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FATAL CUTANEOUS MYCOSIS IN TENTACLED SNAKES (*ERPETON TENTACULATUM*) CAUSED BY THE *CHRYSOSPORIUM* ANAMORPH OF *NANNIZZIOPSIS VRIESII*

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Abstract: The fungus *Chrysosporium* anamorph of *Nannizziopsis vriesii* was identified as the cause of fatal, multifocal, heterophilic dermatitis in four freshwater aquatic captive-bred tentacled snakes (*Erpeton tentaculatum*). Pale, 1- to 4-mm focal lesions involving individual scales, occurred primarily on the head and dorsum. Histology showed multifocal coagulation necrosis of the epidermis, with marked heterophilic infiltration without involvement of the underlying dermis. Septate, irregularly branched hyphae, and clusters of 4- to 8- by 2- to 3-μm rod-shaped cells (arthroconidia) were present within the lesions and in a superficial crust. Failure to maintain an acidic environment was likely a predisposing factor in the development of these lesions.

Key words: *Chrysosporium* anamorph of *Nannizziopsis vriesii*, dermatitis, *Erpeton tentaculatum*, fatal dermatomycosis, fungus, tentacled snake.

INTRODUCTION

Dermatitis is the most frequently reported manifestation of fungal disease in reptiles.[12] The majority of reports concern snakes, and most of the fungi identified as causing cutaneous mycosis are opportunists of environmental origin.[4,12] Many of these fungi are considered part of the normal cutaneous microflora of captive reptiles.[10]

Several reports have implicated the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) as the cause of outbreaks of dermatitis in captive reptiles.[7,9,11] Brown crusty skin lesions have been described in three species of chameleons (*Chamaeleo*),[9] and fatal multifocal dermatitis has been seen in brown tree snakes (*Boiga irregularis*)[7] and saltwater crocodiles (*Crocodylus porosus*).[11]

The ascomycete *N. vriesii* (phylum Ascomycota, order Onygenales) was isolated originally from the skin and lung of *Ameiva* sp., a lizard.[1] Although the sexual (meiotic) stage and *Chrysosporium* mitotic stage (anamorph) of this fungus usually occur together, only the anamorph has been isolated from reptiles with lesions. The fungi described as *Chrysosporium* or *Trichophyton* in some reports of reptilian mycotic disease may, in fact, have been the CANV, a morphologically similar but less well-recognized organism.[9]

Previously reported reptiles infected with the CANV were either wild-caught,[7,9] hatchlings,[11] or involved in experimental trials,[7,11] suggesting that stress associated with capture and transport, crowding, or suboptimal temperature (or all) was a contributory factor. The CANV has been isolated from both terrestrial[7,9] and semiaquatic[11] reptiles. This report describes an outbreak of mycotic dermatitis in the fully aquatic tentacled snake (*Erpeton tentaculatum*), a freshwater aquatic species from Southeast Asia.[2]

CASE REPORTS

Four 10-mo-old captive-bred tentacled snakes were acquired from another zoo and maintained together within a 100-L fresh water aquarium equipped with an external filter in a quarantine facility at the Toronto Zoo. Temperature was maintained at 28°C and pH was 8.0–8.3. The snakes were fed live local baitfish three times weekly, and they ate well from a few days after arrival. The animals weighed 58.0, 63.5, 63.3, and 63.0 g, and each measured approximately 30 cm from snout to tail.

Two months after arrival, all four snakes developed multiple, small pale yellow-white skin lesions, particularly on the head and dorsum. Initially, the lesions appeared as 1- to 4-mm foci involving the tips of individual scales or, more often, entire scales. The lesions were swabbed and cultured on blood and MacConkey agar, yielding growth of several *Pseudomonas* species. Skin scrapings revealed assorted bacteria and sloughed keratino-

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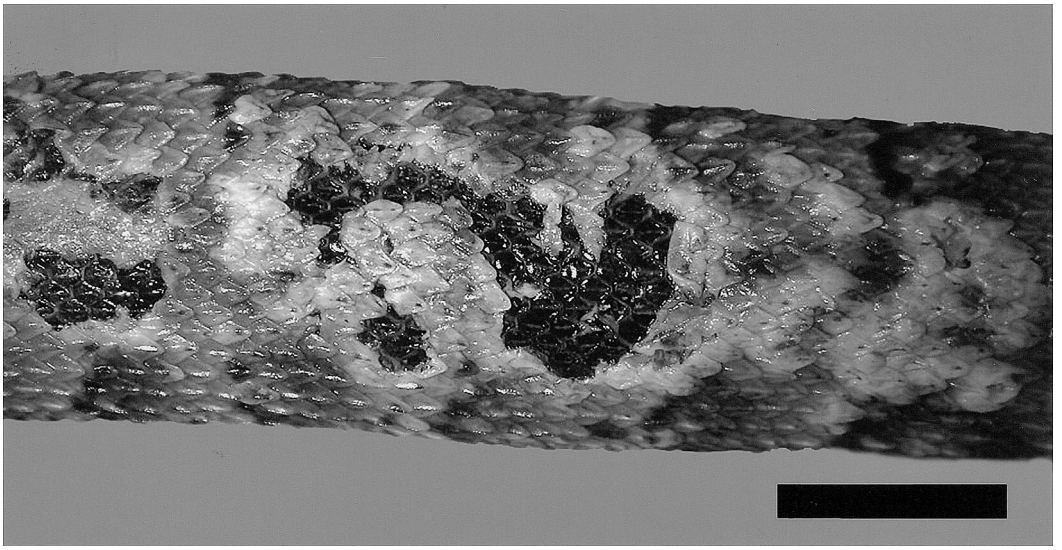

Figure 1. Gross appearance of the skin of a tentacled snake with cutaneous mycosis. Bar = 1 cm.

cytes. On the assumption that the lesions were bacterial in origin, the snakes were treated with ceftazidime (Fortaz, GlaxoSmithKline, Mississauga, Ontario, Canada L5N 6L4; 50 mg/kg, q 72 hr, s.c. for 2 wk), and the lesions were swabbed once daily with a povidone iodine solution (Betadine, Purdue Pharma, Pickering, Ontario, Canada L1W 3W8). The snakes shed frequently (approximately every 14 days), and although immediately after each ecdysis, the skin appeared normal, new lesions became visible 1–2 days later. Because the disease did not progress for 3 mo, no further treatment was attempted. Then, within a period of 14 days, the lesions increased in size and number and the snakes died, about 4 mo after the original onset of symptoms and 6 mo after their arrival.

The gross postmortem findings were similar in all snakes. The skin was dull gray with multiple, scattered to coalescing erosive yellow-white areas measuring up to 5 mm (Fig. 1). The superficial crust could be easily removed, exposing small foci of creamy exudate. The lesions did not extend below the epidermis. There was moderate subcutaneous edema. The jugular and hepatic veins were noticeably congested, and there was generalized edema, particularly in the cranial half of the body. Clear fluid was present in the celomic cavities, and three animals (snakes 1, 3, and 4) showed pronounced pulmonary edema with intratracheal fluid accumulation. In snakes 2 and 3, a few 20- to 40-mm tapeworms were present in the intestine. The livers of all the snakes were pale and friable, and the liver of snake 1 had a mottled appearance with multiple pale areas.

Representative samples of all major organs were collected in 10% buffered formalin, processed routinely for histopathology, sectioned at 6 μm, and stained with hematoxylin and eosin. Selected skin specimens were also stained with methenamine silver and periodic acid–Schiff.

Samples of skin, lung, heart, liver, kidney, and spleen were collected aseptically for aerobic bacterial culture. Additional skin samples were collected and frozen at −20°C. When fungal elements suggestive of the CANV were seen by histopathology, frozen skin samples from snakes 2, 3, and 4 were sent to the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, Canada for isolation and identification of the fungus. Skin specimens were dissected and plated onto biplates containing phytone yeast extract agar (PYE) in one half and mycosel agar (MYC) in the other (Difco, Becton, Dickinson and Co., Sparks, Maryland 21152, USA). Plates were incubated at 30°C and examined for growth every other day. White fungal colonies visible under a dissecting microscope and emanating from the skin sample were subcultured onto MYC. Fungal growth on skin samples heavily colonized with bacteria was purified by transferring some mycelium into acidified broth (24 hr) and then subculturing from broth onto MYC. Isolates were accessioned in the UAMH as 10199 (snake 3), 10200 (snake 4), and 10201 (snake 2). Colonial and microscopic features

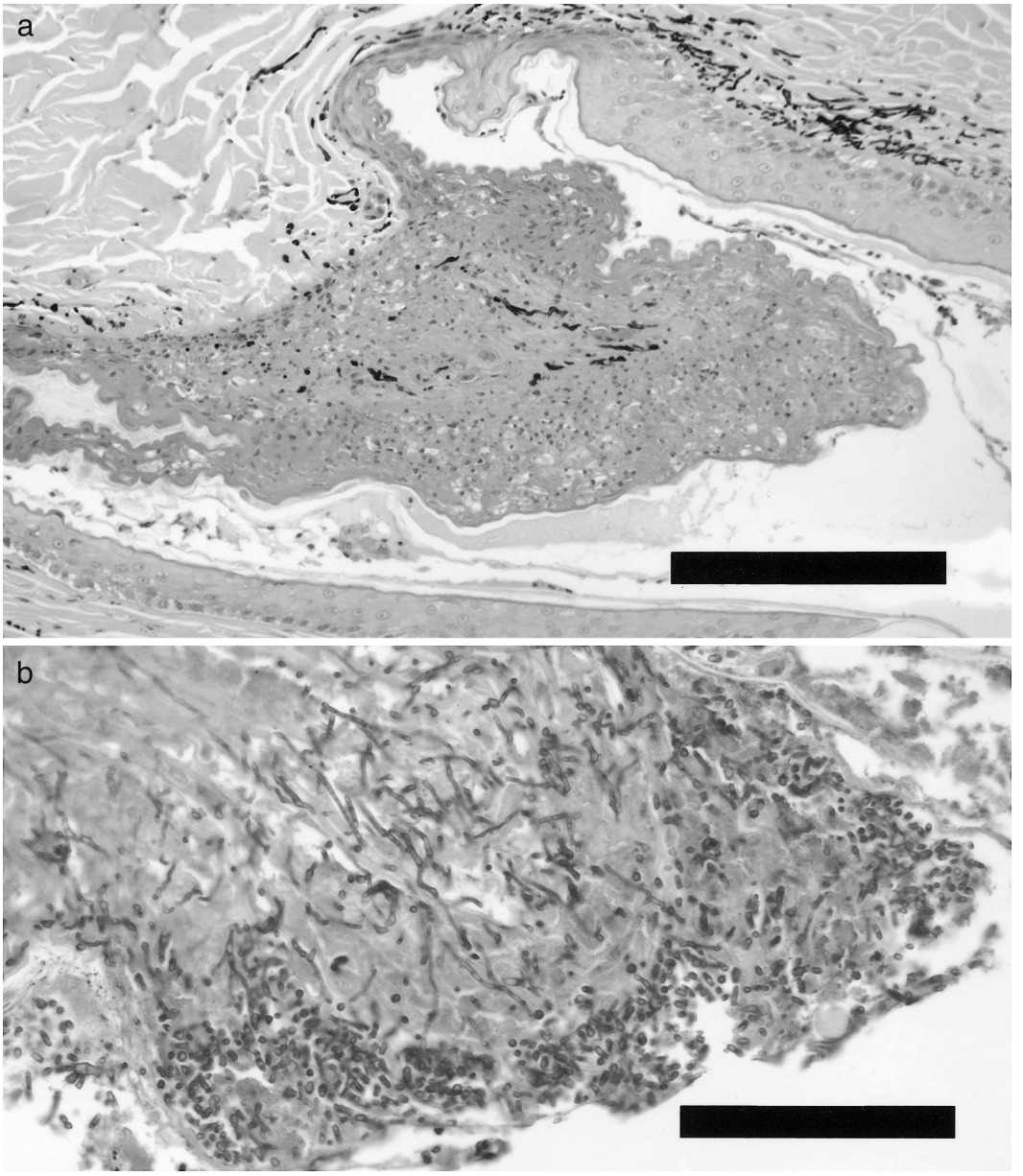

Figure 2. **a.** Photomicrograph of the skin of a tentacled snake showing full-thickness coagulation necrosis of the epidermis with marked heterophilic infiltration. H&E. Bar = 250 μm. **b.** Detail showing the crust composed of serum, cellular debris, and clusters of arthroconidia. Note the fungal hyphae of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* deeper within the lesion. PAS. Bar = 150 μm.

were examined on potato dextrose agar (PDA; Difco), PYE and MYC, oatmeal salts agar (OAT),[6] and in slide culture preparations[6] at 30°C. Ability to grow at 35°C was assessed on PDA. Physiologic characteristics were evaluated on bromcresol purple–milk solids–glucose agar (BCP-MS-G, recipe[6]) and Christensen urea broth (Difco) as described previously.[7,10,11]

Histologic findings were similar in all cases. In the skin, there were multiple foci of necrosis and inflammation scattered throughout the sections (Fig. 2a). Larger 1- to 2-mm lesions had full-thickness coagulation necrosis of the epidermis with marked heterophilic infiltration, whereas smaller 20- to 40-μm lesions were confined to the superficial layers. The underlying dermis showed minimal

inflammation. On the surface of most ulcers was a crust composed of serum, cellular debris, and clusters of 4- to 8- by 2- to 3- μm arthroconidia. Fungal hyphae occurred within the deeper lesions (Fig. 2b). Hyphae were septate, irregularly branched, and 2–3 μm wide. In unaffected areas, there was moderate vacuolization of the epidermis. Snake 4 was completing a shed, and in some areas the necrotic lesions appeared confined to the external layer, whereas the underlying growing epidermis was proliferative and thickened.

Various lesions were identified in the internal organs of all snakes, but no fungal hyphae were present. Livers showed moderate, diffuse macrovesicular hepatic lipidosis. Snake 1 also had several irregular foci of mild biliary proliferation and mononuclear infiltration randomly scattered through the liver. Snake 3 had scattered foci of intrahepatic granulopoiesis and minimal scattered heterophilic myocarditis. Kidneys showed moderate interstitial edema and mild thickening of the glomerular basement membranes. The pancreatic portions of the splenopancreata were practically devoid of zymogen, and the splenic portion of snakes 1, 2, and 4 showed scattered extramedullary granulopoiesis. The lungs of snakes 1, 3, and 4 were edematous and showed mild heterophilic infiltration. Snake 3 had a 120-μm thin-walled granuloma within the intestinal wall containing a larval cestode characterized by an inverted protoscolex. Snake 2 had a single 100-μm loosely arranged granuloma of unknown origin in the gastric tunica muscularis.

Standard aerobic bacterial culture was conducted on samples of skin, lung, heart, liver, spleen, and kidney. High numbers of one or more of *Aeromonas* sp., *Pseudomonas putrefaciens*, and *Staphylococcus aureus* were cultured from the external skin surface of all snakes, but swabs from the inner aspect of the skin of snakes 3 and 4 grew no bacteria. Lower numbers of the same bacterial species and *Escherichia coli* were cultured from lung, liver, and kidney of snakes 1 and 3, whereas cultures from snakes 2 and 4 yielded no significant growth.

The fungus identified as the CANV was cultured from the three referred skin samples but only from samples plated onto MYC. On PYE, there was overgrowth by the rapidly growing contaminant *Trichoderma* sp. Growth of CANV was inhibited when skin was heavily colonized with bacteria, but subculture into acidified broth successfully eliminated the bacteria. When subcultured onto PDA at 30°C, colonies reached diameters of 6.5–7 cm in 21 days and were yellowish-white, powdery and flat, or raised centrally because of downy overgrowth. There was no growth at 35°C. Reactions on BCP-MS-G after 10–13 days included moderate to profuse growth, no pH change, and clearing of milk solids behind the colony. Urease was weakly positive (pale pink) in 4–7 days and strongly positive (fuchsia) by 10 days. Isolates UAMH 10200 and 10201 grown on OAT were powdery with patches of downy overgrowth (Fig. 3). Microscopic examination of two areas revealed a preponderance of aleurioconidia in the powdery sector (''A'') and of arthroconidia in the downy sector (''B''). Colonies subcultured as A and B elicited slightly different physiologic reactions when retested. The aleurioconidial A type was strongly urease positive and showed strong clearing of the milk solids on BCP-MS-G after 13 days, whereas the arthroconidial B type was weakly urease positive and showed less clearing of the milk solids.

Microscopic examination of slide culture preparations revealed pyriform conidia (aleurioconidia) formed at the ends of short stalks, or sessile, i.e., produced directly on the sides of the hyphae (Fig. 4). Conidia were 4–8 μm long by 2–3.5 m wide. Hyphae also fragmented to form intercalary arthroconidia measuring 4–8 μm long by 2–3.5 μm wide. No teleomorph (meiotic stage) was formed.

DISCUSSION

The CANV was confirmed as the etiologic agent of dermatitis in the tentacled snakes on the basis of the histologic findings of necrotic lesions infiltrated by hyaline, septate, branching hyphae, the presence of characteristic arthroconidia in the epidermis and in crusts above the ulcers, and by the isolation of the same fungus from skin samples of the three snakes cultured. The bacterial isolates are considered to constitute secondary infection, with bacterial septicemia probably constituting the immediate cause of death in snake 3. However, osmotic imbalance associated with cutaneous damage caused by the fungus was the postulated cause of death in the remaining snakes. Integrity of the integument is of paramount importance to aquatic species, and epidermal defects may have led to passive influx of water causing edema, anasarca, and ultimately death. This mechanism was proposed as the cause of death in hatchling Florida softshell turtles affected by ulcerative epidermitis caused by a *Mucor* sp.[5]

The source of the CANV exposure for reptiles and the factors contributing to onset of infection in reptiles are not yet clear,[7,9,11] although the organism has been recently demonstrated as a primary pathogen for reptiles under experimental conditions.[8] The CANV appears to have a low prevalence in the environment of captive reptiles as judged by its iso-

Figure 3. Gross appearance of fungal growth from UAMH 10200 (snake 4) on oatmeal salts agar after 35 days at 30°C showing powdery colonies overlaid with tufts of downy mycelium.

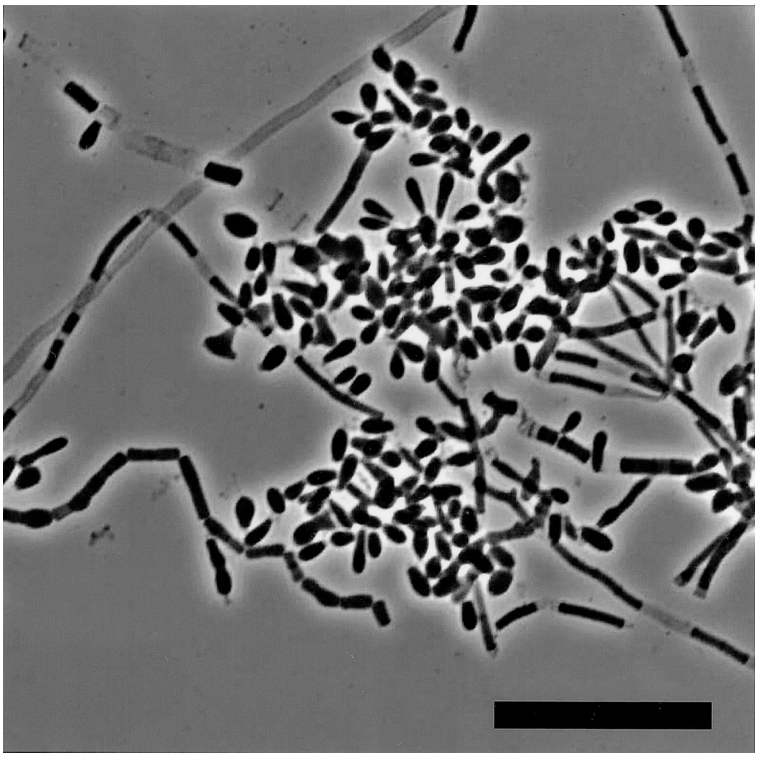

Figure 4. Photomicrograph showing the *Chrysosporium* anamorph of *Nannizziopsis vriesii* from slide culture preparations. Note pyriform conidia (aleurioconidia) formed at the ends of short stalks. Bar = 40 μm.

lation only once from samples of freshly shed skin from 127 healthy captive squamates.[10] Cultures of exuvia collected aseptically from animals in 42 institutions yielded 742 fungal isolates belonging to 50 genera. The most prevalent fungi were those known as common environmental saprophytes including *Aspergillus* spp., *Penicillium* spp., *Paecilomyces lilacinus*, and members of the zygomycetes (*Mucor*, *Syncephalastrum*, etc.). In the cases in this study, the tank and the furnishings were disinfected with 1% bleach before the arrival of the snakes. Apart from the fish they were fed, the snakes had no contact with organic material or other animals.

In two previous outbreaks, the clinical progression of the disease was relatively short compared with the protracted course in these tentacled snakes. Four brown tree snakes died 3–14 days after the onset of clinical signs,[7] and a group of 48 hatchling saltwater crocodiles succumbed 14–23 days after lesions were first noted.[11] In both instances, the affected areas of skin were treated topically with iodine-based antiseptics.[7,11] Although there was no noticeable improvement in the tree snakes, there was a marked reduction in mortality in the hatchling crocodiles, which were also treated with dilute formalin baths. In the cases in this study, the topical iodine treatment, in combination with the very frequent shedding, appears to have slowed the progression of the infection and postponed the snakes' deaths. On the basis of impression smears made from lesions in these snakes, fungal disease was not suspected. Skin biopsies possibly would have allowed for in vivo diagnosis and more specific treatment.

Aquatic snakes can be challenging to manage in captivity; water temperature, hardness, pH, and ammonia levels are critical factors. Because wild tentacled snakes inhabit slow-moving acidic streams,[2] maintaining water at pH 6–6.5 has been recommended for captives.[2,3] The affected snakes were housed in water of pH $\geq$ 8, which may have predisposed them to infection. No cases of fungal dermatitis have been seen in another group of snakes kept under similar conditions but in water of pH $<$ 7.

The CANV has been recognized as a potential pathogen of both terrestrial and semiaquatic reptiles. This report demonstrates its significance in a fully aquatic species and emphasizes the need for further studies regarding the taxonomy and pathogenicity of this fungus and for prevention of infection. Although reptile isolates of the CANV have been considered to belong to a single species on the basis of morphologic similarities, molecular work in progress suggests that the CANV appears to represent a species complex and its members may be allied to specific hosts (Sigler, unpubl. data). The isolates from these tentacled snakes differed from those isolated previously from reptiles by growing faster at 30°C, demonstrating a greater prevalence of intercalary arthroconidia, and producing few undulate lateral hyphae.

LITERATURE CITED

1. Apinis, A. E. 1970. Concerning *Rollandia*. Trans. Br. Mycol. Soc. 55: 499–502.

2. Coborn, J. 1991. The Atlas of Snakes of the World. T.F.H. Publications, Neptune, New Jersey.

3. Cox, M. 1991. The Snakes of Thailand and Their Husbandry. Krieger Publishing, Malabar, Florida.

4. Jacobson, E. R. 1980. Necrotizing mycotic dermatitis in snakes: clinical and pathologic features. J. Am. Vet. Med. Assoc. 177: 838–841.

5. Jacobson, E. R., M. B. Calderwood, and S. L. Clubb. 1980. Mucormycosis in hatchling Florida softshell turtles. J. Am. Vet. Med. Assoc. 177: 835–837.

6. Kane, J., R. C. Summerbell, L. Sigler, S. Krajden, and G. Land. 1997. Laboratory Handbook of Dermatophytes. A Clinical Guide and Laboratory Manual of Dermatophytes and Other Filamentous Fungi from Skin, Hair and Nails. Star Publishing Co., Belmont, California.

7. Nichols, D. K., R. S. Weyant, E. W. Lamirande, L. Sigler, and R. T. Mason. 1999. Fatal mycotic dermatitis in captive brown tree snakes (*Boiga irregularis*). J. Zoo Wildl. Med. 30: 111–118.

8. Paré, J. A., K. Coyle, L. Sigler, A. K. Maas, and R. Mitchell. 2003. Pathogenicity of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* for veiled chameleons (*Chamaeleo calyptratus*). Proc. Assoc. Rept. Amphib. Vet. 2003: 9–10.

9. Paré, J. A., L. Sigler, D. B. Hunter, R. C. Summerbell, D. A. Smith, and K. L. Machin. 1997. Cutaneous mycoses in chameleons caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (Apinis) Currah. J. Zoo Wildl. Med. 28: 443–453.

10. Paré, J. A., L. Sigler, K. L. Rypien, and C. F. C. Gibas. 2003. Cutaneous mycobiota of captive squamate reptiles with notes on the scarcity of *Chrysosporium* anamorph of *Nannizziopsis vriesii*. J. Herpetol. Med. Surg. 13: 10–15.

11. Thomas, A. D., L. Sigler, S. Peucker, J. H. Norton, and A. Neilan. 2001. *Chrysosporium* anamorph of *Nannizziopsis vriesii* associated with fatal cutaneous mycoses in the salt-water crocodile (*Crocodylus porosus*). Med. Mycol. 40: 143–151.

12. Zwart, P., and H. D. Schröder. 1985. Mykosen. *In:* Ippen, R., H. D. Schröder, and K. Elze (eds.). Handbuch der Zootierkrankheiten, vol. 1. Akademie Verlag, Berlin, Germany. Pp. 349–364.

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