Phylogeny of mitosporic entomopathogenic fungi: Is the genus *Paecilomyces* polyphyletic?

Miroslav Oborník, Milan Jirku, and David Dolezel

Abstract: We analyzed sequences of the divergent domain at the 5′ end of the large subunit rRNA gene from the mitosporic entomopathogenic fungi *Paecilomyces* sp., *Paecilomyces fumosoroseus*, *Paecilomyces farinosus*, *Paecilomyces lilacinus*, *Verticillium lecanii*, *Verticillium psalliotae*, *Beauveria bassiana*, *Aschersonia* sp., *Aschersonia placenta*, ascomycetous *Cordyceps* sp., and *Cordyceps militaris*. Phylogenetic analysis showed *P. fumosorseus* as the best characterized out of the analyzed species with the *B. bassiana* clade as its sister group. Two of the *P. farinosus* isolates were invariably placed within the *Verticillium* cluster, which also contained *C. militaris*. The only analyzed *P. lilacinus* isolate appeared on the root of the hyphomycetous fungi and was characterized as the most distinct from all the hyphomycetous fungi tested. Polyphyly of the genus *Paecilomyces* was well supported by the Kishino-Hasegawa test. In all trees based on the small subunit rRNA gene sequences obtained from the GenBank™, *V. lecanii*, *V. psalliotae*, *P. fumosoroseus*, *P. tenuipes* and *B. bassiana* form, together with that of *C. militaris*, the best supported cluster in the tree. The rest of *Cordyceps* spp. constitute a distinct clade. Phylogenetic relationships derived from both tested DNA regions show polyphyly of the genus *Paecilomyces* and close relationships among entomopathogenic species of the genera *Verticillium*, *Paecilomyces*, and *Beauveria*.

Key words: *Paecilomyces*, *Verticillium*, *Beauveria*, *Aschersonia*, entomopathogenic fungi, molecular phylogeny, ribosomal RNA genes.

Résumé : Nous avons déterminé les séquences du domaine divergent de l'extrémité 5′ de la grosse sous-unité du gène de l'ARNr chez des champignons entomopathogènes à reproduction asexuée tels le *Paecilomyces* sp., le *Paecilomyces fumosoroseus*, le *Paecilomyces farinosus*, le *Paecilomyces lilacinus*, le *Verticillium lecanii*, le *Verticillium psalliotae*, le *Beauvaria bassiana*, l'*Aschersonia* sp., l'*Aschersonia placenta*, les ascomycètes *Cordyceps* sp. et le *Cordyceps militaris*. Les analyses phylogénétiques ont confirmé que le *P. fumosoroseus* était le mieux caractérisé parmi les espèces étudiées avec le clade *B. bassiana* considéré comme son groupe-sœur. Deux des isolats du *P. farinosus* ont été invariablement placés dans le groupe *Verticillium* qui comprenait aussi le *C. militaris*. Le seul isolat de *P. lilacinus* analysé est apparu sur les racines de champignons hyphomycètes et il s'est révélé comme le moins apparenté et le plus distinct de tous les hyphomycètes étudiés. La polyphylie du genre *Paecilomyces* concordait très bien avec le test de Kishino-Hasegawa. Dans chacun des arbres phylogénétiques basés sur la petite unité du gène de l'ARNr, le *V. lecanii*, le *V. psalliotae*, le *P. fumosoroseus*, le *P. tenuipes* et le *B. bassiana*, don't les séquences ont été obtenues de GenBank ™, formaient, avec le *C. militaris*, le groupe le plus étoffé de l'arbre phylogénétique. Les autres *Cordyceps* spp. constituaient un clade différent. Les parentés phylogénétiques observées dans les deux régions d'ADN vérifiées ont confirmé une polyphylie du genre *Paecilomyces*, ainsi qu'une très forte parenté avec les espèces entomopathogènes des genres *Verticillium*, *Paecilomyces* et *Beauvaria*.

Mots clés : *Paecilomyces*, *Verticillium*, *Beauvaria*, *Aschersonia*, champignons entomopathogènes, phylogénie moléculaire, gènes codant l'ARN ribosomique.

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Introduction

Many different factors limit the use of the entomopathogenic mitosporic fungi in practical biocontrol systems. The unresolved taxonomic issues of mitosporic fungal pathogens of insects represent one of the major obstacles. The lack of a meiosporic life cycle is reflected in the limited range of morphological characters, which in combination with the

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high level of their inducibility, results in the construction of unreliable taxonomic systems. Such systems do not necessarily reflect the evolutionary origin of the analyzed taxa. Although taxonomy and phylogeny are sometimes considered to be rather theoretical fields, they are essential for the practical use of fungi in safe biocontrol. The knowledge of phylogenetic relationships among taxa can be, in the case of the entomopathogenic filamentous mitosporic fungi, extremely important for the following reasons: (*i*) information about the meiosporic states of these fungi is very limited; (*ii*) the narrow range of morphological characters usable in taxonomic classification results in their ambiguous taxonomy; and (*iii*) some members of the genus *Paecilomyces*, namely *Paecilomyces lilacinus* (Thom) Samson, can cause various mycoses in humans (Fletcher et al. 1998; Itin et al. 1998; Ono et al. 1999; ChanTack et al. 1999). A better understanding of phylogeny of the *Paecilomyces* will help to minimize possible risks to human health.

The entomopathogenic fungus *Paecilomyces fumosoroseus* (Wize) Brown & Smith represents a geographically widespread species reported as a pathogen of many different insects (Lepidoptera, Coleoptera, Diptera, Homoptera), including whiteflies (Aleyrodidae). This fungus is able to colonize mites (*Tetranychus urticae*) as well as some fungi (Samson 1974; Osborne and Landa 1992), and as a saprophyte, it can successfully survive in the soil (Samson 1974; Tigano-Milani et al. 1995*b*). Considering its high virulence to many agriculturally important pests, commercial interest has been focused on the development of a biopesticide based on highly virulent isolates of *P. fumosoroseus* (Osborne and Landa 1992).

In the latest revision of the genus, *P. fumosoroseus* and *P. lilacinus* were placed in the same section *Isarioidea*, with *Paecilomyces farinosus* (Holm.: S.F. Gray) Brown & Smith as the type species (Samson 1974). As mentioned above, *P. lilacinus* has been described as a rare fungal pathogen of humans. Moreover, it has caused mycotic infections not only in immunocompromised patients (Itin et al. 1998; ChanTack et al. 1999) but also in an otherwise healthy person (Ono et al. 1999). In consideration of these facts, a precise identification and classification of the *Paecilomyces* isolates used or potentially usable in biocontrol systems is of great importance. Phylogenetic analysis of the target species can help to avoid misidentification, in particular the replacement of *P. lilacinus* with other species of the genus *Paecilomyces*.

Although molecular phylogeny has frequently been studied in fungi (Guadet et al. 1989; Bruns et al. 1991; Boekhout et al. 1994; Moncalvo et al. 1995; Castlebury and Domier 1998), including parasites of plants (Morales et al. 1993; Kusaba and Tsuge 1994; Ward and Adams 1998) and nematode-trapping fungi (Tigano-Milani et al. 1995*a*; Liou and Tzean 1997; Ahren et al. 1998), entomopathogenic fungi stand a bit out of this stream. Major interest has mostly been focused on studies of their intraspecific variability and less frequently their interspecific variability, by use of various DNA markers (Strongman and MacKay 1993; Bidochka et al. 1994; Berretta et al. 1998; Cantone and Vandenberg 1998). Very few of the fingerprinting data obtained were used for the analysis of evolutionary relationships (Tigano-Milani et al. 1995*b*; Hajek et al. 1996; Oborník et al. 1999, 2000). Similarly, in contrast to their practical importance, the entomopathogenic fungi have been subjected to only a handful of phylogenetic studies (Curran et al. 1994; Fukatsu et al. 1997; Bidochka et al. 1999; Oborník et al. 1999, 2000; Nikoh and Fukatsu 2000). For one reason or another, the applied studies of the entomopathogenic fungi seem to be ahead of the knowledge of their taxonomy based on the sequence data. The aim of our work is to help to bridge this gap.

Materials and methods

Entomopathogenic fungi used for the phylogenetic analysis (Table 1) were obtained from the culture collection of entomopathogenic fungi of the Department of Plant Production, University of South Bohemia, where they are stored in the form of alginate prills. The activated prills were used as sporulating cultures to inoculate agar plates (potato dextrose agar). Subsequently, the fresh sporulating cultures were used for DNA extraction.

DNA was extracted according to Tigano-Milani et al. (1995*a*). Polymerase chain reaction (PCR) amplification was performed according to the manufacturer's instructions, using 1 U of DynaZyme II Recombinant Polymerase (Finnzymes, Finland), 10× reaction buffer, 100 μM of each deoxynucleoside triphosphate (Promega, U.S.A.), 25 pmol of each primer, and 10 ng of template DNA. The amplification program consisted of 1 cycle of 94°C for 5 min; 25 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1:15 min; and a final step of 72°C for 5 min. To amplify a divergent domain at the 5′ end of the large subunit (LSU) rRNA gene (Guadet et al. 1989), the following primers were used: NL 1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL 4 (5′-GGTCCGTGTTTCAAGACGG-3′) (Boekhout et al. 1994). PCR products were purified using QIAquick PCR Purification kit (QIAGEN, Germany) and sequenced with the above primers, using the Thermo Sequenase kit version 2.0 (Amersham Life Science, U.K.) and automatic sequencer Perkin Elmer 310 ABI Prism.

Partial LSU rRNA gene sequences from isolates of *Paecilomyces* sp., *P. fumosoroseus*, *P. lilacinus*, *Verticillium lecanii* (A. Zimmerm.) Viégas, *Verticillium psalliotae* Treschow, *Beauveria bassiana* (Balsamo) Vuillemin, *Cordyceps militaris* (L.) Link, *Cordyceps* sp., *Aschersonia* sp., and *Aschersonia placenta* B. and Br. were aligned using ClustalX and were manually corrected. Maximum likelihood (ML) and distance (DS) (model F84) trees were constructed using the PAUP* heuristic options with the Tree bisection–reconnection algorithm as the branch-swapping method (Swofford 1998). Bootstrap supports (500 replications) of ML and DS trees were also determined (Swofford 1998). Trees were rooted using *Neurospora crassa* sequence U40124 as an outgroup. The Kishino-Hasegawa test was performed on unconstrained ML trees and constrained topologies and used to test monophyly of the genus *Paecilomyces* (PAUP*; Swofford 1998).

Partial small subunit (SSU) rRNA gene sequences from *P. fumosoroseus*, *Paecilomyces tenuipes* (Peck) Samson, *V. lecanii*, *V. psalliotae*, *B. bassiana*, *Metarrhizium anisopliae* Metschnikov (Sorokin), *C. militaris*, *Cordyceps capitata* (Holmsk.:Fr.) Link, *Cordyceps ophioglossoides* (Ehrenberg:Fr) Link., *Cordyceps "jezoensis"* (GenBank accession No. AB027319) (Nikoh and Fukatsu 2000), and *Tritirachium* sp. (Table 2) were aligned using ClustalX and were manually corrected. Trees were constructed using the PAUP* heuristic search with ML and DS (F84) as a optimality criterions. Quartet-puzzling supports were determined (1000 replications).

Results and discussion

We sequenced part of the divergent domain at the 5′ end of the LSU rRNA gene (Guadet et al. 1989) from 17 isolates of entomopathogenic fungi (Table 1). The obtained sequences as well as their homologues from other entomopathogenic fungi,

Table 1. Isolates of entomopathogenic fungi used for partial large subunit rRNA-based phylogenetic analysis.

Species and isolate	Accession No.
Aschersonia placenta	
Ap1	AF169319*
Aschersonia sp.	
A28	AF169315*
A31	AF169316*
Ai1a	AF169317*
Ai2b	AF169318*
Beauveria bassiana	
NRRL28020	AF049164*
GB4605	AF245300*
Cordyceps sp.	
F1041	U68129*
Cordyceps militaris	
NRRL28021	AF049166*
GJS 93–51	AF043135*
Paecilomyces sp.	
PA 93	AF173002
PA 94	AF172343
Paecilomyces farinosus	
PFA 2169	AF172341
PFA 2179	AF172342
PFA2122	AF172340
Paecilomyces fumosoroseus	
PA 144	AF170074
PA 162	AF170075
PA 164	AF170076
PA 176	AF170077
PA 190	AF170078
PA 206	AF170079
PA 207	AF170080
PA 208	AF170081
Paecilomyces lilacinus	
PLL 2003	AF172339
Verticillium lecanii	
PA 98	AF172338
PA 196	AF172335
VLE 2002	AF172336
VLE 2007	AF172337
NRRL 26541	AF049175*
NRRL 28023	AF049176*
ATCC 46578	U17421*
ATCC 58909	U17414*
Verticillium psalliotae	
NRRL 26542	AF049177*
NRRL 26999	AF049178*

Table 2. Entomopathogenic fungi used for small subunit rRNA-based phylogenetic analysis.

Species and isolate	Accession No.
Beauveria bassiana	
IFO 4848	AB027336
Cordyceps capitata	AB027318
Cordyceps "*jezoensis*"	AB027319
Cordyceps militaris	AB027333
Cordyceps ophioglossoides	AB027321
Cordyceps tricentri	AB027330
Cordyceps sp.	
97003	AB027329
Metarrhizium anisopliae	
IFO5940	AB027337
Paecilomyces fumosoroseus	
DCPO3	AB032475
Paecilomyces tenuipes	D85136
Verticillium lecanii	
NRRL 28023	AF049156
NRRL 26541	AF049155
Verticillium psalliotae	
NRRL 26999	AF049158
NRRL 26542	AF049157
Tritirachium sp.	
IAM14522	AB003951

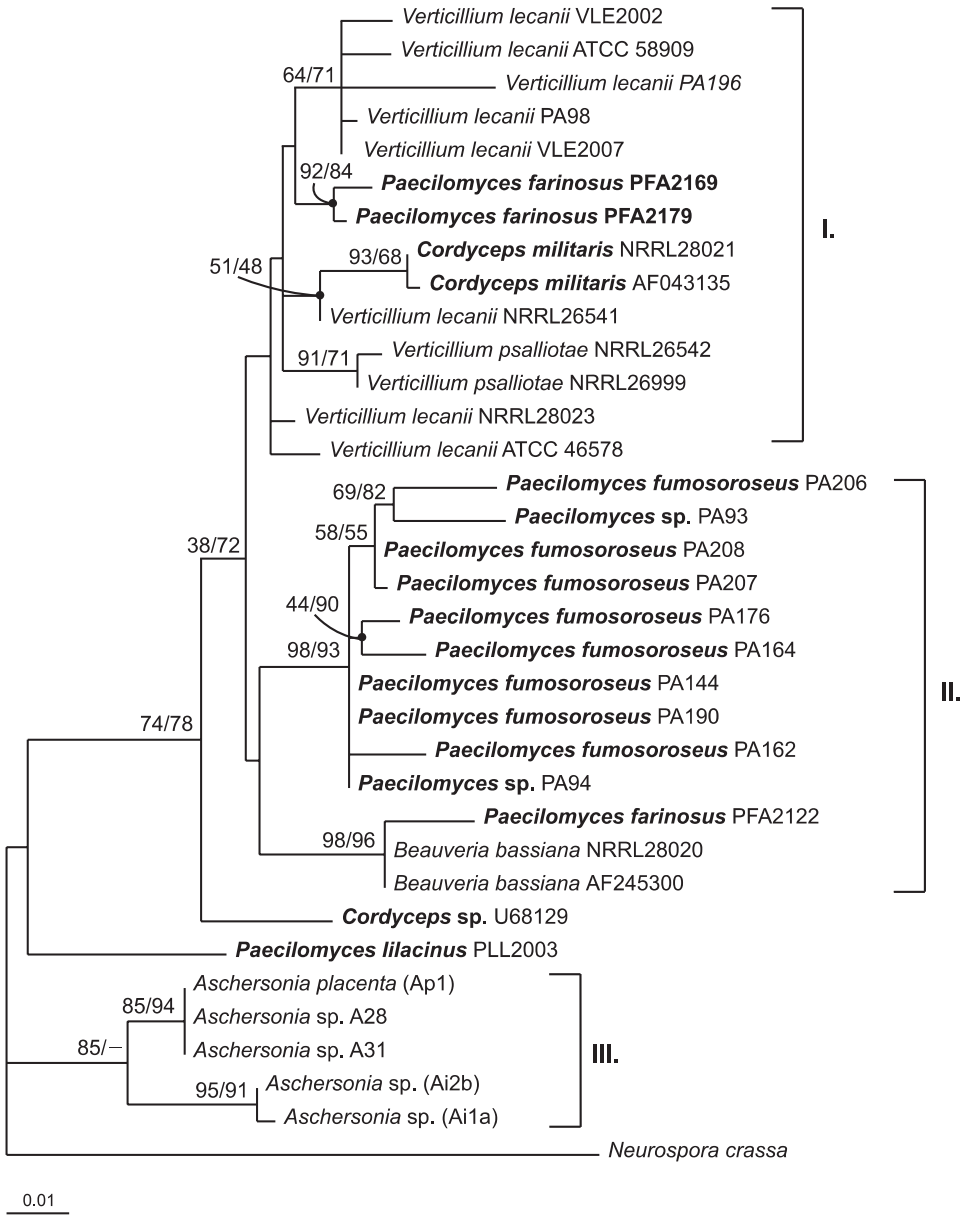
available in the GenBank™ (Table 1), were subjected to a phylogenetic analysis. We also analyzed partial SSU rRNA gene sequences from 15 isolates of entomopathogenic fungi, obtained from the GenBank™ (Table 2).

The analyzed isolates form three groups in all the constructed LSU rRNA-based trees (Fig. 1). The first clade (Clade I.) (Fig 1) was composed of the *Verticillium* isolates. Surprisingly, two of the *P. farinosus* strains (PFA2169, PFA2179) appeared invariably within this clade, while the third isolate of *P. farinosus* was strongly affiliated to *B. bassiana.* The second cluster (Clade II.) contained all isolates of *P. fumosoroseus* with the *B. bassiana* branch as a sister group, and the third cluster was composed of the *Aschersonia* isolates (Clade III.). Two isolates of *C. militaris* appeared within the *Verticillium* cluster; however, *Cordyceps* sp. (U681129) forms an outgroup to both the *Verticillium* and *P. fumosoroseus* clusters. The last member of the genus *Paecilomyces, P. lilacinus,* was characterized as the most distinct species from all hyphomycetous fungi tested. ML analysis placed *P. lilacinus* on the root of hyphomycetous fungi, while the DS analysis affiliated it with the *Aschersonia* clade (data not shown). Such affinity of *P. lilacinus* to the *Aschersonia* cluster is not supported by Kishino-Hasegawa test (Fig. 2C and Table 3) and is probably artefactual.

The SSU rRNA-based phylogenetic analysis placed the tested fungi into two major groups (Fig. 3). All *Verticillium*, *Paecilomyces*, and *Beauveria* isolates formed a highly supported clade together with the ascomycetous entomopathogen *C. militaris*. Branches whithin this cluster are relatively short, especially in the case of *V. lecanii*, *V. psalliotae*, and *P. fumosoroseus.* The genus *Paecilomyces*, represented by *P. fumosoroseus* and *P. tenuipes*, is polyphyletic, however; the internal structure of this cluster is not well supported. On the other hand, polyphyly of this genus is stable regardless of the optimality criterion used (data not shown).

According to the phylogeny based on partial LSU rRNA, *P. fumosoroseus* constitutes a monophyletic group clearly distinct from all other analyzed species (Fig. 1). All tested isolates of *P. fumosoroseus* were originally classified by the

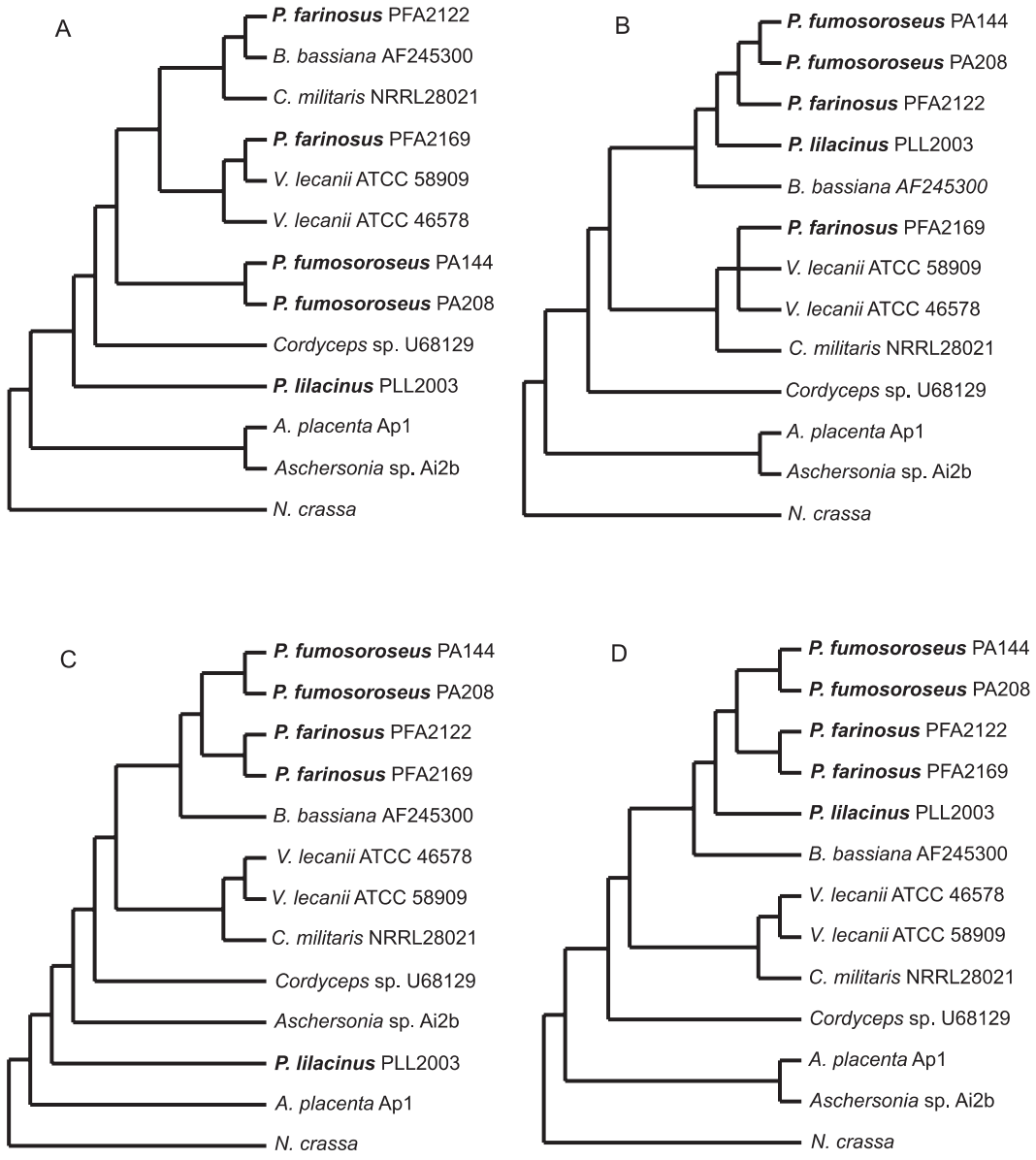
Fig. 1. Maximum-likelihood tree inferred from the divergent domain at the 5′ end of the LSU rRNA gene. Alignment used consisted of 513 characters: 378 characters were constant, 67 characters were parsimony uninformative, and the number of parsimony-informative characters was 69. Bootstrap supports (maximum likelihood/distance) are included.



use of morphological characters only. Such classification was correct, and morphology itself is evidently sufficient for the identification and classification of *P. fumosoroseus*. The LSU rRNA-based analysis of the genus *Paecilomyces* showed that *P. fumosoroseus* is a very well-defined species, and therefore, the danger of its replacement with *P. lilacinus* is not high. Moreover, the *P. lilacinus* sequence is apparently the most divergent when compared with other analyzed mitotic hyphomycetous fungi (Fig. 1). On the other hand, our dataset contain just one isolate of *P. lilacinus*, and only an inclusion of other isolates may confirm its phylogenetic position. The polyphyletic character of the genus *Paecilomyces* reflected by the different positions of *P. fumosoroseus*, *P. farinosus*, and *P. lilacinus* radically questions the present taxonomy of these fungi. The fact that *P. farinosus* is placed within the *Verticillium* clade, makes its use as a type species of the section *Isarioidea* puzzling. For a long time, *P. farinosus* had been considered as the anamorph of *C. militaris*. Cultural studies showed that the anamorph of *C. militaris* is in fact *Verticillium*-like (Samson 1974). It is well demonstrated here (Fig. 1) that *P. farinosus* forms a compact clade together with *Verticillium* and *C. militaris*, and this group is firmly distinct from the second clade composed of *P. fumosoroseus* and *B. bassiana*. The position of *P. farinosus* within the *Verticillium* cluster also testifies to a close relationship between the genera *Paecilomyces* and *Verticillium*. The position of the third *P. farinosus* strain (PFA2122) questions identification and classification of this isolate.

The SSU rRNA-based trees supported the polyphyly of

Fig. 2. Maximum-likelihood tree topologies tested using the Kishino-Hasegawa test. (A) No constraints; (B) *Paecilomyces farinosus* PFA2169 within the *Verticillium* clade, other *Paecilomyces* are monophyletic; (C) *Paecilomyces lilacinus* within the *Aschersonia* clade, other *Paecilomyces* are monophyletic; (D) all *Paecilomyces* are monophyletic.



the genus *Paecilomyces*, the close relationships between the genera *Paecilomyces*, *Verticillium*, and *Beauveria*, and their affinity to *C. militaris* as well (Fig. 3), while the plant pathogenic fungus *Verticillium dahliae* was very distinct from the other *Verticillium* isolates. Polyphyly of the genus *Verticillium* has been described recently (Bidochka et al. 1999). Moreover, within the genus *Verticillium*, the ability to colonize and infect insects has apparently evolved more than once (Bidochka et al. 1999). In the case of *Paecilomyces* spp., the situation is probably analogous. On the other hand, a close relation between entomopathogenic mitotic fungi can also be caused by convergent evolution rather than by their common origin. However, the convergent scenario is, in our opinion, disqualified by the outgroup position in all SSU rRNA trees of another mitotic entomopathogenic fungus *M. anisopliae*. Moreover, the fact that the *Verticillium–Paecilomyces–Beauveria* cluster is firmly affiliated with *C. militaris* and is clearly distinct from the other analyzed *Cordyceps* species makes a common origin of these mitotic fungi more plausible. A recently published phylogeny of *Cordyceps* spp. placed all tested mitotic entomopathogenic fungi in one group together with *C. militaris* (Nikoh and Fukatsu 2000). We suggest that this topology, which is supported by both tested nuclear rRNA regions and also by the analysis based on mitochondrial genes (Nikoh and Fukatsu 2000), can be explained by the ability of a common meiotic ancestor to form morphologicaly different mitotic states.

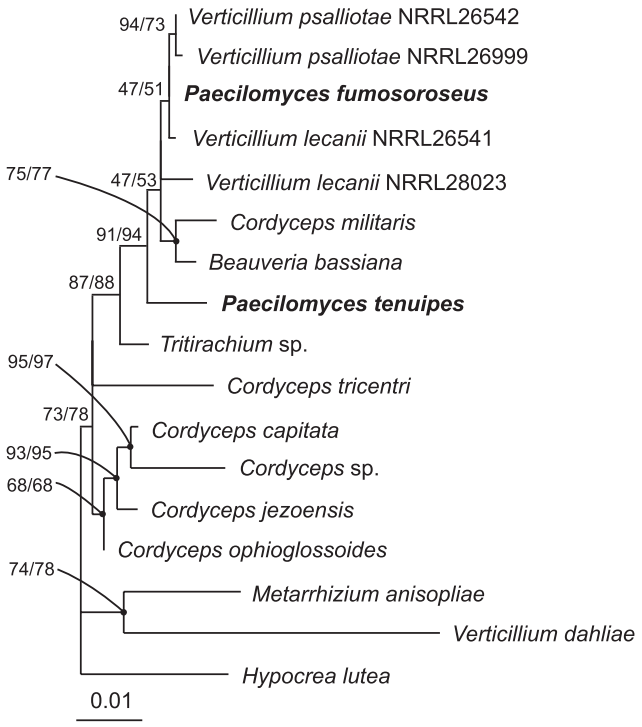
Our data underline the necessity of the taxonomic revision of the entomopathogenic mitosporic fungi by the use of molecular techniques (Samson 1995). Such a revision would

Table 3. Kishino-Hasegawa test for tree topologies A–D (Figure 2) using maximum likelihood and maximum parsimony as an optimality criterions.

	Kishino-Hasegawa test							
	Maximum likelihood				Maximum parsimony			
Topology	–ln L	Diff –ln L	*T*	*P*	Length	Length diff	*T*	*P*
A	1579.43816	(best)	—	—	157	(best)	—	—
B	1651.91749	72.47932	4.2768	<0.0001	180	23	4.1973	<0.0001
C	1665.92195	86.48379	4.8310	<0.0001	185	28	4.7643	<0.0001
D	1651.61539	72.17723	3.6603	0.0003	176	19	3.5683	0.0004

Note: *P* values lower than 0.05 indicate significant difference.

Fig. 3. Maximum-likelihood quartet-puzzling tree inferred from partial small subunit rRNA gene sequences. Tree was constructed out of alignment containing 985 characters, from which 868 were constant and 34 were parsimony informative. Quartet-puzzling supports (maximum likelihood/distance) are included.



not only shed more light on their evolution, but would be extremely important for the introduction of microbial biopreparations into practical biocontrol systems.

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References

Ahren, D., Ursing, B.M., and Tunlid, A. 1998. Phylogeny of nematode-trapping fungi based on 18S rDNA sequences. FEMS Microbiol. Lett. **158**: 179–184.

Berretta, M.F., Lecuona, R.E., Zandomeni, R.O., and Grau, O. 1998. Genotyping isolates of the entomopathogenic fungus *Beauveria bassiana* by RAPD with fluorescent labels. J. Invertebr. Pathol. **71**: 145–150.

Bidochka, M.J., McDonald, M.A., St Leger, R.J., and Roberts, D.W. 1994. Differentiation of species and strains of entomopathogenic fungi by random amplified polymorphic DNA (RAPD). Curr. Genet. **25**: 107–113.

Bidochka, M.J., St Leger, R.J., Stuart, A., and Gowanlock, K. 1999. Nuclear rDNA phylogeny in the fungal genus *Verticillium* and its relationship to insect and plant virulence, extracellular proteases and carbohydrases. Microbiology (Reading, U.K.), **145**: 955–963.

Boekhout, T., Kurtzman, C.P., O'Donell, K., and Smith, M.T. 1994. Phylogeny of the yeast genera *Hanseniaspora* (Anamorph *Kloeckera*), *Dekkera* (Anamorph *Brettanomyces*), and *Eeniella* as inferred from partial ribosomal DNA nucleotide sequences. Int. J. Syst. Bacteriol. **44**: 781–786.

Bruns, T.D., White, T.J., and Taylor, J.W. 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. **22**: 525–564.

Cantone, F.A., and Vandenberg, J.D. 1998. Intraspecific diversity in *Paecilomyces fumosoroseus*. Mycol. Res. **102**: 209–215.

Castlebury, L.A., and Domier, L.L. 1998. Small subunit ribosomal RNA gene phylogeny of *Plasmodiophora brassicae.* Mycologia, **90**: 102–107.

ChanTack, K.M., Thio, C.L., Miller, N.S., Karp, C.L., Ho, C., and Merz, W.G. 1999. *Paecilomyces lilacinus* fungemia in an adult bone marrow transplantant recipient. Med. Mycol. **37**: 57–60.

Curran, J., Driver, F., Ballard, J.W.O., and Milner, R.J. 1994. Phylogeny of *Metarhizium*: analysis of ribosomal DNA sequnce data. Mycol. Res. **98**: 547–552.

Fletcher, C.L., Hay, R.J., Midgley, G., and Moore, M. 1998. Onychomycosis caused by infection with *Paecilomyces lilacinus*. Brit. J. Dermatol. **139**: 1133–1135.

Fukatsu, T., Sato, H., and Kuriyama, H. 1997. Isolation, inoculation to insect host, and molecular phylogeny of an entomogenous fungus *Paecilomyces tenuipes*. J. Invertebr. Pathol. **70**: 203–208.

Guadet, J., Julien, J., Lafay, J.F., and Brygoo, Y. 1989. Phylogeny of some *Fusarium* species as determined by large-subunit rRNA sequence comparison. Mol. Biol. Evol. **6**: 227–242.

Hajek, A.E., Hodge, K.T., Liebherr, J.K., Day, W.H., and Vandenberg, J.D. 1996. Use of RAPD analysis to trace the origin of the weevil pathogen *Zoophtora phytonomi* in North America. Mycol. Res. **100**: 349–355.

Itin, P.H., Frei, R., Lautenschlager, S., Buechner, S.A., Surber, C., Gratwohl, A., and Widmer, A.F. 1998. Cutaneous manifestation of *Paecilomyces lilacinus* infection induced by contaminated skin lotion in patients who are severely immunosuppressed. J. Amer. Acad. Dermatol. **39**: 401–409.

Kusaba, M., and Tsuge, T. 1994. Nuclear ribosomal DNA variation and pathogenic specialization in *Alternaria* fungi known to produce host-specific toxins. Appl. Environ. Microbiol. **60**: 3055–3062.

Liou, G.Y., and Tzean, S.S. 1997. Phylogeny of the genus *Arthrobotrys* and allied nematode-trapping fungi based on rDNA sequences. Mycologia, **89**: 876–884.

Moncalvo, J.M., Wang, H.H., and Hseu, R.S. 1995. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia, **87**: 223–238.

Morales, V.M., Pelcher, L.E., and Taylor, J.L. 1993. Comparison of the 5.8S rDNA and internal transcribed spacer sequences of isolates of the *Leptosphaeria maculans* from different pathogenicity groups. Curr. Genet. **23**: 490–495.

Nikoh, N., and Fukatsu, T. 2000. Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. Mol. Biol. Evol. **17**: 629–638.

Oborník, M., Stouthamer, R., Meekes, E., and Schilthuittzen, M. 1999. Molecular characterization and phylogeny of the entomopathogenic fungus *Aschersonia* sp. Plant Prot. Sci. **35**: 1–9.

Oborník, M., Klíč, M., and Žižka, L. 2000. Genetic variability and phylogeny inferred from random amplified polymorphic DNA data reflect life strategy of entomopathogenic fungi. Can. J. Bot. **78**: 1150–1155.

Ono, N., Sato, K., Yokomise, H., and Tamura, K. 1999. Lung abscess caused by *Paecilomyces lilacinus*. Respiration, **66**: 85–87.

Osborne, L.S., and Landa, Z. 1992. Biological control of whiteflies with entomopathogenic fungi. Fla. Entomol. **75**: 456–471.

Samson, R.A. 1974. *Paecilomyces* and some allied Hyphomycetes. Stud. Mycol. **6**: 1–19.

Samson, R.A. 1995. Constraints associated with taxonomy of biocontrol fungi. Can. J. Bot. **73**: S83–S88.

Strongman, D.B., and MacKay, R.M. 1993. Discrimination between *Hirsutella longicolla* var. *longicolla* and *Hirsutella longicola* var. *cornuta* using random amplified polymorphic DNA fingerprinting. Mycologia, **85**: 65–70.

Swofford, D.L. 1998. Phylogenetic Analysis Using Parsimony (and Other Methods). Version 4. Sinauer Associates, Sunderland, Mass.

Tigano-Milani, M.S., Samson, R.A., Martins, I., and Sobral, B.W.S. 1995*a*. DNA markers for differentiating isolates of *Paecilomyces lilacinus*. Microbiology (Reading, U.K.), **141**: 239–245.

Tigano-Milani, M.S., Honeycutt, R.J., Lacey, L.A., Assis, R., McClelland, M., and Sobral, B.W.S. 1995*b*. Genetic variability of *Paecilomyces fumosoroseus* isolates revealed by molecular markers. J. Invertebr. Pathol. **64**: 173–178.

Ward, E., and Adams, M.J. 1998. Analysis of ribosomal DNA sequences of *Polymyxa* species and related fungi and the development of genus and species specific PCR primers. Mycol. Res. **102**: 965–974.