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Ribosomal and RPB2 DNA sequence analyses suggest that *Sporidesmium* and morphologically similar genera are polyphyletic

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ABSTRACT

Sporidesmium and morphologically similar dematiaceous, hyphomycetous genera are characterised by holoblastic phragmoconidia produced on proliferating or non-proliferating conidiophores. They include a number of asexual (anamorphic) genera taxonomically segregated from *Sporidesmium sensu lato* and are similar in having schizolytic conidial secession. The taxonomy of these ubiquitous asexual fungi and their affinities with known *Ascomycetes* are, however, still obscure. This study incorporates a phylogenetic investigation, based on the LSU nu-rDNA and RNA polymerase II second largest subunit (RPB2) gene sequence, to assess the possible familial placement of *Ellisembia*, *Linkosia*, *Repetophragma*, *Sporidesmiella*, *Sporidesmium* and *Stanjehughesia*, and justify whether anamorphic characters are proper phylogenetic indicators. Phylogenies provide conclusive evidence to suggest that *Sporidesmium* is not monophyletic and species are phylogenetically distributed in two major ascomycete classes, *Dothideomycetes* and *Sordariomycetes*. Morphologies currently used in their classification have undergone convergent evolution and are not phylogenetically reliable. The possible teleomorphic affinities of these anamorphic genera are discussed in light of morphology and molecular data. As these anamorphs, in most cases, are the sole known morph of the holomorph, it is proposed that in the absence of or failure to detect their teleomorphic phase, the anamorph names should be used for the holomorph.

Introduction

Pleomorphism in the Kingdom *Fungi* results in the occurrence of morphologically distinct, visibly unconnected asexual (anamorphic) and sexual (teleomorphic) phases, which are of the same fungal species and may occur at different times or in different habitats (Burnett 2003; Puja *et al.* 2006). It is thought that many fungi only occur as an anamorphic state (Hennebert 1993) and have lost their ability to form a teleomorph (Seifert & Gams 2001). The apparent absence of sexual reproduction in almost one-fifth of the fungi (Pringle *et al.* 2005) requires continuation of the dual classification system

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in fungi (Seifert & Samuels 2000). The anamorphic classification system, however, has led to an artificial classification of asexual (anamorphic) fungi. An interesting example is *Sporidesmium* and morphologically similar genera. They include several dematiaceous, hyphomycetous genera taxonomically segregated from *Sporidesmium sensu lato*. These anamorphic fungi produce holoblastic phragmoconidia on proliferating or non-proliferating conidiophores and are known to have schizolytic conidial secession (Ellis 1958, 1971, 1976; Kirk 1982; Subramanian 1992; Hernández-Guitiérrez & Sutton 1997; Shoemaker & Hambleton 2001; Wu & Zhuang 2005). They occur predominantly in their anamorphic phase, as saprobes on rotten wood, dead branches, and decaying leaves of various plant species. Some species are also important plant pathogens (Ellis 1971, 1976; Guo 1989; Wu & Zhuang 2005).

Sporidesmium was described to accommodate *Sporidesmium atrum*, which featured 'conidia with 3–5 transverse septa borne singly at the ends of short conidiophores' (Ellis 1958). In the absence of the type material or any authentic collection of *S. atrum*, Ellis (1958) considered *S. ehrenbergii* to be conspecific to the type. Responding to a view that *Sporidesmium* is heterogeneous (Hughes 1979), Kirk (1982) introduced *Sporidesmiella*, for those species with few septa and cuneate conidia. The generic circumscription of *Sporidesmium* was further narrowed in a major rearrangement by Subramanian (1992), who proposed several novel anamorphic genera, including *Ellisembia*, *Penzigomyces*, *Repetophragma*, and *Stanjehughesia*, based on conidial ontogeny (Table 1). This approach was further modified by Hernández-Guitiérrez and Sutton (1997) who described *Imimyces* and *Linkosia*, and Shoemaker and Hambleton (2001) who introduced *Imicles*. Recently, Wu and Zhuang (2005) proposed a new generic concept by combining *Sporidesmium* and *Penzigomyces* into one genus as *Sporidesmium*, and merging *Imicles* with *Ellisembia*. The present generic delineation is based on the absence/presence of conidiophore, type of conidiophore proliferation and conidial septation (Table 1).

Molecular phylogenetic studies on this group are limited to a recent report of Réblová and Winka (2001). Based on phylogenetic analyses of the LSU nu-rDNA sequences, Réblová and Winka (2001) reported that *Ellisembia folliculatum* (as *Sporidesmium folliculatum* and anamorphic *Lecythothecium duriligni*) and *Stanjehughesia hormiscioides* (as *Sporidesmium hormiscioides* and anamorphic *Umbrinosphaeria caesariata*) are phylogenetically linked to the *Chaetosphaeriaceae*. However, available morphological data, either based on cultural studies or association of two fungi on same substrate, reveal that *Sporidesmium* and morphologically similar genera may have teleomorphic associations with five ascomycete families within the *Dothideomycetes* and *Sordariomycetes* (Table 2). Therefore, the taxonomic utility of morphological characters used to delimit the genera is questionable (Réblová 1999). The present study, based on phylogenetic analyses of partial sequences of LSU nu-rDNA and RPB2 gene, aims to: (1) investigate phylogenetic affinities of *Sporidesmium* and morphologically similar genera with known ascomycete families and, (2) assess the phylogenetic importance of morphological characters used to delimit the genera.

Materials and methods

Fungal isolates and DNA extraction

Most of the fungal isolates used in the study were procured from the Culture Collection in Novozymes China, Beijing. For genetic uniformity, all the isolates used in this study were obtained by single spore isolation method (Choi *et al.* 1999; Wu & Zhuang 2005). Isolates used, together with accession numbers, are listed in Table 3. Isolates were grown on potato-dextrose agar (PDA) and malt-extract agar (MEA) for two to four weeks and total genomic DNA was extracted from mycelia following the protocols as outlined by Jeewon *et al.* (2003, 2004) and Cai *et al.* (2005, 2006).

DNA amplification and sequencing

DNA amplification was performed by PCR. For partial LSU nu-rDNA amplification, LROR and LR5 primers (Vilgalys & Hester 1990) were used. The fRPB2-5F and fRPB2-7cR primers were used for the amplification of partial RPB2 gene (Liu *et al.* 1999). The amplification reaction for partial LSU nu-rDNA and partial RPB2 gene was performed in a 50 µl reaction volume as outlined by Jeewon *et al.* (2004): 1 × PCR buffer, 0.2 mM d'NTP, 0.3 µM of each primer; 1.5 mM $MgCl_2$, 0.8 units Taq Polymerase and 5–10 ng DNA. The PCR thermal cycle for partial LSU nu-rDNA amplication was as follows: 95 °C for 3 min, followed by 34 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 30 s and elongation at 72 °C for 1 min, with a final extension step of 72 °C for 10 min (Vilgalys & Hester 1990). The PCR thermal cycle for partial RPB2 gene amplification consisted of 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 2 min and elongation at 72 °C

Table 1 – Morphology of *Sporidesmium* and similar genera

Genus	Conidiophore	Conidiophore proliferation	Conidial septation	Ref.
Sporidesmium	Present	Lacking/percurrent	Euseptate	1, 3, 5.
Ellisembia	Present	Lacking/percurrent	Distoseptate	3, 5
Repetophragma	Present	Annellidic	Euseptate	3
Sporidesmiella	Present	Annellidic	Distoseptate	2
Stanjehughesia	Absent	-	Euseptate	3
Linkosia	Absent	-	Distoseptate	4

1: Ellis (1958); 2: Kirk (1982); 3: Subramanian (1992); 4: Hernández-Guitiérrez & Sutton (1997); 5: Wu & Zhuang (2005).

Table 2 – The anamorph–teleomorph associations of *Sporidesmium* and morphologically similar genera

Anamorph	Teleomorph	Family	Ref.[a]
Class: *Dothideomycetes*			
Sporidesmium kielmeyerae	*Akaropeltella kielmeyerae*	*Micropeltidaceae*	4, 9
Sporidesmium lycii	*Cucurbitaria varians*	*Cucurbitariaceae*	6
Sporidesmium sp.	*Eupelte rapaneae*	*Asterinaceae*	1, 6
Sporidesmium sp.	*Eupelte amicta*	*Asterinaceae*	6
Sporidesmium sp.	*Placosoma nothopanacis*	*Asterinaceae*	5, 6
Class: *Sordariomycetes*			
Ellisembia adscendens	*Miyoshiella triseptata*	*Chaetosphaeriaceae*	7, 9
Ellisembia bambusicola	*Miyoshiella fusispora*	*Chaetosphaeriaceae*	9
Ellisembia folliculatum	*Lecythothecium duriligni*[b]	*Chaetosphaeriaceae*	10
Sporidesmium scutellare	*Eriosphaeria aggregata*	*Trichosphaeriaceae*	3
Sporidesmium sp.	*Chaetosphaeria capitata*	*Chaetosphaeriaceae*	8
Stanjehughesia hormiscioides	*Umbrinosphaeria caesariata*[b]	*Chaetosphaeriaceae*	10
Stanjehughesia larvata	*Miyoshiella larvata*	*Chaetosphaeriaceae*	2, 9

a 1: Ellis (1958); 2: Hino & Katumoto (1961); 3: Müller & Munk (1964); 4: Farr (1972); 5: Hughes (1978); 6: Sivanesan (1984); 7: Shoemaker & White (1985); 8: Sivanesan & Chang (1995); 9: Réblová (1999); 10: Réblová & Winka (2001).
b Well-established anamorph–teleomorph connections by culture-based and DNA-mediated studies.

for 1.5 min, with a final extension step of 72 °C for 10 min (Liu *et al.* 1999). The PCR products, spanning approximately 900 bp (partial LSU nu-rDNA) and 1200 bp (partial RPB2), were checked on 1% agarose electrophoresis gels stained with ethidium bromide. The PCR products were then purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham Biosciences, Buckinghamshire, UK; product code: 27-9602-01). DNA sequencing was performed using the above-mentioned primers in an Applied Biosystem 3730 DNA analyser at the Genome Research Centre, The University of Hong Kong.

Table 3 – Newly generated sequences: taxon, isolate code/s, and GenBank accession number

Taxon[a]	Isolate code/s[b]	GenBank accession numbers	
		LSU nu-rDNA	RPB2
Ellisembia sp. (1)	NN43946	DQ 408568	
Ellisembia sp. (2)	HKUCC 10558 (NN8247B)	DQ 408565	DQ435088
E. adscendens	HKUCC 10820 (NN44654)	DQ 408561	DQ435092
E. bambusicola	HKUCC 3578	DQ 408562	
E. brachypus	HKUCC 10555 (NN8080B)	DQ 408563	DQ435083
E. calyptrata	HKUCC 10821 (NN44042)	DQ 408564	DQ435085
E. leonensis	HKUCC 10822 (NN44360)	DQ 408566	DQ435089
E. minigelatinosa	NN47497	DQ 408567	DQ435090
Linkosia fusiformis	HKUCC 10824 (NN43150)	DQ 408571	DQ435084
L. multiseptum	HKUCC 10825 (NN43190)	DQ 408572	DQ435091
Linkosia. sp.	HKUCC 10826 (NN47479)	DQ 408573	
Morrisiella indica	HKUCC 10827 (NN44710)	DQ 408578	DQ435082
Neosporidesmium sp.	HKUCC 10562 (NN8069)	DQ 408579	DQ435076
Repetophragma goidanichii	HKUCC 10828 (NN43193)	DQ 408574	DQ435078
R. inflatum	NN42958	DQ 408576	
R. ontariense	HKUCC 10830 (NN43780)	DQ 408575	DQ435077
Sporidesmiella fusiformis	HKUCC 10831 (NN43083)	DQ 408577	DQ435079
Sporidesmina malabarica	NN43118	DQ 408580	
Sporidesmium australiense	HKUCC 10833 (NN44068)	DQ 408554	DQ435080
S. macrurum	HKUCC 2740	DQ 408555	DQ435086
S. obclavatulum	HKUCC 10834 (NN42863)	DQ 408556	
S. pachyanthicola	HKUCC 10835 (NN44070)	DQ 408557	
S. parvum	HKUCC 10836 (NN45992)	DQ 408558	
S. tengii	HKUCC 10837 (NN44362)	DQ 408559	
S. tropicale	HKUCC 10838 (NN44474)	DQ 408560	
Stanjehughesia polypora	NN 47796	DQ 408569	DQ435087
S. vermiculata	HKUCC 10840 (NN42952)	DQ 408570	DQ435081

a The fungal descriptions are available in Wu & Zhuang (2005).
b Abbreviations: HKUCC, Hong Kong University Culture Collection; NN, Novozymes China Culture Collection.

Sequence alignment and phylogenetic analyses

For each fungal strain, sequences obtained from the respective primers (LROR & LR5; fRPB2-5F & fRPB2-7cR) were aligned in Clustal X (Thomson *et al.* 1997). Forty-four new sequences were generated in this study (Table 3). In total, three datasets were analysed: dataset based on LSU nu-rDNA sequences (dataset I), dataset based on RPB2 gene sequences (dataset II), and dataset based on combined LSU nu-rDNA and RPB2 gene sequences (dataset III). Alignments were manually adjusted to allow maximum alignment and minimise gaps in Se-Al v2.0a11 (Rambaut 1996).

Phylogenetic analyses were performed in PAUP* 4.0b10 (Swofford 2002). Ambiguously aligned regions were excluded from all analyses. Unweighted parsimony (UP) and weighted parsimony (WP) analyses were performed. Gaps were treated as missing data and as fifth characters to increase the probability of finding the most parsimonious tree/s. WP analyses were also performed using a symmetric step matrix generated with the program STMatrix v2.2, by which the relative frequencies of nucleotide substitutions were calculated and converted into costs of changes (Francois Lutzoni and Stefan Zoller, Department of Biology, Duke University, USA). Trees were inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics [tree length (TL), CI, RI, related consistency index (RC), homoplasy index (HI), and log Likelihood (-ln L)] were calculated for trees generated under different optimality criteria. Clade stability was assessed in BS analyses with 1000 replicates, each with 10 replicates of random stepwise addition of taxa. Kishino-Hasegawa tests (Kishino & Hasewaga 1989) were performed in order to determine whether trees were significantly different. Trees were figured in Treeview (Page 1996).

The best-fit model of evolution was determined by Modeltest v3.7 (Posada & Crandall 1998). ML analyses were conducted but had to be aborted due to the extreme computational time required by the huge dataset of 101 taxa. PPs (Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002) were determined by MCMC sampling in MrBayes 3.1 (Huelsenbeck & Ronquist 2001), using above estimated model of evolution. Six simultaneous Markov chains were run for 1M generations and trees were sampled every 100th generations (resulting 10K total trees). The first 1K trees that represented the burn-in phase of the analyses were discarded and the remaining 9K were used for calculating PP in the majority rule consensus rule tree. These analyses were repeated five times starting from different random trees to ensure trees from the same tree space were being sampled during each analysis. The statistical congruence of the sequence datasets was tested for dataset III using the partition homogeneity test (Farris *et al.* 1995; Huelsenbeck *et al.* 1996) as implemented in PAUP*. The three datasets have been submitted to TreeBASE (www.treebase.org) and the accession details are as follows: SN2819-11068 (LSU nu-rDNA dataset); SN2819-11069 (RPB2 dataset); SN2819-11070 (combined LSU nu-rDNA and RPB2 dataset).

Results

LSU nu-rDNA phylogenies

The LSU nu-rDNA dataset (dataset I) consisted of 101 taxa, including 27 newly generated sequences belonging to nine anamorphic genera [*Ellisembia* (8), *Linkosia* (3), *Morrisiella* (1), *Neosporidesmium* (1), *Repetophragma* (3), *Sporidesmiella* (1), *Sporidesmina* (1), *Sporidesmium* (7) and *Stanjehughesia* (2)]. The other reference taxa included members of known ascomycete families of the *Dothideomycetes* and *Sordariomycetes*, and *Ostropa barbara* was the designated outgroup (Table 4). However, there seems to be some nomenclatural problems with the *Ostropa barbara* deposited in GenBank (AY584642 and AY584686). This taxon has been referred to the *Ostropales* (*Lecanoromycetes*) but based on recent phylogenies it was found to cluster within the *Leotiomycetes* (Lutzoni *et al.* 2004). Lutzoni *et al.* (2004) pointed out that this might be due to misidentification of the fungus and the sequences deposited in GenBank might not actually come from *Ostropa barbara*. A number of different analyses were undertaken with other members of the *Leotiomycetes* and phylogenies recovered were essentially identical in topology (details not shown). Therefore, we consider that it is phylogenetically appropriate to use *Ostropa barbara* as an outgroup. The final dataset comprised 875 characters. Likelihood-ratio test in Modeltest v3.7 suggested that the best-fit model of evolution for this dataset was TrNef + I + G. Eighty-five characters from the ambiguous regions were excluded in the analyses. In UP analysis, when gaps were treated as missing data, there were 390, 116 and 284 constant, variable parsimony-uninformative and parsimony informative characters, respectively and the analysis resulted in 41 trees. When gaps were treated as fifth character, there were 372, 130 and 288 constant, variable parsimony-uninformative and parsimony informative characters, respectively, and this analysis resulted in 88 trees. However, WP using estimated transition:transversion ratios from STMatrix and treating gaps as fifth character yielded only one tree. Based on KH test, these UP or WP trees were not significantly different (details not shown). The single parsimonious tree (TL = 4285.18, CI = 0.326, RI = 0.736, RC = 0.240, HI = 0.674, -ln L = 29905.8750) with *Ostropa barbara* as outgroup generated from WP and treating gaps as fifth character is shown in Fig 1. BS values (equal to or above 50 %) based on 1K replicates are shown on the upper branches. Values of the PP from MCMC analyses are represented as thickened branches on the tree.

Twenty-four isolates belonging to six 'core genera' (*Ellisembia*, *Linkosia*, *Repetophragma*, *Sporidesmiella*, *Sporidesmium* and *Stanjehughesia*) were found to be associated with members of two ascomycete classes, *Dothideomycetes* and *Sordariomycetes* (Fig 1). Within the *Sordariomycetes*, *E. brachypus*, *Ellisembia* sp. (1), *Morrisiella indica*, *Linkosia* sp. and *Stanjehughesia vermiculata* nested within the *Chaetosphaeriales* clade. *E. brachypus* clustered with *Lecythothecium duriligni* (anamorph: *E. folliculatum*) with 100 % statistical support and *Ellisembia* sp. (1) was found to be basal to the *Chaetosphaeriaceae*. However, in another analysis incorporating a broader taxon sampling across the *Chaetosphaeriaceae*, *Ellisembia* sp. (1) clustered with

Table 4 – Additional sequences used in the analyses obtained from GenBank

Taxon	GenBank accession numbers	
	RPB2	LSU nu-rDNA
Acremonium alternatum		U57349
Anguillospora longissima		AY204597
Annulatascus triseptatus	AY780148	AY346257
Annulusmagnus triseptatus		AY590287
Apiospora sinensis		AY083831
Aquaticola ellipsoidea		AY590290
A. hyalomura		AY590291
Arthopyrenia salicis		AY538339
Arthrinium phaeospermum		AY083832
Ascitendus austriacus		AY590294
A. triseptatus		AY094186
Ascolacicola austriaca		AF261067
Bimuria novae-zelandiae		AY016356
Botryosphaeria rhodina	AF107802	AY928054
B. ribis		AY004336
Byssothecium circinans		AY016357
Camarops amorpha	AY780156	AY780054
C. ustulinoides		AY346267
Cenococcum geophilum	AY485616	
Chaetosphaerella phaeostroma	AY780172	AY695264
Chaetosphaeria biapiculata		AF466065
C. conirostris		AF466066
C. lentomita		AF178548
C. luquillensis		AF466074
C. minuta		AF466075
C. ovoidea	AY780173	
Chaunopycnis alba		AF245296
Chloridium lignicola		AF178544
Cochliobolus heterostrophus		AY544645
Cordyceps militaris	AY545732	AY184966
C. ophioglossoides		AB027367
Cryptadelphia groenendalensis		AY281104
C. polyseptata		AY281102
Curvularia brachyspora	AF107803	AF279380
Diamantinia citrina		AY346278
Diaporthe phaseolorum		AY346279
Diatrype disciformis		U47829
Didymella bryoniae	AF107801	
D. cucurbitacearum		AY293792
Dothidea insculpta	AF107800	
Eutypa sp.	AY780176	AY083825/AY346280
Fusoidispora aquatica		AY780365
Geosmithia lavendula		D88325
Gliocladium flavofuscum	AF545547	
Halosphaeria appendiculata		U468851
Helicomyces roseus		AY787932
Hypocrea crassa	AY481587	
H. rufa		AY489726
Hypomyces chrysospermus		AB027385
Hypoxylon fragiforme		AY083829
Karstenula rhodostoma		AY789933
Lasiosphaeria ovina		AY436413
Lecythothecium duriligni		AF261071
Lepteutypa cupressi		AF382379
Letendraea helminthicola		AY016362
Linocarpon appendiculatum	AY780183	
Lojkania enalia		AY016363
Melanochaeta hemipsila	AY780184	AF466083/AY346292
Melanomma radicans	AY485625	U43479
Melanopsammella vermicularioides		AF466087
Microascus trigonosporus	AF107792	U47835
Microxyphium citri		AY004337
Munkovalsaria appendiculata		AY772016
Mycosphaerella cryptica		AF309585
M. punctiformis	AY485626	
M. suttonii		AF309587
Myrothecium gramineum		AY283538
Nectria cinnabarina	AF545567	AF193237
N. mariannaeae		AY283553
Neurospora pannonica	AY780185	AY780070
Niesslia exilis		AY489718
Ostropa barbara	AY584686	AY584642
Oxydothis frondicola		AY083835
Pestalotiopsis versicolor		AF382357
Phaeosphaeria avenaria		AY544684
Pleospora herbarum	AF107804	
P. herbarum var. *herbarum*		AF382386
P. iqbalii		AY787931
Pseudocercospora sp.		AY598900
Rhamphoria delicatula		AF261068
Scytalidium dimidiatum	AY485627	
Seiridium cardinale		AF382377
Seynesia erumpens	AY641073	AF279410
Sordaria fimicola	AY780194	AY681160/AY780079
Spirosphaeria cupreorufescens		AY616236
Sporormiella minima	AF107805	
Striatosphaeria codinaeaphora		AF466088
Thyridium chrysomallum	AY780193	
Tubeufia amazonensis		AY787938
Umbrinosphaeria caesariata		AF261069
Valsa ceratosperma		AF408387
Venturia hanliniana		AF050290
Westerdykella ornata		AY853401
Xylaria curta		U47840
Zopfiella tetraspora		AY999108

Lecythothecium duriligni (anamorph: *E. folliculatum*) and *E. brachypus* (results not shown). *Morrisiella indica, Linkosia* sp. and *Stanjehughesia vermiculata* constituted a well-supported subclade and nested among *Chaetosphaeria* species.

E. bambusicola, *E. minigelatinosa*, *Ellisembia* sp. (2) and *Sporidesmium parvum* clustered together and formed a strongly supported clade with *Fusoidispora aquatica* as a sister taxon (but with no statistical support; clade A in Fig 1). WP analyses also placed *E. adscendens* and *E. leonensis* as sister group to *Cryptadelphia groenendalensis* and *C. polyseptata*, but this relationship did not receive any support (*E. adscendens* and *E. leonensis*, however, clustered with high confidence). Phylogenies also indicated that *Sporidesmium tropicale* was basal to other members of the *Annulatascaceae* as it was paraphyletic to *Aquaticola* species. *Linkosia multiseptum* clustered together with *Rhamphoria delicatula*. However, all three subclades connecting these anamorphic hyphomycetes to other members of the *Annulatascaceae* were either weakly or not supported.

Within the *Xylariales* clade, which was supported by 92 % BS support, *Sporidesmina malabarica* and *Stanjehughesia polypora* formed a strongly supported subclade basal to other members of the *Diatrypaceae* and *Xylariaceae*, while *Sporidesmium macrurum* was a sister taxon to the *Amphisphaeriaceae* and *Apiosporaceae* (Fig 1). *Repetophragma inflatum* also formed part of the *Xylariales* clade but its relationship with other

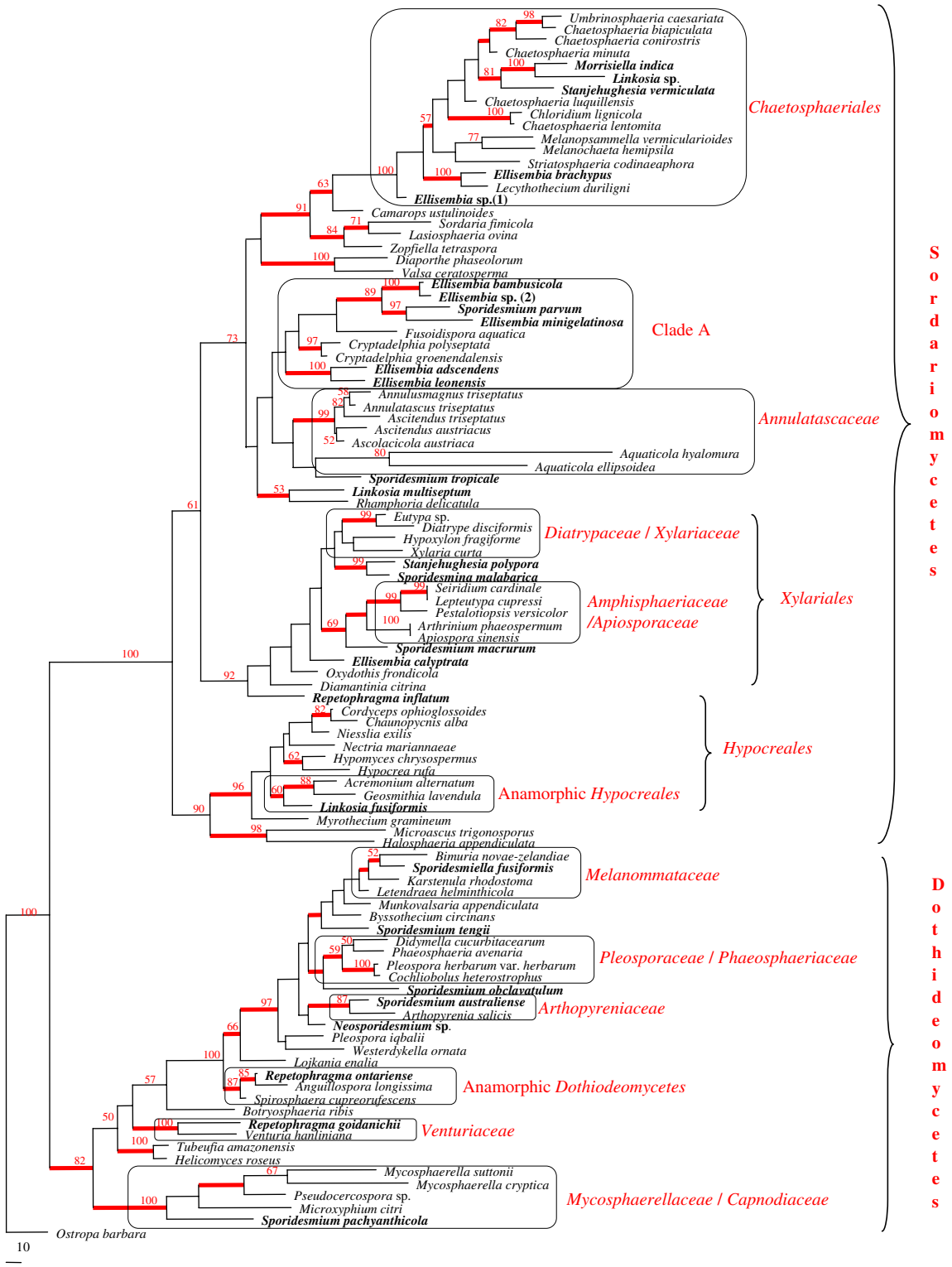

Fig 1 – Phylogram of the most parsimonious tree generated based on the LSU nu-rDNA sequences (TL = 4285.18, CI = 0.326, RI = 0.736, RC = 0.240, HI = 0.674, -ln L = 29905.8750). Data were analysed with random addition sequence, WP and treating gaps as fifth character. Values above the branches are parsimony BS (equal or above 50 %). Thickened branches represent significant Bayesian PP (equal or above 95 %). The tree is rooted with *Ostropa barbara*.

Xylariales members could not be properly assessed. Only one taxon, *Linkosia fusiformis* clustered with moderate BS support to other anamorphic *Hypocreales* such as *Acremonium alternatum* and *Geosmithia lavendula*.

Within the *Dothideomycetes*, *Sporidesmiella fusiformis* clustered with *Bimuria novae-zelandiae* (*Melanommataceae*) (with 99 % Bayesian support), while *S. tengii* was basal to the melanommataceous-clade. *S. obclavatulum* grouped with members

of the *Pleosporaceae* and *Phaeosphaeriaceae*. *S. australiense* showed strong phylogenetic affinities with *Arthropyrenia salicis* (100 % Bayesian support), while *Neosporidesmium* sp. was paraphyletic to *Arthropyrenia salicis* and *S. australiense*. *Repetophragma ontariense* showed strong phylogenetic affinities with anamorphic *Dothideomycetes*, i.e., *Anguillospora longissima* and *Spirosphaera cupreorufescens*. *Repetophragma goidanichii* clustered with *Venturia hanliniana* with 100 % statistical support. *Sporidesmium pachyanthicola* fitted in well (with high statistical support) within the *Mycosphaerellaceae* and *Capnodiaceae* clade.

RPB2 phylogenies

The RPB2 dataset (dataset II) consisted of 17 newly sequenced taxa, 27 taxa from GenBank and 1085 characters. Likelihood-ratio test in Modeltest v3.7 suggested that the best-fit model of evolution for this dataset was TrN + I + G. Two-hundred and seventy-eight characters from the ambiguous regions were excluded in the analyses. In UP analyses, there were 310, 37 and 460 constant, variable parsimony-uninformative and parsimony informative characters, respectively. When gaps were treated as missing data, UP analysis resulted in one tree. When gaps were treated as fifth character, UP analysis yielded 13 trees. WP analysis using estimated transition:transversion ratios from STMatrix and treating gaps as fifth character yielded only one tree. Based on KH test, these trees generated under these different criteria were not significantly different (details not shown). The single parsimonious tree (TL = 8372.08, CI = 0.214, RI = 0.400, RC = 0.086, HI = 0.786, -lnL = 28326.9688) generated from WP and treating gaps as fifth character is shown in Fig 2. The branching patterns with respect to the placement of ingroup taxa were similar to those obtained from the LSU nu-rDNA phylogenies, although some of the clades/subclades were less resolved.

Phylogenies of the combined LSU nu-rDNA and RPB2 dataset

The combined dataset of LSU nu-rDNA and RPB2 sequences (dataset III) comprised 1971 characters, out of which 255 ambiguously aligned characters were excluded in the analyses. Likelihood-ratio test in Modeltest v3.7 suggested that the best-fit model of evolution for this dataset was TrNef + I + G. In UP analysis and treating gaps as missing data, there were 821, 123 and 672 constant, variable parsimony-uninformative and parsimony informative characters, respectively, and the analysis resulted in one tree. When gaps were treated as fifth character, there were 814, 130 and 672 constant, variable parsimony-uninformative and parsimony informative characters, respectively and this analysis also resulted in one tree. WP analysis using estimated transition:transversion ratios from STMatrix and treating gaps as fifth character yielded only one tree. Based on KH test, these trees were not significantly different (details not shown). Treating gaps as fifth character under both criteria resulted in trees with similar topologies. The single parsimonious tree (TL = 8485.72, CI = 0.310, RI = 0.433, RC = 0.134, HI = 0.690, -lnL = 29905.8750) generated from WP and treating gaps as fifth character is shown in Fig 3. Phylogenies derived from this combined dataset are similar to those derived from individual datasets. There seems, however, to be better statistical support for the major clades, although the relationships of ingroup taxa towards other members are still either weakly or not supported.

Discussion

Previous taxonomic studies have revealed intimate associations of *Sporidesmium* and morphologically similar genera with taxonomically diverse teleomorphic taxa, within the *Dothideomycetes* and *Sordariomycetes* (Table 2). Most of the associations were based on circumstantial evidence, such as occurrence of the anamorph and teleomorph on the same substrate. Two species of *Sporidesmium* have been reported to be associated with thyriothecia of *Eupelte amicta* and *E. rapaneae* (*Asterinaceae*, *Dothideomycetes*). However, these connections are yet to be proven through cultures (Sivanesan 1984). Similarly, Shoemaker and White (1985) reported an association of *Ellisembia adscendens* and *E. bambusicola* with *Miyoshiella triseptata* and *M. fusispora* (*Chaetosphaeriaceae*, *Sordariomycetes*), respectively, but neither the anamorphs nor the suggested teleomorphs grew on culture medium. Such associations on the same substrate do not convincingly prove anamorph–teleomorph connections.

There has been only one report using culture-based and molecular phylogenetic studies, which have established anamorph–teleomorph connections among *Sporidesmium* and morphologically similar genera (Réblová & Winka 2001). Our study provides an evolutionary perspective of *Ellisembia*, *Linkosia*, *Repetophragma*, *Sporidesmiella*, *Sporidesmium* and *Stanjehughesia*, and reveals familial or ordinal affiliations of these anamorphic fungi. This study also investigates whether morphological characters currently used for generic delimitation are phylogenetically significant. One of the most important findings of this study is that *Sporidesmium* and morphologically similar genera are not monophyletic as they are distributed among two major fungal classes within the *Ascomycota* (Figs 1–3).

Phylogenetic affiliation within the Sordariomycetes

The connections of *Ellisembia folliculatum* and *Stanjehughesia hormiscioides* with *Lecythothecium duriligni* and *Umbrinosphaeria caesariata*, respectively, and their phylogenetic affinities with the *Chaetosphaeriaceae* were revealed following culture-based and DNA-mediated studies (Réblová & Winka 2001). In our study, *Ellisembia brachypus*, *Ellisembia* sp. (1), *Linkosia* sp., *Morrisiella indica* and *Stanjehughesia vermiculata* cluster within the *Chaetosphaeriales* (Fig 1). It is interesting that *Linkosia* sp., *Morrisiella indica* and *Stanjehughesia vermiculata* form a strongly supported subclade, paraphyletic to *Stanjehughesia hormiscioides* (anamorphic *Umbrinosphaeria caesariata*) and *Chaetosphaeria* species. The phylogenetic association of *Linkosia* sp., *Morrisiella indica* and *Stanjehughesia vermiculata* is concordant with their morphology. All three taxa are similar in having phragmoconidia produced from conidiogenous cells on highly reduced conidiophores or apparently no conidiophores.

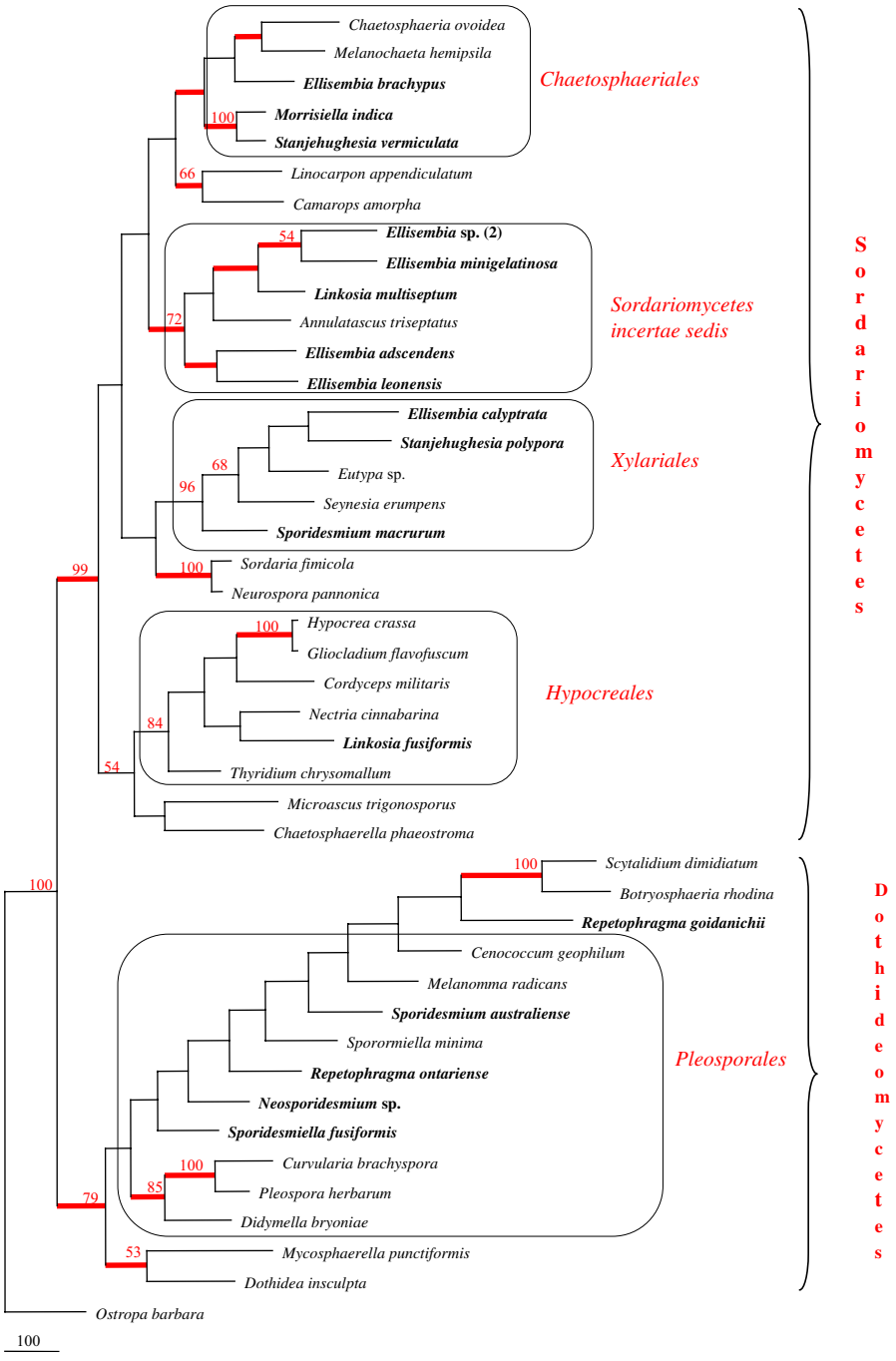

Fig 2 – Phylogram of the most parsimonious tree based on RPB2 gene sequences (TL = 8372.08, CI = 0.214, RI = 0.400, RC = 0.086, HI = 0.786, -lnL = 28326.9688). Data were analysed with random addition sequence, WP and treating gaps as fifth character. Values above the branches are parsimony BS (equal or above 50 %). Thickened branches represent significant Bayesian PP (equal or above 95 %). The tree is rooted with *Ostropa barbara*.

Morrisiella indica, however, differs from *Linkosia* sp. and *Stanjehughesia vermiculata* in having synnematous conidiomata.

Morphological and ecological observations support the phylogenetic affiliation of some *Sporidesmium* species and morphologically similar genera within the *Chaetosphaeriaceae*. *Chaetosphaeria* and *Sporidesmium* species and morphologically similar genera are commonly found on decomposing plant tissues (Fernández & Huhndorf 2005; Wu & Zhuang 2005), thus exploiting similar microhabitats. *Exserticlava* is one of the ten anamorphs of *Chaetosphaeria* (Fernández & Huhndorf 2005) and it shares morphological features with *Ellisembia*, i.e., distoseptate phragmoconidia and schizolytic conidial secession. The phragmoconidia of *Exserticlava* are, however, produced polyblastically (Hughes 1979). Sivanesan and Chang (1995) also reported an association of a *Sporidesmium* anamorph with the type of *Chaetosphaeria capitata*. However, they

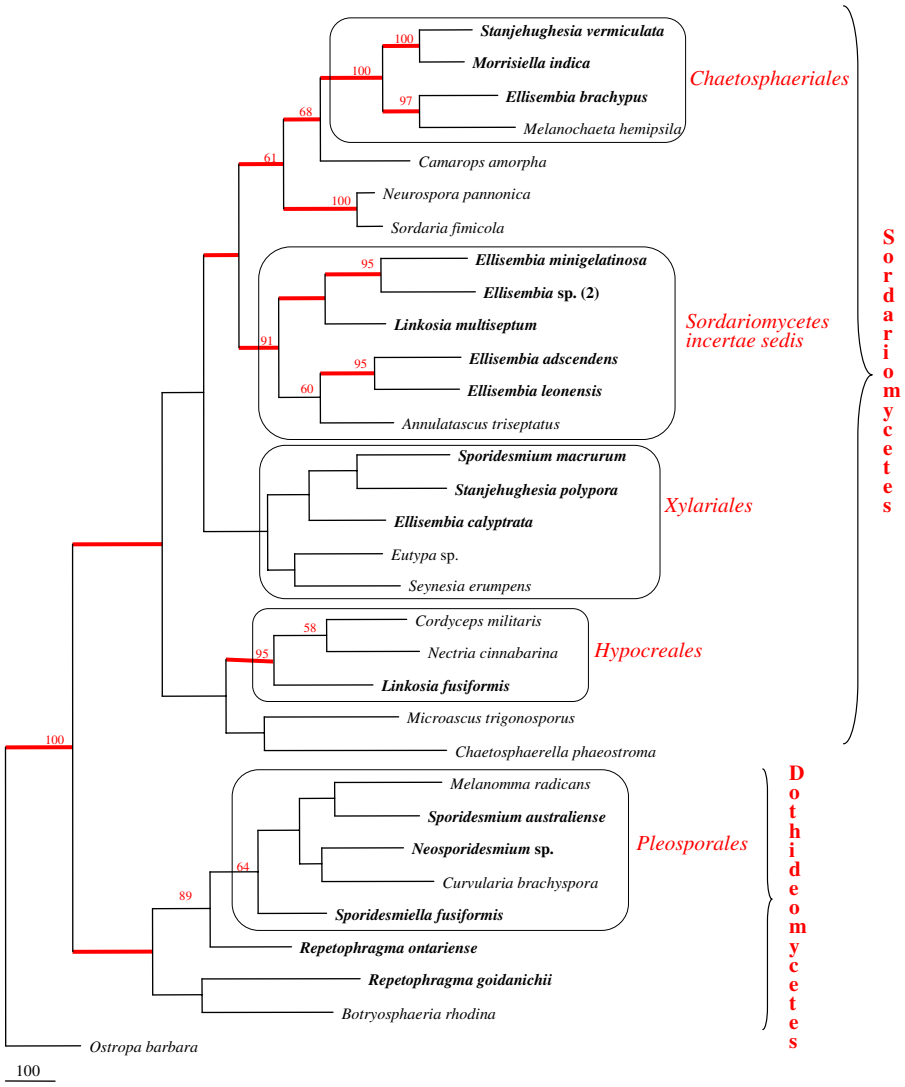

Fig 3 – Phylogram of the most parsimonious tree based on combined LSU nu-rDNA and RPB2 gene sequences (TL = 8485.72, CI = 0.310, RI = 0.433, RC = 0.134, HI = 0.690, -lnL = 29905.8750). Data were analysed with random addition sequence, WP and treating gaps as fifth character. Values above the branches are parsimony BS (equal or above 50 %). Thickened branches represent significant PP (equal or above 95 %). The tree is rooted with *Ostropa barbara*.

observed no common mycelial link, and no proper connection was made. Réblová and Winka (2001) reported a phialidic *Chloridium*-like synanamorph of *Stanjehughesia hormiscioides* (anamorphic *Umbrinosphaeria caesariata*). This fact is an indication that chaetosphaeriaceous anamorphs with phialidic conidia, e.g. *Cacumisporium*, *Catenularia*, *Cylindrotrichum*, *Chalara*, *Chloridium* (Réblová & Winka 2001; Huhndorf & Fernández 2005), may also have *Sporidesmium*-like synanamorphs.

Ellisembia bambusicola, *E. minigelatinosa*, *Ellisembia* sp. (2) and *Sporidesmium macrurum* cluster within clade A (Fig 1) and form a relatively well-supported subclade as a sister group to *Cryptadelphia groenendalensis*, *C. polyseptata*, and *Fusoidispora aquatica*. These taxa are similar in having phragmoconidia produced on proliferating or non-proliferating conidiophores. *Cryptadelphia*, tentatively placed in the *Trichosphaeriaceae*, has *Brachysporium* anamorphs (Réblová & Seifert 2004). *Ellisembia* is similar to *Brachysporium* in having dematiaceous phragmoconidia, but differs mainly in the type of conidial secession (schizolytic *versus* rhexolytic). *Fusoidispora*, a freshwater genus, shares a close phylogenetic proximity with members of the *Annulatascaceae* (Vijaykrishna *et al.* 2005). Its taxonomic position, however, is still unclear and it has not been connected to any anamorph. *Ellisembia adscendens* and *E. bambusicola*, the suggested anamorphs of *Miyoshiella* species (Shoemaker & White 1985; Réblová 1999), currently placed in the *Chaetosphaeriaceae*, are not phylogenetically related to the *Chaetosphaeriaceae* (Fig 1, clade A). A detailed study on *Miyoshiella* species and their anamorphs is needed to resolve this issue, as these anamorphic cultures did not produce any teleomorphs.

In this study, the phylogenetic relationship of *Sporidesmium tropicale* with *Aquaticola* (*Annulatascaceae*) is not statistically supported. There is no reported anamorphic connection of *Aquaticola* species. *Ascolacicola* (*Annulatascaceae*), however, has been connected to *Trichocladium* (Ranghoo & Hyde 1998)

anamorphs. *Trichocladium* shares few morphological characters with *Sporidesmium*, except the holoblastic production of conidia. In this study, *Rhamphoria delicatula* (*Trichosphaeriaceae*) (Réblová & Winka 2001) is found to be closely related to *Linkosia multiseptum*. Müller and Munk (1964) reported an association of *Sporidesmium scutellare* with *Eriosphaeria aggregata* (*Trichosphaeriaceae*). Although such a close association on a substrate is unreliable evidence, it may be suggestive of a connection. The association of *Arthrinium* with *Apiospora* on the same substrate is indicative of them being two different phases of the same life cycle and this has been confirmed by DNA sequence analyses (Smith *et al.* 2003). Likewise, Réblová and Seifert (2004) frequently observed *Brachysporium* spp. with an ascomycete in fresh/herbarium collections. The LSU nu-rDNA comparison using single-spore isolates of *Brachysporium nigrum* and *Cryptadelphia groenendalensis* revealed that the two taxa are closely related and differed by a single nucleotide (Réblová & Seifert 2004). Further molecular phylogenies and anamorph–teleomorph connection studies are required to clarify the relationship of *Sporidesmium* and morphologically similar genera within the *Annulatascaceae* and *Trichosphaeriaceae*.

In our study, *Sporidesmina malabarica* is found to be closely related to *Stanjehughesia polypora* with high statistical support. *Sporidesmina malabarica* is similar to *Stanjehughesia polypora* except for features such as synnematous conidiomata and production of a mixture of euseptate and distoseptate phragmoconidia. The phylogenetic significance of synnematous conidiomata is questioned here and such a character is also common to other anamorphs, which are distributed among the Ascomycetes. *Sporidesmium* species are known to produce *Selenosporella* synanamorphs (Wu & Zhuang 2005). Interestingly, *Selenosporella* has been connected to teleomorphs of the *Xylariales*: *Eutypa spinosa* (*Diatrypaceae*) (Glawe & Rogers 1986), *Iodosphaeria ripogoni* (Samuels *et al.* 1987) and *Oxydothis selenosporellae* (*Xylariales incertae sedis*) (Samuels & Rossman 1987). It is therefore credible that *Stanjehughesia polypora* and *Sporidesmina malabarica* are phylogenetically linked to the *Diatrypaceae* and *Xylariaceae* and *Ellisembia calyptrata* shares close affinities with *Oxydothis frondicola*. The possible placement of *Sporidesmium macrurum* within the *Apiosporaceae*/*Amphisphaeriaceae*, however, does not concur with morphological data. *Apiosporaceae* has *Arthrinium* and *Cordella* anamorphs (Hyde *et al.* 1998), while the *Amphisphaeriaceae* is connected to coelomycetous anamorphs (Kang *et al.* 2002; Jeewon *et al.* 2003).

It is interesting that *Acremonium alternatum*, *Geosmithia lavendula* and *Linkosia fusiformis* form a well-supported subclade within the *Hypocreales*. *Acremonium* anamorphs are produced in more than 20 ascomycete genera across many orders (Kendrick & Murase 1994). *Geosmithia* is a polyphyletic genus with affinities to hypocrealean and eurotialean fungi (Ogawa *et al.* 1997; Kolarík *et al.* 2004). Future studies on anamorph–teleomorph connections may reveal *Sporidesmium*-like anamorphs among some hypocrealean *Ascomycetes*, which are already known to produce more than 34 different anamorphs (Kendrick & Murase 1994).

Phylogenetic affinities within the Dothideomycetes

Based on morphological examination of specimens, several researchers have observed a close association of *Sporidesmium*-like taxa with several bitunicate species on the same substrate. This has prompted taxonomists to hypothesise that *Sporidesmium* may be connected to *Akaropeltella* (*Micropeltidaceae*) (Farr 1972), *Cucurbitaria* (*Cucurbitariaceae*) (Sivanesan 1984), *Eupelte* (*Asterinaceae*) (Ellis 1958; Sivanesan 1984) and *Placostroma* (*Asterinaceae*) (Hughes 1978; Sivanesan 1984). Molecular data generated in this study clearly show that there is an intimate phylogenetic link between *Sporidesmium* and morphologically similar genera, with members of the *Pleosporales* or other related *Dothideomycetes*. There is no evidence for a connection between *Sporidesmiella fusiformis* and *Bimuria novae-zelandiae* or *Karstenula rhodostoma*, however, the molecular data indicate that *Sporidesmiella fusiformis* may belong in the *Melanommataceae*.

Sporidesmium obclavatulum shows phylogenetic affinities with the *Pleosporaceae* and *Phaeosphaeriaceae* (*Pleosporales*; Fig 1). Other hyphomycetous anamorphs that have teleomorphic associations within the *Pleosporales* include *Curvularia*, *Dendryphiopsis* and *Helminthosporium* (Sivanesan 1984; Hughes 1978). *Dendryphiopsis atra*, anamorphic *Kirschsteiniothelia aethiops* (*Pleosporaceae*), occasionally produces a terminal 2–6-septate phragmoconidium on a main non-proliferating or percurrently proliferating conidiophore, which usually bears one or two lateral branches, as in *Sporidesmium* (Hughes 1978). However, *Dendryphiopsis atra* differs from *Sporidesmium* in having monotretic conidiogenous cells, i.e., each enteroblastic conidiogenous cell produces a solitary conidium by protrusion of the inner wall through one channel in the outer wall (Ellis 1976). *Sporidesmium* is similar to *Helminthosporium* in having dematiaceous phragmospores. *Helminthosporium*, however, differs from *Sporidesmium* is having polytretic conidiogenous cells, i.e., each enteroblastic conidiogenous cell produces an acropetal chain of conidia by protrusion of the inner wall through several channels in the outer wall (Ellis 1976). *Curvularia*, as in *Sporidesmium*, also produces dematiaceous phragmoconidia, however, in the former, conidiogenous cells are polytretic, as in *Helminthosporium* (Ellis 1976). The association of *Repetophragma goidanichii* with *Venturia hanliniana*, however, is surprising as previously reported venturiaceous anamorphs (*Cladosporium*, *Fusicladium*, *Pollaccia*, *Spilocea*) (Sivanesan 1977) share few morphological similarities with *Sporidesmium*.

In this study, *Repetophragma ontariense*, *Anguillospora longissima* (the suggested anamorph of a polyphyletic genus *Massarina*) (Willoughby & Archer 1973; Liew *et al.* 2002) and *Spirosphaera cupreorufescens* formed a well-supported subclade within the *Dothideomycetes* (Fig 1). *Anguillospora*, an aquatic hyphomycete genus, is polyphyletic. Eight species of *Anguillospora* are distributed among the *Dothideomycetes*, *Leotiomycetes* and *Orbiliomycetes* (Belliveau & Bärlocher 2005). *Spirosphaera cupreorufescens*, an aeroaquatic fungus, produces terminal, holoblastic conidia on mononematous conidiophores, as in *Repetophragma*. However, the conidia in *Spirosphaera cupreorufescens* are unique, being irregular globose, formed by spirally interwoven filaments. *Spirosphaera* is a polyphyletic genus, which is spread among *Leotiomycetidae sensu lato* and the *Dothideomycetes* (Voglmayr 2004).

The phylogenetic affinities of *Repetophragma goidanichii* and *Sporidesmium pachyanthicola* with *Botryosphaeria* (Figs 1–3) and the *Mycosphaerellaceae* (Fig 1), respectively, are likewise interesting as these two polyphyletic teleomorphic genera produce

more than 15 and 23 anamorphic forms, respectively (Verkley *et al.* 2004; Barber *et al.* 2005; Schubert & Braun 2005; Phillips *et al.* 2006). It is believed that the probability of finding dothideomycetous teleomorphs of the *Sporidesmium*-type is high, as these fungi, like many other anamorphic *Dothideomycetes*, e.g., *Curvularia*, *Helminthosporium*, produce single, terminal phragmoconidia, that could sometimes be dictyoseptate or enteroblastic (monotretic or polytretic). This is the first phylogenetic study that has established a phylogenetic link between *Sporidesmium*-type fungi with the *Dothideomycetes*.

Convergent evolution of morphological characters

The morphological delimitation of *Sporidesmium* and similar morphological genera is based mainly on conidium ontogeny (Table 1). The presence or absence of euseptation of conidia is one important character that has been used to delimit genera. According to Réblová (1999), however, electron microscopic studies of conidiation (Luttrell 1963; Cole & Samson 1979) reveal that the initial stages of differentiation of distoseptate conidia are the same as those of euseptate conidia. The distinction is demonstrated during maturation where the outer layer of euseptate conidia breaks down, while in distoseptate conidia it remains intact. *Sporidesmina malabarica* has been reported to produce a mixture of euseptate and distoseptate conidia (Subramanian & Bhat 1987; Wu & Zhuang 2005). The segregation of *Linkosia* and *Stanjehughesia* from *Sporidesmium sensu lato*, based on presence or absence of conidiophores, has also been questioned by Réblová (1999). Hughes & Illman (1947) and Réblová (1999) reported taxonomically unacceptable variations in conidiophores in *Stanjehughesia larvata* (as *Sporidesmium larvatum*) grown under different conditions. Réblová (1999) reported macronematous, multiseptate conidiophores of *Stanjehughesia larvata* (as *Sporidesmium larvatum*) covering the perithecia of *Miyoshiella larvata*. However, in culture, the conidiophores were reduced to single 0–1-septate conidiogenous cells. Hughes & Illman (1947) reported *Stanjehughesia larvata* (syn. *Sporidesmium larvatum*) with several-septate, ampulliform conidiophores, or conidiophores that had terminal proliferating conidiogenous cells.

The present study shows that the phenotypes of *Sporidesmium* and morphologically similar genera have multiple evolutionary origins. Therefore, many of the morphological characters currently used to delimit the genera do not appear to be phylogenetically significant. These morphologically similar anamorphic taxa have evolved on more than one occasion. There have been numerous reports on paraphyly and polyphyly of anamorphic genera (e.g. Seifert *et al.* 2000; Tsui *et al.* 2006). Anamorphic phenotypes, in genera such as *Acremonium* (Rossman 2000), *Anguillospora* (Belliveau & Bärlocher 2005), *Chalara* (Paulin & Harrington 2000), *Embellisia* (Pryor & Bigelow 2003), *Geosmithia* (Ogawa *et al.* 1997; Kolarík *et al.* 2005), *Graphium* (Okada *et al.* 2000), *Phialophora* (Gams 2000), *Spirosphaera* (Voglmayr 2004) and *Sporidesmium* may have been conserved and maintained in many fungal lineages by natural selection. The structural and functional adaptations of these phenotypes in different ecological niches could be one of the reasons in favour of their selection.

The present study shows that generic delineation of *Sporidesmium* and morphologically similar genera has resulted in an artificial assemblage of phylogenetically unrelated anamorphic fungi. We can not, however, ignore the anamorphic names just because they are not yet associated with any known teleomorph. Anamorphs, in the absence of their teleomorph, are the sole known morph of the holomorph. Therefore, we favour the continuation of known species names of *Sporidesmium* and similar genera. It is proposed that in the absence of or failure to detect a sexual phase of these anamorphic fungi, the anamorphic names should be used for the holomorph.

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