Mycol. Res. **109** (5): 581–589 (May 2005). © The British Mycological Society
doi:10.1017/S0953756205002741 *Printed in the United Kingdom.*

On the relationships of *Paecilomyces* sect. *Isarioidea* species[1]

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Received 21 May 2004; accepted 14 February 2005.

Phylogenetic relationships of *Paecilomyces* sect. *Isarioidea* species were analysed using the β-tubulin gene and ITS rDNA. Maximum parsimony analyses showed that the section does not form a natural taxonomic group and is polyphyletic within the *Hypocreales*. However, a group was recognized, designated as the *Isaria* clade, to be monophyletic comprising of the following *Paecilomyces* species: *P. amoeneroseus*, *P. cateniannulatus*, *P. cateniobliquus*, *P. cicadae*, *P. coleopterorus*, *P. farinosus*, *P. fumosoroseus*, *P. ghanensis*, *P. javanicus* and *P. tenuipes*. Some of these species have teleomorphs in *Cordyceps*.

INTRODUCTION

The genus *Paecilomyces* as conceived by Bainier (1907) is only monophyletic within the order *Eurotiales* when characterised by a *Byssochlamys* teleomorph using an 18S phylogeny (Luangsa-ard, Hywel-Jones & Samson 2004). Such species conform to the sect. *Paecilomyces* which was recognised by Samson (1974) for thermophilous species with eurotialean associations. Following the expansion of the genus *Paecilomyces* by Brown & Smith (1957), Samson (1974) recognised mesophilic species previously placed in *Isaria* or *Spicaria* and with mostly insect associations as a distinct sect. *Isarioidea* within that genus. An 18S mRNA-based phylogeny confirmed that sect. *Isarioidea* is not eurotialean (Luangsa-ard *et al*. 2004) demonstrating that the current generic concept of *Paecilomyces* is not monophyletic even at the ordinal level.

Luangsa-ard *et al*. (2004) also suggested that sect. *Isarioidea* was not a monophyletic assemblage. *P. inflatus* could not be linked with other mesophilic species with hypocrealean affinities as it grouped with the order *Sordariales* (Luangsa-ard *et al*. 2004). Since Luangsa-ard *et al*. (2004) found that *P. inflatus* had affinities with the *Sordariales*, we chose to omit this species from our current work and focus on species accepted to be within the *Hypocreales*. The work presented here aimed to determine if an entomogenous habit for sect. *Isarioidea* is a monophyletic characteristic within the *Hypocreales*. With a view to considering whether the genus name *Isaria* should be resurrected for such species, we use a β-tubulin- and an ITS-derived phylogeny to examine the relationships of sect. *Isarioidea* within the *Hypocreales*, omitting species from *Paecilomyces* sect. *Paecilomyces*, as well as *P. inflatus*.

MATERIALS AND METHODS

Partial β-tubulin sequences of 34 strains and sequences of ITS1-5.8S-ITS2 rDNA of 37 strains of *Paecilomyces* and its associated hypocrealean teleomorphs *Torrubiella* and *Cordyceps* were analysed using maximum parsimony. Morphologically similar species in the genera *Mariannaea, Metarhizium* and *Nomuraea* were also included in the analyses (Table 1).

DNA extraction and PCR amplification

Genomic DNA was extracted from fresh mycelia using a Fast DNA Kit (BIO 101 Systems, Vista, CA). CSL-Y buffer was used and extractions were processed in a FastPrep machine (FastPrep FP 120, BIO 101 Systems) for 30 s at speed 4.5.

[1] Dedicated to John Webster on the occasion of his 80th birthday.
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Table 1. List of cultures used in this study.

Taxon	Strain[a]	Host/Substratum	ITS rDNA	β-tubulin gene
Akanthomyces sp.	BCC 1570	Pentatomid bug, Thailand	–	AY624238
	BCC 1566	Pentatomid bug, Thailand	–	AY624239
Cordyceps cf *takaomontana*	BCC 1409	Lepidopteran larva, Thailand	AY624198	AY624240
Isaria japonica	BCC 2808	Lepidopteran larva, Japan	AY624199	AY624241
	BCC 2821	Lepidopteran larva, Japan		AY624242
	BCC 2787	Lepidopteran larva, Japan	AY624200	
Mariannaea elegans var. *elegans*	CBS 132.41	Culture contaminant of Polyporus, South Africa	–	AY624243
M. elegans var. *punicea*	CBS 239.56	Soil, Zaire	AY624201	–
M. camptospora	CBS 209.73	Forest soil, The Netherlands	AY624202	AY624244
Metarhizium flavoviride	BCC 7672	Homoptera (non-cicadae) adult, Thailand	AY624203	AY624248
	BCC 11959	Coleoptera larva, Thailand	–	AY624249
M. cylindrosporae	BCC 1762	Homoptera (cicada) adult, Thailand	AY624204	AY624247
Metarhizium sp.	BCC 12692	Coleoptera larva, Thailand	–	AY624246
Nomuraea rileyi	CBS 806.71	*Trichoplusia ni*, USA	AY624205	AY624250
Paecilomyces	CBS 107.73	Coleopteran pupa	AY624168	AY624207
amoeneroseus	CBS 729.73	Nitidulidae, Ghana	AY624169	AY624208
P. carneus	CBS 239.32	Dune sand, France	AY624171	AY624209
	CBS 399.59	Forest soil	AY624170	AY624210
P. cateniannulatus	CBS 152.83	Coleopteran adult	AY624172	AY624211
P. cateniobliquus	CBS 153.83	*Adoxophyes privatana*	AY624173	AY624212
P. cicadae	BCC 2574	Cicada nymph, Thailand	AY624175	–
P. cinnamomeus	CBS 398.86	Living leaf of *S. jambus*	AY624174	AY624213
	CBS 828.88	Coccidae	–	AY624214
P. coleopterorum	CBS 102.73	Lampyridae, France	AY624176	AY624215
	CBS 110.73	Coleopteran larva, Ghana	AY624177	AY624216
P. farinosus	CBS 111113	Denmark	AY624181	AY624219
	CBS 541.81	Small spider, Galapagos Is.	AY624180	AY624218
	CBS 262.58	Garden soil, UK	AY624179	AY624217
	CBS 240.32	Lepidopteran pupa, The Netherlands	AY624178	
P. fumosoroseus	CBS 107.10	France	AY624184	AY624222
	CBS 244.31	Butter, Ireland	AY624182	AY624220
	CBS 375.70	Food, Japan	AY624183	AY624221
P. ghanensis	CBS 105.73	Lepidopteran pupa	AY624185	AY624223
P. javanicus	CBS 134.22	*Stephanoderis hampei*, Indonesia	AY624186	AY624224
	CBS 994.73	Coleopteran pupa	AY624187	AY624225
P. lilacinus	CBS 284.36	Soil	AY624189	AY624227
	CBS 432.87	Egg mass of Meloidogyne, Peru	–	AY624228
	CBS 431.87	Egg mass of Meloidogyne, Philippines	AY624188	–
	CBS 940.73	*Aethus* sp., Ghana		
	BCC 2012	Cydnid bug, Thailand	AY624190	–
			–	AY624226
P. marquandii	CBS 182.27	Soil	AY624193	AY624229
P. niphetodes	CBS 229.73	Wood of *Fagus sylvatica*, The Netherlands	AY624191	AY624231
	CBS 364.76	Soil on a rock	AY624192	AY624230
P. penicillatus	CBS 448.69	Rotting mushroom	AY624194	AY624232
P. tenuipes	ARSEF 5135	Lepidopteran pupa	AY624196	AY624234
	CBS 997.73	Lepidopteran larva, The Netherlands	AY624195	AY624233
P. viridis	CBS 348.65	*Chameleo lateralis*, Madagascar	AY624197	AY624235
	CBS 659.71	*Chameleo lateralis*	–	AY624236
Torrubiella luteorostrata	BCC 9617	Homoptera, Thailand	AY624206	AY624237

[a] CBS, Centraalbureau voor Schimmelcultures, Utrecht; BCC, National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok; ARSEF, ARS Collection of Entomopathogenic Fungi, Ithaca.

For the amplification of the partial β-tubulin and ITS rDNA sequences the following primers and PCR conditions were used: primers bt2a and bt2b (Glass & Donaldson 1995) for the β-tubulin gene with these conditions: 3 min at 94 °C; denaturation 1 min at 94 °, annealing 1 min 30 s at 58 °, extension 2 min at 72 ° (35 cycles) and 8 min at 72 °. The primers for the amplification of ITS rDNA were ITS 4 and ITS 5 (White *et al.* 1990) with these conditions: 2 min at 96 °; denaturation 1 min at 96 °, annealing 1 min at 55 °, extension 2 min at 72 ° (35 cycles) and 8 min at 72 °. All amplifications were carried out in 50 µl volume consisting of 1 × PCR buffer, 200 µM each of the four dNTPs, 2.5 mM $MgCl_2$, 1 U SuperTaq polymerase (HT Technologies, Cambridge, UK) and 0.5 µM of each primer. Amplifications were performed using a GeneAmp PCR System 9700 (AB Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands).

DNA visualisation, quantification and purification

The PCR products were visualised by ethidium bromide staining after gel electrophoresis of 5 µl of the product in 1 % agarose gel. Quantification of the PCR products was done using a standard DNA marker of known size and weight. Products with clear bands were purified using GFX columns (DNA and Gelband Purification Kit; Amersham Biosciences, Uppsala).

DNA sequencing

DNA sequence reactions were carried out using BigDye Terminator Sequencing Kit (Applied Biosystems) The SeqMan computer programme was used for compilation and editing of the forward and reverse sequences generated from each template.

Sequence alignment and phylogenetic analysis

A preliminary alignment of the sequences was performed using ClustalW incorporated in BioEdit v. 5.06 (Tom Hall, Department of Microbiology, North Carolina State University, Raleigh, NC) using default parameters. The sequence alignment was manually optimised using BioEdit and SeAl. Alignment gaps were treated as missing data in the analysis. Alignments are available upon request.

Phylogenetic analyses were performed on an Apple Power-Macintosh G4 using maximum parsimony as implemented in PAUP* (version 4.0b10; Swofford 1999). Heuristic searches with 1000 random-addition sequence replicates was performed using TBR branch-swapping and MulTrees option in effect. Uninformative characters were excluded from the analysis. To test the robustness of the branches tree scores for consistency index (CI), rescaled consistency index (RC), homoplasy index (HI) and retention index (RI) were computed. Successive weighting was carried out to ensure the stability of the weights and to evaluate the consistency of the characters. Characters were re-weighted until the tree scores were stable. Relative support for the resulting trees was obtained from bootstrap analyses (Felsenstein 1985) using 500 heuristic searches with groups occurring at 50 % or greater frequencies being retained in the consensus trees. All heuristic searches were performed using a tree-bisect.-reconnection (TBR) branch-swapping algorithm with 10 random sequence addition replications and MulTrees option in effect.

RESULTS

β-tubulin gene phylogeny

For the β-tubulin dataset, 44 sequences with a length of *ca* 433 bp appeared. The nectriaceous genus *Mariannaea* was used as outgroup. In the β-tubulin analysis, therefore, 14 available species of *Paecilomyces* sect. *Isarioidea* (Samson 1974) were included along with two more recently described species, *P. cateniobliquus* and *P. cateniannulatus*, which by their phenotypic characters and ecology would appear to belong in sect. *Isarioidea*. Also, representatives of *Nomuraea* and *Metarhizium* were included to allow comparisons of green-spored *Paecilomyces* with these genera.

For the analysis of the β-tubulin sequences, 246 characters were excluded as being parsimony uninformative while 187 characters were retained as informative. The β-tubulin analysis resulted in 41 MPT's with branch lengths of 750. The resulting phylogram (Fig. 1) was based on a final dataset of 44 taxa with three forming the *Mariannaea* outgroup and the remaining 41 taxa the ingroup. Of the 41 ingroup taxa there were 34 *Paecilomyces* isolates including two teleomorph isolates with accepted *Paecilomyces* anamorphs. This tree enjoyed good support (CI = 0.484; RI = 0.729; RC = 0.353; HI = 0.516).

Of the 16 *Paecilomyces* species included in the β-tubulin analysis, all had an 84 % split from the nectriaceous outgroup in a broadly clavicipitaceous clade (Fig. 1). Within this major clade was a barely supported clade (54 %) consisting of two species, *P. penicillatus* and *P. niphetodes*. We considered these two species to have an uncertain affinity with the *Clavicipitaceae* within the *Hypocreales*. Four other species of *Paecilomyces* sect. *Isarioidea* grouped in largely unsupported clades that included other genera with *Paecilomyces*-like features (e.g. catenate conidia) such as *Nomuraea* and *Metarhizium*. Within this clade, however, there was a 100 % supported split for *P. cinnamomeus* with *Torrubiella luteorostrata*.

The remaining ten species were in two well-supported clades. One species, *P. lilacinus* was linked with a strain that was provisionally identified as *Isaria takamizusanensis* in a clade that had 99 % support (Fig. 1). This clade (Clade L) is characterised by purplish conidia. The remaining nine species formed a distinct and terminal clade (Clade I) with 94 % support. Clade I was characterised by a generally entomogenous (in the broad sense of including spider pathogens) and isarioid habit. It centred on the accepted epitype isolate of *P. farinosus*, the type species of sect. *Isarioidea* (Hodge *et al.* 2005)

ITS phylogeny

For the ITS dataset we used 44 sequences of *ca* 550 bp covering the ITS1-2 regions and including partial but unusable sequences of the flanking 18S and 28S regions. The ITS analysis had two *Mariannaea* sequences as the outgroup and the other 42 representing the ingroup. For the ingroup there were 34 isolates identified as *Paecilomyces*/*Isaria* plus three associated teleomorphs. The tree length was 924 with a CI of 0.581; RI = 0.837; RC = 0.486; HI = 0.419. With this, 121 trees were retained. The ingroup was not constrained but still resulted in a 100 % split for the *Hypocreaceae* and *Clavicipitaceae* from the *Mariannaea* outgroup (Fig. 2).

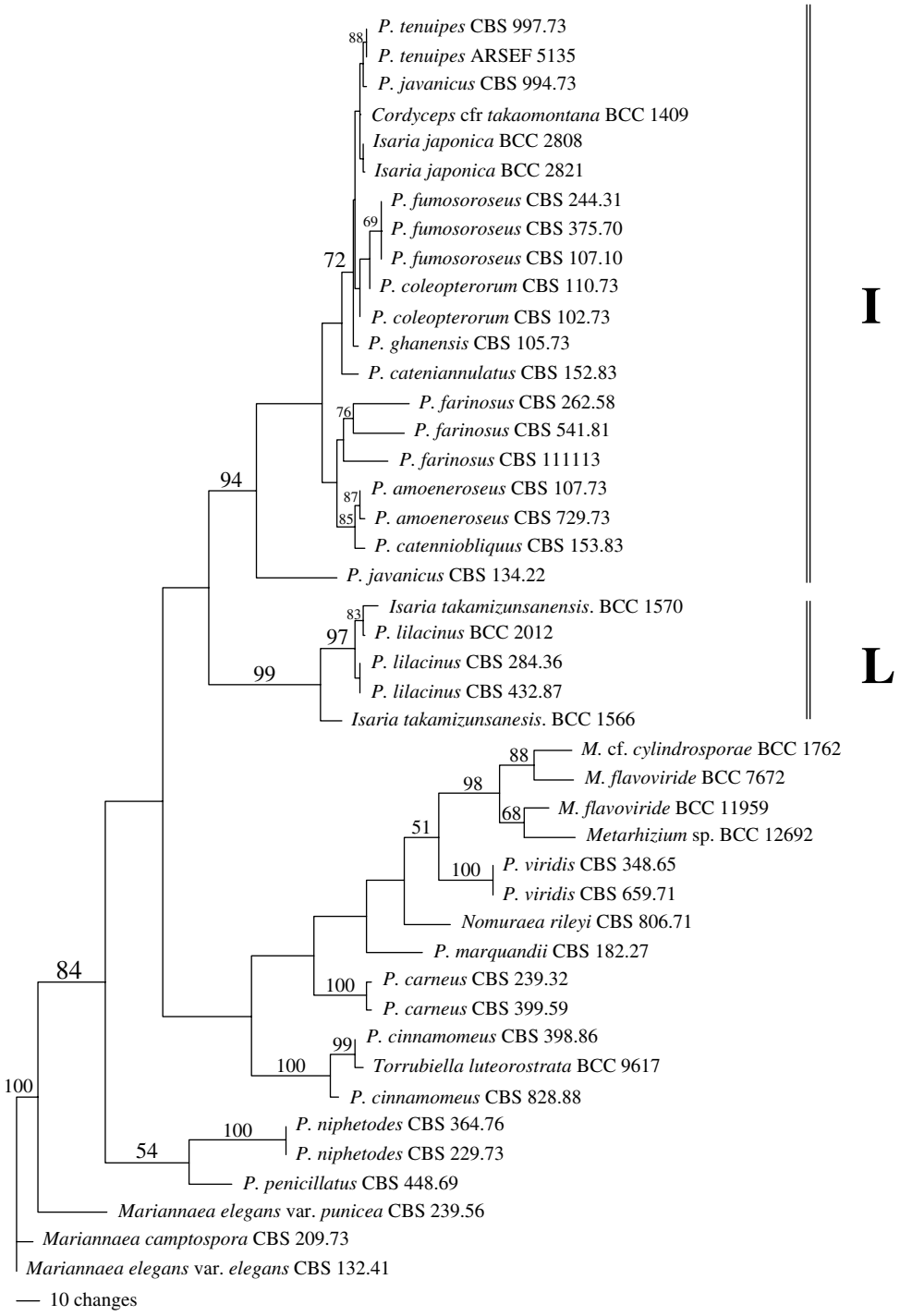

Fig. 1. One of the most parsimonious trees representing relationships of species found in *Paecilomyces* sect. *Isarioidea* inferred from maximum parsimony analysis of the β-tubulin gene (partial sequence) (CI = 0.484; RI = 0.729; RC = 0.353; HI = 0.516). Phylogram of the best tree according to the Kishino-Hasegawa test. The numbers above the branches represent the bootstrap values based on 500 replicates with values over 50% shown. I, *Isaria* clade; and L, *Paecilomyces* species with purple conidia.

Three *Paecilomyces* isolates that an 18S phylogeny had shown to have affinities with the *Hypocreaceae* were basal to the *Clavicipitaceae* clades although they still had an 84% split from the nectriaceous outgroup.

As with the β-tubulin phylogeny, there were 17 (16 for the β-tubulin phylogeny) species of *Paecilomyces* that were separated from the nectriaceous outgroup using an ITS phylogeny (Fig. 2). With the ITS phylogeny this split had 100% bootstrap support. Similarly, *P. penicillatus* and *P. niphetodes* were basal with uncertain association to the *Clavicipitaceae*. Also, four species that grouped (but unsupported) with *Nomuraea* and *Metarhizium* in the β-tubulin phylogeny had a 91% support for a grouping with *Nomuraea* and *Metarhizium* in the ITS phylogeny (Fig. 2). No ITS sequence was available for *Isaria takamizusanensis*.

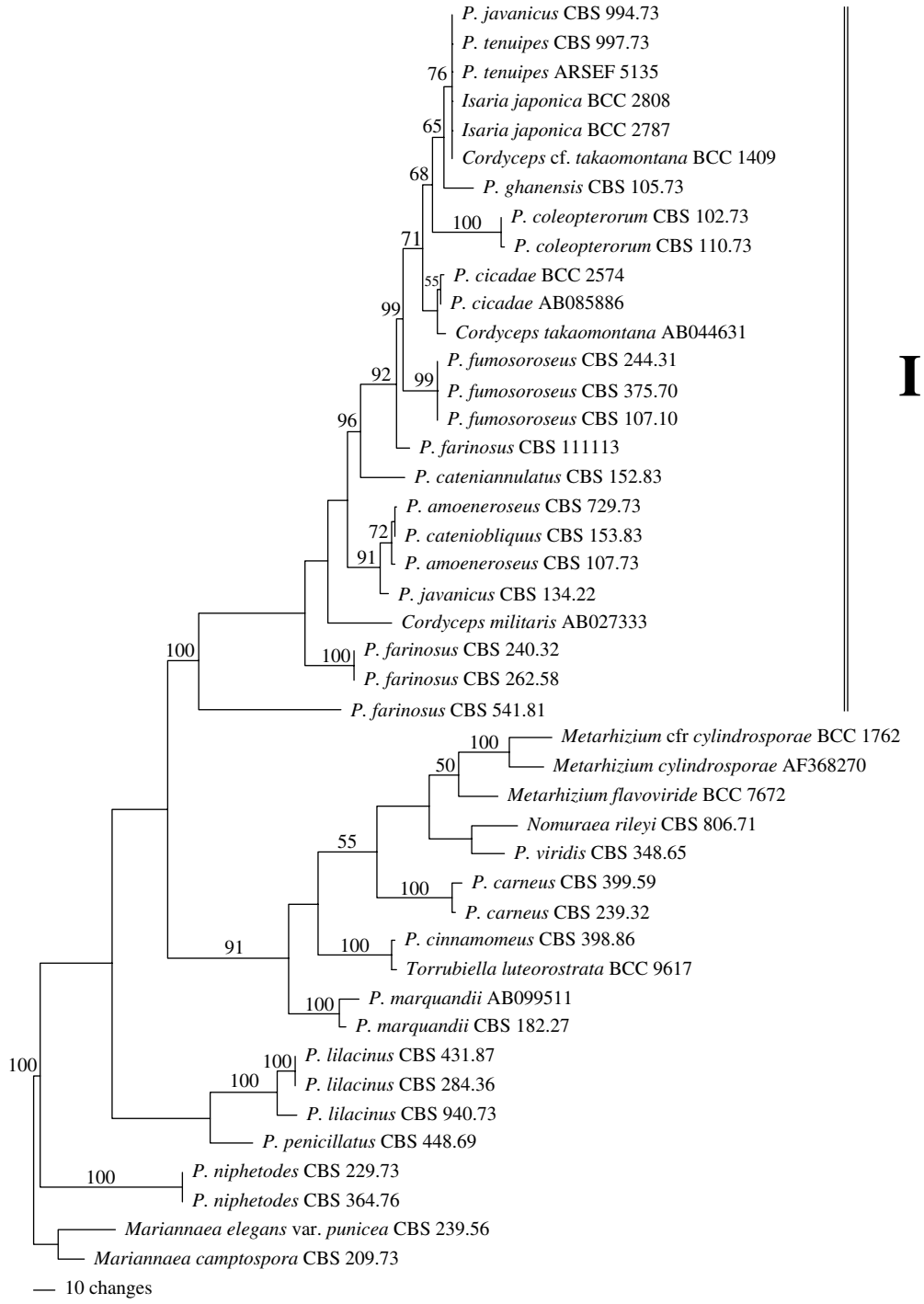

Fig. 2. One of the most parsimonious trees representing relationships of species found in *Paecilomyces* sect. *Isarioidea* inferred from maximum parsimony analysis of the ITS1-5.8S-ITS2 rDNA (CI = 0.581; RI = 0.837; RC = 0.486; HI = 0.419). The numbers above the branches represent bootstrap values based on 500 replicates with values over 50 % shown. I, *Isaria* clade.

However, with the ITS phylogeny, the *P. lilacinus* isolates were basal in an unsupported clade with *P. penicillatus* (Fig. 2). The ITS phylogeny also had a major clade that had 100 % support and included the nine species of Clade I in the β-tubulin phylogeny plus *P. cicadae* which was not available in the β-tubulin phylogeny. This clade also included three *Cordyceps* sequences including *C. militaris*, the type species of the genus *Cordyceps*. Within the ITS-based Clade I there was better support for the various clades (Fig. 2).

DISCUSSION

The significant points on which the ITS and β-tubulin phylogenies are based may be summarised as follows. First, the 18S phylogeny of Luangsa-ard *et al.* (2004)

demonstrated 100% bootstrap support for an *Isarioidea*/*Paecilomyces* split at the section level, as conceived by Samson (1974), supporting the distinctions based on mesophilic/thermophilic characters. However, sects *Paecilomyces* (thermophilic) and *Isarioidea* (mesophilic) appeared to be polyphyletic, although a monophyletic grouping of *Paecilomyces* based on the teleomorph *Byssochlamys* was recognised (Luangsa-ard *et al*. 2004). Luangsa-ard *et al*. (2004) also determined that sect. *Isarioidea* was split across two orders, most species were placed in the order *Hypocreales* and *P. inflatus* had affinities with the order *Sordariales* (although its inclusion in this order was not certain).

Many species placed in sect. *Isarioidea* are pathogens of invertebrates. With our study we sought to determine if there was a monophyletic assemblage of entomogenous species within the *Hypocreales*. As mentioned above, the non-entomogenous *P. inflatus* was omitted from this work and requires further study to determine its placement in respect of the *Sordariales* and other related orders.

The 18S phylogeny (Luangsa-ard *et al*. 2004) suggested that the genus *Mariannaea* was basal to hypocrealean members of sect. *Isarioidea*, probably with nectriaceous links. Samson (1974) considered *Mariannaea* to be morphologically similar to *Paecilomyces*. Therefore, isolates of this genus were considered an appropriate outgroup. In our β-tubulin analysis we had 16 species of *Paecilomyces* representing the order *Hypocreales*, while for the ITS analysis we had 17 species available.

The hypothesis under investigation, that there is a monophyletic grouping of insect-associated *Paecilomyces* sect. *Isarioidea* species cannot be demonstrated on the basis of β-tubulin and ITS phylogenies presented here. Our molecular analysis demonstrates that insect-associated *Paecilomyces* are polyphyletic within the order *Hypocreales*. However, in the analyses presented there is a defined clade (Clade I, Figs 1–2), within the *Hypocreales*, which can be based on *P. farinosus* isolates, especially a Danish isolate (CBS 111113) that equates to the type locality for what Holm (1781) described as '*Ramaria farinosa*'.

Clade I includes ten species with insect associations. However, two invertebrate-associated species, *P. lilacinus* and *P. cinnamomeus*, were not placed in Clade I. *P. lilacinus* isolates grouped together with another pink-spored species provisionally identified as *Isaria takamizusanensis* in the β-tubulin phylogeny. *P. cinnamomeus* grouped with *Torrubiella luteorostrata*, which Hywel-Jones (1993) had shown to be an anamorph-teleomorph assemblage. Three non-entomogenous species of *Paecilomyces* sect. *Isarioidea* were also in the same clade as *P. cinnamomeus*, as well as being part of a clade that included the green-spored anamorph genera *Nomuraea* and *Metarhizium*. Also, *P. penicillatus* and *P. niphetodes* both appeared basal to the other *Paecilomyces* of sect. *Isarioidea* which supports the observation that with an 18S phylogeny these two species seemed to have affinities to the *Hypocreaceae* rather than the *Clavicipitaceae*. These species require further study to determine their true placement with respect to each other and are not considered further here.

Clade I has a teleomorph link to *Cordyceps s. lat*. In the ITS phylogeny *C. militaris* was placed in Clade I suggesting a close relationship between it and '*Isaria*', even though the early belief of *C. militaris* being the teleomorph of *P. farinosus* was rejected (Petch 1936). This species linkage was possibly due to the inclusion of *C. militaris* and *P. farinosus* in the same paper by Holm (1781). Petch (1936), however, rightly concluded that the anamorph of *Cordyceps militaris* was a '*Cephalosporium*'. Oborník, Jirkuz & Dolezel (2001) demonstrated a close link between *Paecilomyces* and *Verticillium* (the '*Cephalosporium*' of Petch). A recent revision of *Verticillium* sect. *Prostrata* places the anamorph of *Cordyceps militaris* in *Lecanicillium* (Gams & Zare 2001).

Although four isolates of *P. farinosus* were included in the ITS analysis, in our work these did not form a monophyletic group. Three *P. farinosus* isolates were basal to Clade I, and ironically were close to *C. militaris* while the isolate selected as representing the species (Luangsa-ard *et al*. 2004) was separated from other *P. farinosus* isolates by a clade that includes the ex-type isolates of *P. javanicus*, *P. amoeneroseus* and *P. cateniobliquus* (Figs 1–2).

Three *P. farinosus* sequences were available for the β-tubulin phylogeny. In contrast to the ITS phylogeny, CBS 262.58 and CBS 541.81 grouped, albeit unsupported, with CBS 111113. *Isaria farinosa* was treated as the type for *Isaria* by Clements & Shear (1931). De Hoog (1972) noted that Clements & Shear (1931) were the first to propose *I. farinosa* as the type concluding 'if this were accepted, the younger genus *Paecilomyces* would become a synonym of *Isaria*'. Similarly, Samson (1974) concluded that if *I. farinosa* was an acceptable lectotype then 'because of priority, *Isaria* should be used for all *Paecilomyces* species'. Although not explicitly stated, de Hoog (1972) and Samson (1974) did not consider this acceptable and chose to reject *I. farinosa* as possible lectotype for the genus *Paecilomyces*.

Based on our previous work (Luangsa-ard *et al*. 2004) we can demonstrate no phylogenetic link between *Paecilomyces* as conceived by Bainier (1907) and based on *P. variotii*, and any of the mesophilic *Isaria* isolates from Clade I based on *I. farinosa* as conceived by Holm (1781). Therefore, our phylogenies lead us to conclude that while *I. farinosa* would not be an appropriate lectotype for the genus *Paecilomyces* as used by Brown & Smith (1957) it is an acceptable lectotype for an '*Isaria*' clade (Clade I) as identified from the β-tubulin gene and ITS phylogenies presented here.

Apart from the tenuous link to *C. militaris* and its *Lecanicillium* anamorph, Clade I has a clear association with a *Cordyceps* provisionally identified as

C. takaomontana. Nikoh & Fukatsu (2001) confirmed in an 18S phylogeny the proximity of *C. takaomontana* and *P. tenuipes*, which had become an accepted link by default (Kobayasi 1981, Zongqi 1994). However, *P. tenuipes* was the only *Paecilomyces* used in their analysis; their solate NHJ5946 was a sulphur-yellow *Cordyceps* provisionally identified as *C. takaomontana* since it produced an anamorph that conformed to *P. tenuipes s. lat*. In both the β-tubulin and the ITS phylogenies the Thai isolate of *C. takaomontana* grouped in a clade containing Thai and Japanese isolates of *P. tenuipes* as well as the isolate of *P. tenuipes* from the type locality in New York.

Nikoh & Fukatsu (2001) also produced an 18S–28S sequence of an isolate of *C. takaomontana* (AB044637) from which we were able to secure a useable sequence for inclusion in the ITS phylogeny. Nikoh & Fukatsu (2001) placed their isolate of *C. takaomontana* in a group which they referred as a 'moth clade' that included *Cordyceps militaris*, *C. pruinosa* and *P. tenuipes*. However, in our ITS analysis the Nikoh & Fukatsu sequence grouped (but without support) with two isolates that were identified with *P. cicadae*. One of these was a Thai isolate while the other was from Japan. This brings into question the recognition of clades based on host insects at the order level. We feel the current global dataset of available sequences is too limited to make such conclusions.

The Thai strain of *P. cicadae* was isolated from a cicada nymph and compares well with the ITS sequence (AB085886) which was obtained from Japanese material (Yokoyama, Yamagishi & Hara 2004). In the ITS phylogeny both isolates grouped together with 55% support and were on a clade with 99% support that included *P. coleopterorum*, *P. fumosoroseus*, *P. ghanensis*, *P. javanicus* and *P. tenuipes*. This clade also included the two isolates identified as *Cordyceps takaomontana* and two isolates identified as '*Isaria japonica*'.

The two isolates of *I. japonica* from *Lepidoptera* in Japan group with *P. tenuipes*, and more recent Japanese identifications recognise the synonymy of Samson (1974). Significantly, the ITS tree suggested that the six isolates in the 'tenuipes' clade are identical, yet one of these (CBS 994.73) was identified as *P. javanicus*. This identity is resolved as follows. The position of *P. javanicus* is fixed by the ex-type isolate of the species (CBS 134.22), which appears as part of a well-supported, more basal clade that includes the ex-type isolates of *P. amoeneroseus* and *P. cateniobliquus*. The isolate identified by Samson (1974) as *P. javanicus* (CBS 994.73) is, from the ITS phylogeny, identifiable as *P. tenuipes* based on the designated ex-type isolate of ARSEF5135 from the type locality (see above).

Significantly, in discussing *P. javanicus*, Samson (1974) stated that 'Although *P. javanicus* is usually mononematous on insects, synnematous conidiogenous structures were observed in CBS 994.73 in pure culture'. He concluded 'These characters show that *P. javanicus* is closely related to *P. tenuipes*'. From our molecular phylogenetic study, we must conclude that the name *P. javanicus* that Samson (1974) applied to CBS 994.73 would be better replaced by *P. tenuipes*, especially, as the host was a lepidopteran pupa – a typical host for *P. tenuipes*. Also, our phylogeny suggests that the true *P. javanicus* is not close to *P. tenuipes*, apart from its inclusion in the '*Isaria*' clade recognised in our study.

We consider it significant that most of the mesophilic *Paecilomyces* species which were placed in the genus *Isaria* fall within the currently recognised '*Isaria*' clade, and help to establish this as a monophyletic group. We therefore accept Clade I as representing a separate anamorph genus *Isaria*. Importantly, Clade I can be considered invertebrate-pathogenic. Members of the genus are pathogenic to insects of the orders *Coleoptera*, *Homoptera* (cicada nymphs primarily) and *Lepidoptera* (larvae). It is also pathogenic to spiders (*Araneae*). In Thailand, *Paecilomyces* species are frequently found on spiders and these have all been identified as *P. javanicus*. However, within Clade I there were three isolates identified as *P. javanicus*. Significantly, this clade has no associations with leaf-associated *Homoptera*. Most species are specific in their host associations, although *P. fumosoroseus* appears to have a wider host range which has made it a popular candidate in biocontrol studies (Oborník *et al*. 2001). In terms of morphologies, the *Isaria* clade is characterised by synnematous growth and conidial colours that are white or pink in the case of *P. fumosoroseus*.

We conclude that Clade I is largely entomogenous, with some isolates found on various substrates including indoor air and food. The most commonly reported species, *P. farinosus* and *P. fumosoroseus*, are the ones with the lowest host-fidelity. We do not feel it is important to attribute any significance on the non-entomogenous origin of, for instance CBS 244.31 (from butter).

In conclusion, sect. *Isarioidea* is not monophyletic and there is not a monophyletic grouping for all the invertebrate-associated species. However, a clear clade emerged (Clade I) which included entomogenous species previously considered to belong in *Isaria*. It seems appropriate then, that the genus *Isaria* should be taken up again but restricted to members of Clade I. Hodge *et al*. (2005) have discussed the nomenclatural status and history of the generic name *Isaria* and conclude that *Isaria* Fr. 1832: Fr. is a valid generic name that has previously been typified with *I. farinosa* (Holmsk.: Fr.) Fr. 1832 A lectotype illustration and an epitype specimen of *I. farinosa* was designated. Consequently, Gams *et al*. (2005) proposed to conserve the name *Isaria* and as typified by *I. farinosa*.

TAXONOMY

Isaria Pers., *Syn. Meth. Fung.*: 687 (1801): Fr., *Syst. Mycol.* **3**: 270 (1832); *nom. cons. prop.*

Conidiophores mono- or synnematous, usually consisting of several verticillate branches, each bearing a dense whorl of phialides. Synnemata often branched with apical sporulating structures. *Phialides* consisting of a cylindrical or swollen basal portion, terminating in a thin often long neck, producing divergent conidial chains. *Conidia* one- or rarely two-celled, smooth-walled, hyaline. *Colonies* bright coloured, white, yellow, pale green, pink, red or purple. *Hyphae* hyaline to slightly pigmented, rough- or smooth-walled. *Chlamydospores* present in some species.

Teleomorph: *Cordyceps*, often absent in culture.

Type species: *Ramaria farinosa* Holmsk. 1781.

Isaria farinosa (Holmsk.) Fr., *Syst. mycol.* **3**: 271 (1832): Fr.

Ramaria farinosa Holmsk., *K. Danske Vidensk. Selsks. Skr., Nye Samling* **1**: 279 (1781).

Paecilomyces farinosus (Holmsk. : Fr.) A. H. S. Br. & G. Sm, *Trans. Br. mycol. Soc.* **40**: 50 (1957).

Isaria amoenerosea P. Henn., *Hedwigia* **41** (Beiblatt): 66 (1902).

Paecilomyces amoeneroseus (P. Henn.) Samson, *Stud. Mycol.* **6**: 27 (1974).

Isaria cateniannulata (Z. Q. Liang) Samson & Hywel-Jones, **comb. nov**.

Basionym: *Paecilomyces cateniannulatus* Z. Q. Liang, *Acta Phytopathol. Sin.* **11**: 10 (1981).

Isaria cateniobliqua (Z. Q. Liang) Samson & Hywel-Jones, **comb. nov**.

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Isaria cicadae Miquel, *Bull. Sci. phys. nat. néerl., sér.* 2, **10**:378 (1838).

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Isaria coleopterora (Samson & H. C. Evans) Samson & Hywel-Jones, **comb. nov**.

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Isaria fumosorosea Wize, *Bull. int. Acad. pol. Sci. Lett., classe sci. math. nat.* **1904**: 721 (1905).

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Isaria ghanensis (Samson & H. C. Evans) Samson & Hywel-Jones, **comb. nov**.

Basionym: *Paecilomyces ghanensis* Samson & H. C. Evans, *in* Samson, *Stud. Mycol.* **6**: 46 (1974).

Isaria javanica (Frieder. & Bally) Samson & Hywel-Jones, **comb. nov**.

Basionym: *Spicaria javanica* Frieder. & Bally, *Meded. Koffiebessenboeboek Fonds* **6**: 146 (1923).

Paecilomyces javanicus (Frieder. & Bally) Samson, *Stud. Mycol.* **6**: 41 (1974).

Isaria tenuipes Peck, *Rep. N. Y. St. Bot.* **31**: 49 (1879).

Paecilomyces tenuipes (Peck) Samson, Stud. Mycol. **6**: 49 (1974).

Additional species

Paecilomyces ramosus Samson & H. C. Evans 1974 and *P. xylariiformis* (Lloyd) Samson 1974 probably also belong to *Isaria*, but both are only known from dried herbarium material and no molecular analyses have been carried out. The remaining species of *Paecilomyces* formerly placed in sect. *Isarioidea* (i.e. *P. suffultus*, *P. marquandii*, *P. lilacinus*, *P. cinnamomeus*, *P. viridis*, *P. niphetodes*, *P. sulphurellus*, *P. puntonii*, *P. inflatus*, *P. carneus*, and *P. penicillatus*) need to be further studied to determine their true relationships with each other and with other anamorphs within the *Clavicipitaceae*.

ACKNOWLEDGEMENTS

This work was supported by the Biodiversity Research and Training Program in Thailand (BRT Grant No. BRT 143008) with co-financing from the National Center for Genetic Engineering and Biotechnology (BIOTEC). Theresa Kwong is thanked for allowing us to use the β-tubulin sequences for the *Metarhizium* clade. Julian Mitchell is thanked for his long-continued support and counselling to J. J. L. and N. L. H.-J. Richard Humber is thanked for providing a *P. tenuipes* isolate from the New York type locality.

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