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Phylogeny of the appendaged coelomycete genera: *Pseudorobillarda*, *Robillarda*, and *Xepiculopsis* based on nuclear ribosomal DNA sequences

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Abstract – A molecular study of selected species of the genera *Pseudorobillarda, Robillarda* and *Xepiculopsis* was undertaken to determine their phylogenetic relationships. Based on the analysis of the LSU, SSU and ITS nrDNA, *Xepiculopsis graminea* belongs in the *Hypocreales*, grouping with *Myrothecium* and *Didymostilbe* (and its sexual morph *Peethambara*) with species of *Stachybotrys* in a sister clade, but cannot be assigned to any known family. *Robillarda sessillis* clusters with members of the *Amphisphaeriaceae* (*Xylariales*) but shows no affinity with any genus in that family. *Pseudorobillarda* species formed a monophyletic group within the *Pleosporomycetidae* (*Dothideomycetes*), but do not show affinity with any family or order. This study confirms that *Pseudorobillarda* and *Robillarda* are phylogenetically distantly related and a monographic treatment is warranted to resolve the position of other species assigned to these genera. Moreover, molecular data supports the introduction of a new family within the *Hypocreales* for a well supported clade with the genera *Didymostilbe, Myrothecium, Stachybotrys* and *Xepiculopsis*.

DNA phylogeny / *Pseudorobillarda* / *Robillarda* / systematics / *Xepiculopsis*

INTRODUCTION

Currently some 2,873 asexual *Ascomycota* and *Basidiomycota* genera are known, while for 1,728 (60.15%) of these genera no sexual morph link has been established (Hyde *et al.*, 2011). For example, 90% of freshwater aquatic hyphomycetes are not yet connected with sexual states. With the advent of molecular studies a number of asexual genera have been linked to families, orders or classes (Rungjindamai *et al*., 2008; Abdel-Wahab *et al*., 2010; Diederich *et al*., 2012). Traditionally asexual fungi have been linked to their sexual states when observing them growing together e.g. *Lecythothecium duriligni* and its asexual state *Sporidesmium* (Reblova & Winka, 2001); or by culture techniques e.g.

Nereiospora cristata and *Monodictys pelagica* (Mouzouras & Jones, 1985). Shenoy *et al*. (2007) reviewed the use of DNA sequence-data on the taxonomy of asexual fungi and Shenoy *et al*. (2010) established the phylogenetic affinities of the fungal asexual states *Bahusutrabeeja*, *Diplococcium*, *Natarajania*, *Paliphora*, *Polyschema*, and *Spadicoides*. In an ongoing investigation of tropical coelomycetes (Sivichai & Jones, 2003; Plaingam *et al*., 2003; Pinruan *et al*., 2004, 2008; Somrithipol *et al*., 2006a,b, 2007, 2008; Pinnoi *et al*., 2007; Rungjindamai *et al*., 2008; Jones *et al*., 2008), we have employed molecular data to determine their phylogenetic relationships. For this study we have selected three coelomycetes, with no known sexual morphs, for investigation (Hyde *et al*., 2011).

Besides several well-known genera, such as, *Colletotrichum* (Wikee *et al*., 2011a), *Phomopsis* (Udagaya *et al*., 2011) and *Phyllosticta* (Wikee *et al*., 2011b), the identification of most coelomycetes, a group of asexual fungi comprising circa 1,000 genera and 7,000 species, is based exclusively on morphological characters, which often overlap (Sutton, 1980; Naj Raj, 1993; Kirk *et al*., 2008). Identification of genera such as *Pestalotiopsis* and *Pestalotia*; *Pseudorobillarda* and *Robillarda* are contentious with consequent transfer from one genus to the other (Naj Raj, 1993; Jeewon *et al*., 2003a, b; Maharachchikumbura *et al*., 2011; Tempesta *et al*., 2011, Zhang *et al*., 2012). In these cases colour, degree of septation and possession and number of conidial appendages determine the genera. Apart from genera of economic importance, the phylogenetic affiliation of coelomycetes generally remains largely unresolved. It is important that these are studied at the molecular level as they may also help to resolve missing lineages of ascomycetes and basidiomycetes (Hibbett *et al*., 2007; Rungjindamai *et al*., 2008; Sri-indrasutdhi *et al*., 2010; Réblova *et al*., 2011).

The three genera selected are *Pseudorobillarda, Robillarda,* and *Xepiculopsis* which possess ellipsoidal conidia with appendages, but differ in conidial appendage ontogeny (Plaingam, 2002; Plaingam *et al*., 2005). Conidia of *Robillarda* species are always 1-septate while septation of *Pseudorobillarda* species ranges from non- to 4-septate. Moreover, conidial appendages in *Robillarda* are always apical, while those of *Pseudorobillarda* can be apical or basal. In both genera appendages are formed by out growths of the conidial cell-wall (Plaingam, 2002), while in *Xepiculopsis* the apical appendage is formed by inversion of a sheath (Plaingam, 2002). No sexual morphs are known for these three genera (Hyde *et al*., 2011).

The genera *Robillarda* and *Pseudorobillarda* are often confused as they both possess septate conidia with polar appendages and many *Robillarda* species have been transferred to *Pseudorobillarda* (e.g. *Robillarda phragmitis* Cunnell, *R. agrostidis* R. Sprague, *R. jaczewski* Girz., *R. muehlenberiae* R. Sprague) (Nag Raj, 1993). These transfers are made as there is no real consensus as to the reliability of the selected morphological characters used by different taxonomists. Morphological features that can be used to distinguish these two genera include scanning (SEM) and transmission electron microscopy (TEM) of conidial ontogeny and especially of the appendages. In *Pseudorobillarda* the conidial cell wall is two layered, the thin outer cell wall layer (10-15 nm) forming the appendages with one pair apical and the second pair subapical (Plaingam, 2002) (Figs 3-7). Conidia in *Robillarda* develop holoblastically from the conidiogenous cells, all appendages are apical and cellular, but development of the appendages has not been studied at the TEM level (Nag Raj, 1993; Plaingam, 2002).

Currently 15 species are listed for *Pseudorobillarda* (http://www.indexfungorum.org/names/Names.asp), with *P. phragmitis* (Cunnell) M. Morelet the type species (Morlet, 1968), and based on *Robillarda phragmitis* Cunnell,

described from submerged dead stems of *Phragmitis communis* (L.) Trin.. In *Pseudorobillarda* conidiogenesis is phialidic and conidia have polar appendages (Morlet, 1968; Nag Raj, 1993). Plaingam *et al*. (2005) reviewed the taxonomy of the genus, and described a new species *P. siamensis* Plaingam, Somrith. & E.B.G. Jones from senescent leaves collected in Khao Yai National Park, Thailand and fully illustrated the three species studied here.

The type species of *Robillarda* is *R. sessilis* (Sacc.) Sacc., and 35 names are listed for the genus (http://www.indexfungorum.org/names/Names.asp). However, many *Robillarda* species have been transferred to the genus *Pseudorobillarda* (Morlet, 1968). The genus is characterized by holoblastic conidiogenous cells, 1-septate conidia, and with a separate apical cell modified into branched appendages (Figs 8-9).

Nag Raj (1993) described two new genera: *Xepicula* and *Xepiculopsis*, based on *Myrothecium* species with cupulate conidiomata. He regarded the circumscription of the genus *Myrothrecium* as to broad. The two genera were separated based on the morphology of the cupulate conidiomata and the presence of setae on the excipulum. The type of *Xepiculopsis* is *X. perpulchra* Naj Raj (Nag Raj, 1993). He also transferred *Myrothecium gramineum* Lib. to this new genus, but with reservations, as there was confusion with the type material deposited by Libert (1837), in that it contained more than one fungus. Conidiogenesis in *X. graminea* (Lib.) Naj Raj is phialidic, and conidia are unicellular with a polar appendage formed by the fragmentation and inversion of a sheath (Plaingam, 2002).

The sexual morphs of most coelomycetes are ascomycetes, although a few are known to have basidiomycetous morphs. Some species have clamp connections e.g. *Fibulocoela*, *Ellula* and *Pycnovellomyces*, although they have not been linked to a specific sexual genus (Nag Raj, 1993). Rungjindamai *et al*. (2008) however, demonstrated that *Chaetospermum* (*Sebacinales*), *Guilia* (*Corticiales*) and *Mycotribulus* (*Agaricales*) species were basidiomycetes based on LSU and ITS sequence data. For most coelomycetes with appendaged conidia, their sexual morphs are unknown, however a significant number have been linked by culture studies: *Acrocalymma medicaginis* (*Massarina*), *Chaetoconis polygoni* (*Cerio-spora*), *Mastigosporella hyalina* (*Wustneiopsis*), and *Seimatosporium* (*Disco-stroma*) to name but a few (Naj Raj, 1993).

The objectives of this study are to 1) distinguish between the genera *Pseudorobillarda* and *Robillarda*, and 2) resolve the familial and ordinal status of the genera *Pseudorobillarda*, *Robillarda* and *Xepiculopsis,* based on ribosomal DNA sequence data.

MATERIALS AND METHODS

Specimen collection, culture maintenance and fungal cultivation

Five coelomycetes collected in Thailand were studied: three species of *Pseudorobillarda* (*P. siamensis, P. sojae* Uecker & M.M. Kulik and *P. texana* Naj Raj), *Robillarda sessillis* and *Xepiculopsis graminea*. Plaingam *et al.* (2005) detailed the taxonomy of the three *Pseudorobillarda* species studied in this paper. Details of collection sites, substratum and isolate accession codes are listed in

Table 1. Collection site, substrata and GenBank accession number of *Robillarda sessilis, Pseudorobillarda siamensis, P. sojae, P. texana* and *Xepiculopsis graminea* sequenced in this study (In this study strains of the generic types was used for the selected genera)

Taxa	*Original code*	*Source**	*Substratum and geographical origin*	*Date of isolation*	*GenBank accession number*		
					SSU	*LSU*	*ITS*
Pseudorobillarda siamensis N. Plaingam, Somrith. & E.B.G. Jones (2005) (Type strain of species)	SFC00795	BCC12531	Fallen dicotyledonous leaf, Khao Yai National Park, Nakon Nayok	19 July 2000	FJ825365	FJ825375	FJ825370
P. sojae Uecker & Kulik (1986)	SFC01947	BCC20495	Dead leaf, Khao Yai National Park, Nakhon Ratchasima	20 March 2006	FJ825366	FJ825376	FJ825371
P. texana Nag Raj (1993)	SFC00866	BCC12535	Fallen dicotyledonous leaf, Khao Yai National Park, Prachin Buri	18 August 2000	FJ825367	FJ825377	FJ825372
Robillarda sessilis (Sacc.) Sacc. (1880) (Type species)	SFC00858	BCC13393	Leaf of *Eucalyptus camaldulensis*, Kasetsart University, Bangkok	2 April 2003	FJ825368	FJ825378	FJ825373
Xepiculopsis graminea (Lib.) Nag Raj (1993) (Type species)	SFC00785	BCC9458	Grass leaf, Banphkong, Chachaensao	4 July 2001	FJ825369	FJ825379	FJ825374

*BCC = BIOTEC Culture Collection, Pathumthani, Thailand.
Pseudorobillarda phragmitis (Cunnell) M. Morlet (Sequence of type species from GenBank See Table 2).

Table 1. All strains are deposited in the BIOTEC Culture Collection (BCC). Accession numbers of sequences used in the phylogenetic analyses are deposited in GenBank and listed in Table 2 (see online supplementary material). Strains of the type species of the genera *Pseudorobillarda*, *Robillarda* and *Xepiculospis* been have been sequenced in this study, along with the type strain of *P. siamensis.*

Genomic DNA extraction and PCR amplification

The coelomycete strains were grown on potato dextrose agar (PDA), transferred to potato dextrose broth (PDB), and incubated without agitation at 25°C for 2 weeks for DNA extraction. Mycelium was harvested, washed with warm sterilized distilled water, frozen at –80°C for 1-2 hours, and the fungal biomass ground into fine powder with sterilized mortar and pestle. DNA was extracted using CTAB lysis buffer (O'Donnell *et al*., 1997) and incubated at 65°C for 1 hour. The mixture was extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1). The upper liquid phase was precipitated with 7.5 M ammonium acetate and absolute ethanol and kept at –20°C for at least 30 min. Extracted DNA was washed twice with 70% ethanol, air dried and the DNA resuspended in 50 µl TE buffer.

Partial nuclear small subunit (SSU) and nuclear large subunit (LSU) regions of rDNA were amplified with gene specific primers: NS1, NS2, NS3, NS4, NS5, NS6 and LROR, LR2, LR3, LR7, respectively (White *et al*., 1990; Bunyard

et al., 1994) using FINNZYMES, DyNAzyme™ II DNA polymerase kit (Cat No F-551S, Finnzymes, Espoo, Finland). The amplification cycles were performed following White *et al*. (1990) and Bunyard *et al*. (1994) with a DNA Engine DYAD ALD 1244 thermocycler (MJ Research, Inc, Waltham, MA). The PCR products were purified with NucleoSpin® Extract DNA purification kit (Cat. No. 740 609.50, Macherey-Nagel, Duren, Germany) following the manufacturer's instruction and then sequenced by Macrogen Inc. (Seoul, Korea) using the same primers as for amplification.

Sequence alignment and phylogenetic analysis

A BLAST search was employed to obtain the closest matched sequences in the GenBank database (Altschul *et al*., 1990). The SSU and LSU rDNA sequences were multiple aligned along with other related sequences obtained from GenBank using Clustal W 1.6 (Thompson *et al.*, 1994) and adjusted manually where necessary using BioEdit 7.5.0.3 (Hall, 2006).

The aligned dataset was subsequently analysed using maximum parsimony in PAUP* 4.0b10 (Swofford, 2002), for the most parsimonious trees (MPTs). Heuristic searches algorithm with tree-bisection-reconnection (TBR) branch swapping, 100 replicates of random stepwise sequence addition, were performed. Gaps were treated as missing data and given equal weight. The tree length, consistency indices (CI) and retention indices (RI) were calculated for each tree generated. The Kishino-Hasegawa (K-H) test was used for estimation of the best tree topology (Kishino and Hasegawa 1989).

Bayesian phylogenetic inference was calculated with MrBayes 3.0b4 with general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Huelsenbeck & Ronquist, 2001). Four Markov chains were run from random starting trees for 5.000.000 generations and sampled every 100 generations. The first 500.000 generations were discarded as burn-in of the chain. A majority rule consensus tree of all remaining trees was calculated.

Statistical support for the internal branches was estimated by bootstrapping analysis (Felsenstein, 1985) with 1000 replications (10 replicates of random stepwise sequence addition, TBR branch swapping) and posterior probabilities were performed. The maximum parsimony bootstrap values (≥ 50%) and Bayesian posterior probabilities (≥ 0.95) are shown above and below the tree branches, respectively. The rDNA sequences, consisting of SSU and LSU were submitted into the GenBank database and the new sequences generated for this investigation are shown in Table 1.

RESULTS

SSU and LSU phylogeny of three coelomycete genera

A phylogenetic tree was constructed from a dataset consisting of nuclear small subunit (SSU) and nuclear large subunit (LSU) sequences. This alignment was combined from two fragments of 1,409 bps for SSU and 1,377 bps for LSU. The data set contained 107 sequences with *Peziza vesiculosa* and *P. proteana* (*Pezizales*) as outgroup taxa. The DNA insertion of SSU and LSU sequences

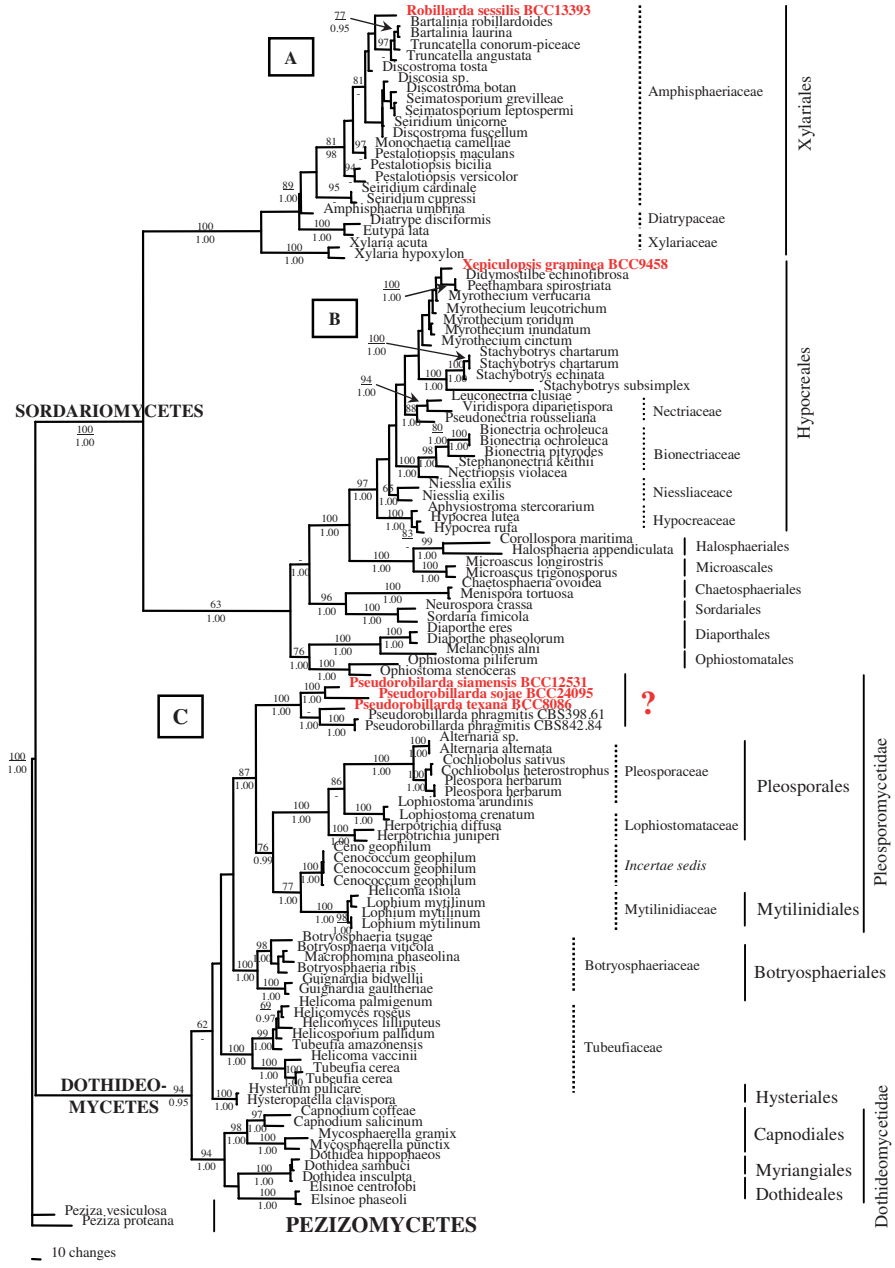


Fig. 1. A single parsimonious tree inferred from SSU and LSU rDNA sequences of *Pseudorobillarda* species (*P. siamensis, P. sojae* and *P. texana*), *Robillarda sesillis* and *Xepiculopsis graminea.* The MP BS value (≥ 50%) and Bayesian PP (≥ 0.95) are shown above and below the branches, respectively (tree length = 3,853 steps, CI = 0.389, RI = 0.823). Coelomycetes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position. The sources of culture and accession numbers of sequence retrieved from GenBank are shown in Table 2.

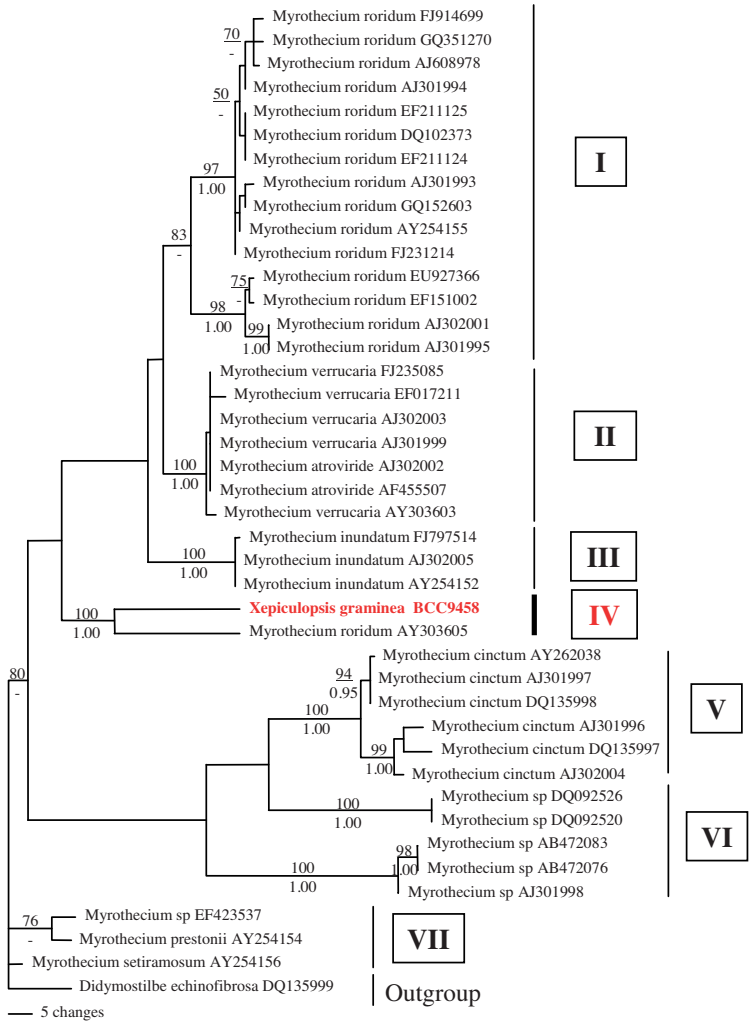
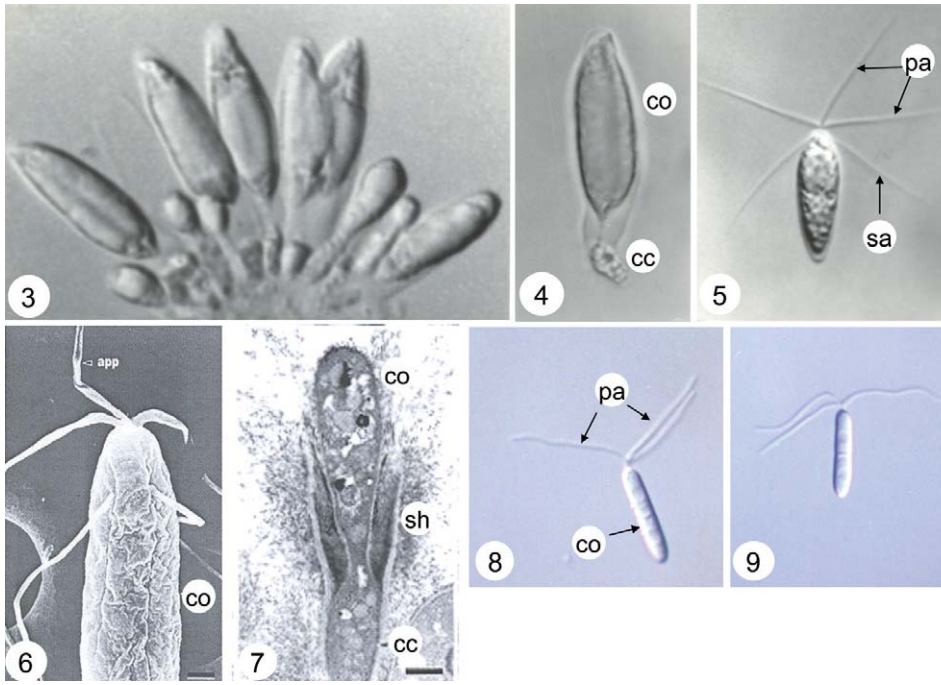


Fig. 2. One of 12 MPTs from ITS rDNA sequence of *Xepiculopsis graminea*. The MP BS value (≥ 50%) and Bayesian PP (≥ 0.95) are shown above and below the branches, respectively (tree length = 431 steps, CI = 0.717, RI = 0.918). Coelomycetes sequenced in this study are printed in bold. Bar = number of changes per nucleotide position. The accession numbers of sequence retrieved from GenBank are shown on phylogenetic tree.

were present in *Pseudorobillarda* species. Insertion fragments of SSU and LSU regions of *P. siamensis* BCC12531 were observed one at 1148-1621 (473 bps) and another at 902-1343 (441 bps), respectively. Furthermore three and one insertion regions were present in SSU and LSU regions of *P. texana* BCC12535 at 534-924 (381 bps), 1530-1924 (394) and, 2050-2125 (75 bps) for SSU and 889-1358 (469 bps) for LSU. Inclusion and exclusion of all insertion regions had no effect on the tree topology. Therefore the insertion regions of *Pseudorobillarda* species were removed in all analyses.



Figs 3-9. Conidial ontogeny of *Pseudorobillarda siamensis*. **3-5.** Light micrographs. **3.** Conidiopores, phialidic arising from the base of the pycnidium. **4.** Conidium budded from conidiogenous cell with immature conidium. **5.** Mature conidium with three polar appendages (pa) and two sub polar appendages (sa). **6.** SEM micrographs of mature conidium (co) with three polar appendages (app) and two subpolar appendages **7.** TEM micrographs of conidium formation. Conidum (co), sheath (sh) and conidiogenous cell (cc). **8-9.** Light micrograph of mature conidia of *Robillarda sessilis* with three polar appendages.

A total of 2,786 characters, 793 are parsimony informative, 207 are parsimony uninformative and 1,786 are constant characters. The phylogenetic analysis based on maximum parsimony criterion and Bayesian inference resulted in the same tree topology in major orders and classes of fungi, therefore the maximum parsimonious tree is shown.

Fig. 1 shows a single parsimonious tree estimated from the combined datasets. Within the two major classes *i.e.* the *Sordariomycetes* and *Dothideomycetes*, only minor swapping was found in the tree resulting from the two analyses. In order to solve the taxonomic position of our five coelomycete strains, taxa from those classes were incorporated into the dataset. Within the *Sordariomycetes*, 60 representative taxa from all major orders: *Chaetosphaeriales*, *Diaporthales, Sordariales* (*Sordariomycetidae*); *Halosphaeriales, Hypocreales, Microascales*, *Ophiostomatales* (*Hypocreomycetidae*); and the *Xylariales* (*Xylariomycetidae*), while 43 taxa from eight orders of the *Dothideomycetes*, comprising *Botryosphaeriales, Capnodiales, Dothidiales, Hysteriales, Incertae sedis*, *Myriangiales*, and *Pleosporales*, were analyzed. Our isolates *i.e. Robillarda sessillis, Pseudorobillarda siamensis, P. sojae, P. texana* and *Xepiculopsis graminea* grouped with taxa in different orders distant from one another. *Robillarda sessillis* and *X. graminea* were placed within the Sordariomycetidae with 100% BS

and 1.00 PP, while the three *Pseudorobillarda* species grouped with other *Dothideomycetes* with 94% BS and ≤ 0.95 PP.

In this investigation, *Xepiculopsis* nested within the *Hypocreales* with 97% BS and 1.00 PP and formed a clade with *Peethambara spirostriata* and *Didymostilbe echinofibrosa* (Fig. 1 subclade B). This subclade has phylogenetic affinity with several *Myrothecium* species. Furthermore, *Stachybotrys* species were closely allied in a sister clade with moderate support (72% BS and 1.00 PP). Other families within the *Hypocreales* (*Bionectriaceae, Nectriaceae, Niessliaceae* and *Hypocreaceae*) formed a separate clade.

Robillarda sessillis falls within the *Xylariales* with 100% BS and 1.00 PP (Fig. 1 subclade A) and the *Amphisphaeriaceae* with 91% BS and 0.98 PP linked with the *Diatrypaceae* and *Xylariaceae*. Although *R. sesillis* grouped within the *Amphisphaeriaceae*, it showed no affinity with the genera included in the analysis (*Amphisphaeria, Bartalinia, Discosia, Discostroma, Monochaetea, Pestalotiopsis, Seiridium* and *Truncatella*).

Three *Pseudorobillarda* species (*P. siamensis, P. sojae* and *P. texana*) were phylogenetically related with members of the *Dothideomycetes* with 94% BS and ≤ 0.95 PP (Fig. 1 subclade C). The three species of *Pseudorobillarda*, *P. siamensis*, *P. sojae* and *P. texana,* formed a monophyletic clade with 100% BS and 1.00 PP, although they could not be assigned to any family or order in the *Dothideomycetes* and appear to represent a distinct lineage within that subclass.

ITS phylogeny of *Xepiculopsis graminea*

Our sequence of *X. graminea* was aligned along with several sequences of *Myrothecium* species with *Didymostilbe echinofibrosa* DQ135999 as the outgroup. A total of 639 characters, 465 are constant characters, 17 are parsimony uninformative and 157 are parsimony informative. Our analysis yielded 12 maximum parsimonious trees (MPTs). The best MPT is shown in Figure 2 and *Myrothecium* formed seven different clades (I-VII). *Xepiculopsis graminea* had an affinity with *Myrothecium roridum* AY303605 with high support (100% BS). However other sequences of *M. roridum* grouped together in a separate clade (clade I). Other species of *Myrothecium* (*M. atroviride* (Berk. & Broome) M.C.Tulloch*, M. cinctum* (Corda) Sacc.*, M. inundatum* Tode and *M. verrucaria* (Alb. & Schwein) Ditmar) are distributed in difference lineages with high support.

DISCUSSION

Our study places the genera *Xepiculopsis* and *Robillarda* in the *Sordariomycetidae*, while *Pseudorobillarda* species group in the *Dothideomycetidae*. This clearly demonstrates that, based on strains of the generic type species of the selected taxa, *Robillarda* and *Pseudorobillarda* are not phylogenetically related. Although a strain of the generic type, *Robillarda sessilis*, grouped within the *Amphisphaeriaceae*, *Xylariales*, a strong relationship with a specific genus could not be made. The strains of *Pseudorobillarda* species could not be referred with confidence to any family, even an order, but grouped within a clade comprising the *Mytilinidiaceae* (*Hysteriales*), and the *Lophiostomataceae* and *Pleosporaceae* (*Pleosporales*), which all group within the *Pleosporomycetidae*,

even though the generic type was included. Suetrong *et al.* (2009), in a larger data set and four gene analysis, showed that the three *Pseudorobillarda* species referred to in this study grouped with *Farlowiella carmichaeliana* with weak support, but could not be assigned to any family in the *Dothideomycetes*.

Naj Raj (1993) regarded the generic concept for *Myrothecium* too broad with sporodochial, synnematous and cupulate forms. He thus separated *Myrothecium* species with cupulate conidiomata into two new form genera *Xepicula* and *Xepiculopsis*, the former with septate conidiomatal setae and lacking exipular elements. Both genera possess phialidic conidiogenesis cells with flaring collarettes and conidia that are fusiform to ellipsoidal, unicellular pale olivaceous smooth with polar mucoid appendages formed by fragmentation of a sheath (Plaingam, 2002).

In this study *Xepiculopsis graminea,* the type species of *Xepiculopsis* (Nag Raj, 1993), formed a well supported clade with *Didymostilbe echinofibrosa* and its sexual morph: *Peethambara spirostriata*, *Myrothecium* and *Stachybotrys* species in the *Hypocreales*. It has been suggested that *Myrothecium* and *Peethambara* are allied to the *Bionectriaceae* (Rossman *et al.*, 2001). However, data from a five-gene study indicated an "undiscovered sister lineage to all other families in the *Hypocreales* (*Bionectriaceae, Clavicipitaceae, Hypocreaceae, Nectriaceae* and *Niessliaceae*)" (Casterburry *et al*., 2004). It may not be surprising that *X. graminea* groups with this hypocrealean clade as it was originally described as *Myrothecium gramineum*. The genus *Xepicula* is based on *X. leucotricha*, originally described as *Myrothecium leucotrichum*. From our SSU and LSU data, a putative strain of *X. leucotricha* groups within the main *Myrothecium* clade; and should be referred to that genus.

Taxonomy

Myrothecium leucotrichum (Peck) Tulloch, Mycol. Pap. 130: 12, 1972.

≡ *Excipula leucotricha* Peck, Rep. St. Mus. N.Y. 29: 49, 1878

≡ *Amerosporium leucotrichum* (Peck) Saccardo, Syll. Fung. 3: 682, 1884.

≡ *Xepicula leucotricha* (Peck) Nag Raj, In Coelomycetous anamorphs with appendage-bearing conidia. 980: 1993.

= *Myrothecium jollymannii* Preston, Trans. Br. Mycol. Soc. 31: 272, 1948.

= *Myrothecium indicum* Rama Rao, Antonie van Leuwenhoek 29: 180, 1963.

Notes: Our molecular data does not support the introduction of the genus *Xepicula* by Nag Raj (1993) and *X. leucotricha* is regarded as synonym of *M. leucotrichum*. The taxonomic placement of *Xepiculopsis graminea* cannot be resolved at this time and further collections of it and *Myrothecium roridum* are required. Our study shows that morphological data alone is insufficient for the classification of coelomycetes due to their pleomorphism and demonstrates that molecular sequences are a better indicator of phylogenetic relationships.

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