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# The New Species Concept in Dermatophytes—a Polyphasic Approach

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**Abstract** The dermatophytes are among the most frequently observed organisms in biomedicine, yet there has never been stability in the taxonomy, identification and naming of the approximately 25 pathogenic species involved. Since the identification of these species is often epidemiologically and ethically important, the difficulties in dermatophyte identification are a fruitful topic for modern molecular biological investigation, done in tandem with renewed investigation of phenotypic characters. Molecular phylogenetic analyses such as multilocus sequence typing have had to be tailored to accommodate differing the mechanisms of speciation that have produced the dermatophytes that are commonly seen today. Even so, some biotypes that were unambiguously considered species in the past, based on profound differences in morphology and pattern of infection, appear consistently not to be distinct species in modern molecular analyses. Most notable among these are the cosmopolitan bane of nails and feet, *Trichophyton rubrum*, and the endemic African agent of childhood tinea capitis, *Trichophyton soudanense*, which are effectively inseparable in all analyses. The molecular data require some reinterpretation of results seen in conventional phenotypic tests, but in most cases, phylogenetic insight is readily integrated with current laboratory testing procedures.

**Keywords** Dermatophytes · Taxonomy · Molecular identification · Morphological identification · Species concept

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## Introduction: Why Dermatophyte Biosystematics and Identification are Important (Medical and Scientific Aspects)

The dermatophytes belong to the small category of disease organisms that almost every human alive will be infected by at some point over the course of his or her lifetime. Over USD $500,000,000 per year is spent worldwide for drugs targeted against dermatophytoses [1]. Dermatophyte species are closely related to each other phylogenetically (see below), and drugs that are effective against one species are generally effective against others as well [2]. There are a few exceptions to this generality: for example, *Trichophyton verrucosum* and "*Trichophyton mentagrophytes* var. *granulosum*" (the modern identity

uncertain of the isolates so identified in the study is uncertain) have limited susceptibility to fluconazole [3], a drug that is by no means the most commonly used in treating dermatophytosis. Even discounting such occasional minor differences in susceptibility, however, identification of individual dermatophyte species causing infection remains important for several reasons.

First, dermatophytosis is often connected with epidemiological circumstances promoting reinfection [1, 4]. For example, *Microsporum canis* commonly indicates a cat (rarely a dog) as a persistent inoculum source, while *Microsporum gypseum*, causing similar lesions, indicates contact with contaminated soil. An outbreak of *Microsporum audouinii* infection in a school may indicate inoculum from an index patient who has recently travelled from an endemic area such as central Africa. In various cases, sources of potentially reinfective inoculum must be dealt with in dermatophytosis or else therapy runs a high risk of proving futile.

Second, the actual treatment regimens may differ for different dermatophyte species: for example, *Trichophyton tonsurans* in tinea capitis tends to require shorter treatment times than *M. canis*. The latter fungus to some extent evades drug exposure by forming arthroconidia outside the hair shaft, while the former forms arthroconidia inside the hair shaft where contact with conventional anti-fungal drugs is relatively high [2].

Third, especially in onychomycosis, culture and species identification may be needed to distinguish dermatophytes from non-dermatophytic species causing dermatophytosis-like infection that does not respond to anti-dermatophyte therapy. The classic example is dermatophytosis-like infection by *Neoscytalidium dimidiatum*. Non-pathogenic fungi superficially resembling dermatophytes, such as *Trichophyton terrestre*, *Aphanoascus fulvescens* and *Myriodontium keratinophilum* regularly grow from dermatophytic and psoriatic lesions as well as nails infected by other non-dermatophytes, and these entities must also be recognized as non-dermatophytes [1].

It is critical to note, though, that the extent to which these factors recommending dermatophyte identification are emphasized in real medical practice depends on socio-economic considerations. For example, since the great majority of dermatophytes causing onychomycosis are anthropophilic and connected to fomites primarily in the home environment, some authorities recommend avoiding the cost of species identification whenever outgrowth of a dermatophyte-like fungus has been confirmed with a positive result on Dermatophyte Test Medium (DTM) [5]. This recommendation does not specifically ask physicians using DTM in the office to check microscopically for non-pathogenic dermatophytoids (most commonly *T. terrestre*) or *Chrysosporium*-like organisms producing false-positive results on DTM, and is primarily justified statistically, based on overall cure rates for large numbers of patients. Clearly, then, differing ethical approaches [6] in dermatologic mycology may have a profound effect on whether or not organism identification is recommended. Utilitarian ethics (greatest good for the greatest number of people [6]) appear to justify rapid and inexpensive approaches misdiagnosing some patients but efficiently curing the majority, while Kantian ethics (absolute duty to each individual patient to perform a correct diagnosis wherever possible [6]) mandate a relatively meticulous approach to disease confirmation. Dermatophytosis is not a life-threatening affliction and the consequences of false-positive misdiagnosis generally amount to less than $1,000 in drug and medical costs plus the nuisance and risk of side effects involved in therapy. Organism identification in dermatologic mycology is clearly most important to Kantian practitioners who strive to avoid such misdiagnoses. A clear discussion of these issues is helpful in resolving many widely differing recommendations in the literature, and thus clarifying the practical value of accurate dermatophyte taxonomy.

Scientific investigation adds another dimension to the interest in dermatophyte identification. The dermatophytes occupy a unique niche as the only fungi other than *Pneumocystis* spp. primarily subsisting as communicable disease agents of mammals or birds. The course of evolution that led to the dermatophytes' *modus vivendi* appears to have been highly distinctive [6]. One particularly unusual feature is that crossing over to pathogenesis of hosts lacking regular contact with a particular type of humid soil habitat tends to eliminate the possibility of sexual reproduction completely, leading to purely asexual evolution [7–9]. The processes of speciation in dermatophytes, especially anthropophilic dermatophytes, appear to have been anomalous, and species themselves may be difficult to define [7, 10]. Thus,

dermatophytes pose an ongoing problem not only in practical species identification but also in theoretical species conceptualization. One of the main objects in the study of dermatophytes, then, is to come up with species concepts that are both scientifically defensible and practically useable.

## Brief Historical Overview of Dermatophyte Classification Schemes and Changing Species Concepts (Morphological, Biological, Phylogenetic)

Early descriptions of dermatophytes were often ambiguous in regard to whether the object of description was a fungus per se or a mycotic disease condition. For example, P.H. Malmsten's 1845 description of *T. tonsurans* [11] was heavily based on clinical signs as well as fungal structures seen in host materials; the drawings included in the article show only infected hairs and follicular structures. By the time Sabouraud compiled his master review *Les Teignes* [12], considerably greater emphasis was placed on structures seen in culture. Taxonomy above the species level, however, remained rooted in clinical findings. Sabouraud [12], for example, placed all the agents of favus-like diseases—diverse species we now know as *Trichophyton schoenleinii*, *Trichophyton mentagrophytes sensu stricto* (agent of murine favus), *Microsporum gallinae* and *M. gypseum*—in the genus *Achorion.* At the same time, however, he made observant remarks such as "As for *Achorion gypseum*, its (reproductive) organs place it among the '*Microsporums*' of animals… (suggesting a provisional placement of) the '*Achorions*' adjacent to the '*Microsporums*' ..." Mycological characters seen in culture were recognized, but given lower taxonomic priority than clinical features. Moreover, great emphasis was placed on the exact form of primary cultures. This emphasis, however, led to some species such as *T. tonsurans* being split into multiple species: Sabouraud himself coined not fewer than 10 synonyms for different primary isolate forms of *T. tonsurans* [4].

The main template of twentieth century dermatophyte morphotaxonomy was fashioned by Emmons [13]. Emmons made it quite clear that "in classifying species of the ringworm fungi, which are *fungi imperfecti*, one must depend on a study of vegetative structures and conidia." Besides terminating the clinically based genus *Achorion*, he reduced the number of recognized species to 19, and listed 35 synonymous names. His classification, however, made synonyms of some entities that were later recognized as distinct species whether examined morphologically, physiologically or genetically. These organisms were *Microsporum persicolor* (as *Trichophyton persicolor*) and *Microsporum fulvum*; *Trichophyton equinum* was excluded from discussion without comment. Even in this reductive taxonomy, the modern concept of *T. tonsurans sensu stricto* remained split into four species.

Further order was brought to dermatophyte phenetic taxonomy by the physiological investigations of Centers for Disease Control group with their investigations of hair perforation [14] and growth factor responses [15]. These tests finally led to the unification of *T. tonsurans* and distinct recognition of other debated fungi such as *T. equinum*. Other enzymatic reactions useful in phenetic classification were discovered later, such as urease activity [16, 17] and glucose repression of alkalogenic proteolysis on Bromocresol purple (BCP) milk solids glucose agar [18, 19]. These tests all greatly clarified species concepts and facilitated identification, though they also engendered some questionable taxa based on variants, such as *Trichophyton raubitschekii* for urease-positive "granular" *Trichophyton rubrum* types [20], and the literally hairsplitting *T. tonsurans* var. *sulfureum* subvar. *perforans* for *T. tonsurans* isolates retaining the ability to perforate hair in vitro [21].

Biological species concepts entered the picture with the modern rediscovery of dermatophyte teleomorphs by Dawson and Gentles [22] and Stockdale [23]. Several geophilic and zoophilic dermatophytes, as well as related non-pathogenic dermatophytoids like *T. terrestre* and *Trichophyton ajelloi*, were found to produce sexual states in the genera *Arthroderma* or *Nannizzia* (later reduced to synonymy with *Arthroderma*). The charting of dermatophyte sexual patterns began to take an unusual course after Stockdale [24] discovered that members of many apparently non-mating species could be induced to reveal their mating type in an incomplete mating reaction with testers of *Arthroderma simii*. Most of the recognized asexual species could be typed in this manner and demonstrated to be descended from a single ancestral

mating type. For example, *T. rubrum* could be shown to be (−) in mating type while the highly similar *Trichophyton megninii* (currently considered a synonym of *T. rubrum*) was (+). Just a few important species, such as *Epidermophyton floccosum* and *T. soudanense* (also currently considered synonymous with *T. rubrum*), resisted typing with this system and remain of unknown mating type status. Summerbell [25] eventually pointed out the obvious ecological factor linking the asexual, unifactorial species: they all infected animals (including *Homo sapiens*) not maintaining a soil-based burrow or den habitation suitable for the sexual processes forming *Arthroderma* teleomorphs to take place on shed hair or similar keratinous debris.

The physiological and mating system information combined with morphological studies gave rise to a stable British/North American school of dermatophyte taxonomy that was summarized in widely distributed identification manuals by Rebell and Taplin [26] and Kane et al. [1].

The DNA base composition studies of Davison et al. [27] began a new era of molecular biological investigations of dermatophytes. Though dermatophyte species proved to be relatively closely related to one another, early molecular tests such as restriction fragment typing [28] were able to distinguish common species. Full entry into the modern era of biosystematics came with the first molecular phylogeny of the dermatophytes and their Arthrodermataceous relatives by Gräser et al. [29], with additional molecular phylogenetic analyses rapidly following [30–35]. All these phylogenies tended to be strongly influenced by the close evolutionary relationships among dermatophyte species that evolved on hosts that are themselves relatively recently evolved, particularly humans as well as animals such as cats, cattle and horses that have undergone rapid selection and habitat change in the course of domestication. The revelation of this close relationship coincided with a significant collapse in the number of recognized species. The asexual anthropophilic dermatophyte *T. tonsurans*, for example, (−) mating type was declared conspecific with the asexual equine dermatophyte *T. equinum*, (+) mating type. Similarly, the sexual *Microsporum vanbreuseghemii* (teleomorph *Arthroderma grubyi*) was synonymized with the radically morphologically different, but closely phylogenetically related, asexual agent of fowl favus, *M. gallinae*.

The most recent phase of dermatophytic taxonomic history is a slight re-expansion of the number of dermatophyte species from the reduction brought about by early molecular phylogeny studies. *T. equinum* was recently unambiguously resegregated from *T. tonsurans* [10, 36, 37] and species that had been questioned as shakily based, such as *M. audouinii* and *T. schoenleinii*, were confirmed in multigene studies as separate entities [37, 38]. On the other hand, some traditionally recognized species, though at least roughly correlated with certain phenetic and clinical features, remain in synonymy even after the examination of multiple genes. For example, *T. soudanense* remains in synonymy with *T. rubrum*, even though the morphotaxonomic concept predominantly consisted of highly visually distinctive isolates that correlated with an equally distinctive epidemiology of endothrix tinea capitis (inclusive of occasional tinea corporis and other non-scalp infection in affected families). It is unclear at the moment what should be done with such entities from the point of view of nomenclature.

## The Basis of Conventional Phenotypic Laboratory Identification

Conventional laboratory identification of dermatophytes still consists primarily of micro- and macromorphological examination of primary isolates, supplemented with physiological tests for atypical isolates in some laboratories in wealthier parts of the globe. The results of direct specimen microscopy are also still important, particularly in tinea capitis. To a very large extent in many industrialized regions, dermatophyte identification has left the investigative scientific sphere and joined the corporate technological sphere, with large private laboratory firms routinely processing high sample volumes. The diagnostic, therefore, tends to be stripped down to the minimal cost necessary to achieve targeted proficiency levels that are based mainly on the ability to identify typical isolates accurately. The strong point of this approach is that the great majority of dermatophytes are typical in outgrowth, and can be readily identified based on conidia, hyphal features, and colony appearance as seen in primary culture on Sabouraud glucose agar [1, 26]. When laboratories add further tests to identify atypical isolates, the most

commonly used appear to be those based on widely commercially available materials, in particular the urease test. To my knowledge, however, there has not been a recent published survey of techniques used in large commercial laboratories conducting dermatologic mycology studies. However, a recent pattern-of-practice survey of all licensed laboratories in Ontario, Canada, showed, for primary dermatophyte isolation, five public health laboratories used five primary isolation media in dermatologic mycology according to a sophisticated protocol choosing the most appropriate one or two for any given specimen type [1], while 11 of 14 private and hospital laboratories deployed at most two media, with two using only a single commercial Sabouraud-cycloheximide medium and one doing only direct microscopy. It is likely that the use of specialized identification media follows similar patterns.

Techniques used and recommended for particular groups of dermatophytes in conventional identification are briefly discussed below. The dermatophytes considered are those for which diagnostic considerations have been significantly affected by recent molecular biological research. Dermatophytes like *E. floccosum* and *T. verrucosum* for which accurate phenotypic identification is the same today as it was in the 1990's, are not discussed. Molecular identifications are discussed in section "Reworking of species concepts in dermatophytes based on multilocus sequence typing (MLST) and population genetic data".

## The *Arthroderma otae* Complex

By far the most common species worldwide in this complex is *M. canis*, the cat dermatophyte. This species has characteristic thick-walled, fusoid-apiculate ('beaked'), rough-walled macroconidia and a lemon-yellow colony pigmentation, making typical isolates among the easiest fungi in the world to identify. The most common identification problem is provided by isolates not producing macroconidia on Sabouraud glucose agar: these isolates can almost always be stimulated to produce these conidia on potato dextrose and other sporulation media, and if subcultured to BCP milk solids-glucose medium will do this while also producing a confirmatory negative reaction for alkalinization of the medium. The main differential diagnostic organism is the closely related *M. audouinii*, which is rarely but regularly transported from African endemic regions to urban areas in the rest of the world. *M. audouinii* lacks the lemon pigment of typical *M. canis*, but can be confused with pale *M. canis* isolates, especially those producing few or no macroconidia or those producing distorted macroconidia (formerly given taxonomic status as *M. canis* var. *distortum*). Though *M. audouinii* may produce atypical, rough-walled, beaked macroconidia with a medial constriction, many isolates remain non-sporulating or produce only microconidia. A high index of suspicion for pale brownish, dermatophyte-like cultures arising from scalp of persons under 19 years old is helpful for presumptive recognition of *M. audouinii*. It is definitively distinguished from atypical *M. canis* either by means of the rapid and specific (but now rarely used) polished rice test [1], where it gives a negative growth response due to an as yet uncharacterized growth factor deficiency, or by the time-consuming and not entirely reliable (best used in tandem with positive and negative quality control inoculations) in vitro hair perforation test, where it gives a negative result contrasting with the positive result seen for typical *M. canis*.

Two rare members of this complex need to be dealt with. First, the nearly extinct *Microsporum ferrugineum*, now restricted to a few rural areas of Asia and Africa, can be recognized by its colonies resembling atypical *M. canis* but lacking conidiation, producing strongly septate "bamboo hyphae" and, most definitively, failing to perforate hair in vitro. Second, the *M. canis* strains adapted to horses, sometimes referred to in the past as *Microsporum equinum*, produce few or no conidia; those produced tend to be very short (1–3 cells long) [1, 26]. The typical horse isolates do not perforate hair in vitro.

## The *Arthroderma vanbreuseghemii*, *Arthroderma benhamiae* and *T. mentagrophytes sensu stricto* Complexes (Isolates Matching the Traditional Concept of *T. mentagrophytes sensu lato*)

The conventional identification of members of the *A. vanbreuseghemii* complex is complicated by a high degree of phenotypic overlap with members of the *A. benhamiae* complex. These two groups taken together were long treated as a single species, i.e., the Emmons [13] concept of *T. mentagrophytes*, and they form a highly recognizable phenotypic range of

morphologies. Zoophilic isolates, mostly from rodents, rabbits, guinea pigs, or secondarily infected domesticated animals (cattle, dogs, etc.), typically form clumps of racemose microconidiation (conidiation *en grappe* or in grape-like arrangements) yielding copious round to broadly clavate conidia. Characteristic spiral appendages are also usually present and are a classic confirmatory identification character. These fungi degrade urea (excepting isolates primarily infecting the European hedgehog, i.e., *Trichophyton erinacei* ss. str., which is urease-negative), perforate hair in vitro, and rapidly alkalinize BCP milk solids glucose agar.

Anthropophilic isolates tend to be velvety to cottony in culture rather than clumpy; velvety cultures retain the micromorphology described for zoophilic isolates above, while cottony cultures tend to produce laterally disposed, relatively thinly clavate microconidia and few or no spiral appendages.

Mating studies conducted worldwide [39] and subsequent molecular surveys [40] have shown that the majority of anthropophilic "*T. mentagrophytes*-like" isolates obtained worldwide are members of the *A. vanbreuseghemii* complex. Anthropophilic-type isolates of *A. benhamiae* have mainly been noted in mating studies from North America and eastern Europe [39]. Thus, particularly in western Europe and Japan, there appears to be a high likelihood that all or nearly all velvety and cottony, anthropophilic isolates resembling the traditional concept of *T. mentagrophytes* will be correctly identifiable as *Trichophyton interdigitale* ss. str., the principal anamorph name attached to the *A. vanbreuseghemii* complex. In areas where anthropophilic isolates mating as *A. benhamiae* have been reported, however, further research is necessary to determine if *T. interdigitale* can be reliably morphologically identified, or if it will be routinely confused with *A. benhamiae* anamorphs of similar appearance and physiology. Though differences in conidial shape have occasionally been noted between *A. vanbreuseghemii* anamorphs and *A. benhamiae* anamorphs, there has been insufficient research to determine if such characters could be used reliably in practical identification. These two anamorph groups are most reliably distinguished using molecular techniques.

In general, zoophilic isolates of *A. vanbreuseghemii* cannot reliably be phenotypically distinguished from typical zoophilic *A. benhamiae* isolates in any part of the world. A phylogenetically separate group of anamorphs, *T. mentagrophytes* ss. str. (formerly *T. mentagrophytes* var. *quinckeanum* [37]) is also nearly impossible to distinguish morphologically in culture from zoophilic *A. vanbreuseghemii* or *A. benhamiae*.

Cultures from human secondary infections, like those directly isolated from mouse favus, tend to be unusually flat and folded on Sabouraud glucose agar, and to have lateral rather than racemose microconidial arrangement [26, 41]. However, atypical isolates of the two morphologically overlapping *Arthroderma* complexes may also be similar in form. Definitive identification of *T. mentagrophytes* ss. str. can only be performed molecularly at this time.

European hedgehog isolates in the *A. benhamiae* complex classified as *T. erinacei* ss. str., can usually be recognized by the combination of negative urease activity and a yellow colony reverse colour on Sabouraud glucose agar. Occasional degenerated isolates matching the now abandoned morphospecies concept of *Trichophyton proliferans* combine the negative urease test and yellow colony reverse with production of fluffy colonies that, in microscopy of substrate hyphae from deep in the agar, reveal the presence of "propagules," defined as multicelled, cylindrical or spindle-shaped structures "giving rise to multiple frond-like hyphae" [26]. African-type *T. erinacei* isolates from the African hedgehog (an increasingly popular house pet) are not morphologically distinguishable from zoophilic *A. benhamiae* or *A. vanbreuseghemii* anamorphs.

## The *Arthroderma simii* complex

*Arthroderma simii* (anamorph *Trichophyton simii*) is one of the most poorly known dermatophyte species. It is mainly known from the Indian subcontinent and allegedly primarily infects monkeys [1]; however, its form and mating strongly suggest association with ground-dwelling animals [25] and it probably has another primary host animal that has not yet been detected. Infections, mostly tinea corporis infections of travelers to India, are rarely but predictably seen in most parts of the world. The fungus can be preliminarily recognized because, though it resembles a zoophilic *A. vanbreuseghemii* or *A. benhamiae*, it produces copious macroconidia not only on primary isolation, but also in subculture. Occasional *A. vanbreuseghemii* or *A. benhamiae* isolates

producing many macroconidia in primary isolation on Sabouraud glucose agar tend to revert to predominantly microconidial reproduction in subculture to the same medium [1, noted as *T. mentagrophytes*]. In classic reference laboratory mycology, it was the only dermatophyte species routinely recognized by mating with tester isolates and confirming high production of well-formed ascospores. Today, molecular confirmation may be more convenient; however, *A. simii* has not yet been well studied molecularly, and the degree of genetic biodiversity it may comprehend is unknown.

As the molecular identification sections below indicate, *A. simii* clusters closely with *T. mentagrophytes* ss. str. and *T. schoenleinii*. The criteria for phenotypic identification of *T. schoenleinii* have not been altered by these molecular studies. Probst et al. [37], however, have shown that known camel-associated isolates of *T. mentagrophytes* ss. str., previously classified as *Trichophyton sarkisovii* and *T. langeronii*, may form favic chandeliers, potentially leading to confusion with *T. schoenleinii*.

## The *Trichophyton rubrum* Complex

The *T. rubrum* complex has the distinction of being the most ancient line of purely asexual filamentous fungi confirmed as yet in mycological studies. As such, it presents an exceptional identification problem, in that, with no process other than natural selection to stabilize and unify phenotype, it has evolved a highly diverse array of phenotypes, some clinically distinct and others merely morphologically aberrant. All phenotypes, as far as we know, have evolved on a single host lineage, the *Homo* lineage leading to modern *H. sapiens*. The most ancient lineages, as was predicted [42], appear to be those associated with the scalp [9], a part of the skin which arguably represents the ancestral hirsute condition of our genus more conservatively than the relatively glabrous skin zones found on most of the lower body. Scalp-associated members of the complex include *Trichophyton violaceum* and a portion of the isolates currently considered to represent African populations of *T. rubrum*. The latter group was mainly referred to as *T. soudanense* in morphotaxonomy, with a few isolates being pigeonholed as *Trichophyton gourvilii*, *T. raubitschekii* and even some urease negative *T. rubrum* isolates. An important character for all these fungi is that most cases derive from endothrix tinea capitis in children, or less commonly from tinea corporis or (very rarely) other tineas in adults who are members of affected families. The great majority of laboratory evaluations begin with hairs showing conspicuous endothrix infection. In *T. violaceum*, there are three prominent phenotypes: (1) classic pan-African and west/central Asian *T. violaceum*, consisting of dense, slow-growing and glabrous (this combination of characters is called "faviform"), predominantly blood-red colonies, sometimes with whitish sectors, and with a uniform stimulation response to thiamine; (2) isolates corresponding to the *T. violaceum* synonym "*Trichophyton glabrum*," similar to typical *T. violaceum* except whitish in colour, mostly coming from the Horn of Africa region (Eritrea, Somalia); and (3) central African isolates corresponding to the synonym "*T. yaoundei*" which are faviform and whitish but often secrete a brown pigment into surrounding Sabouraud glucose agar; these isolates lack the thiamine stimulation response found in other *T. violaceum* lineages. The *T. violaceum* lineages all show an identical effect on BCP milk solids glucose agar, a wide zone of clearing (peptonization of the milk proteins) around the colonies, with some alkalinization of the medium.

In endothrix scalp-infecting African *T. rubrum*, the major phenotype seen is classic *T. soudanense*, which shows flattened but not faviform, radially striate, yellow to blood-red colonies featuring reflexively branched hyphae in the radial striae. These isolates have variable vitamin responses and, in most cases, a negative urease reaction. The much less commonly seen *T. gourvilii* phenotype also forms reflexive branches and is red, vitamin-independent and urease-negative. Both endothrix phenotypes show alkalinization and a small but distinct zone of peripheral clearing on BCP milk solids glucose agar.

*Trichophyton rubrum* also encompasses a lineage causing large-spored ectothrix tinea capitis, corresponding to the morphospecies *T. megninii*. This lineage consists of cottony isolates with blood-red colony reverse, similar in appearance to typical *T. rubrum*, but distinguished by a requirement for exogenous L-histidine, an ability to rapidly alkalinize BCP milk solids glucose agar without producing a peripheral clear zone, and a sometimes weak but detectable urease activity. It is also the only member of the *T. rubrum* complex that is known to be (+) in

mating type; the other elements are either (−) (*T. rubrum* ss. str., *T. violaceum*), or unclassifiable (*T. soudanense*). Currently, it is more common from tinea corporis than from the traditional tinea capitis and tinea barbae, which were both spread by unsterilized barbering instruments [43]. Preliminary recognition as an isolate differing from *T. rubrum* ss. str. is difficult. Most isolates obtained outside Portugal, the endemic area for *T. megninii*, are probably not recognized as distinct from *T. rubrum* ss. str.

Classic *T. rubrum* isolates infecting the lower body encompass a variety of phenotypes coalescing around two main groups, both autotrophs not requiring exogenous growth factors and both showing glucose repression of alkalinization on BCP milk solids glucose agar. One group, characterized as microsatellite group A by Ohst et al. [44], are mostly from Africa and southern Asia, and are mainly granular-textured isolates with copious macro- and microconidia, a blood-red reverse, positive urease, and a strong association with tinea corporis and tinea cruris [45]. Such isolates also rarely cause scalp infection, but do not form endothrix or ectothrix structures. Historically, they have been repeatedly described as new species, but almost all the names given, such as *Trichophyton fluviomuniense*, were nomenclaturally invalid or, lacking type material, soon became names of uncertain application (*nomina dubia*). The most widely used name, *T. raubitschekii*, has actually had more currency in the literature since it was declared synonymous than it had while it was still considered (by some) to designate a separate species [46, 47]. Summerbell [48], who retained *T. soudanense* as a separate species based on its distinct ITS barcode, morphology and epidemiology, referred to *raubitschekii*-like isolates as "Afro-Asiatic *T. rubrum*." This expression, however, would be easily confused with the more recent inclusion by Gräser et al. [9] of *T. soudanense* isolates as part of an "African population" of *T. rubrum*. The continued use of the epithet *raubitschekii* as an informal variant name might, therefore, be advisable for clear communication.

The most commonly seen dermatophyte in much of the world is the microsatellite type B [44], classic *T. rubrum* ss. str., which is typically cottony, relatively sparsely conidial or producing mainly clavate microconidia, and blood red in colony reverse on Sabouraud glucose agar. Isolates in this group, in contrast to *raubitschekii* variant isolates, have lost urease activity. They are strongly associated with infections of the feet, and less commonly with infections of the hands, glabrous skin or groin. Scalp infection is very rare and not associated with endo- or ectothrix structures.

## The Molecular Basis of Current Species Concepts: How Good are the Species Concepts We Recognize Today?

The increasing availability of modern genetic techniques provides microbiologists with extremely powerful tools for accurate organism identification. It is not uncommon in general that when such methods are used, strains not differentiated phenotypically prove to be distinct and thus to belong to unique, as yet undescribed taxa. Or in turn, it may be found that species separated phenotypically have identical genotypes when molecular markers are studied. As has been seen above, within the dermatophytes, the first case has been found to apply to 'aggregate' species such as the taxa formerly treated together as *T. mentagrophytes* sensu Emmons. Out of this complex four distinct taxa, inclusive of an as yet unnamed taxon, have been segregated [30, 37]. The second case applies to several anthropophilic *Trichophyton* species as well as some *Microsporum* species. A good example is seen in the isolates previously called *T. raubitschekii*, *T. soudanense*, *T. gourvilii*, *Trichophyton fischeri* and *Trichophyton kanei*. All of them are now unified in the taxon typified by the ex-neotype isolate of *T. rubrum* [9].

The majority of *Microsporum* species, as well geophilic *Trichophyton* species, are found in phylogenetic examination to be consistent with long-standing morphological species concepts and to an even greater extent with biological species concepts.

In the biological species recognition (BSR), defined as the operational unit of the biological species concept (BSC), species-level boundaries are determined by measuring the decreasing ability of intermated isolates to produce fully viable progeny. As mentioned, in geophilic dermatophytes, the BSR correlates with commonly used molecular species identification markers. None of the teleomorphic species shows more than 97% ITS sequence identity to other species [33, 49–51]. The BSC, however, is

inadequate for the biosystematic analysis of dermatophytes that no longer reproduce by random mating. This applies to the majority of dermatophytes associated with mammals.

A practical application of the phylogenetic species concept, the 'genealogical concordance phylogenetic species recognition' (GCPSR) was introduced by Avise and Ball [52] 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 seldom hybridize with other species (a phenomenon that by contrast often occurs in plants), the threshold of phylogenetic distinction where disconcordant gene histories no longer appear, indicates the beginning of reproductive isolation and hence of species divergence in sexual species. In the case of strictly clonal reproduction, as in the mammalian dermatophytes, the GCPSR is not optimal because there is no incongruence between gene genealogies at any level of diversity. There are, however, theoretical as well as practical reasons to treat at least some of the clonal entities within the dermatophytes as separate species (outlined in Gräser et al. [10]), but, for science to proceed in a meaningful manner, no traditionally recognized entities can be maintained a priori on the basis of clinical and classical laboratory appearance alone.

## Reworking of Species Concepts in Dermatophytes Based on Multilocus Sequence Typing (MLST) and Population Genetic Data

### MLST Marker for Phylogenetic Analysis

MLST is a successful genetic typing approach that was developed in biodiversity studies of bacteria. This technique uses nucleotide sequences from ca. 500 nucleotides of each of 10 or more housekeeping genes to construct phylogenies and to find the limits of incongruencies in order to define species borders [53]. For fungal systematics, however, relatively variable genomic regions, e.g., ITS, intergenic spacers (IGS) between genes and gene introns must be used due to the slow evolution of many organisms of the kingdom. RFLP (restriction fragment length polymorphism) analysis of the ITS region, at least in dermatophytes, does not attain the discriminating potential of sequencing [54].

The power of MLST runs into problems, however, when the species being typed have insufficient genetic variation to allow strains to be differentiated. Such reduced levels of genetic diversity occur as a consequence of certain evolutionary processes, such as recent speciation or, in relatively extreme cases, profuse radiation of species such as is seen in the dermatophytes. In such cases, highly variable loci have to be analysed, e.g., microsatellites.

### Microsatellite Markers for Population Genetic Analysis

Microsatellites have a far higher mutation rate ($10^{-4}$–$10^{-5}$ estimated for yeast) than the $10^{-9}$ rate seen for point mutations in non-coding loci like ITS. This mutability generates high levels of intraspecific diversity [55]. Typing with microsatellites is similar to MLST in that an entire stretch of sequence is surveyed for genetic variation, albeit for length polymorphisms rather than point mutations, and multilocus genotypes are generated and then added to a database. However, the hypervariability of microsatellite loci and their stepwise mode of mutation, creates alleles that might be identical in form, but not identical in descent, (i.e., loci might be homoplaseous). Also, microsatellites found to be polymorphic in one genetically isolated clade could be monomorphic in others [49]. Studies using microsatellites have shown that the problems of both homoplasy and unexpected monomorphism can be overcome by using many microsatellite loci in tandem [9].

#### *Other Markers/Methods for Strain Typing*

A variety of techniques based on variation in fingerprint patterns have also been described as useful for strain typing or even for population genetic studies. AP-PCR, PCR fingerprinting and RAPD are based on a single short primer that amplifies genomic fragments scattered around the chromosomes of the strain. Bands differing in size and intensity are produced. The reliability of these methods depends on using standardized conditions (inclusive of the make of thermocycler). Therefore, it is almost impossible to reproduce these techniques among laboratories. In dermatophytes, these techniques have

been extensively studied. Due to a lack of variability, a result of recent adaptive radiation, only interspecific discrimination is possible [56, 57] in contrast to what is seen in many other fungal species.

## The Phylogeny of the Genus *Arthroderma*

In morphotaxonomy, the family Arthrodermataceae (dermatophytes and *Ctenomyces serratus*) is a monomorphic group characterized by tiny, smooth, essentially oblate ascospores and fully expressed keratinophily [58]. This grouping is also reflected when molecular markers are applied [59, 60]. However, the recognition of four morphological anamorph genera—*Trichophyton*, *Microsporum*, *Epidermophyton* and *Chrysosporium*, disagrees with phylogenetic species concepts. When nuclear DNA targets are examined, not even the genus *Trichophyton* is monophyletic, because it embraces and is thus split up by the genera *Microsporum* and *Epidermophyton* [29, 61]. In addition, some *Chrysosporium* species are interspersed among the geophilic *Trichophyton* species. These species are not at all related to the type species of *Chrysosporium*, *C. merdarium*, and there is no basis for them retaining them under their current genus name.

### *The Phylogeny of the Geophilic Dermatophytes*

The paraphyletic grouping of anthropophilic and zoophilic species in the genus *Trichophyton* in one clade and geophilic species in another supports the view that ecology has been a particularly strong driver of the evolution in dermatophytes. The 31 geophilic taxa in the family Arthrodermataceae include 11 *Trichophyton*, 6 *Chrysosporium* and 13 *Microsporum* anamorphs (Table 1, Fig. 1). Some of them are not discriminated by anamorphic characters, e.g., isolates grouped within *T. terrestre* (3 teleomorphic species) and *M. gypseum* (2 teleomorphic species). This circumstance indicates once more that the MSC (morphological species concept) can be inadequate. In turn, ascospore morphologies as well as the genetic makeup of the organisms show a clear distinction among the five taxa [33, 50, 51, 58, 61, 62].

Recently a new geophilic *Trichophyton* species, *T. eboreum* was described by Brasch and Gräser [50]. Its morphology was close to *T. terrestre*, but the ITS sequence was very distantly related to that of any known taxon in the genus. One year later, again with the help of molecular methods, the teleomorph of this species was discovered and described as *Arthroderma olidum* by Campbell et al. [62]. The anamorphic species was isolated from an African immigrant suffering from tinea pedis. Phylogenetic analysis, however, clustered *T. eboreum* amongst the geophilic dermatophytes. The grouping was confirmed by the consistent isolation of the species from badger burrows [62]. This finding supports the usefulness of the phylogenetic species concept.

## The Basis of Phylogenetic Species Concepts n the Dermatophyte Species Associated with Mammals

### *The* Arthroderma otae *Complex*

This complex is composed of three close related species, *M. canis*, *M. audouinii* and *M. ferrugineum*. This grouping obtains support from a variety of genomic markers, including nuclear markers such as the ITS, *mep*B, and 3′ non-coding ubiquitin (Ub) regions, as well as mitochondrial markers such as the intergenic spacers between the *ATP9* and *COXII* gene and the NADH subunits 1 and 3 (N3 and N1) [32, 38]. Also, high resolution methods such as AP-PCR or PCR fingerprinting have been applied with success for species differentiation in this complex [56, 57] whereas RFLP analysis of the whole mtDNA was shown to be overly conservative, enabling species recognition only for *M. audouinii* [63]. Other nuclear markers applied for species delineation in the dermatophytes, e.g., actin gene intron, partial topoisomerase gene and the ribosomal LSU may be promising candidates for this complex [64–67]. The evidence regarding the usefulness of these markers, however, is deficient, since none of the studies cited included all three species.

Two of the three species, *M. audouinii* and *M. ferrugineum*, reproduce strictly clonally and, in association with humans, appear to have evolved in different geographic niches, Africa and Asia. *M. canis* is distributed worldwide and is associated with cats and horses, primarily. Microsatellite markers have indicated that multiple populations exist with both clonal and sexual reproduction. Strains with horse population hosts are unevenly distributed

**Table 1** Synonymized taxa and a typical accession number (ITS sequence) shown for every species of the current concept

| New species concept | Accession number | Synonymized taxa |
|---|---|---|
| Mammal associated dermatophytes | | |
| *Trichophyton equinum* | EF067316 | All varieties of *T. equinum* |
| *T. tonsurans* | EF043270 | All varieties of *T. tonsurans* |
| *T. interdigitale* (anthropophilic) | AF168124 | *T. mentagrophytes* var. *goetzii*, *interdigitale*, *nodulare*, *T. krajdenii* |
| *T. interdigitale* (zoophilic) | AY062119 | *T. mentagrophytes* var. *mentagrophytes*, *granulosum*, *T. verrucosum* var. *autotrophicum* |
| *Arthroderma vanbreuseghemii* | Z98014 | Identical |
| *T. mentagrophytes* | Z97995 | *T. mentagrophytes* var. *quinckeanum*, *T. langeronii*, *T. sarkisovii* |
| *T. schoenleinii* | Z98011 | Identical |
| *A. simii*/*T. simii* | Z98017 | Identical |
| *T. concentricum* | Z98012 | Identical |
| *T. verrucosum* | Z98003 | All varieties of *T. verrucosum* |
| *T. erinacei* | Z97997 | *T. mentagrophytes* var. *erinacei* |
| *A. benhamiae* | Z98015 | Identical |
| *T. anamorph* of *A. benhamiae* | Z98016 | *T. mentagrophytes* var. *granulosum* |
| *T. rubrum* | Z97993 | *T. fischeri*, *T. kanei*, *T. raubitschekii* |
| *T. rubrum* (African population) | AF170473 | *T. raubitschekii*, *T. soudanense*, *T. gourvilii*, *T. megninii* |
| *T. violaceum* | AJ270811 | All varieties of *T. violaceum*, *T. yaoundei* |
| *Epidermophyton floccosum* | AJ000629 | Identical |
| *Microsporum audouinii* | AJ000622 | *M. langeronii*, *M. rivalieri* |
| *M. canis* | AJ000619 | *M. distortum*, *M. equinum* |
| *M. ferrugineum* | AJ252336 | Identical |
| Geophilic *Microsporum* species | | |
| *A. gypseum*/*M. gypseum* | AJ970141 | *M. appendiculatum* |
| *A. incurvatum*/*M. gypseum* | AJ970153 | Identical |
| *A. fulvum*/*M. fulvum* | AJ000627 | *K. longifusus*, *M. boullardii*, *M. ripariae* |
| *A. persicolor*/*M. persicolor* | AJ000615 | Identical |
| *A. grubyi*/*M. gallinae* | AJ000612 | *M. vanbreuseghemii* |
| *A. borellii*/*M. amazonicum* | AJ877220 | Identical |
| *A. racemosum*/*M. racemosum* | AJ970146 | Identical |
| *A. cajetanum*/*M. cookei* | AJ970145 | Identical |
| *A. obtusum*/*M. nanum* | AJ970149 | Identical |
| *A. cookiellum* | AM000034 | Identical |
| *A. corniculatum* | AJ000612 | Identical |
| *M. praecox* | AJ970148 | Identical |
| *M. duboisii* | AJ970142 | Identical |
| Geophilic *Trichophyton* species | | |
| *A. gertleri*/*T. vanbreuseghemii* | AJ877210 | Identical |
| *A. gloriae*/*T. gloriae* | AJ877209 | Identical |
| *A. ciferrii* | AJ877217 | Identical |
| *A. flavescens*/*T. flavescens* | AJ877219 | Identical |
| *A. uncinatum*/*T. ajelloi* | AJ877212 | All varieties of *T. ajelloi*, *E. stockdaleae* |

**Table 1** continued

| New species concept | Accession number | Synonymized taxa |
|---|---|---|
| *A. quadrifidum*/*T. terrestre* | AJ877214 | Identical |
| *A. lenticulare*/*T. terrestre* | AJ877211 | Identical |
| *A. insingulare*/*T. terrestre* | AJ000606 | Identical |
| *A. olidum*/*T. eboreum* | AJ876907 | Identical |
| *A. ciferrii*/*T. georgiae* | AJ877217 | Identical |
| *A. melis*/*T. melis* | AJ877216 | Identical |
| *T. thuringiense* | AJ877215 | Identical |
| *T. phaseoliforme* | AJ970152 | Identical |
| *A. multifidum*/*Chrysosporium* sp. | AJ877218 | Identical |
| *A. tuberculatum*/*Chrysosporium* sp. | AJ877221 | Identical |
| *A. curreyi*/*Chrysosporium* sp. | AJ877223 | Identical |
| *A. cuniculi*/*Chrysosporium* sp. | AJ000609 | Identical |
| *Chrysosporium vespertilium* | AJ007846 | Identical |
| *Ctenomyces serratus*/*Chrysosp.* | AJ877222 | Identical |

among the genetic populations: the majority belongs to a single clonal population [49].

*The* Arthroderma vanbreuseghemii *Complex*

Three globally distributed anamorph species are associated with this teleomorphic complex, namely *T. tonsurans*, *T. equinum* and *T. interdigitale* [10]. *T. tonsurans* is anthropophilic while *T. equinum* is associated with horses. *T. interdigitale* is the only species among dermatophytes where profound ecological disjunctions are found within a single species. This taxon unifies anthropophilic and zoophilic strains. This situation, however, is not new: some varieties of the former *T. mentagrophytes* complex, e.g., *T. m.* var. *interdigitale*, var. *nodulare*, and var. *goetzii* (all taxonomically invalid names) were associated with humans, while other variant names such as *T. m.* var. *mentagrophytes* and var. *granulosum* were used for zoophilic isolates. Recent studies undertaken by Michel Monod and Yvonne Gräser's groups suggest that the zoophilic strains in *T. interdigitale* are associated, at least in European veterinary practices, with cats [68], and that a genotypic discrimination using ITS may be possible [unpublished data]. Other animal hosts that have regularly yielded zoophilic *T. interdigitale* (usually listed as *A. vanbreuseghemii*) well confirmed by competent mating studies and/or by molecular techniques include rats [69, 70], mice [71] chinchilla [71, 72], hamsters [70, 72, 73], dogs [70, 74, 75], sea lions [70, 73], guinea pigs [69], rabbits [75] and squirrels [73]. Phenotypically, the zoophilic *T. interdigitale* strains corresponding to the old variety '*T. m.* var. *granulosum*' and the former type concept of *T. m.* var. *mentagrophytes*, are indistinguishable from strains phylogenetically belonging to *A. benhamiae*. *A. benhamiae* strains, however, are more restricted in host species range, and are especially strongly associated with rabbits and guinea pigs [39, 76]. ITS, LSU, and the intron of the heat shock protein gene support this grouping, as do PCR fingerprinting analyses [30, 37, 57, 67]. In LSU and RAPD studies, the discrimination of *T. tonsurans* from *T. equinum* has unfortunately not been investigated. In Ninet's study using sequencing of the D2 domain of the LSU, *T. mentagrophytes* types I and II correspond to the anthropophilic *T. interdigitale* strains while type III is identical with the zoophilic *T. interdigitale*. Type IV refers to *A. benhamiae* strains [67, Drouot et al., unpublished data]. RFLP analysis of the mtDNA as well as of the ITS and chitin synthase I sequences were too conservative to discriminate among *T. tonsurans*, *T. interdigitale* and *T. equinum* [35, 54, 77].

*The* Arthroderma simii *Complex*

The anamorph of *A. simii* is *T. simii*, which is reputed to be associated with monkeys [4]. Two other species are closely related to this holomorphic species, a

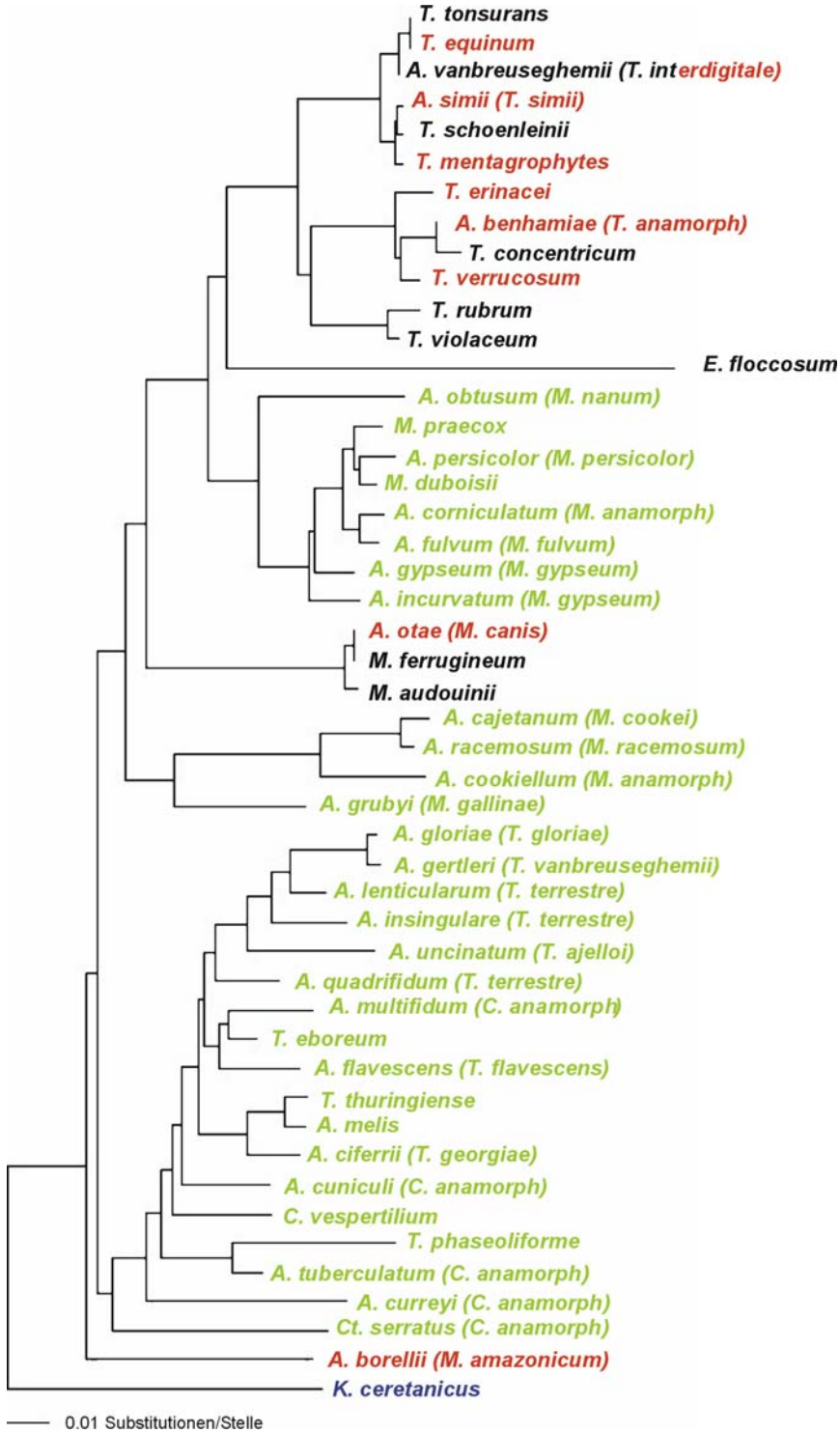


**Fig. 1** Phylogenetic relationship of all validly described dermatophyte species based on ITS sequence data. For tree construction, the Kimura 2 parameter model and the Neighbour Joining method were used. *Keratinomyces ceretanicus* was used as outgroup species. Black coloured are anthropophilic species, red coloured are zoophilic species and green coloured are geophilic species

zoophile rooted by the neotype of *T. mentagrophytes* (CBS 318.56) and an anthropophile, *T. schoenleinii*. Both species seem to be endemic in identical areas and are nowadays restricted to Asia, The Near and Middle East and Africa. The zoophilic *T. mentagrophytes* (the former variety '*quinckeanum*' of the

*T. mentagrophytes* complex), though historically attributed to mouse favus [41] seems not to be most prominently associated with mice or other rodents, but rather with camels. In our lab, fresh isolates from rodents (inclusive of mice) have never corresponded to *T. mentagrophytes* in molecular investigations. In our previous studies [37], just one of the isolates studied (SJ ED 0001) was recorded as being from mouse. Considerably larger numbers of isolates consistent with this genotype have been deposited in collections worldwide in connection with species descriptions of strains isolated from camels, namely *T. langeronii* from dromedaries in Saudi Arabia and *T. sarkisovii* from Bactrian camels in Kazakhstan. These species revealed ITS sequences identical with that of the neotype of *T. mentagrophytes* [37]. *T. schoenleinii* was recently found to be relatively common in western China [78]. Bactrian camels are also widespread in this area, which borders on Kazakhstan. This may explain the close phylogenetic relatedness of *T. schoenleinii* and *T. mentagrophytes*, assuming a host jump from camels to humans. Based on the scenario that dermatophytes begin as sexual organisms associated with ground-dwelling animals, then host-jump to become asexual organisms on non-ground-dwelling animals [25], *T. mentagrophytes* may have evolved from a heavily conidial rodent-based lineage to a camel lineage (bactrian form visually recognizable as a faviform, *T. verrucosum*-like colony according to [79] and then to a distinct, highly faviform human lineage, namely *T. schoenleinii*. Currently, the grouping of the *T. mentagrophytes* lineage is supported by MLST markers, such as ITS, *TRI2*, *TRI4*, and *ATP9*/*CytII*, as well as by RAPD analysis [31, 37, 57]. RFLP of the mtDNA as well as of the ITS region and the topoisomerase II gene are unable to discriminate the species of this complex [54, 66, 77]. The *CHS* gene has never been applied for discrimination among the species of this complex.

### *The* Arthroderma benhamiae *Complex*

Among the members of the *A. benhamiae* complex, we find two varieties within the former *T. mentagrophytes* complex that have now obtained species status, *T. m.* var. *granulosum* and *T. m.* var *erinacei*. *T. erinacei* is zoophilic and has hedgehogs as host species. Within *T. erinacei*, however, two races have been distinguished on the basis of mating experiments. *T. erinacei* strains associated with the African hedgehog species *Aterelix albiventris*, native to Central Africa, belong genotypically to the African race, while those associated with the European hedgehog *Erinaceus europaeus*, cluster with the European race [80].

As mentioned earlier, the '*T. m.* var. *granulosum*' type of *A. benhamiae* (which lacks a binomial anamorphic species name, but is correctly called the *Trichophyton* anamorph of *A. benhamiae*) are morphologically indistinguishable from similar strains of *A. vanbreuseghemii*. Strains of both species are distributed worldwide, but strains of *A. benhamiae* are very likely to have a more restricted range of host species (rabbits and guinea pigs). The third zoophilic species in the *A. benhamiae* complex is *T. verrucosum*, which is associated with cattle but not sheep. The former variety '*T. verrucosum* var. *autotrophicum*,' which has Karakul and related Asiatic sheep as a host turned out to be associated with the *A. vanbreuseghemii* complex [30]. This association was supported by ITS sequencing of sheep isolates recently collected in China [78]. Originally *T. v.* var. *autotrophicum* strains were isolated from Karakul sheep's in South West Africa [81]. The only anthropophilic species in this complex is *Trichophyton concentricum*, which is endemic to certain indigenous populations in South East Asia and the Pacific islands (e.g., Indonesia, Melanesia, and New Guinea). Many genomic markers, like ITS, IGS of the mtDNA (*ATP9*/*COXII*) are supporting this concept [30]. RFLP analysis of the ITS restricted with *Mva*I did not distinguish *T. concentricum* and *T. erinacei*. *T. verrucosum* appeared to be distinguished, but related *A. benhamiae* strains were not investigated [54]. Clinically, the zoophilic species mainly cause highly inflammatory tinea capitis, tinea corporis or tinea faciei while *T. concentricum* causes a characteristic clinical picture, tinea imbricata.

### *The* Trichophyton rubrum *Complex*

The *T. rubrum* complex, as mentioned above, consists of two anthropophilic species, *T. rubrum* and *T. violaceum* [9, 82]. This complex has no close related teleomorphic species suggestive of clonal reproduction for quite a long time [10]. This mode of reproduction gets evidence by a population genetic

study using microsatellite markers [8, 9]. A recent study [9] showed that *T. rubrum* is geographically substructured into two populations, one of African origin (population 2) and one found in the rest of the world (population 1). The former mainly causes tinea corporis and capitis, while the latter is associated with tinea pedis and onychomycosis. It should be stressed that the African population of *T. rubrum* is not entirely isomorphous with the former species *T. soudanense* because strains without reflexive branching hyphae and with urease production (concept of *T. raubitschekii*) also grouped with this population. Correspondingly, population 1 contained some urease positive strains along with the prevalent negative strains [9, 44].

*Trichophyton violaceum* is clearly different: it is also endemic in Africa and causes mainly mild forms of tinea capitis. The following markers support the grouping of the *T. rubrum*/*violaceum* complex into just two species: ITS (inclusive of RFLP analysis), LSU (D2 domain), IGS of the mtDNA, *ATP9*/*COXII*, *HSP* gene intron, chitinase gene, topoisomerase gene, RAPD analysis and microsatellites [9, 54, 57, 66, 67, 82–85].

## Recommendations for Routine and Reference Laboratories

As gold standard for the identification of atypical or difficult dermatophyte isolates in reference laboratories, ITS sequencing is recommended. It is the only marker for which a complete database exists that can be used for dermatophyte identification (in NCBI/EMBL and SmartGene; a software package designed by a Swiss company). A 'barcode' database at the CBS which will be available for the public is build up at the moment. Attention is needed, however, to the correctness of species names given in public databases, since the sequences deposited represent many viewpoints and historical periods. Therefore, we designate a 'barcode' (recommended, verified correct) sequence accession number each species (Table 1). In several cases, only one or two polymorphic nucleotides (signature nucleotides) separate two species, such as *M. ferrugineum* and *M. canis* (in ITS2), *T. equinum* and *T. tonsurans* (in ITS1 [36]) and the zoophilic and anthropophilic strains of *T. interdigitale*. Sequences for all dermatophyte species are not available for LSU or topoisomerase gene. The *CHS* gene and SSU are too conservative for species recognition.

The drawback for PCR fingerprinting methods in diagnosis is their reliance on culture, which is often unsuccessful in examinations of dermatophytoses [86]. For routine laboratories, where molecular methods enter more and more, ITS sequencing represents the most valuable means by which species identification can be performed directly from the clinical specimens [87, 88]. Direct sequencing from specimens is applicable, in principle, to other methods and markers as well; however, a complete barcode sequence database is only available for ITS. For all other genomic regions, the extent to which closely related species can be distinguished is not known. At the moment, only sequencing is able to differentiate all species at once. All other methods, e.g., ELISA-PCR [89] depend on the number of gene probes designed for identification. The test designed by Beifuß et al. allows identification of only five common species. Close related species e.g., *T. tonsurans* and *T. equinum* are not to distinguish from *T. interdigitale*. Bio-Advance's ONYCHODIAG kit employs just a single probe to detect all dermatophytes as a group, inclusive of non-pathogenic geophiles and related chrysosporia [90]. This makes the distinction of zoophiles from anthropophiles impossible, a factor that is important in therapy. Another advantage of ITS sequencing is that most other fungal species (non-dermatophytes) involved in dermatomycoses can also be identified [88]. Routine laboratories that rely entirely on conventional diagnostics should be aware that they will have a *T. mentagrophytes* complex problem and that misidentifications are always possible.

It is recommended to strictly rely on the new species concepts, at least when using molecular identification methods. Given that the former *T. mentagrophytes sensu lato* consists of four species that are distinguished at many molecular targets, it would be very confusing to use the name *T. mentagrophytes* for all isolates [65, 66, 89]. The question would arise: which *T. mentagrophytes* is meant? In turn, species that have been synonymized (e.g., *T. megninii* and other members of the T. *rubrum* complex) are not reliably differentiated by any molecular target, inclusive of microsatellites. These

factors should be kept in mind by anyone performing dermatophyte identification.

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