Medical Mycology May 2006, **44**, 199–209

Taylor & Francis
Taylor & Francis Group

Review

Dermatophytes: recognizing species of clonal fungi

Y. GRÄSER*, S. DE HOOG† & R. C. SUMMERBELL†

**Institut für Mikrobiologie und Hygiene, Department of Parasitology (Charité), Humboldt University, Berlin, Germany, and †Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands*

Now that molecular data have forever changed our perspective on the anthropophilic and zoophilic dermatophyte species, the concepts of these species needs re-evaluation. In this paper, main concepts (morphological, biological (BSC), phylogenetic and genealogical concordance phylogenetic species recognition (GCPSR)) are compared. While in geophilic dermatophytes the application of the BSC works well for species distinction and is supported by molecular data, it is not applicable for the anthropophilic and zoophilic dermatophytes where the majority of species reproduce purely asexually. Also, the application of GCPSR (an operational method to define the limits of species using molecular, multi-locus data) is problematic. GCPSR can be applied in recombining fungi even when recombination is infrequent and fungi lack phenotypic sexuality. In truly clonal fungi, however, no incongruities in multi-locus data are found, and thus separation of species may be difficult. In fungi this problem is currently taken to be non-existent, since clonality is supposed to lead to extinction. In the medically relevant, host-associated dermatophytes, however, is reason to suggest that clonal dermatophyte lineages are able to maintain ongoing populations and to follow independent evolutionary trajectories. We distinguish seasonal, short-lived and long-lived clonal species. The final goal of a species concept, in the dermatophytes as well as in other fungi, is to provide a taxonomic system that reflects the evolution of the fungal species so that the underlying biological trends elucidated in this way may be brought forward to help to guide the clinician in applying optimal therapy and prophylaxis. The application of the different species concepts may have an enormous impact on the nomenclature of dermatophytes, directly affecting the quality of communications with care providers.

Keywords dermatophytes, *Trichophyton*, *Microsporum*, taxonomy, evolution, ITS rDNA, microsatellites, clonal fungi

Introduction

The correctness of a number of taxonomic changes made in dermatophyte taxonomy on the basis of molecular data is still a matter of debate [1–4]. With the present article we will critically evaluate the contributions of molecular biology to dermatophyte diagnostics and to understanding of dermatophyte pathogenicity. This will include some examples where published molecular decisions were not optimal, e.g., the unification of *T. equinum* and *T. tonsurans*, but which already have been corrected by the original authors [5]. This demonstrates that interpretations stemming from molecular data are not infallible. Species distinction in medical mycology prior to molecular input has led, however, to more than 3000

Received 26 September 2005; Accepted 31 January 2006
Correspondence: Yvonne Gräser, Institut für Mikrobiologie und Hygiene, Dorotheenstr. 96, 10117 Berlin, Germany. Tel: +49 030 450524066; Fax: +49 030 450524902; E-mail: Yvonne.graeser@charite.de

DOI: 10.1080/13693780600606810

superfluous names. Even though most of these were abandoned long before the molecular era, some superfluous names still exist in modern times, showing that the combination of cultural phenotype and clinical data are by no means *a priori* unambiguous. This underscores that there is not a single data set that answers all possible questions, and that a polyphasic approach is compulsory.

The mechanisms of pathogenicity used by a microbe infecting a particular plant or vertebrate host are not easily established. Molecular tools may contribute towards revealing the clinical potential of a given strain. In a parallel example from phytopathology, Schürch *et al*. [6], using *in vitro* and field experiments, have been able to show that alterations or deletion of a single gene (NIP1) can block pathogenicity of strains of *Rhynchosporium secalis* in barley where the intact gene causes leaf necrosis but the mutated or deleted gene does not. Although all isolates of this fungus are classified in a single species they obviously have a different host range. Similarly, phylogenetic studies of *Aspergillus flavus* in its plant pathogenic role have shown that it consists of two subgroups, called groups I and II, and morphological studies indicated that it consists of two morphological groups based on sclerotium size. *Aspergillus oryzae*, an industrially important lineage that is not a plant pathogen, is nested within group I [7]. From the population genetics point of view, *A. oryzae* is merely a variant of *A. flavus*. It is distinctive in character by virtue of its lack of aflatoxin production, allowing it to be safely used in food production, but the non-functionality of these genes is merely due to mutations in some of the key regulatory genes of the aflatoxin pathway [8]. A situation where just a few mutations are involved in differences in pathogenicity may also apply in dermatophyte species. For example, a difference of this nature may distinguish *Microsporum canis*, pathogenic on cats, from its very closely related offshoot '*Microsporum equinum*' on horses. Until a virulence gene responsible for the trait (pathogenicity on a particular host) is discovered, the clinical potential of a strain(s) can be evaluated by examining the isolate(s) in a taxonomic framework inclusive of ecological, phenetic and genetic data. Preferably this research should not be merely inductive, adding characters until a satisfactory overview is obtained, but rather should be performed deductively, driven by hypotheses on fungal micro- and macro-evolution. The final goal is to provide a taxonomic system that reflects the evolution of the fungal species and that may help to guide the clinician in applying optimal therapy and prophylaxis. As we will show in this text, application of different species concepts may have an enormous impact on the nomenclature of dermatophytes, directly affecting the quality of communications with care providers.

Species concepts in dermatophytes

Most of the mammal-associated dermatophytes have evolved only recently (50 Mya), and consequently the differences between entities are often relatively small and not easily discerned [9]. Dispersal mechanisms are dependent on hosts that may be sedentary. For example, for much of the history of the human species, groups strongly tended to remain within limited geographic areas, e.g., continents or subcontinents. Gene flow, being linked to population size, is then limited. Neutral mutations drift to fixation, and thus metapopulations within a single fungal species can be structured. With human migration as seen in recent times, however, anthropophilic species and intraspecific variants can be rapidly distributed over large geographic areas, leading to a near-absence of polymorphism within widely dispersed entities.

Depending on the set of characters applied, species known to be distinct may even appear to be identical to one another. This may occur with molecular markers as well as with phenetic characters, but the best known examples involve phenetic characters. As an example of phenotypic identity that was long known to be illusory, *Trichophyton mentagrophytes* in the traditional concept of C.E. Emmons [10] was used for many years as an 'aggregate' species name for the anamorph of two teleomorphic species, *Arthroderma benhamiae* and *A. vanbreuseghemii*. These species were recognized as separate in terms of the biological species concept (BSC) [11] and were later shown to be strongly phylogenetically separate in molecular studies focusing on the ribosomal Internal Transcribed Spacer (ITS) region. In contrast, *Microsporum canis*, *M. audouinii* and *M. ferrugineum* are phenetically distinct and were long recognized as separate anamorph species. Though in initial molecular studies they appeared highly similar in ITS sequences and in mitochondrial DNA analysis and could have been conceived as variants within the same species, they were soon reconfirmed as separate species by the finding of polymorphisms found in non-coding regions and microsatellite markers [2,12,13].

Which molecular targets are good for species delineation?

In filamentous fungi outside the anthropophilic and zoophilic dermatophytes, ITS is commonly used for

species distinction [14–16], while microsatellites are generally used at the population/strain rather than the species level [17,18]. This demonstrates that at least some of the dermatophyte species are still in an early state of evolution, having accumulated small numbers of mutations in DNA regions that are not under direct selection as pathogenicity factors. In addition, our recently published and still unpublished studies have shown that the distribution patterns of rare microsatellite polymorphisms found in certain anthropophilic dermatophyte species, notably the *T. rubrum* complex, do not necessarily correlate with morphospecies that have previously been proposed [19]. For example, many isolates molecularly placed into *T. rubrum* microsatellite T1 group A [19] have the positive urease activity and heavy macroconidiation that led to the segregation of *Trichophyton raubitschekii* from *T. rubrum* [20]. Some members of T1 group A, however, are morphologically identical to conventional *T. rubrum*, isolates of which usually belong to T1 group B [19]. This shows that the concept of a separate *T. raubitschekii* cannot be genetically supported. In general, however, no marker can be regarded as *a priori* revealing standard taxonomic levels of diversity, and molecular data have to be carefully evaluated in each fungal group before decisions can be taken.

Why there may be conflict between molecular and morphological species concepts?

The genetic background of most morphological differences among classical dermatophyte species is not known. Given the low degree of genetic divergence distinguishing several long-recognized morphospecies, it may be hypothesized that some of these types are based on widely occurring, degenerative single-gene mutations. For example, a change in mycelial branching rate can transform a fast-growing, diffuse fungal colony type into a slow-growing, compact 'faviform' type. A remarkable mutation in *Microsporum fulvum* interfering with conidial maturation leads to a Christmas-tree-shaped conidial apparatus; a strain with this appearance was described as a separate species, *Keratinomyces longifusus*. If additional, visually inapparent mutations correlate with such visible morphological changes, the possibility exists that such entities will differ in clinical body site or host predilection, and perhaps even in antifungal susceptibility. The aim of molecular studies should therefore be to integrate the data in a polythetic taxonomic system, and to evaluate whether in some cases the biosystematic significance of phenetic and clinical differences may have been overestimated.

The question of whether morphological distinction may be assigned too much significance is not unique to dermatophytes. For example, *Polycytella hominis*, a fungus with highly distinctive morphology, was classified as the sole member of a distinct genus, but was recently recognized to be merely a mutant of *Pseudallescheria boydii* [21]. The highly characteristic meristematic fungus *Fissuricella filamenta* was demonstrated to be a variant of *Trichosporon asteroides* [22]. The supposedly neurotropic genus *Dissitimurus* is probably based on a degenerate strain of the common opportunist and saprobe *Bipolaris hawaiiensis* [15].

Application and limitation of the biological species concept (BSC) to dermatophytes

Molecular methods can help to determine which of the observed phenotypically-distinguishable entities are indeed biological units that maintain population continuity and undergo evolution in an environmental niche, whether or not this niche is a mammalian host. Fundamental to the molecular approach to these matters is a series of concepts about fungal evolution and speciation that serve as a conceptual framework. First, ideally but also somewhat primitively, the biological species concept (BSC), popular in the 1960s and 1970s, held that well-defined fungal species should consist of heterothallic strains forming interbreeding communities reproductively isolated from other such groups. If mating types are equally distributed and mating is strictly at random, the species is in equilibrium and gene flow is not disrupted over time. In reality, however, a wide diversity of mechanisms involving fungus, host and environment tend to generate perpetual imbalance. For example, one mating type may become much more common than the other. The conceptual delimitation of species and the establishment of exact points of genetic divergence signifying the genesis of species-level boundaries implies that we understand the speciation processes that occur when fungi are evolving in their natural niches. Our conceptual models of various kinds fit the underlying biological realities better in some cases than in others. Biological species recognition (BSR; the operational version of the BSC) provides a good example. In the 1960s, *in vitro* mating experiments and Stockdale's compatibility tests [23] became the classical approach to dermatophyte taxonomy. These criteria worked well with profusely interbreeding species, particularly the geophilic ones. Species-level boundaries were determined by the decreasing ability of intermates to

produce fully viable progeny. In geophilic species, biological species concepts can be correlated with commonly used molecular species identification markers, as none of the geophilic teleomorphic species shows more than 97% ITS sequence identity [24,25]. The difficulty in finding any parallel to this situation in mammal-associated dermatophytes, particularly those in which mating has been consistently demonstrated not to occur is one of the limitations of the BSC. The biological species concept is inadequate for the biosystematic analysis of dermatophytes that no longer reproduce by random mating.

The limitations of the genealogical concordance phylogenetic species recognition (GCPSR) in dermatophytes

Molecular data come into play to determine the degree of distinction among closely related species with decreased or negligible sexuality. In the phylogenetic species concept (PSC; 26), a species is the smallest recognizable cluster of individuals sharing a common monophyletic lineage, or a cluster of organisms that share a derived character state. Decisions on the limits of species, however, remain arbitrary. Trees can be influenced by the quality of the information used, e.g., by whether or not the data set includes all relevant sequence types. In dermatophytes, the molecular distinction of phenotypic and clinical entities can be particularly problematic, as the number of polymorphic sites is low in most of the markers usually investigated by fungal systematists. To deal with cases of this nature in all branches of eukaryotic biology, Avise [27] introduced a multi-gene approach, combining the tracing of gene genealogies with a determination of the degree to which linkage among multiple genetic markers within subclades was attenuated by recombination. This 'genealogical concordance phylogenetic species recognition' (GCPSR) was used as an operational method to define the limits of species. The use of several independent gene genealogies enables the congruently changed genes, with a shared phylogenetic history, to be distinguished from incongruent genes derived from introgression or intrapopulational recombination. In the fungal kingdom, where sexual species do not commonly hybridize with other distinct species (a phenomenon often occurring in plants), the level of phylogenetic distinction, where non-concordant gene histories no longer appear, indicates the beginning of reproductive isolation and hence of species divergence in sexual species. The applicability of GCPSR to fungi has been supported by recent studies showing agreement between phylogenetic concordance-based and biological species in *Neurospora* [28].

In sexual species, there are two basic mechanisms by which reproductive isolation is followed by the disruption of gene flow, leading to formation of a separate species. Allopatric speciation results from the genetic drifting-apart that occurs when two populations are separated by long-lasting geographic barriers. Sympatric speciation is a supposedly less common mechanism, resulting from the occupation of a new host or other distinct habitat by some individuals within a population. Normally these processes take some time and occur gradually. In neutrally evolving DNA markers, including non-coding regions such as introns, intergenic spacers and microsatellites, variation is accumulated over time. The low degree of genetic variability observed in many anthropophilic and zoophilic dermatophyte species is classically explained by the founder effects that most probably gave rise to these evolutionarily recently diverged populations [29]. GCPSR can still be applied, even in cases where recombination is infrequent or unknown.

The GCPSR and clonal reproduction

There is a concern that GCPSR is not optimal in the case of strictly clonal reproduction, because then there is no incongruence between gene genealogies at any level of diversity. Genotypes are found unaltered in the next generations. As there is no sexuality, any incidental mutation in neutral markers might, by chance, have arisen at the time an ecological isolation occurred generating a profoundly different new phenotype, conceivable as a separate species. No interpretation of molecular differences is thus possible without a clear understanding about whether or not the group under investigation is capable of sexuality or some parallel form of recombination, such as parasexuality. In addition, an understanding of the link between phenotype and habitat is salient, in order to allow understanding of which types of phenotypic variation, corresponding to discernible but perhaps subtle differences in the sequences of biosystematically studied genetic markers, may represent the emergence of a natural population that is distinct both qualitatively and in epidemiological or ecological statistics.

Taylor *et al*. [30] argue that strictly clonal fungi with a long evolutionary history seem to be rare, perhaps due to a more frequent than expected occurrence of processes such as parasexuality and mitotic recombination (processes which, however, have rarely been

directly demonstrated to occur in nature). A large share of the observed clonality seems to be seasonal and is rapidly outcompeted by recombined genotypes. If an organism were strictly clonal, the lack of recombination would make it vulnerable to environmental change, since it would be less able than a sexual species to rapidly adapt unless its effective population size (N^e; see [31]) was very large, harbouring a wide array of pre-existing mutations that might by chance be newly advantageous in changing circumstances. A high effective population size would also tend to be needed to protect clonal species against the adverse effects of 'Müller's Ratchet', the inherent tendency for non-recombining lineages to be weakened by the slow accumulation of deleterious mutations [32]. Since such large population sizes may be difficult to attain, most clonal lineages, if they persist over evolutionary time, are much more likely to be short-lived than are species with full or partial meiotic recombination, or with significant levels of mitotic recombination [30]. The dichotomy between long-established sexual lineages on the one hand and seasonal and evolutionarily short-lived asexual lineages on the other may hold true for most of the human fungal pathogens and opportunists that are acquired from the environment. Anthropophilic and non-soil-associated [33] zoophilic dermatophytes, however, have a different mode of transmission, being spread primarily either directly from host to host or spread via fomites where no significant environmental reproduction occurs. Since conventional sexual processes cannot occur either on the host or in the dormant material in fomites, this pattern of spread tends to enforce the generation and dissemination of clones. Recently emerged clones remain closely related to their teleomorphic ancestor (see examples 1 and 2). Although such clonal lineages among the anthropophilic dermatophytes may have existed for many human generations, they are probably mainly short-lived in evolutionary terms, having existed for a shorter period of time than *Homo sapiens* (60,000 years) itself. However, if they are able to maintain beyond seasonality, there is no reason to exclude the possibility that such clonal lineages in theory can become quite remote from the (perhaps extinct) teleomorph species from which they ultimately derived (see example 3).

Clones in dermatophytes may be the result of processes referred to as 'sudden speciation' [31], a phenomenon that in various types of organisms can be brought about by processes such as polyploidization or chromosomal rearrangements, or by any phenomenon leading to a decrease of random mating. In dermatophytes, it has been proposed [29] that such events can occur when strains of different mating type within a single species become genetically distinct from one another due to predominantly asexual reproduction and rarity of sexual mating over time, and then a lineage arising from just one of the two mating types is able to switch to stably infecting a new animal host species on an ongoing basis. Rapid speciation entails little or no change in genetic composition at the allelic level except in the very small proportion of genes directly selected for or against in the context of the host switch. In some cases, the clonal offshoots of interbreeding communities may still be able to mate, but may simply have become sympatrically or geographically inaccessible to their potential partners. This appears to be the case, for example, with the European race of the hedgehog dermatophyte, *Trichophyton erinacei ss. str*. This population, which has as host the European hedgehog *Erinaceus europeus*, consists of a single mating type that is physiologically differentiated (e.g., urease-negative) in a way suggestive of relatively long evolutionary isolation from its closest relatives, the potentially sexually reproducing African race of *T. erinacei* colonizing the African four-toed hedgehog *Aterelix albiventris* [34], and the African race of *Arthoderma benhamiae*. It remains, however, able to mate *in vitro* with testers of the African race of *Arthroderma benhamiae* [35,36].

The longevity of clonal offshoots is an essential issue in dermatophyte taxonomy, as these offshoots may account for a large share of the observed variation, contrasting with the situation in most eukaryotic organisms where much of the variation seen can be taken to be sexual. If clones reproductively isolated from their sexual relatives are short-lived and relatively rare, as is proposed by Taylor *et al*. [30], then entire clonal clades up to but not including the presumed last common ancestor of the clonal and recombining sister groups, could be defined as a species (Fig. 1a). In the anthropophilic dermatophytes this would mean a considerable reduction of the number of species recognized, since species like *T. violaceum* and *T. rubrum* clearly have arisen as offshoots within large, complex clades where no sexuality is known. However, since the offshoots within clonal dermatophyte lineages are able to maintain ongoing populations and to follow independent evolutionary trajectories, their recognition as separate entities has seemed feasible to several generations of mycologists (Fig. 1b). The issue of how such subdivisions in clonal lineages can be taxonomically conceptualized following molecular study is discussed below using practical examples.

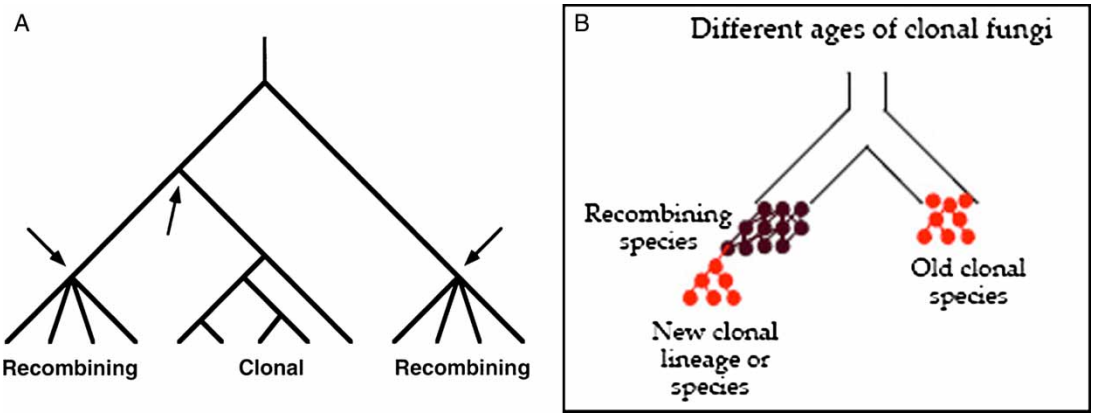

Fig. 1 (A) Recognizing species with consensus trees made of multiple gene genealogies. For recombining species, the interface between incongruence (shown as a polytomy with its lack of resolution) and congruence of gene genealogies can be used to define the limit of the species. Clonal species would include all individuals subsequent to the last common ancestor of the clonal and recombining species. Reprinted from Taylor J, Jacobson D, Fisher M. The evolution of asexual fungi: reproduction, speciation and classification. *Annu Rev Phytopathol* 1999; **37**: 197–246 [30]. (B) Different ages of clonal fungi. The figure is published with permission of J. Taylor, Berkeley, CA.

Delimitation of micro-species in dermatophytes

Example 1. Clonal offshoots can have their own evolutionary fates: examples of recently emerged clones

The anamorphic *Trichophyton* and *Microsporum* species associated with humans are evolutionarily linked to only a small number of sexually reproducing species, viz. *A. benhamiae*, *A. vanbreuseghemii*, *A. simii* and *A. otae*. If clonal offshoots such as those found in this complex are evolutionarily short-lived, as claimed by Taylor *et al.* (30), the probability should be very high that functionally or completely non-mating anamorphs will be virtually genetically identical to anamorphs successfully mating to form the teleomorph of the most closely-related sexual-mating population. This is, however, not the case. Teleomorphic species are found to be genetically close to independently evolving anamorphic offshoots, but are rarely strictly identical with them even in relatively conservative phylogenetic loci such as ITS. This strongly suggests that clonal offshoots indeed emerge infrequently and may drift away from their ancestors over the relatively long evolutionary term.

Short-lived entities between and within epidemics as defined by Taylor *et al.* [30, pp. 216–217] may accumulate single mutations but should remain part of their ancestral interbreeding community. *Microsporum audouinii* and *M. ferrugineum* are offshoots of the interbreeding species *Arthroderma otae*, but each of them shows significant genetic distance from *A. otae* in common phylogenetic marker loci (Table 1; Fig. 2). Allopatric speciation took place, where the *M. ferrugineum* lineage became resident in Asia and *M. audouinii* in Africa [13]. The clonal offshoots acquired differences in morphology, physiology, ecology and distribution. The time period over which this occurred is reflected in the phylogenetically studied portion of the genotype. Apparently, significant evolution took place after one of the mating types became inaccessible after the shift from a predominantly zoophilic life cycle to an anthropophilic strategy [13]. Multilocus sequence data showed that the entities are reproductively isolated, at a

Table 1 Genotypes of clonal offshoots and corresponding teleomorphs, as inferred from single-strand conformational polymorphism (SSCP) patterns and sequences of genomic DNA markers.

	ITS*	ATP/CYTII	N3/N1	UB/VAR	MP	MS(GT)n (AGA)n	AM (AC4, 9, 12)
Arthroderma otae/*Microsporum canis*	A	A	A	A	A	A	A
M. equinum	A	A	A	A	A	A	A
M. audouinii	B	B	B	B	B	B	B
M. ferrugineum	C	C	C	C	C	C	C

*Sequenced gene and other molecular character designations: ITS, internal transcribed spacer of nuclear ribosomal DNA; ATP/CYTII, intergenic spacer between the adenosine triphosphatase 9 and the cytochrome oxidase subunit II gene of the mitochondrial (mt)DNA; N3/N1, intergenic spacer between the NADH hydrogenase gene subunits 3 and 1 of the mtDNA; UB/VAR, 3' non-coding region of the ubiquitin gene of the nuclear DNA; MP, putative metalloproteinase; MS, microsatellite markers (repeat motif); AM, anonymous markers.

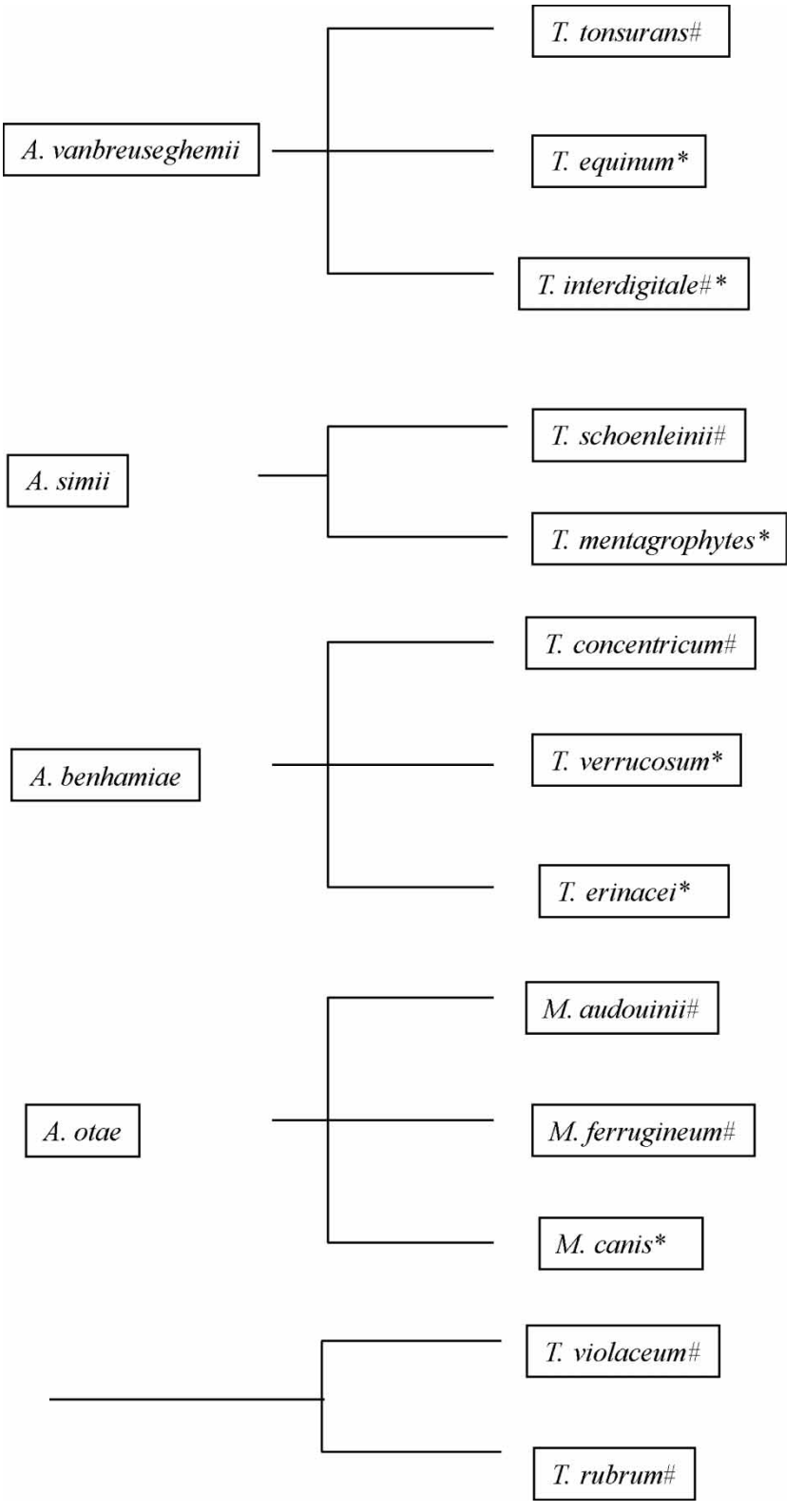

Fig. 2 Schematic presentation of the current species concept in dermatophytes. The close relationships of the teleomorphic species (*Arthroderma*) to the clonal offshoots is shown (# = anthropophilic species, * = zoophilic species). Geophilic species were omitted.

significant distance to each other. *M. audouinii* was just as well-established on humans 170 years ago [37] as it is in its remaining endemic areas today. Some of the clonal offshoots may survive and evolve over more than a few seasons and thus may be regarded as species.

The boundaries where emerging offshoots must be considered to have separated from their ancestral-type relatives and should be recognized as species are difficult to determine. In GCPSR, submergence within the ancestral-type species should be assumed when random mating can still take place. However, in the Arthrodermataceae, teleomorphs are likely to be lost gradationally by increasing preponderance of one mating type. As soon as an entity consists of a single mating type and no recombination of any kind takes place, it should be categorized as a separate albeit clonal species despite potential *in vitro* interbreeding.

Example 2. Interbreeding communities and host shifts

The zoophilic, asexual lineage *T. mentagrophytes ss. str*. as delineated by Probst *et al*. [5], appears to be derived from the teleomorph *Arthroderma simii*, and was originally associated with mice and camels (Fig. 2). It underwent a host shift from mice to camels that was not correlated with any polymorphisms in neutral markers and genes [5]. Bactrian camel offshoots, though superficially quite different in appearance from *T. mentagrophytes*, have few and gradational distinguishing features. In contrast, with the emergence of *T. schoenleinii* from within this lineage, after a switch to the human host, was accompanied by genetic changes (Table 2; Fig. 2). This human-associated offshoot is phenetically recognizable by several distinct morphological and physiological features, e.g., a complete lack of conidiation.

The sexual species with the largest number of asexual offshoots is *A. vanbreuseghemii*. Host shifts to humans are less clearly accompanied by genetic changes in *T. interdigitale*, which is therefore a lineage that includes strains from humans but also from other mammals. The anthropophilic *T. tonsurans* is very close to the horse-associated *T. equinum*, and both in turn are close to *T. interdigitale*. *T. tonsurans* and *T. equinum*, apart from being of different mating type [36], are only phylogenetically distinguishable when population genetics parameters are used that are generally considered to indicate intraspecific levels of variation in other fungi (Fig. 2). Slight differences in the patterns seen in Single Strand Conformational Polymorphism (SSCP) analysis supported the distinction of the entities when tests were done using the intergenic spacer between the ATPase, the cytochrome oxidase of the mtDNA and the intron of the nuclear heat shock protein (HSP) gene (Table 3). Physiological features (nicotinic acid requirement in *T. equinum* and not in *T. tonsurans*) have long been known [20]. However, polymorphisms that would corroborate the distinction of *T. equinum* var. *autotrophicum* from the type variety (the former is autotrophic while the latter requires exogenous nicotinic acid) were not observed using the genome regions listed.

Example 3. Clonal offshoots in clades without known sexuality; long-lived clonal species

No teleomorph is known in the *T. rubrum*/*T. violaceum* clade, which is quite distant from any other dermatophyte group (Fig. 2). Thus the ancestral interbreeding community cannot be determined with certainty. These anamorphic species are thus likely to have been in existence much longer than the clonal species within the *M. canis* complex. On the basis of ITS data, clinical picture (tinea capitis *vs*. the remaining forms of tinea including onychomycosis) and epidemiological data, a number of described species related to *T. violaceum* and *T. rubrum* were reduced to synonymy [3]. There was some conflict among the various molecular analyses used, with microsatellite DNA markers showing that the entities *T. gourvilii* and *T. soudanense* group somewhat closer to *T. rubrum* than to *T. violaceum* [19], while earlier ITS analyses had suggested that these entities were closer to *T. violaceum* [3]. The structure of the data set (Table 4) suggests recombination within a

Table 2 Genotypes in the *Arthroderma simii* clade.

	ITS*	Tri2	Tri4	ATP/COXII
A. simii	A	ND	ND	A
T. mentagrophytes	B	B-G†	B	B
T. schoenleinii	C	A	C	C

*Sequenced gene designations as in Table 1 except Tri2, a subtilisin gene family (sub6) member encoding a secreted proteinase; Tri4,a member of the prolyl oligopeptidase family of serine proteinases. †Genotypes that do not correspond to strains isolated from mice or camels.

Table 3 Genotypes of clonal offshoots in the *T. interdigitale*/*T tonsurans* clade.

	ITS*	ATP/CYTII	HSP
T. interdigitale (human)	A	A	A
T. tonsurans	B	B	B
T. equinum (incl. var. *autotrophicum*)	B'†	C	C

*Sequenced gene designations: as in Table 2. † B' = a single nucleotide difference.

Table 4 Genotypes of clonal offshoots in the *Trichophyton rubrum*/*T. violaceum* clade.

	ITS*	ATP/CYTII	HSP	MS (GT)n
T. violaceum	A	A	A	A
T. yaoundei	A	A	A	B
T. gourvilii	A	B	B	C
T. soudanense	A	B	B	C
T. kanei	B	B	B	C
T. raubitschekii	B	B	B	C
T. fischeri	B	B	B	D
T. rubrum	B	B	B	D

*Sequenced gene designations: as in Table 1 except HSP, intron of the gene of the heat shock protein.

single, anthropophilic species consisting of geographically separated populations. The five microsatellite markers investigated so far (unpublished data) have revealed ca. 30 genotypes within the *T. rubrum* clade (defined to include the synonymous taxa *T. fischeri*, *T. soudanense*, *T. gourvilii*, *T. rodhainii*, *T. raubitschekii*, *T. kanei* and *T. fluviomuniense*). These genotypes grouped in three clusters corresponding to geographic origin (Africa, Asia and Europe/the Americas) in conjunction with sympatric isolation (in this case consisting of occupation of a different ecological niche on the human body). One group of genotypes, mostly isolated from Africa and mostly corresponding to the traditional concept of *T. soudanense*, clearly had an association with tinea capitis, whereas a second cluster with the majority of strains from Europe and America mainly caused tinea pedis or onychomycosis. The third group, predominantly Asian but also with some African representatives, partly but not entirely corresponded to the previous concept of *T. raubitschekii* and had an association with chronic tinea corporis and tinea cruris despite also being isolated from some tinea pedis, tinea unguium and tinea capitis cases [19,38].

Example 4. Limits of segregation

Some entities lack genetic support, even when highly variable microsatellite loci are analyzed. *Microsporum equinum* from horses is generally regarded to be phylogenetically identical to *M. canis*, despite its host differences (Table 1) [13] and relatively marginal phenotypical distinctions, viz., few and short macroconidia and some minor physiological apomorphies as noted by Kane *et al*. [39]. This implies that some dermatophyte species can act as generalists on furred animals, and not every series of infections on a new host will lead to an evolutionary process; moreover, even when such processes are occurring on domesticated animals transported to new geographic areas around the world, they may be detected at an extremely early stage. Mere occurrence on different hosts is not to be interpreted as a series of fully accomplished host shifts. A parallel evolutionary story involving horses and onygenalean fungal pathogens is that of *Histoplasma capsulatum* var. *farciminosum*. This morphologically and epidemiologically distinct variety, deviating markedly from other known types of *H. capsulatum*, caused a contagious skin disease of horses mainly in Egypt and nearby areas of northeast Africa. Surprisingly, however, it was found in the multilocus genotyping studies of Kasuga *et al*. [40] to contain offshoots of three distinct phylogenetic species within the *H. capsulatum* species complex, the most common of which was a South American genotype that was probably new to both the Old World continents and horses some time after the European colonization of South America.

The question then becomes which features can be regarded to be significant as indicators of species status. Since mutations are maintained in clonal lineages, each distinguishable type might in principle be classifiable as a separate clonal species following the classic phylogenetic species concept. Kawasaki *et al*. [41] showed that a single-ascospore isolate with a *T. verrucosum*-like colony morphology was produced in the progeny of a mating cross conducted between two morphologically typical *Arthroderma benhamiae* isolates. Though this mutant lacked the distinguishing growth factor deficiencies of *T. verrucosum* (thiamine and usually inositol) and was not shown to have any *T. verrucosum*-like abilities in regard to maintaining ongoing subclinical infections on cattle, this suggests at least that the so-called 'favic' growth form considered so indicative of *T. verrucosum* in *in vitro* growth (and also of *T. violaceum*, *T. schoenleinii*, and the Asian sheep offshoot of *A. vanbreuseghemii*, formerly called *T. verrucosum* var. *autotrophicum*) can arise through meiotic recombination or mutation at any moment. Probably either a widely distributed gene responsible for this phenotype is involved, or else certain (as yet uncharacterized) genes responsible for normal colony growth and hyphal branching in dermatophytes are relatively vulnerable to acquiring mutations conferring this phenotype.

Example 5. Quality of data

Species have occasionally been described on the basis of only a single isolate. This may be justified when the taxon is obviously remote from any known entity, but dermatophytes have been heavily sampled worldwide for a century, and strongly genetically isolated single isolates are rather exceptions, such as the recent

description of *T. eboreum* [25]. In respect to groups with high degrees of phenotypic similarity, a conservative approach is advocated where new cryptic species should be formally introduced only after it is shown that the new species concepts remain intact when a large sample of related biodiversity has been added to the analysis, or when ITS data show a large phylogenetic distance to the most closely related species. As a potentially debatable example, we reduced *Microsporum riparae* [42] to synonymy because the ITS sequence of the type strain, CBS 529.71, proved to be highly similar (98%) to that of the morphologically similar *M. fulvum* [24]. The ITS sequences of *M. gallinae* and *M. vanbreuseghemii* were found to be 100% identical, contradicting the apparently profound morphological and mating-competence divergences between these fungi and showing the power of molecular analysis to reveal hidden relationships among the dermatophytes. These synonymizations are supported by the observation that all sexually reproducing species of the geophilic *Microsporum* and *Trichophyton* clade as well as the included asexual species (e.g., *M. praecox*, *M. duboisii*, *T. thuringiense* and *T. phaseoliforme*) are more distantly related to their nearest taxonomically recognized neighbor (less than 97% ITS similarity) than *M. ripariae* or *M. vanbreuseghemii* were to their respective nearest neighbors. Such analogistic comparisons among biologically different subclades are not always 'the end of the story' in biosystematics, but at the very least, it can be said that at present there is inadequate genomic support to re-propose *M. ripariae* or *M. vanbreuseghemii* as species distinct from their genetically near-identical sibling-phenotypes.

Conclusions

There are theoretical as well as practical reasons to treat at least some of the clonal entities within the dermatophytes as separate species (Fig. 2), but no entities can be maintained *a priori* on the basis of clinical and classical laboratory appearance alone [43]. When the observed phenetic polymorphism is based on a widely occurring single mutation, as may be the case in the distinction between *T. equinum* var. *equinum* and *T. equinum* var. *autotrophicum*, no correlated evolution has taken place and thus any taxon based on this mutation has no biomedical predictive value, other than whatever useful epidemiological information may be gleaned from the chance occurrence of geographical founder effects (such as the association of *T. equinum* var. *autotrophicum* with Australia and New Zealand). Characters that are known to be variable in dermatophytes should be attributed lower value than relatively invariant characters, since the different character states may be observed in some members of every species. In order to be taxonomically significant, mutations should be relatively unique, and should correspond with other, independent markers indicating reproductive isolation. The study of morphology, ecological niche, clinical features and disruption of gene flow should be combined in a polyphasic approach.

The main reason given by Taylor *et al*. [30] for not formally recognizing clonal offshoots from species known to interbreed is the assumption that the clones are short-lived. From the above discussion of dermatophytes, it is evident that clonal offshoots with a separate evolutionary history do exist and should be reflected in the taxonomic system. They can be conceived as separate, albeit clonal, species, notwithstanding the vestigial potential of at least some of them for *in vitro* interbreeding, or they can be redisposed as varieties of the nearest recombining ancestor. The limit where species-level separation in clonal lineages can be inferred lies well above the level of difference seen in a single, commonly occurring mutation, but there is no exact amount of molecular difference that unambiguously justifies the recognition of a formal entity.

Acknowledgements

We thank J. W. Taylor for helpful comments and fruitful discussion.

References

1 Gräser Y, Kuijpers A, Presber W, De Hoog S. Molecular taxonomy of *Trichophyton mentagrophytes* and *T. tonsurans*. *Med Mycol* 1999; **37**: 315–330.
2 Gräser Y, Kuijpers AF, El Fari M, Presber W, de Hoog GS. Molecular and conventional taxonomy of the *Microsporum canis* complex. *Med Mycol* 2000; **38**: 143–153.
3 Gräser Y, Kuijpers AFA, Presber W, De Hoog GS. Molecular taxonomy of the *Trichophyton rubrum* complex. *J Clin Microbiol* 2000; **38**: 3329–3336.
4 Woodgyer A. The curious adventures of *Trichophyton equinum* in the realm of molecular biology: a modern fairy tale. *Med Mycol* 2004; **42**: 397–403.
5 Probst S, De Hoog GS, Gräser Y. Development of DNA markers to explore host shifts in dermatophytes. *Stud Mycol* 2002; **47**: 57–74.
6 Schürch S, Linde CC, Knogge W, *et al*. Molecular population genetic analysis differentiates two virulence mechanisms of the fungal avirulence gene NIP1. *Mol Plant Microbe Interact* 2004; **17**: 1114–1125.
7 Geiser DM, Dorner JW, Horn BW, *et al*. The phylogenetics of mycotoxin and sclerotium production in *Aspergillus flavus* and *Aspergillus oryzae*. *Fungal Genet Biol* 2000; **31**: 169–179.
8 Kusumoto K, Nogata Y, Ohta H. Directed deletions in the aflatoxin biosynthesis gene homolog cluster of *Aspergillus oryzae*. *Curr Genet* 2000; **37**: 104–111.

9 Harmsen D, Schwinn A, Weig M, *et al*. Phylogeny and dating of some pathogenic keratinophilic fungi using small subunit ribosomal RNA. *J Med Vet Mycol* 1995; **33**: 299–303.
10 Emmons CW. Dermatophytes: natural groupings based on the form of the spores and accessory organs. *Arch Dermatol Syphilol* 1934; **30**: 337–362.
11 Takashio M. Une nouvelle forme sexuée du complexe *Trichophyton mentagrophytes*, *Arthroderma vanbreuseghemii*, sp. nov. *Ann Parasitol Hum Comp* 1973; **48**: 713–732.
12 Kawasaki M, Aoki M, Ishizaki H. Phylogenetic relationships of some *Microsporum* and *Arthroderma* species inferred from mitochondrial DNA analysis. *Mycopathologia* 1995; **30**: 11–21.
13 Kaszubiak A, Klein S, De Hoog GS, *et al*. Population structure and evolutionary origins of *Microsporum canis*, *M. ferrugineum* and *M. audouinii*. *Inf Gen Evol* 2004; **4**: 179–186.
14 Berbee ML, Pirseyedi M, Hubbard S. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 1999; **91**: 964–977.
15 De Hoog GS, Vicente V, Caligiorne RB, *et al*. Species diversity and polymorphism in the *Exophiala spinifera* clade containing opportunistic black yeast-like fungi. *J Clin Microbiol* 2003; **41**: 4767–4778.
16 Groenewald M, Kang J-C, Crous PW, *et al*. ITS and β-tubulin phylogeny of *Phaeoacremonium* and *Phaeomoniella* species. *Mycol Res* 2001; **105**: 651–657.
17 Wickstead B, Ersfeld K, Gull K. Repetitive elements in genomes of parasitic protozoa. *Microbiol Mol Biol Rev* 2003; **67**: 360–375.
18 Dettman JR, Taylor JW. Mutation and evolution of microsatellite loci in *Neurospora*. *Genetics* 2004; **168**: 1231–1248.
19 Ohst T, De Hoog GS, Presber W, *et al*. Origins of microsatellite diversity in the *Trichophyton rubrum-T. violaceum* clade (dermatophytes). *J Clin Microbiol* 2004; **42**: 4444–4448.
20 Kane J, Summerbell R, Sigler L, *et al*. *Laboratory handbook of dermatophytes*. Belmont, CA: Star Publishing Company, 1997.
21 Borman AM, Campbell CK, Linton CJ, *et al*. *Polycytella hominis* is a mutated form of *Scedosporium apiospermum*, as shown by gene sequence analysis. *Med Mycol* 2006; **44**: 33–39.
22 Guého E, Smith MT, De Hoog GS, *et al*. Contributions to a revision of the genus *Trichosporon*. *Antonie van Leeuwenhoek* 1992; **61**: 289–316.
23 Stockdale PM. Sexual stimulation between *Arthroderma* simii and other dermatophytes. In: Vanbreuseghem R, De Vroey C (eds). *Sexuality and Pathogenicity of Fungi*. Paris, New York: Masson, 1981: 57–68.
24 Gräser Y, De Hoog GS, Kuijpers AFA. Recent advances in the molecular taxonomy of dermatophytes. In: Kushwaha RKS, Guarro J (eds). *Biology of Dermatophytes and other Keratinophilic Fungi. Revta Iberoam Micol* 17 (Suppl.). País Vasco, Spain: Bilbao, 2000: 17–21.
25 Brasch J, Gräser Y. *Trichophyton eboreum sp.nov*. isolated from human skin. *J Clin Microbiol* 2005; **43**: 5230–5237.
26 Avise JC, Ball RM. Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surv Evol Biol* 1990; **7**: 45–67.
27 Avise JC. *Molecular Markers, Natural History and Evolution*. New York: Chapman & Hall, 1994: 511.
28 Dettman JR, Jacobson DJ, Taylor JW. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evol Int J Org Evol* 2003; **57**: 2703–2720.
29 Summerbell RC. What is the evolutionary and taxonomic status of asexual lineages in dermatophytes? *Stud Mycol* 2002; **47**: 97–101.
30 Taylor JW, Jacobson DJ, Fisher MC. The evolution of asexual fungi: Reproduction, speciation and classification. *Annu Rev Phytopathol* 1999; **37**: 197–246.
31 Burnett J. *Fungal Populations & Species*. Oxford: Oxford University Press, 2003: 348.
32 Bidochka MJ, De Koning J. Are teleomorphs really necessary?: Modelling the potential effects of Muller's Ratchet on deuteromycetous entomopathogenic fungi. *Mycol Res* 2001; **105**: 1014–1019.
33 Summerbell RC. Form and function in the evolution of dermatophytes. In: Kushwaha RKS, Guarro J (eds). *Biology of Dermatophytes and other Keratinophilic Fungi. Revta Iberoam Micol* 17 (Suppl.). País Vasco, Spain: Bilbao, 2000: 30–43.
34 Takahashi Y, Sano A, Takizawa K, Fukushima K, Miyaji M, Nishimura K. The epidemiology and mating behavior of *Arthroderma benhamiae* var. *erinacei* in household four-toed hedgehogs (*Atelerix albiventris*) in Japan. *Nippon Ishinkin Gakkai Zasshi (Japan J Med Mycol)* 2003; **44**: 31–38.
35 Vanbreuseghem R, Takashio M. The groups of *Arthroderma benhamiae* var. *erinacei*. In: Vanbreuseghem R, De Vroey C (eds). *Sexuality and Pathogenicity of Fungi*. New York: Masson, Paris, 1981: 118–127.
36 Summerbell RC, Weitzman I, Padhye A. The *Trichophyton mentagrophytes* complex: biological species and mating type prevalences of North American isolates, and a review of the worldwide distribution and host associations of species and mating types. *Stud Mycol* 2002; **47**: 75–86.
37 Gruby M. Recherches sur la nature, le siège et le développement du porrigo decalvans ou phytoalopécie. *Comp Rend Acad Sci* 1843; **17**: 301.
38 Kane J, Krajden S, Summerbell RC, *et al*. Infections caused by *Trichophyton raubitschekii*: clinical and epidemiological features. *Mycoses* 1990; **33**: 499–506.
39 Kane J, Padhye AA, Ajello L. *Microsporum equinum* in North America. *J Clin Microbiol* 1982; **16**: 943–947.
40 Kasuga T, White TJ, Koenig G, *et al*. Phylogeography of the fungal pathogen *Histoplasma capsulatum*. *Mol Ecol* 2003; **12**: 3383–3401.
41 Kawasaki M, Mochizuki T, Ishizaki H, *et al*. Ascospore-derived isolate of *Arthroderma benhamiae* with morphology suggestive of *Trichophyton verrucosum*. *Med Mycol* 2004; **42**: 223–228.
42 Hubalek Z, Rush-Munro FM. A dermatophyte from birds: *Microsporum ripariae* sp. nov. *Sabouraudia* 1973; **11**: 287–292.
43 De Hoog GS, Guarro J, Gené J, *et al*. *Atlas of Clinical Fungi*, 2nd ed. Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili, Utrecht/Reus, 1126 pp., 2000.