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Survey for the *Chrysosporium* Anamorph of *Nannizziopsis vriesii* on the Skin of Healthy Captive Squamate Reptiles and Notes on their Cutaneous Fungal Mycobiota

Jean A. Paré[1], DMV, DVSc, DACZM, Lynne Sigler[2], MSc
Krystal L. Rypien[2,3], BSc, Connie-Fe C. Gibas[2], MSc

1. Department of Surgical Sciences, School of Veterinary Medicine
University of Wisconsin, 2015 Linden Drive West, Madison, WI 53706, USA

2. University of Alberta Microfungus Collection and Herbarium
Devonian Botanic Garden, Edmonton, Alberta, T6G 2E1, Canada

3. Krystal L. Rypienís present address: Department of Ecology and Evolutionary Biology
Corson Hall, Cornell University, Ithaca, NY 14853-2701, USA

Abstract: The *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) is a fungus that has been implicated in several recent cases of reptile dermatomycoses. A survey was conducted to investigate whether this fungus was present on the skins of healthy squamate reptiles. Skin was collected as aseptically as possible from actively shedding lizards (n = 36) or from freshly shed snake exuvia (n = 91) and placed on fungal culture media for selective recovery of cycloheximide-tolerant fungi. The CANV was cultured from only one animal, an African rock python, *Python sebae*. Fungi belonging to 50 genera were identified from 127 reptiles: *Aspergillus* spp., *Penicillium* spp., and *Paecilomyces lilacinus* were most frequently isolated. Keratinophilic fungi isolated from reptiles did not belong to zoophilic or anthropophilic species, inferring that the potential for acquisition of dermatophytosis from handling squamate reptiles is low.

Key words: reptiles, fungi, mycobiota, mycoses, *Chrysosporium* anamorph of *Nannizziopsis vriesii*, *Paecilomyces lilacinus*.

Introduction

The *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) is a fungus that has been implicated in several outbreaks of skin disease in reptiles (Paré, *et al*, 1997, Nichols, *et al*, 1999, Thomas, *et al*, 2002). In addition to published cases, this fungus has been recovered on day geckoes, *Phelsuma* sp., a ball python, *Python regius*, a corn snake, *Elaphe guttata*, a milk snake, *Lampropeltis triangulum*, and a garter snake, *Thamnophis* sp., all with skin lesions (Sigler and Paré, unpublished data). The source of the fungus has not been clearly identified (Paré, *et al*, 1997, Nichols, *et al*, 1999, Thomas, *et al*, 2002). Whether the CANV is a common environmental fungus, or part of the normal reptile skin microflora is unknown. We solicited samples of skin of healthy squamate reptiles from zoological and veterinary institutions to determine the prevalence of the CANV on the integument of healthy captive reptiles.

Materials and Methods

In October 2000, sample submission request packages were forwarded to 183 institutions located in the United States and Canada. Solicited institutions included North American veterinary schools that offer an exotic animal clinical service, all American Zoo Association-accredited zoological facilities that held more than ten reptiles in their collections, and randomly selected private veterinary clinics listed in the Association of Reptilian and Amphibian Veterinarians directory. Each package included a cover letter, sampling instructions, a sample submission data sheet, and six precut waxed paper envelopes for shipment of samples. Each institution was asked to submit two 2.5 x 2.5 cm samples of shed skin from three different healthy-looking squamate reptiles, ideally from distinct enclosures. We defined healthy-looking as free of cutaneous lesions or any obvious disease, and in good body condition. Samples were to be collected as aseptically as possible from an actively shedding lizard or snake, or from a freshly (overnight) shed snake exuvium. Samples were returned to the University of Wisconsin in Madison (UW), Wisconsin, USA, where one was retained, while the duplicate sample collected from the same animal was forwarded to the University of Alberta in Edmonton (UA), Alberta, Canada. Packages returned after June 1, 2001 were not analyzed. Samples were set up for fungal culture at both facilities using the same protocol, and results of culture were pooled. Skin samples were cut into six small sections using sterile forceps and scissors, and these were placed onto the surface of

Mycosel® agar (Mycosel Agar, Becton Dickinson and Company, Cockeysville, MD) containing chloramphenicol (0.0005%), and cycloheximide (0.04%). Medium containing cycloheximide is used routinely to selectively isolate cycloheximide-tolerant dermatophytes, *Chrysosporium* species, and related fungi within the ascomycete order Onygenales, and to inhibit more rapidly growing saprotrophic fungi (Kane, *et al*, 1997). Plates were incubated at 28°C (82.4°F), and were observed over a period of five weeks for fungal colonies. A fungus was recorded as present if it appeared on any of the six pieces of skin cultured. The Fleiss quadratic 95% confidence interval was calculated for each prevalence value.

Fungi were identified on the basis of colonial features and on microscopic observation of conidiation and fruiting bodies (Kane, *et al*, 1997). The main objective was to determine the presence of the CANV, for which the colonial and microscopic characteristics have been described (Paré, *et al*, 1997, Nichols, *et al*, 1999, Thomas, *et al*, 2002). *Nannizziopsis vriesii* is a member of the ascomycetes (order Onygenales) in which initial growth in culture is that of the *Chrysosporium* anamorph (asexual stage). Colonies are white and powdery, with a pale to yellowish reverse, and the hyphae produce small club-shaped conidia and arthroconidia. Isolates demonstrating similar characteristics, including all *Chrysosporium* and dermatophyte-like fungi, were subcultured and identified to species. Other fungi were identified at least to genus and often to species. A representative isolate of each keratinophilic onygenalean fungus (*Chrysosporium*, *Malbranchea*, and *Microsporum* species), and selected other isolates were deposited in the University of Alberta Microfungus Collection and Herbarium (UAMH).

Results

Of the 183 solicited institutions, 42 returned skin samples before the deadline, for a return rate of 23%. Results are based on skin samples from 36 lizards in 20 species encompassing eight families, and 91 snakes belonging to 39 species in seven families (Table 1).

From the 127 skin samples (254 culture plates), 742 fungal isolates belonging to 50 genera were identified (Table 2). Thirty-three *Chrysosporium* isolates were obtained from 31 different reptiles (26%). Only one *Chrysosporium* isolate, from an African rock python (sample UW101B), met all the CANV criteria and was deposited as UAMH 9985. Twenty-two isolates were *C. zonatum*. Other *Chrysosporium* isolates included the *C. anamorph* of *Aphanoascus fulvescens* (four animals), *C. evolceanui* (three animals), *C. keratinophilum* (two animals), and the *C.* anamorph of *Arthroderma tuberculatum* (one animal). The only dermatophytes isolated were *Microsporum gypseum* and *M. boullardii*, from one and three reptiles respectively.

Next to the prevalent *Penicillium* (78%) and *Aspergillus* (69%) species, the zygomycetes were the most commonly occurring fungi (57%): *Syncephalastrum racemosum* and *Mucor* species were found on 19 and 18% of samples, respectively, while *Rhizopus* and *Cunninghamella* were recovered from 8 and 4% of samples. *Paecilomyces lilacinus* occurred on 24% of samples. *Trichosporon* species were found on 11% of samples, and three of the isolates were identified as *T. asahii.*

Discussion

A single isolate of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (UAMH 9985) was recovered from 127 squamate reptiles, for a total prevalence of 0.8%, inferring that this fungus is not a common constituent of the cutaneous microflora of healthy captive squamate reptiles. This isolate was obtained from scales of the dorsal aspect of an overnight exuvium of a juvenile African rock python. The snake had recently been purchased from a dealer by a southwestern zoological institution, and was being held in quarantine. Newspaper was used for substrate in the enclosure. At the time of collection of the skin specimens, the python appeared healthy, but it failed to adapt, ate poorly, and died four months after arrival. Necropsy findings were consistent with starvation, and there was no evidence of infectious disease on histopathology. Isolate UAMH 9985 is the first CANV isolate obtained from a reptile not demonstrating cutaneous lesions (Sigler and Flis, 1998, Sigler and Paré, unpublished data). All other known isolates of the CANV have come from reptiles demonstrating severe, often fatal dermatomycosis (Paré, *et al*, 1997, Nichols, *et al*, 1999, Thomas, *et al*, 2002, Sigler and Paré, unpublished data). The results of this study suggest that there is a very low prevalence of the CANV on the skins of healthy captive squamate reptiles. In contrast, results document a high prevalence of common environmental fungi (e.g., *Aspergillus*, *Penicillium*, *Paecilomyces*, zygomycetes, *Trichosporon*) on the reptile integument and many of these fungi have been incriminated as occasional agents of opportunistic dermatomycoses in reptiles. Further studies are needed to determine the source of exposure to the CANV and the factors that contribute to onset of infection.

Species of *Chrysosporium* and *Malbranchea* are regularly encountered in surveys of fungi recovered from animal skin and hair (Carmichael, 1976, Kane, *et al*, 1997, Sigler and Sigler and Flis, 1998). *Chrysosporium zonatum*, the most prevalent *Chrysosporium* species in this survey, was first described in 1989 from soil in Northeast Africa (Al-Musallam and Tan, 1989), and has since been isolated from soils, dung, sewage sludge, river sediments, and a lesion on poultry in Asia, Europe, and southern North America (Sigler, *et al*, 1998). In humans, *C. zonatum* caused disseminated infection in a boy with chronic granulomatous disease (Roilides, *et al*, 1999) and non-invasive pulmonary disease in an adult male (Hayashi, *et al*, 2002). The high recovery rate of *C. zonatum* from reptile skin in comparison to other chrysosporia was unexpected but this fungus is thermotolerant and grows over a wide range of temperatures. It may therefore survive and proliferate in the captive environment of reptiles since a thermal gradient is typically provided. *Aphanoascus fulvescens* is an uncommon agent of dermatomycosis in animals and humans (Kane, *et al*, 1997, de Hoog, *et al*, 2000). *Chrysosporium queenslandicum*, recently reported as the cause of a disseminated infection in a garter snake, *Thamnophis* sp., (Vissiennon, *et al*, 1999), was not recovered. The *Malbranchea* species isolated from reptile skins are chiefly known from soil, animal hair, and bird feathers and nests (Sigler and Carmichael, 1976). The *Malbranchea* anamorph of *Uncinocarpus reesii* and *Malbranchea chrysosporoidea* have been cultured from rodent lungs, but without evidence of infection (Sigler and Carmichael, 1976). *M. aurantiaca* has

Species		Number
LACERTILIA		
Agamidae:	*Chlamydosaurus kingii*, frilled lizard	1
	Pogona vitticeps, Inland bearded dragon	2
Anguidae:	*Ophiosaurus apodus*, European glass lizard or sheltopusik	1
Chamaeleonidae:	*Chamaeleo calyptratus*, Yemenese or veiled chameleon	1
	Furcifer pardalis, panther chameleon	1
Gekkonidae:	*Eublepharis macularius*, leopard gecko	3
	Phelsuma madagascariensis grandis, giant Madagascar day gecko	2
	Uroplatus henkeli, Henkel's leaf-tailed gecko	1
Helodermatidae:	*Heloderma horridum*, Mexican beaded lizard	2
	Heloderma suspectum, Gila monster	1
Iguanidae:	*Basiliscus plumifrons*, plumed or green basilisk	1
	Brachylophus fasciatus, Fiji banded iguana	1
	Crotaphytus collaris, collared lizard	1
	Cyclura nubila lewisi, Grand Cayman iguana	1
	Iguana iguana, green iguana	8
Scincidae:	*Corucia zebrata*, Solomon Island skink	3
	Tiliqua rugosus, shingleback skink	1
	Tiliqua scincoides, Eastern blue-tongued skink	2
Varanidae:	*Varanus exanthematicus*, savannah monitor	2
	Varanus griseus, desert monitor	1
OPHIDIA		
Boidae:	*Acrantophis dumerili*, Dumeril's Madagascan boa	1
	Boa constrictor, red-tailed boa or boa constrictor	7
	Boa constrictor ortoni, Peruvian red-tailed boa or boa constrictor	1
	Bothrochilus boa, Bismarck ringed python	2
	Corallus caninus, emerald tree boa	2
	Corallus enydris, garden tree boa	1
	Epicrates cenchria, rainbow boa	3
	Epicrates cenchria alvarezi, Argentinian rainbow boa	1
	Epicrates cenchria cenchria, Brazilian rainbow boa	1
	Eunectes notaeus, yellow anaconda	1
	Liasis mackloti savuensis, Savu Island python	1
	Lichanura trivirgata, rosy boa	1
	Lichanura trivirgata gracia, desert rosy boa	2
	Morelia spilota cheynei, jungle carpet python	1
	Morelia viridis, green tree python	1
	Python curtus brongersmai, red blood python	1
	Python molurus bivittatus, Burmese python	2
	Python regius, royal or ball python	3
	Python sebae, African rock python	3
	Sanzinia madagascariensis, Madagascar tree boa	1
Colubridae:	*Drymarchon corais couperi*, Eastern indigo snake	2
	Elaphe guttata, corn snake	3
	Elaphe guttata emoryi, Great Plains rat snake	1
	Elaphe guttata guttata, corn snake	7
	Elaphe obsoleta spiloides, gray rat snake	1
	Lampropeltis getulus californiae, California kingsnake	2
	Lampropeltis getulus getulus, Eastern kingsnake	1
	Lampropeltis getulus goini, Goin's kingsnake	1
	Lampropeltis mexicana thayeri, variable kingsnake	1
	Lampropeltis triangulum amaura, Louisiana milksnake	1
	Lampropeltis triangulum annulata, Mexican milksnake	1
	Lampropeltis triangulum hondurensis, Honduran milksnake	3
	Lampropeltis triangulum sinaloae, Sinaloan milksnake	1
	Lampropeltis triangulum elapsoides, scarlet kingsnake	2
	Lampropeltis triangulum stuarti, Stuart's milksnake	1
	Lampropeltis triangulum triangulum, Eastern milksnake	1
	Lamprophis aurora, Aurora house snake	1
	Pituophis melanoleucus affinis, Sonoran gopher snake	1

Table 1. Taxa of reptiles from which skin samples were submitted for survey of mycobiota .

been isolated from lizard dung (Sigler and Carmichael, 1976, Sigler and Flis, 1998). *M. filamentosa*, an uncommon species recorded previously only from soil in Argentina and Africa, was recovered from a twin-spotted rattlesnake, *Crotalus pricei*, and a frilled lizard, *Chlamydosaurus kingii*, in this survey. Further study showed that *M. filamentosa* is the anamorph of a newly described species of *Auxarthron* (Sigler, *et al*, 2003). The only dermatophytes recovered were *Microsporum boullardii* and *M. gypseum*, both considered geophilic species (Kane, *et al*, 1997). *Microsporum boullardii* is uncommonly recorded in the literature. First isolated from soil in Guinea, and characteristically associated with the African continent (de Hoog, *et al*, 2000), it was recovered in this survey from three North American species, a Southern copperhead, *Agkistrodon contortrix contortrix*, a timber rattlesnake, *Crotalus horridus*, and a collared lizard, *Crotaphytus collaris*, likely all wild-caught specimens. *Microsporum gypseum* was cultured from the skin of a frilled lizard. This dermatophyte occasionally causes dermatophytosis in rodents, cats, dogs, and rarely in humans (Kane, *et al*, 1997, de Hoog, *et al*, 2000). This survey failed to identify any *Trichophyton* species or any zoophilic or anthropophilic dermatophyte from healthy captive squamate reptiles. This provides some basis to suggest that the risk of acquiring dermatophytosis from handling of reptiles is minimal.

Because our objective was to recover *Chrysosporium* species, and the CANV in particular, we used an isolation medium containing cycloheximide for selective recovery of cycloheximide-tolerant fungi. While many saprotrophic fungi are strongly inhibited on media containing this compound, their growth is not entirely suppressed, and their recovery provided some insight as to the normal cutaneous fungal microflora of healthy reptiles. The most prevalent fungal genera were *Penicillium, Aspergillus,* and *Paecilomyces*. Species within each of these genera have been identified previously in the literature as occasional causes of cutaneous mycosis in reptiles (Austwick and Keymer, 1981, Migaki, *et al*, 1984, Tappe, *et al*, 1984, Heard, *et al*, 1986, Maslen, *et al*, 1988, Schildger, *et al*, 1991, Cork and Stockdale, 1994, Cheatwood, *et al*, 1999, Martinez-Silvestre and Galan, 1999, Jacobson, *et al*, 2000). *Paecilomyces lilacinus*, the predominant *Paecilomyces* species cultured in this survey, has been incriminated in several cases of systemic mycosis in squamate reptiles (Austwick and Keymer, 1981, Schildger, *et al*, 1991), in chelonians (Herad, *et al*, 1986) and particularly in crocodilians (Austwick and Keymer, 1981, Maslen, *et al*, 1988). It was strongly tolerant of cycloheximide, and grew particularly profusely from skin samples of all three banded sea kraits, *Laticauda colubrina*. *Paecilomyces lilacinus* is a common contaminant in the warm and humid environment of crocodile pens (Thomas, *et al*, 2002) and along with some *Fusarium* species, is especially prevalent in aquatic habitats where fatty meats are used for food sources (Thomas, *et al*, 2002).

Zygomycetes (e.g., *Mucor*, *Syncephalastrum*, *Rhizopus*, *Cunninghamella*), occasional agents of zygomycosis (Austwick and Keymer, 1981, de Hoog, *et al*, 2000, Jacobson, *et al*, 2000), were isolated with regularity. The high prevalence of *Syncephalastrum racemosum*, from 18% of samples,

Table 2. Frequency of isolation on Mycosel agar of fungal genera obtained from actively or freshly shed skin samples of 127 squamate reptiles.

Genera	Frequency of isolation (%), (95% C.I. %)
Penicillium:	99 (78%), (69.4-84.6)
Aspergillus including *Emericella* teleomorph:	87 (69%), (59.6-76.3)
Paecilomyces:	44 (35%), (26.6-43.7)
(*P. lilacinus*)	30 (24%), (16.7-32.1)
Chrysosporium:	33 (26%), (18.8-34.7)
(*C. zonatum*)	22 (17%), (11.4-25.3)
(*C.* anamorph of *Aphanoascus fulvescens*)	4 (3%), (1.0-8.4)
(*C. evolceanui*)	3 (2%), (0.6-7.3)
(*C. keratinophilum*)	2 (1.6%), (0.3-6.1)
(*C.* anamorph of *Arthroderma tuberculatum*)	1 (0.8%), (0.04-5.0)
(*C.* anamorph of *Nannizziopsis vriesii*)	1 (0.8%), (0.04-5.0)
Zygomycota:	73 (57%), (48.4-66.1)
Mucor	24 (19%) (12.7-27.0)
Syncephalastrum racemosum	23 (18%) (12.1-26.1)
Rhizopus	10 (8%) (4.1-14.4)
Cunninghamella	5 (4%) (1.5-9.4)
Other (*Absidia, Rhizomucor* and unidentified)	6 (4%) (1.9-10.4)
Scopulariopsis including *Microascus* teleomorph:	29 (23%) (16.1-31.3)
Chaetomium:	19 (15%) (9.5-22.6)
(*C. globosum*)	16 (13%) (7.6-19.9)
Cladosporium:	18 (14%) (8.8-21.7)
Acremonium:	16 (13%) (7.6-19.9)
Malbranchea:	15 (12%) (7.0-19.0)
(*M. aurantiaca*)	5 (4%) (1.5-9.4)
(*M.* anamorph of *Uncinocarpus reesii*)	4 (3%) (1.0-8.4)
(*M. filamentosa*)	2 (1.6%) (0.3-6.1)
(*M. chrysosporoidea*)	2 (1.6%) (0.3-6.1)
Others (*M. sclerotica, M.* sp.)	2 (1.6%) (0.3-6.1)
Alternaria:	13 (10%) (5.8-17.2)
Trichosporon:	11 (9%) (4.6-15.3)
Sporothrix (including *Ophiostoma* teleomorph):	6 (5%) (1.9-10.4)
Fusarium:	5 (4%) (1.5-9.4)
Gymnascella (*G. marginospora*)	4 (3%) (1.0-8.4)
Ochroconis (*O. humicola*)	4 (3%) (1.0-8.4)
Microsporum:	4 (3%) (1.0-8.4)
(*M. boullardii*)	3 (2%) (0.6-7.3)
(*M. gypseum*)	1 (0.8%) (0.04-5.0)
Geotrichum:	3 (2%) (0.6-7.3)
Cephalotrichum, Exophiala, Sesquicillium, Metarhizium, Pseudallescheria:	2 (1.6%) (0.3-6.1)
Chlamydosauromyces punctatus	1 (0.8%) (0.04-5.0)
Amauroascus, Aphanocladium, Arthrographis, Botrytis, Curvularia, Engyodontium, Exophiala, Geomyces, Myriodontium, Oidiodendron, Ovadendron, Phialophora, Phoma, Trichothecium, Trichoderma, Verticillium, unidentified yeast	*1 (0.8%) (0.04-5.0)*

was unanticipated and may reflect the strong tolerance of this zygomycete to cycloheximide. This fungus was associated with dermal lesions in two animals in a retrospective study of crocodiles with skin disease but without evidence of causality (Buenviaje, *et al*, 1998).

The yeast-like fungus *Trichosporon*, also commonly incriminated as the agent of mycoses in reptiles (Austwick and Keymer, 1981, Schildger, *et al*, 1991, Buenviaje, *et al*, 1998, Jacobson, *et al*, 2000), as well as fungi belonging to the genera *Cladosporium, Alternaria, Scopulariopsis, Acremonium,* and *Chaetomium* were also isolated with consistency. Although *Fusarium* species have been reported as the cause of both superficial and deep mycosis in reptiles (Austwick and Keymer, 1981, Frelier, *et al*, 1985, Holz and Slocombe, 2000, Jacobson, *et al*, 2000, Rose, *et al*, 2001), they were recovered infrequently in this survey. The remaining isolates belonged to 33 other genera, including one new genus of ascomycetes (Table 2). *Chlamydosauromyces punctatus* was isolated from skin samples from the head and tail of a 7-year-old male frilled lizard housed at the San Diego zoo (Sigler, *et al*, 2003).

An average of 4.2 different genera of fungi was cultured from a single reptile, often with more than one species per fungal genus (e.g., two species of *Penicillium* or *Aspergillus*). The high number of fast-growing, saprotrophic fungi isolated from the skins of healthy reptiles, even on a selective (cycloheximide) medium, indicates that caution should be exerted when interpreting fungal culture results from cutaneous lesions in a sick reptile. The number of fungal genera isolated from any given individual reptile ranged from 15 in one Mexican beaded lizard, *Heloderma horridum*, to zero in two different corn snakes and in a Central American bushmaster, *Lachesis muta stenophrys*. Heavy bacterial contamination was a factor in failure to recover fungi from two of the three latter specimens. We noted a weak but discernible trend for reptile skins from the same institution to yield similar microfungi. For example, *Chrysosporium zonatum* was cultured from the skin of a garden tree boa, *Corallus enydris*, a Solomon Island skink, *Corucia zebrata* and a rosy boa, *Lichanura trivirgata*, from one institution, a Southern copperhead, a desert monitor lizard, *Varanus griseus*, and an Eastern indigo snake, *Drymarchon corais couperi*, from a second institution, a Gila monster, *Heloderma suspectum*, a tiercepelo fer-de-lance viper, *Bothrops asper*, and a green iguana, *Iguana iguana*, from a third institution, and an African sedge viper, *Atheris nitschei*, a Madagascar tree boa, *Sanzinia madagascariensis*, and a Bismarck ringed python, *Bothrochilus boa*, from a fourth institution. While it was cultured from every animal sampled in these four institutions, *C. zonatum* was only isolated 11 other times from the remaining 115 reptiles. Although such a trend could be attributed to the use of the same contaminated instruments when collecting or handling specimens, it is probably more a result of enclosure furniture or substrate, which are likely to be consistent within the same institution. The fungal microflora of reptiles is likely to parallel closely that of the substrate they live in.

The variety of fungi isolated from the skins of healthy reptiles emphasizes the importance of histopathology in the diagnosis of fungal dermatological disease. Microscopic features of fungal elements within lesions need be consistent or compatible with the morphological features of the species of fungus isolated from the lesion if a causal relationship is to be established. In cases of CANV mycosis, for example, arthroconidiation is often observed in tissues whereas such a finding is incompatible with a diagnosis of *Aspergillus* infection even if *Aspergillus* is isolated from the lesion.

Although the data from this survey are limited, they provide an indication that the CANV is not commonly found on healthy animals. How animals come into contact with the CANV, and the factors that lead to infection by this fungus are not understood. Further studies are needed to determine the conditions and factors that govern infection of reptiles by this fungus.

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