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## ***Baipadisphaeria* gen. nov., a freshwater ascomycete (Hypocreales, Sordariomycetes) from decaying palm leaves in Thailand**

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*Baipadisphaeria spathulospora* gen. et sp. nov., a freshwater ascomycete is characterized by black immersed ascomata, unbranched, septate paraphyses, unitunicate, clavate to ovoid asci, lacking an apical structure, and fusiform to almost cylindrical, straight or curved, hyaline to pale brown, unicellular, and smooth-walled ascospores. No anamorph was observed. The species is described from submerged decaying leaves of the peat swamp palm *Licuala longicalycata*. Phylogenetic analyses based on combined small and large subunit ribosomal DNA sequences showed that it belongs in Nectriaceae (Hypocreales, Hypocreomycetidae, Ascomycota). *Baipadisphaeria spathulospora* constitutes a sister taxon with weak support to *Leuconectria clusiae* in all analyses. Based on morphological and phylogenetic evidence, a new genus is proposed.

**Key words** – *Licuala* – Nectriaceae – peat swamp – tropical fungi

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### **Introduction**

Continued studies of saprobic fungi on the peat swamp palm *Licuala longicalycata* have yielded a number of new ascomycetes: *Jahnula appendiculata* (Jahnulales) (Pang et al. 2002, Pinruan et al. 2002), *Submersisphaeria siamense* (Sordariales) (Pinnoi et al. 2004), *Phruensis brunneispora* (Diaporthales) (Pinruan et al. 2004a), and *Flammispora bioteca* (Pinruan et al. 2004b). A further new species with immersed, dark-brown, globose ascomata, clavate to ovoid asci with fusiform and hyaline to pale brown unicellular ascospores was collected on *L. longicalycata*. This taxon could not be assigned to any genus, although many features indicated the Hypocreales. The goals of this study were to 1) to fully characterize

and describe the morphology of this freshwater peat swamp taxon; 2) use morphology and phylogenetic analyses (LSU and SSU rDNA data) to elucidate the phylogeny and taxonomy of the new taxon.

### **Materials and Methods**

#### **Collection and isolation**

Submerged material of the palm *Licuala longicalycata* was collected from Sirindhorn Peat Swamp Forest, Narathiwat, southern Thailand on 12 May 2001, and 15 February 2002. The material was returned to the laboratory, incubated in plastic boxes on damp tissue paper and examined under stereo and compound microscopes. Type material has

been deposited in the BIOTEC Bangkok Herbarium (BBH) and cultures deposited in the BIOTEC Culture Collection (BCC). Single-spore isolations were made on cornmeal agar (CMA) with added antibiotics to suppress bacterial growth following the method of Choi et al. (1999). All observations, including photographic documentation, were of material mounted in water and examined with a differential interference microscope.

### **Growth of fungi, DNA extraction, amplification and sequencing**

Fungal cultures were maintained on CMA at 25°C. The fungus was grown in potato dextrose broth (PDB) at 25°C, and the biomass harvested, washed with sterile distilled water, frozen at -20°C and ground with a mortar and pestle. DNA was extracted using CTAB method (O' Donnell et al. 1997). The small and large subunit ribosomal DNA (SSU and LSU rDNA) were amplified, using i-Taq DNA Polymerase (iNtRON Biotechnology, Korea Cat. No. 25022.3) in Thermo Cycler (MJ Research DYDD ALD 1244). These two regions were amplified with several primers i.e. NS1, NS4, NS5, NS6 (for SSU) and LROR and LR7 (for LSU). The primer pairs and amplification cycles were performed following White et al. (1990), Bunyard et al. (1994), and Landvik (1996). The PCR products were purified using a NucleoSpin<sup>R</sup> Plant DNA purification kit (MACHEREY-NAGEL, Catalogue No. 740 570. 50), then sequenced by the Macrogen Inc. (Korea) using the same primers for amplifications.

### **Phylogenetic analyses**

Two strains of *Baipadisphaeria spathulospora* (BCC 16119, BCC 20906) were sequenced to confirm their monophyly. Small and large subunit rRNA sequences were aligned individually using MUSCLE 3.6 (Edgar 2004) and manually adjusted using BioEdit 7.0.4.1 (Hall 2005). The alignment was entered into PAUP\* 4.0b10 (Swofford 2002). Sequences of combined SSU and LSU rDNA of *B. spathulospora* were analyzed with several sequences of the Sordariomycetes obtained from the GenBank database. Phylogenetic trees of combined datasets were constructed based on the published data (Rossman et al. 2001,

Castlebury et al. 2004, Schoch et al. 2007) and details of all of the sequences presented in Table 1. Moreover, LSU sequences of *B. spathulospora* were compared and analyzed with several members of the Nectriaceae and accession numbers of sequences retrieved from GenBank are shown on the tree. Sequences of *B. spathulospora* generated in this study were submitted to GenBank and the accession numbers listed in Table 2.

Phylogenetic trees were generated using unweighted maximum parsimony and Bayesian inference methods. For the maximum parsimony analysis we used heuristic searches with a stepwise starting tree, a random stepwise addition of 100 replicates and tree-bisection-reconnection (TBR) branch-swapping algorithm, with gaps treated as missing data. Bootstrap analysis was performed with full heuristic searches on 1,000 replicates, stepwise addition of sequence, 10 replicates of random addition of taxa, TBR branch-swapping algorithm. Bayesian phylogenetic inference was performed with MrBayes 3.0b4 with general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Huelsenbeck & Ronquist 2001). Four Markov chains were run from random starting trees for 5 M generations and sampled every 100 generations. Statistical supports consisting of maximum parsimony bootstrap (% BS) values ( $\geq 50\%$ ) and Bayesian posterior probabilities (PP) ( $\geq 0.95$ ) for the internal branches was estimated and shown above and below the tree branches, respectively.

## **Results**

### **Phylogeny of *Baipadisphaeria spathulospora* based on combined dataset of SSU and LSU sequences**

In order to determine the taxonomic placement of *Baipadisphaeria spathulospora*, 1,031 bases of SSU and 1,212 bases of LSU sequences were initially searched through BLAST search tool. Phylogenetic investigation demonstrated that overall major orders within the Sordariomycetes are well supported. Four major subclasses within the Sordariomycetes comprising Hypocreomycetidae, Sordariomycetidae Spaturosporomycetidae and Xylario-

**Table 1.** SSU and LSU rDNA sequences included in this analysis, which were obtained from GenBank.

Subclass	Order	Family	Taxon	Source	GenBank accession number		
					SSU	LSU	
Hypocreomycetidae	Hypocreales	Bionectriaceae	<i>Bionectria ochroleuca</i>	CBS 114056	AY489684	AY489716	
			<i>Bionectria ochroleuca</i>	AFTOL-ID 187	DQ862044	DQ862027	
			<i>Bionectria pityrodes</i>	ATCC 208842	AY489696	AY489728	
			<i>Hydropisphaera erubescens</i>	ATCC 36093	AY545722	AY545726	
			<i>Hydropisphaera peziza</i>	CBS102038	AY489698	AY489730	
			<i>Nectriopsis violacea</i>	CBS424.64	AY489687	AY489719	
			<i>Ochronectria calami</i>	CBS125.87	AY489685	AY489717	
			<i>Roumegueriella rufula</i>	CBS 346.85	DQ522561	DQ518776	
			<i>Stephanonectria keithii</i>	CBS114057	AY489695	AY489727	
			<i>Stilbocrea macrostoma</i>	CBS114375	AY489693	AY489725	
			Clavicipitaceae	<i>Balansia henningsiana</i>	GAM 16112/AEG 96-27a	AY489683	AY489715
				<i>Claviceps purpurea</i>	GAM 12885	AF543765	AF543789
				<i>Cordyceps cardinalis</i>	OSC 93609	AY184973	AY184962
	<i>Epichloë typhina</i>	ATCC 56429		U32405	U17396		
	Nectriaceae	<i>Haematonectria haematococca</i>		CBS 114067	AY489697	AY489729	
		<i>Leuconectria clusiae</i>		ATCC 22228	AY489700	AY489732	
		<i>Persiciospora africana</i>	ATCC64691	AY015620	AY015631		
		<i>Pseudonectria rousseliana</i>	CBS114049	AF543767	U17416		
		<i>Viridispota diparietispora</i>	ATCC MYA 627	AY489703	AY489735		
	Niessliaceae	<i>Niesslia exilis</i>	CBS357.70	AY489686	AY489718		
		<i>Niesslia exilis</i>	CBS560.74	AY489688	AY489720		
	Hypocreaceae	<i>Hypocrea lutea</i>	ATCC 208838	AF543768	AF543791		
		<i>Hypocrea rufa</i>	CBS 114374	AY489694	AY489726		
<i>Hypomyces polyporinus</i>		ATCC76479	AF543771	AF543793			
<i>Sphaerostilbella berkeleyana</i>		CBS 102308	AF543770	U00756			
<i>Verticillium incurvum</i>		CBS 460.88	AF339600	AF339551			

**Table 1 (Continued).** SSU and LSU rDNA sequences included in this analysis, which were obtained from GenBank.

Subclass	Order	Family	Taxon	Source	GenBank accession number	
					SSU	LSU
Hypocreomycetidae	Lulworthiales	Lulworthiaceae	<i>Linda obtusa</i>	IFO 31317b	AY879002	AY878960
			<i>Linda obtusa</i>	CBS 113030	AY879001	AY878959
	Microascales		<i>Lulworthia uniseptata</i>	CBS 167.60	AY879034	AY878991
			<i>Corollospora maritima</i>	JK 4834	U46871	U46884
			<i>Doratomyces stemonitis</i>	CBS 127.22/AFTOL ID-1380	DQ836901	DQ836907
			<i>Halosphaeria appendiculata</i>	CBS 197.60	U46872	U46885
			<i>Lignicola laevis</i>	JK 5180A	U46873	U46890
	Microascales		<i>Microascus trigonosporus</i>	CBS 218.31	DQ471006	DQ470958
			<i>Nimbospora effusa</i>	JK 5104A	U46877	U46892
			<i>Nohea umiumi</i>	JK 5103F	U46878	U46893
			<i>Juncigena adarca</i>	JK 5548A	EF027720	EF027727
	Incertae sedis		<i>Swampomyces aegyptiacus</i>	CY2973	AY858943	AY858950
			<i>Swampomyces clavatispora</i>	LP83	AY858945	AY858952
			<i>Torpedospora radiata</i>	AFTOL-ID 751	DQ470999	DQ470951
<i>Camarops microspora</i>			CBS 649.92	DQ471036	AY083821	
<i>Camarops ustulinoides</i>			DEH 2164	DQ470989	DQ470941	
Sordariomycetidae	Bolineales	Bolineaceae	<i>Diaporthe eres</i>	CBS 109767	DQ471015	AF408350
			<i>Diaporthe phaseolorum</i>	NRRL 13736	L36985	U47830
	Diaporthales	Diaporthaceae	<i>Leucostoma niveum</i>	AR 3413/AFTOL2125	DQ862050	AF362558
			<i>Valsa ambiens</i>	AR 3516/AFTOL2131	DQ862056	AF362564
			<i>Ophiostoma piliferum</i>	CBS 158.74	DQ471003	DQ470955
	Ophiostomatales	Ophiostomataceae	<i>Ophiostoma stenoceras</i>	CBS 139.51	DQ836897	DQ836904
			<i>Gelasinospora tetrasperma</i>	CBS 178.33	DQ471032	DQ470980
	Sordariales	Sordariaceae	<i>Neurospora crassa</i>		X04971	AF286411
			<i>Sordaria fimicola</i>	CBSC 15-5973	AY545728	AY545724
			<i>Xylaria acuta</i>	ATCC56487	AY544719	AY544676
Xylariomycetidae	Xylariales	Xylariaceae	<i>Xylaria hypoxylon</i>	OSC 100004	AY544692	AY544648

**Table 2.** Isolates and accession numbers of *Baipadisphaeria spathulospora*.

Taxa	Source	Substratum and geographica origin	Date of isolation	GenBank accession number	
				SSU	LSU
<i>B. spathulospora</i> (W32A)	BCC16119	<i>Licuala longicalycata</i> , Sirindhorn peat swamp forest	15 May 2001	M134241	HM134243
<i>B. spathulospora</i> (W32B)	BCC20906	<i>Licuala longicalycata</i> , Sirindhorn peat swamp forest	20 Feb 2002	M134242	HM134244

mycetae, were incorporated and analyzed. The results showed that *B. spathulospora* had a phylogenetic affinity with members of the Hypocreomycetidae with high support (95% BS and 1.00 PP) (data not shown). A combined SSU and LSU dataset was undertaken to identify the higher order classification of *B. spathulospora*. This dataset consisted of 51 taxa from the seven major orders of the Sordariomycetes (Bolineales, Diaporthales, Hypocreales, Lulworthiales, Microascales, Ophiostomatales, and Sordariales), with two sequences from the Xylariales as the outgroup. Of the 2,347 total characters, 1,658 were constant, 149 parsimony uninformative and 540 parsimony informative. The trees obtained from all analyses yielded identical topology, confirming the position of the new taxon in the Hypocreales.

To consider the familial levels within the Hypocreales, various families, consisting of the Bionectriaceae, Clavicipitaceae, Hypocreaceae, Nectriaceae, and Niessliaceae, were compared with *B. spathulospora*. *B. spathulospora* is positioned in the Nectriaceae (Hypocreales, Hypocreomycetidae, Sordariomycetes) in all analyses.

Maximum parsimony yielded three equally most parsimonious trees (MPTs), and differed in the minor branch arrangement in the Hypocreales and Microascales. The K-H test was applied to these trees and resulted in the first tree having the best topology (Fig. 8). Bayesian inference provided a topology similar to other analyses, with minor difference in branch swapping within the Hypocreales and Lulworthiales. However, this difference does not affect the position of *Baipadisphaeria spathulospora*. Therefore, only MPT from maximum parsimony analysis is shown in this paper.

The two *B. spathulospora* strains are monophyletic (99% BS and 0.98 PP), and grouped together within the Nectriaceae with strong support. These two isolates grouped firmly with *Leuconectria clusiae* as the most closely related genus and species, with 81% BS and <0.95 PP support (Fig. 8). The *B. spathulospora* strains and the sister group *Leuconectria* formed a clade with several members within the Nectriaceae clade comprising *Haematonectria haematococca*, *Viridispora diarietispora*, *Persiciospora africana*, and *Pseudonectria rousseliana* with good support (62% BS and 1.00 PP).

#### Phylogeny of *Baipadisphaeria spathulospora* based on LSU sequences

LSU sequences of the *B. spathulospora* strains were compared with several taxa within the Nectriaceae with two taxa of the Bionectriaceae as the outgroup. Of the 853 total characters, 713 were constant, 28 parsimony uninformative, and 112 parsimony informative. Maximum parsimony analysis gave 6 MPTs with minor swapping within the Nectriaceae. Bayesian inference also yielded the same tree topology with maximum parsimony, the latter is shown (Fig. 9).

It clearly shows that *B. spathulospora* belongs to the Nectriaceae with high support (100% BS and 1.00 PP). *Nectria* species are polyphyletic and dispersed in at least three subclades which consist of Nectriaceae clade I, Nectriaceae clade II, and *Haematonectria* (Fig. 9). The *Baipadisphaeria* strains grouped together with high support (94% BS and 1.00 PP) (Nectriaceae clade 1) and formed a clade with *Leuconectria grandis* (EU031441) with *Leuconectria clusiae* (U17412, AY489732) as a sister group.

***Baipadisphaeria* Pinruan gen. nov.**

MycoBank MB 518245

Ascomata immersa vel semi-immersa, coriacea, ostiolata, solitaria. Asci octospori, unitunicati, clavati vel ovoid, apedicellati, sine apical ring, cum paraphyses. Ascospores 3–4-seriate, fusiforme vel cylindrici, hyalinae vel pale brunneae, aseptatae, laevia.

Ascomata immersed, or semi-immersed, coriaceous, ostiolate, solitary. Asci 8-spored, unitunicate, clavate to ovoid, apedicellate, apically narrow and rounded, lacking any apical structure. Paraphyses elongate, unbranched, hyphal-like. Ascospores 3–4-seriate, fusiform to cylindrical, straight or curved, hyaline to pale brown, aseptate, smooth-walled.

Etymology – from Thai ‘Baipad’, referring to the fan leaves of a palm; and Latin ‘sphaeria’, referring to the rounded ascomata.

Typus generis – *Baipadisphaeria spathulospora* U. Pinruan

Cultura ex-typus – BCC 16119.

***Baipadisphaeria spathulospora* Pinruan sp. nov.**

Figs 1–7

MycoBank: MB 518246

Ascomata 220–250 µm diam, solitaria vel scattered, immersa, subglobosa, coriacea, ostiolata. Peridium ad 45 µm crassum, cella crassitunicatum et textura angularis. Paraphyses 5–7 µm crassa. Asci 75–100 × 30–45 µm, clavati vel ovoidea, unitunicati, apedicellati, sine apical ring. Ascospores 49.5–55 × 8–12.5 µm, fusiformes vel cylindrici, hyalinae vel pale brunneae, aseptatae, guttulatae.

Ascomata 220–250 µm diameter, solitary and scattered, dark brown, immersed, subglobose, no ascomatal setae or hyphae, coriaceous, ostiolate, paraphyses lacking. Peridium up to 45 µm thick, comprising two layers: outer layer of brown, angular cells, and an inner layer of elongate, hyaline cells. Paraphyses septate, hyphal-like, unbranched, extending beyond the asci, 5–7 µm wide at the base. Asci 75–100 × 30–45 µm ( $\bar{x}$  = 84 × 28.5 µm, n = 15), 8-spored, clavate to ovoid, unitunicate, apically narrow and rounded, lacking any apical structure and apedicellate. Ascospores 49.5–55 × 8–12.5 µm ( $\bar{x}$  = 51.5 × 10 µm, n = 20), overlapping 3–4-seriate, fusiform to almost cylindrical, some narrower in the center, straight or curved, hyaline to pale

brown, unicellular, smooth-walled, with 4–5 large guttules.

Holotype – Thailand, Narathiwat, Sirindhorn Peat Swamp Forest, on submerged trunk of *Licuala longicalycata*, 12 May 2001, U. Pinruan (Wah32A) in BBH, living culture BCC16119.

Anamorph – unknown.

**Discussion**

Within the Hypocreomycetidae, the major orders and families have been mostly defined based on morphological characteristics (Castlebury et al. 2004). The Nectriaceae is a large family comprising some 57 genera and about 646 species, while the genus *Nectria* has some 89 species (Kirk et al. 2008). Classically the Nectriaceae is characterized by: ascomata bright coloured, membranous, superficial, lacking prominent necks, perithecial, that change colour in KOH; asci narrowly clavate to cylindrical, with an apical pore, and a short pedicel; ascospores hyaline, unicellular to 1-septate, smooth, most with a hyphomycete anamorph. However, *Baipadisphaeria* lacks some of these key characters in having immersed ascomata, apedicellate asci, lacking an apical pore and no known anamorph. Many other genera also do not conform to the features outlined above, e.g. *Halonectria* (Hypocreales incertae sedis, long necks, ascomata immersed in the substratum, no known anamorph, lignicolous and marine) (Jones et al. 2009), and *Vittatispora* (Melanosporales, perithecia with long necks, often submerged in the substratum) (Chaudhary et al. 2006). With the advent of sequence data and a redefining of critical taxonomical criteria, many *Nectria* species have been referred to new genera, e.g. *Cyanonectria* (based on *Nectria cyanostoma*) (Samuels et al. 2009), and *Leuconectria* (based on *Pseudonectria clusiae*) (Rossman & Samuels 1993).

Sequence data clearly establishes *Baipadisphaeria* as a member of the Nectriaceae, Hypocreales with moderate support and with *Leuconectria clusiae* as a sister group in the combined LSU/SSU data set, or *L. grandis* in the LSU dataset. However, these two genera share few morphological features in common. They differ in ascomatal colour, position in the substratum, ascus



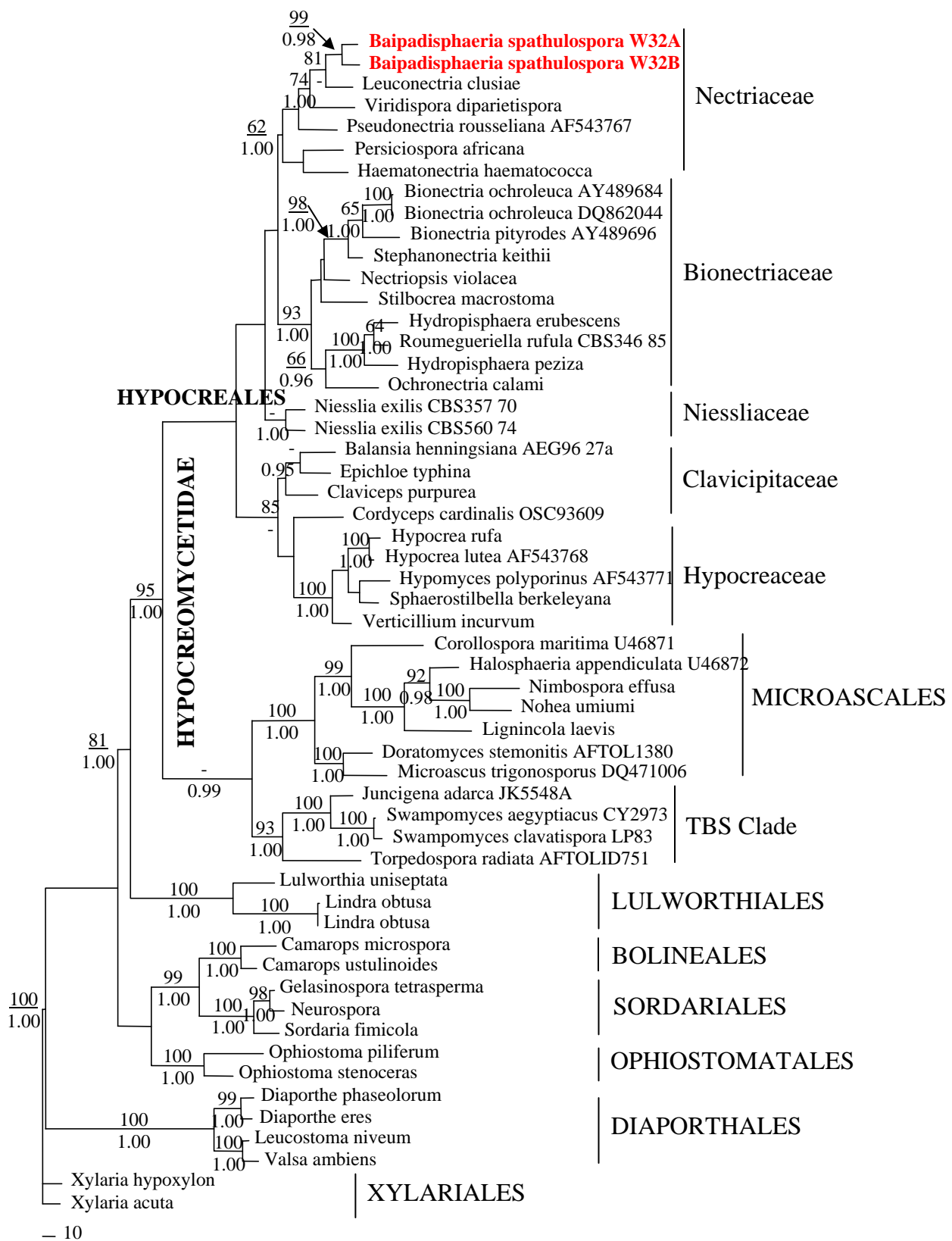
**Figs 1–7** – Light micrographs of *Baipadisphaeria spathulospora* (from holotype). **1** Ascomata immersed in substratum. **2** Peridium that comprises several layers of cells. **3** Paraphyses and ascus. **4,5** Apedicellate asci. **6,7** Ascospores. – Bars **1, 2** 100  $\mu\text{m}$ . **3–7** 10  $\mu\text{m}$ .

morphology, ascospore measurements and anamorph. They also differ in the substrata they grow on (*B. spathulospora* on the palm *Licuala longicalycata*, *Leuconectria clusiae* isolated from soils or decaying fruits) and habitat (*B. spathulospora* freshwater, *L. clusiae* terrestrial).

The elongated ascospores of *Baipadisphaeria* invite comparison with a number of genera in the Phyllachoraceae: *Brobdingnagia*, *Ophiodothella*, and *Phyllachora* (Hyde & Cannon 1999). *Brobdingnagia* has large oblate ascomata formed within distinct stromatic tissue which is absent in *Baipadisphaeria*. *Phyllachora* also has pronounced elongate ascospores, but asci have an apical ring that is

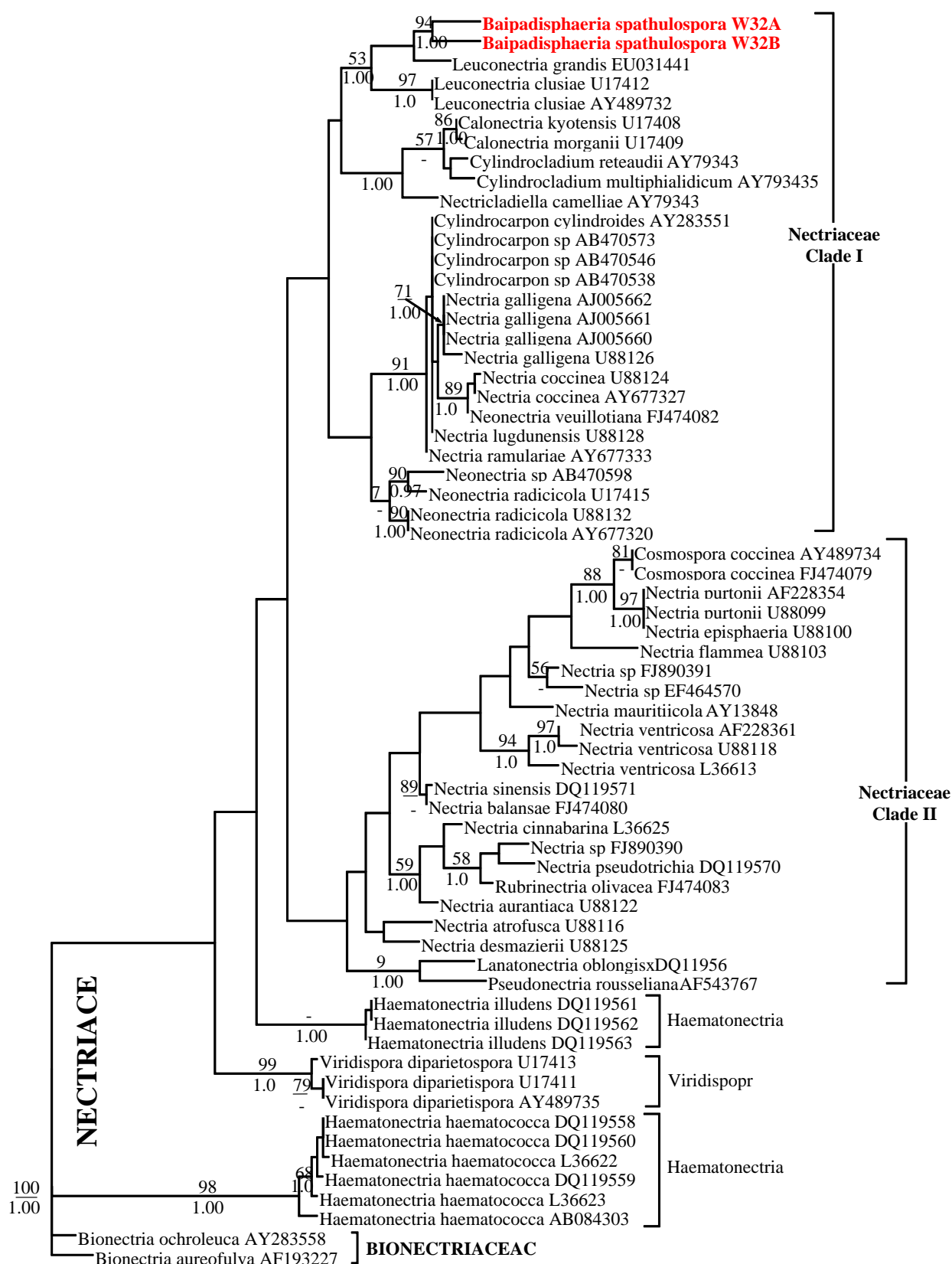
lacking in *Baipadisphaeria*, while in *Ophiodothella* ascospores are filiform, but *Baipadisphaeria* is distinguished from it by a combination of characters, very large ascospores ( $51.5 \times 10 \mu\text{m}$ ), and asci lacking an apical ring. Phylogenetic results also indicate that *Baipadisphaeria* has no affinity with the Phyllachorales (Figs. 8, 9).

Within the Hypocreales there is great diversity in ascoma and ascospore morphology, as highlighted by ascospore phenology of bryophilous members of the order (Döbbeler 2005). Morphologically ascospores of *Baipadisphaeria spathulospora* resemble those of *Nectria muscivora*, a necrotrophic parasite on acrocarpus mosses. Members of the



**Fig. 8.** One of the two most parsimonious trees inferred from unweighted maximum parsimony analysis from combined dataset, consisting of SSU and LSU rDNA sequences. Bootstrap values higher than 50% from maximum parsimony and posterior probabilities higher than 0.95 from Bayesian analysis are given above and below the branches, respectively. Scale bar indicates 10 step changes (tree length = 2386 steps, CI = 0.498, RI = 0.564).





**Fig. 9.** One of the six most parsimonious trees inferred from unweighted maximum parsimony analysis from LSU rDNA sequences. Bootstrap values higher than 50% from maximum parsimony analysis and posterior probabilities higher than 0.95 from Bayesian analysis are given above and below the branches, respectively. Scale bar indicates 10 step changes (tree length =405 steps, CI =0.440, RI =0.803)

Nectriaceae may be stromatic, superficial, often hardly papillate and coloured; peridium is generally membranous, paraphyses present or absent or deliquescent; asci cylindrical, thin-walled, not fissitunicate, often with a minute J-apical ring; ascospores variously shaped, septate, hyaline to yellow to pale brown with no appendages or gelatinous sheath, usually with anamorphs that are hyphomycetous (Kirk et al. 2001). Morphologically *Baipadisphaeria* shares many of these characteristics with Nectriaceae, but differs in its black immersed ascomata, well developed septate paraphyses, large unicellular ascospores and no known anamorph. Like many nectrioid species, *Baipadisphaeria* is saprobic.

Diversity studies have revealed the existence of many new genera that cannot be accommodated in established genera, even orders. This is exemplified by the new ascomycetes recovered by us from palms at the Siridhorn Peat Swamp Forest, Narathiwat province, Thailand. Despite molecular data, their assignment to higher order classification was not resolved. However, only when such new taxa are described (and supported by molecular data) will we be able ultimately to classify them with confidence. Similarly, Schoch et al. (2007) have pointed out that the taxonomic position of many clades in the Hypocreomycetidae can not be resolved e.g. TBM clade.

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### References

Bunyard BA, Nicholson MS, Royse DJ 1994 – A systematic assessment of *Morchella*

using RFLP analysis of the 28S ribosomal RNA gene. *Mycologia* 86, 762–772.

Castlebury LA, Rossman AY, Sung GH, Hyten AS, Spatafora JW 2004 – Multigene phylogeny reveals new lineage for *Stachybotrys hartarum*, the indoor air fungus. *Mycological Research* 108, 864–872.

Chaudhary P, Campbell J, Hawksworth DL, Sastry KN 2006 – *Vittatispora*, a new melanosporeous genus from Indian soil. *Mycologia* 98, 460–467.

Choi YW, Hyde KD, Ho WH 1999 – Single spore isolation of fungi. *Fungal Diversity* 3, 29–38.

Döbbeler P 2005 – Ascospore diversity of bryophilous Hypocreales and two new hepaticolous Nectria species. *Mycologia* 97, 924–934.

Edgar RC 2004 – MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32, 1792–1797.

Hall T 2005 – BioEdit version 7.0.4.1, Department of Microbiology, North Carolina State University. <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>

Huelsenbeck JP, Ronquist F 2001 – Mr.Bayes: Bayesian inference of phylogenetic diversity. *Bioinformatics (Oxford)* 17, 754–755.

Hyde KD, Cannon PF. 1999 – Fungi causing tar spots on palms. *Mycological Papers* 175, 1–114.

Jones EBG, Sakayaroj J, Suetrong S, Somrithipol S, Pang K-L. 2009 – Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. *Fungal Diversity* 35, 1–203.

Kirk PM, Cannon PF, David JC, Stalpers JA 2001 – Ainsworth and Bisby's Dictionary of the Fungi, 9<sup>th</sup> edn. CAB International, Oxon.

Kirk PM, Cannon PF, David WM, Stalpers JA 2008 – Ainsworth and Bisby's Dictionary of the Fungi, 10<sup>th</sup> ed. CAB International, Oxon.

Landvik S 1996 – *Neolecta*, a fruit-body-producing genus of the basal ascomycetes, as shown by SSU and LSU rDNA

- sequences. *Mycological Research* 100, 199–202.
- O'Donnell K, Cigelnik E, Weber NS, Trappe JM 1997 – Phylogenetic relationship among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. *Mycologia* 89, 48–65.
- Pang KL, Abdel-Wahab MA, Sivichai S, El-Sharouny HM, Jones EBG 2002 – *Jahnulales* (Dothideomycetes, Ascomycota): a new order of lignicolous freshwater ascomycetes. *Mycological Research* 106, 1031–1042.
- Pinnoi A, Pinruan U, Hyde KD, Lumyong S 2004 – *Submersisphaeria palmae* sp. nov. and key to the genus and notes on *Helicoubisia*. *Sydowia* 56, 72–78.
- Pinruan U, Jones EBG, Hyde KD 2002 – Aquatic fungi from peat swamp palms: *Jahnula appendiculata* sp. nov. *Sydowia* 54, 242–247.
- Pinruan U, Sakayaroj J, Hyde KD, Jones EBG 2004a – Aquatic fungi from peat swamp palm: *Phruensis brunneispora* and its hyphomycete anamorph. *Mycologia* 96, 1161–1168.
- Pinruan U, Sakayaroj J, Hyde KD, Jones EBG 2004b – *Flammispora* gen. nov., a new freshwater ascomycete from decaying palm leaves. *Studies in Mycology* 50, 381–386.
- Rossmann AY, McKemy JM, Pardo-Schultheiss RA, Schroers H-J 2001 – Molecular studies of the Bionectriaceae using large subunit rDNA sequences. *Mycologia* 93, 100–110.
- Rossmann AY, Samuels GJ 1993 – *Leuconectria clusiae* gen. nov. and its anamorph *Gliocephalotrichium bulbilium* with notes on *Pseudonectria*. *Mycologia* 85, 685–704.
- Samuels GJ, Lu BS, Chaverri P, Candoussau F, Fournier J, Rossmann AY 2009 – *Cyanonectria*, a new genus for *Nectria cyanostoma* and its *Fusarium* anamorph. *Mycological Progress* 8, 49–58.
- Schoch CL, Sung G-H, Volkmann-Kohlmeyer B, Kohlmeyer J, Spatafora JW 2007 – Marine fungal lineages in the Hypocreomycetidae. *Mycological Research* 111, 154–162.
- Swofford DL. 2002 – Phylogenetic analysis using parsimony (and other methods) Version 5. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.
- White TJ, Bruns T, Lee S, Taylor J 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocol: A guide to methods and applications* (eds Innis MA, Gelfand DH, Sninsky JS, White, TJ). San Diego: Academic Press, 315–322.