

## Tinea nigra by *Hortaea werneckii*, a report of 22 cases from Mexico

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**Abstract:** Tinea nigra is a superficial mycosis caused by *Hortaea werneckii*. It is an infrequent asymptomatic infection that affects human palms and soles, and is mostly observed in tropical countries. We evaluate retrospectively twenty-two confirmed cases of tinea nigra from a total of eleven yr (1997–2007) and discuss the epidemiology, clinical features and treatment of this disease. In twelve cases, adults were involved, in 10, children. In nineteen cases the disorder was located on palms of hands and in three on soles of feet. In all cases, the obtained isolates were morphologically identified as *Hortaea werneckii* and the identification of ten isolates was retrospectively confirmed with the help of sequences of the internal transcribed spacer regions of the ribosomal DNA. The patients received topical treatment with Whitfield ointment, ketoconazole, bifonazole, or terbinafine. Treatment with keratolytic agents and topical antifungals was effective.

**Key words:** *Hortaea werneckii*, keratolysis, melanized fungi, superficial mycosis, tinea nigra, tinea palmaris.

### INTRODUCTION

Tinea nigra is a superficial mycosis caused by the melanized, yeast-like fungus *Hortaea werneckii* (Horta) Nishimura & Miyaji, formerly incorrectly classified in genera such as *Cladosporium*, *Cryptococcus*, *Exophiala* and *Phaeoannelomyces* (McGinnis *et al.* 1985, de Hoog *et al.* 2000). It is an infrequent, asymptomatic infection, limited to tropical and subtropical countries. Most typically, it affects palms of hands but is occasionally found on other parts of the body. Symptoms include hyperchromic plaques, in which the fungus may or may not live in commensalism with other organisms (Hughes *et al.* 1993, Bonifaz 2001, Gupta *et al.* 2003). The disorder has longtime been regarded as an infection and therefore *Hortaea werneckii* was classified as a BioSafety Level 2 organism (Anon 2004). However, de Hoog & Gerrits van den Ende (1992) and Göttlich *et al.* (1992) noted that tinea nigra is subclinical, only dead keratin cells on the skin being colonized. No keratinolysis can be observed and the adhesion to human hands is to be explained by the hydrophobic character of the yeast cells. It has been proven that the natural habitat of the fungus comprises hypersaline environments due to its halophilic behaviour (Zalar *et al.* 1999, Plemenitaš *et al.* 2008). The present article is a retrospective report of cases of tinea nigra, its epidemiological, clinical, and therapeutic features, as well as a review of the disorder.

### MATERIAL AND METHODS

This is an eleven-yr retrospective study (1997–2007) of confirmed clinical cases of tinea nigra (Table 1). Each patient underwent clinical examinations and laboratory tests such as direct KOH (20 %) analysis and culturing on Sabouraud glucose agar without or with antibiotics (Mycobiotic, Difco Co), incubated at 28 °C for 8–30 ds. Each of the strains was identified using macro- and

microscopical features. The identification of ten strains was verified with sequences of the internal transcriber spacer regions (ITS) of the rDNA. Methods for DNA extraction and sequencing were those of Badali *et al.* (2008). Sequences were compared using a black yeast molecular database maintained at the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

After diagnosis, the patients received Whitfield ointment topical treatment (salicylic acid 3 %, benzoic acid 2 %) twice daily for 15 ds, or one of the following antifungals as creams: ketoconazole 2 % (Nizoral), bifonazole 1 % (Mycospor) and terbinafine 1 % (Lamisil). Subsequently, follow-up of patients was performed for 1–2 mos after application of the last dose. Cure was defined as the absence of clinical signs and negative culturing (Table 2).

### RESULTS

Twenty-two confirmed cases of tinea nigra were reviewed and patient data recorded (Table 1). The ITS region of ten strains was identical with the ITS of the ex-type strain of *Hortaea werneckii*, CBS 107.67. Table 1 shows the strain numbers and the main demographical data of the patients. Twelve out of 22 patients were adults and the remaining were adolescents and children. Mean overall age was 20.7 yr; mean age of the adult patients was 27.3 yr, and 11.2 yr for the adolescents/children. All adults were farmers. Most of the adolescents/children were in school-age. Most patients originated from rural areas in tropical and humid regions characterized by abundant subtropical and tropical vegetation. Several of them reported to have close contact with plants and grasses. Eleven cases originated from coastal zones where patients may have been in contact with substrata of high salinity: the majority of them lived near the sea, salt-marsh or river estuaria. The remaining 11 patients came from metropolitan zones and did not provide any specific information (Fig. 1). The

**Table 1.** Demographic data of 22 patients with tinea nigra.

Case nr	Age (yr)	Gender	Disease duration (mos)	Geographical zone	Occupation	Predisposing factor	Disease location	Direct exam	Culture	CBS Number
1	23	M	2	MA	Farmer	-	Palm	+	<i>H. werneckii</i>	-
2	9	M	1	MA	Junior	-	Palm	+	<i>H. werneckii</i>	-
3	25	F	2	LC Tampico	Home	Hyperhydrosis, running on beach	Sole	+	<i>H. werneckii</i>	CBS 123043
4	18	M	4	MA	Student	Hyperhydrosis	Palm	+	<i>H. werneckii</i>	CBS 123041
5	40	F	3	MA	Home	Hyperhydrosis	Palms (bilateral)	+	<i>H. werneckii</i>	-
6	9	M	2	LR Tabasco	Junior	-	Palm	+	<i>H. werneckii</i>	-
7	11	M	2	LR Tabasco	Junior	-	Palm	+	<i>H. werneckii</i>	-
8	20	M	8	MA	Farmer	-	Palm	+	<i>H. werneckii</i>	CBS 123046
9	15	F	2	MA	Home	Hyperhydrosis	Palm	+	<i>H. werneckii</i>	CBS 122348
10	2	F	1	LC Tapachula Chis	None	-	Palm	+	<i>H. werneckii</i>	-
11	28	M	2	MA	Home	-	Palm	-	<i>H. werneckii</i>	CBS 122344
12	25	F	8	MA	Farmer	Hyperhydrosis	Palm	+	<i>H. werneckii</i>	CBS 123044
13	16	M	3	LC, Tampico	Junior	Hyperhydrosis	Foot (interdigital)	+	<i>H. werneckii</i>	CBS 123045
14	8	F	1	MA	Junior	-	Palm	+	<i>H. werneckii</i>	-
15	20	M	1.5	MA	Student	Hyperhydrosis	Palm	+	<i>H. werneckii</i>	-
16	28	F	2	LC. Cabo San Lucas BC	Farmer & worker	Saltpan worker	Palm	+	<i>H. werneckii</i>	-
16	28	F	2	LC. Cabo San Lucas BC	Farmer & worker	Saltpan worker	Palm	+	<i>H. werneckii</i>	-
17	12	M	1	LC, LR Veracruz	Junior	-	Palm	+	<i>H. werneckii</i>	CBS 123042
18	15	F	3	LC, LR Veracruz	Junior	-	Palm	+	<i>H. werneckii</i>	-
19	12	F	2	MA	Home	-	Palm	+	<i>H. werneckii</i>	-
20	61	M	18	LC Tampico	Farmer and fisher	-	Palm	+	<i>H. werneckii</i>	CBS 122342
21	30	F	2	LC, LR Salinacruz Oax	Junior	Hyperhydrosis, running on beach	Sole	+	<i>H. werneckii</i>	CBS 122340
22	28	M	6	LR Acapulco	Student	Hyperhydrosis	Palm	+	<i>H. werneckii</i>	-

MA = Metropolitan area; LC = living near coast; LR = living near river.

**Table 2.** Summary of treatment of tinea nigra.

Topical	No. of cases	No. of applications per d	Mean treatment duration	Cases / Response
Whitfield's ointment	11	2	18 ds	10/ Cure 1 /Relapse*
Ketoconazole	4	2	15 ds	4 /Cure
Bifonazole	4	1	12 ds	4/Cure
Terbinafine	2	1	15 ds	2 /Cure
None	2	-	-	2 / Spontaneous cure

\*Treated later with bifonazole.

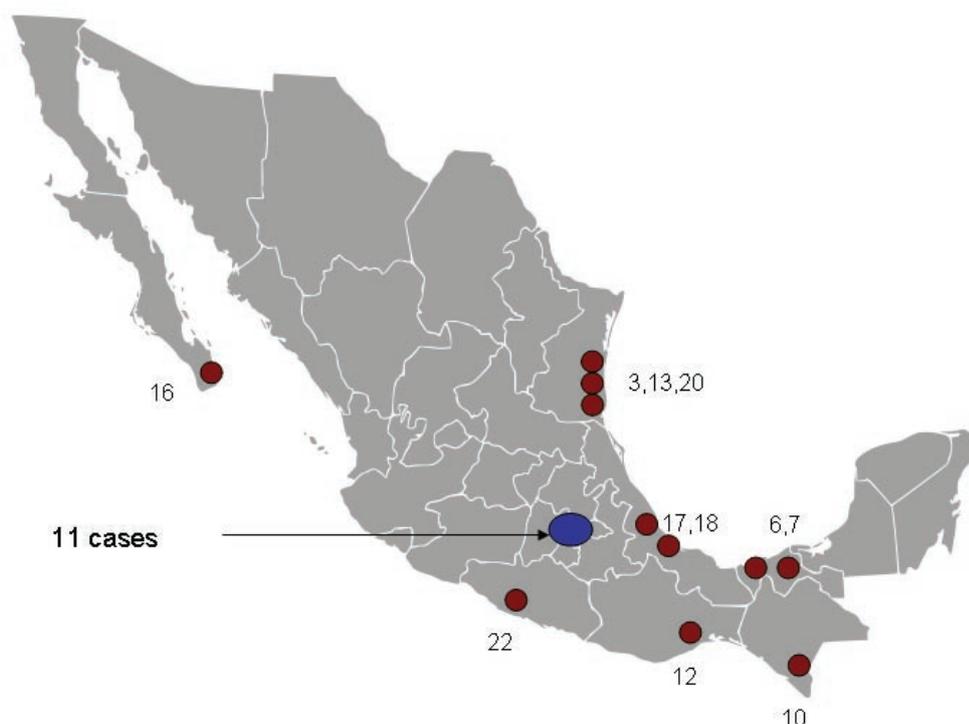


Fig. 1. Map showing the distribution of cases analysed.

majority of patients were without apparent specific predisposing factors for fungal infection. Palm hyperhidrosis was present in 6/22 cases; one case reported professional activities in a saltpan. Two patients used to run barefoot along the beach. The clinical location of tinea nigra was the palms in most cases; in 17 out of 18 cases the infection was unilateral and one was bilateral (Figs 2A–C). Of the cases located on the feet, two occurred on the sole (Fig. 2D) and one was located in three interdigital spaces (Fig. 2E). These three patients reported sole hyperhidrosis; two of them used to walk barefoot along the beach.

Direct examinations were positive in 21/22 cases (95.4 %; Figs 2F–H). All patients had a positive culture that was preliminarily identified as *Hortaea werneckii*; active growth was evident on average after 5.5 ds (Figs 2–4). Biopsies were taken in two patients (due to confusion with nevi); however, perivascular infiltrates were reported in both cases and short fungal filaments could be found on the stratum corneum.

In view of generating a retrospective report, various types of treatment were applied (Table 2). Eleven cases were treated with Whitfield ointment. Treatment failed in one case, which is why ketoconazole cream 2 % was applied successively. Using the antifungals ketoconazole 2 %, bifonazole 1 %, and terbinafine 1 %, all patients achieved clinical cure within 12–18 ds, with a mean total treatment period of 15 ds. Two patients were not treated with any medication due to uncertain initial diagnosis, but presented spontaneous cure was achieved within about two mos.

## DISCUSSION

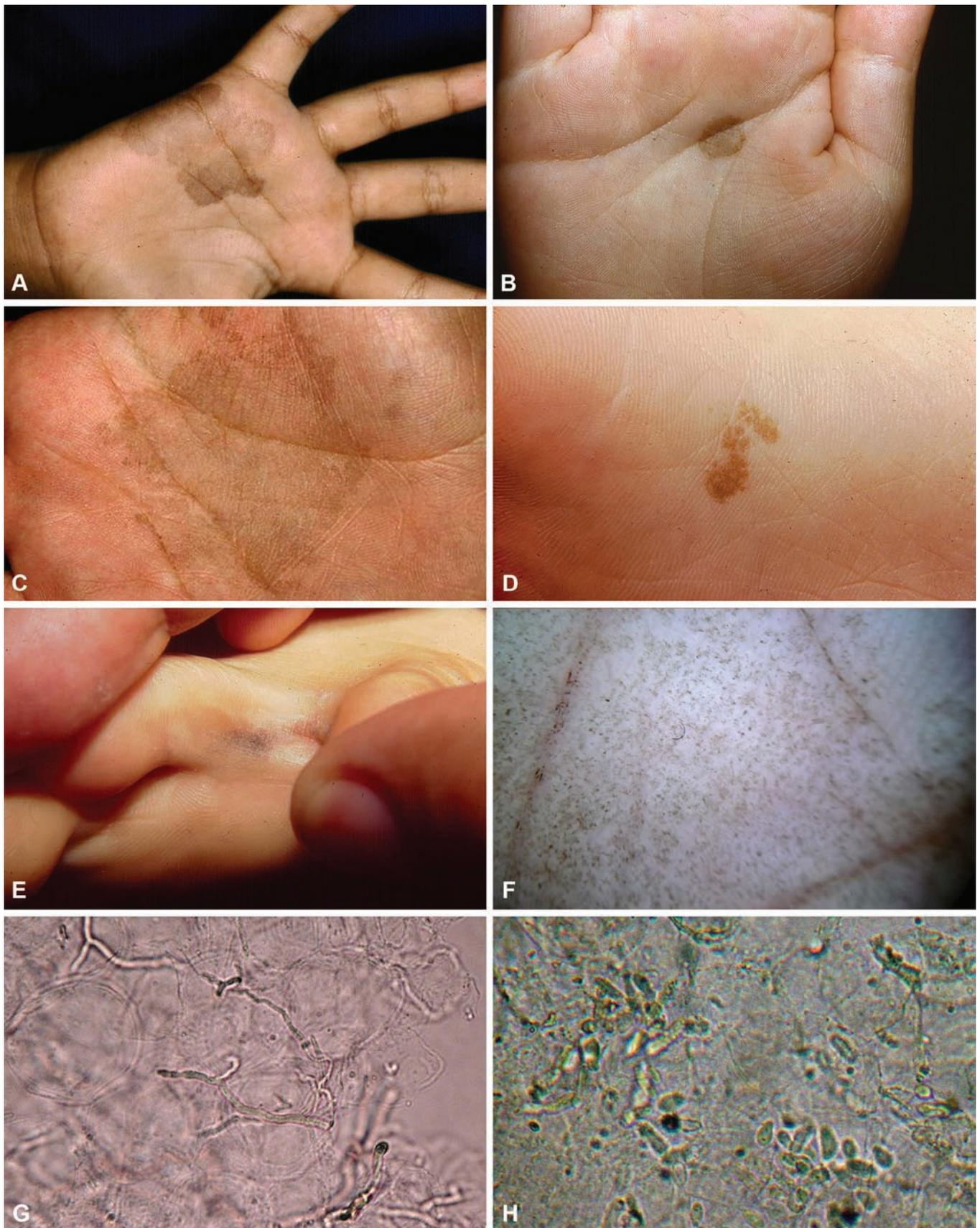
*Hortaea werneckii* is best known from (sub)tropical climates and lives in environments with reduced water activity such as sea water, natural or man-made salt pans (Zalar *et al.* 1999), occasionally it can also be present in house dust (Uezato *et al.* 2006). It is a halophilic species, having the capacity to support high salt

concentrations (3–30 % NaCl). Its prevalence reaches a peak in highly saline water of crystallization ponds (Gunde-Cimerman *et al.* 2000). Therefore this microorganism has been used as a model to study these conditions of extremotolerance, for example, its role of oxidative stress, osmotic adaptation and melanization (Petrovič 2006, Kogej *et al.* 2007).

The melanized, polymorphic and yeast-like fungus *Hortaea werneckii* may be difficult to recognize by morphological characters. Its relatively restricted, black primary cultures, 1 µm wide annellated zones and one-septate conidia facilitate its specific identification. ITS sequences show limited variation, and the species is clearly distinct from other, closely related taxa (Zalar *et al.* 1999). No teleomorph of this fungus has been found. It is known to be phylogenetically affiliated to the order *Capnodiales* (Crous *et al.* 2007a).

In some regions of Venezuela, *Stenella araguata* Syd. has been reported as a causative agent of tinea nigra (Perez *et al.* 2005). This species is known from only two occasions: the original specimen caused leaf spots on vegetation (*Pithecellobium lanceolatum*, *Mimosaceae*) and its taxonomic status is now unclear. Another isolate came from a case of tinea nigra (Crous *et al.* 2007b). The ITS of the latter, CBS 105.75, was sequenced (EU019250) and mentioned twice under invalid name "*Catenulostroma castellanii*" (Crous *et al.* 2007a,b). Based on ITS rDNA data, the strain CBS 105.75 is clearly different from *H. werneckii* (G.S. de Hoog, unpublished data), but judging from rDNA large subunit data (Crous *et al.* 2007a) the species is a close relative and might represent a second agent of tinea nigra. As long as its identity with the type of *S. araguata* is pending, it is difficult to attribute a taxonomic name to this fungus.

Tinea nigra is an uncommon discolouration of the skin. Most reports originate from tropical, humid climate zones. Cases from Latin America have been reported from Panama, Colombia, Venezuela, Brazil and Mexico (Chang & Arenas 1983, Durán *et al.* 1983, Severo *et al.* 1994, Pegas *et al.* 2003, Perez *et al.* 2005), while Asian reports came from India, Sri Lanka, Myanmar



**Fig. 2.** A. Extensive tinea nigra palmaris (case 1; Table 1); B. Localized tinea nigra (case 9, CBS 122348; Table 1); C. Close-up of a pigmented macula (case 20, CBS 122342; Table 1); D. Tinea nigra plantaris, panoramic; E. Tinea nigra interdigital (case 13, CBS 123045; Table 1); F. Tinea nigra under dermatoscopy, multiple pigmented lesions; G. Direct examination of pigmented and septate hyphae (KOH, 40 $\times$ ); H. Direct examination of short filaments and multiple blastoconidia (KOH, 40 $\times$ ) (case 20, CBS 122342, Table 1).

and Polynesia (Uezato *et al.* 2006). The disorder is rare in Europe (Hughes *et al.* 1993, Reid 1998) and the United States (Burke 1993, Shanon *et al.* 1999, Tseng *et al.* 1999). Predisposing factors associated with the condition are hyperhydrosis (Severo *et al.* 1994, Bonifaz 2001, Padilla *et al.* 2002) and presence in coastal sea areas or hypersaline environments, where the causative agent may be picked up from the natural habitat (de Hoog & Gerrits van den Ende 1992).

Our 22 cases present a large series and, unlike other published reports (for example, Severo *et al.* 1994, Perez *et al.* 2005), cover a long follow-up period. Our cases are clinically and demographically similar to the ones reported from other continents (Uezato *et al.* 2006). Tinea nigra is considered a rare disease. According to statistics in our hospital in Mexico City, D.F., it accounts for 0.085 % of all mycoses. The frequency of the disorder may be higher, but due to its asymptomatic nature and the possibility of spontaneous cure, patients seldom reach the doctor's office. This is the case only when patients worry because of confusion with other pigmented skin diseases, such as melanoma.

The disorder has no preference for age categories (Table 1), cases equally occurring in adults and children, and no gender differences are observed (Durán *et al.* 1993, Pegas *et al.* 2003). Course of the infection according to our data was 1–18 mos, with a mean incubation time of 3.8 mos. According to the patient's information, we think that the developing lesions become visible within 15–30 ds. The lesions are invariably flat, not elevated and without inflamed margins; they consist of pigmented, mostly brownish, irregular and asymptomatic macules and with well-defined borders, and are covered by fine scaling. Some patients



Fig. 3. Culture of *Hortaea werneckii* on SGA. Two phases: yeast-like phase with moist colonies, and filamentous colony (case 11, CBS 122344; Table 1).



Fig. 4. Multiple blastoconidia with central septa (KOH, 40×). (case 20, CBS 122342; Table 1).

report that throughout the day a change in colour of the macules can be observed: early in the morning macules can appear in more intense pigmentations but they fade as the day goes by. This is probably due to the fact that the fungus is cleared from the hands as a result of daily activities. Only one patient in our study, presented with erythema, also reported moderate itching as was noted in other studies (Hughes *et al.* 1993, Perez *et al.* 2005).

Tinea nigra has a major clinical relevance because it can be mistaken for various types of nevi. In some reports it even was misidentified as melanomas. Also some of our cases, seen at external dermatology services, were initially diagnosed as various types of nevi (Hall & Perry 1998, Tseng *et al.* 1999). With this incorrect diagnosis, two of the cases underwent biopsies, which lead to the observation of fungal elements in the stratum corneum with a discrete perivascular infiltrate. Differential diagnosis is essential, due to the extremely different prognosis of the various conditions. The tinea nigra macule denotes the superficial growth of the fungus, strictly limited to the stratum corneum. This may be revealed by dermatoscopy, which is a technique allowing the observation of fungal elements with certainty, particularly when flanked with microbiological tests (Smith *et al.* 2001; Fig. 2F).

The palms of hands constitute the major location of the disease, which explains the often-used name “tinea nigra palmaris”. Most cases are unilateral but also bilateral infections can be observed (Tseng *et al.* 1999), probably resulting from autoinoculation (Fig. 2A). In our study, in 19/22 cases the disorders were located on the palms and 3 on the soles. Surprisingly, one of the sole infections was located in the interdigital spaces (Fig. 2E) and had a discrete pigmentation (case 13, strain CBS 123045; see Table 1), which led to suspect dermatosis neglecta caused by the chronic deposition of dirt or filth (Ruiz-Maldonado *et al.* 1999). We are unaware of any case of a tinea nigra located in the interdigital spaces. Recently a similar condition caused by chaetothyrialean black yeast-like fungi was reported (Badali *et al.* 2008), differing by the fact that the etiologic fungus concerned, *Cladophialophora saturnica* showed invasive behaviour, while *Hortaea werneckii* is strictly commensal. The two cases located on the plantar region were also informative, as they concerned patients with the habit of running barefoot along the seaside, an environment with high salinity and probably the natural niche of the fungus. Both cases had a clinical history of hyperhydrosis probably resulting in saline plantar cutaneous conditions and adhesion of the fungus (Fig. 2C; Göttlich *et al.* 1995). It is important to comment on case 16 (Table 1), an irregular worker in saltpans, that is considered the natural environment of *H. werneckii*. It is probable that more saltpan-workers carry the disorder but due to the asymptomatic nature of the disease do not seek medical help (Gunde-Cimerman *et al.* 2000).

Recently Ng *et al.* (2005) reported the isolation of *H. werneckii* from the serum and a splenic abscess of a human patient. This is an exceptional case, in which the fungus behaved as an opportunistic pathogen and was the causative agent of a systemic phaeohyphomycosis.

Given the taxonomic uncertainties mentioned above, confirmation of identification with microbiological and molecular tests is compulsory. Direct KOH examinations provide us with quick information and demonstrate the short, tortuous, thick, light brown hyphae, which may occasionally be darkened, and sometimes present short filaments and yeast-like cells. The pigmentation of hyphae unambiguously distinguishes tinea nigra from various other types of dermatophytoses or skin infections (Hughes *et al.* 1993, Gupta *et al.* 2003). The use of chlorazol black (Feuilhade de Chauvin 2005) as a clearing solution is not recommended because

this reagent stains fungal elements black, which interferes with distinction from dermatophyte hyphae. In our study nearly all cases were diagnosed by direct examination and were confirmed by culture. Colonies of *Hortaea werneckii* grow on a standard media within 5–8 ds. They are initially black with a creamy appearance and later become filamentous. This morphological transition is characteristic for *H. werneckii*, but is also known in *Exophiala* (*Chaetothyriales*). Filamentous isolates may be mistaken for some chaetothyrialean fungi or for *Cladosporium* spp. Conidia appear as pigmented yeast cells with a dark central septum, the outer wall later becoming thick-walled, heavily pigmented. Conidia finally germinate with hyphae resulting in yeast-like colonies that gradually change over into filaments to complete the anamorph life cycle.

Molecular diagnostics of *H. werneckii* were developed by Uijthof *et al.* (1994) using PCR-fingerprinting techniques and by Zalar *et al.* (1999) using sequencing of rDNA ITS. Uezato *et al.* (1989) applied molecular diagnostics in clinical practice. Abliz *et al.* (2003) developed specific primers on the basis of ITS data and validated the primers by a comparison with 42 other melanized fungal species, including chaetothyrialean agents of cutaneous and subcutaneous disease.

The treatment of tinea nigra is very simple and effective. Most cases resolve with only keratinolytic agents like urea, salicylic acid and Whitfield ointment, applied once or twice a day (Sayegh-Carreno *et al.* 1989, Bonifaz 2001). Most of the cases in our report were managed with Whitfield ointment. Mean treatment duration (Table 2) was approximately 15 ds; two cases presented spontaneous cure. Most topical antifungals are also effective. Good treatment results have been reported using miconazole (Marks *et al.* 1980), ketoconazole (Chang & Arenas 1983, Burke 1993), bifonazole (Meisel 1984), terbinafine (Shanon *et al.* 1999) and ciclopirox olamine (Rossen & Lingappan. 2006). There is even a report of oral itraconazole therapy (Gupta *et al.* 1997), which is not recommended for this commensal fungus. Most cases in our study resolved with topical therapy within two weeks.

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