

Phylogeny and morphology of *Neodeightonia palmicola* sp. nov. from palms

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Palm fungi are a taxonomically diverse group. Recent collections of fungi from palms in northern Thailand resulted in the discovery of a new species of *Neodeightonia*, herein described as *N. palmicola*. This new species is distinct in having hyaline, aseptate ascospores surrounded by a remarkable sheath. This study compares this new species with related taxa of *Botryosphaeria* and *Neodeightonia* using morphological and molecular characteristics. Sequence data show our species is more closely related to *Neodeightonia subglobosa* (the type species of the genus) than to *Botryosphaeria* species. Morphological and molecular features of the new species are described.

Keywords: ascomycetes, Botryosphaeriaceae, ITS, LSU, SSU, taxonomy.

Palm fungi are a taxonomically diverse group, with more than 1500 described species, which include representatives from almost all major fungal classes (Fröhlich & Hyde 2000, Taylor & Hyde 2003). The ascomycetes which have been described from palms are a very diverse assemblage. In 2000, 794 species of ascomycetes distributed in 278 genera and 79 families had been described from palms (Fröhlich & Hyde 2000, Hyde *et al.* 2000, Taylor & Hyde 2003) and many more have recently been described (Pinruan *et al.* 2008, 2010, Pinnoi *et al.* 2010).

Botryosphaeria-like species and their anamorphs have previously been recorded from palms (Fröhlich & Hyde 1995, 2000, Hyde *et al.* 2000, Taylor & Hyde 2003, Phillips *et al.* 2008), including six *Botryosphaeria* species (Taylor & Hyde 2003). *Botryosphaeria* Ces. & De Not. was introduced by Cesati & De Notaris (1863). Species of *Botryosphaeria* are cosmopolitan and occur on a wide range of monocotyle-

donous, dicotyledonous and gymnosperm hosts, on woody branches, herbaceous leaves, stems and culms of grasses, or on twigs and in the thalli of lichens (Barr 1987, Denman *et al.* 2000, Mohali *et al.* 2007, Lazzizzera *et al.* 2008, Marincowitz *et al.* 2008). Taxa range in habit from being saprobic, to parasitic or endophytic (Smith *et al.* 1996, Denman *et al.* 2000, Slippers & Wingfield 2007, Huang *et al.* 2008, Pérez *et al.* 2010) and can cause die-back and canker diseases of numerous woody hosts (von Arx 1987, Phillips *et al.* 2007, Slippers *et al.* 2007, Alves *et al.* 2008, Lazzizzera *et al.* 2008, Marincowitz *et al.* 2008, Zhou *et al.* 2008, Phillips & Alves 2009, Pérez *et al.* 2010). *Botryosphaeria* has been shown to be a polyphyletic genus comprising several teleomorphic and anamorphic groups such as *Barriopsis*, *Dothidotthia*, *Dothiorella*, *Gibberella*, *Guignardia*, *Lasiodiplodia*, *Lisea*, *Phaeobotryon*, *Phaeobotrosphaeria* and *Neodeightonia* (Saccardo 1877, Punithalingam 1969, von Arx & Müller 1975, Barr 1989, Hyde 1995, Slippers *et al.* 2004a, Crous *et al.* 2006, Alves *et al.* 2008, Lazzizzera *et al.* 2008, Phillips *et al.* 2008, Abdollahzadeh *et al.* 2009, Pitt *et al.* 2010).

Neodeightonia C. Booth was introduced by Booth (1969). However, von Arx & Müller (1975) transferred the type, *N. subglobosa* to *Botryosphaeria*, reducing *Neodeightonia* to synonymy under *Botryosphaeria*. Phillips *et al.* (2008) reinstated this genus, which is distinguishable from *Botryosphaeria* morphologically (based on the dark, 1-septate ascospores) and phylogenetically and described a new species *N. phoenicum*.

In this study, we collected an interesting fungus from palms in Chiang Rai, Thailand. The taxon fits well with the current concept of *Neodeightonia*, and can be distinguished from *Botryosphaeria* by the uniloculate ascomata and aseptate ascospores surrounded by a well-developed sheath (von Arx & Müller 1954, Punithalingam 1969, Hyde *et al.* 2000, Taylor & Hyde 2003, Alves *et al.* 2004, Phillips *et al.* 2005, 2008). Critical morphological examination showed that this palm fungus differs from all previously described species in *Neodeightonia* (Punithalingam 1969, Phillips *et al.* 2008), and we therefore described it here as a new species.

Materials and Methods

Isolation and identification

Palm fronds were collected from Khun Korn Waterfall, Chiang Rai Province, Thailand. Samples were processed and examined following the methods described in Hyde *et al.* (2000) and Taylor & Hyde (2003).

Isolations were made from single ascospores. Ascomata were cut through horizontally and the contents were transferred to a drop of sterile water on a flamed microscope slide. A portion of this was taken

and spread over a few square centimeters of a plate of 2 % water agar (WA). The Petri dish bearing the spores was incubated at 25 °C overnight. The next day individual germinating spores were transferred to fresh plates of PDA (Alves *et al.* 2006). To induce sporulation, isolates were grown on 2 % water agar (WA) (Biolab, S.A) with sterilized pine needles placed onto the medium, at 25 °C near-UV light.

Observations and photomicrographs were made from material mounted in water using Nikon ECLIPSE 80i microscope. Measurements were made with the Tarosoft (R) Image Frame Work. From measurements of 50 conidia and ascospores the mean, standard deviation and 95 % confidence intervals were calculated. Dimensions are given as 95 % confidence limits with minimum and maximum dimensions in parentheses. Dimensions of other fungal structures are given as the range of at least 20 measurements. Mean values are in brackets with 'n' being the number of measurements. The holotype is deposited at the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Fungi isolated in our study were deposited at Mae Fah Luang University Culture Collection (MFLUCC) and BIOTEC Culture Collection (BCC).

DNA extraction, PCR amplification and sequencing

Fungal isolates were grown on PDA for 7 d at 25 °C in the dark. Genomic DNA was extracted from the fresh mycelium using Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) according to the manufacturer's protocol (Hangzhou, P.R. China).

DNA amplification was performed by polymerase chain reaction (PCR). Primer pairs NS1 and NS4 as defined by White *et al.* (1990) were used to amplify a region spanning of the small subunit rDNA. LROR and LR5 primer pairs as defined by Vilgalys and Hester (1990) were used to amplify a segment of the large subunit rDNA. Primer pairs ITS4 and ITS5 as defined by White *et al.* (1990) were used to amplify the internal transcribed spacers. The amplifications were performed in a 50 µl reaction volume as follows: 1X PCR buffer, 0.2 mM dNTP, 0.3 µM of each primer; 1.5 mM MgCl₂, 0.8 units Taq Polymerase and 5–10 ng DNA (Cai *et al.* 2009). The amplification conditions were as follows: initial denaturation of 5 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 48 °C and 90 s at 72 °C, and a final extension period of 10 min at 72 °C (Phillips *et al.* 2008). The PCR products were checked on 1 % agarose electrophoresis gels stained with ethidium bromide.

PCR products were then purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27-9602-01). The sequences were carried out by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, P.R. China). For each fungal strain, sequences obtained for

Table 1. – Isolates considered in this phylogenetic study.

Accession no.	Species	Host	Collector	Locality	GenBank		
					ITS	SSU	LSU
CBS678.88	<i>Botryosphaeria corticola</i>				AY259104		DQ377847
CBS112545	<i>B. corticola</i>	<i>Quercus suber</i>	M.E. Sánchez	Spain, Cádiz	AY259089		DQ377848
CBS112549	<i>B. corticola</i>	<i>Q. suber</i>	A. Alves	Portugal, Aveiro	AY259100	EU673206	AY928051
CMW7999	<i>B. dothidea</i>	<i>Ostrya sp.</i>	B. Slippers	Crociifisso Switzerland	AY236948		
CMW8000	<i>B. dothidea</i>	<i>Prunus sp.</i>	B. Slippers	Crociifisso, Switzerland	AY236949	DQ677998	AY928047
CMW9075	<i>B. dothidea</i>	<i>Populus nigra</i>	G. J. Samuels	New Zealand	AY236950		
KJ93.12	<i>B. dothidea</i>	<i>Prunus sp.</i>	K.A. Jacobs	District of Columbia, USA	AF027746		
KJ93.23	<i>B. dothidea</i>	<i>Syringa vulgaris</i>	K.A. Jacobs	Maryland, USA.	AF027751		
KJ94.26	<i>B. dothidea</i>	<i>Populus persica</i>	P.L. Pusey	Japan	AF027749		
CBS115041	<i>B. iberica</i>	<i>Q. ilex</i>	J. Luque	Spain, Aragon	AY573202	EU673155	AY928053
CBS110299	<i>B. lutea</i>	<i>Vitis vinifera</i>	A.J.L. Phillips	Portugal, Oeiras	AY259091	EU673148	AY928043
CMW9076	<i>B. lutea</i>	<i>Malus domestica</i>	S.R. Pennycook	New Zealand	AY236946		
GS97-59	<i>B. mamane</i>	<i>Sophora chrysophylla</i>	D.E. Gardner	Hawaii	AF246930		
CMW4049	<i>B. prava</i>	<i>Eucalyptus grandis</i>	M.J. Wingfield	Sumatra, Indonesia	AY236937		
CMW9080	<i>B. prava</i>	<i>Populus nigra</i>	G.J. Samuels	New Zealand	AY236942		
CMW9077	<i>B. prava</i>	<i>Actinidia deliciosa</i>	S.R. Pennycook	New Zealand	AY236939		
CMW994	<i>B. prava</i>	<i>Malus sylvestris</i>	G.J. Samuels	New Zealand	AY243395		

KJ94.09	<i>B. ribis</i>	<i>Melaleuca quin-querervia</i>	M.B. Rayachhetry	Florida	AF027743	
CMW7054	<i>B. ribis</i>	<i>Ribes rubrum</i>	N.E. Stevens	New York	AF241177	
CMW7772	<i>B. ribis</i>	<i>Ribes</i> sp.	B. Slippers, G. Hudler	New York	AF236935	AY928044
CMW7773	<i>B. ribis</i>	<i>Ribes</i> sp.	B. Slippers, G. Hudler	New York	AF236936	DQ246263
IMI63581b	<i>B. sarmentorum</i>	<i>Ulmus</i> sp.	E.A. Ellis	New York	AY573212	EU673158 AY928052
CBS110496	<i>Diplodea porosum</i>	<i>V. vinifera</i>	A.J.L. Phillips	South Africa	AY2590093	EU67317 DQ377894
WAC12533	<i>Lasiodiplodia crassispora</i>	<i>Santalum album</i>	T.I. Burgess, B. Dell	Kununurra, Australia	DQ103550	DQ377901
CMW14077	<i>L. gonubiensis</i>	<i>Syzygium cordatum</i>	D. Pavlic	Eastern Cape, S. Africa	AY639595	DQ377902
CBS116459	<i>L. pseudotheobromae</i>	<i>Gmelina arborea</i>	J. Carranza-Velásquez	Costa Rica,	EF622077	EU673199 EU673256
CBS124.13	<i>L. theobromae</i>	<i>Unknown</i>	J.J. Taubenhaus	U.S.A	DQ458890	EU673195 AY928054
CBS111530	<i>L. theobromae</i>	<i>Leucospermum</i> sp.	J.E. Taylor	Hawaii	EF622074	
CBS169.34	<i>Neodeightonia phoenicum</i>	<i>Phoenix dactylifera</i>	H.S. Fawcett	California	EU673338	EU673203 EU673259
CBS122528	<i>N. phoenicum</i>	<i>Phoenix dactylifera</i>	F. Garcia,	Spain	EU673340	EU673205 EU673261
CBS123168	<i>N. phoenicum</i>	<i>P. canariensis</i>	M. Rojo	Spain	EU673339	EU673204 EU673260
CBS448.91	<i>N. subglobosa</i>	<i>Keratomycesis in eye</i>		United Kingdom	EU673337	DQ377866
MFLUCC10 0822	<i>N. palmicola</i>	<i>Arenga westerhoutii</i>	J.K. Liu	Thailand	HQ199221	HQ199223 HQ199222
MFLUCC10 0823	<i>N. palmicola</i>	<i>Caryota urens</i>	J.K. Liu, R. Phookamsak	Thailand	HQ199224	HQ199226 HQ199225

Abbreviations of isolates and culture collections: BCC = BIOTEC Culture Collection, Bangkok, Thailand; CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; IMI = CABI Bioscience, Egham, UK; KJ = Jacobs and Rehner 1998; MFUCC = Mae Fah Luang University Culture Collection; WAC = Department of Agriculture Western Australia Plant Pathogen Collection, Perth, Australia.

the respective primers (ITS4 and ITS5, LROR and LR5, NS1 and NS4) were manually aligned to obtain an assembled sequence using Bioedit (Hall 1999). The reference nucleotide sequences of ITS, LSU and SSU regions of various genera *Botryosphaeria*, *Diplodia*, *Lasiodiplodia* and *Neodeightonia* were obtained from GenBank (Table 1).

Phylogenetic analysis

Sequences were aligned using ClustalX v. 1.83 (Thompson *et al.* 1997). The alignments were checked visually and improved manually where necessary. Phylogenetic analyses were performed by using PAUP v. 4.0b10 (Swofford 2002) for Maximum-parsimony (MP) analyses and MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003) for Bayesian analyses. Trees were rooted to *Diplodia porosum* and visualized with TreeView (Page 1996).

Maximum-parsimony analyses were performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis & Bull 1993).

The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run for 1 000 000 generations and trees were sampled every 100th generation (resulting 10 000 total trees). The first 2000 trees, which represented the burn-in phase of the analyses, were discarded and the remaining 8000 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai *et al.* 2006, 2008).

Results

Phylogenetic analysis

The ITS, LSU and SSU sequences for the isolates studied were combined and aligned with 32 sequences of 15 taxa retrieved from GenBank, representing a selection of genera and species in the *Botryosphaeriaceae* (Table 1). Incomplete portions at the ends of the sequences were excluded from the analyses. The combined dataset after alignment consisted of 1943 characters including gaps. A partition homogeneity test in PAUP was not significant ($P = 0.47$) indicating that the individual datasets were congruent and produced trees with the similar

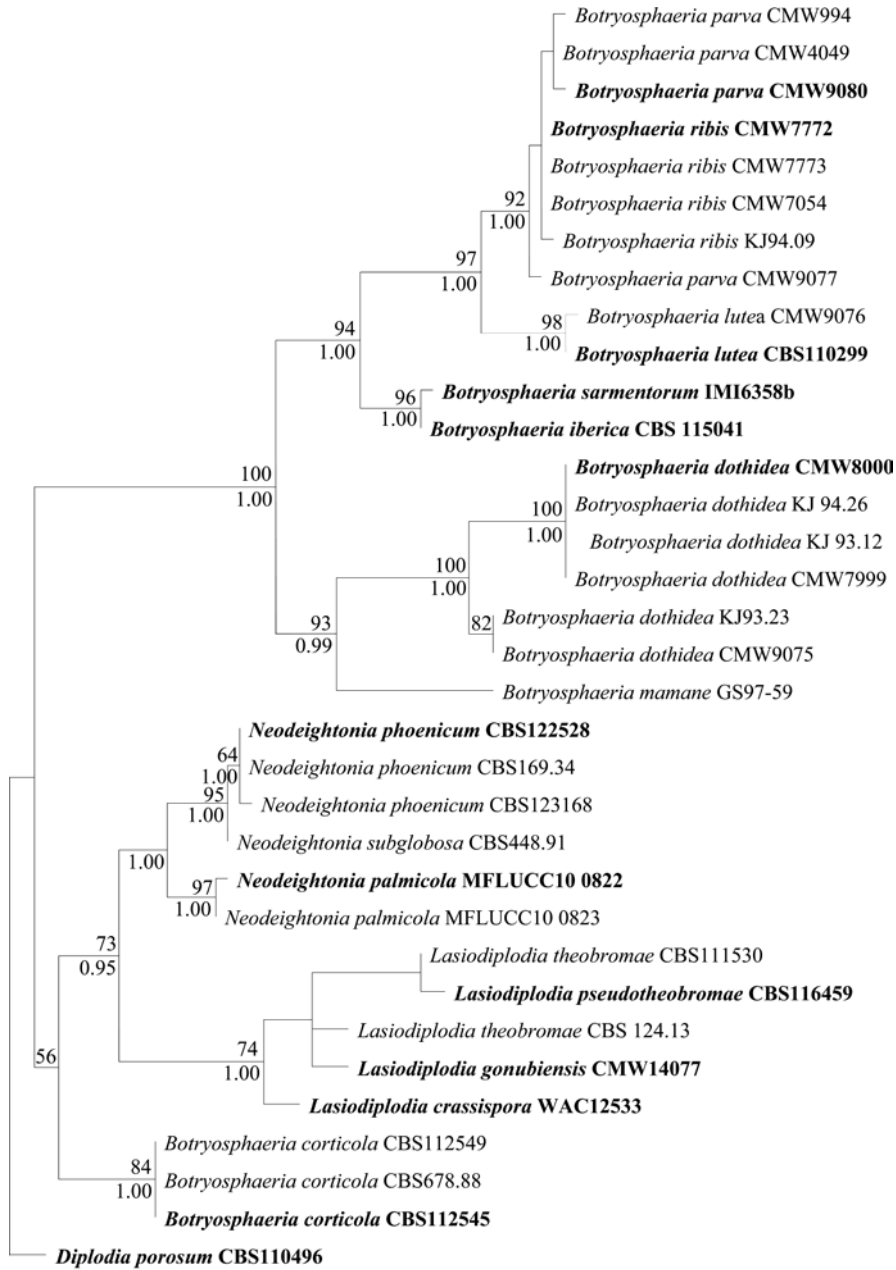


Fig. 1. – Phylogenetic tree generated from parsimony analysis based on combined ITS rDNA sequences, LSU and SSU. Data were analyzed with random addition sequence and treating gaps as missing data. MP bootstrap values $\geq 50\%$ and Bayesian values $\geq 95\%$ are shown above and below branches, respectively. The tree is rooted with *Diplodia porosum*. The type species and new species are in bold face.

topology. Therefore the three datasets were combined in a single analysis. Of the 1943 characters, 1798 were constant, while 29 were variable and parsimony-uninformative. Maximum parsimony analysis of the remaining 95 parsimony informative characters resulted in a single tree with TL = 160, CI = 0.802, RI = 0.944, RC = 0.757 and HI = 0.198. The overall topology of the 50 % majority rule consensus tree of 8000 trees sampled in the Bayesian analysis had a similar topology as the MP tree, which is presented in Fig. 1 with bootstrap support above the branches. The Bayesian trees are therefore not shown, but the Bayesian values are marked below branches of parsimony tree (Fig. 1).

Isolates obtained in this study clustered in the *Neodeightonia* clade and were distinct from the *Botryosphaeria* and *Lasiodiplodia* clades. The two isolates from palms formed a sister group to *N. subglobosa* and *N. phoenicum* within the *Neodeightonia* clade with high bootstrap support (97 %, Fig. 1).

Taxonomy

Based on morphological characteristics as well as DNA phylogeny, we conclude that the fungus collected from palms in Thailand is distinct from *N. subglobosa* and *Botryosphaeria* spp. compared in our study. Our data also indicate that this fungus should reside in *Neodeightonia* as a new taxon. We provide the following description for this new species.

Neodeightonia palmicola J.K. Liu, R. Phookamsak and K.D. Hyde., **sp. nov.**—Figs. 2,3

Mycobank: MB 518804

Etymology: Referring to the host on which the fungus was collected.

Ascomata globosa vel subglobosa, 180–230 µm high, 270–420 µm diametro, immersa vel erumpescentia, uniloculata. Ostiolis centralibus. Pseudoparaphyses hyalines, filiformes, septatis. Asci 110–210 (–225) × 17–22.5 (–24) µm, cylindricae to clavatae, stipitate, bitunicate, 8-sporae. Ascosporae 23–32 × 8–13 µm, ellipsoideae vel fusiformes, unicellulares, hyalinae vel sheath. Pycnidia subimmersa, solitaria, globosa vel subglobosa, black, mycelio tecta, usque ad 240 µm diametro. Paraphyses cylindricae, non septatae, hyalinae. Cellulae conidiogenae holoblasticae, cylindricae, hyalinae. Conidia primaria hyalinae, unicellulares, ellipsoideia vel obovoid-eia, contentu granulati, apice rotundata, interdum basi truncata. Conidia senia cinnamomescentia vel brunnescentia, unum ad tria septa formantia.

HOLOTYPUS: THAILAND, Chiang Rai Prov., Muang District, Khun Korn Waterfall, on dead leaves of *Arenga westerhoutii*, 18 Dec 2009, J.K. Liu, JKA0022 (MFLU10 0407); ex-type culture MFLUCC10 0822.

Ascomata 180–230 µm high (excluding the neck), 270–420 µm diam., uniloculate, immersed to erumpent in host tissue, globose to subglobose, brown to dark brown, rounded at the base. Ostiole cir-

cular, central, papillate. Peridium 26–55 μm wide, comprising several layers of brown-walled cells, the outer stratum of 1–3 cells comprising thick, dark brown walls, the inner layer 3–5 cells, *textura angularis* comprising pale brown to hyaline, thin-walled cells. Pseudoparaphyses thin-walled, hyaline, frequently septate, often constricted at the septa, up to 3–5 μm diam. Asci (–80)110–210 (–225) \times 17–22.5 (–24) μm (\bar{x} = 154.2 \times 20.5 μm , n = 25), 8-spored, bitunicate, fissitunicate, endotunica thick-walled, clavate to cylindrical-clavate, stipitate, apically rounded, with a well developed ocular apical chamber (3.7–7.3 μm , n = 20), arising from the base of ascoma. Ascospores 23–31.5 \times 8.5–12.5 μm , (\bar{x} = 27 \times 10 μm , n = 50), obliquely uniseriate or irregularly biseriate, ellipsoidal-fusiform or fusiform, widest in the middle, both ends obtuse, 1-celled, aseptate, hyaline, smooth, thin-walled, with bipolar germ pores, surrounded by a complex sheath. Pycnidia (formed on WA on sterilized pine needles within 21–28 days) uniloculate, semi-immersed, solitary, globose, covered by mycelium, up to 240 μm wide, wall 4–8 cell layers thick, composed of dark brown thick-walled *textura angularis*, becoming thin-walled and hyaline toward the inner region. Paraphyses cylindrical, aseptate, hyaline. Conidiogenous cell holoblastic, cylindrical to subcylindrical, hyaline, 9–20 \times 3–6 μm . Conidia initially hyaline, unicellular, ellipsoid to obovoid, thick-walled with granular content, rounded at apex, occasionally truncate at base. Aging conidia becoming cinnamon to sepia, forming one septa, 17.5–24.5 \times 9.5–12.5 μm (\bar{x} = 21.5 \times 11.0 μm , n = 50).

In Culture: Circular sterile isolate, fast growing (50 mm in 4 days); Colour: The first two days colony is white and after 4 days becoming brownish grey (5F2); raised, fluffy, fairly dense, and aerial with an erose edge.

Distribution: Chiang Rai, Thailand.

Other material examined: THAILAND, Chiang Rai Prov., Muang District, Khun Korn Waterfall, on living leaves of *Caryota urens.*, 22 Jul 2009, R. Phookamsak, RP0004 (MFLU10 0409) – (culture MFLUCC10 0824); on dead leaves of *Caryota urens.*, 18 Dec 2009. J.K. Liu, JKC0004 (MFLU10 0408) – (culture MFLUCC10 0823).

Note: *Neodeightonia palmicola* has asci and ascospores of similar shape to *N. subglobosa*, the type species of the genus. The two taxa are also similar in uniloculate ascomata morphology and septa pseudoparaphyses. Despite these similarities, there are other characters that can readily differentiate two species. *N. palmicola* has larger ascospores (23–31.5 \times 8.5–12.5 μm vs. 20–26 \times 7–10 μm .) and asci (110–210 (–225) \times 17–22.5 (–24) μm vs. 110–130 (–140) \times 16–20 μm). Most importantly, *N. palmicola* has hyaline; aseptate ascospores surrounded by a remarkable well developed sheath which is different to *N. subglobosa* where the mature ascospores are described as brown and 1-septate. Furthermore, their anamorph characters are different. *N. palmicola* has ellip-

soid to obovoid, 1-septate, conidia (17.5–24.5 × 9.5–12.5 μm), which are larger than that of *N. subglobosa* with spherical to subglobose, aseptate, conidia (9–12 × 6–9 μm).

The isolates from palms, irrespective of whether they were derived from single ascospores or conidia, were morphologically similar. Colonies on PDA formed abundant aerial mycelium that was initially white but turned dark olivaceous after 3–4 d at room temperature. The re-

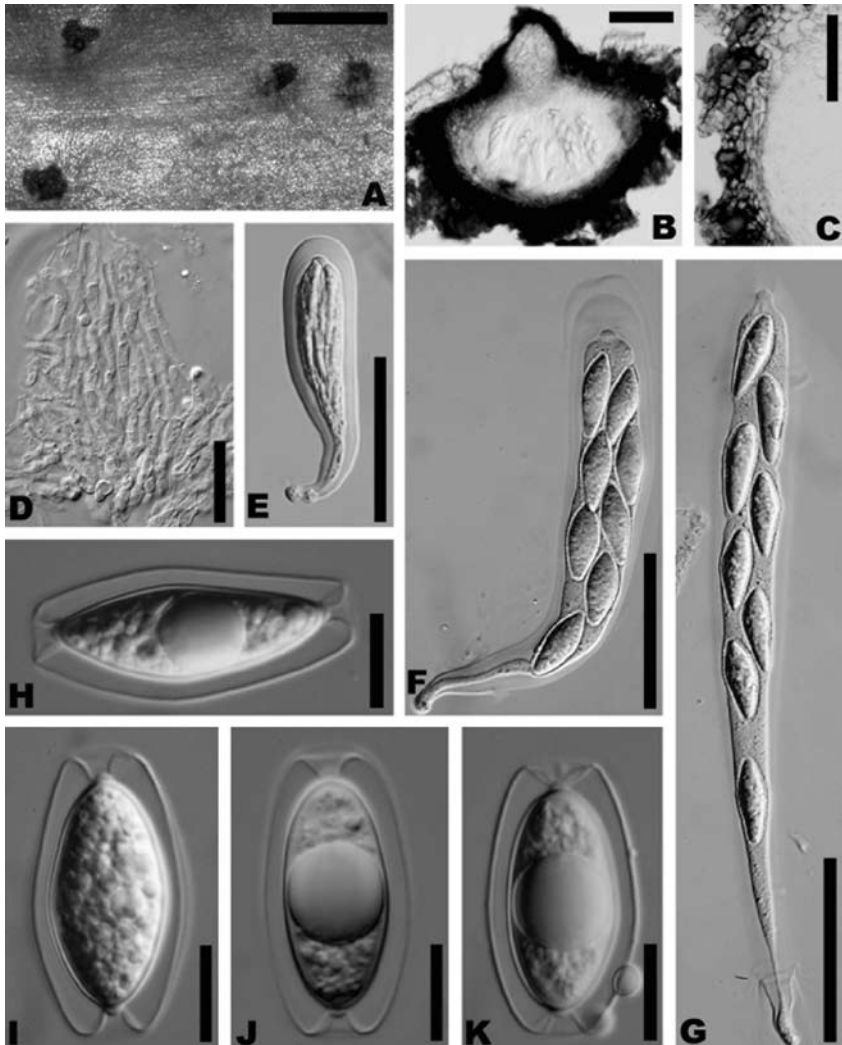


Fig. 2. – *Neodeightonia palmicola* (Holotype). **A.** Appearance of ascomata on host surface. **B.** Section of ascoma. **C.** Section of peridium comprising a few cells layers of textura angularis. **D.** Pseudoparaphyses. **E–G.** Ascus. **H–K.** Ascospores with a well developed sheath. Scale bars: A = 1 mm, B,C = 100 μm, D = 30 μm, H– K = 10 μm.

verse side was almost black in older cultures. Pycnidia appeared on the pine needles on WA within 21–28 d, the conidia (Fig. 3. F–J) were similar to *Diplodia corticola*, the anamorph of *B. corticola* and *N. phoenicum* in shape and colour. These isolates, however, differed from *D. corticola* in having markedly smaller conidia ($17.5\text{--}24.5 \times 9.5\text{--}12.5 \mu\text{m}$),

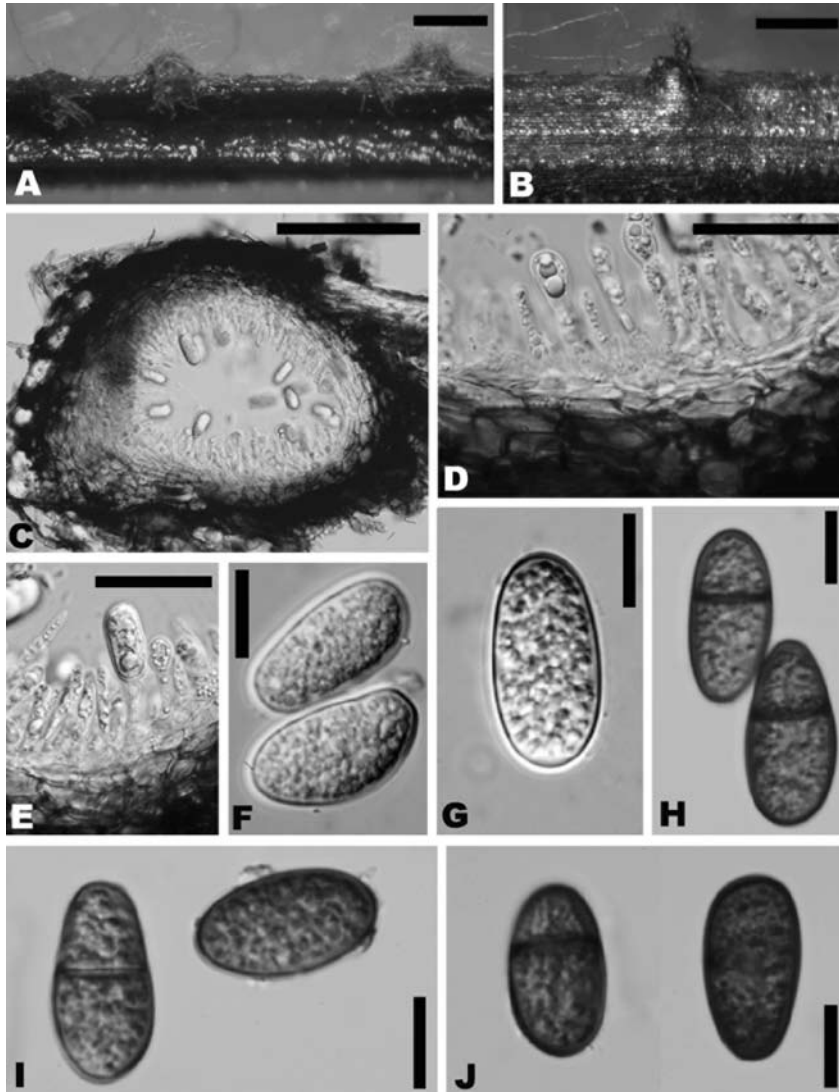


Fig. 3. – **A–J.** *Neodeightonia palmicola* (Holotype). **A–B.** Conidiomata on pine needles. **C:** Section of conidioma. **D–E:** Conidiogenous cells. **F–G:** Young conidia. **G–J.** Mature conidia with septa. Scale bars: A,B = 500 μm . C = 100 μm . D,E = 30 μm . G–J = 10 μm .

while those of *D. corticola* are mostly (23.7–) 29.6–30.3(–46.1) × (9.1–) 13.4–13.8(–20.5) μm (Alves *et al.* 2004), and is distinguished from *N. phoenicum* by the absence of conidial striations (Phillips *et al.* 2008).

Discussion

Botryosphaeria is a widespread genus comprising approximately 36 species (Kirk *et al.* 2008). The taxonomy of *Botryosphaeria* is very confusing and the genus is in need of monographing. Several similar genera have been moved and transferred to the genus *Botryosphaeria* (Saccardo 1877, von Arx & Müller 1975, Phillips *et al.* 2008). Recent studies on *Botryosphaeria* have employed molecular methods to reveal phylogenetic relationships among species (Jacobs & Rehner 1998) and to help resolve species complexes (Smith *et al.* 1996, Denman *et al.* 2000, Alves *et al.* 2004, Slippers *et al.* 2004a, Slippers *et al.* 2004b, Crous *et al.* 2006, Phillips *et al.* 2008). Molecular species concepts are narrower than traditional morphology based taxa (Kirk *et al.* 2008). By employing the molecular and traditional morphological approaches, some genera have been emended and reinstated, and some new genera were introduced, such as *Barriopsis*, *Phaeobotryon* and *Neodeightonia* (Crous *et al.* 2006, Phillips *et al.* 2008, Abdollahzadeh *et al.* 2009).

Neodeightonia is a genus supported by molecular and morphological data (Phillips *et al.* 2008, Abdollahzadeh *et al.* 2009). Three species were described in *Neodeightonia*, i.e. *N. phoenicum*, *N. ramulicola* and *N. subglobosa*, while, *N. ramulicola* has been transferred to *Dothidotthia ramulicola* (Barr 1989). The new data on morphology of the teleomorph and anamorph, as reported in this paper, reveal further distinctions from other genera in the *Botryosphaeriaceae*, namely the hyaline; aseptate ascospores surrounded by a well developed sheath and uniloculate ascomata. Our molecular data (Fig. 1) indicates that the new taxon groups with *N. phoenicum* and *N. subglobosa* in a well supported clade (97 %).

Palms continue to be a source of many new species (Fröhlich & Hyde 2000, Hyde *et al.* 2000, Taylor & Hyde 2003, Pinnoi *et al.* 2006, Pinruan *et al.* 2007, Rungjindamai *et al.* 2008) and it is not surprising that *Neodeightonia palmicola* has been found on the palms *Arenga westerhoutii* and *Caryota urens* in Thailand. *N. phoenicum* was also described from the palm *Phoenix* sp. while *N. subglobosa* was found on a *Bambusa* species.

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References

- Abdollahzadeh J., Goltapeh E.M., Javadi A., Shams-Bakhsh M., Zare R., Phillips A.J.L. (2009) *Barriopsis iraniana* and *Phaeobotryon cupressi*: two new species of the *Botryosphaeriaceae* from trees in Iran. *Persoonia* **23**: 1–8.
- Alves A., Correia A., Luque J., Phillips A.J.L. (2004) *Botryosphaeria corticola*, sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph, *Diplodia mutila*. *Mycologia* **96**: 598–613.
- Alves A., Correia A., Phillips A.J.L. (2006) Multi-gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. *Fungal Diversity* **23**: 1–15.
- Alves A., Crous P.W., Correia A., Phillips A.J.L. (2008) Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Diversity* **28**: 1–13.
- Arx JA. von., Müller E. (1954) Die Gattungen der amersporen Pyrenomyceten. *Beitrage zur Kryptogamenflora der Schweiz* **11(1)**: 1–434.
- Arx JA. von, Müller E (1975) A re-evaluation of the bitunicate ascomycetes with keys to families and genera. *Studies in Mycology* **9**: 1–159.
- Arx JA. von (1987) Plant pathogenic fungi. *Berlin, Stuttgart*.
- Barr M.E. (1987) *Prodomus* to the class *Loculoascomycetes*. Published by the author, *Amherst, MA*.
- Barr M.E. (1989) The genus *Dothidotthia* (Botryosphaeriaceae) in North America. *Mycotaxon* **34**: 517–526.
- Cai L., Jeewon R., Hyde K.D. (2006) Phylogenetic investigations of Sordariaceae based on multiple gene sequences and morphology. *Mycological Research* **110**: 137–150.
- Cai L., Guo X.Y., Hyde K.D. (2008) Morphological and molecular characterisation of a new anamorphic genus *Cheirosporium*, from freshwater in China. *Persoonia* **20**: 53–58.
- Cai L., Wu W.P., Hyde K.D. (2009) Phylogenetic relationships of *Chalara* and allied species inferred from ribosomal DNA sequences. *Mycological Progress* **8**: 133–143.
- Cesati V., Notaris G. de (1863) Schema di classificazione degli sferiacei italici aschigeri più o meno appartenenti al genere *Sphaeria* nell'antico significato attribuitogli da Persoon. *Commentario della Società Crittogamia Italiana* **1, 4**: 177–240.
- Crous P.W., Slippers B., Wingfield M.J., Rheeder J., Marasas W.F.O., Philips A.J.L., Alves A., Burgess T.I., Barber P.A., Groenewald J.Z. (2006) Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* **55**: 235–253.
- Denman P.W., Taylor J.E., Kang J.C., Pascoe I., Michael J. (2000) An overview of the taxonomic history of *Botryosphaeria*, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology* **45**: 29–140.

- Fröhlich J., Hyde K.D. (1995) *Guignardia candeloflamma* sp. nov. causing leaf spots of *Pinanga* spp. *Mycological Research* **99**: 110–112.
- Fröhlich J., Hyde K.D. (2000) Palm microfungi. *Fungal Diversity Research Series* **3**: 1–393.
- Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.
- Hillis D.M., Bull J.J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**: 182–192.
- Huang W.Y., Cai Y.Z., Hyde K.D., Corke H., Sun M. (2008) Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. *Fungal Diversity* **33**: 61–75.
- Huelsenbeck J.P., Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Hyde K.D (1995) Fungi from palms. XX. The genus *Guignardia*. *Sydowia* **47**: 180–198.
- Hyde K.D., Taylor J.E., Fröhlich J. (2000) Genera of Ascomycetes from palms. *Fungal Diversity Research Series* **2**: 1–247.
- Jacobs K.A., Rehner S.A. (1998) Comparison of cultural and morphological characters and ITS sequences in anamorphs of Botryosphaeria and related taxa. *Mycologia* **90**: 601–610.
- Kirk P.M., Ainsworth G.C., Cannon P.F., Minter D.W. (2008) Ainsworth & Bisby's dictionary of the fungi. CAB Intl.
- Lazzizzera C., Frisullo S., Alves A., Lopes J., Phillips A.J.L. (2008) Phylogeny and morphology of *Diplodia* species on olives in southern Italy and description of *Diplodia olivarum* sp. nov. *Fungal Diversity* **31**: 63–71.
- Marincowitz S., Groenewald J.Z., Wingfield M.J., Crous P.W. (2008) Species of Botryosphaeriaceae occurring on Proteaceae. *Persoonia* **21**: 111–118.
- Mohali S., Slippers B., Wingfield M.J. (2007) Identification of *Botryosphaeriaceae* from *Eucalyptus*, *Acacia* and *Pinus* in Venezuela. *Fungal Diversity* **25**: 103–125.
- Nylander J.A.A. (2004) MrModeltest 2.0. Program distributed by the author. *Evolutionary Biology Centre, Uppsala University*.
- Pérez C.A., Wingfield M.J., Slippers B., Altier N.A., Blanchette R.A. (2010) Endophytic and canker-associated *Botryosphaeriaceae* occurring on non-native *Eucalyptus* and native *Myrtaceae* trees in Uruguay. *Fungal Diversity* **41**: 53–69.
- Page R.D.M. (1996) TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357–358.
- Phillips A.J.L., Alves A., Correia A., Luque J. (2005) Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. *Mycologia* **97**: 513–529.
- Phillips A.J.L., Crous P.W., Alves A. (2007) *Diplodia seriata*, the anamorph of “*Botryosphaeria*” *obtusa*. *Fungal Diversity* **25**: 141–155.
- 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.
- Phillips A.J.L., Alves A (2009) Taxonomy, phylogeny, and epitypification of *Melanops tulasnei*, the type species of *Melanops*. *Fungal Diversity* **38**: 155–166.
- Pinnoi A., Lumyong S., Hyde K.D., Jones E.B.G. (2006) Biodiversity of fungi on the palm *Eleiodoxa conferta* in Sirindhorn peat swamp forest, Narathiwat, Thailand. *Fungal Diversity* **22**: 205–218.
- Pinnoi A., Phongpaichit P, Jeewon R, Tang A.M.C., Hyde K.D., Jones E.B.G. (2010) Phylogenetic relationships of *Astrocystis eleiodoxae* sp. nov. (Xylariaceae). *Mycosphere* **1**: 1–9.

- Pinruan U., Hyde K.D., Lumyong S., McKenzie E.H.C., Jones E.B.G. (2007) Occurrence of fungi on tissues of the peat swamp palm *Licuala longicalycata*. *Fungal Diversity* **25**: 157–173.
- Pinruan U., Sakayaroj J., Hyde K.D., Jones E.B.G. (2008) *Thailandiomyces bisetulosus* gen. et sp. nov. (Diaporthales, Sordariomycetidae, Sordariomycetes) and its anamorph *Craspedodidymum*, is described based on nuclear SSU and LSU rDNA sequences. *Fungal Diversity* **29**: 89–98.
- 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**: 53–63.
- Pitt W.M., Huang R., Steel C.C., Savocchia S. (2010) Identification, distribution and current taxonomy of Botryosphaeriaceae species associated with grapevine decline in New South Wales and South Australia. *Australain Journal of Grape Wine Research* **16**: 258–271.
- Punithalingam E. (1969) Studies on Sphaeropsidales in culture. *Mycological Papers* **119**: 1–24.
- Rannala B., Yang Z. (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* **43**: 304–311.
- Ronquist F., Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572.
- Rungjindamai N., Pinruan U., Choeyklin R., Hattori T., Jones E.B.G. (2008) Molecular characterization of basidiomycetous endophytes isolated from leaves, rachis and petioles of the oil palm, *Elaeis guineensis*, in Thailand. *Fungal Diversity* **33**: 139–161.
- Saccardo P.A. (1877) Fungi veneti novi vel critici vel Mycologiae Venetae addendi. *Michelia*: 1–72.
- Slippers B., Crous P.W., Denman S., Coutinho T.A., Wingfield B.D., Wingfield M.J. (2004a) Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* **96**: 83–101.
- Slippers B., Fourie G., Crous P.W., Coutinho T.A., Wingfield B.D., Carnegie A.J., Wingfield M.J. (2004b) Speciation and distribution of *Botryosphaeria* spp. on native and introduced Eucalyptus trees in Australia and South Africa. *Studies in Mycology* **50**: 343–358.
- Slippers B., Wingfield M.J. (2007) *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* **21**: 90–106.
- Slippers B., Smit W.A., Crous P.W., Coutinho T.A., Wingfield B.D., Wingfield M.J. (2007) Taxonomy, phylogeny and identification of *Botryosphaeriaceae* associated with pome and stone fruit trees in South Africa and other regions of the world. *Plant Pathology* **56**: 128–139.
- Smith H., Wingfield M.J., Crous P.W., Coutinho T.A. (1996) *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86–88.
- Swofford D.L. (2002) PAUP: phylogenetic analysis using parsimony, version 4.0 b10. *Sunderland, MA: Sinauer Associates, U.S.A.*
- Taylor J.E., Hyde K.D. (2003) Microfungi of tropical and temperate palms. *Fungal Diversity Research Series* **12**: 1–459.
- Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**: 4876.
- 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.

- White T.J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR protocols: a guide to methods and applications: 315–322. Academic Press, San Diego, USA.
- Zhaxybayeva O., Gogarten J.P. (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics* **3**: 4.
- Zhou X.D., Xie Y.J., Chen S.F., Wingfield M.J. (2008) Diseases of eucalypt plantations in China: challenges and opportunities. *Fungal Diversity* **32**: 1–7.

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