

Sequence data reveals phylogenetic affinities of fungal anamorphs *Bahusutrabeeja*, *Diplococcium*, *Natarajania*, *Paliphora*, *Polyschema*, *Rattania* and *Spadicoides*

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Abstract Partial 28S rRNA gene sequence-data of the strains of the anamorphic genera *Bahusutrabeeja*, *Diplococcium*, *Natarajania*, *Paliphora*, *Polyschema*, *Rattania* and *Spadicoides* were analysed to predict their phylogenetic relationships and taxonomic placement within the *Ascomycota*. Results indicate that *Diplococcium* and morphologically similar genera, i.e. *Spadicoides*, *Paliphora* and *Polyschema* do not share a recent common ancestor. The type species of *Diplococcium*, *D. spicatum* is referred to *Helotiales* (*Leotiomycetes*). The placement of *Spadicoides bina*, the type of the genus, is unresolved but it is shown to be closely associated with *Porosphaerella* species, which are sister taxa to *Coniochaetales* (*Sordariomycetes*). Three *Polyschema* species analysed in this study represent a

monophyletic lineage and are related to *Lentithecium fluviatile* and *Leptosphaeria calvescens* in *Pleosporales* (*Dothideomycetes*). DNA sequence analysis also suggests that *Paliphora intermedia* is a member of *Chaetosphaeriaceae* (*Sordariomycetes*). The type species of *Bahusutrabeeja*, *B. dwaya*, is phylogenetically related to *Neodeightonia* (= *Botryosphaeria*) *subglobosa* in *Botryosphaeriales* (*Dothideomycetes*). Monotypic genera *Natarajania* and *Rattania* are phylogenetically related to members of *Diaporthales* and *Chaetosphaeriales*, respectively. Future studies with extended gene datasets and type strains are required to resolve many novel but morphologically unexplainable phylogenetic scenarios revealed from this study. It is increasingly becoming evident that a fungal

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lineage may include a mosaic of anamorphs, teleomorphs and pleomorphs whose morphologies may not always be correlated. It is therefore suggested that where possible all new species descriptions, whether teleomorphic, anamorphic or pleomorphic, should include DNA sequence-data to facilitate amalgamation of anamorphic and pleomorphic genera in a single phylogenetic classification system.

Keywords Ascomycetes · Asexual fungi · Hyphomycetes · Molecular phylogeny · Taxonomy

Introduction

The phenomenon of pleomorphism in fungi, i.e. occurrence of dual or multiple morphological forms of a fungal species in different time and space has resulted in considerable difficulties in developing a stable phylogenetic classification system in kingdom *Fungi* (Shenoy et al. 2007). This has partially resulted from the establishment of the dual system of classification in which sexual and asexual fungal forms are grouped separately. In this arrangement, more than 20,000 anamorphs have been grouped in a pigeon-hole-like system of genera, mainly based on anamorphic characters (Shenoy et al. 2007). The practice of a dual classification goes against the principle of natural classification. Anamorphic genera are regularly described by fungal taxonomists to facilitate practical purposes such as identification, but with little consideration for the principle of natural groupings in classification. Recent efforts in fungal gene sequence-data analysis have shown that many ‘well-established’ anamorphic genera are polyphyletic (e.g. Seifert et al. 2000; Shenoy et al. 2006, 2007; Crous 2009; Crous et al. 2009b; Jones et al. 2009). There is a warranted need to revisit the taxonomy of anamorphic genera and refine their taxonomy based on a polyphasic approach as demonstrated in recent studies (Groenewald et al. 2008; Aveskamp et al. 2008, 2009, 2010). This paper presents our recent studies on molecular phylogeny of seven anamorphic genera that include members of the *Diplococcium*-complex viz. *Diplococcium* Grove (1885), *Spadicoides* S. Hughes (1958), *Paliphora* Sivanesan and B. Sutton (1985), and *Polyschema* H.P. Upadhyay (1966) and three anamorphic genera reported from India viz. *Bahusutrabeeja* Subramanian and Bhat (1977), *Natarajania* Pratibha and Bhat (2005) and *Rattania* Prabhugaonkar and Bhat (2009).

Diplococcium and *Spadicoides* have similar conidial ontogeny. In both genera conidiogenous cells are terminal or intercalary, and polytretic with several unthickened conidiogenous loci (pores). Conidial ontogeny is holoblastic and the conidiogenous pores are easily visible after conidial secession. The conidia are acropleurogenous, dry, dematiaceous, usually thick-walled, 0–5-septate and may

have thick, black or brown bands at the septa. The type species of *Diplococcium* (i.e. *D. spicatum* Grove) and *Spadicoides* (i.e. *S. bina* S. Hughes) differ mainly in branching of conidiophores and catenation of conidia. *Diplococcium spicatum* produces catenate conidia on branched conidiophores, whereas *Spadicoides bina* produces solitary conidia on unbranched conidiophores (Hughes 1958; Ellis 1963; Sinclair et al. 1985; Goh and Hyde 1996). The conidiophore branching was considered to be taxonomically more important at the generic level than conidial catenation by earlier mycologists (e.g. Ellis 1963, 1971a, b, 1972; Wang 1976; Wang and Sutton 1982). Sinclair et al. (1985), however, stated that at the generic level, conidiophore branching is taxonomically less important than conidial catenation, and emended the generic description of *Spadicoides* to include species that produce solitary conidia from conidiophores that may be simple or branched. This generic delineation based on a single anamorphic character has been accepted by several authors (e.g. Kuthubutheen and Nawawi 1991; Bhat and Kendrick 1993; Goh and Hyde 1996, 1998; Ho et al. 2002).

Information on teleomorphs of *Diplococcium* and *Spadicoides* members is limited and ambiguous. Available taxonomic information from morphological and culture-based studies suggests that *Diplococcium* species might have connections with phylogenetically divergent sexual morphologies. The suggested sexual forms include *Helminthosphaeria* (*Sordariomycetes*) (Bisby 1938; Ellis 1971b; Sutton 1973; Subramanian 1983; Samuels et al. 1997; Goh and Hyde 1998; Réblová 1999b) and *Othia* (*Dothideomycetes*) (Subramanian and Sekar 1989). There is one report that, based on circumstantial evidence such as growth of the anamorph on ascomata, suggested *Tengiomyces* as a possible sexual form of *Spadicoides* (Réblová 1999a). These reports indicate that members of *Diplococcium*-*Spadicoides* are possibly derived from phylogenetically distant lineages. Phylogenetic significance of conidial catenation in *Diplococcium*-*Spadicoides* taxonomy is, therefore, doubtful and questionable. DNA sequence-analysis is employed in this study to determine the phylogenetic relationships and possible taxonomic placement of these asexual fungi within known ascomycetes.

Paliphora is similar to *Spadicoides* in conidial ontogeny but produces hyaline, euseptate conidia (Sivanesan and Sutton 1985), whereas *Polyschema* differs from *Spadicoides* mainly in having monotretic or polytretic conidiogenous cells borne on micronematous conidiophores (Upadhyay 1966; Ellis 1971a; Ho et al. 2002). Inclusion of *Paliphora* and *Polyschema* in this study aims to examine whether morphological similarities between these two genera and *Spadicoides* correspond to the DNA-based phylogenetic groupings. This paper also deals with phylo-

genetic placement of three hyphomycetous genera, *Bahusutrabeeja*, *Natarajania* and *Rattania* reported from India. *Bahusutrabeeja* was described to accommodate a hyphomycetous fungus that produces unicellular, hyaline, pear-shaped to subglobose phialoconidia with several evenly distributed appendages (Subramanian and Bhat 1977). This species has been frequently reported from aquatic habitats in Hong Kong and Guang Dong areas of China (Tsui et al. 2001; Wu and McKenzie 2003). Recently, this species has been collected from Goa on leaves of *Mallotus philippinensis* (family *Euphorbiaceae*). *Natarajania* is characterized by branched conidiophores with terminal, phialidic conidiogenous cells bearing a distinct collar-canal and producing slimy, dark-brown, smooth conidia (Pratibha and Bhat 2005). *Rattania* includes a sporodochial fungus with monoblastic conidiogenous cells that produce slimy, fusiform, curved conidia bearing tiny setulae at both ends (Prabhugaonkar and Bhat 2009). There are, however, no known reports on teleomorphs of *Bahusutrabeeja*, *Natarajania* and *Rattania*.

The present study, based on phylogenetic analyses of partial 28S rRNA gene sequence-data, aims to facilitate possible amalgamation of these seven anamorphic genera in ascomycete taxonomy.

Materials and methods

Fungal isolates and DNA extraction

Taxa in this study along with their culture numbers, host/substrate details, place of collection and NCBI-GenBank accession numbers of partial 28S rRNA gene sequences are listed in Table 1. The fungal isolates were grown on potato-dextrose agar (PDA) and malt extract agar (MEA) for 2–4 weeks. Genomic DNA from fungal mycelia was extracted based on: 1) phenol-chloroform method as outlined in Cai et al. (2005) or 2) Fungal/Bacterial DNA Kit following the manufacturer's protocols (Zymo Research, catalogue number D6005).

Polymerase Chain Reaction (PCR) amplification and DNA Sequencing

DNA amplification was performed by PCR. The LROR-LR5 (White et al. 1990) primer-pairs were used to amplify partial 28S rRNA gene region. Amplification reactions were performed in a 50 μ l reaction volume as outlined in Shenoy et al. (2006). The thermal cycle 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. The PCR products

spanning approximately 900 bp for partial 28S rRNA gene were checked on 1% agarose electrophoresis gel stained with ethidium bromide. PCR products were then purified using commercial kits (Amersham Biosciences, UK, catalogue number 27-09602-01; Qiagen, Valencia, USA, catalogue number 28706). DNA sequencing was performed using the above-mentioned primers in an Applied Biosystem 3130/3730 analyzer at the Genome Research Centre, The University of Hong Kong and at the Central DNA sequencing facility of Institute of Microbial Technology (CSIR), Chandigarh, India.

Sequence alignment and phylogenetic analyses

Sequences obtained from the respective primers were aligned using Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, USA) and the consensus sequences were deposited in NCBI-GenBank. Fifteen new sequences were generated in this study (Table 1). Each consensus sequence was subjected to a NCBI-BLAST search to verify its identity. Additional sequences retrieved from NCBI-GenBank and their accession numbers are listed in Fig. 1. Sequences were assembled and aligned using the web interface of MAFFT (Katoh et al. 2005) available at <http://mafft.cbrc.jp/alignment/server>, optimised by eye and manually corrected when necessary in MEGA4 (Tamura et al. 2007; Kumar et al. 2008).

Ninety-nine ambiguously aligned characters and the characters from intron regions were delimited and excluded from all analyses. The likelihood model parameters were estimated with MrModeltest version 2.1 (Nylander 2004). Maximum likelihood analyses were performed using GARLI version 0.96 (Zwickl 2006) with the default parameters except that the number of independent search replicates was set to 5. The resultant best-tree with a lowest likelihood ratio was chosen and edited in MEGA 4. Branch support was estimated by performing 100 bootstrap replicates (Felsenstein 1985) in GARLI. The resulting trees were fed into PAUP version 4b10 (Swofford 2002) to obtain a majority rule consensus tree.

Bayesian posterior probabilities (PP) for each internode were calculated with a Metropolis-coupled Markov Chain Monte Carlo (MCMC) sampling method as implemented in MrBayes version 3.1 (Huelsenbeck and Ronquist 2001). Six simultaneous Markov chains were run for one million generations (resulting 10K total trees). The first 2,000 trees were discarded and the remaining 8,000 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 space were being sampled during each analysis.

Table 1 Taxa in this study along with their strain numbers, host/substrate, place of collection and GenBank accession numbers of partial 28S rRNA gene sequences

Taxon	Strain no.	Host/substrate ^a	Place of collection	GenBank Acc. No.
<i>Bahusutrabeeja dwaya</i>	MTCC 9680 (= GUFCC 4904)	<i>Mallotus philippinensis</i> , leaf	India, Goa, Colem	HM171320
<i>Diplococcium asperum</i>	CBS 139.95	Apple leaf	Italy	EF204493
<i>Diplococcium spicatum</i>	CBS 162.47	<i>Alnus glutinosa</i> , bark	Info. not available	EF204484
<i>Diplococcium spicatum</i>	CBS 852.73	Wine cork	France, Château Latour Labatut, Mtg St. Emilion	EF204496
<i>Natarajania indica</i>	MTCC 9659 (= GUFCC 5240) ^b	<i>Antiaris toxicaria</i> , dead leaf	India, Goa, Canacona, Netravali	HM171321
<i>Paliphora intermedia</i>	CBS 199.95	<i>Buchenavia capitata</i> , leaf	Cuba	EF204500
<i>Paliphora intermedia</i>	CBS 896.97 ^c	Leaf litter	Australia, Queensland; Lamington National Park	EF204501
<i>Polyschema larviformis</i>	CBS 463.88	Soil	Turkey, Izmir	EF204503
<i>Polyschema congolensis</i>	CBS 542.73 ^b	Soil	Zaire, Ndjili, Kinshasa	EF204502
<i>Polyschema terricola</i>	CBS 301.65 ^b	Soil under <i>Saccharum officinarum</i> , 25 cm depth	Brazil, Recife	EF204504
<i>Rattania setulifera</i>	MTCC 9698 (=GUFCC 15501) ^b	<i>Calamus thwaitesii</i> , leaf	India, Goa, Dhoothsagar	HM171322
<i>Spadicoides atra</i>	CBS 489.77	<i>Quercus petraea</i> , branch	Czech Republic, forest Lánská obora	EF204506
<i>Spadicoides bina</i>	CBS 113708	<i>Picea abies</i>	Sweden, Uppland, Dalby par., Jerusalem	EF204507
<i>Spadicoides verrucosa</i>	CBS 128.86 ^b	<i>Bambusa</i> sp., old fungi on leaf	India, Andhra Pradesh; Adilabad	EF204508
<i>Spadicoides xylogena</i>	CBS 310.31	<i>Agave sisal</i>	Info. not available	EF204509

CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; MTCC Microbial Type Culture Collection and Gene Bank (CSIR-IMTECH), Chandigarh, India; GUFCC Fungus Culture Collection of Goa University, India

^a As per CBS Fungi Database; ^b Ex-type strain; ^c Isotype strain

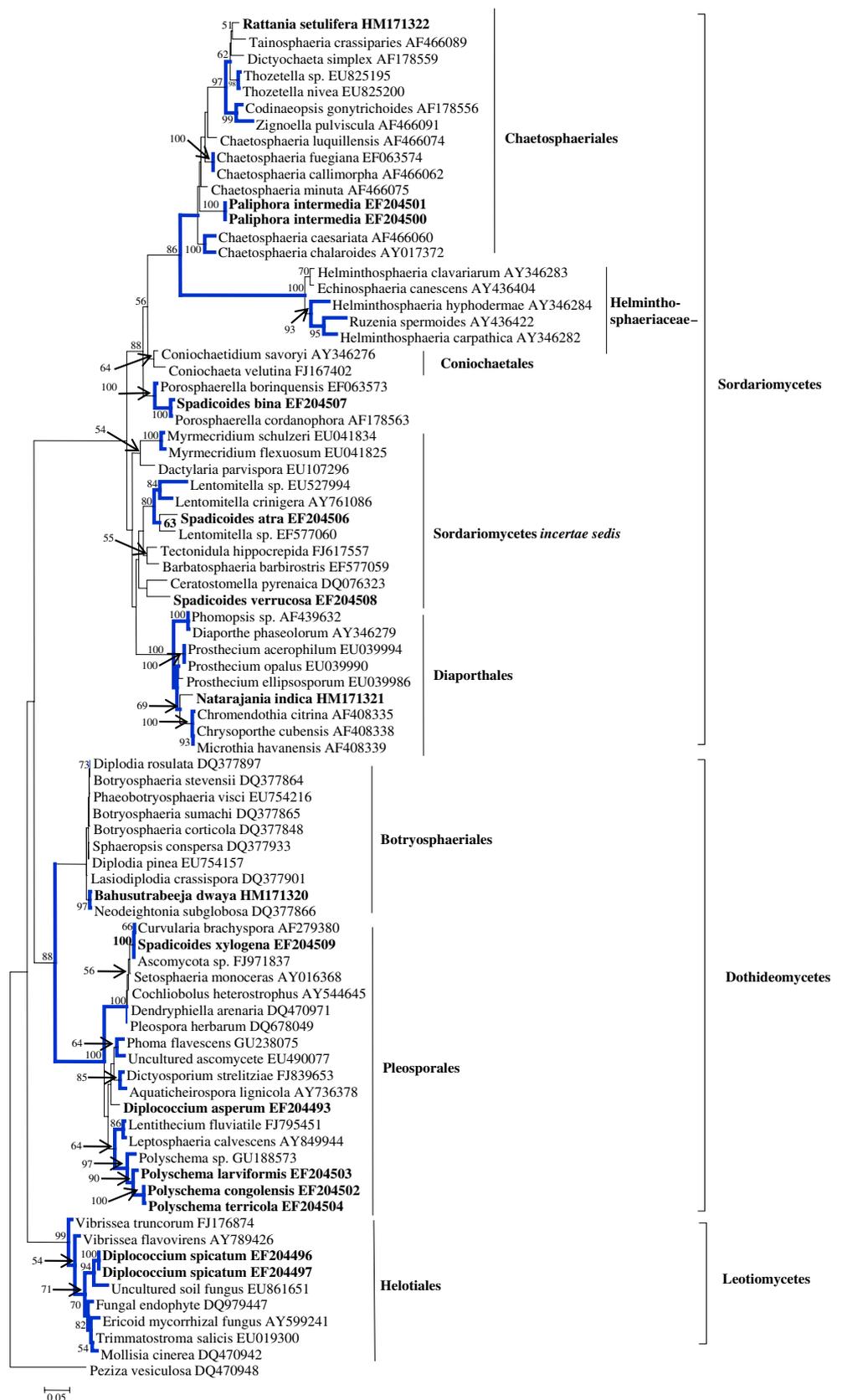
Results

The 28S rRNA gene sequence dataset consisted of 83 taxa including 15 newly generated sequences those belong to seven anamorphic genera (Fig. 1 and Table 1). The other reference taxa included members of known families of *Dothideomycetes*, *Leotiomycetes* and *Sordariomycetes* (Fig. 1). After excluding 99 ambiguously aligned characters (including a few characters from introns regions), the final dataset comprised 902 characters. There were 494, 368 and 40 constant, parsimony-informative and autapomorphic characters, respectively. Likelihood ratio test in MrModeltest suggested the best fit-model of evolution for this dataset was GTR+I+G. The best-tree from ML analyses with a lowest likelihood ratio of -9127.379 is shown in Fig. 1. Bootstrap values (equal to or above 50%) based on 100 replicates are shown on the upper branches. Values from PP (equal to or above 95%) from MCMC analyses are represented as thickened branches on the tree.

Three strains of *Diplococcium* analysed in this study showed phylogenetic affinities with members of *Dothideomycetes* and *Leotiomycetes* (Fig. 1). *Diplococcium spicatum* clustered with an uncultivable soil fungus (GenBank no. EU861651), sister to an ericoid mycorrhizal fungus (GenBank no. AY599241), a fungal endophyte (GenBank no. DQ979447), *Mollisea cinerea* and *Trimmatostroma salicis*. The *Helotiales* clade on the whole received strong statistical support. The *D. asperum* strain, however, clustered within *Pleosporales* (*Dothideomycetes*). It was found to be basal to a clade containing *Aquatichirospora lignicola*, *Dictyosporium sterilitiae*, *Phoma flavescens* and an uncultured ascomycetous strain (GenBank no. EU490077). This clustering pattern, however, received less than 50% bootstrap support.

Spadicoides species clustered with members of *Dothideomycetes* and *Sordariomycetes* (Fig. 1). *Spadicoides atra* formed a strongly supported monophyletic lineage with *Lentomitella* species within the *Sordariomycetes* clade.

Fig. 1 A maximum likelihood (ML) tree generated based on partial 28S rRNA gene sequence-data (-lnL = 9127.379). Bootstrap values (equal to or above 50%) based on 100 replicates are shown on the upper branches. Values from PP (equal to or above 95%) from MCMC analyses are represented as thickened branches on the tree. The tree is rooted with *Peziza vesiculosa*



Spadicoides verrucosa clustered with *Ceratostomella pyrenaica*, sister to a clade containing *Barbatosphaeria barbirostris* and *Tectonidula hippocrepida*. This cluster, however, received less than 50% bootstrap support. *Spadicoides bina* clustered with *Porosphaerella borinquensis* and *P. cordanophora* with high support and this clade is paraphyletic to *Coniochaeta velutina* and *Coniochaetidium savoyi*. *Spadicoides xylogena*, however, belongs to the *Pleosporales* clade (Fig. 1). It grouped with strong statistical support with *Curvularia brachyspora* and an unidentified ascomycete species (GenBank no. FJ971837).

Paliphora intermedia strains formed a distinct lineage within *Chaetosphaeriales* but its position within the order was not resolved (Fig. 1). *Polyschema* species from this study formed a strongly supported monophyletic group with *Polyschema* sp. (GenBank no. GU188573), and are related to *Lentithecium fluviale* and *Leptosphaeria calvescens* within *Pleosporales*. *Bahusutrabeeja dwa* clustered with *Neodeightonia* (= *Botryosphaeria*) *subglobosa* with 97% bootstrap and 100% PP support within *Botryosphaeriales* (*Dothideomycetes*). *Natarajania indica* clustered with *Chromendothia citrina*, *Cryphonectria cubensis* and *Microthia havanensis* within *Diaporthales* (*Sordariomycetes*). This clustering arrangement, however, received only 69% bootstrap support. *Rattania setulifera* is part of a moderately supported clade with *Taniosphaeria crassiparies*, *Dictyochoeta simplex*, and *Thozetella species* within *Chaetosphaeriales*.

Discussion

Diplococcium is one of the earliest described hyphomycetes genera. The type species *D. spicatum* was originally described from rotten wood in Sutton Coldfield, United Kingdom. Unfortunately, no holotype was designated in the protologue (Grove 1885). In the absence of any authenticated type material or living culture of the fungus, it is challenging to reconstruct the phylogeny of *Diplococcium*. The two non-type strains of *D. spicatum* (CBS162.47 and 852.73) employed in this study are phylogenetically related to an uncultured soil fungus (GenBank no. EU861651 and deposited by Nemergut et al. 2008) within *Helotiales* (*Leotiomyces*) (Fig. 1). This association is ecologically relevant as *Diplococcium spicatum* is known to inhabit similar ecological niches such as decomposing plant material in contact with soil (Goh and Hyde 1998). *Diplococcium spicatum* has also been reported from the indoor environment at a considerably higher level (avg. CFU/g = 24850) (Scott 2001). Although Maximum Likelihood analysis suggests a close evolutionary relatedness between *Diplococcium spicatum*, *Mollisia cinerea* and *Trimmatostroma salicis*, there are few morphological

characters to support this. *Mollisia cinerea*, the type species of the genus, produces a phialidic anamorph (Crous et al. 2003), unlike *Diplococcium*. Though *Diplococcium spicatum* and *Trimmatostroma salicis* (a sporodochial anamorph) are morphologically similar in producing catenate conidia, *T. salicis* differs from the former in having meristematic, arthric conidiogenous cells (Ellis 1971a).

Grouping of *Diplococcium asperum* within *Pleosporales* (*Dothideomycetes*) proves that *Diplococcium* does not represent a natural grouping. In this scenario, there is a need to re-collect *D. spicatum* from its type locality and redefine the species boundary based on DNA sequence-data. Though the observed association of *D. asperum* (CBS 139.95; isolated from apple leaf) with *Aquaticheirospora lignicola*, *Dictyosporium sterilitziae*, *Phoma flavescens* and an uncultured ascomycetes strain (GenBank no. EU490077) was not statistically supported (Fig. 1), *D. asperum* has 96% sequence identity with these taxa and shares similar ecological niches (decomposing plant materials/soil) (Kodsueb et al. 2007; Hollister 2008; Crous et al. 2009a; Aveskamp et al. 2010).

Diplococcium may also have phylogenetic associations with other fungal orders/families. Several *Helminthosphaeria* species have been associated with *Diplococcium* anamorphs but these associations are yet to be confirmed through culture/ DNA-based methods (e.g. Goh and Hyde 1998; Samuels et al. 1997; Réblová 1999b; Huhndorf et al. 2004). Though we have involved members of *Helminthosphaeriaceae* (including *H. clavariarum*, the presumed teleomorph of *D. clavariarum*) in our analysis, *D. spicatum* and *D. asperum* did not show a close phylogenetic relationship with this family (Fig. 1). It still remains to be probed whether *Diplococcium* and *Helminthosphaeria* are two spore stages of a single fungus or if *Diplococcium* leads a fungicolous lifestyle on the latter. The culture-based teleomorphic connection of *D. pulneyense* with *Othia pulneyensis* (*Dothideomycetes*) (Subramanian and Sekar 1989) has to be re-examined. The type material of *Othia*, based on which *Diplococcium pulneyense* was connected to *Othia*, has been found to actually represent a *Botryosphaeria*-like species (Phillips et al. 2005; Crous et al. 2006). It is subject to future studies whether *Diplococcium pulneyense* belongs to *Botryosphaeriales* or not. There is one report on the *Selenosporella* synanamorph of *Diplococcium hughesii* (Wang and Sutton 1998). *Selenosporella* is known to be polyphyletic (Seifert et al. 2000) and as on 17 June 2010, there are no DNA sequence-data available under this generic name in NCBI-GenBank to verify any phylogenetic connection between the two.

This study reveals some novel phylogenetic scenarios with respect to placement of *Spadicoides* species (Fig. 1). The placement of the type species of *Spadicoides*, *S. bina* with *Porosphaerella* species near *Coniochaetales* is

intriguing. *Porosphaerella* species are known to produce *Cordana* and morphologically similar *Pseudobotrys* anamorphs. These anamorphs produce conidiogenous loci resembling spinules, unlike *S. bina* that produces conidiogenous pores (Hughes 1958; Fernández and Huhndorf 2004). There are few comparable morpho-taxonomic characters that can explain the relationship between the known *Porosphaerella* anamorphs and *Spadicoides*. This is the first report of a *Spadicoides* anamorph in the *Porosphaerella* lineage. It is clear from this study that *Spadicoides atra* is a part of the *Lentomitella* lineage (*Sordariomycetes*) that is known to produce *Phaeoisaria*-like anamorphs in culture. *Spadicoides* and *Phaeoisaria* anamorphs are morphologically similar in having solitary, acropleurogenous conidia, but the latter is known to produce denticulate conidiogenous cells (Réblová 2006; Huhndorf et al. 2008), unlike *Spadicoides*. The phylogenetic placement of *S. verrucosa* needs to be resolved in future studies as its association with *Ceratostomella pyrenaica* received low statistical support (Fig. 1). Phylogenetic association of *Spadicoides* species with the presumed teleomorph *Tengiomyces* (Réblová 1999b) could not be tested due to lack of DNA sequences of *Tengiomyces* in public DNA databases.

The placement of *Spadicoides xylogena* within the *Pleosporales* can be explained with the available morphological data. *Spadicoides* shares similar conidial ontogeny with the known anamorphic *Pleosporales* such as *Alternaria*, *Bipolaris*, *Curvularia*, *Dendrophyion*, *Dendrophyiopsis*, *Dendryphiella*, *Drechslera*, *Exserohilum*, *Helminthosporium*, *Scolecobasidium* and *Ulocladium* (Sivanesan 1984; Schoch et al. 2006). *Spadicoides* is morphologically similar to these anamorphs in having tetric/polytetric conidiogenous cells. In this study, *Spadicoides xylogena* shares a close phylogenetic relationship with *Curvularia brachyspora*. Both of them have solitary acropleurogenous conidia produced on polytetric conidiogenous cells but *C. brachyspora* differs in having conidia produced on sympodial conidiogenous cells (Ellis 1971a).

The *Spadicoides*-like anamorphs *Paliphora* and *Polyschema* were not related to the four *Spadicoides* species included in this study. Members of *Polyschema* appear to represent a distinct lineage within *Pleosporales* (Fig. 1). Their close affinities with *Lentithecium fluviatile* and *Leptosphaeria calvescens* and correct placement within *Pleosporales* (Fig. 1) should be tested using more strains from diverse ecological habitats as well as anamorphs of *L. fluviatile* and *L. calvescens*. *Paliphora intermedia* constitutes a distinct lineage within *Chaetosphaeriales* (Fig. 1). This result represents a novel phylogenetic scenario in which a polytetric anamorph is reported within *Chaetosphaeriales* that has been known to include mainly phialidic (Réblová and Winka 2001; Huhndorf and Fernández 2005; Fernández

et al. 2006) and a few *Sporidesmium*-like anamorphs (Réblová 1999b; Shenoy et al. 2006). We are also reporting *Rattania* as a new introduction in the anamorph-rich *Chaetosphaeriales* clade. *Rattania setulifera* shows a close phylogenetic relationship with phialidic anamorphs such as *Codinaea* (i.e. anamorphic *Taniosphaeria*) and *Dictyochaeta*. Both *Rattania* and *Codinaea* produce conidia with tiny setulae at both ends but the former produces monoblastic conidiogenous cells, unlike *Codinaea* and *Dictyochaeta* (Fernández and Huhndorf 2005).

A close phylogenetic relationship of *Bahusutrabeeja dwaya* with *Neodeightonia* (= *Botryosphaeria*) *subglobosa* is interesting. The latter is known to produce a *Diplodia*-like coelomycetous anamorph (Crous et al. 2006). *Bahusutrabeeja* is morphologically different from *Sphaeriopsis* in having phialidic conidiogenous cells that produce unicellular conidia with 8–12 slender appendages (Subramanian and Bhat 1977). The monotypic genus *Natarajania* is related to members of the *Cryphonectriaceae* in *Diaporthales* that is known to include mainly coelomycetous anamorphs (Gryzenhout et al. 2006). *Natarajania* is one of the few known hyphomycetes in *Diaporthales* and its correct placement warrants further investigation.

Conclusion

Many of the novel phylogenetic scenarios revealed from this study cannot easily be explained with available morphological data. Recent studies have revealed the presence of fungal lineages which are mosaic of anamorphs, teleomorphs and pleomorphs and with little morphological-data in support of their phylogenetic proximity (Shenoy et al. 2006, 2007). Fungal taxonomists should, therefore, voluntarily include (where possible) DNA sequence-data in all new species description, whether teleomorphic, anamorphic or pleomorphic. This would facilitate a taxonomic link of anamorphic genera in a single phylogenetic classification system.

The GenBank sequence data must also be used with caution. Cai et al. (2009) and Hyde et al. (2009) showed that more than 86% of names used for *C. gloeosporioides* sequences were wrongly applied. We also wonder how many of the generic names may be wrong? We presently have few options but to accept GenBank names as being probably correct, but in future we hope that more and more taxa will be epitypified so that conclusions of the type made from studies of this sort can be confidently made.

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