Appl Microbiol Biotechnol (2012) 95:339–344
DOI 10.1007/s00253-012-4165-2

MINI-REVIEW

Aspergillus and *Penicillium* identification using DNA sequences: barcode or MLST?

Stephen W. Peterson

Received: 28 March 2012 / Revised: 9 May 2012 / Accepted: 10 May 2012 / Published online: 27 May 2012

Abstract Current methods in DNA technology can detect single nucleotide polymorphisms with measurable accuracy using several different approaches appropriate for different uses. If there are even single nucleotide differences that are invariant markers of the species, we can accomplish identification through rapid DNA-based tests. The question of whether we can reliably detect and identify species of *Aspergillus* and *Penicillium* turns mainly upon the completeness of our alpha taxonomy, our species concepts, and how well the available DNA data coincide with the taxonomic diversity in the family Trichocomaceae. No single gene is yet known that is invariant within species and variable between species as would be optimal for the barcode approach. Data are published that would make an MLST approach to isolate identification possible in the most well-studied clades of *Aspergillus* and *Penicillium*.

Keywords Barcode · MLST · Species concept · Trichocomaceae

Introduction

Aspergillus and *Penicillium* species are widespread and common, and particular species impact human activities either positively or negatively. *Aspergillus versicolor* is a cosmopolitan species often used as an indicator species for sick building syndrome (Anderson et al. 2011). Additionally it produces the mycotoxin sterigmatocystin (Veršilovskis and Saeger 2010) in several commodities. *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus* (Walsh et al. 2011), and *Talaromyces* (syn. = *Penicillium*) *marneffei* (Sudjaritruk et al. 2012) are recognized opportunistic pathogens of humans, especially those with weakened immune systems. *Aspergillus niger* is used for the production of enzymes and citric acid (e.g., Dhillon et al. 2012), *Aspergillus oryzae* is used to produce soy sauce, *Penicillium roqueforti* ripens blue cheeses and penicillin is produced by *Penicillium chrysogenum* (e.g., Xu et al. 2012). Heat-resistant *Byssochlamys* species can grow in pasteurized fruit juices (Sant'ana et al. 2010). These genera along with a few others comprise the family Trichocomaceae. Because of their impact on our lives, it is often important to have accurate identifications of these species.

Traditional taxonomic studies rely primarily on phenotypic analysis (e.g., Raper and Fennell 1965). Sequence analysis for taxonomic purposes in *Aspergillus* and *Penicillium* began in the late 1980s (e.g., Edman et al. 1986; Olsen et al. 1986; Dupont et al. 1990; Logrieco et al. 1990) using rRNA sequences, and advanced to analysis of protein-coding genes soon after PCR methods (Glass and Donaldson 1995), thermal cycler units, dye-based sequence reactions, and automated sequence readers became readily available. The Barcode of Life Consortium (http://connect.barcodeoflife.net/group/fungi) and PubMLST (http://pubmlst.org) provide frameworks for advancing the use of DNA for species identifications. In this communication, I discuss some of the factors affecting the possibility of detecting and identifying the species of the Trichocomaceae using DNA sequences.

What is a species

At one time it was generally accepted that species resulted from a special act of creation, but Darwin argued in the

S. W. Peterson (✉)
Bacterial Foodborne Pathogens and Mycology Research Unit,
National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture,
1815 North University Street,
Peoria IL 61604, USA
e-mail: stephen.peterson@ars.usda.gov

1860 s that individuals were mutable, mutations were heritable, and that natural selection could change the essential character of the species. Since then the nature of species and how to detect the limits of each has been a regular topic of debate (e.g., de Queiroz 2007; Doolittle and Bapteste 2007; Ereshefsky 2011). How we define and recognize species is the critical issue in DNA-based identification.

In a colloquial sense, a species is an essentialistic entity, meaning that a set of characteristics or properties exist, all of which any individual of the species must possess, and these properties or characteristics define the species. The set of useful or harmful characteristics is the practical reason for knowing species and the set of characteristics may be simple or complex. For Link in 1809, *A. flavus* was defined by the possession of the aspergillum and yellow-green color, while in 1869 *A. niger* Tiegh. was defined by the aspergillum and dark brown to black color. On the basis of a far more sophisticated phenotypic analysis, Raper and Fennell (1965) recognized 148 distinct *Aspergillus* taxa. Pitt et al. (2000) accepted 185 taxa in *Aspergillus* 35 years later. Some of the species number increase came from discovery of germplasm not seen before and some came from the reexamination and splitting of previously known but variable species. Raper and Thom (1949) accepted 139 *Penicillium* species, while 51 years later Pitt et al. (2000) accepted 239 species.

As a matter of formality, fungal species are established under the rules of botanical nomenclature. The requirements are that a type specimen must be permanently preserved in a publicly accessible herbarium, a description of the features that make this new species identifiable must be formulated and the description and the designation of the type must be published in a non-ephemeral medium. Different techniques have been introduced to aid taxonomic decisions such as SEM (Kozakiewicz 1989), secondary metabolite analysis (Frisvad and Filtenborg 1989), or single locus DNA sequence analysis (Peterson 2000; Samson and Frisvad 2004), but the morphology of the type specimen has always held the position of primacy in defining a fungal species.

A significant shift in taxonomic philosophy was published by Taylor et al. (2000). They proposed adoption of Simpson's (1951) evolutionary species concept, "…a phyletic lineage (ancestral-descendent sequence of interbreeding populations) evolving independently of others, with its own separate and unitary evolutionary role and tendencies…" for fungi. Rather than rely on morphological distinctions seen in a type specimen, they endorsed genealogical concordance phylogenetic species recognition (GCPSR) as the method to identify the species. Recent studies have validated the view of Taylor et al. (2000). Dettman et al. (2003, 2006) have correlated GCPSR with mating potential in the genus *Neurospora*. In Trichocomaceae, Peterson and Horn (2009) using GCPSR analysis showed that the morphologically defined *Penicillium cinnamopurpureum* included elements of six phylogenetically distinct species. Perrone et al. (2011) recognized the cryptic species *Aspergillus awamorii* on the basis of phylogenetic analysis of DNA sequence data and refer to the other eight described species in the "*A. niger* aggregate" as morphologically indistinguishable. Soares et al. (2012) described three new species in the *A. flavus* clade on the basis of genealogical concordance analysis of DNA sequences, even though those species were barely distinguishable morphologically from *A. flavus*. Morphological analysis sometimes leads to the grouping together of disparate species due to convergence of characters, and in other cases morphological divergence does not occur as quickly as genotypic divergence and the species are morphologically indistinguishable. Genealogical concordance phylogenetic analysis is a consistent measure of genetic divergence and an objective means of asserting the limits of species.

Nomenclature

Nomenclature rules for fungi are defined in the International Code of Botanical Nomenclature (ICBN). The ICBN is updated periodically with the most recent changes due to be published in summer 2012. The question of nomenclature arises because *Aspergillus* and *Penicillium* are pleomorphic fungi, living either solely as the anamorphic (asexual) phase, the teleomorphic (sexual) phase, or the holomorph (both phases) and some species have only one known state. Major monographers of *Aspergillus* and *Penicillium* (Raper and Thom 1949; Raper and Fennell 1965) in the middle of the twentieth century believed that asexual morphology revealed phylogenetic relationships and placed all fungi with *Penicillium* anamorphs in that genus whether they also made a teleomorph or not. They used the same philosophy in their study of *Aspergillus*. However, following nomenclatural rules that gave precedence to names based on sexual reproductive morphology, Benjamin (1955) segregated certain sexually reproducing *Penicillium* species into the genus *Talaromyces*, Stolk and Samson (1971) renamed some *Penicillium* species into the genus *Hamigera*, and Stolk and Scott (1967) moved some *Penicillium* species into *Eupenicillium* based on sexual reproduction.

Using the same nomenclature, Horn et al. (2009) discovered the teleomorph of *A. flavus* and introduced the name *Petromyces flavus* for the fungus. Peterson (1992) gave the names *Aspergillus thermomutatus* and *Neosartorya pseudofischeri* to a single species opportunistically causing human disease that displays both *Aspergillus* and *Neosartorya* morphologies. In a naming system based solely on phenotypic distinctions and dried herbarium-type specimens, the assignment of closely related species into different genera on the basis of whether they possess a teleomorph or not was a

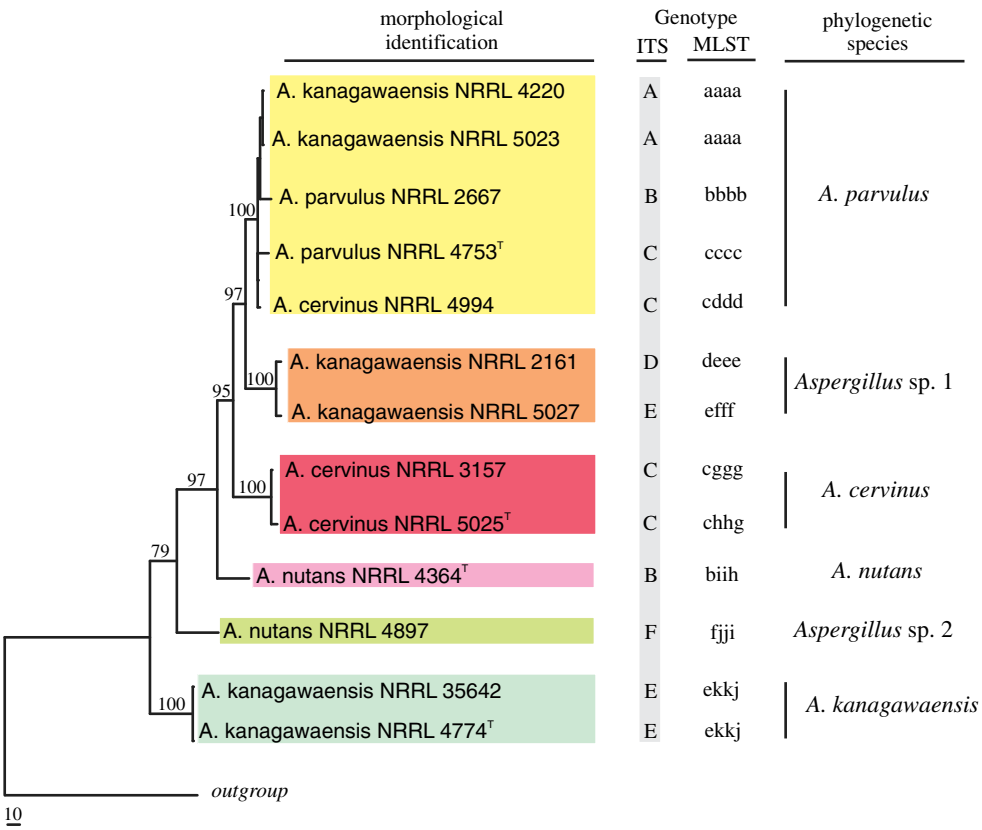

Fig. 1 Maximum parsimony tree of 13 *Aspergillus* section *Cervini* isolates using sequence data from four loci, *numbers above tree nodes* are bootstrap values. The morphological identification for each isolate is written out in the *colored boxes*. The *colored boxes* show the limits of species determined from GCPSR analysis of four-locus DNA data (Peterson 2008). Nuclear ITS, beta tubulin, calmodulin, and RNA polymerase beta genotypes are given arbitrary letter designations and listed by the isolates. ITS genotypes are shared by different species and do not always uniquely identify species; protein-coding loci are variable in some species but species do not share genotypes and each MLST genotype correctly identifies the isolates

rational way to deal with identification. Consequently taxonomists of the Trichocomaceae have created a system of dual names for most species in the family.

The most recent changes to the nomenclatural code (Knapp et al. 2011; McNeil and Turland 2011; Norvell 2011) allow phylogenetically related organisms to be treated together under a single generic name even when they display different morphs and it now allows anamorphic and teleomorphic names to compete equally on the basis of priority. Name changes, based solely on compliance with nomenclatural rules, will likely occur in the Trichocomaceae based on the application of DNA sequence-based phylogenetic analysis (e.g., Samson and Frisvad 2004; Houbraken et al. 2007; Peterson 2000; Peterson 2008; Varga et al. 2011). Samson et al. (2011) applied the single name philosophy to one major clade of Trichocomaceae consolidating *Talaromyces* and *Penicillium* subgenus *Biverticillium* species under the name *Talaromyces*, regardless of whether the species form the *Talaromcyes* state or not. They rely on phylogenetic rather than phenotypic analysis of relatedness to guide naming. The other primary clade with *Penicillium* morphology contains *Penicillium* species from three subgenera and *Eupenicillium* species. Some authors have started treating this clade under the singular name *Penicillium* (Houbraken et al. 2011; Peterson et al. 2011). The application of phylogenetic analysis and unitary naming will result in stable, reliable, and intuitively understandable taxonomy.

Barcodes

Barcoding initially used cytochrome oxidase 1 (*COI*) for barcode identification of animals. Seifert et al. (2007) tested the applicability of COI for barcoding species of *Penicillium* subg. *Penicillium* (Samson and Frisvad 2004) and found that not all species possessed distinct genotypes at the COI locus. Seifert (2009) and Eberhardt (2010) have discussed

Table 1 Phenotypic species identification, phylogenetic species identifications, ITS barcode genotypes and ITS, BT, CF, and RPB MLST genotypes for isolates of *Aspergillus* section *Cervini*

	ITS genotype	MLST genotype
Phenotypic species		
A. kanagawaensis	A, D, E,	aaaa, deee, efff, ekkj
A. parvulus	B, C	bbbb, cccc,
A. cervinus	C	cddd, cggg, chhg
A. nutans	B, F	biih, fjji
Phylogenetic species		
A. parvulus	A, B, C	aaaa, bbbb, cccc, cddd
Aspergillus sp. 1	D, E	deee, efff
A. cervinus	C	cggg, chhg
A. nutans	B	biih
Aspergillus sp. 2	F	fjji
A. kanagawaensis	E	ekkj

some of the candidate loci for a fungal barcode. Schoch et al. (2012) have a detailed proposal to use internal transcribed spacer (ITS) region as the universal fungal barcode locus. While limitations exist for this molecule, the ease of amplification, primer universality, and very good level of sensitivity make the prospects for correct or near-correct identification in the entire fungal kingdom likely. Some of the Trichocomaceae species can be barcode identified using ITS but for others the ITS barcode is problematic.

Peterson (2008) as part of a larger study examined 13 isolates of four phenotypically defined species placed in *Aspergillus* section *Cervini* by Raper and Fennell (1965). That study used multi-locus DNA sequence data and GCPSR analysis to define species. Of six isolates phenotypically identified initially as *Aspergillus kanagawaensis* (Fig. 1), two are *A. kanagawaensis*, two belong in *Aspergillus parvulus*, and two other belong in an undescribed species; of the three isolates morphologically identified as *Aspergillus cervinus*, two are in *A. cervinus* and one is in *A. parvulus*; of the two *Aspergillus nutans* isolates, one is *A. nutans* and one represents an undescribed species. Phenotypic analysis fails to correctly identify these isolates and species.

Using the phenotypic taxonomy of Raper and Fennell (1965), the phylogenetic systematics of Peterson (2008) and arbitrarily assigning a letter to each unique ITS region genotype (Table 1, Fig. 1), phenotypic *A. kanagawaensis* isolates displayed three genotypes, *A. cervinus* isolates displayed one genotype, *A. parvulus* isolates display two genotypes, and *A. nutans* isolates display two ITS genotypes. However, *A. parvulus* and *A. cervinus* share ITS genotype C, and *A. parvulus* and *A. nutans* share the ITS genotype B rendering identification in either case uncertain. In terms of phylogenetic species, *A. kanagawaensis* isolates have one ITS genotype, *A. cervinus* isolates have one ITS genotype, *A. nutans* isolates have one ITS genotype, *A. parvulus* isolates have three ITS genotypes, undescribed sp.1 has two ITS genotypes, and undescribed sp.2 has one ITS genotype. Using phylogenetic species concepts, *A. cervinus* and *A. parvulus* share ITS genotype C, and *A. kanagawaensis* and *Aspergillus* sp. 1 share genotype E, rendering identification uncertain. Using the data from *Aspergillus* section *Cervini*, (Table 1, Fig. 1) and ITS, beta tubulin, calmodulin, and DNA-dependent RNA polymerase beta loci, each of the species (either phenotypic or phylogenetic) have MLST genotypes that are not overlapping between species, rendering a specific identification for each isolate.

The protein-coding region genotypes often display polymorphisms within species, and genotypes are not shared between species making them a potential target for barcoding. However the lack of primer sequence universality (Schoch et al. 2012) and presence of variable sized introns limits the application of protein-coding regions for all fungi barcoding.

MLST

Maiden et al. (1998) proposed a DNA sequence multi-locus sequence typing system for isolates of *Neisseria meningitidis*. The system is appealing in fungi too because DNA sequences are measurably accurate and unambiguous, easily transmittable via the web, and can be compared at a central worldwide data center. MLST is not confounded by different locus-associated rates of evolution nor does it rely on a single locus that could be affected by horizontal gene transfer. Balajee (2008) suggested a two-tiered identification system for species of *Aspergillus*, particularly the medically important species. Sectional or group identification would be based on ITS sequences, and species or infraspecific taxa would be identified on the basis of protein-coding gene sequences. This has the positive value of being able to take advantage of MLST schemes for medically important species that have been worked out using various loci. Short et al. (2011) produced a six-locus MLST scheme for *Fusarium* species found in hospital environments. Balmas et al. (2010) produced a MLST system for studying soil species of *Fusarium*.

At this time, the alpha taxonomy of the Trichocomaceae is well advanced, and current investigations are mostly utilizing GCPSR analysis of DNA sequence data to determine species limits in revisions of the sections. The data largely exist for a three- or four-locus identification system in non-medically important *Aspergillus* species using beta tubulin, calmodulin, DNA-dependent RNA polymerase, and nuclear ITS loci. MLST will provide an accurate and reliable DNA sequence-based identification system for the Trichocomaceae.

Acknowledgments The author gratefully acknowledges the skilled technical support provided by Amy E. McGovern. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

References

Anderson B, Frisvad JC, Sondergaard I, Rasmussen IB, Larsen LS (2011) Associations between fungal species and water-damaged building materials. Appl Environ Microbiol 77:4180–4188

Balajee SA (2008) DNA based methods for species identification in the genus *Aspergillus*. In: Varga J, Samson RA (eds) *Aspergillus* in the genomic era. Wageningen Academic, Wageningen, pp 261–276

Balmas V, Migheli Q, Scherm B, Garau P, O'Donnell K, Ceccherelli G, Kang S, Geiser DM (2010) Multilocus phylogenetics show high levels of endemic fusaria inhabiting Sardinian soils (Tyrrhenian Islands). Mycologia 102:803–812

Benjamin CR (1955) Ascocarps of *Aspergillus* and *Penicillium*. Mycologia 47:669–687

De Queiroz K (2007) Species concepts and species delimitation. Syst Biol 56:879–886

Dettman JR, Jacobson DJ, Turner E, Pringle A, Taylor JW (2003) Reproductive isolation and phylogenetic divergence in *Neurospora*: comparing methods of species recognition in a model Eukaryote. Evolution 57:2721–2741

Dettman JR, Jacobson DJ, Taylor JW (2006) Multilocus sequence data reveal extensive phylogenetic species diversity within the *Neurospora discreta* complex. Mycologia 98:436–446

Dhillon GS, Brar SK, Kaur S, Verma M (2012) Rheological studies during submerged citric acid fermentation by *Aspergillus niger* in stirred fermentors using apple pomace ultrafiltration sludge. Food Bioprocess Technol. doi:10.1007/s11947-011-0771-8

Doolittle WF, Bapteste E (2007) Pattern pluralism and the tree of life hypothesis. Proc Natl Acad Sci (USA) 104:2043–2049

Dupont J, Dutertre M, Lafay J-F, Roquebert MF, Brygoo Y (1990) A molecular assessment of the position of *Stilbothamnium* in the genus *Aspergillus*. In: Samson RA, Pitt JI (eds) Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum, New York, pp 335–342

Eberhardt U (2010) A constructive step towards selecting a DNA barcode for fungi. New Phytol 187:266–268

Edman JC, Kovacs JA, Masur H, Santi DV, Elwood HJ, Sogin ML (1986) Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. Nature 334:519–522

Ereshefsky M (2011) Mystery of mysteries: Darwin and the species problem. Cladistics 27:67–79

Frisvad JC, Filtenborg O (1989) Terverticillate penicillia: chemotaxonomy and mycotoxin production. Mycologia 81:837–861

Glass NL, Donaldson G (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330

Horn BW, Moore GG, Carbone I (2009) Sexual reproduction in *Aspergillus flavus*. Mycologia 101:423–429

Houbraken J, Due M, Varga J, Meijer M, Frisvad JC, Samson RA (2007) Polyphasic taxonomy of *Aspergillus* section *Usti*. Stud Mycol 59:107–128

Houbraken J, López-Quintero CA, Frisvad JC, Boekhout T, Theelen B, Franco-Molano AE, Samson RA (2011) *Penicillium araracuarense* sp. nov., *Penicillium elleniae* sp. nov., *Penicillium penarojense* sp. nov., *Penicillium vanderhammenii* sp. nov. and *Penicillium wotroi* sp. nov., isolated from leaf litter. Int J Syst Evol Microbiol 61:1462–1475

Knapp S, McNeil J, Turland NJ (2011) Fungal Nomenclature. Changes to publication requirements made at the XVIII International Botanical Congress in Melbourne—what does e-publication mean for you? Mycotaxon 117:509–515

Kozakiewicz Z (1989) *Aspergillus* species on stored products. Mycol Pap 161:1–188

Logrieco A, Peterson SW, Wicklow DT (1990) Ribosomal RNA comparisons among taxa of the terverticillate penicillia. In: Samson RA, Pitt JI (eds) Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum, New York, pp 343–356

Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG (1998) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci (USA) 95:3140–3145

McNeil J, Turland NJ (2011) Major changes to the Code of Nomenclature—Melbourne, July 2011. Taxon 60:1495–1497

Norvell LL (2011) Melbourne approves a new CODE. Mycotaxon 116:481–490

Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR, Stahl DA (1986) Microbial ecology and evolution: a ribosomal RNA approach. Annu Rev Microbiol 40:337–365

Perrone G, Stea G, Epifani F, Varga J, Frisvad JC, Samson RA (2011) *Aspergillus niger* contains the cryptic phylogenetic species *A. awamori*. Fungal Biol 115:1138–1150

Peterson SW (1992) *Neosartorya pseudofischeri* sp. nov. and its relationship to other species in *Aspergillus* section *Fumigati*. Mycol Res 96:547–554

Peterson SW (2000) Phylogenetic analysis of *Penicillium* based on ITS and LSU-rDNA sequences. In: Samson RA, Pitt JI (eds) Classification of *Penicillium* and *Aspergillus*: integration of modern taxonomic methods. Harwood, Reading, pp 163–178

Peterson SW (2008) Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. Mycologia 100:191–212

Peterson SW, Horn BW (2009) *Penicillium parvulum* and *Penicillium georgiense*, sp. nov. isolated from the conidial heads of *Aspergillus* species. Mycologia 101:71–83

Peterson SW, Orchard SS, Menon S (2011) *Penicillium menonorum*, a new species related to *P. pimiteouiense*. IMA Fungus 2:121–125

Pitt JI, Samson RA, Frisvad JC (2000) 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. Harwood Academic, Amsterdam, pp 9–50

Raper KB, Fennell DI (1965) The genus *Aspergillus*. Williams and Wilkins, Baltimore

Raper KB, Thom C (1949) A manual of the penicillia. Williams and Wilkins, Baltimore

Samson RA, Frisvad JC (2004) *Penicillium* subgenus *Penicillium*: new taxonomic schemes, mycotoxins and other extrolites. Stud Mycol 49:1–260

Samson RA, Yilmaz N, Houbraken J, Spierenburg H, Seifert KA, Peterson SW, Varga J, Frisvad JC (2011) Phylogeny and nomenclature of the genus *Talaromyces* and taxa accommodated in *Penicillium* subgenus *Biverticillium*. Stud Mycol 70:159–184

Sant'Ana AS, Simas RC, Almeida CAA, Cabral EC, Rauber RH, Mallmann CA, Everlin MN, Resenthal A, Massaguer PR (2010) Influence of package, type of apple juice and temperature on the production of patulin by *Byssochlamys nivea* and *Byssochlamys fulva*. Int J Food Microbiol 142:156–163

Schoch CL, Seifert KA, Huhndorf S, Robert V, Souge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Natl Acad Sci (USA) doi:10.1073/pnas.1117018109; Available at: www.pnas.org/cgi/doi/10.1073/pnas.1117018109

Seifert KA (2009) Progress towards DNA barcoding of fungi. Mol Ecol Resour 9(suppl 1):83–89

Seifert KA, Samson RA, deWaard JR, Houbraken J, Lévesque CA, Moncalvo J-M, Louis-Seize G, Hebert PDN (2007) Prospects for fungus identification using *CO1* DNA barcodes, with *Penicillium* as a test case. Proc Natl Acad Sci (USA) 104:3901–3906

Short DPG, O'Donnell K, Zang N, Juba JH, Geiser DM (2011) Widespread occurrence of diverse human pathogenic types of the fungus *Fusarium* detected in plumbing drains. J Clin Microbiol 49:4264–4272

Simpson GG (1951) The species concept. Evolution 5:285–298

Soares C, Rodrigues P, Peterson SW, Lima N, Venâncio A (2012) Three new species of *Aspergillus* section *Flavi* isolated from almonds and maize in Portugal. Mycologia. doi:10.3852/11-088

Stolk AC, de Scott B (1967) Studies on the genus *Eupenicillium* Ludwig. I. Taxonomy and nomenclature of Penicillia in relation to their sclerotioid ascocarpic states. Persoonia 4:391–405

Stolk AC, Samson RA (1971) Studies on *Talaromyces* and related genera I. Hamigera gen. nov. and Byssochlamys. Persoonia 6:341–357

Sudjaritruk T, Sirisanthana T, Sirisanthana V (2012) Immune reconstitution inflammatory syndrome from *Penicillium marneffei* in an

HIV-infected child: a case report and review of literature. BMC Infect Dis 12:28

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol 31:21–32

Varga J, Frisvad JC, Kocsubé S, Bankovics B, Tóth B, Szigeti G, Samson RA (2011) New and revisited species in *Aspergillus* section *Nigri*. Stud Mycol 69:1–17

Veršilovskis A, Saeger SD (2010) Sterigmatocystin: occurrence in foodstuffs and analytical methods—an overview. Mol Nutr Food Res 54:136–147

Walsh TJ, Wissel MC, Grantham KJ, Petraitiene R, Petraitis V, Kasai M, Francesconi A, Cotton MP, Hughes JE, Greene L, Bacher JD, Manna P, Salomoni M, Kleiboeker SB, Reddy SK (2011) Molecular detection and species-specific identification of medically important *Aspergillus* species by real-time PCR in experimental invasive pulmonary aspergillosis. J Clin Microbiol 49:4150–4157

Xu X, Yang J, An Y, Pan Y, Liu G (2012) Over-expression of pcvA involved in vesicle–vacuolar fusion affects the conidiation and penicillin production in *Penicillium chrysogenum*. Biotechnol Lett 34:519–526