# Morphological varieties of *Trichophyton rubrum* clinical isolates

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## Variedades morfológicas de aislamientos clínicos de *Trichophyton rubrum*

**Resumen.** *Trichophyton rubrum* es el dermatofito antropofílico causante de micosis superficiales aislado con mayor frecuencia en todo el mundo. Diversas variedades morfológicas de este dermatofito han sido reportadas, lo cual en algunas ocasiones hace difícil su identificación**.** Nuestro objetivo fue identificar y determinar la frecuencia de variedades morfológicas entre los aislados de *T. rubrum* en México. Se incluyeron ciento treinta y un aislamientos obtenidos de pacientes con dermatofitosis. La variedad morfológica fue determinada por las características macro y microscópicas de los aislamientos crecidos en tres diferentes medios, por la producción de ureasa y por la prueba de perforación del pelo *in vitro*. Se identificaron seis variedades morfológicas, de las cuales las más frecuentes fueron "Y", vellosa típica y flava. Los resultados indican que aunque *T. rubrum* presenta una amplia variedad morfológica, solamente tres variedades predominan en México, lo cual podría indicar alguna diferencia en su capacidad infectiva.

**Palabras clave:** Dermatofitosis, dermatofitos.

**Abstract.** *Trichophyton rubrum* is the worldwide most frequently isolated anthropophilic dermatophyte causing superficial mycosis. Several morphological varieties of this dermatophyte have been reported, making its identification sometimes difficult. Our aim was to identify and to determine the frequency of the different morphological varieties among the *T. rubrum* isolates in Mexico. One hundred and thirty one isolates, obtained from patients with dermatophytosis were studied. The different morphological varieties were evaluated by their macroscopic and microscopic features of isolates growing on three different media, by the urease production and by their *in vitro* hair perforation capability. Using these criteria, six morphological varieties were identified, being the most frequent the "Y", typical downy, and *flava* varieties. In this study we found that although *T. rubrum* presents several morphological varieties, only three of them were more frequently found among isolates of Mexico, which could indicate differences in their capacity to cause infection.

**Key words:** Dermatophytosis, dermatophytes.

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## Introduction

*Trichophyton rubrum* is an anthropophilic dermatophyte worldwide distributed causing the vast majority of the mycotic skin and nails infections [1,6, 7,11] and rarely on the scalp [13]. This fungus is identified mainly by its morphological characteristics and some biochemical tests [5]. Classically, it has been considered that a primary isolate obtained from a dermatophytosis-suggestive lesion characterized by a cottony colony, with deep wine-red

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pigment on the reverse, fine filaments, and few microconidia corresponds to *T. rubrum*. However, because of its wide morphological diversity, frequently in the clinical mycological laboratory there are sometimes doubts to determine with certainty the identity of this fungus. This diversity, reported a while ago by numerous investigators [10], was used to propose concepts such as "*Trichophyton rubrum* complex" [3], and also to suggest new *Trichophyton* species, which led to the creation of *T. rubrum* varieties, and to develop schemes to facilitate their identification and differentiation from other dermatophytes.

Numerous investigators dealing with the study of dermatophytes have made important contributions to the knowledge of this group of fungal pathogens [9]. Rebell and Taplin recorded the morphological characteristics of many dermatophytes, now considered as the bases for the identification of the main species [9]. In the following years, Kane and Fisher [5] developed more laborious identification schemes in response to the appearance of more complex dermatophyte varieties and the difficulties to properly separate them. In Australia, Kaminski [4] proposed a more summarized scheme based on the macroscopic and microscopic morphological characteristics, and some biochemical tests. Specifically for *T. rubrum*, this author considered two types of strains: the granular and the downy. The granular type was subdivided in two varieties (*rhodainii* and African), and the downy in seven varieties ("Y", *flava*, "P", melanoid, hyperpigmented, uncolored, and dysgonic). Her study was based on the culture characteristics of *T. rubrum* varieties in six different media, on the urease production, and the ability to perforate hair *in vitro*.

The present study was designed to determine the morphological types and varieties of *T. rubrum* among Mexican patients with *T. rubrum* infections. With this in mind, a significant number of clinical isolates were investigated considering the scheme proposed by Kaminski [4].

## Materials and methods

**Isolates:** We studied 131 *T. rubrum* isolates obtained from patients that sought medical attention in one of four dermato-mycological health care centers (School of Medicine, UNAM; Hospital General "Darío Fernández Fierro", ISSSTE; Instituto Nacional de Ciencias Médicas y Nutrición; Hospital de Especialidades, CMN Siglo XXI, IMSS). These patients presented lesions suggestive of dermatophytosis in the glabrous skin, scalp or nails, and had not received any antifungal treatment. Age, gender, and other potential opportunistic factors were recorded. According to the affected anatomical areas, the lesions were classified in localized (one anatomic region) or in disseminated (two or more anatomical regions).

**Mycological study:** Scales were removed from the affected regions for their mycological study that consisted in direct microscopic examination and culture on Sabouraud's dextrose agar (SDA) with and without antibiotics (chloramphenicol and cycloheximide). After two weeks of incubation at 25°C, the colonies suggestive of *T. rubrum* were microscopically examined with lactophenol blue.

**Morphological study:** The macroscopic characteristics used to identify *T. rubrum* on SDA were: cottony- or powdery-like, white or light beige, flat or elevated colony, with or without pigment on the reverse of the colonies under investigation. The microscopic study was made on microcultures using the same medium. The microscopic evaluated characteristics were: fine, regularly septate mycelium; clavate or piriform, scarce or abundant microconidia; eventually clavate or cylindrical, multipseptate, smooth thin wall, some with terminal appendages macroconidia; and hyphal structures similar to macroconidia (closterospores).

**Determination of the morphological variety:** To investigate the morphological varieties, all isolates were grown on Lactritmel agar, 5% sodium chloride agar, and 1% peptone agar. The hair perforation test was also performed and the urease production was determined on Christensen's agar. When taxonomic doubt existed between *T. rubrum* and *T. mentagrophytes*, a culture on bromocresol purple-milk solids-glucose medium was performed. The culture media above mentioned were elaborated in according to reference 5.

## Results

One hundred and thirty one clinical isolates highly suggestive of being *T. rubrum* were included; from these, 123 (88.6%) were confirmed. Three isolates were identified as *T. mentagrophytes*, two as *T. tonsurans*, two as *Chrysosporium carmichaelii,* and one as *Chrysosporium* sp.

The clinical and epidemiological data from the 123 patients infected with *T. rubrum* were: 48% women and 52% men; ages between 10 and 81 years-old. The main topography of the localized lesions corresponded to toe nails and soles (81.5%); the disseminated form was found in the lower extremities (from the groin to the feet, 50%), trunk and extremities (32%). One or more opportunistic factors were found in 36 cases; 16 with diabetes mellitus, 8 with rheumatoid arthritis, 6 with lupus erythematous systemic (LES), 2 with ankylosing spondylitis, 1 with leukemia, and 3 cases of association (diabetes mellitus and rheumatoid arthritis; arthritis and LES; and diabetes mellitus and LES).

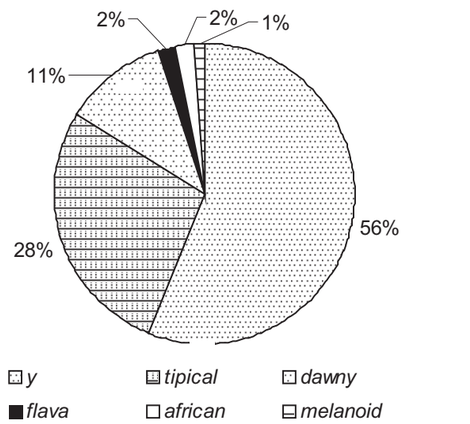


Figure 1. Distribution of the *Trichophyton rubrum* morphological varieties observed in 114 isolates obtained from patients with dermatophytosis.

From the 123 *T. rubrum* isolates, the morphological varieties and frequencies depicted in Figure 1 were determined in 114 isolates; of these, 112 corresponded to the downy type and only 2 to the granular type (var. *african*)**.** The nine remainder isolates did not show morphological characteristics compatible with the scheme proposed by Kaminski (six of them corresponded to patients with opportunistic factors), and were, therefore, separated for further analyses. The 114 isolates corresponded to 96 cases of the localized form and 18 to the disseminated form. Most of the isolates corresponded to the "*Y*" variety followed by the typical downy (TD) variety, which was defined as such when their characteristics did not correspond to the morphological varieties but they were compatible with the general description of downy type on SDA. The correlation between the morphological variety of *T. rubrum* versus age, gender, or risk factors revealed that none of these factors influences the distribution of the varieties. Figure 2 depicts cultures of *T. rubrum* grown in the different media, showing the main characteristics considered to determine the morphological variety according to the Kaminski scheme [4]. Figure 3 depicts the microscopic aspect of both morphological types: the downy type presented piriform microconidia and, generally, no macroconidia were observed; in the granular type, abundant clavate microconidia were observed as well as cylindrical macroconidia with a smooth wall, multiseptate; in the var. *african*, appendages were frequently observed at one of their ends. Microscopically the downy type varieties do not show large differences among them.

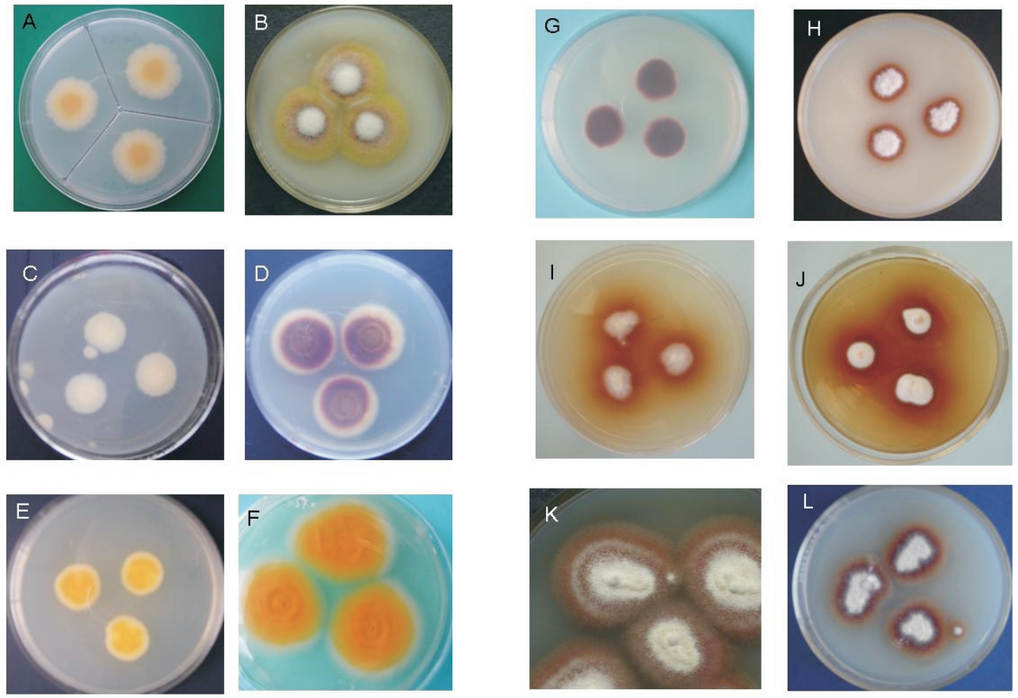


Figure 2. Macroscopic aspect of *T. rubrum* morphological varieties grown on differential media. A: var. "Y", peptone agar, reverse (note the yellow pigment); B: var. "Y", SDA, reverse (note the yellow and red pigments); C: var TD, peptone agar, reverse (note the absence of pigment); D: var. TD, SDA, reverse (note the wine-red pigment); E: var. *flava*, peptone agar, reverse (note the yellow pigment); F: var. *flava*, SDA, reverse (note the yellow pigment); G: var. "P", peptone agar, reverse (note the purple pigment); H: var. "P", Lactrimel agar, obverse (note the wine-red pigment); I: var. *melanoid*, peptone agar, obverse (note the brown diffusible pigment); J: var. *melanoid*, SDA, obverse (note the brown diffusible pigment); K: var. *african*, Lactrimel agar, obverse (note the granular aspect); L: var. *african*, Lactrimel agar, obverse (note the wine-red pigment).

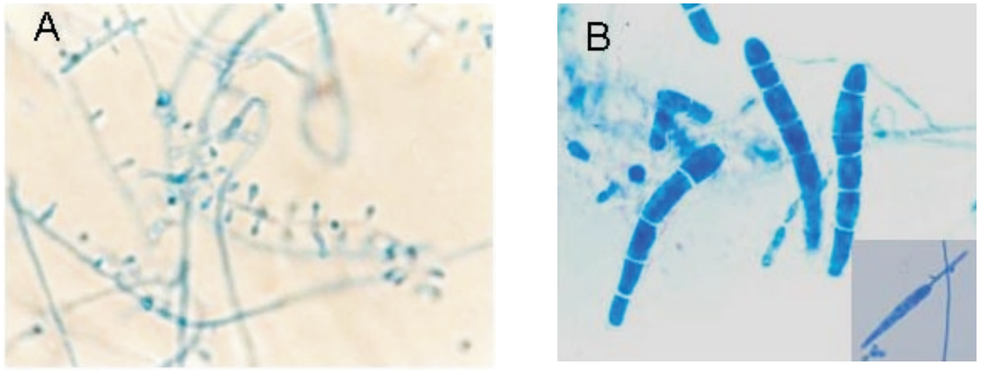


Figure 3. Microscopic morphology of the *T. rubrum* types strains grown on SDA. A: downy type (piriform microconidia, lateral to the hypha); B: granular type (thin macroconidia, clavate-shaped, multiseptate, smooth wall, usually presenting terminal appendages).





## Discussion

The precise classification of some fungi affecting the skin, hair, or nails has been problematic for most of clinical mycologists. In our experience, it is difficult sometimes to differentiate *T. rubrum* from some *T. tonsurans*, *Chrysosporium* or even from *T. mentagrophytes* strains, particularly when only the morphological characteristics of the primo-isolates are considered. Occasionally, deep wine-red pigment producing isolates suggestive of *T. rubrum* are observed, but further microscopic and biochemical studies some times show that these macroscopic attributes actually correspond to *T. mentagrophytes*.

The main characteristic considered for *T. rubrum* identification is the production of deep wine red pigment, although the pigment can vary in color (yellow, purple, orange, or even melanoid) [10,14]. The ability for pigment production is probably associated to the availability of some nutrients like dextrose as considered by Kane *et al*. [5] and as demonstrated in Figures 1C and 1D, where TD strain grown in peptone agar did not develop the pigment, whereas on SDA it produced a deep wine red pigment. However, the pigment production is not always related with the nutrients availability; in our experience different isolates growing on the same medium can develop different pigments. Probably, the ability to produce or not pigments could also be observed in clinical cases, as referred by Perrin and Baran [8]. These investigators described two cases of longitudinal melanonychia caused by *T. rubrum* producer of melanoid pigment. However, the clinical experience of other mycologists does not refer the nail coloration as a characteristic feature indicating infection with a specific *T. rubrum* variety, since pigmentation has been attributed to infection by other dermatophytes and yeasts or bacteria [2,12].The *T. rubrum* morphological varieties most often found in this study were "Y", TD, and "*flava*"; it is not known whether these varieties possess some particular virulence factor favoring their high prevalence in clinical cases. In this study, the comparison of diverse conditions, such as immunosuppressive factors, age, gender, and extension of the lesions did not reveal differences in the predominant varieties. Although our data in Figure 1 agree with the scheme proposed by Kaminski [4], it is noteworthy to observe that some times, the same strain grown in triplicate showed morphological variability, conducing to eliminate it from the data final tabulation and indicating the need to start this kind of studies with monospore cultures to limit the probability of these changes, although the natural infection probably does not occur with monospore strains.

The molecular studies performed up to now to validate the morphological and physiological studies have not been conclusive for *T. rubrum*, which is also the case in other taxonomically related species. Gräser *et al*. [3] found that diverse *Trichophyton* species, such as *T. rodhainii*, *T. raubitschekii*, *T. pedis*, *T. rubrum*, *T. kanei,* and *T. fischeri*, possess the same genetic pattern. Although this could be related to anomalies such as the selection of genetic markers, despite these controversial findings, molecular biology is a new powerful tool that could be helpful in the phylogenetic classification of fungal species.

The morphological taxonomical scheme used in this study did not properly discriminate all the investigated isolates. However, considering the wide variability shown by *T. rubrum* isolates, it is possible that no scheme be totally inclusive. Molecular studies might become of importance for the classification of *T. rubrum* varieties by using more sensitive techniques than those used up to now.

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