

Yellow fungus and related diseases in Australian reptiles

Fact sheet

Introductory statement

Yellow fungus disease in lizards occurs as a result of infection with *Nannizziopsis guarroi*. Similar disease conditions identified in Australian reptiles are linked to related fungi in the *Nannizziopsis* and *Paranannizziopsis* genera (Sigler et al. 2013; Paré and Sigler 2016). *Ophidiomyces ophiodiicola* is the causative agent of **snake fungal disease**, an emerging disease of free-ranging snakes in North America which is also occasionally identified globally in captive snakes, including in Australia (Sigler et al. 2013; Stchigel et al. 2013; Lorch et al. 2016; Paré and Sigler 2016). It has not been reported in wild Australian snakes.

Aetiology

Yellow fungus disease in inland bearded dragons (*Pogona vitticeps*) and green iguanas (*Iguana iguana*) is caused by *N. guarroi*. Similar dermatomycoses in a variety of reptilian species are caused by other species in the *Nannizziopsis* and *Paranannizziopsis* genera (Paré and Sigler 2016). The aetiologic agent of **snake fungal disease** (previously thought to be *Chrysosporium* anamorph of *Nannizziopsis vriessi*; CANV), has now been identified as *O. ophiodiicola*, the only known species of the *Ophidiomyces* genus (Sigler et al. 2013; Stchigel et al. 2013)¹. These fungal genera fall into the family Onygenaceae of the order Onygenales (Sigler et al. 2013; Stchigel et al. 2013).

Natural hosts

Nannizziopsis spp. have been reported in a wide range of lizard species, crocodiles and humans (Paré and Sigler 2016). *Paranannizziopsis* spp. have been reported in lizards, snakes and tuatara (*Sphenodon punctatus*)

¹ The fungal agent formerly known as the *Chrysosporium* anamorph of *Nannizziopsis vriessi* (CANV) has undergone extensive taxonomic reclassification, resulting in its division into a number of different species within three genera namely *Nannizziopsis*, *Paranannizziopsis* and *Ophidiomyces* (Sigler et al. 2013; Stchigel et al. 2013).

(Paré and Sigler 2016). *O. ophioidiicola* has been identified in a wide range of snakes (Lorch et al. 2016; Paré and Sigler 2016).

World distribution

Cases of *Nannizziopsis* spp. and *Paranannizziopsis* spp. infection in reptiles have been reported in Africa, Asia, Europe, North America and Oceania (Sigler et al. 2013; Stchigel et al. 2013; Paré and Sigler 2016). *O. ophioidiicola* is considered a recently emerging disease of wild snakes in North America, but has been identified in captive snakes since the 1980s in Europe, North America and Australia (Sigler et al. 2013; Stchigel et al. 2013; Paré and Sigler 2016).

Occurrences in Australia

Species identified in Australia include *N. barbata*, *N. crocodile*, *P. australasiensis* and *O. ophioidiicola* (Thomas et al. 2002; Johnson et al. 2011; Sigler et al. 2013; Paré and Sigler 2016).

Outbreaks of *N. crocodili* occurred on two separate occasions in 1994 and 1997 in saltwater crocodiles (*Crocodylus porosus*) sourced from the same crocodile farm in northern Queensland (Thomas et al. 2002; Paré and Sigler 2016).

In 2008-2009 an outbreak of what is now identified as *N. barbata* was reported in a group of captive coastal bearded dragons (*P. barbata*) in New South Wales (Johnson et al. 2011; Paré and Sigler 2016). A separate case was diagnosed in a captive coastal bearded dragon (Johnson et al. 2011; Sigler et al. 2013). A very similar isolate to *N. barbata* has been obtained from a free-ranging eastern water dragon (*Physignathus lesueurii*) with dermal lesions (Paré and Sigler 2016).

P. australasiensis was cultured from lesions on two file snakes (*Acrochordus arafura*) housed in captivity in Victoria (Paré and Jacobson 2007; Sigler et al. 2013; Paré and Sigler 2016).

O. ophioidiicola has been diagnosed in captive snakes in Australia, including a file snake in Queensland and a broad-headed snake in South Australia (McLelland et al. 2010; Sigler et al. 2013; Paré and Sigler 2016). There is also suggestion that mycotic dermatitis attributed to *Geotrichum candidum* in three captive carpet snakes in Queensland may have in fact been caused by *O. ophioidiicola* (McKenzie and Green 1976; Paré and Sigler 2016).

Anecdotal evidence suggests that clinical cases consistent with yellow fungus disease and snake fungal disease may have been occurring in captive snakes and lizards in Australia for several decades (R Woods, A Reiss pers. comm. Aug 2018).

Epidemiology

N. guarroi as well as other members of the *Nannizziopsis* and *Paranannizziopsis* genera appear to be primary, obligate pathogens, rather than opportunists, as fungal pathogens have been traditionally considered (Mitchell and Walden 2013). Koch's postulates have been fulfilled in experimental infection of veiled chameleons (*Chamaeleo calypttratus*) with *N. guarroi* (Paré et al. 2006). In captivity, suboptimal environmental factors may make reptiles more susceptible to these infections and the pathogens are contagious between animals in close proximity (Thomas et al. 2002; Paré and Sigler 2016). Although cases

have been identified in free-ranging reptiles, there is little available information on the epidemiology in the wild (Paré and Sigler 2016).

O. ophiodiicola is now well recognised as a pathogen of free-ranging snakes, particularly in North America. Experimental infections have fulfilled Koch's postulates and have demonstrated action as a primary pathogen (Allender et al. 2015a; Lorch et al. 2015). The fungus has not been identified on the skin of healthy snakes, but is found in copious amounts in the lesions of affected snakes, indicating that it is not part of the cutaneous mycobiota (Allender et al. 2011; Allender et al. 2015b; Paré and Sigler 2016). Although the fungus does not grow at temperatures less than 15 C, snakes undergoing brumation may be susceptible during the autumnal decline and spring rise in temperatures, when the snakes' immune systems are depressed (Lorch et al. 2016; Paré and Sigler 2016). Small skin lesions colloquially termed "hibernation" sores or blisters that have been recognised in snakes for many years also test positive for the fungus, suggesting infection has been occurring historically, but severity of clinical disease (at least in some incidences) may have been increasing in recent years. The cause of this increased severity (from small healing sores to a fatal disease) is unclear (Lorch et al. 2016; Paré and Sigler 2016).

Clinical signs

Lesions are similar for all pathogens, although appear to vary in severity depending on the host species and likely other environmental factors. Grossly, lesions are initially yellow, and then thicken to form brown, hyperkeratotic, necrotic plaques. These plaques may crack and seep exudate, or slough to reveal whitish pink, swollen dermis. Lesions are often found on the head and around the mouth but can occur anywhere on the body and can involve a whole limb in the case of lizards (Paré and Jacobson 2007; Lorch et al. 2016; Paré and Sigler 2016).

Dermatomycoses due to *Nannizziopsis* spp. are slowly progressive and often fatal skin diseases. Affected reptiles range in body condition from poor to good. Skin lesions progress over several months from dry and yellow to hyperkeratotic plaques to exudative and necrotic ulcers (Figure 1) (Johnson et al. 2011; Paré and Sigler 2016). In bearded dragons (*Pogona* spp.), the mouth and face are commonly affected but lesions may occur anywhere on the body (Bowman et al. 2007; Abarca et al. 2009; Johnson et al. 2011; Le Donne et al. 2016; Schmidt-Ukaj et al. 2016). Infection is often fatal in bearded dragons, with infection extending to muscle, bone and internal tissues including liver, heart, kidney, lungs and intestine (Bowman et al. 2007; Johnson et al. 2011; Masters et al. 2016; Paré and Sigler 2016; Schmidt-Ukaj et al. 2016). In iguanas, infection is often identified in the hind limbs and tail (Han et al. 2010; Kahraman et al. 2015). Hatchling saltwater crocodiles infected with *N. crocodili* developed multiple leathery plaque-like lesions on or under the scales, which could be peeled away to reveal white or red tissue. Infection was often fatal (Thomas et al. 2002).

P. australiensis in tuatara causes skin lesions similar to those described above. In tuatara, lesions resolved with treatment in all reported cases. In a coastal bearded dragon, the same agent resulted in fatality, with similar progression of disease as that described for *Nannizziopsis* spp. (Masters et al. 2016). In file snakes, infection results in disseminated punctate or circular, whitish lesions across the epidermis (Paré and Jacobson 2007; Sigler et al. 2013; Paré and Sigler 2016).

O. ophiodiicola in snakes causes yellow to brown, thickened, necrotic crusts, mainly affecting the head also the scales of the body, most often on the underside of the snake. In milder cases, the processes of ecdysis (slough) can resolve the infection, but if deeper tissues are affected, disease may recur post-slough. Systemic infection with involvement of bone and lung has been reported, but is rare (Lorch et al. 2016).



Figure 1. Yellow fungus disease in a captive coastal bearded dragon *Pogona barbata* (Courtesy R Johnson).

Diagnosis

Definitive diagnosis of skin or systemic disease associated with fungi in the *Nannizziopsis*, *Paranannizziopsis* and *Ophidiomyces* genera requires both a) identification of the organism through culture and sequencing or PCR and b) histopathology identifying fungal elements within lesions. Reptile skin plays host to many fungal elements, so presence of the organism alone is not synonymous with disease (Paré and Sigler 2016). Identification of the organism using matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS), which may be more accurate than sequencing, has been described but may not yet be available in Australia (Schneider et al. 2017).

Clinical pathology

Although not pathognomonic, cytology of sticky tape preparations or impression smears may reveal presence of conidia or arthroconidia suggestive of a fungus in the *Nannizziopsis*, *Paranannizziopsis* or *Ophidiomyces* genera (Le Donne et al. 2016; Paré and Sigler 2016). Conidia are 5-8 μm long by 3-5 μm wide, clavate to ovoid to cylindrical and surrounded by a thin capsule. Arthroconidia are arranged in rows of up to 7 conidia, separated by thin septae (Le Donne et al. 2016).

Pathology

Grossly, lesions are as described above. In systemically affected bearded dragons at necropsy, collection of pale yellow, gelatinous material can be found within the coelomic cavity and pericardial sac and granulomatous changes have been noted in the liver (Bowman et al. 2007) (C Sangster 2018, personal communication). Histologic lesions include granulomatous fungal dermatitis, myositis, osteomyelitis,

hepatitis, nephritis, coelomitis, myocarditis and pneumonia (Bowman et al. 2007; Johnson et al. 2011; Paré and Sigler 2016; Schmidt-Ukaj et al. 2016). Hyphae found within granulomas and in the keratin layer are 2-4 µm wide, septate and exhibit haphazard branching. Arthroconidia on the surface of the epidermis is suggestive, but not pathognomonic for fungi in this order (Paré and Jacobson 2007).

Differential diagnoses

Other dermatomycoses, bacterial dermatitis, stomatitis and osteomyelitis should be excluded from the list of differential diagnoses. Previously, fungi from this order have been misidentified as *Trichophyton* spp. or as other fungal species including *Geotrichium* spp. or *Trichosporon* spp. (Bowman et al. 2007; Johnson et al. 2011; Paré and Sigler 2016).

Laboratory diagnostic specimens

Multiple skin biopsies of dermal lesions, half placed in 10% neutral buffered formalin for histopathology and half submitted fresh (or less desirably frozen) for PCR and /or culture (Paré and Sigler 2016).

Skin samples pulled from lesions can be submitted for PCR and culture. Swabs should be avoided as the fungus is difficult to culture from these samples (Paré and Sigler 2016).

Sections of multiple internal organs in 10% neutral buffered formalin and fresh/frozen are recommended if systemic disease is suspected (C Sangster pers. comm. July 2018).

Laboratory procedures

Histopathological examination should include H&E and either PAS or Grocott-Gomori's methylene silver stain for fungal identification (C Sangster pers. comm. July 2018).

Samples for culture are best treated with enrofloxacin to limit bacterial overgrowth, plated on Mycosel™ agar (Becton, Dickinson and Company, Franklin Lakes, NJ) and incubated at 30°C. White powdery colonies should be subcultured, to prevent bacterial overgrowth, and speciated by sequencing (Paré and Sigler 2016).

Treatment

Medical treatment of confirmed cases involves systemic antifungals and topical antifungal or antiseptic solutions (Paré and Sigler 2016). Susceptibility testing of *N. guarroi* has revealed sensitivity to voriconazole and terbinafine, but less so to itraconazole (Van Waeyenberghe et al. 2010; Paré and Sigler 2016). Serum biochemistry should be monitored for signs of liver toxicity. Surgical excision or debridement of lesions should be carried out if possible and in conjunction with medical therapy (R Johnson pers. comm. 2009).

Prevention and control

Prevention of *Nannizziopsis* spp., *Paranannizziopsis* spp. and *O. ophiodiicola* infection in captive reptiles should focus on reducing the fungal load, with attention being paid to regular substrate changes and good hygiene in captive situations. Providing optimal husbandry conditions, including species-appropriate temperature gradients, hydration/humidity and nutrition are important steps in prevention and control (Paré and Sigler 2016). In captive reptiles, infection appears to be more common at low ambient temperatures (R

Johnson 2009, personal communication). Affected individuals should be isolated and biosecurity measures followed as the organism can act as a contagious, primary pathogen (Paré and Sigler 2016).

Surveillance and management

Wildlife disease surveillance in Australia is coordinated by Wildlife Health Australia. The National Wildlife Health Information System (eWHIS) captures information from a variety of sources including Australian government agencies, zoo and wildlife parks, wildlife carers, universities, industry and members of the public. Coordinators in each of Australia's States and Territories report monthly on significant wildlife cases identified in their jurisdictions. NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. Please contact admin@wildlifehealthaustralia.com.au. There are currently no targeted surveillance programs for reptile fungal diseases. There are a small number of cases reported in eWHIS including a suspected case in a wild broad-shelled turtle *Chelodina expansa* (no culture or PCR).

We encourage those with laboratory confirmed cases of this condition in native Australian or feral animals to submit this information to the national system for consideration for inclusion in the national database. Please contact us at admin@wildlifehealthaustralia.com.au.

Research

Molecular differentiation of the taxonomy of this order of fungi has significantly advanced knowledge in this area, allowing identification of morphological and physiological properties, host trends and sensitivity patterns for many new species (Paré and Sigler 2016). Large gaps in the epidemiology of these pathogens, particularly in free-ranging reptiles, remain. This is particularly significant in the case of *O. ophioidiicola*, which is an emerging disease resulting in significant losses of free-ranging snakes in North America (Lorch et al. 2016). *Nannizziopsis* and *Paranannizziopsis* spp. that appear to be specific to Australasia have been identified, but only in low numbers of animals. Further studies are warranted to understand fully the origin and nature of these organisms, and their significance as primary pathogens in reptiles in Australia, both captive and free living. To date, reports in free-ranging Australian reptiles have been opportunistic, with no research investigating incidence, host range or epidemiology of disease related to these pathogens, illustrating a significant knowledge gap.

Human health implications

Previously, infection with what was termed CANV had been reported in humans, generally in immunosuppressed patients (Steininger et al. 2005; Paré and Sigler 2016). However, the recent molecular characterisation work has revealed these cases to be caused by species of *Nannizziopsis* that are distinct from those found in reptiles. The risk of zoonotic transmission of *O. ophioidiicola* or *Nannizziopsis* spp. and *Paranannizziopsis* spp. from reptiles to humans is not considered high, although it is considered possible in immunocompromised patients (Paré and Sigler 2016).

Conclusions

In Australia, outbreaks of infection with fungi from the *Nannizziopsis* and *Paranannizziopsis* genera have occurred in farmed crocodiles, captive bearded dragons and captive file snakes. Sporadic cases of *O. ophioidiicola* have occurred in captive Australian snakes. Overseas these fungi have been found to be significant primary pathogens of lizards and snakes. As the fungus is easily spread by contact, wildlife carers

and veterinarians caring for captive and free-living reptiles need to be vigilant in preventing the spread of these pathogens. Further work is recommended to better understand the epidemiology of the diseases, and possible risks to Australian native reptiles.

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To provide feedback on this fact sheet

We are interested in hearing from anyone with information on this condition in Australia, including laboratory reports, historical datasets or survey results that could be added to the National Wildlife Health Information System. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to admin@wildlifehealthaustralia.com.au. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

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