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## Botryosphaeriaceae from palms in Thailand - Barriopsis archontophoenicis sp. nov, from Archontophoenix alexandrae

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#### Abstract

During our studies of palm fungi in Thailand we identified a new species of *Barriopsis* on a petiole of *Archontophoenix alexandrae*, which we introduce herein as *B. archontophoenicis*. The new species is compared with other species in the genus *Barriopsis* and differs in its epapillate ostiole and smaller ascospores. Phylogenetic analyses of combined ITS, LSU, SSU and TEF1- $\alpha$  sequence data also show the species to be distinct.

Key words - ascospores - molecular phylogeny - morphology - palm fungi - taxonomy

#### Introduction

Hyde and co-authors have been studying the fungi on palms since 1988 (Hyde 1988, Hyde et al. 2000, Fröhlich and Hyde 2000, Taylor & Hyde 2003) and this paper is a continuation of that research. In earlier studies, all taxa were described and arranged in the Ascomycota based on morphology. This approach was, however, subjective and many taxa were wrongly placed or placed in Ascomycota genera *incertae sedis*. It is therefore essential that these palm fungi are recollected, epitypified where needed, isolated and sequence data obtained so that the palm fungi can be placed in a natural taxonomic framework (Ariyawansa et al. 2014a, b, Jayasiri et al. 2015). In this paper, we introduce a new *Barriopsis* species collected on *Archontophoenix* in Thailand. It is characterized on the basis of morphology and DNA sequence data. The new species is illustrated and compared with other species in the genus.

#### **Materials & Methods**

#### Collection, isolation and identification

Fresh material of *Archontophoenix alexandrae* was collected from Chiang Mai, Thailand in 2014. Fungal structures were examined with a Motic SMZ 168 series stereomicroscope and photographed with an Axio camera on a Zeiss Discover V8 stereomicroscope. Micromorphological structures were photographed with a Canon 600D camera on a Nikon ECLIPSE 80i microscope. Fungal structures were measured with Image Frame Work (IFW) version 0.9.7. Photoplates were made with Adobe Photoshop CS3 Extended version 10. Isolations were made from single ascospores following the method of Chomnunti et al. (2014). Growth rates were recorded by measuring colony diam. after 7 days of incubation at 25°C on MEA medium. The holotype specimen is deposited in Herb. MFLU and ex-type culture in MFLUCC at Mae Fah Luang University, Chiang Rai, Thailand. Facesoffungi and Index Fungorum numbers are registered as outlined in Jayasiri et al. (2015) and Index Fungorum (2016).

#### **Fungal DNA extraction and PCR reaction**

Genomic DNA was extracted from fresh mycelium grown on MEA for two weeks using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) (Phukhamsakda et al. 2015). Specific rDNA regions were amplified with different gene primers, i.e. LROR and LR5 to amplify the large subunit rDNA (LSU) (Vilgalys & Hester 1990), NS1 and NS4 to amplify region of nuclear small subunit rDNA (SSU), ITS5 and ITS4 to amplify the internal transcribed spacers (ITS) (White et al. 1990), and a fragment of translation elongation factor 1- $\alpha$  (TEF1- $\alpha$ ) was amplified and sequenced using EF1-526F and EF1-1567R (Jacobs et al. 2004). The amplification was performed with an initial denaturing step of 5 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s annealing at 52°C, 56°C for ITS and TEF1- $\alpha$  respectively, then 1 min at 72°C, and a final extension of 7 min at 72°C (Doilom et al. 2014). PCR products were checked on 1% agarose electrophoresis gels stained with ethidium bromide. PCR products were sequenced by Shangkai Majorbio Biopharm Technology Co, Ltd, China.

#### Sequence alignment and phylogenetic analyses

DNA sequences were checked with BioEdit (Hall 1999) and MEGA6 (Tamura et al. 2013). A BLAST search with the LSU sequence data was used to reveal the closest matching taxa in Botryosphaeriaceae (Liu et al. 2014, Phookamsak et al. 2015). Multiple sequence alignments were performed MAFFT alignment with (Katoh et al. 2013) online (http://www.ebi.ac.uk/Tools/msa/mafft/). ITS, LSU, SSU and TEF sequences datasets were first analyzed separately and then the individual datasets were concatenated into a combined dataset and prepared in Mega v.6 (Tamura et al. 2013). Data were converted from fasta to nexus format with Clustal X (Thompson et al. 1997). Maximum-parsimony (MP) analysis was done with PAUP v. 4.0b10 (Swofford 2002) and robustness of the branches was determined with 1000 bootstrap replicates along with 1000 of max-trees. Maximum likelihood analysis was performed by RAxMI GUIv.0.9b2 (Kishino & Hasegawa 1989, Silvestro & Michalak 2010). The search strategy was set to rapid bootstrapping at 1000 and the analysis carried out using the GTR-GAMMA model of nucleotide substitution. The number of replicates was inferred using the stopping criterion. Bootstrap values greater than 50% were accepted. The model of evolution was determined with MrModeltest 2.2 (Nylander 2004) and posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001). Bayesian posterior probabilities (BYPP) greater than 95% were accepted. The phylogenetic tree was visualized with Tree View32 (Page 1996).

**Table 1** GenBank accession numbers of the sequences used in phylogenetic analysis.

Crossing rooms		GenBank accession number			
Species name	strain	ITS	LSU	SSU	TEF
Alanphillipsia aloeicola	CBS:138896	KP004444	KP004472	-	-
Alanphillipsia aloeigena	CPC 21286	KF777137	KF777193	-	-
Alanphillipsia aloes	CPC 21298	KF777138	KF777194	-	-
Alanphillipsia aloetica	CPC 21109	KF777139	KF777195	-	-
Alanphillipsia euphorbiae	CPC 21628	KF777140	KF777196	-	-
Barriopsis	MFLUCC 14-1164	KX235306	KX235307	KX235308	KX235305
archontophoenicis					
Barriopsis fusca	CBS 174.26	EU673330	NG_042419	EU673182	EU673296
Barriopsis iraniana	IRAN1448C	KF766150	KF766318	KF766231	FJ919652
Barriopsis iraniana	IRAN1451C	FJ919668	-	-	FJ919657
Barriopsis tectonae	MFLUCC 12-0381	KJ556515	-	-	KJ556516
Diplodia mutila	CBS 112553	AY259093	-	EU673213	AY5/3219
Diplodia mutila	CBS 230.30	DQ458886	EU673265	EU6/3214	DQ458869
Diplodia seriata	CBS 112555	AY259094	-	KF766244	AY573220
Diplodia seriata	CBS 119049	DQ458889	EU673266	EU6/3216	DQ458874
Diplodia spegazziniana	CBS 302.75	EU6/3319	EU6/3238	EU6/3168	EU6/3286
Dothiorella moneti	MUCC505	EF591920	EF591937	-	EF591971
Dothiorella moneti	MUCC507	EF591922	EF591939	-	EF591973
Dothiorella sarmentorum	IMI 63581b	AY573212	-	-	AY573235
Dothiorella sarmentorum	CBS 115038	AY573206	DQ377860	KF766248	AY573223
Lasiodiplodia gonubiensis	CMW14077	AY639595	-	-	DQ103566
Lasiodiplodia gonubiensis	CMW14078	AY639594	-	-	DQ103567
	/CBS115812				
Lasiodiplodia margaritacea	CBS122519 /CBS	EU144050	-	-	EU144065
	116355				
Lasiodiplodia margaritacea	CBS 122519	EU144050	-	-	EU144065
Lasiodiplodia margaritacea	CBS 122065	EU144051	-	-	EU144066
Lasiodiplodia	CBS 116459	EF622077	EU6/3256	KF/662/9	EF622057
pseudotheobromae		<b>EE</b> (22.001		<b>EX (50</b> 100	
Lasiodiplodia	CBS 447.62	EF622081	EU673255	EU6/3198	EF622060
pseudotheobromae					
Lasiodiplodia theobromae	CBS 164.96	AY640255	NG 042460	EU6/3196	AY640258
Lasiodiplodia theobromae	CBS 124.13	DQ458890	AY928054	EU6/3195	DQ458875
Neodeightonia palmicola	MFLUCC 10-0822	HQ199222	HQ199221	HQ199223	-
Neodeightonia palmicola	MFLUCC10-0823	HQ199224	HQ199225	HQ199226	-
Neodeightonia phoenicum	CBS 122528	KF/66198	EU673261	KF/66285	EU673309
Neodeightonia phoenicum	CBS 123168	EU673339	EU673260	EU673204	EU673308
Neodeightonia phoenicum	CBS 169.34	EU6/3338	EU673259	EU6/3203	EU673307
Neodeightonia subglobosa	CBS 448.91	EU6/333/	DQ377866	KF/66286	EU6/3306
Phaeobotryon cupressi	IRAN1455C	FJ919672	-	-	FJ919661
Phaeobotryon cupressi	IRAN1456C	FJ919670	-	-	FJ919659
Phaeobotryon mamane	CPC 12440	EU673332	NG 042459	EU6/3184	EU673298
Phaeobotryon mamane	CPC 12442	EU6/3333	DQ377899	FU(72146	EU6/3299
Pseudofusicoccum	CBS 11/448	KF/66223	DQ377931	EU6/3146	-
stromaticum	CDC 117440		D0077000	FU(72147	
Pseudofusicoccum	CBS 11/449	-	DQ377932	EU6/314/	
stromaticum	CD 0 117000	1 32005554	NG 042420	VF766010	1 3/005550
Spencermartinsia viticola	CBS 11/009	A 1 905554	NG 042420	KF/00313	A Y 905559
Sphaeropsis citrigena	ICMP 16812	EU6/3328	NG 042458	EU6/3180	EU673294
Sphaeropsis citrigena	ICMP 16818	EU6/3329	EU6/324/	EU6/3181	EU6/3295
Sphaeropsis eucalypticola	MELUCC 11-05/9	JA040802	NG 042/27	JA040833	JA04080/
Sphaeropsis eucalypticola	MITLUUU II-0004	JA0408U3	JA040820	JA040830	JA040808
spnaeropsis porosa	CBS 110496/ STE-U 5132	кг/00210	DQ377894	EU0/31/9	A I 343340
Sphaeropsis visci	CBS 186.97	KF766211	EU754216	EU754117	EU673293
Sphaeropsis visci	CBS 100163	EU673324	EU754215	EU754116	EU673292

**Abbreviation:** CBS: Centraalbureau voor Schimmelcultures, The Netherlands; CPC: Collection of Pedro Crous housed at CBS; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan; IMI: CABI Bioscience, Egham, UK; CMW: M.J. Wingfield, FABI, University of Pretoria, South Africa; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, New Zealand; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa.

#### Results

#### Phylogeny

The combined dataset of 47 taxa with *Pseudofusicoccum stromaticum* (*Botryosphaeriaceae*) as the outgroup taxon contained 3505 characters including gaps. Of these, 2729 were constant, 181 were variable and parsimony-uninformative and 595 were parsimony informative. Phylogenetic trees were generated by maximum parsimony (MP), maximum likelihood (ML) and Bayesian posterior probabilities (BYPP). The equally weighted maximum parsimony tree resulted in a single tree of 1415 steps, a consistency index (CI) of 0.728, a retention index (RI) of 0.848, a rescaled consistency index (RC) of 0.618 and homoplasy index (HI) of 0.272. Phylogenetic analysis indicated eight major clades in *Botryosphaeriaceae* (Fig. 1). The new species *Barriopsis archontophoenicis* clustered with all other *Barriopsis* species in a well-supported clade.

Barriopsis archontophoenicis Konta, Boonmee & K.D. Hyde, sp. nov.

Figs 2-4

Index Fungorum number: IF552104 Facesoffungi number: 02097

Etymology - The specific epithet refers to the host genus Archontophoenix.

Holotype - MFLU: 15-0015

Saprobic on woody tissue of Archontophoenix alexandrae. Sexual morph on fresh specimen: Ascostromata 103–194  $\mu m$ , high  $\times$  99–124  $\mu m$  diam. ( $\overline{x} = 132 \times 120 \mu m$ , n = 10), solitary to aggregated, immersed, erumpent at maturity, uniloculate, subglobose, minutely papillate, some with a short neck, and slightly flattened at the base. Ostiole epapillate. Peridium 5-14 um wide, composed of outer layers of black, thick-walled, cells of *textura angularis*, inner layers hyaline and thin-walled. Hamathecium comprising numerous, 2-3 µm wide, hypha-like, septate, cellular pseudoparaphyses. Asci 70–144 × 17–25  $\mu m$  ( $\bar{x} = 109 \times 20 \mu m$ , n = 10), 8-spored, bitunicate, cylindrical-clavate, short-pedicellate, apically rounded, with an ocular chamber. Ascospores  $17-24 \times 7-10 \ \mu m$  ( $\overline{x} = 22 \times 9 \ \mu m$ , n = 20), bi-seriate, ellipsoid to ovoid, broad at the middle, aseptate, initially hyaline, becoming dark brown at maturity, with a large central guttule, without terminal apiculi, smooth-walled, with a distinct mucilaginous sheath. Sexual morph formed in culture after 6 months of incubation: Ascostromata 206–478 µm high × 322–435 µm diam. ( $\overline{x} = 321 \times 336 \ \mu m$ , n = 10), superficial, covered by mycelium, solitary or gregarious, scattered, uniloculate, globose to subglobose, dark brown to black. Ostiole and Hamathecium not apparent. Peridium comprising several layers of textura angularis, outer layers dark-brown and thick-walled, inner layers hyaline and thin-walled. Ascospores  $20-36 \times 10-16 \ \mu m$  ( $\overline{x} = 32 \times 14 \ \mu m$ , n = 20), ellipsoid to ovoid, initially reddish-brown, becoming dark brown at maturity, 2-septate, with two large guttules in center, thick-walled, smooth-walled, ellipsoid to obovoid, with terminal apiculi at both ends. Asexual morph in culture: Coelomycetous. Conidiomata stromatic, pycnidial, superficial, dark brown to black, covered with dense mycelium, on PDA mostly uniloculate, individual or aggregated, thick-walled, ostiolate. Ostiole central, circular, epapillate. Paraphyses arising from the conidiogenous layer, extending above the level of developing conidia, thin-walled, hyaline, usually aseptate, and tip rounded. Conidiophores absent. Conidiogenous cells  $6-11 \times 3-4 \mu m$ , hyaline, thin-walled, smooth, cylindrical, holoblastic. Conidia  $26-34 \times 14-17 \mu m$  $(\bar{x} = 30 \times 16 \ \mu m, n = 10)$ , thick-walled, initially hyaline, aseptate with longitudinal striations on immature conidia, oval, both ends broadly rounded.



**Fig. 1** – Maximum Parsimony (MP) tree derived from a combined ITS, LSU, SSU and TEF1- $\alpha$  sequence dataset. Bootstrap support values for maximum likelihood (ML, red), maximum parsimony (MP, black) greater than 60% and Bayesian posterior probabilities (BYPP, blue) greater than 0.95 are given at the nodes. The tree is rooted with *Pseudofusicoccum stromaticum*. The strain numbers are mentioned after the species names. The new species is indicated in blue and ex-type strains are indicated in black bold.

Culture characters – Ascospores germinating on MEA within 24 hours and germ tube produced from one end of the ascospore. Colonies on MEA and PDA fast growing, reaching 7–8.5 cm diam. after 4 days at 25°C, white at the edge, grey in the middle with strong radiations outwards. After 24 days of incubation, colonies on MEA and PDA become grey-olivaceous, spongy, hyphae septate, branched, smooth, producing conidiomata and after 6 months of incubation, producing ascomata and ascospores.



**Fig. 2** – *Barriopsis archontophoenicis* (MFLU: 15–0015, **holotype**) a Palm *Archontophoenix alexandrae.* b Appearance of ascostromata on host substrate. c Close up of ascostroma erumpent through the host surface. d Peridium. e Pseudoparaphyses. f Section of ascostromata. g Immature ascus. h–j Mature asci. k–p Ascospores. q Germinating ascospore. Scale bars: b = 500  $\mu m$ , c = 200  $\mu m$ , d = 50  $\mu m$ , e, g–j = 20  $\mu m$ , f, k–q = 10  $\mu m$ .



**Fig. 3** – *Barriopsis archontophoenicis* (ex-type culture) a Conidiomata on PDA media after 26 days. b, c Close up of conidiomata. d–h Conidia developing on conidiogenous cells. f. Developing conidia and paraphyses. i–l, Immature conidium. m Hyaline, immature, striate conidia. n Mature conidium. o Brown, mature, striate conidia. Scale bars:  $a = 1,000 \ \mu m$ ,  $b-c = 500 \ \mu m$ ,  $d-o = 10 \ \mu m$ .

Material examined – THAILAND, Chiang Mai Province, Mushroom Research Centre, on dead woody palm (*Archontophoenix alexandrae* (F. Muell.) H.Wendl. & Drude, *Arecaceae*), 15 August 2014, S. Konta P02a (MFLU 15–0015, **holotype**, HKAS92527, **isotype**; ex-type living culture, MFLUCC 14–1164, MUCL 55901).

Notes – *Barriopsis archontophoenicis* is introduced as a novel species in *Barriopsis* based on morphology of the sexual and asexual morphs and phylogenetic analyses. Conidia of *B. archontophoenicis* are longer than in *B. fusca*, shorter than *B. tectonae* but similar to those of *B. iraniana*. Conidia of *B. iraniana* become 1–3 septate while conidia of *B. archontophoenicis* remain aseptate even long after they are formed. Ascospores of *B. archontophoenicis* are smaller than in *B. fusca* and *B. tectonae*. No sexual morph has been reported for *B. iraniana*. Phylogenetically *B. archontophoenicis* is most closely related to *B. iraniana*, but forms a distinct lineage.

#### Discussion

In this paper we introduce *Barriopsis archontophoenicis* as a new species. Phylogenetically it forms a distinct lineage sister to *B. iraniana*. Morphologically these two species are very similar but the aseptate conidia of *B. archontophoenicis* differentiate it from *B. iranaiana*, which has 1–3 septate conidia.

*Barriopsis* is a member of the family *Botryosphaeriaceae* (Botryosphaeriales, Liu et al. 2014) with *B. fusca* (N.E. Stevens) A.J.L. Phillips et al. as the type species (Phillips et al. 2008). There are presently two other accepted species, *B. iraniana* Abdoll et al. (Abdollahzadeh et al. 2009, Phillips et al. 2013) and *B. tectonae* Doilom et al. (Doilom et al. 2014) in the genus. The sexual morph of *Barriopsis* has brown aseptate ascospores that are widest in the middle, and lack terminal apiculi (Phillips et al. 2008). This feature was considered unique to *Barriopsis* differentiating it from all other genera in the *Botryosphaeriaceae* (Phillips et al. 2008). This feature was also seen in *B. archontophoenicis*.

Interestingly, *B. archontophoenicis* forms the sexual morph in culture after long periods of incubation up to 6 months. Although this has never been reported for other species in this genus it is not clear if very old cultures have ever been studied. Furthermore, within the *Botryosphaeriaceae* production of the sexual morph in culture is extremely rare and has been reported only for *Neodeightonia globosa* (Punithalingam 1969) and *Neofusicoccum luteum* (Denman 2002).



**Fig. 4** – Sexual state of *Barriopsis archontophoenicis* (from ex-type culture) a Culture on MEA after 14 days. b–f Ascostromata formed in MEA. g Peridium. h Mycelium. i–n Ascospores. Scale bars: b–f = 100  $\mu m$ , g = 50  $\mu m$ , h, i–n = 10  $\mu m$ .

**Table 2** Ascomata, hamathecium, asci and ascospore dimensions, and host range of *Barriopsis* species

Species name	Ascomata	Hamathecium	Asci	Ascospores	Host	Remarks
<i>Barriopsis archontophoenicis</i> MFLU: 15–0015 (specimen)	99–124 μm diam. × 103–194 μm high	$2-3 \ \mu m$ wide	70–144 × 17–25 μm	$17-22 \times 7-10 \ \mu m$	Archontophoenix alexandrae (palm)	This study
<i>Barriopsis archontophoenicis</i> MFLUCC 14-1164 (in culture)	206–478μ <i>m</i> high × 322–435μ <i>m</i> diam.	-	-	$20-36 \times 10-16 \ \mu m$ 2-septate, with terminal apiculi	Archontophoenix alexandrae (palm)	This study
Barriopsis fusca (type species)	(430–)546.5–520 μm diam. × 328–349 μm high	3–4.5 μ <i>m</i> wide	180–125 × 30–36 μm	(30–)31–36.5(–38.5) × (15.5–)16–18.5(– 21) μm	Citrus sp.	Phillips et al. 2008 Phillips et al. 2013 Liu et al. 2012
Barriopsis tectonae	(195–) 280–325 (– 365) µm high × (230– )265–285(–320) µm diam ostiole with periphyses	2.5–6 µm wide	(120–)167–185(–200) ×(28–)30.5–32(–35) μm	(26–)29–30 (–33) × (13–)14.5–15(–17) μm	Tectona grandis (teak)	Doilom et al. 2014

### **Table 3** Conidiogenous cells and conidia dimensions of *Barriopsis* species

Species name	Conidiogenous cells	Conidia	Remark
<i>Barriopsis archontophoenicis</i> MFLUCC 14-1164	6–11 × 3–4 μm	$26-34\mu m \times 14-17 \ \mu m \ (\overline{\mathbf{x}} = 30 \times 16 \ \mu m, \mathbf{n} = 10)$	This study
Barriopsis fusca (type species)	7–12 × 3–5 µm	(20–)23–25(–28) $\mu m \times$ (11–)12–13(–16) $\mu m$	Phillips et al. 2008 Phillips et al. 2013 Liu et al. 2012
Barriopsis tectonae	-	(29–)37–37.5(–38) $\mu m \times 15.5$ –17.5(–18) $\mu m (\bar{x} = 36 \times 17 \ \mu m, n = 10)$	Doilom et al. 2014
Barriopsis iraniana	7–12 × 3–5 µm	$(22.5-)24-30 \ \mu m \times (12.8-)14-18(-21.5) \ \mu m$	Abdollahzadeh et al. 2009

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