Recent advances in the molecular taxonomy of dermatophytes

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Summary The use of characteristics for species distinction in dermatophytes over the last century is outlined. On the basis of molecular data, three main groups are recognized, largely coinciding with ecology and clinical data less so with taxonomic borderlines. One group consists mainly of anthropophilic *Trichophyton* species; the mainly zoophilic *Microsporum* species are paraphyletic to this group. The geophilic species are highly diverse. Adaptation to the human host is accompanied by a gradual loss of sexuality.

Key words Dermatophytes, Phylogeny, ITS

The fungal nature of dermatophytoses was recognized in the first half of the 19th century, when Gruby (1843) [1] described *Microsporum audouinii* from a case of human *tinea capitis.* In the seventy years that followed, the majority of the dermatophyte species presently considered most significant in human disease were introduced. None of these taxa was cultivated, species distinction being primarily based on clinical features. No authentic materials or microscope slides have been preserved. A new reference system should therefore be built up by the indication of neotypes [2].

Sabouraud (1910) [3] was one of the first to systematically grow the etiologic agents on artificial media, thereby introducing a wealth of additional diagnostic criteria in cultural characteristics and morphology. The number of taxa distinguished increased exponentially. However, it was later realized [4] that these biological features are unstable: coloured metabolites, which are characteristic in primary cultures, easily are lost in repeated transfers, while colonies may lose sporulation by formation of fluffy, sterile sectors. This diminishes their value for taxonomy, which necessarily includes the re-examination of old reference strains.

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Nutritional physiology and tolerance tests were therefore introduced [4,5] as a set of methods independent from morphology. The technique was not primarily meant for distinction of new species, but rather as an aid to recognize existing taxa. The test system consisted of a number of agar media for the detection of vitamin requirements, *in vitro* hair perforation, growth on polished rice and presence or absence of urease. Kane and Fischer [6] added supplementary tests such as growth on bromcresol purple casein agar, casamino acids-erythritol-albumin agar and tolerance of sodium chloride. Due to the low discriminatory ability and the frequent incongruency of existing taxonomic borderlines and physiological data, the diagnostic system has become rather complicated [6]. Numerous exceptions and variants were encountered, which often again were introduced as separate microtaxa, down to the level of variety [7] or subvariety [8].

A successful approach to the resolution of the taxonomy of dermatophytes was based on the biological species concept, which defines species as groups of interbreeding populations reproductively isolated from other groups. Particularly geophilic species frequently produce *Arthroderma* teleomorphs in culture. For zoo- and particularly anthropophilic taxa, mating experiments are mostly needed, which are carried out on diluted agar media with additional salts [9,10]. With single-spore isolations, tester strains were developed, which could be used for definite species identification. The methodology is, however, not applicable for routine identification of dermatophytes. Moreover, some species that failed to produce a teleomorph in any crossing, such as *Trichophyton rubrum* and *T. tonsurans*, have recently been proven to be nearly exclusively clonal [11,12] and thus are unlikely to produce ascospores at any time. In close relatives of such species teleomorphs are known to occur at low frequency. The results of molecular studies, discussed below, can help to select strains for crossings with a high probability of positive result. This will lead to an integration of biological and molecular taxonomic concepts.

In daily practice, dermatophyte diagnostics has been solved for the great majority of the strains, but for a small portion of isolates a renewed study of reference

Table 1. Current taxonomy of the family *Arthrodermataceae* based on morphological, ecological and genotypic features.

Current taxonomy Anamorph / Teleomorph	Synonyms
T. tonsurans	*T. areolatum* *T. equinum* var. *autotrophicum* *T. equinum* var. *equinum* *T. floriforme* *T. spadiceum* *T. tonsurans* var. *crateriforme* *T. tonsurans* var. *epilans* *T. tonsurans* var. *sulfureum*
T. balcaneum	*T. abissinicum* *T. immergens* *T. radicosum*
T. interdigitale / A. vanbreuseghemii	*T. batonrougei* *T. candelabrum* *T. krajdenii* *T. mentagrophytes* var. *interdigitale* *T. mentagrophytes* var. *nodulare* *T. mentagrophytes* var. *goetzii* *T. rotundum* *T. verrucosum* var. *autotrophicum*
T. mentagrophytes	*T. depressum* *T. langeronii* *T. mentagrophytes* var. *quinckeanum* *T. papillosum* *T. sarkisovii*
T. simii / A. simii	Identical
T. schoenleinii	Identical
T. erinacei / A. benhamiae	*T. mentagrophytes* var. *erinacei* *T. proliferans*
T. verrucosum	*T. verrucosum* var. *album* *T. verrucosum* var. *discoides* *T. verrucosum* var. *ochraceum* *T. verrucosum* var. *verrucosum*
T. concentricum	Identical
T. bullosum	Identical
T. rubrum	*T. circonvolutum* *T. fischeri* *T. fluviomuniense* *T. kanei* *T. kuryangei* *T. megninii* *T. pedis* *T. pervesii* *T. raubitscheckii* *T. rodhainii* *T. rubrum* var. *nigricans*
T. violaceum	*T. glabrum* *T. gourvilii* *T. soudanense* *T. violaceum* var. *indicum* *T. yaoundei*
M. audouinii	*M. langeronii* *M. rivalieri*
M. canis / A. otae	*M. distortum* *M. equinum*
M. ferrugineum	Identical
E. floccosum	Identical
M. nanum/A. obtusum	Identical
M. praecox	Identical
M. persicolor / A. persicolor	Identical
M. gypseum / A. gypseum	Identical
M. duboisii	Identical
M. sp. */ A. corniculatum*	Identical
M. fulvum / A. fulvum	*K. longifusus* *M. boullardii* *M. ripariae*
M. gypseum / A. incurvatum	Identical
M. cookei / A. cajetani	Identical
M. racemosa / A. racemosum	Identical
M. gallinae / A. grubyi	*M. vanbreuseghemi*
M. amazonicum / A. borelli	Identical
T. gloriae / A. gloriae	Identical
T. vanbreuseghemii / A. gertleri	Identical
T. ajelloi / A. uncinatum	*T. ajelloi* var. *nanum* *E. stockdaleae*
T. terrestre / A. lenticulare	Identical
T. terrestre / A. quadrifidum	Identical
T. terrestre / A. insingulare	Identical
T. flavescens / A. flavescens	Identical
A. melis	Identical
T. georgiae / A. ciferrii	Identical
Chrysosporium sp. */ A. multifidum*	Identical
Chrysosporium sp. */ A. tuberculatum*	Identical
Chrysosporium sp. */ A. cuniculi*	Identical
T. thuringiense	Identical
T. phaseoliforme	Identical
Chrysosporium sp. */ Ctenomyces serratus*	Identical
Keratinomyces ceretanicus	Identical
Chrysosporium sp. */ Arthroderma curreyi*	Identical

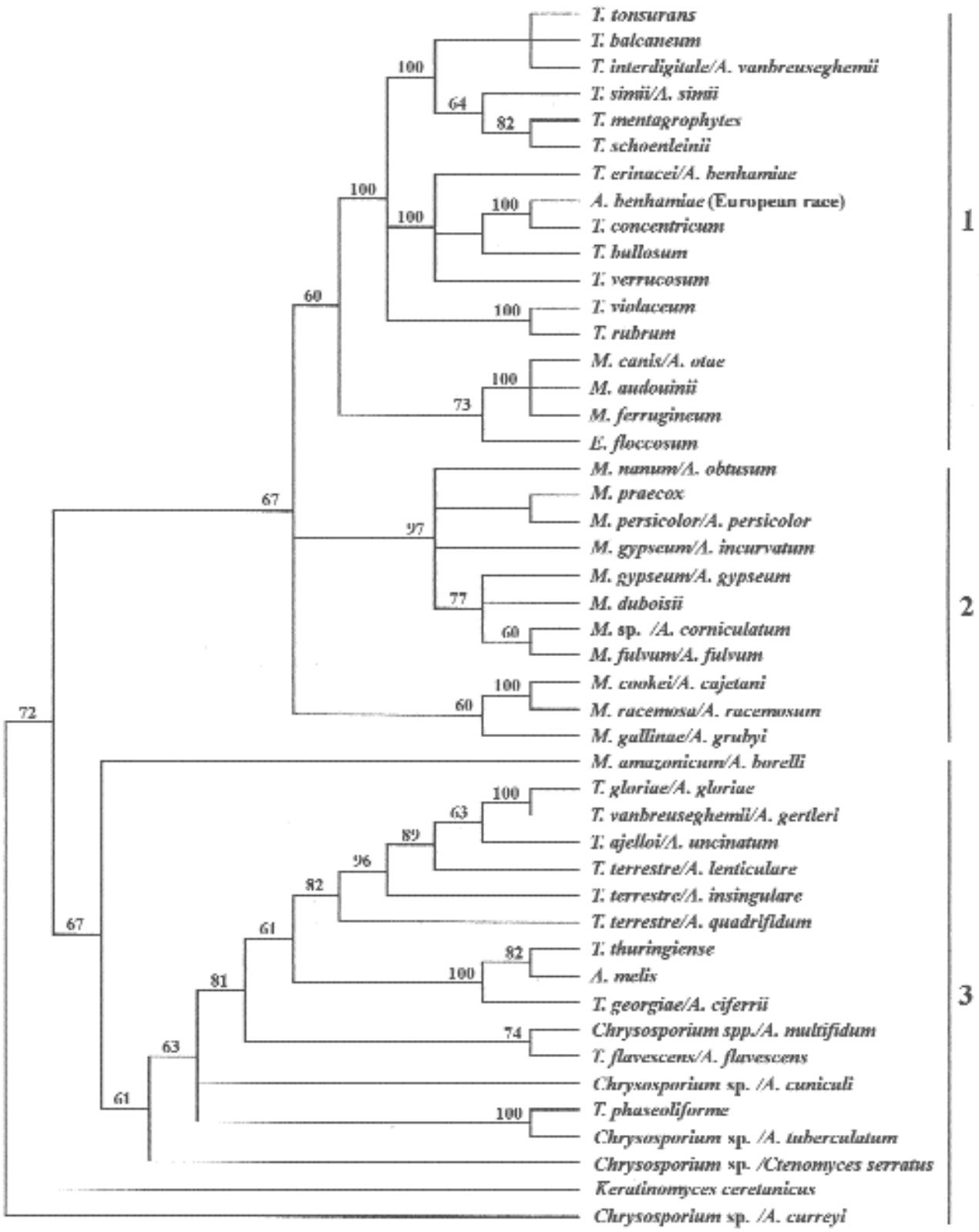

Figure 1. Consensus tree of the family *Arthrodermataceae* obtained by Parsimony analysis using sequences of the ITS1, 5.8 S and ITS2 rDNA regions. Bootstrap values above 60% are shown. Group 1 species are anthropophilic/zoophilic, group 2 species are zoophilic/geophilic and group 3 species are strictly geophilic.

strains is necessary. Collection strains are degenerative and therefore the taxonomy by conventional means has remained to be unsatisfactory. That is one reason why the dermatophytes were among the first fungal groups where molecular methods were applied, viz. nDNA G+C composition and genomic DNA homology [13,14]. These authors noted that the wide spectrum of anthropophilic dermatophyte species recognized at that time actually comprised a complex of very closely related taxa. Attempts to resolve the human-associated dermatophyte taxa by sequencing studies were unsuccessful when relatively invariable genes such as the 18S rDNA domain were used, because it was difficult to establish a hierarchy of species [15]. Subsequent studies, applying a diversity of molecular techniques, all lead to similar conclusions: restriction fragment analyses (RFLP) of the mitochondrial DNA [16,17], sequencing of the chitin synthase gene [18] or hybridization with DNA probes [19] all provided insufficient separation of taxa. Even the application of methods such as RAPD analysis [20], arbitrary primed PCR [21] or PCR fingerprinting [22], which all detect hypervariable DNA elements, were unsuccessful. Infraspecific grouping was nevertheless achieved with these methods, and particularly with IGS rDNA sequencing [23]. But insufficient taxonomic resolution was achieved among several human-associated dermatophyte species (e.g. *T. rubrum* complex, *T. interdigitale* complex, *T. tonsurans / T. equinum* etc.). This lead to the conviction that many anthropophilic taxa, as defined on the basis of classical methods, were very closely related to each other, if not identical.

A clue towards resolving the problem appeared to be the inclusion of a larger number of sufficiently remote species, setting a standard for phylogenetic distances within the group as a whole. Sequencing the variable D2 region of the 26S rDNA [24] revealed that most geophilic taxa were clearly apart from the remaining taxa. In an extended study based on ITS rDNA phylogeny [25] three different groups were found. In contrast to the classical taxonomic system, which unifies the species according their morphological features in three form genera (*Trichophyton*, *Microsporum*, *Epidermophyton*), the molecular grouping rather appeared in agreement with clinical and ecological traits of these species. The anamorph genera were found to be poly- or paraphyletic (Figure 1). Particularly the strictly geophilic species (group 3) showed a high degree of taxonomic and sequence diversity, which is in accordance with their sexual reproduction including a wide variety of *Arthroderma* species. Most of these geophilic species are known from only a limited number of strains, but nevertheless for nearly all a teleomorph has been found. The *Microsporum* species (group 2) constitute a more coherent and morphologically well distinguishable group; the great majority of them is found asymptomatically in the fur of mammals. Only some taxa are known as soil residents. The mainly anthropophilic species of the genus *Trichophyton* (group 1) were found to be paraphyletic to *Microsporum* and are closely akin to each other, thus confirming the results of the studies mentioned above. Gräser et al. [2,26,27] used PCR fingerprinting and AFLP (amplified fragment length polymorphism) concomitantly to calibrate the ITS findings. In main traits the same outcome was obtained, although finer taxonomic distinctions were achieved in the case of PCR fingerprinting and particularly of AFLP.

After an analysis of a large number of reference and clinical strains, it gradually became clear that there is a good correspondence of ITS phylogeny on the one hand and clinical and ecological data on the other, and also an acceptable consistency with morphology. In contrast, some physiological data, such as *Trichophyton*-agars, and cultural characteristics are either undiagnostic or vary at random. This is not problematic when working with primary isolates in routine diagnostics, but for taxonomy these methods have largely become obsolete.

The evolution of the dermatophytes may be supposed to have gone through the following stages. Members of group 3 live in soil and are generally unable to provoke diseases in warm-blooded animals. Some of these taxa are even unable to tolerate temperatures above 30°C [28]. Zoophilic species are carried asymptomatically by furred animals, but through direct transmission to the naked skin of humans they are able to provoke highly inflammatory mycoses. The genus *Trichophyton*, primarily containing species with little-differentiated conidia or being sterile, are frequently found on humans causing acute inflammatory infections, but some of them, such as *T. interdigitale*, *T. concentricum*, *T. violaceum* and *T. rubrum*, have low virulence with little inflammation, and are chronic. The latter group can be regarded as truly anthropophilic, as these species are able to mitigate cellular immune response. They are directly transmitted from human to human. Despite the fact that of some species thousands of strains have been studied, several taxa still lack a known teleomorph. Thus a loss of teleomorph and a tendency towards clonal reproduction seems a likely evolutionary trend in this group. Since the strictly anthropophilic species, including *Epidermophyton floccosum*, are not a monophyletic group: anthropophily seems to have developed several times within the dermatophytes.

The current taxonomic system of the dermatophytes is summarized in table 1.

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