Medical Mycology 2009, **47** (Supplement 1), S13–S20

What is a species in *Aspergillus*?

R. A. SAMSON* & J. VARGA*†

**CBS Fungal Biodiversity Centre, Utrecht, The Netherlands, and †Department of Microbiology, University of Szeged, Szeged, Hungary*

The various species concepts with emphasis on those which can be applied to *Aspergillus* and its teleomorphs are discussed. Any proposed new species should show evidence for evolutionary divergence from other taxa, particularly unique DNA characters at multiple loci and the polyphasic approach was suggested as the 'gold standard' for species delimitation using a combination of multilocus sequence data, morphological, physiological characteristics and ecological data. For species descriptions it is recommended to examine several gene sequences (e.g., ITS, calmodulin, β-tubulin, actin) and submit them to recognized sequence databases. Dual naming of *Aspergillus* taxa with teleomorphs has been recommended where necessary. To avoid confusion, the 'List of Names in Current Use' is recommended as a reference for *Aspergillus* nomenclature. For clinical researchers who depend on one of the names, it was suggested to use the name 'complex' if identification is solely based on morphology, which cannot distinguish between closely related species. A protocol for naming new *Aspergillus* taxa is proposed, which include the deposition of type cultures in at least two recognized culture collections. If type cultures are not available the taxon can be declared invalid.

Keywords *Aspergillus*, nomenclature, phylogenetics, polyphasic taxonomy, species concepts

Introduction

The genus *Aspergillus* has a great impact in various fields of research and many species are important as human and animal pathogens, spoilage agents of food, producers of toxic metabolites or, on the other hand, as useful microorganisms in food fermentation and biotechnological applications [1–3]. Although the genus with more than 260 species has been studied for several centuries, the systematics is still in a state of flux. In the light of recent molecular developments including detailed studies of several *Aspergillus* genomes the question 'what is a species in *Aspergillus*?' has became timely. A good species definition for taxa in common agricultural products has been discussed by Perrone *et al*. [4], while Balajee *et al*. [5] described the importance of the species concept for those taxa which have to be identified for the clinical setting. During an International Workshop held from 12–14 April 2007 in Utrecht, The Netherlands, participants working in various fields of *Aspergillus* research, discussed what an *Aspergillus* species is and how we delimit a species. In addition the current nomenclature of *Aspergillus* together with a protocol for naming a new species was argued. A discussion about the species concept and summary of the important debates and the recommendations are presented here.

The morphological, biological and phylogenetic species concepts

In 1942, Ernst Mayr introduced a new use of 'concept' in regard to species by elevating several different approaches to species identification to the level of concept [6]. Many different species concepts have been proposed since then; Hey [7] counts 24 of them. The most commonly discussed operational species concepts (also called Species Recognition to differentiate these

Received 6 February 2008; Received in final form 7 July 2008; Accepted 19 July 2008

Correspondence: Robert A. Samson, CBS Fungal Biodiversity Centre, PO Box 85167, 3508 AD Utrecht, The Netherlands. E-mail: r.samson@cbs.knaw.nl

DOI: 10.1080/13693780802354011

operational species concepts from the theoretical ones) [8] are the morphological species concept, the biological species concept, and the phylogenetic species concept [8,9]. Among these, the biological species concept treats populations whose members are able to interbreed freely under natural conditions [10,11] as belonging to one species, and is by far the most popular among evolutionists. This concept has been used to delimit several fungal species including Fusaria [12]. The biological species concept requires that members of the same species are sexually cross-fertile and that the progeny of the crosses are both viable and fertile. Consequently, this species concept has only limited value for species definition in Aspergilli, since it can only be applied to heterothallic species. The majority of species in the Trichocomaceae family is homothallic, with nine confirmed exceptions; six of those belong to the genus *Neosartorya*: *N. fennelliae, N.spathulata, N. nishimurae, N. udagawae, N. indohii* and *N. tsurutae* [13–16], one to *Emericella* (*E. heterothallica*) [3], while *Talaromyces derxii* belongs to *Penicillium* subgenus *Biverticillium* [17]. Recently, *Byssochlamys spectabilis* has also been identified as a heterothallic species [18]. The biological species concept – accompanied by morphological data – has been applied for the description of some of these heterothallic Aspergilli including *Emericella heterothallica* [3] and *Neosartorya fennelliae* [15].

The morphological (or phenotypic; [7,19]) species concept is based on the similarity of observable morphological (and usually physiological) characters, e.g., spore size and shape, or cultural characteristics. Most *Aspergillus* species have been described on the basis of these morphological features [3,20]. It is worth noting that most of *Aspergillus* species that were described many decades ago proved to be 'good' species when other approaches have been used to delimit species boundaries.

A phylogenetic species corresponds to a monophyletic group composed of the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent. The phylogenetic species concept can avoid the subjectivity of determining the limits of a species by relying on the concordance of more than one gene genealogy. Mayden [9] called this type of phylogenetic species concept as the Genealogical Concordance Concept, which was proposed by Avise and Ball [21] based on ideas of coalescence and lineage sorting. The term Genealogical Concordance Phylogenetic Species Recognition (GCPSR) has been suggested by Taylor *et al.* [8] and used in later taxonomic research [22–27]. Molecular phylogenetics has uncovered cryptic speciation in a number of taxa [22,28,29], suggesting that morphological characters provide a very broad species concept that does not reflect the true extent of evolutionary divergence and reproductive isolation, as appears to be the rule in fungi [30]. There is usually a very strong correlation between biological species in fungi defined based on laboratory mating tests and those defined based on genealogical concordance principles [25,27].

The role of genomics in species recognition is also important. The availability of multiple genomes from several species of the genus *Aspergillus* allowed examining the demarcation of fungal species at the whole-genome level [31]. Comparative intra- and interspecific analysis of the genomes revealed significant variation in the nature of species boundaries across *Aspergillus*. The values obtained from the comparison between *A. oryzae* and *A. flavus* are remarkably similar to those obtained from an intra-specific comparison of *A. fumigatus* strains, giving support to the proposal that *A. oryzae* represents a distinct ecotype of *A. flavus* and not a distinct species. Although it is unlikely that genomics will come up with a golden rule the application of which will solve all taxonomic problems in Aspergilli, genomic data can aid *Aspergillus* taxonomy by serving as a source of novel and unprecedented amounts of comparative data, and as a resource for the development of additional diagnostic tools.

The polyphasic species concept

Following the introduction of powerful molecular technologies, there has been a tendency to over-evaluate the contribution of (phylo)genetic criteria to the description of species. However, there is no one method (morphological, physiological or molecular) that works flawlessly in recognizing species. To overcome this, a polyphasic approach has been proposed about 35 years ago aiming at the integration of different kinds of data and information (phenotypic, genotypic, and phylogenetic) on microorganisms and essentially indicates a consensus type of taxonomy. The term 'polyphasic taxonomy' was probably first coined by Colwell [32] and is used for the delineation of taxa at all levels [33–35]. In some cases, the consensus classification is a compromise containing a minimum of contradictions. It is thought that the more parameters that will become available in the future, the more polyphasic classification will gain stability. In principle, all genotypic, phenotypic, and phylogenetic information may be incorporated into polyphasic taxonomy. Genotypic information is derived from the nucleic acids (DNA and RNA) present in the cell, whereas phenotypic information is derived from proteins and their functions, different chemotaxonomic markers, and a wide range of other expressed features (Fig. 1). The number of different molecules which have been applied in taxonomic studies is large, and their applications as markers are manifold [36–38]. The polyphasic approach – including biological, morphological and phylogenetic data – resulted in extremely robust species definitions for several *Fusarium* species including *Gibberella circinata* (*Fusarium circinatum*) [39] and *Gibberella konza* (*Fusarium konzum*) [40]. In recent research *Aspergillus* species have been defined based on this polyphasic approach including morphological, molecular, physiological and extrolite data [35,41–51]. Physiological characters and extrolite data often revealed differences between previously described 'cryptic species' delimited based solely on phylogenetic data, further strengthening the applicability of the polyphasic approach for species delimitation in Aspergilli [46,52]. Recently, Houbraken *et al*. [18] also successfully applied both the GCPSR and the biological species concept to identify the heterothallic *Byssochlamys spectabilis* as the teleomorph of *Paecilomyces variotii*.

Molecular characters provide the greatest number of variable characters for fungal taxonomy. However, species descriptions should include data from as many sources as possible, comprising morphology, physiology and molecular data, which can be used not only as tools for identifying an isolate, but also for understanding its biology [23].

The use of physiology and extrolites as species characters

In addition to morphological characters, isolates of *Aspergillus* can also be characterized by their profiles of secondary metabolites, by their growth rate at certain temperatures and water activities, their growth on creatine-sucrose agar and the conidial colour. These

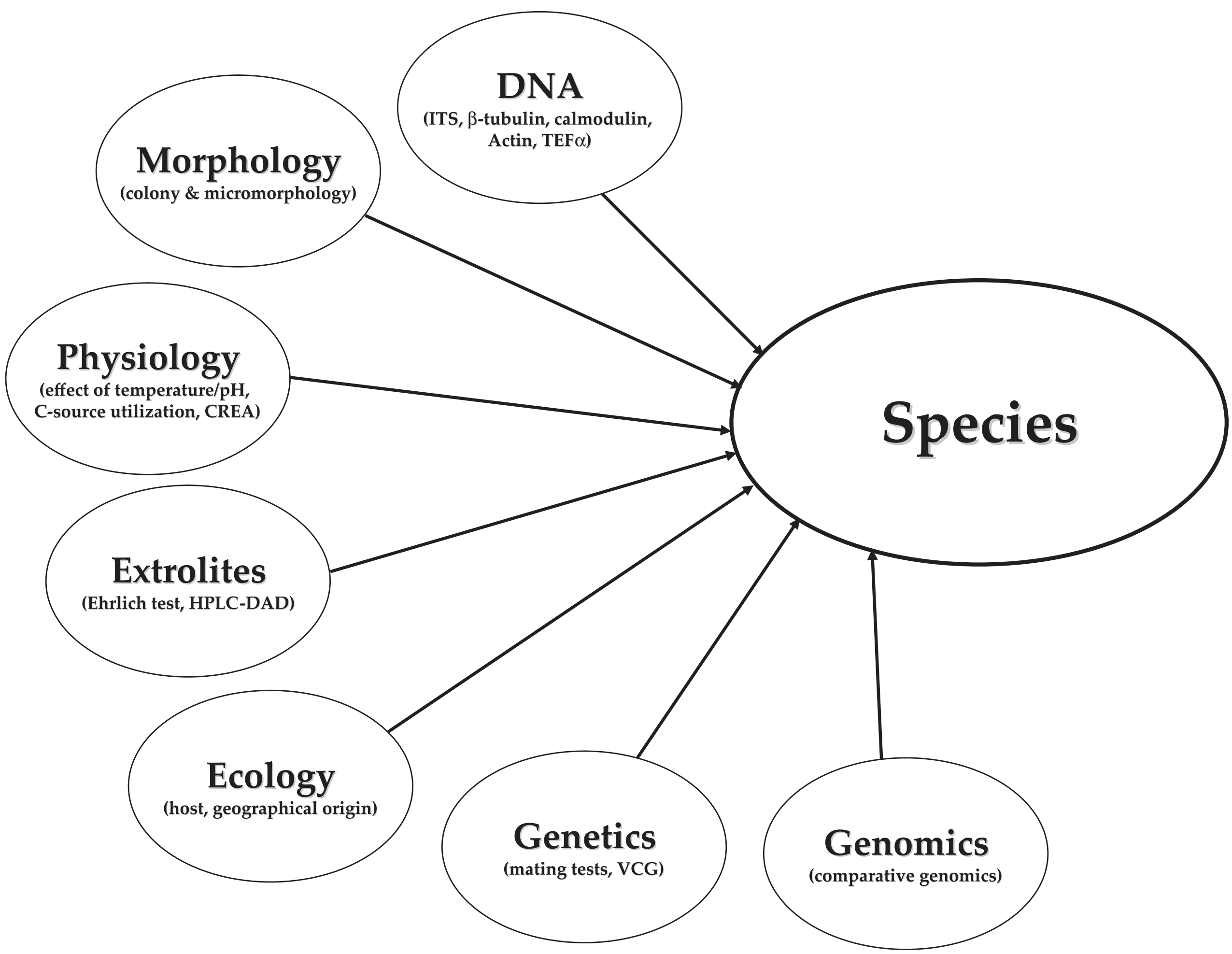

Fig. 1 Scheme showing the various data sets which can be combined for a polyphasic taxonomic classification of an *Aspergillus* taxon.

characters have for example been tested for the identification of members of *Aspergillus* section *Nigri* [48]. Secondary metabolites can be used in species recognition because they have high species specificity [53,54]. Practically all *Aspergillus* species produce a unique combination of different types of small organic compounds such as polyketides, non-ribosomal peptides, terpenoids as well as many other compounds of mixed biosynthetic origin. Some of these compounds are even unique to a single species. The fact that secondary metabolites are good phenotypic characters for species recognition is supported by the recent studies on full genome sequencing of important Aspergilli concluding that major genomic differences between species are often related to the number and similarity of polyketide synthase and non-ribosomal peptide synthetase genes [55–57]. In filamentous fungi including Aspergilli, genes responsible for secondary metabolite biosynthesis, export, and transcriptional regulation are often found in co-regulated clusters. Most of these clusters have been found to be located in subtelomeric regions, which are often associated with frequent genome rearrangements and deletions [55,56]. A more detailed comparison has showed that most pathways are novel and unique to each organism [58]. The creation of new pathways appears to involve *de-novo* assembly, segmental duplication and accelerated differentiation. The enrichment of secondary metabolite genes in subtelomeric regions in the Aspergilli may facilitate the rapid reorganization and evolution of these genes in a species-specific fashion [59].

Three independent ways of classifying and identifying Aspergilli appear to be applicable: morphology combined with physiology and nutritional features, secondary metabolite profiling and DNA sequencing. These three ways of identifying *Aspergillus* species often point to the same species. This consensus approach can be used initially, but if consensus is achieved it is recommended to combine at least two of these independent ways of characterizing Aspergilli in a polyphasic taxonomy. The chemical combination of secondary metabolites and DNA sequence features has not been explored in taxonomy yet.

Molecular characters for species delimitation: Which and how many genes are required to delimit a species?

During the discussion at the International workshop held in Utrecht (12–14 April 2007), it was suggested that genealogical concordance principles should be tested before the question can be answered of how many genes are needed to define a species. It was agreed that ITS sequences should be determined for later quick identification purposes, although sequence data are not required by the International Code of Botanical Nomenclature (ICBN) [60]. For species delimitation, the polyphasic approach was suggested as the ‘gold standard’ using a combination of multilocus sequence data, morphological, physiological characteristics and ecological data. However, no one character could be used as a ‘gold standard’ to test the null hypothesis that this is not a new species, only applying the whole set of characters will enable us to define new species. For species descriptions it is recommended to examine several (2–3) gene sequences (e.g., ITS, calmodulin, β-tubulin, actin) and submit them to recognized sequence databases. If the description of the new species meets the current conditions of the Botanical Code, it is not possible to reject a species even in the absence of sequence data. However, such an action can be recommended during reviewing manuscripts describing new *Aspergillus* species. Regarding extrolites, it was suggested that a set of 4–8 compounds to be used, rather than a single molecule. The workshop strongly recommended that it would be good to have a database (DNA and other types of characters) for quick identification; characters that are used for identification versus characters that are used to delimit a species should be separated.

Nomenclature of *Aspergillus* and its teleomorphs

The nomenclature of Aspergilli has always been an important issue because applied researchers do not like to see new names or changes in names, in particular for those which are common. Like for all other fungi, the naming of *Aspergillus* species must follow the Rules of the ICBN [60]. In the past however taxonomists have neglected these rules and for example Raper & Fennell [3] only used *Aspergillus* names for sexual taxa and refused to use the correct names for the teleomorphs.

Conservation of names is a slow and often tedious process and requires careful documentation of the problem, and establishment of the reasons for conservation. Any proposal must be approved by the Special Committee on Fungi and Lichens of the ICBN before ratification by a full Botanical Congress, a body that meets only once every six years. Approval by the Special Committee is by no means automatic. For example a proposal for the conservation of the commonly used name *Aspergillus nidulans* was not approved in 1992.

The introduction of molecular taxonomy increased the importance of conservation of species names. Old herbarium specimens, unrecognisable by traditional techniques, can nevertheless often be examined by DNA analysis. European herbaria contain many old *Aspergillus* specimens which are not viable anymore, so that cultural methods cannot be used to obtain a recognizable species, but they may still contain DNA of analysable quality. It is possible that many of the *Aspergillus* names in use are predated by names validly applied to herbarium specimens which are recognizable by molecular techniques. A solution to this type of problem was proposed by compiling a list of names in current use (NCU) of all the names in the fungal family *Trichocomaceae*, including *Aspergillus* and *Penicillium* [61,62] and although it was not formally approved by the Botanical Congress, it was recommended to use the list to stabilize nomenclature in these two important genera.

The concept of 'dual nomenclature', which simply means the use of more than one name for a single taxon, was established in the International Code of Botanical Nomenclature (ICBN) in 1910, to accommodate the problem of naming fungi that exhibit pleomorphic life cycles [63]. Article 59 of the ICBN governs the naming of these fungi. The Article has implications for many common fungi that are holomorphic, i.e., that produce both a teleomorph and an anamorph. Dual nomenclature has permitted the use, for any taxon, of either the teleomorph or the anamorph name as appropriate.

A proposal to abandon dual nomenclature – termed 'one name one fungus' – has a great deal of appeal to the theoretical mycologist. Why should a fungus have more than one name when genetic studies will often determine that it is a single species based on DNA analysis? This topic has been debated at length elsewhere [64–69], but is important here because *Aspergillus* happens to be one of the hardest genera to see a way forward. One group of scientists believes that 'the teleomorph name has precedence in the ICBN, so all species should be named according to the teleomorph with which molecular science indicates they are associated', and this approach is simplistic. First, it is not clear to what teleomorph genus some anamorph species may be associated. This is not a serious problem in *Aspergillus* but becomes very complex with species of *Trichoderma* or *Paecilomyces*. Second, many industrial users of taxonomies are now well familiar with the fact that a teleomorph name on a fungus means ascospores: use of teleomorph names for species without ascospores can only cause loss of information. Third, that approach requires many name changes. It is most unlikely that practical users of taxonomies would ever accept those new names, and confusion would result.

It was suggested at the recent International *Aspergillus* workshop to use the same species name for both the anamorph and teleomorph, although it is not invalid to use a second name according to the Botanical Code. A single culture should have only one name, but again it is not invalid to use a second name. Several participants suggested using *Aspergillus* as the primary name and the teleomorph as a secondary name, but according to the ICBN the second name will be invalid. Another suggestion was to epitypify all new species to prepare for a single name, although again, it is not in agreement with the Botanical Code. Regarding clinical researchers who depend on one of the names, it was suggested to use the name 'complex' if identification is based on morphology, which cannot distinguish between closely related species. The majority of the participants agreed to give preference to the teleomorph name. Researchers working on *Aspergillus* genetics mentioned that in a specific field, a name has a certain meaning, and preferred the name *Aspergillus* for phylogeny and genetics. Another suggestion was to give the name as follows: teleomorph [anamorph genus] if both exist, but it was rejected by most participants because names should not become too long by combining anamorph and teleomorph names. In the final vote, it was accepted by most participants to use dual names where necessary, single name in normal use, depending on the state that is seen, and treat it as a recommendation. Regarding Latin description of new species, it was accepted to have a short Latin diagnosis, followed by a more detailed English description. The majority of the workshop showed a strong interest in the need for a separate fungal nomenclatural code such as the code which the bacteriologists use.

What are the standards for describing species and storing type cultures?

It was proposed that ex type cultures of new species be deposited in 2–3 different internationally recognized culture collections. If there is no type culture available, the *Aspergillus* community has the option of declaring it invalid. There should be a limit to the time (e.g., six months) between publishing and depositing in collections. These suggestions are not accommodated by the

Botanical Code, but were accepted as recommendations for good practice. New taxa should always be compared with ex type cultures of related species.

Aspergillus databases

Databases are critical for identification and biology. It was agreed that different specialized databases are needed for key identification purposes, with good links between databases. However, each database requires funding and curation. The use of a Wikipedia approach for the databases was questioned because of a lack of quality control, although such an approach could be used for general information regarding protocols and media. It was suggested to use the *Aspergillus/Penicillium* website (http://www.aspergilluspenicillium.org) as a clearinghouse by linking to other sites. It was accepted that links should show the focus of each individual database, and there is a need for links to other communities as well. The databases that are linked to the *Aspergillus* website should be of high quality. Some overlap between databases is not a real problem. Regarding a simple database for species identification, it was suggested to include basic sequences for identification, pictures and links to media protocols. Although such a database already exists, it is in a more complicated form.

Conclusions

The recommendations of an international panel of mycologists working in various fields of *Aspergillus* research for criteria of species delimitation are unique in mycology. To answer the question formulated in the title, a species in the *Aspergillus* genus should be delimited based on a combination of criteria including molecular, morphological, physiological and other characteristics, i.e., by using the polyphasic approach. It is also unique that a protocol is proposed how to describe a species with all its conditions of formal descriptions and depositing type cultures. The opinions of the *Aspergillus* researchers may deviate from those working with other fungal groups, but it is important to note that the applied aspects were taken as an important part of their considerations. Hopefully the recommendations, put forward in this paper, will aid in delimiting new taxa in this economically important genus, and help to clarify the evolutionary genetics of Aspergilli. These recommendations for species delimitation, descriptions and identification are an important milestone in *Aspergillus* mycology. A more detailed overview of the recommendations is given by Samson *et al*. [70].

Acknowledgements

The authors are both employees of the Royal Netherlands Academy of Arts and Sciences (Amsterdam, the Netherlands).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1 Varga J, Samson RA (eds). *Aspergillus in the Genomic Era*. Wageningen: Wageningen Academic Publishers, 2008.
2 Samson RA, Hoekstra ES, Frisvad JC (eds). *Introduction to Food and Airborne Fungi.* 7th ed. Utrecht: Centraalbureau voor Schimmelcultures, 2004.
3 Raper KB, Fennell DI. *The Genus Aspergillus*. Baltimore, MD: Williams & Wilkins, 1965.
4 Balajee SA, Houbraken J, Verweij PE, *et al*. *Aspergillus* species identification in the clinical setting. *Stud Mycol* 1997; **59**: 39–46.
5 Perrone G, Susca A, Cozzi G, *et al*. Biodiversity of *Aspergillus* species in some important agricultural products. *Stud Mycol* 2007; **59**: 53–66.
6 Mayr E. *Systematics and the Origin of Species*. Irvington: Columbia University Press, 1942.
7 Hey J. The mind of the species problem. *Trends Ecol Evol* 2001; **16**: 326–329.
8 Taylor JW, Jacobson DJ, Kroken S, *et al*. Phylogenetic species recognition and species concepts in fungi. *Fungal Genet Biol* 2000; **31**: 21–32.
9 Mayden RL. A hierarchy of species concepts: The denouement in the saga of the species problem. In: M. F. Claridge MF, Dawah HA, Wilson MR (eds). *Species: The Units of Biodiversity.* London: Chapman & Hall, 1997: 381–424.
10 Dobzhansky T. *Genetics and the Origin of Species*. Irvington: Columbia University Press, 1937.
11 Wilson EO. *The Diversity of Life*. Cambridge: Harvard University Press, 1992.
12 Summerell BA, Salleh B, Leslie JF. A utilitarian approach to *Fusarium* identification. *Plant Dis* 2003; **87**: 117–128.
13 Takada M, Horie Y, Abliz P. Two new heterothallic *Neosartorya* from African soil. *Mycoscience* 2001; **42**: 361–367.
14 Takada M, Udagawa S. A new species of heterothallic *Neosartorya*. *Mycotaxon* 1985; **24**: 395–402.
15 Kwon-Chung KJ, Kim SJ. A second heterothallic *Aspergillus*. *Mycologia* 1974; **66**: 628–638.
16 Horie Y, Miyaji M, Nishimura K, Franco MF, Coelho KIR. New and interesting species of *Neosartorya* from Brazilian soil. *Mycoscience* 1995; **36**: 199–204.
17 Takada M, Udagawa S. A new species of heterothallic *Talaromyces*. *Mycotaxon* 1988; **31**: 417–425.
18 Houbraken J, Varga J, Rico-Munoz E, Johnson S, Samson RA. Sexual reproduction as a cause of heat resistance in the food spoilage fungus *Byssochlamys spectabilis* (anamorph *Paecilomyces variotii*). *Appl Environ Microbiol* 2008; 1613–1619.
19 Tibayrenc M. The species concept in parasites and other pathogens: a pragmatic approach? *Trends Parasitol* 2006; **22**: 66–70.
20 Thom C, Church MB. *The Aspergilli*. Baltimore: Williams & Wilkins, 1926.

21 Avise JC, Ball RM. Principles of genealogical concordance in species concepts and biological taxonomy. In: Futuyma D, Antonovics J (eds). *Oxford Surveys in Evolutionary Biology.* Oxford: Oxford University Press, 1990: 45–67.
22 Geiser DM, Pitt JI, Taylor JW. Cryptic speciation and recombination in the aflatoxin-producing fungus *Aspergillus flavus. Proc Natl Acad Sci USA* 1998; **95**: 388–393.
23 Geiser DM, Klich MA, Frisvad JC, *et al*. The current status of species recognition and identification in *Aspergillus. Stud Mycol* 2007; **59**: 1–10.
24 Dettman JR, Jacobson DJ, Turner E, Pringle A, Taylor JW. Reproductive isolation and phylogenetic divergence in *Neurospora*: comparing methods of species recognition in a model eukaryote. *Evolution* 2003; **57**: 2721–2741.
25 O'Donnell K, Cigelnik E, Nirenberg HI. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* 1998; **90**: 465–493.
26 O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genet Biol* 2004; **41**: 600–623.
27 O'Donnell K. Molecular phylogeny of the *Nectria haematococca–Fusarium solani* species complex. *Mycologia* 2000; **92**: 919–938.
28 Pringle A, Baker DM, Platt JL, *et al*. Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus *Aspergillus fumigatus. Evolution* 2005; **59**: 1886–1899.
29 Balajee SA, Gribskov JL, Hanley E, Nickle D, Marr KA. *Aspergillus* lentulus sp. nov., a new sibling species of *A. fumigatus. Eukaryot Cell* 2005; **4**: 625–632.
30 Geiser DM. Practical molecular taxonomy of fungi. In: Lange L, Tkacz J (eds). *Advances in Fungal Biotechnology for Industry, Medicine and Agriculture.* New York: Kluwer Academic Publishers, 2004: 1–12.
31 Rokas A, Payne G, Fedorova ND, *et al*. What can comparative genomics tell us about species concepts in the genus *Aspergillus*? *Stud Mycol* 2007; **59**: 11–17.
32 Colwell RR. Polyphasic taxonomy of the genus *Vibrio*: numerical taxonomy of *Vibrio cholerae, Vibrio parahaemolyticus,* and related Vibrio species. *J Bacteriol* 1970; **104**: 410–433.
33 Murray RGE, Brenner DJ, Colwell RR, *et al*. Report of the ad hoc committee on approaches to taxonomy within the Proteobacteria. *Int J Syst Bacteriol* 1990; **40**: 213–215.
34 Frisvad JC, Samson RA. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. *Stud Mycol* 2004; **49**: 1–174.
35 Hong SB, Cho HS, Shin HD, Frisvad JC, Samson RA. Novel *Neosartorya* species isolated from soil in Korea. *Int J Syst Evol Microbiol* 2006; **56**: 477–486.
36 Rossello-Mora R. Opinion: the species problem, can we achieve a universal concept? *Syst Appl Microbiol* 2003; **26**: 323–326.
37 Uilenberg G, Goff WL. Polyphasic taxonomy. *Ann NY Acad Sci* 2006; **1081**: 492–497.
38 Vandamme P, Pot B, Gillis M, *et al*. Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev* 1996; **60**: 407–438.
39 Nirenberg HI, O'Donnell K. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* 1998; **90**: 434–458.
40 Zeller KA, Summerell BA, Bullock S, Leslie JF. *Gibberella konza* (*Fusarium konzum*) sp. nov. from prairie grasses, a new species in the *Gibberella fujikuroi* species complex. *Mycologia* 2003; **95**: 943–954.
41 Hong SB, Go SJ, Shin HD, Frisvad JC, Samson RA. Polyphasic taxonomy of *Aspergillus fumigatus* and related species. *Mycologia* 2005; **97**: 1316–1329.
42 Hong SB, Shin HD, Hong JB, *et al*. New taxa of *Neosartorya* and *Aspergillus* in *Aspergillus* section *Fumigati*. *Antonie van Leeuwenhoek* 2007; **93**: 87–98.
43 Houbraken J, Due M, Varga J, *et al*. Polyphasic taxonomy of *Aspergillus* section. *Usti Stud Mycol* 2007; **59**: 107–128.
44 Noonim P, Mahakarnchanakul W, Varga J, Frisvad JC, Samson RA. Two new species of *Aspergillus* section *Nigri* from Thai coffee beans. *Int J Syst Evol Microbiol* 2008; **58**: 1727–1734.
45 Perrone G, Varga J, Susca A, *et al*. *Aspergillus uvarum* sp. nov., an uniseriate black *Aspergillus* species isolated from grapes in Europe. *Int J Syst Evol Microbiol* 2008; **58**: 1032–1039.
46 Pildain MB, Frisvad JC, Vaamonde G, *et al*. Two new aflatoxin producing *Aspergillus* species from Argentinean peanuts. *Int J Syst Evol Microbiol* 2008; **58**: 725–735.
47 Samson RA, Hong SB, Peterson SW, Frisvad JC, Varga J. Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph, *Neosartorya*. *Stud Mycol* 2007; **59**: 147–203.
48 Samson RA, Noonim P, Meijer M, *et al*. Diagnostic tools to identify black Aspergilli. *Stud Mycol* 2007; **59**: 129–146.
49 Varga J, Due M, Frisvad JC, Samson RA. Taxonomic revision of *Aspergillus* section *Clavati* based on molecular, morphological and physiological data. *Stud Mycol* 2007; **59**: 89–106.
50 Varga J, Frisvad JC, Samson RA. Polyphasic taxonomy of *Aspergillus* section *Candidi* based on molecular, morphological and physiological data. *Stud Mycol* 2007; **59**: 75–88.
51 Varga J, Kocsubé S, Tóth B, *et al*. *Aspergillus brasiliensis* sp. nov., a biseriate black *Aspergillus* species with world-wide distribution. *Int J Syst Evol Microbiol* 2007; **57**: 1925–1932.
52 Larsen TO, Smedsgaard J, Nielsen KF, *et al*. Production of mycotoxins by *Aspergillus lentulus* and other medically important and closely related species in section *Fumigati*. *Med Mycol* 2007; **45**: 225–232.
53 Frisvad JC. The use of high-performance liquid chromatography and diode array detection in fungal chemotaxonomy based on profiles of secondary metabolites. *Bot J Linnean Soc* 1989; **99**: 81–95.
54 Larsen TO, Smedsgaard J, Nielsen KF, Hansen ME, Frisvad JC. Phenotypic taxonomy and metabolite profiling in microbial drug discovery. *Nat Prod Rep* 2005; **22**: 672–693.
55 Galagan JE, Calvo SE, Cuomo C, *et al*. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae. Nature* 2005; **438**: 1105–1115.
56 Nierman WH, Pain A, Anderson MJ, *et al*. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus. Nature* 2005; **438**: 1151–1156.
57 Pel HJ, de Winde JH, Archer DB, *et al*. Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. *Nature Biotechnol* 2007; **25**: 221–231.
58 Nierman WC, Fedorova N. Subtelomeric diversity as a major force in evolution of *Aspergillus* secondary metabolism and virulence pathways. In: *3rd Advances Against Aspergillosis*, Miami, 16–19 January, 2008. San Diego: University of California, 2008: 77.

59 Carbone I, Ramirez-Prado JH, Jakobek JL, Horn BW. Gene duplication, modularity and adaptation in the evolution of the aflatoxin gene cluster. *BMC Evol Biol* 2007; **7**: 111.
60 McNeill J, Barrie FR, Burdet HM, *et al*. *International Code of Botanical Nomenclature (Vienna Code)*. Adopted by the 17th International Botanical Congress Vienna, Austria, July 2005. Regnum Vegetabile 146. Ruggell, Lichtenstein: ARG Gantner Verlag KG, 2006.
61 Pitt JI, Samson RA. Species names in current use in the *Trichocomaceae* (Fungi, Eurotiales). In: Greuter W (ed.)., *Names in Current Use in the Families Trichocomaceae, Cladoniaceae, Pinaceae, and Lemnaceae.* Königstein, Germany: Koeltz Scientific Books, 1993: 13–57 (*Regnum Vegetabile* 128: 13–57).
62 Pitt JI, Samson RA, Frisvad JC. List of accepted species and their synonyms in the family *Trichocomaceae.* In: Samson RA, Pitt JI (eds). *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*. Amsterdam: Harwood Publishers, 2000: 9–49.
63 Cline E. Implications of changes to Article 59 of the International Code of Botanical Nomenclature enacted at the Vienna Congress, 2005. *Inoculum* 2005; **56**: 3–5.
64 Gams W. Towards a single scientific name for species of fungi: a rebuttal. *Inoculum* 2005; **56**: 1–3.
65 Gams W, Korf RP, Pitt JI, Hawksworth DL, Berbee ML, Kirk PM (edited by Seifert K). Has dual nomenclature for fungi run its course? The Article 59 debate. *Mycotaxon* 2003; **88**: 493–508.
66 Hawksworth DL. Limitation of dual nomenclature for pleomorphic fungi. *Taxon* 2004; **53**: 596–598.
67 Rossman AY, Samuels GJ. Towards a single scientific name for species of fungi. *Inoculum* 2005; **56**: 1–3.
68 Martin GW. The genus *Aspergillus. Science* 1946; **103**: 116–117.
69 Thom C, Raper KB. *Aspergillus* or what? *Science* 1946; **103**: 735.
70 Samson RA, Varga J, Witiak SM, Geiser DM. The species concept in *Aspergillus*: recommendations of an international panel. *Stud Mycol* 2007; **59**: 71–74.

This paper was first published online on iFirst on 28 February 2009.