

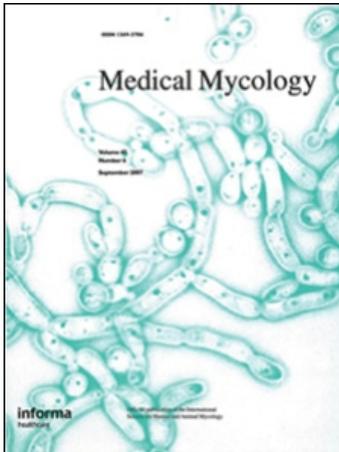
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SHORT COMMUNICATION

**Re-evaluation of the synonymy between *Keratinomyces ceretanicus* and *Trichophyton ajelloi***

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In 1987, *Keratinomyces ceretanicus* was reduced to synonymy with *Trichophyton ajelloi* based on mating between the putative ex-type culture, ATCC 58594, and minus mating strains of *Arthroderma uncinatum*. Although we confirm that ATCC 58594 produces fertile ascospores when mated with *A. uncinatum*, we demonstrate that this strain is not representative of the type of *K. ceretanicus* and conclude that *K. ceretanicus* should be maintained as a separate taxon.

On the basis of one living strain and another collection from Spanish soils, Punsola & Guarro [6] described *Keratinomyces ceretanicus* characterized by its slow growth rate, optimum growth temperature less than 20°C, sensitivity to cycloheximide, predominantly 11–14-celled, narrowly fusiform to pencil-shaped macroconidia, and absence of microconidia. Padhye *et al.* [5] placed *K. ceretanicus* in synonymy with *Trichophyton ajelloi* based on the production of fertile ascospores when ATCC 58594, a strain purportedly derived from the type of *K. ceretanicus*, was mated with (–) mating type strains of *Arthroderma uncinatum*. Padhye *et al.* reported that ATCC 58594 produced smooth, thick-walled cylindro-fusiform 7–12-celled macroconidia, moderate numbers of microconidia, and grew optimally at 25°C rather than 17°C. These characteristics differed significantly from those reported in the original description of *K. ceretanicus* and with our observations of another strain, also derived from the holotype, which had been deposited in 1986 in the University of Alberta Microfungus Collection and Herbarium as UAMH 5384. Since two additional strains of *K. ceretanicus* were available, we conducted a comparative study of colonial and microscopic morphology, optimum growth temperatures and mating reactions between these isolates, ATCC 58594, and four strains of *A. uncinatum* studied by Padhye *et al.*

The isolates examined included: *K. ceretanicus* (FFBA 328, holotype consisting of dried culture deposited at the Faculty of Medicine, Reus (=IMI 311889); UAMH 5384, ex-type culture, soil, Spain, J. Cuarro; UAMH 6412 and 6414, soil, Chile, J. Cano). ‘*K. ceretanicus*’ (CDC B-4562 derived from ATCC 58594, presumptive ex-type culture, obtained from Dr A. Padhye, Center for Disease Control, Atlanta, GA (=UAMH

6480); ATCC 58594, obtained from the American Type Culture Collection). *A. uncinatum* (UAMH 2929 and 3271, monospore isolates (+) tester strains; UAMH 2930 and 3272, monospore isolates (-) tester strains).

Colonial and microscopic morphologies were examined for all isolates on Sabouraud glucose agar (SGA) (Difco Laboratories, Detroit, MI) without antibiotics at 18°C and 25°C and on Mycosel agar (myc) (Baltimore Biological Laboratories, Cockeysville, MD). Colony diameters were measured at 14 days and colony colours were determined using the Methuen Handbook of Colour [4].

For mating studies, strains of *A. uncinatum* and '*K. ceretanicus*' (CDC B-4562) were grown on SGA for 21 days at 25°C, whereas strains of *K. ceretanicus* were grown on SGA for 28 days at 18°C. A conidial suspension was prepared for each strain in a glass vial containing approximately 25 ml of sterile distilled water. From each vial, 2.5 ml was pipetted into glass petri dishes (3 cm diam) containing garden soil mixed with fragments of horse hair (pre-sterilized by autoclaving together for 20 min at 121°C on 2 successive days). All strains were grown alone and crossed in all possible combinations. The plates were incubated in the dark at 18°C, kept humidified by addition of sterile water, and examined periodically for formation of fertile ascomata. A cross was considered negative if no fertile ascomata were produced after 9 weeks.

Results of our mating experiments (Table 1) concurred with Padhye *et al.* [5] that CDC B-4562 (as ATCC 58594) produced fertile ascomata when mated with (-) mating type strains of *A. uncinatum* (UAMH 2930 and 3272). Although purportedly derived from the type specimen, CDC B-4562 behaved differently from UAMH 5384, which failed to mate with any tester strain of *A. uncinatum*. None of the strains identified as *K. ceretanicus* produced ascomata with mating strains of *A. uncinatum*, or in crosses among themselves or in self-self pairings.

The three strains of *K. ceretanicus* also differed from CDC B-4562 and isolates of *A. uncinatum* in their optimal growth temperatures, growth rates, sensitivity to cycloheximide, colony colour and microscopic morphology. Growth rates at 18°C and 25°C are summarized in Table 1. The three strains of *K. ceretanicus* grew slowly at both tem-

TABLE 1. Comparison of growth rates and results of mating tests between *A. uncinatum* and *K. ceretanicus*

Strain	Colony diam. (mm) SGA (14 days)		Colony diam. (mm) myc (14 days)		Mating type <sup>a</sup>	Produced fertile ascomata with
	18°C	25°C	18°C	25°C		
<i>A. uncinatum</i>						
2929	28	55	27	50	+	2930, 3272
2930	35	52	42	55	-	2929, 3271
3271	29	53	32	52	+	2930, 3272
3272	28	48	30	50	-	2929, 3271
' <i>K. ceretanicus</i> '						
B-4562 <sup>b</sup>	27	39	28	46	+	2930, 3272
<i>K. ceretanicus</i>						
5384 ex-type	8	5	NG <sup>c</sup>	NG	Unknown	None
6412	8	10	5	8	Unknown	None
6414	10	7	NG	NG	Unknown	None

<sup>a</sup> Reference [5], and University of Alberta Microfungus Collection Catalogue of Strains.

<sup>b</sup> Derived from ATCC 58594, putative ex-type.

<sup>c</sup> No growth (colony less than 2 mm diam.).

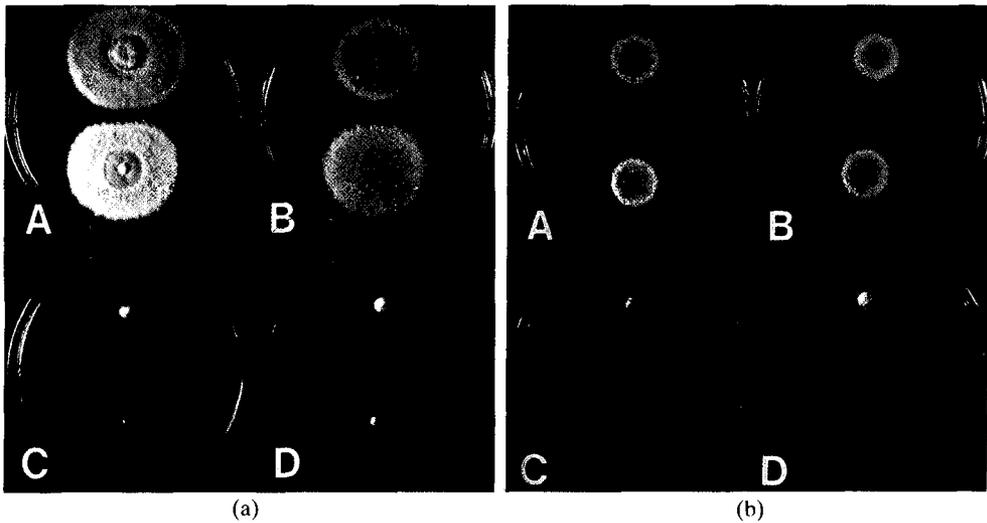


FIG. 1. Colonies after 14 days at 25°C (a) and 18°C (b) on split plates containing SGA (top) and myc (bottom). A = UAMH 2929 *A. uncinatum*; B = CDC B-4562 (as ATCC 58594) '*K. ceretanicus*'; C = UAMH 5384 ex-type *K. ceretanicus*; D = UAMH 6414 *K. ceretanicus*.

peratures, but growth was slightly enhanced at 18°C for two of the three strains. In contrast, all strains of *A. uncinatum* (including CDC B-4562) grew rapidly, and growth was faster at 25°C than at 18°C (Fig. 1a, b). Colonies of the former were pale to vivid yellow (2A1–3A8) with a white margin at 18°C, whereas *A. uncinatum* was light orange, greyish or brownish orange (5A4/6–7D7). On a medium containing 400 µg ml<sup>-1</sup> cycloheximide (myc), strains of *A. uncinatum* were not inhibited, whereas two of three *K. ceretanicus* isolates were strongly inhibited (Table 1; Fig. 1a, b).

The macroconidia of CDC B-4562 were typical of *A. uncinatum* (Fig. 2) in being thick-walled with 7–10 cells, but distinct from those of *K. ceretanicus* (Fig. 3) which dif-

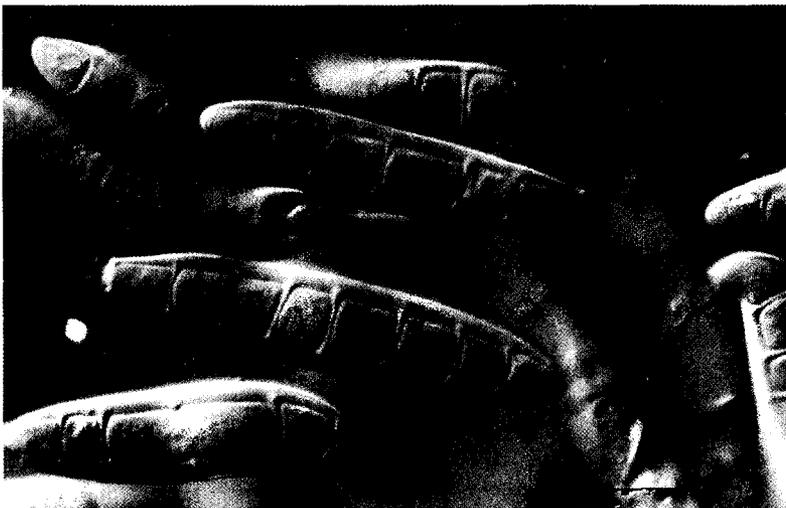


FIG. 2. Macroconidia of '*K. ceretanicus*' CDC B-4562 (as ATCC 58594). Bar = 10 µm.

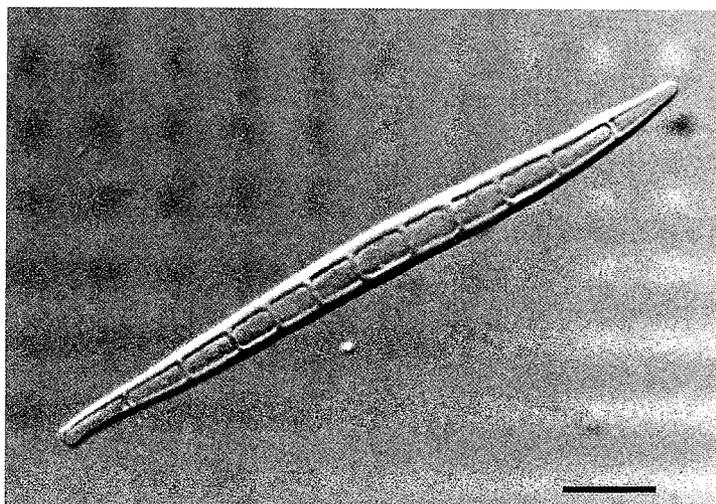


FIG. 3. Macroconidia of *K. ceretanicus* UAMH 5384, ex-type. Bar = 10  $\mu\text{m}$ .

ferred in being fusiform to pencil-shaped, narrower (4–5  $\mu\text{m}$  in width), tapered at the tip, and predominantly 11–14-celled. When growing on soil with hair, the cluster of macroconidia of *K. ceretanicus* appeared white, rather than cream coloured as in the former. No microconidia were observed in strains of *K. ceretanicus*.

The culture obtained directly from the American Type Culture Collection as 58594 proved to be *Hormographis ramirezii* [3], an arthroconidial hyphomycete unrelated to *K. ceretanicus* or *T. ajelloi*. A subculture of UAMH 5384 was deposited in the ATCC as 66969.

We conclude that CDC B-4562 derived from ATCC 58594 does not represent the ex-type culture of *K. ceretanicus*, but rather a misidentified *A. uncinatum*. While negative crosses between tester strains of *A. uncinatum* and *K. ceretanicus* are not conclusive evidence that the species are distinct, the much slower growth rate, lower optimum temperature for growth, sensitivity to cycloheximide and differences in macroconidium morphology suggest that *K. ceretanicus* should be retained as a separate taxon.

Punsola & Guarro [6], following in part the criteria of Vanbreuseghem [7] and von Arx [2], opted to place their species in *Keratinomyces*, despite arguments [1] for placing the genus into synonymy with *Trichophyton*. *K. ceretanicus* is unusual when compared with *Trichophyton* for its psychrophilic growth habit and sensitivity to cycloheximide. It also lacks microconidia, but this character does not exclude it from *Trichophyton*, a form-genus based on production of macroconidia.

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