

A beta-tubulin gene (*benA*) is widely used in taxonomy and identification of *Aspergillus* spp. and other Fungi. Across *Aspergillus* spp. There is either one (*benA*) or two beta-tubulin paralogs (*benA* and *tubC*). The risk of contemporary use of sequences of paralogous genes with non-homologous function in the same phylogenetic analysis is well known. It is evident that it had happened repeatedly in *Aspergillus* section *Nigri*. It is alarming that conventional primers for amplification of partial *benA* sequence can specifically amplify *tubC* paralog in some species. In this work, both paralogs were characterised in a set of species. The beta-tubulin primers in use were revised and new, more *benA* specific primers were designed. Applicability of some markers such as base composition, codon usage and length of introns for distinguishing -tubulin paralogs *benA* and *tubC* is tested. A large study on molecular diversity of 349 isolates of *Aspergillus* (PCR-fingerprint, sequence data - ITS, *benA*, *rpb2*, *caM*) originating from Czech culture collections and from clinical material is also included. 82 species were identified, together with nine tentative new taxa belonging to sections with high economic impact - *Nigri*, *Fumigati* or *Aspergillus* (*Eurotium* spp.). Five species from Section *Aspergillus* could be synonymised with existing taxa. A study on molecular diversity of clinical isolates ($n=200$) is innovative in sense of the geographical origin, Central Europe, where is remarkable lack of similar reports. It also brings support for presence of several rare, little known or new species recognized as mycotic agents. This is the first comprehensive study providing reliable identification of such large set of aspergilli from both superficial and deep infections.