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The 'species complex' issue in clinically relevant fungi: A case study in *Scedosporium apiospermum*

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ABSTRACT

The genus *Scedosporium* currently comprises six species, *Scedosporium apiospermum*, *Scedosporium boydii*, *Pseudallescheria angusta*, *Scedosporium minutisporum*, *Scedosporium dehoogii*, and *Scedosporium aurantiacum*, most of which can be distinguished with the primary fungal DNA barcode, the ITS1/2 region of the rDNA gene cluster. In the present study, four additional genetic loci were explored from a phylogenetic point of view enabling a barcoding approach based on K2P pairwise distances to resolve the taxa *Scedosporium*. We included partial γ-actin (*ACT*), β-tubulin (*BT2*), elongation factor 1α (*TEF1*), and the small ribosomal protein 60S L10 (L1) (*RP60S*). Phylogenetic inference of each marker individually showed that four out of six species within *Scedosporium* can be distinguished unambiguously, while strains of *S. apiospermum*, *S. boydii*, and *P. angusta* showed occasional recombination, and accordingly, no genealogical concordance between markers was obtainable. We defined *S. apiospermum*, *S. boydii*, and *P. angusta* as the '*S. apiospermum* species complex' since observed differences were not consistent between lineages, and no clinical differences are known between entities within the complex. While *BT2* revealed the best performance among the genetic loci tested at the lineage level, barcoding of the ITS region is sufficient for distinction of all entities in *Scedosporium* at the species or 'complex' level.

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Introduction

The term 'species complex' is suggestive to cover groups of organisms or lineages that are taxonomically closely related or even difficult to distinguish. However, no clear definition of the category 'species complex' exists so far and some clarity in the use of the term is urgently needed. The best-known use of 'species complex' for the kingdom *Fungi* is in the genus *Fusarium*, where 'species complexes' were introduced (O'Donnell *et al.* 2012) as an alternative to the subgeneric 'sections' as currently in use in genera like *Aspergillus* (Geiser *et al.* 2007) or *Trichoderma* (Bissett 1991; Druzhinina *et al.* 2005); in *Fusarium* the older, phenotypic sections did not match with phylogeny. The current species complexes in *Fusarium* are monophyletic, together encompass all species known in the respective genera, and hence such species complexes can be viewed as taxonomic categories. However, in other cases a 'species complex' just describes a selected group of entities that are difficult to distinguish from each other and/or classification of such groups is yet unclear. For example, some genetically diverse strains with unclear taxonomic status were listed as '*Aspergillus viridinutans* species complex' (Hong *et al.* 2005). Bensch *et al.* (2012) grouped series of closely related molecular siblings in *Cladosporium* as 'species complexes' under the name of their original phenotype name such as '*Cladosporium herbarum* complex' or '*Cladosporium cladosporioides* complex'; only few of the siblings within these complexes revealed deviant ecological characteristics.

In addition to taxonomic criteria, species complexes have also been defined for divergent practical reasons, one of which may be clinical or industrial significance. Howard *et al.* (2011) suggested to list all well-described clinical species of the *Aspergillus* section *Nigri* as '*Aspergillus niger* complex' due to absence of differences in antifungal susceptibility profiles. Some authors even united groups of unrelated fungi (Reedy *et al.* 2009) that were as yet unclassified (Manamgoda *et al.* 2012).

A further reason to aggregate species as a 'species complex' is unsettled taxonomy. For example, *Cryptococcus neoformans*, a potentially fatal pathogenic yeast, was initially divided into two varieties, var. *neoformans* and var. *gattii*. Katsu *et al.* (2004) united separate lineages within *C. neoformans* as the '*C. neoformans* complex' using the primary barcoding ITS locus of rDNA. Subsequently, Kwon-Chung & Varma (2006) brought the var. *gattii* to species level due to the significant divergence of ecological, biochemical, and molecular characteristics. After a long debate, these molecular siblings recently have been proposed as seven separate species in the '*C. neoformans* complex' (Hagen *et al.* 2015). This is an example of a species aggregate with entities that are closely related but appear to differ in some clinically relevant parameters. This was also the case in the '*Candida parapsilosis* complex', where the original species proved to have higher antifungal susceptibility than more recent molecular siblings (Treviño-Rangel Rde *et al.* 2012).

Scedosporium (being preferred over its sexual state name, *Pseudallescheria*; Lackner *et al.* 2014b) is a genus of ubiquitous ascomycetous fungi causing a wide array of human infections. Among the genus *Scedosporium*, *Scedosporium apiospermum*, and *Scedosporium boydii* are clinically relevant, being the second most common clinical moulds in cystic fibrosis, after *Aspergillus fumigatus*. Currently, an increasing incidence of infections caused by these species has been noticed, presently mainly in immunocompromised hosts (Tammer *et al.* 2011). Two prevalent species are currently recognized, *S. apiospermum* and *S. boydii*, for which as yet not unambiguous diagnostic parameters are available and which are often taken together as a 'complex'.

Thus, the term 'species complex' may (a) stand for a fixed taxonomic category below the genus level, (b) indicate some closely related strains with uncertain taxonomic status, or it may (c) stand for divergent species that for practical reasons are not precisely identifiable. The aim of the present study is to provide clarity and consistency for the term 'species complex' in medical mycology. Cases (a) and (c) are conceptually clear, just differing in their practical bias: taxonomically valid groups, which are either identified or are not distinguished. Here we focused on the most problematic situation (b), where data as yet obtained is insufficient to describe entities within the 'species complex' properly, and use *Scedosporium* as an example.

Materials and methods

Strains

Members of the genus *Scedosporium* were studied by the analysis of five gene fragments and compared with previously published AFLP profiles (Lackner *et al.* 2014a); the same set of strains was used in all partitions. The ten populations distinguished by AFLP were used as reference, in accordance with Lackner *et al.* (2014a), including *Pseudallescheria minutispora*, *Scedosporium dehoogii*, and *Scedosporium aurantiacum*. Thus, a total of 65 strains were analysed, including 19 strains of *Scedosporium apiospermum*, 23 strains of *Scedosporium boydii*, nine strains of *S. dehoogii*, seven strains of *Pseudallescheria angusta*, three strains of *Scedosporium minutisporum* and three strains of *S. aurantiacum*. A single isolate of *Pseudallescheria desertorum* (CBS 489.72) was used as outgroup. All of them were obtained from the reference collection of the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (CBS-KNAW), Utrecht, The Netherlands. All available type strains were included. Stock cultures were maintained on slants of 2 % malt extract agar (MEA) at 24 °C. Meta data on origin and sources of isolation are listed in Supplementary Table 1.

DNA extraction

DNA was extracted following the CTAB protocol that was described previously (Lackner *et al.* 2014a). Quality of genomic DNA was verified by running 2 μL DNA sample in a 1.0 % agarose gel. DNA sample was quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE, U.S.A.), and was stored at −20 °C until further use.

DNA amplification and sequencing

Five gene regions were amplified for inclusion in the multilocus sequence data analysis, i.e. partial the γ-actin (*ACT*) gene, β-tubulin (*BT2*), elongation factor 1α (*TEF1*), the small ribosomal protein 60S L10 (L1) (RP60S L10), and the ITS region.