

European Culture Collections' Organisation

30th Annual Meeting, 16-17 June 2011, Utrecht



Conference Proceedings

Three new aflatoxigenic species of *Aspergillus* section *Flavi* isolated in Portugal

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Three new aflatoxin-producing species isolated from two Portuguese commodities, almonds and maize, are described. A polyphasic approach based on phenotypic (morphology and extrolites) and molecular characterizations was used to identify these isolates as belonging to new species.

From the study of the twenty two isolates obtained in maize and almonds, two were identified as *Aspergillus flavus*, six as *A. minisclerotigenes* and four as *A. parasiticus*. Examination of morphological characters combined with the analyses of aflatoxins and CPA production and molecular data revealed that ten of the *Aspergillus* section *Flavi* strains that were isolated do not match that of described species of the section. The multilocus analyses were performed using different primers for DNA regions presented in Table 1.

Molecular characterization showed that *A. mottae*, *A. sergii* and *A. transmontanensis* are well supported by phylogenetic analyses of the combined data (Figure 1). *Aspergillus transmontanensis* and *A. sergii* are phylogenetically related to *A. parasiticus*. *Aspergillus mottae* is phylogenetically ancestral to *A. flavus* and *A. parasiticus*.

Table 1 - Details of the primers used and target region

Primer pair	Target region	Sequences
Bt2a-Bt2b	Beta tubulin gene	5'GGTAACCAAATCGGTGCTGCTTTC3' 5'ACCCTCAGTGTAGTGACCCTGGC3'
Cf1-Cf4	Calmodulin gene	5'GCCGACTCTTTGACYGARGAR3' 5'TTTYTGATCATRAGYTGGAC3'
I5-D2r	ITS and partial 1su-rDNA	5'GGAAGTAAAAGTCGTAACAAGG3' 5'TTGGTCCGTGTTTCAAGACG3'
Mf-Mr	<i>Mcm7</i> gene	5'ACIMGIGTITCVGAYGTHAARCC3' 5'GAYTTDGCACIACCIGGRTCWCCCAT3'
M1f-M1r	Mating type gene	5'ATTGCCCATTTGGCCTTGAA3' 5'TTGATGACCATGCCACCAGA3'
M2f-M2r	Mating type gene	5'GCATTCATCCTTTATCGTCAGC3' 5'GCTTCTTTTCGGATGGCTTGCG3'
5F-7R	RNA polymerase	5'GAYGAYMGWGATCAYTTYGG3' 5'GAYTGRTRTRTGRTRCGGGAAVGG3'
Tsr1-Tsr2	<i>Tsr1</i> gene	5'CCACGC TCA TTC AA ATCTTC T 3' 5'CCGGTAGTTCGACCACTCGCATA3'

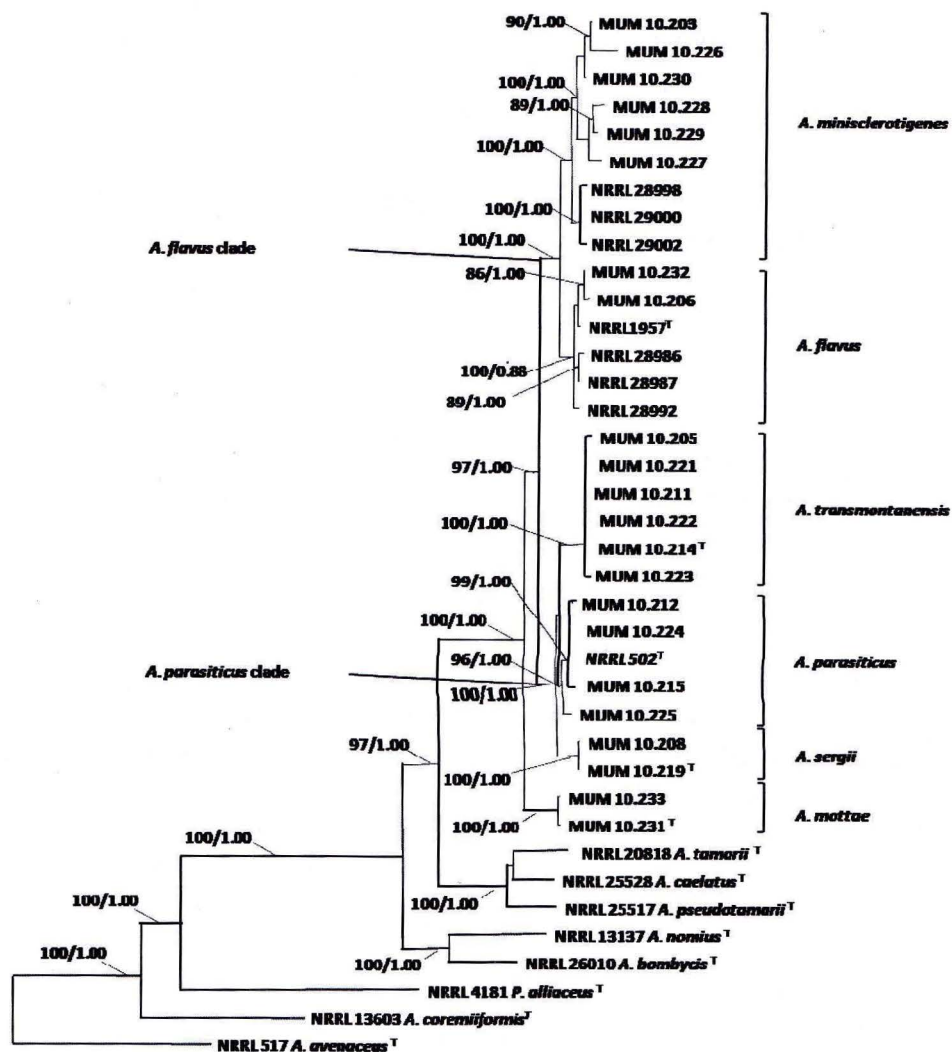


Figure 1. Phylogenetic tree calculated from combined *BT2*, *CF*, *Mcm7*, *RPB2* and *TSR1* data. Of 3746 total characters 2485 are constant, 618 variable characters are parsimony-uninformative, 643 characters are parsimony informative. There were more than 100 most parsimonious trees with CI = 0.7692 and RC = 0.5980. Bootstrap values/Bayesian posterior probabilities are placed above internodes. Bootstrap values below 70 were not considered.

Therefore three new species are proposed *Aspergillus mottae*, *Aspergillus sergii* and *Aspergillus transmontanensis*.

Acknowledgements

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned. USDA is an equal opportunity provider and employer. Célia Soares was supported by a grant SFRH/BD/37264 / 2007 from FCT-Portugal.