

## South Florida microfungi: *Castanedospora*, a new genus to accommodate *Sporidesmium pachyanthicola* (Capnodiales, Ascomycota)

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**Abstract** – The taxonomic status and phylogenetic placement of *Sporidesmium pachyanthicola* in Capnodiales (Dothideomycetes) are revisited based on a specimen collected on the petiole of a dead leaf of *Sabal palmetto* in south Florida, U.S.A. New evidence inferred from phylogenetic analyses of nuclear ribosomal DNA sequence data together with a broad taxon sampling at family level suggest that the fungus is a member of Extremaceae and therefore its previous placement within the broadly defined Teratosphaeriaceae was not supported. A new genus *Castanedospora* is introduced to accommodate this species on the basis of its distinct morphology and phylogenetic position distant from Sporidesmiaceae sensu stricto in Sordariomycetes. The holotype material from Cuba was found to be exhausted and the Florida specimen, which agrees well with the original description, is selected as epitype. The fungus produced considerably long cylindrical to narrowly obclavate conidia in culture strongly resembling those of *Sporidesmajora pennsylvaniensis*, another sporidesmium-like, capnodiaceous anamorph. However, phylogenetic analyses show that they are not congeneric and the latter belongs to the family Phaeothecoidiellaceae.

**Anamorphic / palmicolous / phylogeny / saprobic**

## INTRODUCTION

The generic concept of *Sporidesmium* Link and its segregated genera based on morphological features such as conidial septation, presence or absence of conidiophores and the pattern of extension of the conidiogenous cells (Subramanian, 1992; Hernández & Sutton, 1997; Seifert *et al.*, 2011) was early recognized to be diagnostically valuable but schematic and phylogenetically unsound (Reblová, 1999). Shenoy *et al.* (2006) conducted the first comprehensive molecular study of

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sporidesmium-like taxa revealing that they are polyphyletic and belong to the ascomycete classes Dothideomycetes and Sordariomycetes while the phenotypic characters used to delimit sporidesmium-like genera were not phylogenetically significant. More recently, considerable progress has been made in clarifying their systematic position with the resurrection of the family Sporidesmiaceae Fr., typified by the genus *Sporidesmium*, for a distinct monophyletic clade incertae sedis within Sordariomycetes (Su *et al.*, 2016). This clade contains eight *Sporidesmium* species morphologically similar to *S. ehrenbergii* M.B. Ellis, the generic lectotype. Neither this species nor the original generic type, *S. atrum* Link (Ellis, 1958; Hughes, 1979), were included in the phylogenetic analyses of the family due to the absence of an ex-lectotype strain or a type material, respectively. These authors treated *Ellisembia* Subram. as a synonym of *Sporidesmium* based on molecular data and introduced the family Distoseptisporaceae K.D. Hyde & McKenzie, typified by *Distoseptispora* K.D. Hyde, McKenzie & Maharachch., to accommodate ellisembia-like taxa with relatively short conidiophores and long cylindrical, distoseptate conidia that clustered outside Sporidesmiaceae. They also erected *Pseudosporidesmium* K.D. Hyde & McKenzie to accommodate *S. knawiae* Crous (Crous, 2008) based on its distinct morphology and phylogenetic placement within the subclass Xylariomycetidae in the Sordariomycetes. This taxon was recently placed in its own family Pseudosporidesmiaceae Crous along with a second species and *Repetophragma inflatum* (Berk. & Ravenel) W.P. Wu (Crous *et al.*, 2017). Hyde *et al.* (2016) and Yang *et al.* (2017) further described several *Sporidesmium* and *Distoseptispora* species within their respective families. Zhang *et al.* (2017) collected the first sexual morph of *Sporidesmium*, *S. thailandense* W. Dong, H. Zhang & K.D. Hyde, on submerged wood in Thailand. The fungus produces immersed ascomata with an erumpent neck and long cylindrical, unitunicate asci containing 8 fusiform, 3-septate, obliquely uniseriate ascospores. It did not exhibit an associated anamorph but clustered within Sporidesmiaceae with high support. Two monotypic, sporidesmium-like genera named *Sporidesmioides* J.F. Li, R. Phookamsak & K.D. Hyde and *Pseudostanjeuhughesia* J. Yang & K.D. Hyde (Li *et al.*, 2016; Yang *et al.*, 2017) were introduced within Torulaceae (Pleosporales, Dothideomycetes) and incertae sedis within the subclass Diaporthomycetidae (Sordariomycetes), respectively. The taxonomic and phylogenetic status of further taxa such as *S. australiense* M.B. Ellis and *S. tropicale* M.B. Ellis were clarified by these authors based on multi-gene sequence data. Finally, estimation of divergence times for taxa within Ascomycota and particularly for Sordariomycetes using molecular clock methods led Hyde *et al.* (2017) to suggest that Sporidesmiaceae and its sister family Papulosaceae might be upgraded to ordinal status under the name Sporidesmiales.

Taxonomic and biodiversity studies on saprobic microfungi associated with dead plant debris in south Florida revealed several new or poorly studied sporidesmium-like taxa (Delgado, 2008, 2009, 2010, 2013, 2014). One of them is *S. pachyanthicola* R.F. Castañeda & W.B. Kendr., first described from dead leaves of *Pachyanthus poiretii* Griseb. in neighboring Cuba (Castañeda & Kendrick, 1990). The fungus is characterized by a simple sporidesmium-like morphology consisting of long narrowly obclavate or long cylindrical, mutiseptate conidia with truncate bases and short conidiophores without percurrent extensions. Florida collections were made on rachides and petioles of dead leaves of *Sabal palmetto*, the cabbage palm, and on unidentified dead bark (Delgado, 2009). The fungus has also been reported on dead branches of *Eucalyptus* sp. from subtropical China (Wu & Zhuang, 2005) and recently on dead bamboo stems from Nicaragua (Delgado & Koukol, 2016). Shenoy *et al.* (2006) first assessed the phylogenetic relationships of

*S. pachyanthicola* based on a strain (HKUCC 10835) obtained from the Chinese specimen mentioned above but without details on the isolate's morphology or growth characteristics. Using LSU sequence data they found that the fungus belongs to Dothideomycetes where it grouped in a highly supported clade with a few members of the families Mycosphaerellaceae and Capnodiaceae. Subsequently, this single LSU sequence was included in studies of dothideomycetous or capnodiaceous fungi using a more extensive taxon sampling or to design specific primers for amplification of the LSU locus (Ma *et al.*, 2016). These studies eventually refined the phylogenetic position of *S. pachyanthicola*. The fungus was further placed in Teratosphaeriaceae, a large family segregated from the polyphyletic Mycosphaerellaceae (Crous *et al.*, 2007), within a moderately to highly supported clade together with *Staminwardia suttonii* Crous & Summerell and *Pseudoramichloridium brasiliandum* (Arzanlou & Crous) Cheew. & Crous (Arzanlou *et al.*, 2007; Yang *et al.*, 2010). It was placed also in a poorly supported group together with *Neohortaea acidophila* (Hölker, Bend, Pracht, Tetsch, Tob. Müll., M. Höfer & de Hoog) Quaedvli. & Crous within Teratosphaeriaceae (Crous *et al.*, 2009b). More recently, Hernández *et al.* (2017) placed it within Capnodiales incertae sedis sister to *Catenulostroma* species in Teratosphaeriaceae without statistical support and based on a limited taxon sampling at family level.

While isolating microfungi from samples collected in south Florida, *S. pachyanthicola* was recovered again and successfully cultivated on agar medium. In the present paper the current generic status of the fungus in *Sporidesmium* and its phylogenetic position in Capnodiales are reassessed in the light of new morphological, cultural and molecular evidence, based on a strain other than the one employed by Shenoy *et al.* (2006). Additionally, a hypothetical relationship of this species with *Sporidesmajora pennsylvaniensis* Batzer & Crous, a morphologically similar sporidesmium-like anamorph in Capnodiales (Yang *et al.*, 2010), was tested using molecular data.

## MATERIALS AND METHODS

### *Morphological and cultural study*

Dead leaves of *Sabal palmetto* were first washed off under running tap water and cut in smaller pieces for incubation at room temperature in a plastic container followed by periodical observations. The strain of *S. pachyanthicola* studied here was recovered around colonies of another fungus isolated under these conditions and growing on a 2% Malt Extract Agar (MEA) plate after seven days at 25°C. Once detected it was transferred aseptically to another MEA plate and incubated under similar conditions until sporulation was observed. Single-spore isolates were subsequently obtained following Choi *et al.* (1999). Colonies were subcultured on MEA and Potato Dextrose Agar (PDA) and descriptions are based on one month old cultures. The voucher specimen source of the isolate was reexamined to confirm the presence of the fungus on natural substrate. Fungal structures from both in vitro and in vivo conditions were mounted in lactophenol cotton blue and examined under an Olympus BX45 compound microscope. Minimum, maximum, 5th and 95th percentile values were calculated based on n = 50 measurements of each structure at 1000× magnification and outliers are given

in parentheses. Line drawings were made using a drawing tube (Carl Zeiss, Oberkochen, Germany). Epitype and isoepitype specimens in the form of semi-permanent slides and dried cultures were deposited in the Herbarium of the U.S. National Fungus Collections (BPI) and the Illinois Natural History Survey Fungarium (ILLS), respectively. An ex-epitype living culture is maintained in the Westerdijk Fungal Biodiversity Institute (CBS). Fungal and host plant names are cited throughout the text following Index Fungorum or the International Plant Names Index ([www.ipni.org](http://www.ipni.org)), respectively. Herbaria or culture collection acronyms are cited according to Index Herbariorum (<http://sweetgum.nybg.org/science/ih/>).

#### DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from a 2-week old fungal isolate grown on MEA using a DNeasy® Mini Plant extraction kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. Subsequent methods on PCR amplification and Sanger sequencing were carried out according to Promputtha & Miller (2010). The complete internal transcribed spacer (ITS) region and partial nuclear ribosomal large subunit (LSU) were amplified in separate reactions using the primer sets ITS1F-ITS4 and LROR-LR6, respectively (Vilgalys & Hester, 1990; White *et al.*, 1990; Gardes & Bruns, 1993; Rehner & Samuels, 1995). PCR products were sequenced using these primers along with LR3 and LR3R on an Applied Biosystems 3730XL high-throughput capillary sequencer (Applied Biosystems, Foster City, CA) at the W. M. Keck Center of the University of Illinois Urbana-Champaign. Consensus ITS and LSU sequences were assembled with Sequencher 5.1 (Gene Codes Corp., Ann Arbor, Michigan) and deposited in GenBank.

#### Taxon sampling and phylogenetic analyses

An ITS-LSU concatenated dataset including closest hits from megablast searches of the newly generated sequences of *S. pachyanthicola* in GenBank was prepared for analysis. The available LSU sequence DQ408557 belonging to the Chinese strain HKUCC 10835 was retrieved and added to the dataset. Additional sequences from related capnodiaceous families used in previous phylogenetic studies (Crous *et al.*, 2007, 2009b; Quaedvlieg *et al.*, 2014) were also included (Table 1). To test the hypothesis whether *S. pachyanthicola* shares phylogenetic affinities with the morphologically similar *Sporidesmajora pennsylvaniensis*, sequences from this and related taxa (Yang *et al.*, 2010; Hongsanan *et al.*, 2017) were downloaded and incorporated in the dataset. *Cladosporium macrocarpum* (Cladosporiaceae, Capnodiales) was used as outgroup. Sequences from each region were aligned separately using MAFFT v7.310 (Katoh *et al.*, 2002; Katoh & Standley, 2013) on the online server which automatically selected the FFT-NS-i strategy for both datasets. Alignments were visually examined, manually edited and concatenated in MEGA v6.06 (Tamura *et al.*, 2013). Both maximum likelihood (ML) and Bayesian inference (BI) approaches were employed to reconstruct phylogenetic relationships among taxa. ML analysis was conducted using RAxML v8.2.10 (Stamatakis, 2014) implemented on the CIPRES Science Gateway server (Miller *et al.* 2010) and employing the GTRGAMMA model. Branch support values (BS) were estimated using the rapid bootstrapping algorithm with 1000 replicates and clades with  $BS \geq 70\%$  were considered well supported (Hillis & Bull, 1993). jModeltest 2.1.10 v20160303 (Darriba *et al.*, 2012), also on the CIPRES Gateway, was used to obtain

Table 1. Strains included in this study and their Genbank accession numbers. New sequences generated during this study are in bold

Taxon	Strain	Country of origin	GenBank accession numbers	Reference
			LSU	
			ITS	
<i>Amycosphaerella africana</i>	CBS 110843	South Africa	KF901702	KF902049 Quaedvlieg <i>et al.</i> (2014)
<i>Batcheloromyces leucadendri</i>	CBS 110892	South Africa	—	EU019246 Crous <i>et al.</i> (2007)
<i>Batcheloromyces proteae</i>	CBS 110696	South Africa	JF746163	EU019247 Crous <i>et al.</i> (2007)
<i>Capnobotryella renispora</i>	CBS 214,90	Japan	AY220612	GU214398 Crous <i>et al.</i> (2009b)
<i>Capnobotryella renispora</i>	CBS 215,90	Japan	AY220613	GU214399 Crous <i>et al.</i> (2009b)
<i>Castanodospora pachyantica</i>	CBS 140347	U.S.A.	<b>MH036004</b>	This study
<i>Castanodospora pachyantica</i>	HKUCC 10835	P.R. China	—	DQ408557 Shenoy <i>et al.</i> (2006)
<i>Catenulostroma chromoblastomycesum</i>	CBS 597,97	Zaire	—	EU019251 Crous <i>et al.</i> (2007)
<i>Catenulostroma lignicola</i>	FMR 11491	Spain	KY853429	KY853489 Hernández <i>et al.</i> (2017)
<i>Chaetothyriina guttulata</i>	MFLUCC15-1080	Thailand	KX372277	KU358917 Hongsanan <i>et al.</i> (2017)
<i>Chaetothyriina guttulata</i>	MFLUCC15-1081	Thailand	KX372276	KU358914 Hongsanan <i>et al.</i> (2017)
<i>Cladosporium macrocarpum</i>	MFLUCC15-0383	Thailand	KX372275	KU710171 Singtripop <i>et al.</i> (2016)
<i>Dissocionium aciculare</i>	CBS 299,67	Turkey	EF679372	EF679372 Schubert <i>et al.</i> (2007)
<i>Extremus adstrictus</i>	CBS 342,82	Germany	AF172308	EU019266 Crous <i>et al.</i> (2007)
<i>Extremus antarcticus</i>	CBS 118292	Spain	AY559346	KF310022 Quaedvlieg <i>et al.</i> (2014)
<i>Extremus antarcticus</i>	CBS 136103	Antarctica	KF309978	GU250360 Quaedvlieg <i>et al.</i> (2014)
<i>Extremus antarcticus</i>	CBS 136104	Antarctica	KF309980	KF310021 Quaedvlieg <i>et al.</i> (2014)
<i>Hortaea thailandica</i>	CBS 125423	Thailand	GU214437	GU214429 Crous <i>et al.</i> (2009b)
<i>Hortaea weinckii</i>	CBS 107,67	Portugal	AJ238468	EU019270 Crous <i>et al.</i> (2007)
<i>Hortaea weinckii</i>	CBS 100496	Greece	AY128703	GU301817 Schoch <i>et al.</i> (2009)
<i>Houjia pomigena</i>	CBS 125224	U.S.A.	AY598885	AY598825 Yang <i>et al.</i> (2010)
<i>Houjia yanglingensis</i>	CBS 125227	U.S.A.	FJ438378	FJ47166 Hongsanan <i>et al.</i> (2017)
<i>Houjia yanglingensis</i>	CBS 125225	P.R. China	GQ433631	GQ433631 Hongsanan <i>et al.</i> (2017)

Taxon	Strain	Country of origin	GenBank accession numbers	Reference
			ITS LSU	
<i>Houjia yanglingensis</i>	CBS 125226	P.R. China	GQ433629	Hongsanan <i>et al.</i> (2017)
<i>Neocatenulostroma germanicum</i>	CBS 53988	Germany	EU019253	Crous <i>et al.</i> (2007)
<i>Neocatenulostroma microsporatum</i>	CBS 110890	South Africa	AY260097	Quaedvlieg <i>et al.</i> (2014)
<i>Neohortaea acidiophila</i>	CBS 113389	Germany	GU214636	Schoch <i>et al.</i> (2009)
<i>Paramycosphaerella madeirensis</i>	CBS 112995	Madeira	AY725553	Hunter <i>et al.</i> (2006)
<i>Paramycosphaerella marksii</i>	CBS 68295	South Africa	DQ267587	Hunter <i>et al.</i> (2006)
<i>Paramycosphaerella parkii</i>	CBS 38792	Brazil	AY626979	Crous <i>et al.</i> (2009c)
<i>Parapenidiella pseudotasmannensis</i>	CBS 111687	Australia	AF310107	Crous <i>et al.</i> (2009b)
<i>Parateratosphaeria bellula</i>	CBS 111700	South Africa	EU019301	Crous <i>et al.</i> (2007)
<i>Passalora zambiae</i>	CBS 112971	Zambia	AY725533	Hunter <i>et al.</i> (2006)
<i>Penophila incerta</i>	CBS 118608	Spain	AY559372	Egidio <i>et al.</i> (2014)
<i>Petrophila incerta</i>	CBS 118287	Spain	AY559337	Egidio <i>et al.</i> (2014)
<i>Phaeothecoidiella illinoiensis</i>	CBS 125223	U.S.A.	GU117897	Yang <i>et al.</i> (2010)
<i>Phaeothecoidiella illinoiensis</i>	CMG TN1 2.4E1d	U.S.A.	GU117898	Yang <i>et al.</i> (2010)
<i>Phaeothecoidiella missouriensis</i>	CBS 125222	U.S.A.	AY598878	Yang <i>et al.</i> (2010)
<i>Phaeothecoidiella missouriensis</i>	CBS 118959	U.S.A.	GU117899	Yang <i>et al.</i> (2010)
<i>Pseudoramichloridium brasiliatum</i>	CBS 28392	Brazil	EU041797	Arzanlou <i>et al.</i> (2007)
<i>Pseudoramichloridium henryii</i>	CPC 13122	Australia	KF901533	Quaedvlieg <i>et al.</i> (2014)
<i>Pseudoteratosphaeria flexuosa</i>	CBS 111012	Colombia	AF309603	Hunter <i>et al.</i> (2006)
<i>Pseudoteratosphaeria secundaria</i>	CBS 115608	Brazil	DQ303018	Crous <i>et al.</i> (2007)
<i>Ramichloridium apiculatum</i>	CPC 12310	South Korea	GU214687	Crous <i>et al.</i> (2009b)
<i>Ramularia endophylla</i>	CBS 113265	The Netherlands	AY490763	Verkley <i>et al.</i> (2004)
<i>Repetophragma zigopetalii</i>	VIC 42946	Brazil	KU609074	Buyck <i>et al.</i> (2017)
<i>Schizothryrium pomi</i>	CBS 40661	The Netherlands	EF134949	Baizer <i>et al.</i> (2008)

<i>Schizothyrium pomi</i>	CBS 22857	Italy	EF134947	Baizer <i>et al.</i> (2008)
<i>Sonderhenia eucalypticola</i>	CPC 11252	Spain	DQ303064	Crous <i>et al.</i> (2009b)
<i>Sporidesmajora pennsyhantensis</i>	CBS 125229	U.S.A.	FJ438379	Yang <i>et al.</i> (2010)
<i>Staninwardia suttonii</i>	CBS 120061	Australia	DQ923555	Summerell <i>et al.</i> (2006)
<i>Stomiopeltis versicolor</i>	GA3 23C2b	U.S.A.	FJ438375	Díaz <i>et al.</i> (2010)
<i>Teratoramularia kirschneriana</i>	CBS 113093	Taiwan	GU214669	Videira <i>et al.</i> (2016)
<i>Teratosphaeria cryptica</i>	CBS 110975	Australia	GQ852682	Crous <i>et al.</i> (2009c)
<i>Teratosphaeria dimorpha</i>	CBS 120086	Australia	DQ923528	Summerell <i>et al.</i> (2006)
<i>Teratosphaeria fibrillosa</i>	CBS 121707	South Africa	EU707862	Quaedvlieg <i>et al.</i> (2014)
<i>Teratosphaeria macowanii</i>	CBS 111029	South Africa	AY260096	Crous <i>et al.</i> (2009a)
<i>Teratosphaeria maxi</i>	CPC 12943	South Africa	DQ885898	Crous & Groenewald (2006)
<i>Teratosphaeria nubilosa</i>	CBS 116005	Australia	AY725572	Crous <i>et al.</i> (2009b)
<i>Teratosphaeria stellenboschiana</i>	CBS 116228	South Africa	AY725518	Crous <i>et al.</i> (2007)
<i>Uwebraunia dekeri</i>	CBS 111272	South Africa	AY725551	Crous <i>et al.</i> (2009b)
<i>Uwebraunia dekeri</i>	CBS 567.89	The Netherlands	AF173309	Crous <i>et al.</i> (2007)
<i>Vermiconia antarctica</i>	CBS 136107	Antarctica	KF309982	Quaedvlieg <i>et al.</i> (2014)
<i>Vermiconia calcicola</i>	CBS 140080	Italy	KP791759	Isola <i>et al.</i> (2016)
<i>Vermiconia calcicola</i>	CCFEE 5947	Italy	KP791763	Isola <i>et al.</i> (2016)
<i>Vermiconia flagrans</i>	CBS 118283	Spain	—	Quaedvlieg <i>et al.</i> (2014)
<i>Vermiconia foris</i>	CBS 136106	Italy	KF309981	Quaedvlieg <i>et al.</i> (2014)
<i>Zygomphiala cryptogama</i>	CBS 125658	U.S.A.	FJ425208	Díaz <i>et al.</i> (2010)
<i>Zygomphiala wisconsinensis</i>	CBS 125659	U.S.A.	FJ425209	Díaz <i>et al.</i> (2010)

Abbreviations: **CAP**: Centro de Recursos Microbiológicos, Universidade Nova de Lisboa, Caparica, Portugal; **CBS**: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CCFEE**: Culture Collection of Fungi from Extreme Environments, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy; **CMG**: Culture collection of Markt Gleason at Iowa State University, Ames, IA, USA; **CPC**: Culture collection of P.W. Crous; **FMR**: Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Reus, Spain; **GA3**: Deposited at Department of Plant Pathology & Microbiology, Iowa State University, Ames, IA, USA; **HKUCC**: The University of Hong Kong Culture Collection, Hong Kong, PR. China; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **VIC**: Departamento de Fitopatología, Universidade Federal de Viçosa, Viçosa, Brazil.

the best fitting substitution model for BI analysis which choose GTR+I+G using the Akaike Information Criterion. Bayesian inference was performed with MrBayes v3.2.6 (Ronquist & Huelsenbeck, 2003; Ronquist *et al.*, 2012) and consisted of two independent runs of 10 million generations with four Markov Chain Monte Carlo chains set to stop when standard deviation of split frequencies decreased below 0.01 and trees sampled every 100th generation. The first 25% of trees were removed as burn-in and Bayesian posterior probabilities (BPP) for each node were estimated from the 50% majority rule consensus of the remaining trees. Convergence of runs was further diagnosed in Tracer v1.6.0 (Rambaut *et al.*, 2014) and clades receiving  $BPP \geq 95\%$  were considered statistically significant (Alfaro *et al.*, 2003). Trees were viewed in FigTree v1.4.2. (Rambaut, 2009) and edited in MEGA or Inkscape ([inkscape.org](http://inkscape.org)).

## RESULTS

### *Phylogenetic analyses*

The newly obtained LSU sequence from the Florida strain of *S. pachyanthicola* CBS 140347 was 278 bp longer than the one retrieved online from strain HKUCC 10835 isolated in China. They were 99.5% identical when comparing their overlapping region of 823 bp and differed only by 3 bp and 1 gap. An ITS sequence from the Chinese strain was not available for comparison. The final ITS-LSU dataset consisted of 69 sequences representing 56 taxa including the outgroup and 1974 nucleotide characters, 635 from the ITS alignment and 1339 from the LSU alignment. The RaxML and BI phylogenies were identical in topology and the best-scoring ML tree from the RaxML analysis is shown in Fig. 1. Effective Sample Size values of all relevant parameters were  $> 200$  as verified in Tracer indicating adequate sampling of the posterior distribution (Drummond *et al.*, 2006; Drummond & Rambaut, 2009). The backbone of the tree representing the order Capnodiales was strongly supported in the Bayesian analysis (1.0 BPP) but without BS support. Clades corresponding to the different capnodiaceous families included in the analyses were recovered as monophyletic with high support in both trees except Teratosphaeriaceae, similar to previous phylogenetic studies (Crous *et al.*, 2009b; Yang *et al.*, 2010; Quaedvlieg *et al.*, 2014). Both strains of *S. pachyanthicola* clustered with maximum support (100% BS, 1.0 BPP). They were sister to *Neohortaea acidophila* CBS 113389 also with high support (99% BS, 1.0 BPP). The three strains occurred within a highly supported monophyletic clade (99% BS, 1.0 BPP) formed by members of the family Extremaceae and including the type genus and species *Extremus adstrictus* (Egidi & Onofri) Quaedvlieg & Crous. *Sporidesmajora pennsylvaniensis* CBS 125229 grouped with high support (99% BS, 1.0 BPP) with species of another sporidesmium-like genus, *Houjia* G.Y. Sun & Crous, represented by *H. pomigena* Batzer & Crous and *H. yanglingensis* G.Y. Sun & Crous. They occurred within a monophyletic clade with maximum support (100% BS, 1.0 BPP) representing the family Phaeothecoidiellaceae K.D. Hyde & Hongsanan and including *Phaeothecoidiella missouriensis* Batzer & Crous, its type genus and species.

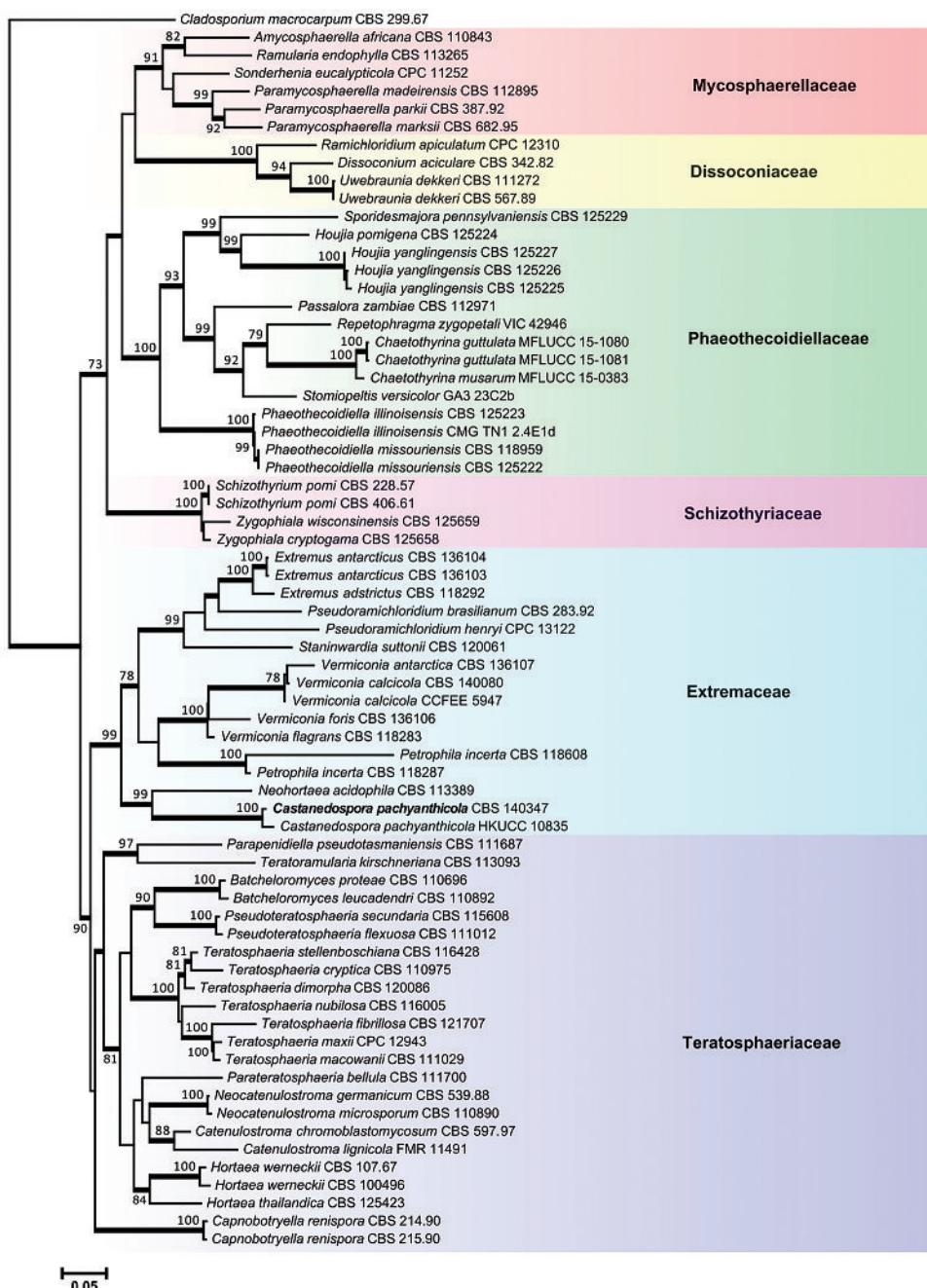


Fig. 1. RaxML phylogenetic tree (ML likelihood = -18908.58) constructed from a concatenated ITS-LSU dataset of 69 sequences belonging to selected families in Capnodiales showing the placement of *Castanedospora pachyanthica*. The name of the new strain obtained during this work is highlighted in bold. Numbers above branches represent ML bootstrap support values  $\geq 70\%$  and thickened branches indicate posterior probabilities  $\geq 95\%$ .

### Taxonomy

***Castanedospora* G. Delgado & A.N. Mill., gen. nov.**

Mycobank: MB 824583

Hyphomycetous. *Colonies* with mycelium partly superficial, partly immersed, composed of branched, septate, pale brown to brown, smooth or finely verruculose hyphae. *Conidiophores* macronematous, mononematous, simple, cylindrical, smooth, brown, septate, without percurrent extensions. *Conidiogenous cells* monoblastic, integrated, terminal, cylindrical or doliform, determinate, brown, apex truncate. *Conidia* long narrowly obclavate or subcylindrical, attenuated toward the apex, straight or flexuous, multiseptate, pale brown to brown, smooth, apex rounded, basal cell conico-truncate.

Type species: *Castanedospora pachyanthicola* (R.F. Castañeda & W.B. Kendr.) G. Delgado & A.N. Mill.

*Etymology:* Named in honor of Dr. Rafael F. Castañeda-Ruiz, Cuban mycologist who first described this fungus and who has extensively contributed to the study of tropical hyphomycetes.

***Castanedospora pachyanthicola* (R.F. Castañeda & W.B. Kendr.) G. Delgado & A.N. Mill. comb. nov.**

Figs 2-3

Mycobank: MB 824584

Basionym: *Sporidesmium pachyanthicola* R.F. Castañeda & W.B. Kendr., Univ. Waterloo Biol. Ser. 33: 45, 1990.

*Colonies* growing saprotrophically on natural substrate, inconspicuous, hairy. *Mycelium* partly superficial, partly immersed, composed of branched, septate, smooth, pale brown to brown hyphae, 1.5-2 µm wide. *Conidiophores* macronematous, mononematous, cylindrical or subcylindrical, straight or flexuous, simple, smooth, brown, 1-7-septate, (8) 10-26 (37) × 3.5-5 µm, often slightly bulbous at their base and up to 6 µm wide, without percurrent extensions. *Conidiogenous cells* monoblastic, integrated, terminal, cylindrical, determinate, brown, 5-7 × 3-4 µm, apex truncate. *Conidia* narrowly obclavate or subcylindrical, attenuated toward the apex, straight or flexuous, 9-33-euseptate, rarely constricted at one septum, pale brown to brown becoming subhyaline or hyaline towards the apex, smooth, (36) 43-162 (188) × 3-5 µm, apex rounded, 1.5-2 µm wide, basal cell conico-truncate. *Teleomorph* unknown.

*Colonies* on MEA slow growing, reaching 13-18 mm diam. after one month at 25°C, circular, gray, felty, raised 2-3 mm, margin entire, reverse black. *Colonies* on PDA similar to MEA, slow growing, reaching 17-19 mm diam. after one month at 25°C, sporulation late, starting after two months on both MEA and PDA. *Mycelium* composed of subhyaline to pale brown, septate, branched, smooth or finely verruculose hyphae, 1.5-3 (4) µm wide. *Conidiophores* similar to those on natural substrate, solitary or in groups of two, terminal or intercalary on the hyphae, when terminal macro-, semimacro- or micronematous, brown, paler when terminal, 1-8-septate, straight or sometimes flexuous, smooth or verrucose at the base, slightly constricted at septa, (6) 10-45 (60) × 3-6 µm. *Conidiogenous cells* doliform or cylindrical, often terminal on the hyphae and narrowly cylindrical or subcylindrical, 5-8 × 3-4 mm, apex truncate, 2-3 µm wide. *Conidia* long narrowly obclavate to long cylindrical, pale brown, (13) 31-200 (219) septate, (146) 172-825 (942) µm long, 2-5 (6) µm wide, 2-2.5 µm wide at the apex, rarely slightly constricted at one

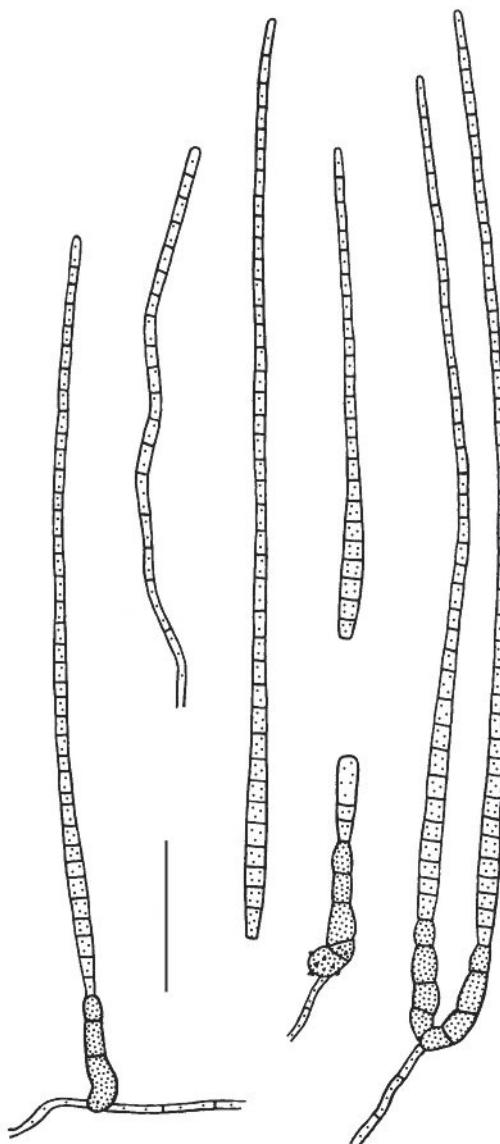


Fig. 2. *Castanedospora pachyanthicola* (from epitype). Hyphae, conidiophores, conidiogenous cells and conidia on MEA.  
Scale bar = 30  $\mu\text{m}$ .

septum, some cells swollen, 0-3-septate and 4.5-6  $\mu\text{m}$  wide, basal cell cylindrical or conico-truncate.

**Typification:** Holotype of *S. pachyanthicola*: Cuba, Pinar del Rio, Viñales, on dead leaves of *Pachyanthus poiretii*, leg. R.F. Castañeda, 27 March 1989, INIFAT C89/138. Epitype designated here: United States, Florida, Broward Co., Fort Lauderdale, 26°12'20.5"N 80°09'50.6"W, 2.9 m asl., on petiole of dead leaf of *S. palmetto* (Walter) Lodd. ex Schult. & Schult. (Arecaceae), 25 January 2014, leg. J.M. Pérez (BPI 892972B; isoepitype ILLS 80801; ex-epitype strain CBS 140347; ex-epitype sequences: ITS MH036004, LSU MH036005).

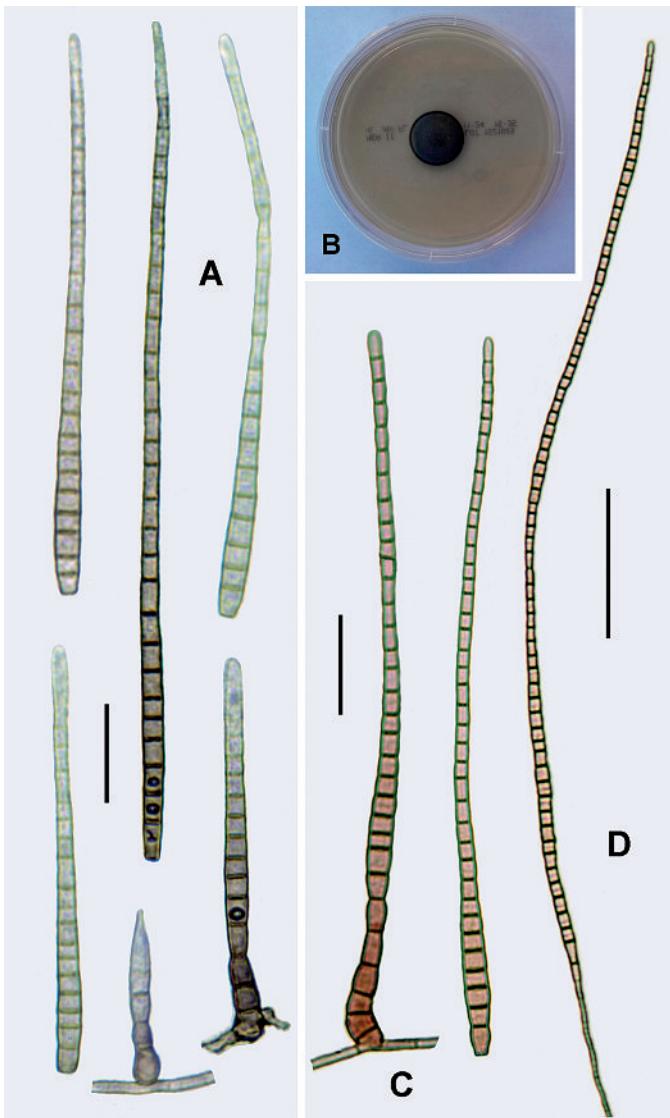


Fig. 3. *Castanedospora pachyanthicola* (from epitype). A. Conidiophores and conidia on natural substrate. B. Colony on MEA after one month at 25°C. C. Conidiophore and conidia on MEA. D. Terminal conidium on the hyphae. Scale bars: A, C = 20 µm, D = 50 µm.

*Notes:* The holotype specimen from Cuba (Castañeda & Kendrick, 1990) has slightly longer conidiophores (30-75 µm) and shorter conidia (50-140 µm) compared with the material studied here on natural substrate. Other specimens previously described from identical palm tree species in Florida (Delgado, 2009) are morphologically similar to the present collection. The Chinese specimen WU 1595 (Wu & Zhuang, 2005) and the source of strain HKUCC 10835 (Shenoy *et al.*, 2006) has shorter conidiophores (20-40 µm) and shorter conidia (35-90 µm). Specimens collected in Nicaragua possessed shorter conidiophores (14-32 µm) and the shortest conidia of all (24-57 µm) with fewer, 5-12 septa in each conidium (Delgado & Koukol, 2016).

## DISCUSSION

The morphological study of the specimen of *S. pachyanthicola* from Florida shows that its characteristics agree well with the protologue of the species despite some minor differences in conidiophore and conidial length. These morphological differences may be attributed to intraspecific variability. The holotype material from Cuba no longer contains conidial structures and the only available microscope slide preparation is lost (Castañeda, pers. comm.). There is no preserved ex-type living culture and therefore the species *S. pachyanthicola* is epitypified with the specimen collected in Florida. On artificial media, the strain CBS 140347 obtained from this specimen often produced conidia reaching more than 900 µm long and having up to 219 septa (Fig. 3D) being considerably longer than conidia on natural substrate. Unfortunately, morphological data are not available for the other existing strain of the fungus, HKUCC 10835, which was not available upon request precluding comparison of morphology and growth characteristics between the two isolates. Such differences in conidiophore and conidial length are apparently not uncommon among sporidesmium-like fungi when growing in artificial conditions. Matsushima (1993) commented on the highly variable length of conidia in *Repetophragma filisporum* (Matsush.) R.F. Castañeda, McKenzie & K.D. Hyde ( $\equiv$  *S. filisporum* Matsush.), shorter on natural substrate (27-70 µm) but longer when growing on a piece of steam-sterilized banana leaf on Corn Meal Agar (50-300 µm). Yang *et al.* (2017) attributed the significant differences in conidial length among specimens of *Distoseptispora multiseptata* J. Yang & K.D. Hyde to the length of incubation. Conidia of the holotype specimen reached up to 380 µm in length while conidia of a second collection measured up to 700 µm in length. According to Wu & Zhuang (2005) most sporidesmium-like taxa can be cultured on Potato Dextrose Agar. However, they are largely underrepresented in international culture collections and only until recently their cultural characteristics started to be recorded on a regular basis with the advent of molecular studies in the group preceded by isolation on agar media. As a result, the taxonomic value of morphological differences between growth under natural and artificial conditions, if any, is still unclear.

Recently, the family Sporidesmiaceae was resurrected in Sordariomycetes for a clade containing several sporidesmium-like taxa morphologically resembling *S. ehrenbergii*, the lectotype species of the genus (Su *et al.*, 2016). This was a major step toward clarifying phylogenetic relationships within the large and polyphyletic genus *Sporidesmium* and its allies. The concept, however, is still a working hypothesis in need of testing pending the availability of fresh collections or isolates of *S. ehrenbergii*, and therefore the phylogeny of the genus still remains unsettled (Crous *et al.*, 2017). Nevertheless, the taxonomic status of phylogenetically distant taxa previously placed in *Sporidesmium* such as *S. pachyanthicola* (Castañeda & Kendrick, 1990) can be reassessed now with more confidence. *Castanedospora* is introduced as a new monotypic genus to accommodate this taxon based on a freshly collected specimen and new morphological, cultural and molecular data. Our phylogenetic analyses confirmed its previously known placement within Capnodiales (Dothideomycetes) (Shenoy *et al.*, 2006), distant from Sporidesmiaceae sensu stricto in Sordariomycetes. They also revealed additional evidence to support its familial position outside the broadly defined Teratosphaeriaceae and within the related Extremaceae. Quaedvlieg *et al.* (2014) introduced Extremaceae to accommodate teratosphaeriaceous asexual fungi known to occur in extreme habitats such as rocks surfaces or within rocks in the Mediterranean region or Antarctica (Ruibal *et al.*,

2009). Current family members are morphologically and ecologically highly diverse being filamentous or yeast-like and ranging from lichenicolous, epiphyllous, and acidophilic or occurring as endophytes, plant pathogens or saprobes. The latter taxa include *Pseudoramichloridium brasiliannum*, isolated from forest soil in Brazil (Arzanlou *et al.*, 2007), or *P. henryi*, isolated from leaf spots of *Corymbia henryi* (S.T. Blake) K.D. Hill & L.A.S. Johnson in Australia (Cheewangkoon *et al.*, 2009), and now *C. pachyanthicola* recorded on miscellaneous plant debris. The family diagnosis included so far sympodially proliferating conidiogenous cells with or without a terminal rachis that can be subdenticulate and conidia rarely with 1-2 transverse septa (Quaedvlieg *et al.*, 2014). With the addition of *Castanedospora*, this concept is expanded to incorporate monoblastic, determinate conidiogenesis and simple, multiseptate conidia. In the present ITS-LSU phylogeny the closest relative of *C. pachyanthicola* was *Neohortaea acidophila* ( $\equiv$  *Hortaea acidophila* Hölker, Bend, Pracht, Tetsch, Tob. Müll., M. Höfer & de Hoog), an extremophilic black yeast isolated from an extract of lignite rock containing humic and fulvic acids at pH 0.6 (Hölker *et al.*, 2004). Quaedvlieg *et al.* (2014) introduced *Neohortaea* to accommodate this acidophilic species in a genus morphologically similar but phylogenetically distinct from *Hortaea* (H.) Nishim. & Miyaji in Teratosphaeriaceae. Surprisingly, the taxon was not included in their extensive multigene trees and its position was left inconclusive. Our phylogeny, on the other hand, supports its placement in Extremaceae distant from other *Hortaea* species in Teratosphaeriaceae such as *H. werneckii* (Horta) Nishim. & Miyaji, the generic type, and *H. thailandica* Crous & K.D. Hyde (Fig. 1). Crous *et al.* (2009b) also showed a close relationship between *N. acidophila* and *C. pachyanthicola* in a single gene analysis using LSU sequence data. Their different morphologies and contrasting ecologies are consistent with the diversity shown in Extremaceae, but additional taxon sampling of family members together with other phylogenetic markers are needed to clarify their intriguing relationship.

A possible affinity between *Castanedospora* and *Sporidesmaja* was not supported in our analyses despite their morphological resemblance in culture and phylogenetic placement in Capnodiales. This monotypic sporidesmium-like genus, typified by *Sp. pennsylvaniensis*, was first isolated from the sooty blotch and flyspeck (SBFS) disease complex on apple in the state of Pennsylvania, U.S.A (Yang *et al.*, 2010). It is characterized by forming long narrowly obclavate, multiseptate conidia when grown on culture media that can reach up to 350  $\mu\text{m}$  on oatmeal agar. Our family-level phylogeny clearly shows that *Castanedospora* and *Sporidesmaja* are not congeneric but phylogenetically distant with the latter belonging to Phaeothecoidiellaceae (Hongsanan *et al.* 2017). This capnodiaceous family was recently introduced for a well-supported clade tentatively named ‘Micropeltidaceae’ by Yang *et al.* (2010) including *Sporidesmaja*. It is based on the genus *Phaeothecoidiella* Batzer & Crous whose two species produce pigmented endoconidia that resemble phragmospores covered by a prominent mucilaginous sheath inside hyphae. *Sporidesmaja* is closely related with *Houjia* (Seifert *et al.*, 2011), another sporidesmium-like but morphologically different genus, within Phaeothecoidiellaceae. Its two species are characterized by conidiophores reduced to conidiogenous cells and short, broadly ellipsoid to subcylindrical or obclavate, transversely and obliquely septate conidia tapering to a cuneiform basal cell possessing a truncate hilum. The family also includes morphologically disparate epiphytic species producing small, flattened thyriothelial ascocarps associated with SBFS diseases of economic fruits such as mango and banana e.g. *Chaetothyridina* Theiss (Singtripop *et al.*, 2016; Hongsanan *et al.*, 2017). Recently, Buyck *et al.* (2017) described *Repetophragma*

*zygopetalii* O.L. Pereira, Meir. Silva & R.F. Castañeda as belonging to the 'Micropeltidaceae' clade mentioned above. In our phylogeny this taxon is actually a member of Phaeothecoidiellaceae (Fig. 1) suggesting that the sporidesmium-like morphology, although still poorly sampled at the ordinal level, might be rather widespread within Capnodiales.

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