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Cutaneous Hyalohyphomycosis Caused by *Fusarium solani* in a Loggerhead Sea Turtle (*Caretta caretta* L.)

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Fusarium solani **was reported as the agent of a cutaneous infection in an injured sea turtle collected in the Mediterranean Sea. The turtle was treated with both a topical 10% solution of iodine in alcohol and ketoconazole. The source of the causal agent was traced to the sand in the tank in which the turtle was maintained. The strain was only sensitive in vitro to amphotericin B and was resistant to 5-fluorocytosine, fluconazole, itraconazole, and ketoconazole.**

Fusarium species are common soil saprophytes and plant pathogens. However, in recent years, they have been reported with increasing frequency as causes of opportunistic infections in humans and in animals (20), including reptiles (1, 6, 11), turtles (8, 17), and other marine animals (5, 13, 19). Among all of the references reviewed, only Rebell (17) mentioned infection of the shells and skin of baby marine turtles from Bimini (Bahamas) produced by *Fusarium solani* (Mart.) Sacc. No other fungal species have been mentioned as a cause of a cutaneous mycosis in turtles. In this paper, a cutaneous hyalohyphomycosis caused by *F. solani* in a subadult loggerhead sea turtle (*Caretta caretta* L.) collected in the Mediterranean Sea is reported.

In September 1996, a subadult (26.3-kg) loggerhead sea turtle (*Caretta caretta* L.) was found floating off the coast of Barcelona, Spain (Mediterranean Sea). The turtle had a fishing hook anchored in the proximal esophagus and a traumatic injury with important loss of shell tissues. The hook was removed by surgical procedures, and debridement of the necrotic shell tissues was carried out. Treatment consisted of supportive therapy and control of secondary infections (amoxicillin, 22 mg/kg of body weight, intramuscularly, once a day, for 8 days, doxycycline, 250 mg, orally once a day for 30 days). During the second month of rehabilitation, the turtle developed several white-scaled skin lesions that were 10 to 35 mm in diameter over the dorsal region of the neck and head (Fig. 1). Samples of skin scrapings of the skin lesions for routine microbial culturing and biopsy specimens were obtained. Histologic sections of biopsy material were stained with hematoxylin and eosin and periodic acid-Schiff stain (PAS). KOH-lactophenol-, hematoxylin and eosin-, and PAS-stained preparations revealed the presence of numerous hyaline septate hyphae in the keratin layers of the stratum corneum (Fig. 2).

Samples were inoculated on Sabouraud glucose agar supplemented with chloramphenicol, blood agar, and MacConkey agar. Cultures on Sabouraud glucose agar supplemented with chloramphenicol yielded numerous vinaceous fungal colonies in pure culture consistent with *Fusarium* sp. Culture of the fungal isolate on potato dextrose agar (14) and synthetic nutrient-poor agar (15) for identification generated bluish-green colonies which presented characteristic conidial structures. The micromorphology showed elongate monophialides bearing oval to kidney-shaped microconidia (Fig. 3). Macroconidia were abundant, stout, thick walled, and generally cylindrical, with dorsal and ventral surfaces parallel for most of their length (Fig. 4). The fungus was identified as *F. solani* according to the description by Nelson et al. (14). Bacteriological cultures yielded *Pseudomonas fluorescens* with the API 20E identification system (API, bioMérieux, Barcelona, Spain).

The turtle was first treated with a topical 10% solution of iodine in alcohol at accessible skin lesions and, afterwards, when the fungal infection was diagnosed, was treated simultaneously with a topical 10% solution of iodine in alcohol and topical ketoconazole. Subsequent susceptibility tests of the strain isolated were performed with antifungal tablets (Neo-

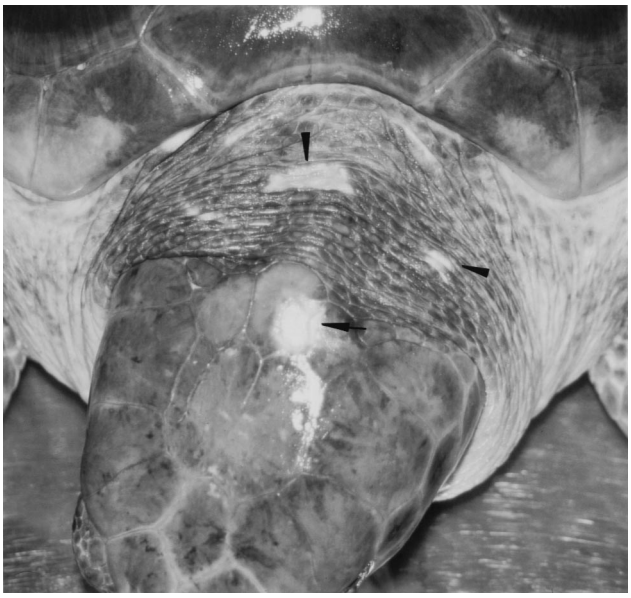

FIG. 1. Lesions on the dorsal regions of the neck and head.

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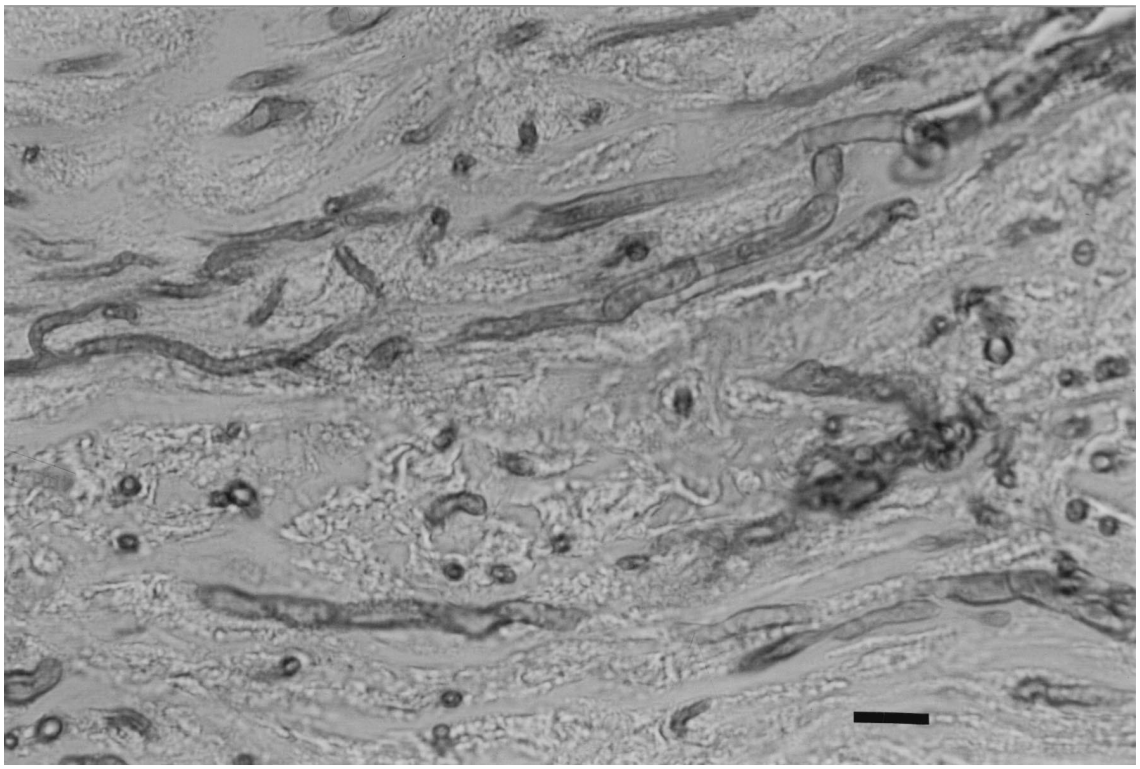

FIG. 2. PAS-stained section of a portion of the tissue biopsy sample (stratum corneum) showing hyphal elements. Bar, 10 μm.

Sensitabs; Rosco Diagnostica, Denmark) and Shadomy agar (2). The strain was sensitive to amphotericin B and was resistant to 5-fluorocytosine, fluconazole, itraconazole, and ketoconazole. The lesions regressed after 6 months of topical treatment with both the 10% solution of iodine in alcohol and ketoconazole.

Sand samples from the tank used to maintain the turtle were obtained for microbial culture with the aim of selectively isolating *Fusarium* spp. living in the habitat of the turtle. For this purpose, the sand samples were inoculated on malt extract agar supplemented with chloramphenicol and malachite green agar 2.5 supplemented with chloramphenicol, a new selective culture medium recently designed in our laboratory for *Fusarium* spp. (3). All of the inoculated plates for both culture media yielded growth of fungal colonies belonging to *Fusarium* sp., although in malt extract agar, colonies belonging to other genera, such as *Aspergillus* sp. and *Penicillium* sp., were isolated. Culture of all of the *Fusarium* isolates on potato dextrose agar and synthetic nutrient-poor agar for identification generated characteristic colonies with conidial structures belonging to *F. solani*.

The origin of this opportunistic infection may be related to the presence of *F. solani* in the tank and to the immunosuppressive state of the turtle due to the traumatic lesions suffered, the surgical treatment applied, and other stress conditions associated with transportation or rehabilitation of these marine animals, which may alter their immunocompetence, as happens in marine mammals (5).

F. solani, like other *Fusarium* spp., is considered to be cosmopolitan in distribution (14). However, in the mycological control sample of the sand of the tank used to maintain the turtle, *F. solani* was the only *Fusarium* species isolated. This species has been also found in beach sands (17), and it has been isolated from marine life as diverse as lobsters and shrimp (20), sharks (19), and gray seals (13). In human infection, a case of an invasive infection produced by *F. solani* associated with an injury by a stingray barb has been described (9).

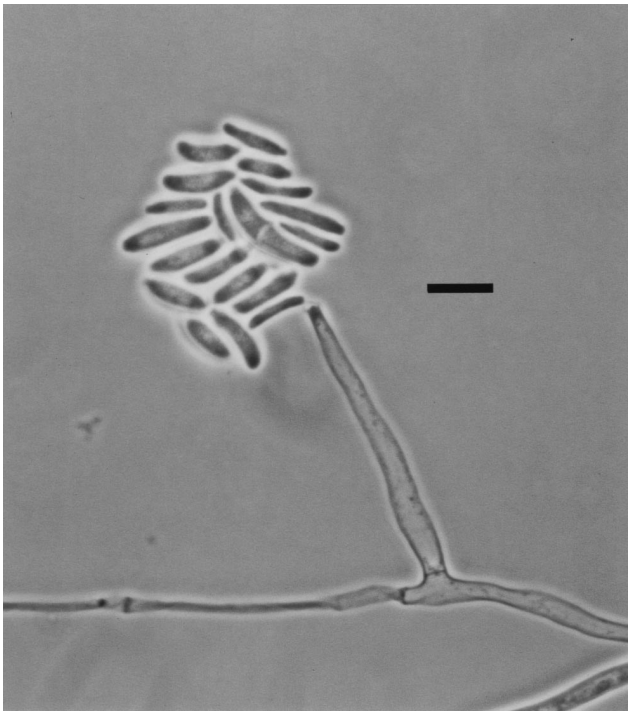

FIG. 3. Characteristic conidiogenous cell and microconidia in false heads of *F. solani*. Bar, 8 μm.

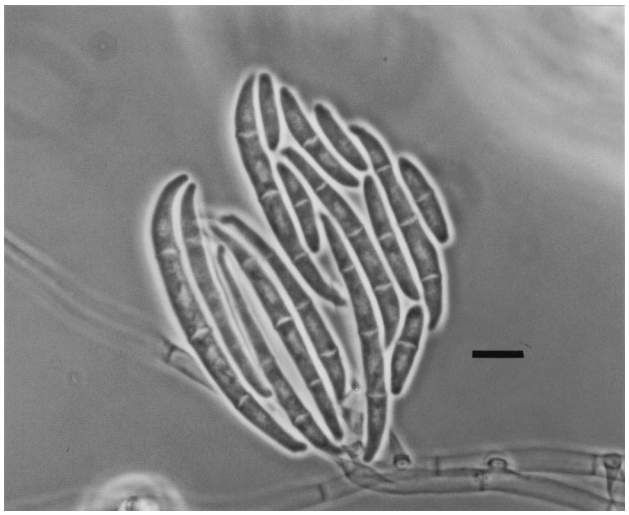

FIG. 4. Characteristic macroconidia of *F. solani*. Bar, 8 μm.

P. fluorescens and other bacteria have been isolated from skin lesions due to biting (traumatic ulcerative dermatitis) in farmed marine turtles. No fungal species was isolated from these lesions (7). Bacterial shell ulceration due to *Pseudomonas* sp. has been reported for a tortoise (10).

It is not easy to judge the efficiency of the topical ketoconazole treatment in the regression of the lesions of the animal studied in our report. There are several factors that are related to the nature of the animal studied. In effect, the special characteristics of the turtle's skin together with aquatic environmental conditions made the skin healing in these animals a slow process. On the other hand, hyalohyphomycosis infections due to *Fusarium* spp. are frequently refractory to antifungal therapy, particularly in granulocytopenic patients and in animal models. Experimental antifungal therapy with amphotericin B, fluconazole, and itraconazole reveals *Fusarium* spp. to be refractory to these compounds (12). *Fusarium* infections in other aquatic animals, such as California sea lions and gray seals, appeared to be refractory to topical as well as systemic antifungal treatment. In some cases, the regression of the lesions seemed to be seasonal and probably is not related to the therapy, being a self-remitting process (13).

Although in general the in vitro antifungal susceptibilities of the different pathogenic fungi can be a valuable guide for the practitioner, reliable antifungal susceptibility testing is still poorly developed, especially for filamentous fungi (16). Recently, some testing conditions have been proposed as guidelines for a reference broth microdilution method (4). Nevertheless, in vitro resistance to different antifungal agents, such as 5-fluorocytosine, ketoconazole, fluconazole, and itraconazole, by *Fusarium* spp. determined by different methods has been repeatedly mentioned (4, 16, 18).

Finally, in the two cited cases of cutaneous hyalohyphomycosis caused by *F. solani* in sea turtles, the source of infection was related to the tank or pool facilities: the water filter in one case (17) and the sand of the tank in our case. It would be advisable to microbiologically control and periodically clean up the water, filters, and/or sand of the tanks or basins used for maintaining these animals, especially when the animals are at a high risk of infection.

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