Wildlife Health AUSTRALIA

Pathogenic skin fungi in Australian reptiles
Fact sheet

Introductory statement

Fungi belonging to the genera *Nannizziopsis*, *Paranannizziopsis* and *Ophidiomyces* (formerly members of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* [CANV] complex) are the cause of skin diseases that may progress to systemic and sometimes fatal disease in a range of reptile species. The disease was formerly referred to as 'yellow fungus disease' due to coloration of the skin lesions. These disease conditions are relatively newly described, suggesting they are 'emerging', although much remains to be learnt about the aetiological agents, epidemiology, presence, and prevalence of these fungal diseases worldwide. The reasons for the apparent emergence of these infections in both free-living and captive reptiles are not understood, however it is likely that global human-assisted movement of reptiles (due to the reptile pet trade) may be a contributing factor (Paré et al. 2020).

In Australia, pathogenic skin fungi have been reported in a range of captive reptile species and in free-living Agamids (dragon lizards) and shingleback lizards (*Tiliqua rugosa*). The focus of this fact sheet is on fungi of the genera *Nannizziopsis*, *Paranannizziopsis* and *Ophidiomyces*.

Aetiology

The genera *Nannizziopsis*, and *Paranannizziopsis* are classified in the family Nannizziopsidaceae of the order Onygenales[1] (Stchigel et al. 2013) and *Ophidiomyces* is classified in the family Onygenaceae (Onygenales) (Sigler et al. 2013).

Nine species of the genus *Nannizziopsis* are associated with skin disease in lizards globally (Sigler et al. 2013; Paré and Sigler 2016; Peterson et al. 2020). *Nannizziopsis barbatae*[2] has 99% nucleotide similarity to *N. crocodili* and is also similar genetically to *N. pluriseptata* (Peterson et al. 2020).

Genus *Paranannizziopsis* (four species) are linked to skin disease in Australasian reptiles including tuatara (*Sphenodon punctatus punctatus*), aquatic file snakes (*Acrochordus* sp.) and coastal bearded dragon (*Pogona*

[1] The fungal agents formerly known as the *Chrysosporium* anamorph of *Nannizziopsis vriesii vriessi* (CANV complex) have been reclassified within the genera Nannizziopsis, Paranannizziopsis and Ophidiomyces (Sigler et al. 2013; Stchigel et al. 2013).

[2] Formerly *N. barbata*

barbata) (Masters et al. 2016; Paré and Sigler 2016). *Ophidiomyces ophidiicola*[3] (formerly *ophiodiicola*; the only species of this genus) is associated with "snake fungal disease" in terrestrial or semiaquatic snake species (Sigler et al. 2013; Stchigel et al. 2013).

Other onygenalean fungi include *Emydomyces testavorans,* which has been associated with lesions in turtles and has no assigned family (Woodburn et al. 2019). *Aphanoascella galapagosensis* has been isolated from a captive Galapagos tortoise with carapace keratitis in the USA (Sutton et al. 2013).

Natural hosts

Nannizziopsis spp. have been reported from infections in a wide range of lizard species, including chameleons, geckos, dragon and iguana lizards, and crocodiles (Paré and Sigler 2016; Peterson et al. 2020). *N. guarroi* is associated with skin disease in bearded dragons (*Pogona vitticeps*) and other lizards in North America (Paré and Sigler 2016). *Nannizziopsis barbatae* has been linked to skin and systemic disease in a range of lizards in Australia (below), including endemic free-living species (Peterson et al. 2020).

Paranannizziopsis spp. have been reported in lizards, snakes and tuatara (Paré and Sigler 2016).

O. ophidiicola has been identified in a wide range of both captive and free-ranging snakes (Sigler et al. 2013; Stchigel et al. 2013; Lorch et al. 2016; Paré and Sigler 2016).

Emydomyces testavorans is reported to be associated with invasive carapace, plastron and skin lesions in captive and free-living aquatic and semi-aquatic turtles in North America (Woodburn et al. 2019).

World distribution

Cases *of Nannizziopsis* spp. and *Paranannizziopsis* spp. infection in captive reptiles have been reported in Africa, Asia, Europe, North America, Australia and New Zealand (Sigler et al. 2013; Stchigel et al. 2013; Paré and Sigler 2016; Peterson et al. 2020). Lesions typical of those associated with *O. ophidiicola* have been reported in captive snakes since the 1980s, primarily in North America but also in Europe. Infection has been identified more recently in wild snakes in North America (Sigler et al. 2013; Stchigel et al. 2013; Lorch et al. 2016; Paré and Sigler 2016; Paré et al. 2020; Snyder et al. 2020).

Occurrences in Australia

Species identified in Australia include *N. barbatae*, *N. crocodili*, *P. australasiensis* and *O. ophidiicola* (Thomas et al. 2002; Johnson et al. 2011; Sigler et al. 2013; Paré and Sigler 2016; Peterson et al. 2020).

N. barbatae has been linked to severe disease in four species of free-living lizards in Australia - see below (Peterson et al. 2020). Other reports of fungal pathogens from these genera in Australia have been in captive reptiles.

Nannizziopsis: cases of severe, proliferative dermatitis, debility and death associated with *N. barbatae* were reported in four species of free-living lizard from at least 8 different locations across Australia, including a significant outbreak among Eastern water dragons (*Intellagama lesueurii*) in Brisbane, Qld, beginning in 2013

[3] Formerly *O. ophiodiicola*

(Peterson et al. 2020). Affected species included a tommy roundhead (*Diporiphora australias*) from the Brisbane areas, an eastern bluetongue skink (*Tiliqua scincoides scincoides*) from Dubbo NSW and a wild shingleback lizard from Perth WA (Peterson et al. 2020). Cases came from at least 8 different locations across Australia. These were the first reports of infection within this genus of fungi in free-living reptiles globally, and *N. barbatae* has not been reported outside Australia. A captive Centralian bluetongue lizard (*Tiliqua multifasciata*), from Vic was also found to be infected. During this study, many other specimens from Australian snakes and lizards (collected since 2010 in Australia) were examined, with the cases reported here the only detections of *N. barbatae* (Peterson et al. 2020).

A fungus very similar to *N. barbatae* (but originally thought to be *N. pluriseptata*) was identified as the cause of skin disease in eight wild-caught but captively housed shingleback lizards (*Tiliqua rugosa*) held in Western Australia in 2019 (WA Wildlife Health Reference Group 2019).

An outbreak (now identified as *N. barbatae*) was reported in a group of captive coastal bearded dragons (*P. barbata*) in NSW in 2008-2009 (Johnson et al. 2011; Paré and Sigler 2016). These were the first known cases of *N. barbatae* in Australia (Peterson et al. 2020). A separate case was diagnosed in another captive coastal bearded dragon (Johnson et al. 2011; Sigler et al. 2013). A isolate very similar to *N. barbatae* was obtained from a free-ranging eastern water dragon (*Physignathus lesueurii*) with skin lesions (Paré and Sigler 2016). *N. barbatae* has not yet been reported outside Australia (Paré et al. 2020).

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 Qld, with lesions and mortalities (Thomas et al. 2002; Paré and Sigler 2016). More recent reports have occurred in captive (zoo) juvenile freshwater crocodiles (*C. johnstoni*) with skin lesions, at a location geographically far removed from the reports in farmed saltwater crocodiles (Hill et al. 2019). *N. crocodili* has not been reported in wild reptiles.

Paranannizziopsis australasiensis was cultured from lesions on two file snakes (*Acrochordus arafurae*) housed in captivity (zoo) in Vic (Paré and Jacobson 2007; Sigler et al. 2013; Paré and Sigler 2016).

Ophidiomyces ophidiicola has been diagnosed only in captive snakes in Australia, including a file snake (*Acrochordus arafurae*) on display in a crocodile farm in Qld (Sigler et al. 2013) and a broad-headed snake (*Hoplocephalus bungaroides*) in a zoo in South Australia, which died with apparent systemic infection (McLelland et al. 2010).

It has been suggested that mycotic dermatitis attributed to *Geotrichum candidum* in three captive carpet pythons (*Morelia spilotes variegate*) in Qld may have been caused by *O. ophidiicola* (McKenzie and Green 1976; Paré and Sigler 2016; Sigler 2021). *Ophidiomyces ophidiicola* has not been reported in wild Australian reptiles.

Anecdotal evidence suggests that clinical cases consistent with *Nannizziopsis* and *Ophidiomyces* fungal disease may have been occurring in captive lizards and snakes in Australia for several decades (Reiss and Woods 2018), although Peterson et al. (2020) reports no positive cases from archived samples from 2010 onwards.

Epidemiology

The epidemiology of these emerging diseases in reptiles is still poorly understood. Ectothermic taxa such as reptiles are considered particularly vulnerable to fungal pathogens and these fungal species appear for the most part to be primary pathogens, rather than opportunists (as fungal pathogens of reptiles have been traditionally considered) (Mitchell and Walden 2013; Peterson et al. 2020).

Nannizziopsis* and *Paranannizziopsis: Koch's postulates have been fulfilled in experimental infection of veiled chameleons (*Chamaeleo calyptratus*) 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). Cases have been identified in free-ranging reptiles, but there is little available information on the epidemiology in the wild (Paré and Sigler 2016; Peterson et al. 2020).

It is not known if *N. barbatae* was introduced to wild lizards through spillover or if it is an endemic fungus that has only been detected recently. It is not yet known if it is truly an "emerging disease" among wild lizards, nor what the factors might be that could be contributing to emergence, however altered host susceptibility or changing environmental conditions have been suggested as possible contributing factors (Peterson et al. 2020).

Ophidiomyces ophidiicola is now well recognised as a pathogen of snakes, including free-ranging species, 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). There is some evidence that some asymptomatic carrier hosts may exist, but are rare (Paré et al. 2020). It is possible that the fungus can be vertically transmitted from dam to offspring (around the time of birth) (Stengle et al. 2019) but transmission routes in general are poorly understood. The fungus is found in copious amounts in the lesions of affected snakes, and is not thought to be 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, or held at suboptimal temperature may be susceptible, if 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). Infection may make snakes more susceptible to predation or other causes of mortality and has been implicated as a cause of wild snake population declines since 2006 (Allender et al. 2015c; Snyder et al. 2020).

Clinical signs

Lesions are similar for all pathogens, although they appear to vary in severity depending on the host species and likely other environmental factors (Peterson et al. 2020). 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; Peterson et al. 2020). Animals may show hyperkeratosis, epidermal hyperplasia, dermal inflammation, necrosis, ulceration, and emaciation.

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; Peterson et al. 2020). 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. australasiensis 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. ophidiicola in snakes mainly affects the head and 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. *Nannizziopsis* fungal disease in a captive coastal bearded dragon *P. 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, in particular the presence of arthroconidia. Reptile skin may host many fungal elements, but confirmed presence of *Nannizziopsis*, *Paranannizziopsis* or *Ophidiomyces* is indicative of disease where hyphae and arthroconidia are present in lesions and the fungus has been isolated or confirmed by PCR. There is increasing evidence that at least some of these fungi (e.g. *N. barbatae*) are obligate pathogens (Paré and Sigler 2016; Peterson et al. 2020). 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. Arthroconidia may be arranged in rows of up to 7 conidia, separated or in a chain (Le Donne et al. 2016; Sigler 2021).

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). 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 skin of reptiles with characteristic lesions is considered pathognomonic for fungi in the Nannizziopsidaceae and *O. ophidiicola*. A tape mount could be used to sample the surface of the lesion. (Paré and Jacobson 2007).

Differential diagnoses

Other dermatomycoses, bacterial dermatitis, stomatitis and osteomyelitis should be excluded from the list of differential diagnoses.

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 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 (Sangster 2018).

Laboratory procedures

Histopathological examination should include H&E and either PAS or Grocott-Gomori's methylene silver stain for fungal identification (Sangster 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 sub-cultured, and speciated by sequencing (Paré and Sigler 2016).

Peterson et al. (2020) recommends an improved method of fungal isolation by modification of the conventional serial dilution technique.

Treatment

Medical treatment of confirmed cases involves systemic antifungals and topical antifungal or antiseptic solutions (Paré and Sigler 2016). There is limited information on successful regimens, and treatment may not be effective or curative (Peterson et al. 2020). 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). Pharmacokinetic studies in bearded dragons and green iguanas suggests terbinafine may reach therapeutic levels in these species after oral administration (McEntire et al. 2020). 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 (Peterson et al. 2020).

Treatment of captive reptiles should also include appropriate cleaning and decontamination of the animal's environment, and the maintenance of simple surroundings to facilitate ongoing hygiene and disinfection. There is currently little information on appropriate disinfections protocols for *Nannizziopsis* sp. however laboratory-based studies on *O. ophidiicola* recommend a minimum of 2 minute environmental exposure to at least 3% bleach or 70% ethanol or a 10 min exposure to 0.16% Roccal, Lysol products, CLR, NPD, or 409 (Rzadkowska et al. 2016).

Prevention and control

Prevention of pathogenic fungal 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. In captive reptiles, infection appears to be more common at low ambient temperatures. Providing optimal husbandry conditions, including species-appropriate temperature gradients, hydration/humidity and nutrition are important steps in prevention and control (Paré and Sigler 2016). Affected individuals should be isolated and biosecurity measures followed as the organism can act as a contagious, primary pathogen (Paré and Sigler 2016).

Prevention and control options for free-living reptiles are limited. Factors that may contribute to increased susceptibility to infection such as environmental degradation, proximity to humans, and other stressors should be addressed wherever possible (Peterson et al. 2020). Appropriate disinfection protocols (see above) should be used for equipment if working with wild reptiles, prior to moving between study sites (Rzadkowska et al. 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. See the WHA website for more information: www.wildlifehealthaustralia.com.au/ProgramsProjects/eWHISWildlifeHealthInformationSystem.aspx#requests.

There are currently no targeted surveillance programs for reptile fungal diseases. There are a number of cases reported in eWHIS including those reported above, and more recent cases in wild lizards in Australia, and an earlier 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). There are significant knowledge gaps related to the incidence, host range and epidemiology of disease related to these pathogens, particularly in free-ranging reptiles (Lorch et al. 2016; Peterson et al. 2020). Further studies are warranted to understand fully the origin and nature of these organisms in the Australian context, and their significance as primary pathogens in both captive and wild reptiles.

Human health implications

Molecular characterisation work has revealed cases of *Nannizziopsis* infection in humans are caused by species that are distinct from those found in reptiles. The risk of zoonotic transmission of pathogenic skin fungi from reptiles to humans is considered low since the temperature range for growth of these reptile associated fungi is generally not compatible with infection in humans (Peterson et al. 2020).

Conclusions

Australia has seen recent confirmed cases of *Nannizziopsis* infection in wild lizards across a broad geographic range, as well as sporadic cases and outbreaks of pathogenic skin fungi in captive crocodiles, lizards and snakes. There is convincing evidence that these fungi are significant primary pathogens of reptiles. Wildlife carers and veterinarians caring for both captive and free-living reptiles need to be vigilant in preventing the spread of these pathogens, as they are easily spread by contact. Further work is needed to improve our understanding of the incidence, host range and epidemiology of these fungal infections, and the risks to Australian native reptiles.

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Acknowledgements

We are extremely grateful to Robert Johnson, Cheryl Sangster, Lynne Sigler, Nicola Peterson and other individuals, agencies and organisations who helped to develop and review this fact sheet.

Updated: August 2021

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.

Disclaimer

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