

I n d e p e n d e n t e x p e r t a s s e s s m e n t
(Critical review)

PhD-thesis: Taxonomy, diversity and clinical relevance of the genus *Aspergillus*

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General assessment

My comments are supposed to be addressed exclusively onto *Aspergillus* topic in which without any hesitation I do see Mr. Hubka as a front leader, outstanding and respected scientist, and a world-renowned taxonomist. Furthermore, in his relatively short research period he already had a deep impact on the field of clinical mycology dealing with superficial infections agents-dermatophytes and/or other fungi important in clinical settings. These are not only my subjective considerations, it is simply and objectively based on research-scientific, publication, teaching and lecturing activities of Mr. Hubka, as all that can be seen from the lists of activities and publications (including chapters in books, invited lectures, reviewing for plenty of journals). In brief, I regard most impressive the number of papers reaching **45 in IF journals**, while in 17 of them being a first author. Four articles were published in *Study in Mycology* with IF 14, one article in *Fungal Diversity* of IF 13,5 and five papers in *Persoonia* with IF 7,5! These journals represent, in fact, the top ones of those dealing with fungal taxonomy. The current 349 citations (H-index 13) will increase in the next years, supporting the value of novelty and impact his and his research group is currently bringing to the mycology field in general. Furthermore, co-authorship with renowned researchers and/or taxonomists from 16 countries (USA, Canada, South Africa, Japan, China, South Korea, The Netherlands, Hungary, Slovenia, France, Turkey, Italy, Spain, *etc.*) are clearly highlighting the overall quality and scientific value of the studies and themes carried out by this “Czech mycology school” spearheaded by Mr. Hubka. As for the studies and resulted publications, research interest of Mr. Hubka is obviously divided onto: (a) *Aspergillus* taxonomy, (b) Dermatophytes – causing superficial, skin and nail, infections, and (c) other agents of human and animal mycoses (ascomycetous, black yeasts and zygomycetous fungi), and finally (d) fungi of indoor and cave environments (generally microfunga).

As can be seen from the list of publications, medical mycology (other than *Aspergillus*) has been dedicated nearly 40% of the studies/publications with IF, while 100% (14) publications

in journals without IF. The dedication for studying clinical fungi, including members of the genus *Aspergillus* occurring in patients of Czech Republic (Hubka *et al.*, 2012. Medical Mycology 50: 601-610), had obviously opened a very complex and unclear taxonomy of morphologically rather confusing, but on the other hand, immensely important group of fungi and thus, providing room for studies and demanding the revision of the genus *Aspergillus*, to which Mr. Hubka contributed the most. His contributions or better to say, definitive revisions, new taxonomic schemes and proposals (taxonomic stability), provided keys towards the treated and accepted species, many newly described species (41!), all that has been now submitted in this outstanding PhD-study that deals with a speciose genus *Aspergillus* P. Micheli ex Haller.

The submitted PhD-work is in a form of compilation of 23 papers-studies published in renowned microbiological and mycological journals and thematically divided, after Introduction and Abstract parts, into 5 areas (parts), as follows:

Part I. Generic concept and nomenclature (5 papers)

Part II. Subgeneric classification (4 papers)

Part III. Species delimitation - biological species concept and interspecies hybridization (7 papers)

Part IV. Taxonomic revisions and species diversity (16 papers)

Part V. Clinical relevance (9 papers)

The PhD-work then further continues with Conclusion and References, lists 23 relevant papers in their published (17), accepted (3) and submitted (3) forms, and finally ends with appendix I (list of taxonomic novelties), II (papers authorships contributions), III (copyright permissions) and IV (cv). In total, the written work contains 713 pages.

The text of the thesis is written in very good scientific style and with excellent English with only few minor typing mistakes, which do not in any case reduce an overall quality of this very valuable PhD-thesis document.

Special Comments and Questions:

Abstract and Introduction: An excellent example for introduction and practically for the entire issue with the genus *Aspergillus* has been already given in Paper II (Studies in Mycology, 2014) including the history of the genus, taxonomic approaches and novelties, methodology – phenotypic and molecular methods, *etc.* Here, in the submitted PhD-thesis, I do personally miss those illustrations depicting a diversity of the morphs (anamorphs) within the genus and perhaps would be of value to include the basic drawings of two different conidiophore structures (*e.g.* uniseriate and biseriate).

Thus my first question/request is to show and define, in illustrative way, the differences in anamorphic forms (micromorphology) present in the genus *Aspergillus*.

To Part I Generic concept and nomenclature

As one of the first authors, Hubka et al. 2013 (Paper I) adopted a single-name nomenclature (following decision of ICPA April 2012, Utrecht, NL) and in their extensive revision study on *Eurotium* (section *Aspergillus*, “*Aspergillus glaucus* group”) legitimately transferred all hitherto known *Eurotium* species into *Aspergillus*. Similarly thus, in followed papers Paper II, (2014) and V (2015), species in *Emericella*, *Chaetosartorya*, *Neosartorya* and *Fennellia* were synonymized with *Aspergillus*.

I need to admit, the previous names belonging to *Eurotium* very typically used mainly in food mycology as a typical funga of dried down products, and species like *E. herbariorum*, *E. amstelodami*, and/or *E. rubrum* were one of the most cited as being reported from such commodities. The same hold true for well-known and commonly used name for *A. nidulans* (*Emericella*) or species previously known as *Neosartorya* spp. *etc.*

My question regarding this new trend of using one fungus = one name system. Is the applicant fully convinced and satisfied with that trend to name those fungi showing two different states in their life cycle (holomorphic) with the only one preferred name (*e.g.* *Aspergillus*)? Second question to that issue: Was the article (Paper IV) entitled “Response to Pitt & Taylor 2016.... by Samson *et al.* accepted manuscript” really necessary? With respect to the way the article has been titled. Isn't there really any place for “a taxonomic compromise”?

To Part II subgeneric classification

Very well done and informative is the table 2 on current infrageneric classification of *Aspergillus* also providing numbers of accepted names and references.

In the PhD thesis text states six subgenera, in the article Hubka *et al.*, 2017 (submitted ms Persoonia – paper X), however, in the introduction part it is stated eight subgenera. This needs to be resolved. For this part of the thesis, I would like to point out the exceptional contribution to *Aspergillus* taxonomy by the author (Paper V = a new section *Jani*; Paper IV = new sections, namely *Petersoni*, *Robusti* and *Tanneri* in subgenus *Circumdati*), establishing 4 new sections!

However, I do personally miss a sort of dichotomous key for those subgenera and their respective sections here in the submitted thesis.

This may be provided by a table form, summarizing any reliable phenotypic characteristics-features setting these particular subgenera and sections (with typical representatives) from each other.

To Part III. Species delimitation - biological species concept and interspecies hybridization

In this special part, the author provides an alternative towards the phylogenetic resolving the species based on recently introduced so-called multispecies coalescent (MSC) model for the delimitation of the species boundaries and for the first time applied for *Aspergillus* taxonomy (namely in Paper VIII - in section *Restricti*; in Paper IX - section *Candidi* and in Paper X – *Fumigati*). Useful and of taxonomic value was including media with elevated sugar or salt content into identification schemes for species in subgenus *Aspergillus*, xerophilic and halophilic taxa, respectively. Outstanding is also the study on β -tubulin paralogous genes published in 2012 in Persoonia = Paper XI revealing a misleading interpretation by using a wrong and barely informative the *tubC* paralog that preferentially amplifies in some species (*e.g.* section *Nigri*) instead of desired the *benA* gene. The author/s provided also a solution how to avoid such misinterpretation (Paper VII and XI).

My question towards this point is what is then a preferable gene in *Aspergillus* to be used for species identification if any research group would have to choose for routine diagnostics only one single gene? Routine means 20-30 (50) amplifications a day.

In the fungal kingdom, in general, the hybridization amongst the different species (interspecific hybridization) is bringing new view on the biological concept of species, and has been hitherto only very rarely successfully established *in vitro*. This and very evident evolutionary tool (of creating new original populations – progeny/ hybrids = new species?) has been demonstrated especially in an excellent study-paper X (submitted manuscript, Persoonia).

The author should comment if it is possible during any routine mycological analysis of environmental or clinical samples to identify any putative *in vivo* (or even *in vitro*) created hybrid/s as such entity or, if our molecular tools nowadays could possibly indicate a new and phylogenetically supported species-taxon?

Part IV. Taxonomic revisions and species diversity

Already mentioned revisions in nearly all subgenera, namely in subg. *Aspergillus* = Paper I, VIII and especially XIV - by Chen *et al.*, 2017 in Studies in Mycology; subg. *Circumdati* = Paper V, VI, IX, XV; subg. *Fumigati* = Paper X, XII, XVI; subg. *Nidulantes* = Paper VII, XVII, XVIII, XIX; subg. *Cremeri* = Paper XX and XX, have resulted in description of 41 *species novum* (**ca 10 % of all known aspergilli!!**), one new combination, 16 type designations and more than 20 synonymizations by the author (or his research group). Thus indeed, very impressive!

In nearly all his taxonomic papers, well thought and organized dichotomous keys (combining phenotypic traits) have been provided.

Since the first revision of *Aspergillus viridinutans* complex (Paper XII, Novakova *et al.*, 2014) accepting 5 species, three years later, additional 5 taxa have been discovered and resolved in *A. viridinutans* clade in (Paper X, Hubka *et al.*, submitted manuscript). Can the author briefly characterize (phenotypically) this medically important group, and also provide a dichotomous key to treat all 10 accepted taxa?

Part V. Clinical relevance

Due to the fact of emerging resistance against the commonly used antifungals, the studies carried out here could in future contribute immensely to the treatment of invasive aspergilloses in terms of reliability, effectiveness for treatment during the particular clinical cases, since such infections are usually very severe, devastating and fatal. Thus the important limit here is the factor time, limiting an overall recovery of the patient from early diagnosis

that prompts a setup of the proper treatment in as short time as possible. At the same time, thus elaboration of so called-antibiograms for every identified clinical isolate is crucial, especially when non-*Aspergillus fumigatus/flavus* infections are of concern (*i.e.* caused by cryptic species, as revealed in Paper XXI during a study of 178 clinical isolates from Czechia, 2012!). In my opinion, to establish a case specific antibiogram for the recovered isolates should always take the highest priority (even regardless of the species identity) and it is no matter which of the methods (EUCAST/CLSI/E-Test/DDM/YO48/) one uses for this drug sensitivity estimation (*in vitro*) - for assessment of MICs. Time plays the main role here and each of the methods gives reliable indication for a recommended treatment.

How much or how deep were the contributions of the applicant in the case studies? Is it a pure mycological work (from obtaining the pure clinical isolate onwards till the MICs assessments) or was the applicant also involved in sampling and further consultancy with the physicians and/or in monitoring of the patients?

Conclusion:

In conclusion of my review I wholeheartedly state that Mr. Hubka submitted an extraordinary valuable document-elaboration dealing with the newest knowledge in mycology, especially taxonomy dedicated to the genus *Aspergillus* that by far exceeds the most demanding scientific criteria for a PhD-work (dissertation).

I do hereby recommend the PhD-thesis of Mr. Vít Hubka for the defense, as the thesis fully meets the requirements for the dissertation work.

Tulln, 28 November, 2017



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