 21 (2–3): 90–106.
 - https://doi.org/10.1016/j.fbr.2007.06.002
- Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57 (5): 758–771.
 - https://doi.org/10.1080/10635150802429642
- Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30 (9): 1312–1313
 - https://doi.org/10.1093/bioinformatics/btu033
- Swofford, D.L. (2003) PAUP: phylogenetic analysis using parsimony, Version 4.0 b10. Laboratory of Molecular Systematics Smithsonian Institution. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3348772 (accessed 1 September 2016)
- Taylor, J.E. & Hyde, K.D. (2003) Microfungi of Tropical and Temperate Palms. *Fungal Diversity Press* 108 (2): 223–223. https://doi.org/10.1017/S0953756204249668
- Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Wu, N., Dissanayake, A.J., Manawasinghe, I.S., Rathnayaka, A.R., Liu, J.K., Phillips, A.J.L., Promputtha, I. & Hyde, K.D. (2021) https://botryosphaeriales.org/, an online platform for up-to-date classification and account of taxa of Botryosphaeriales. Database article ID baab061.
 - https://doi.org/10.1093/database/baab06
- Zhang, W., Groenewald, J.Z., Lombard, L., Schumacher, R.K., Phillips, A.J.L. & Crous, P.W. (2021) Evaluating species in Botryosphaeriales. *Persoonia* 46: 63–115.
 - https://doi.org/10.3767/persoonia.2021.46.03