Molecular Characterization and Phylogeny of the Entomopathogenic Fungus *Aschersonia* spp.*

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Abstract

OBORNÍK M., STOUTHAMER R., MEEKES E., SCHILTHUITTZEN M. (1999): **Molecular characterization and phylogeny of the entomopathogenic fungus** *Aschersonia* spp. Pl. Protect. Sci., **35**: 1–9.

We characterized 23 isolates of the entomopathogenic fungus *Aschersonia* spp. from Mexico, Brazil, Guyana, Trinidad, Venezuela, Columbia, Florida, Malaysia, Thailand, Japan, Philippines, Java and South India using RAPD markers. The data were used to compute the genetic variability and to reconstruct the phylogeny of the genus *Aschersonia*. Relative genetic distances varied from 0.018 (between isolates Aa2 and Ap2) to 0.445 (between isolates A1 and At1). In the constructed phylogenetic tree, isolates were clustered according to their geographical origin. We determined partial 26S ribosomal DNA sequences of five *Aschersonia* isolates (A28, A31, Ai1a, Ai2b – *Aschersonia* spp.; and Ap1– *Aschersonia placenta*) and used them for phylogenetic analysis. Three of the tested isolates were not distinguishable. The tree constructed indicated that isolates Ai1a and Ai2b belong to species distinct from *A. placenta* and *A. aleyrodis*.

Key words: *Aschersonia*; biological control; genetic variability; RAPD; phylogeny; rDNA

Aschersonia spp. (*Coelomycetes, Deuteromycotina*) is referred to as a potential microbial agent to control various species of whiteflies (Aleyrodidae) (SAMSON & MCCOY 1983; FRANSEN *et al.* 1987; MCCOY *et al.* 1988; FRANSEN & VAN LENTEREN 1993). The ability of *Aschersonia* to live as a parasite on insects was first demonstrated at the end of the last century (WEBER 1897). Since then, many *Aschersonia* species have been described (PETCH 1921; MAINS 1959), mostly according to their different morphological characteristics and pigmentation. The specific host range of *Aschersonia* species within insects they parasitize, scale insects (*Coccidae*) and whiteflies (*Aleyrodidae*), is not exactly known. The teleomorph stage of the fungus belongs to the genus *Hypocrella* (*Clavicepitales, Ascomycotina*), forming perithecia (FRANSEN 1987).

Various molecular techniques have been used in the systematics of fungi to assess intra- and interspecific variation, to characterize individual isolates, and to determine phylogenetic relationships. PCR-based DNA markers have been recently used in DNA fingerprinting of a wide range of commercially important fungi (MUTHUMEENASKHI *et al.* 1994; THEODORE *et al.* 1995; FABRE *et al.* 1995; PIPE *et al.* 1995; HUANG *et al.* 1995), including entomopathogenic fungi potentially usable in biocontrol systems (BIDOCHKA *et al.* 1994; TIGANO-MILANI *et al.* 1995a, b). Random Amplified Polymorphic DNA (RAPD) (WELSH & MCCLELLAND 1990; WILLIAMS *et al.* 1990) has been used in DNA fingerprinting of many various microorganisms. It offers advantages for the study of poorly characterized organisms; most significantly, no DNA sequence information is needed, the method is relatively easy and it can provide reproducible results (WELSH & MCCLELLAND 1990; SOBRAL & HONEYCUTT 1994).

Ribosomal DNA sequences have been very frequently applied to study phylogenetic relationships in fungi (BRUNS *et al.* 1991; BOEKHOUT *et al.* 1994; GUADET *et*

*This work was supported by TEMPUS project JEP 4422 and partially supported by the Grant Agency of the Czech Republic, grant No. GA 522 P 141. This work was supported by TEMPUS project JEP 4422 and partially supported by the Grant Agency of the Czech Republic, grant No. GA 522 P 141.

al. 1989; MONCALVO *et al.* 1995). The nuclear rDNA units are present in multiple copies and arranged in very long tandem arrays. Clustered genes coding 18S, 5.8S and 26S rDNAs represent transcription units interrupted by spacers (ITS). Coding sequences are highly conserved and they are mostly used in estimating of phylogenetic relationships at a species and higher level. Highly variable ITS sequences are ideal to study intraspecific variations and phylogeny (BRUNS *et al.* 1991; TAN *et al.* 1994; SHERRIFF *et al.* 1994).

MATERIAL AND METHODS

The *Aschersonia* isolates used in this study, together with their geographical origin and insect host, are shown in Table 1. Fungi were cultivated on PDA (Potato Dextrose Agar) medium as point cultures. We used sporulating cultures for DNA extraction. Isolates Aa4 (a, b, c, d, e) represent one original strain obtained from different culture collections.

DNA for RAPD analysis was isolated using the following procedure: masses of pyknospores and fragments of mycelium were placed in microtubes (0.5ml) and squashed with a sealed Pasteur pipette for approximately 5 min. Then we added a total of 200 µl of 5% CHELEX (Chelex 100 Resin, BIO-RAD Laboratories, USA) in water suspension into each microtube. The tubes were placed in a thermal cycler (Hybaid, OmniGene) for 60 min at 56 °C, then for 5 min at 99 °C. We homogenized the samples (by vortexing for 5 s) and centrifuged them for 2 min at 14 000 rpm; the supernatant was used as a template (WINBERG 1991 – modified).

DNA for amplification of LSU (26S rDNA) region was extracted by the following method: microtubes (0.5ml) were filled to one third with zirconium beads (equal amounts of 0.1 and 0.5 mm beads) and autoclaved. Then we added approximately 50 mm^3 of masses of spores and mycelium, 200 µl of phenol (GIBCO BRL Research Chemicals), 200 µl of Tris-HCl (10mM Tris; 2.5 mM $MgCl_2$, 50 mM KCl, pH 8.5), and 10 µl of SDS (20%). The tubes were placed in a mini-beater (Biospect Products), shaken for 3 min, and centrifuged for 10 min at 12 000 rpm. The top phase was removed to a 1.5ml microtube and 200 µl of chloroform added. We gently shook the mixture until milky and centrifuged it for 5 min at 14 000 rpm. The top phase we transferred into a new

Table 1. Isolates of *Aschersonia* spp. used in this study, their original host and geographical origin

	Isolate	Host	Origin
A1	*Aschersonia* sp.	*Aleyrodidae*	Mexico
A8	*Aschersonia* sp.	*Aleyrodidae*	Malaysia
A13	*Aschersonia* sp.	unknown	Brazil
A15	*Aschersonia* sp.	unknown	Guyana
A17	*Aschersonia* sp.	unknown	Trinidad
A24	*Aschersonia* sp.	unknown	Venezuela
A26	*Aschersonia* sp.	*Aleyrodidae*	Malaysia
A28	*Aschersonia* sp.	*P. azalea*	Japan
A30	*Aschersonia* sp.	unknown	Thailand
A31	*Aschersonia* sp.	unknown	Thailand
A32	*Aschersonia* sp.	unknown	Thailand
Aa1	*Aschersonia aleyrodis*	*Aleyrodidae*	Florida
Aa2	*Aschersonia aleyrodis*	*D. citri*	Japan
Aa4a	*Aschersonia aleyrodis*	*T. vaporariorum*	Columbia
Aa4b	*Aschersonia aleyrodis*	*T. vaporariorum*	Columbia
Aa4c	*Aschersonia aleyrodis*	*T. vaporariorum*	Columbia
Aa4d	*Aschersonia aleyrodis*	*T. vaporariorum*	Columbia
Aa4e	*Aschersonia aleyrodis*	*T. vaporariorum*	Columbia
Ai1a	*Aschersonia* sp.	*D. citri*	Philippines
Ai2b	*Aschersonia* sp.	*D. citri*	Java
Ap1	*Aschersonia placenta*	unknown	South India
Ap2	*Aschersonia placenta*	unknown	unknown*
At1	*Aschersonia turbinata*	unknown	Columbia

*unknown origin, isolate obtained from culture collection in Vilnius, Lithuania

1.5ml microtube, and added 400 µl of sodium acetate (3M). Then we stored the tubes at –20 °C overnight. Next day we centrifuged the tubes in a cold centrifuge for 20 min at 14 000 rpm, washed the pellet twice with 1 ml cold ethanol (70%), dried it in vacuum drier and resuspended it in 50 µl of TE buffer (BREEUWER *et al.* 1992 – modified).

RAPD reactions we performed by using 10-mer primers (Kit B, Operon Technologies Inc. USA) in a total volume of 50 µl containing 5 µl of SUPER Tth reaction buffer, 0.5 U of SUPER Tth Polymerase (HT Biotechnology Ltd. Cambridge, England), 1 µl of dNTPs (10mM solution, Pharmacia LKB Biotechnology, USA) and 2.5 µl of primer (10 ng/µl). To eliminate evaporation we covered the reaction mixture with one drop of mineral oil. The RAPD program profile consisted of 1 cycle of 94 °C for 4 min. and 45 cycles of 94 °C for 1 min., 38 °C for 2 min. and 72 °C for 3 min. We tested 18 primers on three sets of *Aschersonia* isolates (one set of morphologically considerably different isolates, two sets of similar isolates). Manually scored RAPD products of five primers (Table 2) were used to calculate genetic distances and to construct phylogenetic trees using the PAUP program, version 3.1.1., heuristic options (SWOFFORD 1993).

Table 2. Primers used for RAPD amplifications

Primer	Sequence (5′–3′)
OPB 05	TGCGCCCTTC
OPB 06	TGCTCTGCCC
OPB 07	GGTGACGCAG
OPB 08	GTCCACACGG
OPB 14	TCCGCTCTGG

We amplified part of the 26S rDNA by use of SUPER Tth Polymerase (0.5 U/reaction; HT Biotechnology Ltd., Cambridge, England), 1 µl of dNTPs (10mM solution, Pharmacia LKB Biotechnology), 5 µl of 10× reaction buffer (SUPER Tth reaction buffer, HT Biotechnology), 0.4 µl of each primer (100 pmol/µl), and 5 µl of template. PCR reactions we performed in a total volume of 50 µl. 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 °Cv for 1:15 min; and 1 cycle of 72 °C for 5 min. To amplify a divergent domain at the 5' end of the 26S rDNA gene we used the primers (O'DONELL 1993; GUADET *et al.* 1989):

NL 1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL 4 (5′-GGTCCGTGTTTCAAGACGG-3′).

Amplified RAPD products (18 µl) we resolved by gel electrophoresis on a 1.5% agarose gel with 1× TAE buffer stained with ethidium bromide. To estimate the sizes of RAPD products we used lambda DNA digested with *Eco*RI × *Bam*HI × *Hind*III as a molecular marker.

PCR products we cloned (SAMBROOK *et al.* 1989) and sequenced on automatic sequencer 373 DNA Sequencer Stretch (Applied Biosystems). DNA sequences we aligned by use of DAPSA (DNA And Protein Sequence Analysis) program, version 3.3. (University of Cape Town, Harley, E.H., 1994). Phylogenetic trees (including Majority consensus tree 50%) were constructed by using PAUP 3.1.1. (SWOFFORD 1993).

RESULTS

RAPD using five 10-mer primers produced 56 readable and reproducible informative products which we manually scored and used in phylogenetic analyses. We constructed seven most parsimony phylogenetic trees. A majority role consensus tree (50%) we calculated by using the same program. We selected phylogenetic tree No. 2 (Fig. 1) out of seven equally parsimonious phylogenetic trees as the most probable tree according to the history of isolates, and majority role consensus tree (50%) (see Fig. 4). Tree No. 2 differs from the consensus tree in the position of four isolates (A1, A8, A13 and A26). The consensus tree does not show relations among these strains, while tree No. 2 placed isolates into two groups according to their geographical origin (A1 – Mexico + A13 – Brazil, A8 – Malaysia + A26 – Malaysia). We arranged the isolates into five groups according to phylogenetic tree No. 2. The first group contains isolates Aa1 (*A. aleyrodis*, isolated in Florida), A15 (*Aschersonia* sp., Guyana), A17 (*Aschersonia* sp., Trinidad) and A24 (*Aschersonia* sp., Venezuela). The second group is formed by a set of isolates Aa4 (a, b, c, d, e, originally from Columbia) and isolate At1 (*A. turbinata*, Columbia). The third group contains two strains from Thailand (A31, A32, *Aschersonia* spp.) and one strain from South India (Ap1, *Aschersonia placenta*). The fourth group is formed by isolates Aa2 (*A. aleyrodis*, Japan), Ap2 (*A. placenta*, unknown origin), A28 (*Aschersonia* sp., Japan) and A30 (*Aschersonia* sp., Thailand). The last group is more diverse, even the length of branches is much higher than in the others; isolates in this group are in three pairs: A1 (*Aschersonia* sp., Mexico) + A13 (*Aschersonia* sp., Brazil), A8 (*Aschersonia* sp., Malaysia) + A26 (*Aschersonia* sp., Malaysia), and Ai1a (*Aschersonia* sp. Philippines) + Ai2b (*Aschersonia* sp. Java). Genetic distances among isolates (Tables 3) varied from 0.018 (Aa2 × Ap2) to 0.445 (A1 × At1). Isolates of the Aa4 set we classified as identical. All the other strains were distinguishable.

Aligned sequences of the 5'ends of the 26S rDNA (*Saccharomyces cerevisiae* positions 83-451) of strains A28, A31, Ai1a, Ai2b (*Aschersonia* spp.) and Ap1 (*Aschersonia placenta*) are shown in Fig. 2. Of the 351 nucleotides sequenced (reference sequence not included) there were 18 variable sites, and 14 of these were phylogenetically informative (94 variable sites when compared to the reference sequence of *Saccharomyces cerevisiae*).

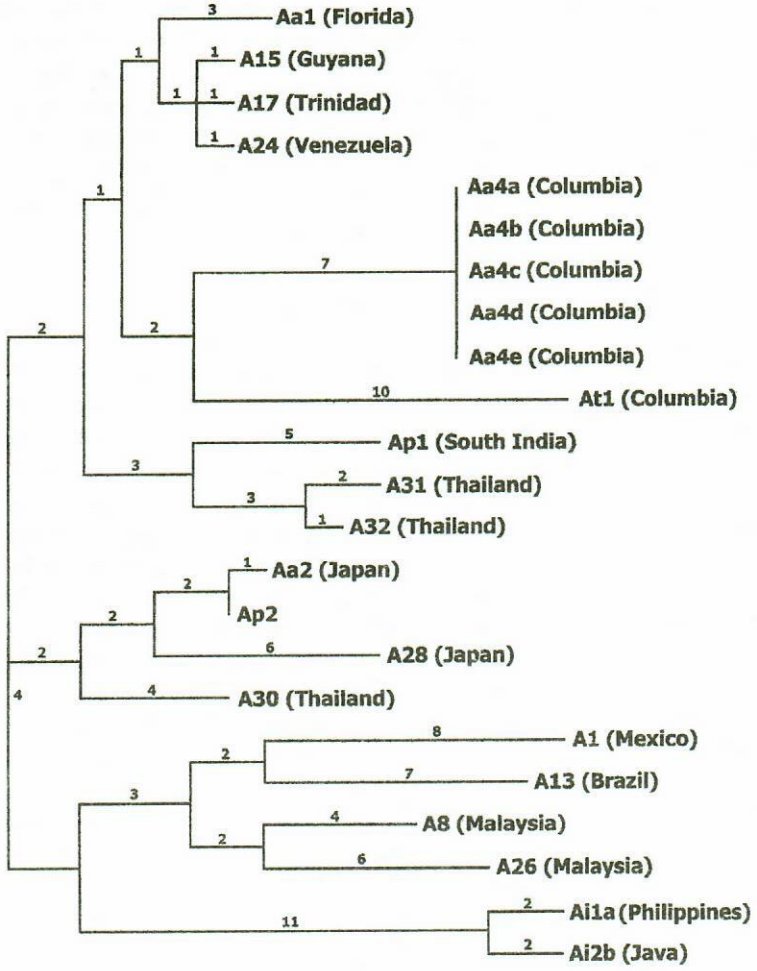

Fig 1. Phylogenetic tree of 24 isolates of entomopathogenic fungus *Aschersonia* spp. inferred from RAPD data. Type of tree – phylogram (PAUP 3.1.1, Heuristic options) (SWOFFORD 1993). Numbers on tree branches indicates number of characteristics supporting each branching

Aligned sequences were used to construct one most parsimony phylogenetic tree (Fig. 3), which placed the isolates into two groups. In the first group we find isolates A28 (*Aschersonia* sp., Japan), A31 (*Aschersonia* sp., Thailand) and Ap1 (*Aschersonia placenta*, South India); the second group is formed by isolates Ai1a (Philippines) and Ai2b (Java) (*Aschersonia* spp.).

DISCUSSION

The phylogenetic tree of *Aschersonia* isolates inferred from RAPD data indicates a close connection between phylogeny and geographical origin. RAPD analysis of another fungal genus parasiting on whiteflies, i.e. *Paecilomyces fumosoroseus* (TIGANO-MILANI *et al.* 1995a; OBORNÍK & LANDA 1997; OBORNÍK *et al.* 1997), did not show any correlation between phylogenetic groups and geographical origin. TIGANO-MILANI *et al.* (1995b) also observed no correlation with geographical origin or hosts in cluster analysis of 28 isolates of *Paecilomyces lilacinus* investigated by AP-PCR markers. The reason for such differences between the tested fungi lies probably in their different biological character. *Aschersonia* spp. is a fungus limited to tropic and subtropic climatic conditions. Wind and air movement can not distribute their spores; probably they are dispersed mostly by moving infected hosts, fungivore mites and water drops. The fungi thus have limited possibilities to spread their infectious units over long distances. *Aschersonia* has also relatively low abilities to survive on the leaf surface without host organisms. Further, the host range they infect is limited to whiteflies and scale insects only (FRANSEN 1986; FRANSEN *et al.* 1987).

On the other hand, *Paecilomyces* (namely *P. fumosoroseus*) is a cosmopolitan fungus, it produces spores that are easily spread by wind, it can survive in the environment as a saprophyte, and a rather large number of various insect and non-insect hosts can be infected (OSBORNE & LANDA 1992).

In the case of *Aschersonia* spp., geographical origin is probably an important factor in the clustering of isolates on the phylogenetic tree. The only exception seems to be a pair of American isolates (A1 – Mexico; A13 – Brazil) which were placed in a phylogenetic group together with isolates from Malaysia (A8; A26), Philippines (Ai1a) and Java (Ai2b). Isolates A1 and A13 probably belong to a

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(83-142)
S.cerevisae   AGTAACGGCG AGTGAAGCGG CAAAAGCTCA AATTTGAAAT CTGGTACCTT C---GGT---
A28           .......... .......... ...C...... .......... ....CC..CC .---..GGGG
A31           .......... .......... ...C...... .......... ....CC..CC .C---.GGGG
Ai1a          .......... .......... ...C...... .......... ....CC...C .CCC..GGGG
Ai2b          .......... .......... ...C....T. .......... ....CC...C .CCC..GGGG
Ap1           .......... .......... ...C...... .......... ....CC..CC .CC--.GGGG

(143-202)
S.cerevisiae  GCCCGAGTTG TAATTTGGAG AGGGCAA-CT TTG-----GG GCCGTTCC-- -TTGTCTGCG
A28           .......... .......C.. ...--.TG.. ...GGCGA.. CG.C....GA G..CC...--
A31           .......... .......C.. ...--.TG.. ...GGCGA.. CG.C....GA G..CC...--
Ai1a          .......... .......C.. ...--.TG.. .CTGGCGA.. TG.C....GA G..CC...--
Ai2b          .......... .......C.. ...--.TG.. .CTGGCGA.. TG.C....GA G..CC...--
Ap1           .......... .......C.. ...--.TG.. ...GGCGA.. CG.C....GA G..CC...--

(203-262)
S.cerevisiae  GTATGTTCCT TGGAACAGGA CGTCATAGAG GGTGAGAATC CCGTGTGGCG AGGAGT----
A28           ---------- --....G... ..C..C.... .......GC. ....C...TC -...CGCCGA
A31           ---------- --....G... ..C..C.... .......GC. ....C...TC -...CGCCGA
Ai1a          ---------- --....G... ..C..C.... .......GC. ....C...TC -...CACCGA
Ai2b          ---------- --....G... ..C..C.... .......GC. ....C...TC -...CACCGA
Ap1           ---------- --....G... ..C..C.... .......GC. ....C...TC -...CGCCGA

(263-322)
S.cerevisiae  -TCTTTGTAA AG-TGCCTTC GAAGAGTCGA GTTGTTTGGG AATGCAGCTC TAAGTGGGTG
A28           GC..C..... ..C.-..... ..C....... ..A....... .....T.... A..A....A.
A31           GC..C..... ..C.-..... ..C....... ..A....... .....T.... A..A....A.
Ai1a          GC..C...G. ..C.-..... ..C....... ..A....... .....T.... ...A....A.
Ai2b          GC..C...G. ..C.-..... ..C....... ..A....... .....T.... ...A....A.
Ap1           GC..C..... ..C.-..... ..C....... ..A....... .....T.... A..A....A.

(323-382)
S.cerevisiae  GTAAATTCCA TCTAAAGCTA AATATTGGCG AGAGACCGAT AGCGAACAAG TACAGTGATG
A28           ...T..GT.T .......... ....CC...C .......... ....C..... ..........
A31           ...T..GT.T .......... ....CC...C .......... ....C..... ..........
Ai1a          ...T..GT.T .......... ....CC...C .......... ....C..... ..........
Ai2b          ...T..GT.T .......... ....CC...C .......... ....C..... ..........
Ap1           ...T..GT.T .......... ....CC...C .......... ....C..... ..........

(383-442)
S.cerevisiae  GAAAGATGAA AAGAACTTTG AAAAGAGAGT GAAAAAGTAC GTGAAATTGT TGAAAGGGAA
A28           .......... ...C..C... .....G.G.. T...C..... .......... ..........
A31           .......... ...C..C... .....G.G.. T...C..... .......... ..........
Ai1a          .......... ...C...... .......G.. T...C..... .......... ..........
Ai2b          .......... ...C...... .......G.. T...C..... .......... ..........
Ap1           .......... ...C..C... .....G.G.. T...C..... .......... ..........

(443-451)
S.cerevisiae  GGGCATTT
A28           .C..TCG.
A31           .C..TCG.
Ai1a          .C..TCAC
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Fig. 2. Alignment of 26S rDNA gene sequences, positions from 83 to 451 for *Saccharomyces cerevisiae*, *Aschersonia* spp. (isolates A28, A31,Ai1a, Ai2b), and *Aschersonia placenta* (Ap1). The sequence of *Saccharomyces cerevisiae* was used as a reference. The dots indicates nucleotides identical to the nucleotides in the reference species, the dashes indicate deletions

Table 3. Relative genetic distances between *Aschersonia* isolates inferred from RAPD data (PAUP 3.1.1.) (SWOFFORD 1993)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. Aa1	–	0.214	0.200	0.182	0.196	0.196	0.196	0.196	0.196	0.312	0.218	0.245	0.089	0.089	0.091	0.259	0.232	0.323	0.161	0.148	0.357	0.357	0.255
2. Aa2	–	–	0.182	0.018	0.232	0.232	0.232	0.232	0.232	0.393	0.273	0.321	0.232	0.196	0.236	0.259	0.161	0.125	0.196	0.130	0.357	0.357	0.382
3. Ap1	–	–	–	0.164	0.218	0.218	0.218	0.218	0.218	0.364	0.222	0.308	0.218	0.182	0.222	0.245	0.225	0.236	0.182	0.167	0.400	0.400	0.345
4. Ap2	–	–	–	–	0.200	0.200	0.200	0.200	0.200	0.382	0.259	0.308	0.200	0.164	0.204	0.226	0.127	0.145	0.200	0.148	0.345	0.345	0.364
5. Aa4a	–	–	–	–	–	0.000	0.000	0.000	0.000	0.411	0.291	0.321	0.179	0.143	0.164	0.333	0.286	0.286	0.286	0.278	0.446	0.446	0.309
6. Aa4b	–	–	–	–	–	–	0.000	0.000	0.000	0.411	0.291	0.321	0.179	0.143	0.164	0.333	0.286	0.286	0.286	0.278	0.446	0.446	0.309
7. Aa4c	–	–	–	–	–	–	–	0.000	0.000	0.411	0.291	0.321	0.179	0.143	0.164	0.333	0.286	0.286	0.286	0.278	0.446	0.446	0.309
8. Aa4d	–	–	–	–	–	–	–	–	0.000	0.411	0.291	0.321	0.179	0.143	0.164	0.333	0.286	0.286	0.286	0.278	0.446	0.446	0.309
9. Aa4e	–	–	–	–	–	–	–	–	–	0.411	0.291	0.321	0.179	0.143	0.164	0.333	0.286	0.286	0.286	0.278	0.446	0.446	0.309
10. A1	–	–	–	–	–	–	–	–	–	–	0.225	0.264	0.304	0.304	0.291	0.315	0.375	0.375	0.446	0.389	0.393	0.393	0.455
11. A8	–	–	–	–	–	–	–	–	–	–	–	0.212	0.236	0.236	0.241	0.189	0.291	0.291	0.345	0.283	0.400	0.400	0.315
12. A13	–	–	–	–	–	–	–	–	–	–	–	–	0.264	0.226	0.231	0.235	0.302	0.302	0.340	0.269	0.340	0.340	0.288
13. A15	–	–	–	–	–	–	–	–	–	–	–	–	–	0.036	0.036	0.241	0.214	0.214	0.214	0.204	0.375	0.375	0.273
14. A17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.036	0.204	0.179	0.214	0.214	0.204	0.375	0.375	0.273
15. A24	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.241	0.182	0.218	0.218	0.189	0.327	0.327	0.241
16. A26	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.204	0.315	0.352	0.308	0.333	0.333	0.377
17. A28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.214	0.286	0.222	0.339	0.339	0.382
18. A30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.214	0.148	0.339	0.339	0.364
19. A31	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.056	0.411	0.411	0.345
20. A32	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.333	0.333	0.315
21. Ai1a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.071	0.382
22. Ai2b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.418
23. At1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

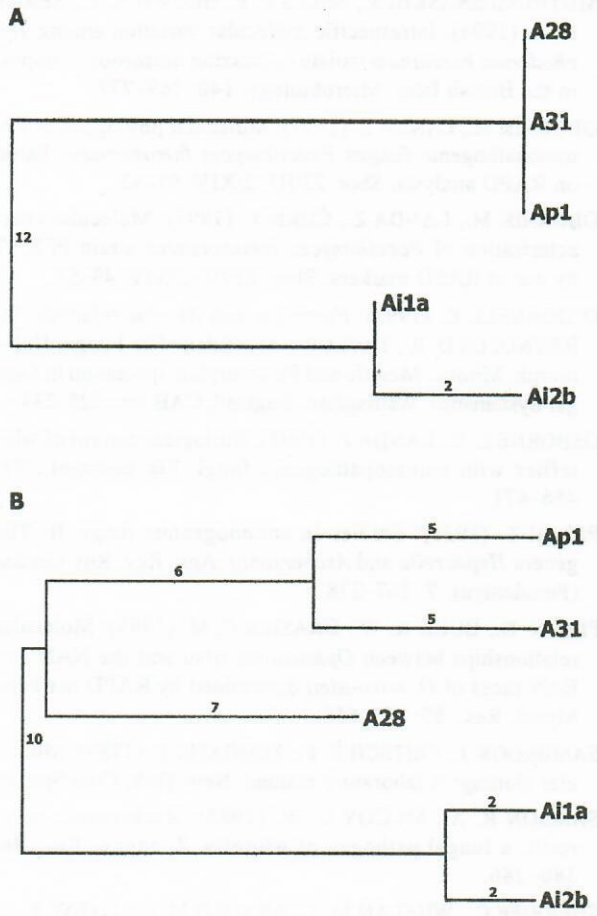

Fig. 3. Phylogenetic trees of five *Aschersonia* isolates inferred from 26S rDNA sequences (**A**) and RAPD data (**B**). Type of trees – phylogram. Numbers on the trees branches indicates number of characteristics supporting each branching (PAUP 3.1.1. Heuristic options, SWOFFORD 1993)

distinct species more related to Asian isolates Ai1a and Ai2b, and they got to the American continent independently from the other American strains. The taxonomic position of isolates identified morphologically only on the genus level can be discussed. American isolates A15 (Guyana), A17 (Trinidad) and A24 (Venezuela) very probably belong to the species *Aschersonia aleyrodis*. Isolates A28 (Japan), A30 (Thailand), A31 (Thailand) and A32 (Thailand) can be classified as *Aschersonia placenta* (since isolates Ap1, A28, and A31 were not distinguishable by use of 26 rDNA region sequences, they very probably belong to the same species). American isolates A1 and A13 belong to an American species distinct from *Aschersonia aleyrodis*. Isolate Aa2, originally identified as *A. aleyrodis*, probably belongs to *A. placenta* (this branching is identical with branching in the majority role consensus tree which is supported by more than 50% of the seven most parsimony trees constructed). It is corresponding also with the geographical origin of the isolate (Japan). As the isolate Ap2 (unknown origin) is related to isolates from Japan (Aa2; A28) and Thailand, it probably also originates from Southern Asia. This isolate we obtained from the culture collection in Vilnius (Lithuania); since the traditional school in the former Soviet Union has worked with isolates from China and Vietnam, this strain was very probably isolated in one of these regions.

The position of isolates in the phylogenetic tree inferred from 26S rDNA sequences suggests that isolates A28 and A31 belong to the same species, *Aschersonia placenta*. The position of isolates Ai1a and Ai2b in both phylogenetic trees (using RAPD and rDNA sequences) indicates distinct species.

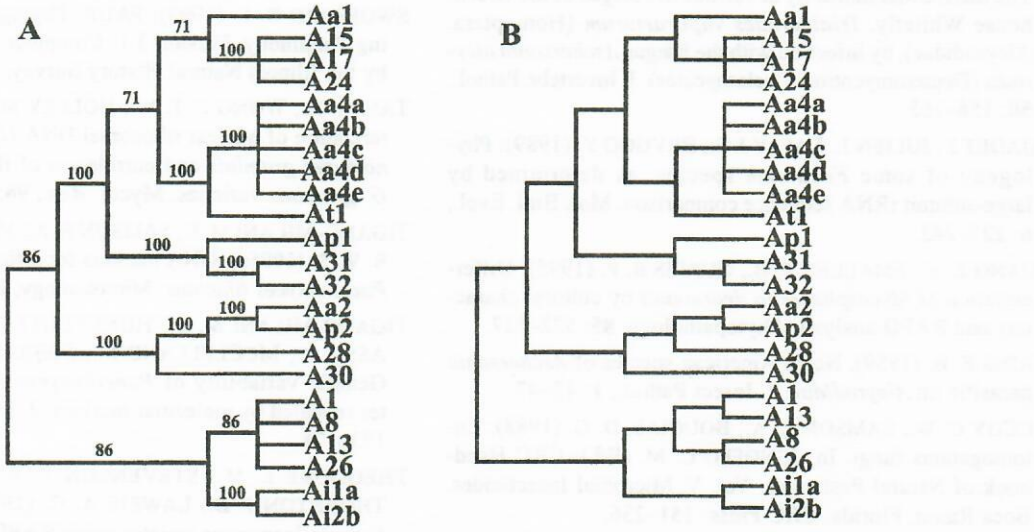

Fig. 4. The comparison of Majority role consensus tree (50%) – **A**, and resulted phylogenetic tree no 2 – **B**. The consensus tree was calculated out of seven most parsimony trees constructed. Numbers on branches shows the frequency of the branching (%) in equally parsimonious trees used for Majority role consensus tree calculation

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Received for publication October 26, 1998
Accepted for publication December 22, 1998

Souhrn

OBORNÍK M., STOUTHAMER R., MEEKES E., SCHILTHUITTZEN M. (1999): **Molekulární charakterizace a fylogeny entomopatogení houby** *Aschersonia* spp. Pl. Protect. Sci., **35**: 1–9.

Pomocí RAPD markerů jsme charakterizovali 23 izolátů entomopatogenní houby *Aschersonia* spp. z Mexika, Brazílie, Guyany, Trinidadu, Venezuely, Kolumbie, Floridy, Malajsie, Thajska, Japonska, Filipín, Jávy a jižní Indie. Data byla použita k zjištění genetické variability a ke sestavení fyogenetického rodokmenu testovaných izolátů rodu *Aschersonia*. Relativní genetické distance se pohybovaly od 0,018 (mezi izoláty Aa2 a Ap2) do 0,445 (mezi izoláty A1 a At1). Izoláty byly v sestaveném fylogenetickém stromu seskupeny v souladu s jejich zeměpisným původem. Provedli jsme také sekvenční analýzu části 268 rDNA u pěti kmenů rodu *Aschersonia* (A28, A31, Ai1a, Ai2b – *Aschersonia* spp., a Ap1 – *Aschersonia placenta*) a získané sekvence jsme použili k fylogenetické analýze. Tři z testovaných izolátů nebylo možné od sebe odlišit. Sestavený strom potvrzuje, že izoláty Ai1s a Ai2b náleží k druhu odlišnému od druhů *A. placenta* a *A. aleyrodis*.

Klíčová slova: *Aschersonia*; biologická kontrola; genetická variabilita; RAPD; fylogeny; rDNA

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