Monograph
On
Dermatophytes

A guide for isolation and identification of dermatophytes, diseases and treatment

By

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2013
Dedication

This monograph is dedicated to my master, friend, teacher and spiritual father Prof. Dr. Dr. Hans Rieth, whom I met for the first time in July 1962, in Travemünde on the occasion of the second meeting of the German-speaking Mycological society, 6 months after my arrival to Germany, and whom I met for the last time in September, 1993 in Greifswald on the occasion of 27th meeting of the society, 5 months before his death. During the 30 years I visited him almost every year, where I always updated my knowledge in mycology.

Mohamed Refai
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1. Introduction and historical\(^{(1),(2)}\)

Dermatophytes are a unique group of moulds which have the capacity to invade keratinized tissue, in man and animals, causing cutaneous infections commonly referred to as tinea or ‘ringworm’. They are dependent on keratin as nutrient source as they can cause its hydrolysis. Dermatophytes affects only skin, hair and nails and remain confined to the dead tissues and do not invade the living part of the tissues. The name dermatophyte consists of two parts, namely “derm” which means skin and “phytes” which means plants. In fact the fungi known as dermatophytes are not plants and are not confined only to the skin, but they can affect also hairs and nails. Consequently, the name is not correct, but it is used because it is tradionally applied to this group of fungi and no other name was proposed.

Remak (1837) detected fungal elements in the scutula in a case of favus and Schoenlein (1839) described the nature of the fungus and recognized its aetiological role in favus. Remak (1845) named the fungus Achorion (the Greek name of scab or scurf) and coined the name of Schoenlein to the fungus as Achorion schoenleinii. Gruby (1843) described small fungal spores surrounding hairs of a patient suffering from ringworm and named the fungus Microsporum audouinii in the honor of his boss Dr. Audouin. Malmsten (1845) described fungal spores inside hairs from cases of ringworm and named the fungus Trichophyton tonsurans (trichos= hair, phytos= plant). This means that in the year 1945 we had three genera of dermatophytes, each with one species. Harz (1870) described a fungus, which he named Trichothecium or Acrothecium floccosum, which was known later as Epidermophyton floccosum. Megnin (1881) described the cause of favus in poultry and named the fungus Achorion gallinae, while Zopf (1890) described and named Trichophyton quinckeanum as the cause of favus in rodents.

Blanchard (1895) described and named two new Trichophyton species, namely Trichophyton megnini and Trichophyton concentricum. Bodin (1902) described Microsporum canis and in 1907 Microsporum gypseum, while Castellani (1909) described and named Trichophyton rubrum.

It is clear that the names of the genera or fungi were coined on clinical basis as in case of Achorion or Epidermophyton or on the type of hair invasion, as in case of Microsporum or Trichophyton. The species took the names of scientists as in case of Schoenlein. Megnin, Audouin and Quinck or the host as in gallinae and canis. With the introduction of solid media by Robert Koch in 1880, morphology of dermatophytes on culture led to names as in case of rubrum and gypseum.
2. Classification of dermatophytes

2.1. Assumed first classifications

a. Assumed classification 1845
This classification contained three genera: Microsporum, Achorion and Trichophyton, each represented by one species.

I. Genus Microsporum (Gruby, 1843)
   *Microsporum audouinii*

II. Genus Achorion (Remack, 1845)
   *Achorion schoenleinii*

III. Genus Trichophyton (Malmsten, 1845)
   *Trichophyton tonsurans*

b. Assumed classification 1870
This classification contained 4 genera, where the new genus Trichothecium was added by Harz.

1. Genus Microsporum (Gruby, 1843)
   *Microsporum audouinii*

2. Genus Achorion (Remack, 1845)
   *Achorion schoenleinii*

3. Genus Trichophyton (Malmsten, 1845)
   *Trichophyton tonsurans*

4. Genus Trichothecium (Harz, 1870)
   *Trichothecium floccosum*

c. Assumed classification 1909
From the above-mentioned data we can imagine the classification of dermatophytes in 1909, i.e. one year before the publication of the famous classification of Sabouraud, would have contained 11 species classified in 4 genera as follows:

1. Genus Microsporum (Gruby, 1843)
   1. *Microsporum audouinii*
   2. *Microsporum canis*
   3. *Microsporum gypseum*

2. Genus Achorion (Remack, 1845)
   4. *Achorion schoenleinii*
   5. *Achorion gallinae*

3. Genus Trichophyton (Malmsten, 1845)
   1. *Trichophyton tonsurans*
   2. *Trichophyton quinckeanum*
   3. *Trichophyton megninii*
   4. *Trichophyton concentricum*
   5. *Trichophyton rubrum*

4. Genus Epidermophyton (Sabouraud, 1908)
   1. *Epidemophyton inguinale*
2.2. Sabouraud’s classification (1910) \(^{(1),(2),(3)}\)

Raimond Sabouraud, one of the best known and most influential of the early medical mycologists, began his scientific studies of the dermatophytes around 1890, culminating in the publication of his classic volume, *Les Teignes*, in 1910. Sabouraud’s contributions included his studies on the taxonomy, morphology, and methods of culturing the dermatophytes and the therapy of the dermatophytoses. The medium that he developed is in use today for culturing fungi (although the ingredients are modified) and is named in his honor, Sabouraud glucose (dextrose) agar.

The classifications of Sabouraud rested on the morphology of the dermatophytes in the skin, hair invasion (endothrix or ectothrix, neo-endothrix, microides and megaspores), the host names (caninum, felineum, equinum, and gallinae), the site of infection (interdigitale, culture morphology as velveticum, tomentosum, violaceum, sulfureum, granulosum etc. The number of dermatophytes in Sabouraud’s classified reached 44 species arranged in three genera (Microsorum: 10, Trichophyton: 30 and Achorion.

The Epidermophyton should be considered the fourth genus in Sabouraud’s classification. However, Sabouraud considered the epidermophyton to be closely related to the dermatophytes, that is why it was named Trichothecium or Acrothecium. Sabouraud considered it as a Trichophyton species and named it *T. intertriginis* in 1905, *T. inguinale* in 1907 and *Epidermophyton inguinale* in 1910.
2.3. **Classification of dermatophytes after Emmons (1934)**

Most of the taxonomic entities were based on irrelevant criteria that led enormous increase of dermatophytes reaching to 118 recognized species. In 1934, Chester Emmons modernized the taxonomic scheme of Sabouraud and others and established a classification of the dermatophytes on the bases of spore morphology and accessory organs. He recognized only the three genera *Microsporum*, *Trichophyton*, and *Epidermophyton* on the basis of botanical principles. Emmons depended on the study of the vegetative structures and conidia to characterize the different dermatophytes, he consequently deleted the clinically based genus Achorion. This led to the reduction of the number of recognized species to 19, and listed the remaining 35 names as synonymous names. He demarcated the three genera on the bases of the macroconidial morphology as follows:

- In *Trichophyton*, the macroconidia, when present, have smooth, usually thin walls and up to 12 septa, are born singly or in clusters and may be elongate and pencil shaped, clavate, or cylindrical. Microconidia, usually more abundant, may be globose, pyriform, or clavate, sessile or stalked, and are borne singly along the sides of the hyphae or in grape-like clusters. The genus was divided into 5 groups and comprised about 10 species.

- In *Microsporum*, the macroconidia are characterized by the presence of rough walls which may be asperulate, echinulate or verrucose. They are spindle-shaped or fusiform, obovate or cylindrofusiform. Microconidia are sessile or stalked and clavate and usually arranged singly along the hyphae. The genus comprised 3 species.

- In case of the genus *Epidermophyton* the macroconidia are broadly clavate with typically smooth, thin to moderately thick walls and one to nine septa. They are usually abundant and borne singly or in clusters. Microconidia are absent. The genus is represented by one species.

![Macroconidia of Trichophyton](image1) ![Macroconidia of Microsporum](image2) ![Macroconidia of Epidermophyton](image3)

The classification of Emmons, however, made synonyms of some dermatophytes that were later recognized as distinct species as *M. persicolor*, *M. fulvum* and *T. equinum*, which was separated from *T. mentagrophytes* by the CDC nutritional tests as distinct species.
2.4. **Classification of dermatophytes after Langeron and Vanbreuseghem (1952)** (1)

Langeron and Vanbreuseghem (1952) classified 27 dermatophytes into 5 genera, namely, Ctenomyces (7), Sabourauditis (6). Trichophyton (12), Langeronia (1) and Epidermophyton (1). Two new genera were established, Ctenomyces for some Trichophyton species and Langeronia for soudanensis, while the genus Microsporum was replaced by the genus Sabourauditis.

<table>
<thead>
<tr>
<th>Ctenomyces</th>
<th>Sabourauditis</th>
<th>Trichophyton</th>
<th>Langeronia</th>
<th>Epidermophyton</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. mentagrophytes</td>
<td>S. audouinii</td>
<td>T. tonsurans</td>
<td>L. soudanensis</td>
<td>E. floccosum</td>
</tr>
<tr>
<td>C. asteroids</td>
<td>S. gypseus</td>
<td>T. sabouraudii</td>
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<tr>
<td>C. granulosus</td>
<td>S. canis</td>
<td>T. sulfurium</td>
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<tr>
<td>C. persicolor</td>
<td>S. gallinae</td>
<td>T. concentricum</td>
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<tr>
<td>C. interdigitalis</td>
<td>S. langeronii</td>
<td>T. quinckeansum</td>
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<tr>
<td>C. radians</td>
<td>S. rivalieri</td>
<td>T. violaceum</td>
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<tr>
<td>C. denticulatus</td>
<td></td>
<td>T. rubrum</td>
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<td></td>
<td></td>
<td>T. ferrugineum</td>
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<td></td>
<td>T. schoenleinii</td>
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<tr>
<td></td>
<td></td>
<td>T. album</td>
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<tr>
<td></td>
<td></td>
<td>T. discoides</td>
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<td></td>
<td></td>
<td>T. megnini</td>
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</tr>
</tbody>
</table>

2.5. **Classification of dermatophytes after Conant et al. (1954)** (5)

The classification of Emmons, was adopted by Conant et al., 1954. It included 14 species and 138 synonyms T. mentagrophytes had 28 synonyms, M. gypseum 22, T. tonsurans 21, M. canis 18 etc. as seen in the following:

1. **Genus Trichophyton Malmsten, 1845**

   A. Gypseum group

   1. **Trichophyton mentagrophytes (Robin) Blanchard, 1996**

   B. Rubrum group

2. **Trichophyton rubrum (Castellani) Sabouraud 1911**
C. Crateriform group

3. *Trichophyton tonsurans* Malmsten 1854  

D. Faviforme group

4. *Trichophyton schoenleinii* (Lebert) Langeron and Milochevitch 1930  
   Synonyms: *Achorion schoenleinii*, *Odium schoenleinii*

5. *Trichophyton concentricum* Blanchard 1896  

6. *Trichophyton ferrugineum* (Ota) Langeron and Milochevitch 1930  
   Synonyms: *Microsporum aureum*, *M. chsencicum*, *M. ferrugineum*, *M. japonicum*, *M. orientale*, *M. ramose*

7. *Trichophyton violaceum* Sabouraud 1902  
   Synonyms: *Achorion violaceum- Favorichophyton violaceum- Trichophyton curri*, *T. glabrum*, *T. gourvili*

8. *Trichophyton verrucosum* Bodin 1902  
   Synonyms: *Trichophyton album*, *T. discoides*, *T. faviforme album*, *T. faviforme discoides*, *T. faviforme ochraceum*

E. Rosaceum group

   Synonyms: *Megatrichophyton rosaceum- Trichophyton rosaceum*, *T. roseum*, *T. vinosum*

10. *Trichophyton gallinae* (Megglin) Silva and Benham 1952  
    Synonyms: *Achorion gallinae- Epidermophyton gallinae- Microsporum tomentosum*, *M. umbonatum*, *M. velveticum- Sabourauditis audouinii- Trichophyton decalvans*

II. Genus Microsporum  Gruby, 1843

1. *Microsporum audouinii* Gruby, 1843  
   Synonyms: *Microsporum depauperatum*, *M. pertenue*, *M. tardum*, *M. tomentosum*, *M. umbonatum*, *M. velveticum- Sabourauditis audouinii- Trichophyton decalvans*

2. *Microsporum canis* Bodin 1902  
   Synonyms: *M. caninum*, *M. equinum*, *M. felineum*, *M. flavescens*, *M. lanosum*, *M. lanuginosum*, *M. niveum*, *M. obesum*, *M. pseudolanosum- Sabourauditis canis*, *S. felineum*, *S. lanosum*

3. *Microsporum gypseum* (Bodin) Guiart and Grigoraki 1928  
   Synonyms: *Achorion gypseum*, *A. series- Closterosporia fulva*, *Cl. Gypseum- Ektotrichophyton nakamurae- Gymnoascus gypseus- Microsporum circuluscentricum*, *M. felineum*, *M. flavescens*, *M. fulvum*, *M. marginatum*, *M. scorteum*, *M. sp. Nakamura*, *M. volvorum*, *M. xanthodes- Sabourauditis felineum*, *S. flavescens*, *S. fulvus*, *S. gypseus*, *S. scorteus- Trichophyton du chien*

III. Epiderophyton Sabouraud, 1910

IV. 1. *Epidermophyton floccosum* (Harz) Langeron and Milochevitch 1930  
   Synonyms: *Achorion floccosum- Epidermophyton clypeiforme*, *E. cruris*, *E. inguinale*, *E. plicatum- Trichophyton cruris, T. inguinale*, *T. intertiginis- Trichothecium floccosum*
2.6. Classification of dermatophytes after Ajello (1962) (6)

Ajello mentioned 8 Microsporum species, 14 Trichophyton species, one Epidermophyton species and added a new genus: Keratinomyces with one species named after his name, K. ajelloi. The group of Ajello in the CDC added the physiological characters in the delineation of closely related species, as hair perforation and nutritional tests.

<table>
<thead>
<tr>
<th>Microsporum</th>
<th>Trichophyton</th>
<th>Keratinomyces</th>
<th>Epidermophyton</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. audouinii</td>
<td>T. concentricum</td>
<td>K. ajelloi</td>
<td>E. floccosum</td>
</tr>
<tr>
<td>M. canis</td>
<td>T. equinum</td>
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<tr>
<td>M. cookei</td>
<td>T. gallinae</td>
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<tr>
<td>M. distortum</td>
<td>T. gourvilli</td>
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<tr>
<td>M. ferrugineum</td>
<td>T. megninii</td>
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<td></td>
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<tr>
<td>M. gypseum</td>
<td>T. mentagrophytes</td>
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<tr>
<td>M. nanum</td>
<td>T. rubrum</td>
<td></td>
<td></td>
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<tr>
<td>M. vanbreuseghemii</td>
<td>T. schoenleinii</td>
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<tr>
<td></td>
<td>T. soudanense</td>
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<td></td>
<td>T. terrestre</td>
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<tr>
<td></td>
<td>T. tonsurans</td>
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<tr>
<td></td>
<td>T. verrucosum</td>
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<td></td>
<td>T. violaceum</td>
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<tr>
<td></td>
<td>T. yaoundei</td>
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</tbody>
</table>

2.7. Classification of dermatophytes after WEITZMAN and SUMMERBELL (1995) (7)

The publications of Dawson, Gentles and Stockdale on dermatophyte teleomorphs revealed that several geophilic and zoophilic dermatophytes, were found to produce sexual states in the genera Arthroderma for Trichophyton species and Nannizzia for Microsporum species. Later, Arthroderma was considered the sole genus representing the sexual phase of both asexually reproducing Microsporum and Trichophyton species. On this basis, Weitzam and Summerbell classified dermatophytes into two groups, anamorph genera and species of dermatophytes, which have no sexual spores and constituted 3 genera, namely Epidermophyton (one species), Microsporum (12 species) and Trichophyton (15 species) and 11 Teleomorph species of the genus Arthroderma, which produce sexual spores and are considered as the perfect state of some Trichophyton and Microsporum species.
I. **Anamorph genera and species of dermatophytes.**

1. **Epidermophyton** Sabouraud 1907  
   *E. floccosum* (Harz) Langeron et Milochevitch 1930

2. **Microsporum** Gruby 1843  
   - *M. audouinii* Gruby 1843  
   - *M. canis* Bodin 1902  
   - *M. equinum* (Delacroix et Bodin) Guegue´n 1904  
   - *M. ferrugineum* Ota 1921  
   - *M. fulvum* Uriburu 1909  
   - *M. gallinae* (Megnin) Grigorakis 1929  
   - *M. gypseum* (Bodin) Guiart et Grigorakis 1928  
   - *M. nanum* Fuentes 1956  
   - *M. persicolor* (Sabouraud) Guiart et Grigorakis 1928  
   - *M. praecox* Rivalier, ex Padhye, Ajello et McGinnis 1987  
   - *M. racemosum* Borelli 1965  
   - *M. vanbreuseghemii* Georg, Ajello, Friedman et Brinkman 1962

3. **Trichophyton** Malmsten 1845  
   - *T. concentricum* Blanchard 1895  
   - *T. equinum* (Matruchot et Dassonville) Gedoelst 1902  
   - *T. gourvillii* Catanei 1933  
   - *T. kanei* Summerbell 1989  
   - *T. megninii* Blanchard 1896  
   - *T. mentagrophytes* (Robin) Blanchard 1896  
   - *T. raubitschekii* Kane, Salkin, Weitzman, Smitka 1981  
   - *T. rubrum* (Castellani) Sabouraud 1911  
   - *T. schoenleinii* (Lebert) Langeron et Milochevitch 1930  
   - *T. simii* (Pinoy) Stockdale, Mackenzie et Austwick 1965  
   - *T. soudanense* Joyeux 1912  
   - *T. tonsurans* Malmsten 1845  
   - *T. verrucosum* Bodin 1902  
   - *T. violaceum* Bodin 1902  
   - *T. yaoundei* Cochet et Doby Dubois 1957 (not validly published)

II. **Teleomorph-anamorph state of dermatophytes**

<table>
<thead>
<tr>
<th>Teleomorph</th>
<th>Anamorph</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arthroderma</strong></td>
<td><strong>Microsporum, Trichophyton</strong></td>
</tr>
<tr>
<td><em>A. benhamiae.</em></td>
<td><em>T. mentagrophytesa</em></td>
</tr>
<tr>
<td><em>A. fulvum</em></td>
<td><em>M. fulvum</em></td>
</tr>
<tr>
<td><em>A. grubyi</em></td>
<td><em>M. vanbreuseghemii</em></td>
</tr>
<tr>
<td><em>A. gypseum</em></td>
<td><em>M. gypseum</em></td>
</tr>
<tr>
<td><em>A. incurvatum</em></td>
<td><em>M. gypseum</em></td>
</tr>
<tr>
<td><em>A. obtusum</em></td>
<td><em>M. nanum</em></td>
</tr>
<tr>
<td><em>A. otae</em></td>
<td><em>M. canis var. canis, M. canis var. distortum</em></td>
</tr>
<tr>
<td><em>A. persicolor</em></td>
<td><em>M. persicolor</em></td>
</tr>
<tr>
<td><em>A. simii</em></td>
<td><em>T. simii</em></td>
</tr>
<tr>
<td><em>A. racemosum</em></td>
<td><em>M. racemosum</em></td>
</tr>
<tr>
<td><em>A. vanbreuseghemii</em></td>
<td><em>T. mentagrophytes</em></td>
</tr>
</tbody>
</table>
### 2.8. Classification of dermatophytes after Simpanya (2000) *(8)*

This classification comprises 18 Microsporum species, 23 Trichophyton species and 2 Epidermophyton species.

<table>
<thead>
<tr>
<th>Microsporum Gruby, 1843</th>
<th>Trichophyton Malmsten, 1845</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. amazonicum</em>, Moraes, Borelli &amp; Feo, 1967</td>
<td><em>T. ajelloi</em> (Vanbreuseghem, 1952)</td>
</tr>
<tr>
<td><em>M. audouinii</em> Gruby, 1843</td>
<td><em>T. concentricum</em> Blanchard, 1895</td>
</tr>
<tr>
<td><em>M. bouillardii</em>, Dominik &amp; Majchrowicz, 1965</td>
<td><em>T. equinum</em>, Matruchot &amp; Dassonville, 1898</td>
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<tr>
<td><em>M. canis</em> Bodin, 1902</td>
<td><em>T. flavescens</em>, Padhye &amp; Carmichael, 1971</td>
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<tr>
<td><em>M. cookei</em> Ajello, 1959</td>
<td><em>T. georgiae</em> Varsavsky &amp; Ajello, 1964</td>
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<tr>
<td><em>M. equinum</em> (Bodin, 1902)</td>
<td><em>T. gloriae</em> Ajello, 1967</td>
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<tr>
<td><em>M. distortum</em> Di Menna &amp; Marples, 1954</td>
<td><em>T. gourvili</em> Catanei, 1933</td>
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<td><em>M. ferrugineum</em> Ota, 1921</td>
<td><em>T. longifusus</em>, Florian &amp; Galgocy, 1964</td>
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<td><em>M. fulvum</em> Uriburu, 1909</td>
<td><em>T. mariati</em> Ajello &amp; Cheng, 1967</td>
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<tr>
<td><em>M. gallinae</em> (Megnin, 1881)</td>
<td><em>T. megninii</em> Blanchard, 1896</td>
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<tr>
<td><em>M. gypseum</em> (Bodin, 1902)</td>
<td><em>T. mentagrophytes</em> (Robin, 1853), Blanchard, 1896</td>
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<tr>
<td><em>M. magellanicum</em>, Coretta &amp; Piantelli, 1977</td>
<td><em>T. mentagrophytes</em> var. interdigitale Priestley, 1917</td>
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<tr>
<td><em>M. nanum</em> Fuentes, 1956</td>
<td><em>T. mentagrophytes</em> var. erinacei Smith &amp; Marples, 1963</td>
</tr>
<tr>
<td><em>M. persicolor</em> (Sabouraud, 1910)</td>
<td><em>T. mentagrophytes</em> var. quinckeanum (Zopf, 1890),</td>
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<tr>
<td><em>M. praecox</em> Rivalieri, 1954</td>
<td><em>T. phaseoliforme</em>, Borelli &amp; Feo, 1966</td>
</tr>
<tr>
<td><em>M. racemosum</em> Borelli, 1965</td>
<td><em>T. rubrum</em> (Castellani, 1910)</td>
</tr>
<tr>
<td><em>M. ripariae</em> Hubalek, &amp; Rush-Munro, 1973</td>
<td><em>T. schönenleini</em>, Remak 1945</td>
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<tr>
<td><em>M. vanbreuseghemii</em> Rious et al., 1964</td>
<td><em>T. soudanense</em> Joyeux, 1912</td>
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<td></td>
<td><em>T. terrestre</em> Durie &amp; Frey, 1957</td>
</tr>
<tr>
<td></td>
<td><em>T. tonsurans</em> Malmsten, 1845</td>
</tr>
<tr>
<td></td>
<td><em>T. vanbreuseghemii</em>, (Florian &amp; Galgocy, 1964),</td>
</tr>
<tr>
<td></td>
<td><em>T. verrucosum</em> Bodin, 1902</td>
</tr>
<tr>
<td></td>
<td><em>T. yaoundei</em>, Cochet &amp; Doby-Dubois, 1957</td>
</tr>
</tbody>
</table>

**Epidermophyton Sabouraud, 1910**

*E. floccosum* (Harz, 1870),

*E. stockdaleae*, Prochaki & Engelhardt-Zasada, 1974

### 2.9. Molecular classification of dermatophytes *(9-15)*

Diagnostic laboratories have traditionally identified dermatophytes based on their colony and microscopic morphology, nutritional and biochemical characteristics, and other factors. Such methods, together with the ecology of an organism (e.g., its adaptation to a particular host) have given rise to a number of species names. However, some organisms that appear to be different species, based on conventional typing and/or ecology, may be very closely related genetically. Furthermore, the traditional typing methods have given rise to a situation where a single anamorph can have two different teleomorphs, suggesting that such “species” actually contain more than one species.

A taxonomic method first proposed in 1999 defined dermatophyte species by genetic techniques, specifically the sequencing of highly variable internal transcribed spacer (ITS) regions of the ribosomal DNA. Some authors have adopted the ITS scheme. Others feel that its adoption is premature and based on limited data. ITS taxonomy has been criticized because it may place organisms into the same species even when they seem to be ecologically distinct based on their...
adaptation to different hosts; zoophilic, anthropophilic or geophilic nature; or distinctive characteristics such as opposite mating types or ability to penetrate hair *in vitro*. In addition, the results of ITS typing may not agree with the results of genetic analyses based on other genes. Some sources also use traditional typing schemes for practical reasons: genetic typing is not widely used in diagnostic laboratories, and some species defined by ITS sequencing can be difficult or impossible to identify by conventional methods. This is especially true for *T. mentagrophytes*.

The following table shows the current taxonomy of the family Arthrodermataceae based on morphological, ecological and genotypic features.

<table>
<thead>
<tr>
<th>Current taxonomy</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anamorph/ Teleomorph</td>
<td></td>
</tr>
</tbody>
</table>
| **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. balcaneeum** | *T. abiissinicum*  
*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. quinckeianum*  
*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. glabrum
T. gourvilli
T. soudanense
T. violaceum var. indicum
T. yaoundei
M. langeronii
M. rivalieri
M. distortum
M. equinum
M. ferrugineum
M. nanum/A. obtusum
M. praecox
M. persicolor/A. persicolor
M. gypseum/A. gypseum
M. duboisii
M. sp./A. corniculatum
M. fulvum/A. fulvum
K. longifusus
M. boullardii
M. ripariae
M. gypseum/A. incurvatum
M. cookei/A. cajetani
M. racemosa/A. racemosum
M. gallinae/A. grubyi
M. amazonicum/A. borelli
T. gloriae/A. gloriae
T. vanbreuseghemii/A. gertleri
T. ajelloi/A. uncinatum
T. ajelloi var. nanum
E. stockdaleae
T. terrestr/ A. lenticulare
T. terrestr/A. quadrifidum
T. terrestr/A. insingulare
T. flavescens/A. flavescens
A. melis
T. georgiae/A. ciferrii
Chrysosporium sp./A. multifidum
Chrysosporium sp./A. tuberculatum
Chrysosporium sp./A. cuniculi
T. thuringiense
T. phaseoliforme
Chrysosporium sp./Ctenomyces serratus
Keratinomyces ceretanicus
Chrysosporium sp./Arthroderma curreyi
Identical

__________________________  __
__________________
__________
2.10. Phylogenetic classification

The mutual phylogenetic relationships of dermatophytes of the genera *Trichophyton*, *Microsporum*, and *Epidermophyton* were demonstrated by using internal transcribed spacer 1 (ITS1) region ribosomal DNA sequences. *Trichophyton* spp. and *Microsporum* spp. form a cluster in the phylogenetic tree with *Epidermophyton floccosum* as an out-group, and within this cluster, all *Trichophyton* spp. except *Trichophyton terrestris* form a nested cluster (100% bootstrap support). Members of dermatophytes in the cluster of *Trichophyton* spp. were classified into three groups with ITS1 homologies, with each of them being a monophyletic cluster (100% bootstrap support).

- **The Arthroderma vanbreuseghemii-Arthroderma simii Complex**:
  - A. vanbreuseghemii,
  - A. simii,
  - Trichophyton mentagrophytes isolates from humans,
  - T. mentagrophytes var. quinckeaneum,
  - Trichophyton tonsurans, and
  - Trichophyton schoenleinii.

- **The Arthroderma benhamiae Complex**
  - Arthroderma benhamiae,
  - T. mentagrophytes var. erinacei, and
  - Trichophyton verrucosum

- **The Arthroderma otae Complex**
  - M. canis,
  - M. audouini and
  - M. ferrugineum

- **The Arthroderma gypseum Complex**
  - Arthroderma fulvum
  - Arthroderma gypseum
  - Arthroderma incurvatum
  - Microsporum gypseum

- **The T. rubrum Complex**
  - Trichophyton rubrum and
  - Trichophyton violaceum

*Trichophyton mentagrophytes complex*

Originally, the *Trichophyton (T.)* mentagrophytes complex distinguished between the anthropophilic subspecies T. mentagrophytes var. interdigitale, T. mentagrophytes var. nodulare (synonym *T. krajdenii*), and T. mentagrophytes var. goetzii and the zoophilic subspecies T. mentagrophytes var. granulosum (rodents), T. mentagrophytes var. erinacei (hedgehog), and T. mentagrophytes var. quinckeaneum (mice). In addition, two sexual species (teleomorph) of this complex are known. These are *Arthroderma (A.)* benhamiae Ajello and Cheng 1967 and *Arthroderma vanbreuseghemii* Takashio 1973. According to recent molecular studies, the species T. mentagrophytes is synonymous with only the zoophilic subspecies *T. mentagrophytes var. quinckeaneum*. The anthropophilic subspecies of *T. mentagrophytes*, as well as many of the zoophilic strains, formerly differentiated as var. mentagrophytes or var. granulosum, are indistinguishable and are now designated *T. interdigitale*. The morphological differentiation between anthropophilic and
zoophilic *T. interdigitale* strains by classical microscopical and biochemical methods is often problematic. In particular, it is impossible to differentiate between the zoophilic strains of *T. interdigitale*, *T. mentagrophytes*, and the *Trichophyton anamorph of A. benhamiae*. In these cases, molecular identification methods may be applied to answer epidemiological, taxonomical and therapeutic questions.

*T. verrucosum* varieties have been eliminated under the current ITS genetic taxonomy, and this organism is redefined to include only the species adapted to cattle. The ITS system reassigns *T. verrucosum* var. *autotrophicum*, which occurs in sheep, to *T. interdigitale*.

A newly proposed taxonomy of *Trichophyton mentagrophytes* and related species was introduced and verified with the grounds for the new classification, phylogenetic analysis, and Templeton's cohesive species concept. So-called asexual species were shown to retain sexual ability and different host preferences were shown not to be comparable to different ecological niches. It was shown that genealogical concordance phylogenetic species recognition (GCPSR) can be applied to so-called asexual Trichophyton species. The results of GCPSR analysis and mating experiments demonstrated that:

- *Arthroderma simii*, *A. vanbreuseghemii*, *T. mentagrophytes var. interdigitale*, and *T. tonsurans* may be considered as single phylogenetic species, and that
- *A. benhamiae*, *T. concentricum*, *T. verrucosum*, and *T. mentagrophytes var. erinacei* may also be considered as single phylogenetic species.

**Trichophyton rubrum complex**\(^{(12)}\)

The members of the *Trichophyton rubrum* complex are the most common agents of dermatomycoses, primarily causing tinea pedis, onychomycosis, tinea corporis, and tinea capitis. *Trichophyton megninii* Blanchard, described in 1896, is the oldest identified taxon in the group. In the 1920s and 1930s, the species was common in Western Europe as an etiological agent of tinea barbae. Now it is endemic in the Mediterranean countries, mainly causing tinea corporis.

The most prevalent species of the complex worldwide is *T. rubrum* (Castellani) Semon. It was described by Castellani in 1910, when all other main dermatophytes had already been known for several decades. The species was suggested to have evolved in the late nineteenth century as a cause of chronic tinea corporis. It has since spread throughout the world as the etiological agent of onychomycosis and tinea pedis.

Another currently predominant species, *Trichophyton violaceum*, was described by Sabouraud in 1902, 6 years after *T. megninii*. This species mostly causes tinea capitis and is distributed particularly in North Africa and the Middle East. The remaining species of the *T. rubrum* complex (*T. circonvolutum*, *T. fischeri*, *T. fluviomuniense*, *T. glabrum*, *T. gourvilii*, *kaniei*, *T. kuryangei*, *T. pedis*, *T. raubitschekii*, *T. rodhainii*, *T. soudanense*, and *T. yaoundei*) are extremely rarely isolated as agents of dermatomycosis, and most of them were described much later, between 1960 and 1990.
Microsporum canis complex

Microsporum canis, Microsporum ferrugineum and Microsporum audouinii are three phylogenetically closely related dermatophytes in the Arthroderma otae complex. They have different ecological niches: the former two are anthropophilic, whilst the latter is a zoophilic species. However, all are involved in human infections, and can cause similar clinical manifestations and be transmitted via human-to-human or animal-to-human routes. Identification/differentiation of these related species is important from an epidemiological point of view.

By far the most common species worldwide in this complex is M. canis, the cat dermatophyte. This species has characteristic thick-walled, fusoid-apiculat ('beaked'), rough-walled macroconidia and a lemon-yellow colony pigmentation, making typical isolates among the easiest fungi in the world to identify. 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 nonsporulating or produce only microconidia. It is definitively distinguished from atypical M. canis either by means of the rapid and specific polished rice test, where it gives a negative growth response due to an as yet uncharacterized growth factor deficiency, or by in vitro hair perforation test, where it gives a negative result contrasting with the positive result seen for typical M. canis.

The ITS2 region constitutes a better target for differentiating members of the M. canis complex (M. audouinii, M. canis and M. ferrugineum), since a few single nucleotide polymorphisms (barcode sequences) were found in the ITS2 region.

Two rare members of this complex are 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 and 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) The typical horse isolates do not perforate hair in vitro.
2.11. Classification of dermatophytes according to their normal habitat.\textsuperscript{(18)}

According to their natural habitat, dermatophytes are classified into:

- **Anthropophilic species**: are restricted to human hosts and produce a mild, chronic inflammation
- **Zoophilic species**: are found primarily in animals and cause marked inflammatory reactions in humans who have contact with infected cats, dogs, cattle, horses, birds, or other animals. This is followed by a rapid termination of the infection.
- **Geophilic species**: are usually recovered from the soil but occasionally infect humans and animals. They cause a marked inflammatory reaction, which limits the spread of the infection and may lead to a spontaneous cure but may also leave scars.

### Anthropophilic
- *E. floccosum*
- *M. audouinii*
- *M. ferrugineum*
- *T. concentricum*
- *T. kanei*
- *T. megninii*
- *T. mentagrophytes* (cottony and velvety)
- *T. raubitschekii*
- *T. rubrum*
- *T. schoenleinii*
- *T. soudanense*
- *T. tonsurans*
- *T. violaceum*
- *T. Yaoundei*

### Zoophilic
- *M. canis* (cats, dogs, etc.)
- *M. equinum* (horses)
- *M. nanum* (pigs)
- *M. persicolor* (rodents)
- *T. equinum* (horses)
- *T. mentagrophytes* (granular) (rodents, rabbits, hedgehogs, etc.)
- *T. simii* (monkeys)
- *T. verrucosum* (cattle)

### Geophilic
- *M. gypseum*
- *T. ajelloi*
- *T. terrestre*
2.12. Classification of dermatophytes according to their primary host and perfect forms\(^{(19)}\)

### Primary host

<table>
<thead>
<tr>
<th>Dermatophytes</th>
<th>Anthoph</th>
<th>Zooph</th>
<th>Geoph</th>
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<tbody>
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<td>Trichophyton</td>
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<td></td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
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<td></td>
<td></td>
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<tr>
<td><em>T. megninii</em></td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td><em>T. tonsurans</em></td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td><em>T. schoenleinii</em></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. concentricum</em></td>
<td>+</td>
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<tr>
<td><em>T. violaceum</em></td>
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<tr>
<td><em>T. soudanense</em></td>
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<tr>
<td><em>T. soudanense</em></td>
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<td></td>
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<tr>
<td><em>T. yaoundei</em></td>
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<tr>
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<tr>
<td>oder <em>granulosum</em></td>
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<td>oder <em>asteroides</em></td>
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<tr>
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<tr>
<td><em>T. simii</em></td>
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<td>(+)</td>
<td>A. uncinatum</td>
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<tr>
<td><em>T. georgiae</em></td>
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<td><em>T. longifusum</em></td>
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<tr>
<td><em>T. terrestre</em></td>
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\[\text{Arthroderma}\]

\[\text{A. benhamiae}\]

\[\text{A. vanbreuseghemii}\]
<table>
<thead>
<tr>
<th>Dermatophytes</th>
<th>Anthoph</th>
<th>Zooph</th>
<th>Geoph</th>
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<tr>
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</table>
3. Morphology of dermatophytes (20-26)

3.1. Macromorphology

Growth occurs on Sabouraud dextrose agar containing chloramphenicol and cyclohexamide (Actidione) and incubated at 30°C for 3-4 weeks. Trichophyton verrucosum grows better at 37°C. Colonies may be circular, asteroid, polygonal or lobulate. Colour of the colony may be white to gray, rosy, vinaceous, reddish, apricot, orange, yellow, violet and the back of the colony varies in colour from yellow to dark red to purplish black. Some dermatophytes need vitamins for growth such as inositol and thiamine (T. verrucosum), nicotinic acid (T. equinum) and histidine (T. megnini). Dermatophytes produce colonies that vary in texture and rate of growth. There are three forms of colonies:

a. The membranous form (glabrous, waxy, humid, faviform): the aerial mycelium is entirely absent and the vegetative mycelium is in compact masses e.g. M. ferrugineum, T. concentricum, T. schoenleinii, T. violaceum and T. verrucosum.

b. The filamentous form (cottony, fluffy, hairy, velvety, woolly): the aerial mycelium is more or less high and dense e.g. E. floccosum, M. audouinii, M. canis, M. distortum, M. nanum and T. rubrum.

c. The granular-powdery form: characterized by excessive conidia and absence of aerial filamentous elements e.g. M. equinum, T. mentagrophytes and T. megnini.

![Forms of colonies of dermatophytes](image)

3.2. Micromorphology

The main structures of dermatophytes are micro and macroconidia, chlamydospores, septated hyphae, racquet hyphae, spirals. The conidia are usually single-celled, sessile or on a stack, single or in groups. The macroconia are spindle-, pencil- or club-shaped. Some dermatophytes consist of only sterile hyphae and rarely produce spores. The sexually reproducing fungi show cleistothecia and ascospores.
The main diagnostic structures in dermatophytes are the macroconidia, which are shown in the following figure.

In case of the genus *Epidermophyton* the macroconidia are broadly clavate with typically smooth, thin to moderately thick walls and one to nine septa. They are usually abundant and borne singly or in clusters. Microconidia are absent. The genus is represented by two species, only *E. floccosum* is pathogenic.

In *Microsporum*, the macroconidia are characterized by the presence of rough walls which may be asperulate, echinulate or verrucose. They are spindle-shaped or fusiform, obovate or cylindrofusiform. Microconidia are sessile or stalked and clavate and usually arranged singly along the hyphae. The genus comprises at least 12 species, *M. audouinii*, *M. canis*, *M. equinum*, *M. ferrugineum*, *M. fulvum*, *M. gallinae*, *M. gypseum*, *M. nanum*, *M. persicolor*, *M. preecox*, *M. racemosum* and *M. vanbreuseghemii*.

In *Trichophyton*, the macroconidia, when present, have smooth, usually thin walls and one to 12 septa, are born singly or in clusters and may be elongate and pencil shaped, clavate, fusiform or cylindrical. Microconidia, usually more abundant, may be globose, pyriform, or clavate, sessile or stalked, and are borne singly along the sides of the hyphae or in grape-like clusters. The genus comprises about 15 species, e.g. *T. concentricum*, *T. equinum*, *T. gourvilli*, *T. kane*, *T. megninii*, *T. mentagrophytes*, *T. rubitschekii*, *T. rubrum*, *T. schoenleinii*, *T. simii*, *T. soudanense*, *T. tonsurans*, *T. verrucosum*, *T. violaceum* and *T. yaoundei*. 
4. Gallery of the commonly isolated dermatophytes\textsuperscript{(20,22,23, 24)}

4.1. \textit{Microsporum audouinii}

Colonies and macroconidia of \textit{Microsporum audouinii}

4.2. \textit{Microsporum canis}

Colonies and macroconidia of \textit{Microsporum canis}
4.3. *Microsporum gypseum*

Colonies and macroconidia of *Microsporum gypseum*

4.4. *Microsporum fulvum*

Colonies and macroconidia of *Microsporum fulvum*

4.5. *Microsporum cookei*

Colony and macroconidia of *Microsporum cookei*
4.6. *Microsporum distortum*

Colony and macroconidia of *Microsporum distortum*

4.7. *Microsporum nanum*

Colony and macroconidia of *M. nanum*

4.8. *Microsporum gallinae*

Colonies and macroconidia of *Microsporum gallinae*
4.9. *Microsporum persicolor*

Colonies and macroconidia of *Microsporum persicolor*

4.10. *Microsporum ferrugineum*

Colonies and macroconidia of *Microsporum ferrugineum*
4.11. *Microsporum equinum*

Colony and macroconidia of *M. equinum*

4.12. *Microsporum amazonicicum*

Colony and macroconidia of *M. amazonicicum*
4.13. *Microsporum boullardii*

Colony and macroconidia of *M. boullardii*

4.14. *Microsporum racemosum*

Colony and macroconidia of *M. racemosum*

4.15. *Microsporum vanbreuseghemii*

Colony and macroconidia of *M. vanbreuseghemii*
4.16. *Trichophyton mentagrophytes*

Colonies, micro- and macroconidia of *T. mentagrophytes*

4.17. *Trichophyton quinckeaneum*

Colonies, micro- and macroconidia of *T. quinckeaneum*
4.18. *Trichophyton rubrum*

Colonies, macro- and microconidia of *Trichophyton rubrum*

4.19. *Trichophyton tonsurans*

Colonies and microconidia of *Trichophyton tonsurans*
4.20. *Trichophyton violaceum*

Colonies and microconidia of *Trichophyton violaceum*

4.21. *Trichophyton megninii*

Colony and microconidia of *Trichophyton megninii*
4.22. *Trichophyton schoenleinii*

Colonies and favic chandliers characteristic of *T. schoenleinii*

4.23. *Trichophyton verrucosum*

Colony, microconidia and chlamydospores of *T. verrucosum*

4.24. *Trichophyton concentricum*

Colonies and sterile hyphae of *T. concentricum*
4.25. *Trichophyton soudanense*

Colony and microconidia of *Trichophyton soudanense*

4.26. *Trichophyton gourvili*

Colonies and microconidia of *Trichophyton gourvili*

4.27. *Trichophyton ajelloi*

Colony and characteristic macroconidia of *T. ajelloii*
4.28. *T. equinum*

Colonies and microconidia of *Trichophyton equinum*

4.29. *Trichophyton georgiae*

Colonies and microconidia of *Trichophyton georgiae*

4.30. *Trichophyton gloriae*

Colonies and macroconidia of *Trichophyton gloriae*
4.31. *Trichophyton erinacei*

Colony and microconidia of *Trichophyton erinacei*

4.32. *Trichophyton terrestre*

Colonies and microconidia of *Trichophyton terrestre*
4.33. *Epidermophyton floccosum*

Colonies and club-shaped macroconidia of *E. floccosum*
5. Diseases caused by dermatophytes

5.1. Diseases caused by dermatophytes in man

The dermatophytosis in man is caused by anthropophilic, zoophilic and geophilic species. The diseases caused by anthropophilic dermatophytes are commonly mild, while those caused by zoophilic dermatophytes are mostly severe and more inflammatory. The diseases caused by dermatophyts are classified according to the body areas that are affected:

a. **Tinea capitis**: is the infection of the scalp and may be classified as scaly ringworm, black-dot ringworm kerion and favus. Infection with *M. audouinii* and *M. canis* is characterized by small-spored ectothrix hair invasion, where the spores are surrounding the hair shaft, and the hairs fluoresce green under the U.V. light. Large-spored ectothrix hair invasion is seen in case of infection with *M. gypseum*, *T. verrucosum* and *T. mentagrophytes*. Infection with *T. violaceum* and *T. tonsurans* is characterized by endothrix hair invasion. The inside of the hair may be fully filled with spores and the hair may break and the remaining part appears as a black dot. In case of favus broad hyphae and air spaces are seen.
**b. Tinea faciei** is seen on the nonbearded parts of the face. It can be caused by a number of anthropophilic and zoophilic organisms including *T. rubrum*, *T. tonsurans*, *T. schoenleinii*, *T. mentagrophytes*, *M. canis* and *T. erinacei*. 
c. **Tinea barbae**: is the infection of the beard and mustache and is caused mainly by *T. verrucosum*, *T. mentagrophytes* and *T. violaceum*.

![Tinea barbae](image)

d. **Tinea corporis**: is the infection of the glabrous skin, usually involving the trunk, shoulders, or limbs, and occasionally the face (excluding the bearded area). It can be caused by all known dermatophytes.

![Tinea corporis](image)

e. **Tinea axillaris**

![Tinea axillaris](image)
f. **Tinea cruris:** is the infection of the groin, perianal, and perineal areas and occasionally the upper thighs. It is commonly caused by *E. floccosum, T. rubrum* and *T. violaceum.*

![Tinea cruris](image)

Tinea cruris

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g. **Tinea manum:** Tinea manuum is a dermatophyte infection that tends to affect the palms of one or both hands, most often caused by anthropophilic dermatophytes, particularly *T. rubrum* but occasional cases may be caused by zoophilic organisms such as *M. canis, T. mentagrophytes, T. verrucosum* and *T. erinacei,* or the geophilic organism *M. gypseum.*

![Tinea manum](image)

Tinea manum
h. **Tinea pedis (“Athlete’s Foot”)**: is the infection of the feet or toes, commonly caused by *T. mentagrophytes, T. rubrum* and *E. floccosum*.

i. **Tinea unguim**: also called onychomycosis, is the infection of the nail plate, commonly caused by *T. rubrum, T. mentagrophytes, E. floccosum, T. violaceum* etc.
5.2. Diseases caused by dermatophytes in animals

a. **Ringworm in cattle:** the common cause is *T. verrucosum* and to a lesser extent is *T. mentagrophytes*. Ringworm begins as scattered, discrete lesions which may develop into large, circumscribed plaques up to 4 inches in diam. These are thickened and covered with a grayish white crusts which are firmly attached to the skin. Spontaneous healing may occur and the lesions become dry, scaly with alopecia and scar formation. Lesions are commonly seen on the face and neck, but also flanks and back may be involved. The initial lesions may be discrete, scaly and alopecic with grayish-white crusts, and tend to appear on the face and neck in calves. Cows and heifers may have lesions more often on the chest and limbs, and bulls on the dewlap and intermaxillary skin. Some areas may become suppurative and thickly crusted. Lesions resembling light brown scabs may also be seen; when these scabs fall off, they leave an area of alopecia.
b. **Ringworm in horses:** The most common clinical picture is that of dry, raised, scaling lesions on any part of the body. *M. equinum* is the dermatophyte responsible for the great majorities of the cases. In horses, most dermatophyte lesions are found in areas of contact with saddles or other tack. They usually begin as small patches of raised hairs, and progress to hair loss, with variable amounts of scaling, erythema, crusting and exudation. Other species such as *M. canis*, *M. gypseum*, *T. mentagrophytes* and *T. verrucosum* can usually be found in equine ringworm. *M. canis* lesions are reported to be milder, in most cases, than *M. equinum*. Kerions may occur in some animals, especially on the face. Miliary dermatitis may also be seen, with small crusted lesions especially on the flanks.

Ringworm in horses
c. **Ringworm in camels:** *Trichophyton langeronii* and *Trichophyton sarkisovii* seem to be adapted to camels. *T. mentagrophytes* and *Microsporum canis* has been reported camelids.

d. **Ringworm in pigs:** dermatophytosis is caused by *M. nanum* and *T. mentagrophytes.* *Microsporum persicolor* and *M. canis* are found occasionally pigs. This organism infects the skin, but does not invade hairs.

e. **Ringworm in monkeys:** Dermatophytosis is caused by *M. canis, M. audouinii, M. distortum,* and *T. mentagrophytes*

f. **Ringworm in sheep and goats:** Dermatophytosis are commonly caused by *Trichophyton verrucosum* The disease is usually seen as circular, alopecic areas with thick scabs on the head, face and non-woolen areas of the legs; however, widespread lesions may be found under the wool when lambs are sheared for showing. In healthy lambs, the disease is usually self-limiting. Other species such as *M. canis, M. gypseum* and *T. mentagrophytes* have been isolated from sheep and goats.
g. **Ringworm in cats and dogs:** infection is caused commonly by *M. canis* and *T. mentagrophytes*. In cats the disease is most often subclinical. The head is most commonly affected, with areas of hair loss around the nose, eyes and ears.

Many cats infected with dermatophytes have few or no lesions. Long-haired adults, in particular, can be subclinical carriers or have only minimal signs, such as patchy areas of short stubble, alopecia, scales or erythematous plaques, visible only on close inspection. More apparent cases tend to be seen in kittens, with the early lesions often found on the face, ears and paws. In addition to focal alopecia and scales, affected areas may develop a thin, grayish white crust or a thick, moist scab. They may or may not be pruritic.

In dogs dermatophytosis is seen most often in puppies. The lesions frequently develop on the face and limbs, although they may occur on any part of the body. *M. canis* tends to appear as small circular areas of alopecia. The hairs are typically broken at the base, giving the appearance of having been shaved. The center of the lesion usually contains pale skin scales in the early stage, giving it a powdery appearance, and the edges are generally erythematous. Vesicles and pustules may also be seen. In later stages, the area is often covered by a crust and the edges swollen. Individual lesions may coalesce to form large, irregular patches. Lesions caused by *T. mentagrophytes* and *T. erinacei* tend to be more thickened and inflammatory than those caused by *M. canis*, while *M. persicolor* typically causes localized or generalized scaling with little erythema and minimal alopecia. Other forms of dermatophytosis can include kerions (localized severe inflammation with swollen, boggy skin oozing pus) and pseudomycetomas. Onychomycosis may occur concurrently with dermatophytosis.
h. **Ringworm in poultry:** *M. gallinae* is the cause. The disease is characterized by a white, mouldy, patchy overgrowth on the comb and wattles. Thick, white crusts often develop. Infection may be caused also by *T. simii*. Other dermatophytes recorded were *M. gypseum*, *T. mentagrophytes* and *T. verrucosum*.

i. **Ringworm in rabbits:** *T. mentagrophytes* is the most common cause of ringworm in rabbits. Focal alopecia, with erythema, crusts, scales and scabs, is initially seen mainly around the eyes, nose, ears and dorsal neck. The lesions may later spread to other areas of the body. The disease is usually self-limiting. *Microsporum canis* is also found regularly in rabbits. *Microsporum persicolor* is found occasionally in rabbits.
j. **Ringworm in rodents:** Most rodents infected with *T. mentagrophytes* are asymptomatic or have few clinical signs. There may be areas of partial or complete alopecia, erythema, scales, and crusts in symptomatic animals. In mice, *T. mentagrophytes, T. quinckeaeum, T. rubrum, T. violaceum, M. gypseum and M. distortum* were recorded. The lesions are often found on the tail.

In guinea pigs, *T. mentagrophytes* is the most frequently isolated dermatophyte and lesions tend to appear first on the face, and then spread to the back and limbs.
k. Ringworm in wild animals

Ringworm was recorded in many wild animals. *T. mentagrophytes, M. canis and M. gypseum* are the most commonly isolated fungi.
6. Diagnosis of diseases caused by dermatophytes

6.1. Wood’s light examination

Under Wood’s light the Microsporum infected hairs fluoresce blue

![Tinea capitis due to *M. canis*](image1)

![fluorescing infected hairs](image2)

6.2. Collection of samples

Suspected lesions should be thoroughly cleaned both mechanically and chemically. The crusts and loosely attached scales and nail particles are to be removed and the underlying surface is then well-cleaned with 70% alcohol using a piece of gauze. Hair stumps are epilated by forceps and skin scrapings by scalpel from the peripheries of the lesion.

6.3. Direct microscopic examination

Skin scrapings, hair or nail particles are placed on a clean slide with few drops of 10-25% KOH solution, or NaOH mixed with 5% glycerol, covered with a coverslip, heated gently and left for at least 30 minutes to 1 hour. The preparation is then pressed gently, the oozing fluid is dried and then examined microscopically for the presence of septate hyphae and arthroconidia characteristic of dermatophytes. Other formulation is 20%KOH-36% dimethyl sulfoxide and two techniques for fluorescence microscopy, the calcofluor white and the Congo red.
Septated hyphae and arthrospores of dermatophyte in skin scrapings

T. verrucosum, large spores

M. canis, small spores

Ectothrix hair invasion

Endothrix hair invasion
6.4. Isolation of dermatophytes

6.4.1. Isolation of dermatophytes from human and animal samples

Part of the samples is embedded into Sabouraud dextrose agar with chloramphenicol and actidione in tubes using a mycological inoculation hook, preferably at the side of the slope and at different sites. It is better to use more than one tube for each sample. The tubes are then incubated at 30°C for 1-4 weeks.

6.4.2. Isolation of dermatophytes from soil samples by hair-baiting method

Dermatophytes growing on hairs distributed on moist soil in hair-baiting

Microscopic examination of a hair showing macroconidia of M. gypseum

Dermatophytes isolated from Egyptian soil by hair-baiting
6.4.3. Isolation of dermatophytes from soil samples by nail-baiting method

6.5. Phenotypic Identification of dermatophytes

6.5.1. Macro- and microscopic morphology
Identification characters include colony texture, pigmentation, growth rate and distinctive morphological structures such as macroconidia, microconidia, spirals, chlamydomspores, etc.
6.5.2. Differentiation of dermatophytes by hair perforation test

The in vitro hair perforation test relies on the development by certain dermatophytes of specialized perforating organs invading detached hairs forming pits at right angles, e.g. *T. mentagrophytes* perforates the hair, while *T. rubrum* does not.

![Hair perforation test](image)

6.5.3. Differentiation of dermatophytes by nutritional tests

Nutritional tests serve to confirm identification, e.g. the inability of *M. audouinii* to grow on rice grains serves to separate it from *M. canis* and *M. gypseum*. *T. verrucosum* has complete requirement for thiamin or a combination of thiamin and inositol. *T. tonsurans* is stimulated by thiamin, and *T. megninii* requires histidine, while *M. equinum* needs nicotinic acid for growth.

6.5.4. Differentiation of dermatophytes by growth on nail agar

| T. rubrum colonies on nail agar (3 w old cultures) | T. mentagrophytes on nail agar | Slav growth, the centre slightly raised and fluffy | Rapid growth, colonies with fine powdery surface around the inoculum site |
6.5.5. Differentiation of dermatophytes by growth in glucose solution

*T. rubrum* growth in glucose solution, on the left: submersed growth, no colour or pH change, on the right, surface growth, half-ball-like colonies, colour changed to dark blue, pH alkaline (30 days old)

6.5.6. Differentiation of dermatophytes by growth in peptone water

Left: *T. rubrum* half-ball-like colonies, right: *T. mentagrophytes*, crateriform growth
On peptone solution (30 days old)
6.6. Molecular identification of dermatophytes

6.6.1. Genomic DNA extraction

a. Extraction of DNA from Dermatophyte cultures

Dermatophyte isolates are grown on SDA at 30°C for 5 days. A small amount of mycelium grown on SDA is placed in DNA lysis buffer (200 mM Tris-HCl [pH 8.0], 0.5% [wt/vol] sodium dodecyl sulfate, 250 mM NaCl, 25 mM EDTA) and crushed with a conical grinder. It is then incubated at 100°C for 15 min and mixed with 150 ml of 3.0 M sodium acetate, kept at -20°C for 10 min, and then centrifuged at 10,000 x g for 5 min. The supernatant is extracted once with phenol-chloroform-isoamyl alcohol (25:24:1 [vol/vol]) and is subsequently extracted once with chloroform. The DNA is precipitated with an equal volume of isopropanol at -20°C for 10 min, washed with 0.5 ml of 99% ethanol, dried, and suspended in 50 ml of ultra-pure water. One microliter of solution is used as the template for PCR.

b. Direct extraction of the DNA from scales

Scales, collected from patients are transferred to a 1.5 ml e-tube. 100 μl-lysis buffer (100 mM Tris-HCl (pH 9.5), 1 M KCl, 10mM EDTA) are added and treated at 100oC for 30 minutes. Proteinase K 50 ng is added and treated for 16 hours at 55oC, followed by 30 minutes at 100oC. After thorough mixing, 100μl P/C/l (phenol:chloroform:isoamyl alcohol =25:24:1, v/v) is added and centrifuged at 13,000 rpm for 15 minutes at 4oC. The supernatant was removed, and an equal amount of isopropanol was added; DNA is concentrated at ~80oC for 1 hour. Purified DNA products are washed twice in 500 ml of 70% ethanol, then centrifuged at 13,000 rpm for 5 minutes. The supernatant is removed and the DNA is dried at 37oC for 20 minutes, dissolved in distilled water, and preserved at -20oC. The concentration of the extracted. DNA is measured with the NanoDrop spectrophotometer ND-1000, and 50 ng is taken for use in PCR.

c. Rapid mini–preparation DNA Extraction for all fungal isolates :

To a 10 ml tube containing 5 ml of lysis buffer (400mM Tris-HCl, pH 8.0, 60 Mm EDTA, pH 8.0, 150 Mm NaCl, SDS 1% w/v), a large portion dermatophyte mycelium grown on SDA in primary culture is added using a sterile toothpick, with which the mycelia are disrupted with a glass homogenizer. The tube is then left at room temperature for 10 min. after adding 1.5 ml of potassium acetate, pH 4.8 (5 M potassium acetate 60ml glacial acetic acid 11.5 ml, distilled water 28.5 ml), the tube is incubated at 90°C for 1 hr. then, vortex mixed briefly, and cellular debris and precipitated protein are removed by centrifugation at > 13000 rpm for 1 min. The supernatant is transferred to another tube and centrifuged again as above. After transferring the supernatant to a new tube, an equal volume of cold isopropanol alcohol is added then, incubated at -20°C/ 1 hr or -80°C / 10 min. The tube is mixed briefly by inversion, centrifuged at > 13000 rpm for 2 min and the supernatant is discarded. The resultant DNA pellet is washed in sufficient amount of ethanol 70% v/v. after centrifugation at 13000 rpm for 1 min, the supernatant is discarded. The DNA pellet is air dried and dissolved in 100 μl of 1X TE; 1 μl of the purified DNA is used in 25-50 μl of PCR mixture.

6.6.2. PCR procedure:

The PCR amplification reaction is performed with a volume of 50 μl. Twenty five μl of PCR Master Mix (2X), Consisted of 1-2 μl (25ng-50ng DNA template) of each sample is added to the PCR master mixture, , 0.5 μl of each primer (10 pmol of each primer), and the remaining volume
Amplification consists of an:
Initial denaturation at 94°C For 4 min
30 cycles of
Denaturation at 94°C for 30 s
Annealing at 55°C for 30 s
Extension at 72°C for 1 min
Final extension at 72°C for 4 min

6.6.3. Multiplex PCR

The reaction mixture of PCR amplification is adjusted to 50μl, which includes 0.1 mM dNTPs, 10X PCR buffer, 0.5 mM primer, 0.6 U Taq polymerase and 50 ng of genomic DNA solution. The Veriti® 96-Well Fast Thermal Cycler (Applied Bio systems, Carlsbad, CA, USA) is used for PCR. For the ITS 1 and 2, the reaction conditions are, as follows: 7 minutes of hot step at 94oC, one minute of denaturation at 95oC, one minute of annealing at 60oC, and one minute of extension at 72oC. The entire process is repeated for 35 cycles, with the final extension at 72oC for 7 minutes. The PCR conditions for the 28S ribosomal RNA are, as follows: 7 minutes of hot step at 94oC, 1 minute of denaturation at 94oC, 30 seconds of annealing at 50oC, and 1 minute of extension at 72oC. The entire process is repeated for 35 cycles, with the final extension at 72oC for 7 minutes. Finally, the PCR conditions of 18S ribosomal RNA are, as follows: 7 minutes of hot step at 94oC, 30 seconds of denaturation at 94oC, 1 minute of annealing at 57.5oC, and 1 minute of extension at 72oC. The entire process was repeated for 35 cycles, with the final extension at 72oC for 7 minutes. The amplified DNA is observed on 3% (w/v) agarose gel in TAE buffer.

6.6.4. PCR-RFLP Procedure:

Total purified PCR product (Amplicon) is digested individually with 20 U of the restriction enzymes Hae III or Bam HI, by 1hr – 16 hr incubation at 37°C in reaction mixture containing 10 μl of PCR product, 18 μl of water, 2.0 μl of 10X Buffer R. The resulting restriction fragments are analyzed by 2% agarose gel electrophoresis and DNA ladder of 1kb is used. Obtained bands are visualized using UV-trans-illuminator and photographed by a digital camera (FUJI 100). Accurate fragment size analysis based on the electrophoretic mobility of the sample relative to the internal standards (100 bp ladder Promega) is achieved by using DNA size analysis GEL PRO software.
6.6.5. Inter Simple Sequence Repeat (ISSR-RAPD):

The simple repeat sequence \((GACA)_4\) is used as a single primer in the PCR amplification. Amplification reactions are performed in volumes of 50 µl containing 25 ng of template DNA. The samples are overlaid with sterile paraffin oil and PCR is performed for 39 cycles of

Denaturation at 94°C for 1 min
Annealing at 50°C for 1 min
Extension at 72°C for 1 min
Final extension at 72°C for 7 min

PCR products (20 µl/sample) are separated by electrophoresis in 1 % agarose gels for 2h at 5V / cm in 0.5x TBE buffer. Amplification products are detected by staining with ethidium bromide and are visualized under UV light. PCR fingerprinting profiles are sized and compared with a scanner and gel image analysis software.
7. Treatment of diseases caused by dermatophytes (77-86)

7.1. Treatment of diseases caused by dermatophytes in man

Topical medications like clotrimazole, butenafine, miconazole, and terbinafine. Systemic medications (oral) like fluconazole, griseofulvin, terbinafine, and itraconizole.

- Tinea corporis (body), tinea manum (hands), tinea cruris (groin), tinea pedis (foot) and tinea faciei (face) can be treated topically.
- Tinea ungium (nails) usually requires oral treatment with terbinafine, itraconizole, or griseofulvin. Griseofulvin is usually not as effective as terbinafine or itraconizole. A lacquer (Penlac) can be used daily, but is ineffective unless combined with aggressive debridement of the affected nail.
- Tinea capitis (scalp) must be treated orally, as the medication must be present deep in the hair follicles to eradicate the fungus. Usually griseofulvin is given orally for 2 to 3 months. Clinically dosage up to twice the recommended dose might be used due to relative resistance of some strains of dermatophytes.
- Tinea pedis is usually treated with topical medicines, like ketoconazole or terbinafine, and pills, or with medicines that contains miconazole, clotrimazole, or tolnaftate. Antibiotics may be necessary to treat secondary bacterial infections that occur in addition to the fungus (for example, from scratching).

7.1.1. TOPICAL THERAPY FOR DERMATOPHYTE INFECTIONS

2. **Butenafine**: 1 % cream Once or twice daily
3. **Ciclopirox (Loprox)**: 1 % cream or lotion Twice daily
4. **Clotrimazole (Lotrimin)**: 1 % cream, solution, or lotion Twice daily
5. **Econazole (Spectazole)**: 1 % cream Once daily
6. **Ketoconazole (Nizoral)**: 1 % cream cream Once daily
7. **Miconazole (Micatin)**: 2% cream cream, spray, lotion, or powder Twice daily
8. **Naftifine (Naftin)**: 1 % cream Once daily, gel Once or twice daily
9. **Oxiconazole (Oxistat)**: 1 % cream or lotion Once or twice daily
10. **Sulconazole (Exelderm)**: 1 % cream or lotion Once or twice daily
11. **Terbinafine (Lamisil)**: 1 % cream or solution Once or twice daily
12. **Tolnaftate (Tinactin)**: 1 % cream, solution, or powder Twice daily
Here are short notes on the most important drugs:

**7.1.2. Azoles:**

The most important azoles are Clotrimazole, Miconazole, Ketokenazol, Itraconazol etc. All the azole antifungals interfere with the cytochrome P450-dependent ergosterol synthesis, whose presence is prerequisite for cell proliferation. The azoles (Bifonazole, Butoconazole, Clotrimazole, Croconazole, Econazole, Fenticonazole, Isoconazole, Omoconazole, Oxiconazole, Sertaconazole, Sulconazole, Terconazole and Tioconazole) are used for topical treatment of dermatomycoses or systematically (Ketoconazole, Itraconazole, Fluconazole, Miconazole) for systemic mycoses. The following azoles are discussed:

**a. Ketoconazole** has been used since the eighties in the systemic therapy of onychomycoses and severe tinea of glabrous skin. The cure rates in most cases are similar to those following griseofulvin. Over time it became clear that ketoconazole can induce hepatitis due to idiosyncrasy, with fatal outcome. For that reason ketoconazole is no longer used for onychomycosis. The approval for systemic application for onychomycoses has been revoked. However it can still be used for skin and hair disease if topical treatment is not effective

**b. Itraconazole** belongs to the newer antifungal drugs and is one of the most important drugs at present for peroral administration in the given context. It is a triazole derivate. In vitro its activity is directed against a variety of different fungi, including dermatophytes, yeasts and some moulds. The mode of action is an inhibition of the biosynthesis of ergosterol, which is essential for generating the fungal cell wall. In vivo itraconazole acts as a fungistatic agent. It has a high affinity to fungal cytochrome-p-450 isoenzymes, which is much higher than the one to human congeners. After peroral administration it can be detected within 24 hours in sweat. The active ingredient accumulates in the stratum corneum, nail material, sebum and vaginal mucosa. 99.8% of itraconazole is bound to plasma proteins. Within one week 35 and 54% respectively are excreted by urine and faeces. Itraconazole has a high affinity to keratin and is highly incorporated into the nail matrix and the nail bed. Effective concentrations of the drug remain present in the nail material for some months. Consequently there is further improvement of the clinical state after discontinuation of the drug. This is the rationale for the option of pulse therapy. Pulse therapy means application of the drug just for one week per month. The result is a lower amount of the applied drug, with similar efficacy. For onychomycosis itraconazole can be given continuously at a dose of 200 mg/d for three months or as pulse therapy with 400 mg/d in two daily doses of 200 mg for one week per month for three to four months.

**c. Fluconazole** is a newer, bis-triazole antifungal drug and suitable for peroral and parenteral administration. It has been used in oropharyngeal and esophageal candidosis or other indications with great success in immunocompromised patients for years. Recently the use of fluconazole has been evaluated for tinea of glabrous skin and onychomycosis. The in vitro activity appears sufficient with a wide variety of fungi encompassing not only yeasts but also dermatophytes. So far very little data are available on the efficacy of fluconazole on mycoses of keratinised tissue, and experience with this therapy is limited.

Fluconazole is well absorbed after peroral application. In contrast to itraconazole, gastric acid, food and antacids or H-2-receptor blocking agents do not influence absorption. After absorption only 11% are bound to plasma proteins, the rest can be found as unbound free molecules. Fluconazole shows a long plasma half life (t 1/2) of 30 hrs. In hair and toenails it can be detected for four to five months
after termination of peroral therapy. Fluconazole is eliminated as unchanged drug through renal excretion. Because of limited data a standard dosage for onychomycosis cannot yet be given. 150 mg/d or 300 mg/d once a week might turn out as the preferred dose in the near future. For tinea of glabrous skin 50 mg/d for two to seven weeks is recommended

7.1.3. Flucytosine (5-Fluorocytosine):

Flucytosine enters the cell via the cytosine permease. It is first deaminated to 5-fluorouracil by cytosine deaminase and subsequently phosphorylated and incorporated into RNA leading to its dysfunction. Another pathway for 5-flourouracil involves the formation of 5-flourodeoxyuridine monophosphate which interferes with DNA synthesis and leads to defective cell division.

7.1.4. Allylamines:

Allylamines are synthetic compounds that contain naphthalene ring and act by inhibition of ergosterol synthesis, e.g. terbinafine and naftifine.

The allylamine terbinafine belongs to the newer antifungal agents as well. It exhibits a primarily fungicidal mode of action. In vitro its activity is directed against a broad range of dermatophytes and moulds as well. It has a lower activity against yeasts. Its in vitro activity against dermatophytes is by more than one order of magnitude higher than the one of other antifungal agents. Terbinafine prevents fungal ergosterol biosynthesis by specific and selective inhibition of fungal squalene epoxidase. This inhibition is followed by a destruction of the fungal cell wall due to the enrichment of toxic squalene. The affinity of terbinafine to cytochrome-p-450 is low in contrast to the azoles. This is one reason for a lower rate of interactions of terbinafine with co-medication in comparison to the azoles. After peroral application terbinafine is bound to plasma proteins for the most part, and maximal plasma concentrations are reached within two hours. The mean terminal plasma half life (t 1/2) after 4 weeks of terbinafine is about three weeks. In the distal part of the nail plate it can be detected within one week. The usual dose of peroral terbinafine is 250 mg/d. For tinea corporis or cruris infections it can be given for 2 to 4 weeks, in tinea pedis for 2 to 6 weeks and in onychomycosis for 6 to 12 weeks.

7.1.5 Griseofulvin

Griseofulvin is a compound synthesised by some Penicillium spec. It has been used for treatment of cutaneous mycoses and onychomycoses since 1959. The only route of application is peroral. The in vitro activity of griseofulvin is limited to dermatophytes. With time griseofulvin has ceased to be the gold standard for systemic therapy. Since the introduction of the newer systemic azoles it is rarely used in the western world. It initiates a fungistatic mechanism of action due to interaction with microtubuli associated proteins and through that, inhibition of fungal cell mitosis. With the ultramicrosize preparation the highest rate of absorption can be found. Even with this best absorbed preparation the cure rates for toenail onychomycoses are lower than 40% despite treatment periods of one year and more is specific for fungi with cell wall containing chitin, mainly dermatophytes. It has no effect on fungi with cellulose cell wall or on yeasts. It inhibits mitosis through interfering with the movement of chromosomes. It causes also curling effect on the hyphae.
7.2. Treatment of diseases caused by dermatophytes in animals

Drugs available to treat dermatophytosis in animals include topical antifungal creams or shampoos, and systemic antifungals. The same treatment principles apply in animals as people; however, practical considerations limit the use of systemic antifungals in some species. Topical drugs are unable to eliminate dermatophytes from within hairs and hair follicles, but they may be effective against organisms in superficial sites (e.g., in the skin), and they can decrease contamination and transmission to others. The optimal treatment in small animals is combined topical and systemic treatment. Systemic antifungals are rarely used in large animals, due to the cost of these drugs and the typically self-limited nature of the disease. The side effects of systemic drugs should also be taken into consideration when choosing a treatment plan. Clipping the hair before treatment is controversial. It may aid the penetration of topical drugs, as well as remove infected hairs. However, it may also result in trauma to the skin and help disseminate the infection. If the animal is clipped, this should be done with care. Some animals such as cattle develop thick crusts, which should be removed by gentle brushing.

8. Prevention and control of diseases caused by dermatophytes

8.1. Hygienic measures

To prevent the introduction of dermatophytes into herds or kennels, newly acquired animals should be isolated and cultured. Wild rodent control can decrease exposure to *T. mentagrophytes*. Some organisms can be acquired by contact with infected soil.

To prevent infected animals from transmitting dermatophytes to others, they should be isolated until the infection has resolved. The premises should be cleaned and disinfected. Some environments (e.g., barns) may be difficult or impossible to decontaminate completely. Animals that have been in contact with the patient should be checked for asymptomatic infections. Some veterinarians use topical antifungals prophylactically for in-contact animals. Dermatophytes can be difficult to eradicate from environments such as kennels, catteries and animal shelters. Successful treatment of these premises must be based on good environmental control, as well as treatment of symptomatically and asymptptomatically infected animals.

8.2. Vaccination

Vaccines are available in some countries for certain organisms, such as *T. verrucosum* and *T. mentagrophytes* in livestock, farmed foxes, chinchillas and rabbits; *T. equinum* in horses; and *M canis* in cats and dogs. In some countries, vaccines have been used in dermatophyte eradication campaigns for cattle. In Norway, there is a program to eradicate *T. verrucosum* from cattle herds by vaccination, disinfection of contaminated stables, isolation of infected animals and good hygiene. In one region of Norway, where 95% of herds participated, the prevalence of cattle ringworm decreased from 70% to 0% over a period of 8 years. In the former Soviet Union, a vaccination campaign reduced the prevalence of *T. verrucosum* in cattle to less than 1% by 1984.

Types of dermatophyte vaccines

- The inactivated vaccines stimulate the CMI, as demonstrated by skin tests and leukocyte migration inhibition tests. Vaccines containing *T. verrucosum* conidia inactivated with formalin have been described for use in cattle.
- **Inactivated vaccine plus adjuvants**: An inactivated vaccine plus adjuvant containing conidia and mycelium of two *T. equinum* strains has been used in the immunization of horses. The vaccine does not prevent the disease, but the lesions are less severe in vaccinated animals compared to non-vaccinated animals.

- **Insol Dermatophyton inactivated vaccine** developed in Boehringer Ingelheim (Switzerland), it is effective in horse, dog and cat, can be used as treatment of the disease, improving the clinical outcome. It contains strains of *T. verrucosum*, *T. mentagrophytes*, *T. sarkisovii*, *T. equinum*, *M. canis*, *M. canis var. distortum*, *M. canis var. obesum*, and *M. gypseum*.

- **Commercial vaccine Feo-O-Vax MC-K1** developed by Fort Dodge in USA. It is an inactivated vaccine containing the mycelium of *M. canis* and an adjuvant. It produces anti-dermatophyte antibody titres similar to those developed in the course of the natural infection, with a low CMI. All vaccinated cats developed the disease after a topical application of *M. canis* conidia; however, the lesions were smaller than those in the control animals. The fact that all of the animals vaccinated had lesions suggests that high titres of antibody against *M. canis* may not be enough for protection against the infection.

- **The inactivated vaccine Dermatovac-IV**. It contains an adjuvant and an optically standardized inactivated suspension of conidia and mycelium of the fungi *M. canis*, *T. equinum*, *M. gypseum* and *T. mentagrophytes*

- **The Ringvac bovis LTF-1301 live vaccine** is the most effective and widely used, marketed by Alpharma, elaborated with the LTF-130 strain of *T. verrucosum*, has a characteristic high level of immunogenicity, low virulence and great stability, has been used effectively in Russia and Norway, administered intramuscularly, stimulates the appropriate immune response (DHS).

- **Permvax-Tricho live vaccine** is marketed in the Czech Republic by Bioveta Ivanovice, contains an attenuated strain of *T. verrucosum*. Triggers a protective immunity status 28 days after the second inoculation, preventing the appearance of the clinical disease for 1 year after vaccination.
9. Materials used for identification of dermatophytes

9.1.1.1. Media used for primary isolation of fungi:

a. Sabouraud dextrose agar (SDA) medium with chloramphenicol:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>40.0 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>250.0 mg</td>
</tr>
<tr>
<td>Agar agar</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Distilled water up to</td>
<td>1000.0 ml</td>
</tr>
</tbody>
</table>

**Preparation:**
The ingredients were suspended in 1000 ml distilled water and mixed thoroughly to give a uniform suspension. The mixture was boiled with frequent agitation. After heating the medium and before autoclaving 250 mg of chloramphenicol were dissolved in 5 ml ethanol and added to the medium. The pH of the medium was adjusted to 6.5 at room temperature and the medium was sterilized by autoclaving at 121°C for 15 minutes.

b. Sabouraud dextrose agar medium with cycloheximide:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>40.0 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Agar agar</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Distilled water up to</td>
<td>1000.0 ml</td>
</tr>
</tbody>
</table>

**Preparation:**
The ingredients were suspended in 1000 ml distilled water and mixed thoroughly to give a uniform suspension. The mixture was boiled with frequent agitation. Cycloheximide was added by dissolving 250mg of powdered cycloheximide in 5 ml acetone. The pH of the medium was adjusted to 6.5 at room temperature and the medium was sterilized by autoclaving at 121°C for 15 minutes.

c. Sabouraud dextrose (SD) broth:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>40.0 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Distilled water up to</td>
<td>1000.0 ml</td>
</tr>
</tbody>
</table>

**Preparation:**
The ingredients were suspended in 1000 ml distilled water and mixed thoroughly to give a uniform suspension. The mixture was boiled with frequent agitation. The pH of the medium was adjusted to 5.7 at room temperature and the medium was sterilized by autoclaving at 121°C for 10 minutes.

d. Rice (Boiled) grain medium:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>08.0 g</td>
</tr>
<tr>
<td>Distilled water up to</td>
<td>25.0 ml</td>
</tr>
</tbody>
</table>

**Preparation:**
Eight grams rice grains were added to 25 ml of distilled water in Erlenmeyer flask. Sterilization by autoclaving at 121°C for 15 minutes was done.
9.2. Stains:

a. India Ink. (Pelikan)

b. Lactophenol Cotton blue stain.

Glycerol 40.0 ml
1% aqueous Cotton blue solution 2.0 ml
Lactic acid 20.0 ml
Phenol crystals 20.0 gm
Distilled water 20 ml

Preparation:
The lactic acid and glycerine were added to the distilled water and mixed thoroughly. The phenol crystals were mixed and heated gently in hot water with frequent agitation until crystals were completely dissolved. 2 ml of 1% cotton blue solution were added and mixed thoroughly.

9.3. Buffers and solutions used for molecular diagnosis:

8.3.1. Buffers and solutions used for DNA extraction from the fungal isolates:

a. Tris EDTA buffer (TE) pH 8.0:

Tris-HCL (Sigma) 10.0 mM
EDTA (Sigma) 1.0 mM
Sterile distilled water 100.0 ml

Preparation:
Tris enzyme (1.2114 g) was added to 0.7322 g EDTA and both were adjusted to pH 8.0 by using 1 N NaOH solution after complete dissociation of the mixture. Then, distilled water was added to a final volume of 1000 ml.

b. Chloroform / Isoamyl alcho (24:1): (Sigma)
A 24 volume of chloroform was mixed with 1 volume of isoamyl alcohol and then stored at room temperature.

c. Phenol / Chloroform / Isoamyl alcohol (25:24:1): (Sigma)
A 25 volume of phenol was mixed with 24:1 volume of chloroform / isoamyl alcohol and then stored at room temperature.

d. Cold Isopropanol solution (Sigma).
   -Ethanol solution (70%) .
   -Cold absolute Ethanol solution (Sigma).

e. Genomic DNA extraction buffers:

   - DNA lysis buffer:

Tris-HCL (Sigma) (pH 8.0) 200.0 mM
EDTA (Sigma) (pH 8.0) 25.0 mM  
SDS (Sigma) 0.5 % w/v  
NaCl 250.0 mM

f. Rapid mini-preparation DNA extraction buffers:

- Universal fungal lysis buffer:

  Tris-HCL (Sigma) (pH 8.0) 400.0 mM  
  EDTA (Sigma) (pH 8.0) 60.0 mM  
  SDS (Sigma) 1 % w/v  
  NaCl 150.0 mM

- Potassium acetate buffer (pH 4.8):

  Potassium acetate (5 M) 60 ml  
  Glacial acetic acid 11.50 ml  
  Sterile distilled water 28.50 ml

g. Buffers and reagents used for PCR Amplification of ITS-Regions:

- (10 X) Tris-borate EDTA buffer (TBE) pH8.4:

  Tris-Base (Sigma) 100.80 gm  
  EDTA (Sigma) (0.5 M pH 8.0) 40.0 ml  
  Boric acid (Sigma) 50.50 gm  
  Sterile distilled water 10.0 L

- PCR Master Mix (Promega)  
- Agrose gel (1.5%).  
- 500 μg / ml Ethidium bromide (1000.0X): (Sigma)

  Only 50 mg of ethidium bromide was dissolved in 100 ml distilled water. The solution was stored in a dark bottle. To achieve the working solution we put it into a melted agarose to reach a final concentration of 0.5μg / ml.

- Gel loading buffer
  0.25% Bromophenol blue  
  0.25% Xylene cyanol FF  
  30.0% Glycerol

  Components are dissolved in sterile D.W. and stored covered with aluminum foil at room temperature.

- (100) bp DNA Ladder Marker (Promega)

h. Buffers and reagents used for RFLP-PCR ITS-Amplicon:

- The restriction endonucleases (MBI Fermentas): e.g.

  1. **Bam HI** isolated from *Bacillus amyloliquifaciens H*
2. **Bsu RI (Hae III)** isolated from *Bacillus subtilis R*

- **Buffer Bam HI** (for 100% Bam HI digestion):
  - Tris-HCL (Sigma) (pH 8.0) 10.0 mM
  - MgCl₂ 5.0 mM
  - KCl 100.0 mM
  - 2-mercaptoethanol 1.0 mM
  - Triton (x-100) 0.02 %
  - BSA 0.10 mg/ml

- (1X) Buffer **Bsu RI (Hae III)** (for 100% Hae III digestion):
  - Tris-HCL (Sigma) (pH 8.5) 10.0 mM
  - MgCl₂ 5.0 mM
  - KCl 100.0 mM
  - BSA 0.10 mg/ml

- (1X) Tris-borate EDTA buffer (TBE).
- Agrose gel (2%).
- DNA Ladder Marker (100) bp (Promega)

### i. Buffers and reagents used for Inter Simple Sequence Repeat (ISSR-RAPD):

- PCR Master Mix (Promega)
- (1X) Tris-Borate EDTA buffer (TBE)
- Nonanchored ISSR primer:

  Universal microsatellite DNA primer (GACA)₄ is a single oligonucleotide complementary to single repetitive sequences present in the target DNA. This repetitive sequences is called microsatellites.

- Agrose gel (1, 1.4, and 2 %)
- 3.1.7.4.5. DNA Ladder Marker (200) bp (Jena Bioscience)

### 9.4. Primers used in dermatophytes

#### a. ITS1, ITS3 and ITS4 primer sequence and their target regions

<table>
<thead>
<tr>
<th>ITS régions PCR primer pair (base séquence)</th>
<th>Amplification target region</th>
</tr>
</thead>
</table>
| ITS1 (5’-TCCGTTAGGTGAACCTGCG-3’)
ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) | The intervening **5.8S rDNA** and the adjacent **ITS1** and **ITS2** regions |
| ITS3 (5’-GCATCGATGAAGAGCGCA-3’)
ITS4 (5’-TCCTCCGCTTATTG ATATGC-3’) | A large portion of the **5.8S rDNA** and adjacent **ITS2** region |
b. Nucleotide sequences of the ITS1-2, 18S ribosomal RNA, and 28S ribosomal RNA sites

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Nucleotide sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Der ITS1-2 Fw</td>
<td>ATCATTAACGCACGCAGGC</td>
</tr>
<tr>
<td>Der ITS1-2 Rv</td>
<td>TGGCCACTCTGCTTTTCGG</td>
</tr>
<tr>
<td>Der 18S Fw</td>
<td>AAGTTGGGTCAAACTCAGGT</td>
</tr>
<tr>
<td>Der 18S Rv</td>
<td>TAGTCCTCCGCAGGT</td>
</tr>
<tr>
<td>Der 28S Fw</td>
<td>ACAGGGATTTGCCGCTCAGTA</td>
</tr>
<tr>
<td>Der 28S Rv</td>
<td>CTTGTTCTGCTATCGGTTC</td>
</tr>
</tbody>
</table>

Species specific ITS 1 &2 primers used by several authors

T. rubrum ITS2 593TTGGCTGCCCATTCGCTAG574
T. tonsurans ITS2 552AAGCCGGAATCGCGGCCTG533
T. violaceum/T. soudanense ITS 2 502CTGCCCATTCGCCAGGAAGC482
T. rubrum/T. soudenense ITS 1 172CTTGGGTGTCTGCTCCTCCGCGCGC513
T. violaceum ITS1 287CTTGGGTGTCTGCTCCTCCGCGCGC627
T. mentagrophytes ITS 1 215AGCCACTAAAGAGAGGCTCGC195
M. gypseum ITS 2 482CCATTCCGCCAGGAGCCGAATC460
M. canis ITS 1 218AGTCCCCCTCAGGCCGCTCC198
E. floccosum ITS2 562CTTCTCCTCCTCCGGGCTGGAAGC539

Species specific primers of dermatophytes

*Epidermophyton floccosum*

TCCATAGGGTGGTTAGTCTGAGCGGT

*M. audouinii/ M. canis/ M. ferrugineum*

TGGCCTAACGCACCATGTATTATTCAGGT
**Microsporum cookie**

GCCTTGACTGGACTCCTTTGTCCG TTAAAT

**Microsporum gallinae**

GGCCTCGTTTTCAATAATTGTCGTTAGAGAAT

**Microsporum gypseum**

CCGGTTTTCTGGCCTAGTTTTAGTTAGGGAT

**Microsporum nanum**

AGGAACGATCAAAAACACGCAGAACAC

*M. audouinii*

MA1_F (5’–CGAAGCGAGCCTCTACGGCAATCCAAAGCAG–3’),

MA1_R (5’–CGAAGCATCTTGCAGGACTCC–3’),

**Trichophyton rubrum**-specific PCR.

universal (uni, 5’TCTTTGAACGCACATTGCACC–3’) a

**Trichophyton rubrum**-specific (Trubrum-rev, 5’CGGTCTGAGGCGGCGTGA3’)

**Set of primers detecting a DNA fragment encoding chitin synthase 1,**

panDerm1 (5’GAAGAAGATTGTCGTTTGCATCGTCTC3’)

panDerm2 (5’CTCGAGGTCAAAAGCAGCCAGAG3’),
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