

Review report by Danny Haelewaters, PhD
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The PhD dissertation by the candidate František Sklenář starts with an abstract in English and Czech, followed by very broadly phrased thesis aims (section 1). The introduction (section 2) first focuses on different species concepts (history, criteria, future current issues in species delimitation (section 2.1 and 2.2)). I found that overview too short to be able to provide nuances. The presentation of species delimitation methods (SDM) provides a clear overview of what is available. Some technicalities are introduced without further explanation. This is the case for, e.g., the “garbage in garbage out” principle. I would have appreciated a fungal example to illustrate. Then the genus *Aspergillus* is introduced from a taxonomy and species criteria point of view in section 2.3. Section 3 of the thesis presents the overall results and discussion making a case for the multispecies coalescent model of multilocus datasets to be used for delimitation in sections and species complexes of *Aspergillus*. Eight papers are presented in the thesis, all already published in top mycological journals (Stud Mycol, Persoonia, Mycologia, Int J Syst Evol Microbiol). The candidate was the first author on 4 of these papers, and contributed to laboratory work, molecular data analysis, preparation of graphs, and manuscript revision of the other 4. There is a discussion of the intraspecific variability in *Aspergillus* and general conclusions (section 4). In all, the thesis marks a significant step forward in the taxonomy of the genus *Aspergillus*, towards a standardization of methodology for species delimitation that could be extrapolated to different groups of fungi. It is also interesting to see an unbiased treatment, with both new species descriptions and synonymies.

Section *Restricti*: An understudied group in the genus. The thesis presents several results: (1) better growth of strains on media with osmotically active substances (compared to MEA), (2) a higher diversity in the section (7 spp. + 14 new spp.), and (3) and an even higher diversity based on eDNA analysis. A clade with three new species was subject to more recent analyses by Hagiuda et al. (2022). It may be that there is ongoing gene flow, interspecific hybridization, or ILS [**first author paper**]. Series *Viridinutantes*: This series includes the human pathogen *Aspergillus fumigatus*. The most interesting result was the discovery of in-vitro interspecific hybridization. One new species was described and two species were synonymized under *A. felix* based on four-locus (for the series) and six-locus (for strains of *A. felix* and related species) datasets. Section *Flavipedes*: These occur in soil and can cause food spoilage. One species was rejected and another was synonymized in ser. *Flavipedes*, and four species were newly described in ser. *Spelaei* (following what is referred to as the “splitting” scenario) [**first author paper**]. For this analysis, the “Delineate” software developed by Sukumaran et al. (2021) was used. Section *Candidi*: This section includes *Aspergillus candidus*, an extremophile, occasional human pathogen, causes food spoilage. An analysis based on sequences of three loci of *A. candidus* strains from indoor and cave environments resulted in the description of a new species. This was supported by SDM and phenotypic characteristics. A second paper revised the entire section, based on the phylogenetic reconstruction of three loci and a comprehensive study of micromorphology. In general, the variability of a characteristic within species is comparable to the total variability in the whole section—resulting in limited taxonomic utility of phenotypic features and a limited number of useful characteristics. The different loci analyzed are incongruent, suggesting recombination and complicating SDM. The choice was made for a “broader” species concept (in contrast to ser. *Spelaei* above), including the description of 2 new species and a new name for a previously invalidly described species. Section *Nidulantes*: This section includes model organism *A. nidulans*. In one paper, six species were newly described from indoor environments based on SDM of a small dataset [**first author paper**]. A second paper revised ser. *Versicolores* within sect. *Nidulantes*. The paper includes as many as 518 strains. A five-locus-based species delimitation supported only 4 species (instead of 17 as recently accepted), which was also supported by phenotypic characteristics. In addition, genomic analysis reveals a high degree of genetic variability in two species, preventing identification to species based on DNA sequence data: different loci identify the same strains as different species [**first author paper**]. Series *Nigri*: This series includes important species in food mycology, biotech, and medicine and has seen a turbulent taxonomic history. A whole genome-scale

ML analysis (5752 orthologous proteins) was performed. Ten STACEY analyses were performed on smaller datasets (200 selected loci, randomly split in 10 groups of 20 loci each). SDM based on 3 loci supported 3 species. STACEY identified 4 likely scenarios with between 3 and 6 species. The scenario with 6 species was retained, partly based on the large intra-specific genetic variability of *A. tubingensis* s.l., the industrial importance of *A. luchuensis* (one of the species within the *A. tubingensis* lineage), and the uncertainty about selection of loci for the three-locus-based SDM.

Specific comments:

I thought the PhD thesis was very well written, with only very few issues I could point out. Some improvements to the language or corrections of typos are presented below:

ABSTRACT

Page 1: "... and their inclusion in a polyphasic taxonomy approach."

AIMS

Page 3: "... the latest methodology of species delimitation into the taxonomy..."

Page 3: "each focusing on the taxonomy of a section of the genus *Aspergillus*."

INTRODUCTION

Page 4: I do not know if your explanation of Figure 1 makes sense. "The use of species criterion 9 in taxonomic study would result in a low number of species (because it delimits only fully separated lineages as distinct species), while species criterion 1 would lead to delimitation of much larger number of species." Is this how de Queiroz intended to interpret this figure?

Page 5: "de Queiroz" without capital D.

Page 11: It is written: "However, molecular identification remains ...". However, delimitation and identification (and recognition) have different goals and require different tools. As shown by Lücking et al. 2020.

Page 12: "Importantly, it has solved the discussion..." This is still very much an ongoing process.

Page 14: I think part of this alternative being unrealistic is the fact that many fungi are difficult to collect and may only be encountered during field expeditions that are logistically and financially difficult to repeat. In this regard, ensuring that intraspecific variability is an ideal but not easily achieved.

Page 14: This statement is not finished: "Although *Aspergillus* species produce various sexual morphs, which were associated with at least 10 teleomorphic names in the era of dual nomenclature."

Page 15: It is written: "The preferred way of *Aspergillus* species identification is by DNA sequencing of marker loci." Is identification correct here? Also species recognition relies on sequencing in the genus?

Page 16: "...from restricted geographic areas." (plural)

Page 16: It is written: "Another way of finding more fungal features usable in taxonomy is by studying the production of secondary metabolites and comparing secondary metabolite spectra between species, ..." Ideally, this should be presented in the past (perfect) tense.

Page 18: typo in the X axis labeling of Figure 9

Page 18: These physiological tests are also used in red yeasts (Haelewaters et al. 2020) and the yeast-like stages of basidiomycetous mycoparasites (Schoutteten et al. 2023)

<https://doi.org/10.3114/fuse.2020.05.12>

<https://doi.org/10.3114/sim.2022.106.02>

RESULTS AND DISCUSSION

Page 19: “Houbraken et al. (2020) added series rank to *Aspergillus* taxonomy and the section *Restricti* was split ...”

Page 22: It would be helpful to also add the section to which this series belongs. More generally, a (schematic) phylogenetic overview of the genus sections and series in the Introduction would be helpful as a guide for the reader.

Page 23: “Series *Olivimuriarum* and *Neonivei* each comprise only a single species, ...”

Page 25: It is written: “We demonstrated that a significant part of the tested species showed elevated MICs to ...” Abbreviations should be introduced upon their first mentioning.

Questions for the candidate:

Q1: Your introduction gives a good overview of criticism to the idea of “species”, species concepts, and species criteria. But it does not address the definition of a species. I am interested to know what is a species to you?

Q2: In fungi, there is the presence of cryptic diversity. Indeed, as you write, this term “cryptic species” has been overused, to the point that authors have also referred to pseudo-cryptic and semi-cryptic species for better reference. The opposite is also true in fungi, the existence of multiple morphological forms within a single phylogenetic species. This is referred to as phenotypic plasticity, sometimes – especially in microfungus parasites – these forms may be restricted to specific microhabitats. Can you comment on this in the light of your discussion on species criteria and taxonomic decisions?

Q3: For some species delimitation methods, recommendations are to use them on single-locus datasets, whereas others recommend multi-locus datasets. What is your opinion on this given your literature review and own experiences in *Aspergillus*?

Q4: I am not personally familiar with the Delineate software. Can you explain how it operates? What analyses does it run?

Q5: In sect. *Nidulantes* ser. *Versicolores*, the case is made that sequences from loci identify a strain as different species. This is explained by recombination between strains of different cryptic species. My question is whether the term “incipient species” (and incipient speciation) could be more appropriate here.

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