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# β-tubulin paralogue *tubC* is frequently misidentified as the *benA* gene in *Aspergillus* section *Nigri* taxonomy: primer specificity testing and taxonomic consequences

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**Abstract**



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β-tubulin (*benA*, *tub-2*) and calmodulin (*caM*) are crucial genes in the taxonomy of *Aspergillus* section *Nigri*. Widely used β-tubulin primers are not specific for the *benA* gene for some taxa and preferentially amplify the *tubC* paralogue. Sequences of the *tubC* paralogue are widely combined with *benA* sequences in recent taxonomical works as well as other works, resulting in incongruent trees. In this study we newly provide *benA* sequences for several ex-type strains, which were characterised using the *tubC* gene only. We designed a highly specific forward primer to *benA* designated *Ben2f* for use in *Aspergillus* section *Nigri*, and tested specificity of numerous primer combinations to β-tubulin paralogs. The primer pairs with the highest specificity to the *benA* gene and functional across species in section *Nigri* includes *Ben2f/Bt2b*, *Ben2f/T22* and *T10/T22*. We also provide tools based on codon usage bias analysis that reliably distinguish both paralogs. Exon/intron arrangement is the next distinctive characteristic, although this tool is not valid outside section *Nigri*. The species identity of taxa from the 'A. *aculeatus* clade' used in previous molecular studies was revised using combined molecular data (ITS, *benA*, *caM*). These data together with two different PCR-fingerprinting methods indicated that *A. japonicus* should be treated as a synonym of *A. violaceofuscus*. Similarly, *A. fijiensis* is reduced to synonymy with *A. brunneoviolaceus*.

**Keywords:** BISERIATE BLACK ASPERGILLI; CODON USAGE; GENE DUPLICATION; INCONGRUENT TREES; PARALOGOUS GENES; PCR FINGERPRINTING; PRIMER DESIGN; UNISERIATE BLACK ASPERGILLI

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