Wildlife disease from a fungal perspective: a look into host specificity and physiology of *Ophidiomyes ophiodiicola* and *Nannizziopsis guarroi*

By

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Do the best you can until you know better.

Then when you know better,

Do better.

— Maya Angelou
Author, Poet,
Civil Rights Activist

Table of Contents

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Dissertation Introduction

Unveiling the mysteries of disease has been a significant core goal of research and technology from time immemorial. Spanning a multitude of different disciplines, the relationship between humanity and disease infinitely cycles between outbreak, discovery, and prevention. Rapid advances in sequencing and genomic technology have given biologists the ability to study the complexity within the epidemiologic triad, colloquially the "disease triad", at the molecular level. Such advances lend to a more holistic view in the relationships between host, pathogen, and environment. However, with complexity comes the muddying of conceptual frameworks of understanding, and a distancing from fundamental physiological experiments of microorganisms. Perhaps inescapably, such advances have prioritized human diseases over other organisms and wildlife diseases essential for environmental health and maintenance that may otherwise hold key insight toward the diseases fervently combatted in humans.

In this dissertation, I lean heavily on the concept of host specificity toward broadening our understanding of fungal physiology in wildlife diseases. I use the understudied fungi *Ophidiomyces ophiodiicola* and *Nannizziopsis guarroi* to enhance the body of literature around the emerging infectious diseases they cause, snake fungal disease and yellow fungus disease, respectively. Each chapter relates its findings to concerns of infectious disease escaping into naïve wildlife populations that could result in potentially unforeseen and long-lasting consequences for a species.

In chapter I, I investigated the pervasive literature assumptions between *N. guarroi* and its associated host, bearded dragons. Specifically, I studied the requirements that consider a fungal pathogen as a direct, primary, infection agent as opposed to an

opportunistic, secondary infection agent. Following Koch's postulates, we confirmed *N. guarroi* could establish an infection in healthy bearded dragons without the necessity of an abrasion, supporting literature assumptions with experimental evidence.

In chapter II, I reexamined the host species for both *O. ophiodiicola* and *N. guarroi* known to be snakes and lizards, respectively. Based on the shared fungal history of these pathogens being a part of the former *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV), I focused on the ability of each fungus to infect the opposite host. I was in part driven to understand if either fungus was truly restricted to their documented hosts. We found evidence that both *O. ophiodiicola* and *N. guarroi* could establish infection in both snakes and lizards, ultimately increasing the host range of each disease and the concern for an increased disease distribution.

In chapter III, I am the first to assemble the genome of *N. guarroi* for a suite of putative genes related to keratin degradation. I focus on the observed ability of *N. guarroi* to infect reptiles without disruptions in the epidermis, identifying the presence of enzymatic tools that could unveil downstream pathogenicity factors and differ from other fungi once a part of the CANV complex.

AUTHOR CONTRIBUTIONS

Chapter I

Savannah Gentry performed the conceptualization of, and conducted, the experiment, wrote the original draft, and edited and submitted the manuscript for publication.

Jeffrey M Lorch contributed to the conceptualization and execution of the experiment and reviewed the manuscript for publication.

Julia S. Lankton performed histopathology and reviewed the manuscript for publication.

Anne Pringle contributed to the conceptualization of the experiment and reviewed the manuscript for publication.

Chapter II

Savannah Gentry performed the conceptualization of, and conducted, the experiment, wrote the original draft, and edited and submitted the manuscript for publication.

Jeffrey M Lorch contributed to the conceptualization and execution of the experiment and reviewed the manuscript for publication.

Julia S. Lankton performed histopathology and reviewed the manuscript for publication.

Anne Pringle contributed to the conceptualization of the experiment and reviewed the manuscript for publication.

Chapter III

Savannah Gentry performed the conceptualization, execution of the bioinformatics analysis, and writing.

Yen-Wen Wang contributed bioinformatics expertise.

Jin Woo Bok performed molecular techniques of the experiment.

Nancy Keller contributed to the conceptualization.

Jeffrey Lorch contributed fungal isolates.

Anne Pring performed writing – review.

Chapter I – Koch's Postulates: Confirming *Nannizziopsis guarroi* as the cause of yellow fungal disease in *Pogona vitticeps*

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ABSTRACT

Nannizziopsis guarroi is an ascomycete fungus associated with a necrotizing dermatitis in captive green iguanas (*Iguana iguana*) and bearded dragons (*Pogona vitticeps*) across both Europe and North America. Clinical signs of the disease include swelling and lesion formation. Lesions develop from white raised bumps on the skin and progress into crusty, yellow, discolored scales, eventually becoming necrotic. The clinical signs are the basis of a colloquial name yellow fungus disease (YFD). However, until now *N. guarroi* has not been confirmed as the primary agent of the disease in bearded dragons. In this experiment, we fulfill Koch's postulates criteria of disease, demonstrating *N. guarroi* as the primary agent of YFD in bearded dragons.

GENERAL AUDIENCE

Nannizziopsis guarroi is a fungus known to cause yellow fungus disease (YFD), which can be fatal for lizard species. However, *N. guarroi* has only been assumed to cause disease in bearded dragons but had yet to be confirmed with experimental support. Using Koch's specific criteria that help determine if a single microbe can cause an infection in an animal host, we demonstrated that *N. guarroi* causes YFD in bearded dragons. Our evidence to support the previous assumptions include observational data of disease

symptoms in the animals during our experiment, studying the tissues from the infected animals, and culturing the pathogenic fungus from the infected tissues. Our findings emphasize the necessity for experimental support toward diseases that remain largely hidden and unfamiliar.

KEYWORDS: Ascomycota, emerging infectious diseases, wildlife, C*hrysosporium* anamoroph of *Nannizziopsis vriesii*, pet trade

INTRODUCTION

Starting in the late 1990s, case reports from veterinary clinics began associating *Nannizziopsis guarroi* with a fungal infection in bearded dragons (*Pogona vitticeps*), termed yellow fungus disease (YFD) (Sigler *et al.* 2013; Paré and Sigler 2016; Paré *et al.* 1997). Although little is known about the natural history of *N. guarroi*, an association of *N. guarroi* with the necrotic mycoses described as YFD in lizard species continues to be reported despite the direct lack of evidence for *N. guarroi* as the agent of disease. As reports of YFD in captive reptiles become more common, particularly among popular species in the pet trade, concern for the potential spread of the disease among wild populations of susceptible animals is increasing.

Clinical signs associated with YFD include lesion formation and swelling: lesions begin as white raised bumps on the skin and develop into crusty, yellow, discolored scales, eventually swelling from inflammation and becoming necrotic at the sites of infection (Le Donne *et al.* 2016; Abarca et al 2010; Schneider *et al.* 2018). However, many have attributed infections associated with *N. guarroi* to other factors compromising host health, for example improper husbandry conditions (Le Donne *et al.* 2016). Case reports associating *N. guarroi* with YFD in captive reptiles dominate the literature and reports

span North America and Europe, but also come from Korea and Australia (Paré *et al.* 1997; Abarca *et al.* 2008, 2009, 2010; Waeyenberghe *et al.* 2010, Han *et al.* 2010, Johnson *et al.* 2011; Schmidt-Ukaj *et al.* 2014, 2016; Bowman *et al.* 2007; Hedley *et al.* 2010; Le Donne *et al.* 2016; Skomorucha *et al.* 2014).

Discovering whether a microbe is the primary agent of disease in a host or hosts is critical to elucidating disease dynamics and identifying appropriate control measures. Fulfilling Koch's postulates enables subsequent investigation into disease spread, origin, and host range. Koch's postulates are standard criteria developed to establish an organism (e.g. a fungus, bacterium, or virus) as the cause of a disease. To satisfy Koch's postulates: (i) the potential agent of disease must be found in an infected host, (ii) the agent must be isolated from the infected host and grown in pure culture, (iii) the isolated agent must cause disease when introduced into a healthy host, and (iv) the agent must be re-isolated from the newly infected and diseased host while a negative control remains uninfected (Koch 1884; Isenberg 1988; Orélis-Ribeiro *et al.* 2010).

Koch's postulates are often used to identify primary agents of disease in wildlife. For example, *Pseudogymnoascus destructans* was confirmed as the primary agent of white-nose syndrome (WNS) in little brown bats by fulfilling Koch's postulates (Lorch *et al.* 2011). Similarly, Koch's postulates were used to confirm *Batrachochytrium dendrobatidis* as the primary agent of chytridiomycosis, a disease causing dramatic population declines and extinctions of numerous amphibian species (Longcore *et al.* 1999; Mitchell *et al.* 2008). Koch's postulates confirmed *Ophidiomyces ophiodiicola* as the primary agent of snake fungal disease (SFD; Lorch *et al.* 2015), and SFD currently

impacts multiple populations of different snake species across North America (Lorch *et al.* 2016; Burbrink *et al.* 2017).

The history of WNS, Chytridiomycosis, and SFD highlights the potential for YFD to spillover into wildlife with potentially devastating effects on naïve populations (Fisher *et al.* 2012). In this context, the apparently widespread distribution of *N. guarroi* and the lack of information on its ecology and physiology is disconcerting. Until recently, *N. guarroi* (formerly *Chrysosporium guarroi*) was considered to be a part of the *Chrysosporium* anamorph of *Nannizziopsis vreisii* (CANV) fungal complex. It is now understood to be a separate species and was described from strains isolated from captive green iguanas (*Iguana iguana*) in Spain (Abarca *et al.* 2008). However, to date, no experiment has tested the assumption that *N. guarroi* is the primary agent of YFD. We aimed to fulfill Koch's postulates with *N. guarroi* by conducting a challenge experiment with captive-bred juvenile bearded dragons to determine if the fungus is the primary agent of YFD. We are motivated in part by the potential for spillover effects from domestic populations into wildlife, particularly, naïve reptile populations (Thompson *et al.* 2009).

MATERIALS AND METHODS

Animal Husbandry

Our experimental protocols, including the protocols for husbandry and euthanasia, were approved by the University of Wisconsin Institutional Animal Care and Use Committee (IACUC; Approval number #V006070IACUC). We conducted live animal experiments at the Charmany Instructional Facility in Madison, Wisconsin, United States. We acquired seven, two- to three-month-old captive-bred bearded dragons (27 g–48 g) from a local vendor. At the Charmany facility, body condition, shedding, and, after

inoculation, infection progression was tracked daily throughout the experiment. Each bearded dragon was housed in an individual plastic tub with a ventilated cover (FIG. 1). Water was available *ad libitum*; shelter and enrichment in the form of climbable sticks were also provided. We randomly designated two bearded dragons as negative controls and isolated them in a separate room while the remaining five were held in a different room for future inoculation with *N. guarroi*. Control and experimental rooms were maintained between 30–35 C with humidity between 30–40%, parameters chosen to optimize fungal growth, and which were appropriate for bearded dragon habitats; no heat gradients were provided within the enclosures. Rooms were kept on a 12 h light/dark cycle and all animals were given eight days to acclimate to their new environment. Research veterinary staff conducted an initial physical and wellness check of the animals and determined all were healthy. We screened all animals for *N. guarroi* prior to the challenge study by swabbing each individual from snout tip to tail end on the dorsal and ventral sides of the body with fine tip rayon swabs (#MW113, Medical Wire & Equipment, Wiltshire, England) moistened with 25 µl of sterile distilled water. Each swab was plated on dermatophyte test media (DTM) and incubated at 24 C for 20 days. All animals were found free of *N. guarroi*.

Inoculation

As an inoculant, we used a pure culture of a strain of *N. guarroi* obtained from the UAMH Centre for Global Microfungal Biodiversity culture collection (UAMH-10352); the culture was originally isolated from a bearded dragon with YFD in 2016 in Madison, Wisconsin, United States, and was grown from a single spore. The isolate was grown on Sabouraurd's Dextrose agar (SDA) and incubated at 24 C for 20 days. A conidial

suspension in phosphate-buffered saline containing 0.5% Tween 20 solution (84 250 conidia/ µl) was prepared as described in Lorch *et al.* (2015). The day of inoculation, the five bearded dragons to be inoculated were gently abraded with sterile sandpaper, creating a 1 cm^2 abrasion area at four sites (snout, dorsal-side neck, ventral-side midbody, and left ventral-side leg around the femoral pits; FIG. 2) following a similar protocol described in Lorch *et al.* (2015); two additional sites were left unabraded (dorsal-side midbody and ventral-side neck; FIG. 2). Each treatment animal was inoculated at each of the six sites with 4 µl of the *N. guarroi* conidial suspension. Following the same abrasion protocol, the negative controls were sham-inoculated with the same, but sterile saline solution.

Euthanasia, Necropsy, Histopathology

We euthanized animals if they developed skin lesions exceeding 1 cm in diameter, if there was a noticeable decline in body condition, or on the final day (day 52) of the experiment. Before euthanasia, each animal was anesthetized in an induction chamber using isoflurane gas. Animals were then injected intracoelomically with 0.1 ml of pentobarbital, a fatal dosage. Decapitation was then used per protocol ensuring death. Animals were necropsied by removing skin samples from each inoculated site (and from sham-inoculated sites), surrounding the 1 cm^2 abraded areas and non-abraded areas; samples were divided for DTM cultures and for histopathological analyses. Skin was removed even if there were no visible gross lesions or other presentation of clinical signs at the inoculated sites. Along with the skin samples, internal organs (heart, lung, kidney, spleen, pancreas, esophagus, stomach, and small and large intestines), decapitated heads, and inoculated legs were fixed in 10% neutral buffered formalin. After fixation,

heads and legs were decalcified in saturated ethylenediaminetetraacetic acid (EDTA) solution. Tissues for histology were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin-eosin (H&E) or periodic acid-Schiff (PAS; FIG. 3) depending on tissue type: internal organs were stained with H&E and skin samples were stained with PAS. Each slide was analyzed for fungal elements (hyphae and/or arthroconidia) by a board-certified veterinary pathologist using a constructed histological scale of none, few, moderate, or many.

Sequencing

Inoculated and sham-inoculated skin samples were cultured on DTM at 30 C for 20 days. Any fungal or bacterial growth from any skin sample was isolated and subcultured on SDA (FIG. 4). To extract fungal DNA from each subculture, a 0.5 cm diameter plug of fungal material was scraped from the subculture using a sterile scalpel and put in a 2 ml screw-cap tube with 3 mm diameter glass beads; tissue was macerated using a mini-beadbeater (Product number: 112011, BioSpec Products, Bartlesville, Oklahoma). Macerated tissues were subsequently placed in 600 µl of cetyltrimethylammonium bromide (CTAB) buffer and incubated at 65 C for 1 h. We then added 600 µl of chloroform isoamyl alcohol to each tube, mixing it with the CTAB and then centrifuging at 13 000 rpm for 10 min. Supernatants were pipetted off and placed in new, sterile 2 ml Eppendorf tubes; we added 600 µl chloroform isoamyl alcohol to each tube and gently rocked tubes on a tipping tray for 10 min. Next, tubes were centrifuged a second time. Following centrifugation, iced 96% EtOH was added at 2x the total sample volume to wash the extracted DNA. We washed each DNA pellet twice and then dried the pellets in a DNA SpeedVac system for 30 min before adding 400 µl of Tris-EDTA (TE)

buffer and storing extracted DNA in the -20 C freezer to be used for downstream PCR. Bacterial DNA was extracted using the Qiagen DNEasy Powersoil kit (Germantown, Maryland) following the manufacturer's protocol.

We conducted PCR for Sanger sequencing on all extracted DNA. For fungi, we amplified the internal transcribed spacer (ITS) region in fungal samples using standard fungal primers, ITS1-F and ITS4 (Manter and Vivanco 2007), EconoTaq Plus 2X Master Mix, and distilled water. PCR reactions used the following parameters: 2 min at 95 C, 15 s at 95 C (30 cycles), 15 s at 49 C, 1 min at 68 C, 5 min at 68 C. For bacteria, we amplified the 16S ribosomal region using primers rp2 and fD1 (Weisburg *et al.* 1991) and PCR reactions used the following parameters: 5 min at 95 C, 1 min at 94 C (25 cycles), 1 min at 59 C, 2 min at 72 C, and 5 min at 72 C.

We submitted the ITS and 16S amplicons to a third-party vendor for Sanger sequencing (Functional Biosciences Madison, Wisconsin), receiving the ab1 files and then visually inspecting and trimming the chromatographs to verify the quality of each of our samples. To determine the species of fungi and bacteria we cultured, we next used BLAST (Basic Local Alignment Search Tool; Altschul *et al.* 1990) to compare our sequences with sequences stored in GenBank (Benson *et al.* 2016). Subsequently, we deposited four sequences into GenBank (acc. No. MT503283, MT503284, MT503285, and MT503223), one of which (MT503283) represents our *N. guarroi* cultures (every culture yielded an identical ITS sequence); sequences are described in more detail in results.

RESULTS

By day 52 post-inoculation, all lizards exposed to *N. guarroi* had developed clinical signs of infection; the negative control animals developed no clinical signs of infection. The initial clinical signs appeared between 15–31 days post-inoculation (FIG. 5). Initial clinical signs were discolored, yellow scales at inoculated sites, often on the ventral midbody and around the femoral pits. Affected areas of skin eventually became swollen, thickened, and darker in coloration. Following are the observed onset of clinical signs for each bearded dragon inoculated with *N. guarroi* summarized from our daily logs; individual animals are identified as BD8N, BD9N, BD10N, BD12N, and BD11N. Animal BD8N developed a single lesion on the ventral side of the neck, 31 days after inoculation. The lesion did not swell or become necrotic by the end of the experiment. Animal BD9N also developed a lesion on the ventral side of the neck 31 days after inoculation which did not swell or become necrotic by the end of the experiment, similar to BD8N. However, BD9N developed an additional lesion on the tail, which was not a site we abraded or inoculated. Animals BD10N and BD12N developed lesions on the ventral midbody and femoral pits 20 days after inoculation both of which progressed into swelling and necrosis at the inoculated sites by the end of the experiment. Animal BD11N developed necrotic lesions on the femoral pits and across the snout 15 days after inoculation, which progressed into the eye causing swelling and a thickening of the scales around the eye. Due to its rapidly declining body condition, BD11N was euthanized 22 days after inoculation and did not reach the end of the experiment. Among the five treated bearded dragons, there was no consistent pattern between abraded and non-abraded sites as lesions formed in both kinds of (but not all) sites. Ecdysis (shedding of the skin) was infrequent during the experiment. Some animals completed a full shed within 2–3 days

while others shed but retained skin remnants for up to 14 days. However, regardless of whether shedding events were complete or incomplete, clinical signs persisted, even after shedding.

Histological analyses confirmed a positive infection in four of the five treated bearded dragons. Fungal elements (hyphae and/or arthroconidia) were present in epidermal, dermal, and/or muscle tissue (FIG. 3; FIG. 5). Microscopically, skin lesions were consistent with those previously reported for YFD in bearded dragons (Bowman *et al.* 2007; Hedley *et al.* 2010; Paré and Jacobson 2007). Lesions were characterized by epidermal ulceration, epidermal and dermal necrosis, and histiocytic to granulomatous dermatitis, panniculitis, and myositis. Lesions contained moderate to large numbers of 3–5 µm diameter, parallel-walled, septate, rarely branching fungal hyphae extending from the epidermis to the underlying muscular layer. Rarely, fungal hyphae were found within blood vessels of the dermis and subcutis. Cylindrical 2 x 3 µm conidia were occasionally present in clusters on the skin surface. In some areas, infection was limited to the subcutis and muscle with minimal involvement of the epidermis and dermis. Mature granulomas as described in previous reports were not seen, probably because of the relatively short duration of the infection trial. In the five treated bearded dragons, none of the internal organ samples had fungal elements, indicating no sign of systemic infection; the negative controls had no fungal elements present in any tissue samples.

Nannizziopsis guarroi was re-isolated in culture from 24 of the 30 skin samples taken from the 30 inoculated sites of the five bearded dragons exposed to the fungus (FIG. 4; TABLE 1). Sequencing data confirmed that every culture of *N. guarroi* was identical to the initial strain used during inoculation. Six of the 30 skin samples (1 from

BD8N, 2 from BD9N, 1 from BD11N, and 2 from BD12N) did not grow fungi when cultured (TABLE 2). *Neosetophoma guiyangensis* was cultured from a single skin sample of one bearded dragon (BD8N; TABLE 2) from which *N. guarroi* was also cultured; we deposited the *N. guiyangensis* sequence into GenBank (MT503284). *Neosetophoma* is a genus of fungal endophytes and saprobes associated with plant hosts and is not known to be pathogenic to animals (Karunarathna *et al.* 2017; Hyde *et al.* 2018). The skin samples from the negative controls never generated *N. guarroi* in culture. However, *Chaetomium globosum* and a *Kocuria sp.* were isolated in culture from one negative control animal (BD1NC; TABLE 2); we deposited sequences of both cultures into GenBank (MT503285 and MT503223, respectively). Neither organism is a known pathogen of bearded dragons; *C. globosum* is a cosmopolitan fungal endophyte and *Kocuria sp.* is a Gram-positive bacterium that occurs on the skin of humans and animals without inciting disease (Wang *et al.* 2016; Kandi *et al.* 2016). The negative control samples containing *C. globosum* and the *Kocuria sp.* did not present any internal or external signs of infection or abnormal clinical signs that would suggest disease from those specific microbes; no histopathological evidence of any of these microbes was seen.

DISCUSSION

By fulfilling Koch's postulates our work definitively demonstrates *N. guarroi* as the primary agent of YFD in bearded dragons: all five treatment animals developed clinical signs of YFD and *N. guarroi* was re-isolated from each animal. While, in this case, Koch's postulates is a useful criteria to determine infection capability of a single microbe in one host species, our experiment cannot elude to what YFD infection could look like in other

potential reptilian species; such findings would require additional challenge experiments with different species.

Nannizziopsis guarroi persisted at the inoculated sites throughout the experiment and was re-isolated from 80% of inoculated sites. However, results of the histopathological analyses and re-isolation were not uniform across each treatment animal or individual inoculation site (TABLE 1). For example, a bearded dragon sometimes did not present histological evidence of infection at an inoculation site from which *N. guarroi* was cultured. Conversely, a bearded dragon sometimes presented histological evidence at a site from which *N. guarroi* was not re-isolated. In the case of one bearded dragon, a lesion formed on the tail, a location that was neither abraded nor inoculated. Our results highlight the variability in clinical signs of YFD: three animals developed swelling and lesions at inoculated sites 20 days after inoculation while two remained clinically normal for 41 days post-inoculation, 4 days before euthanasia. The most likely explanation for both the variability among sites and the two bearded dragons with a late onset of clinical signs is early-stage infection. Had the experiment continued, the early-stage infections associated with a relatively late onset of clinical signs would likely have progressed into a more severe disease.

Observed variability may also be explained by the texture, topography, and coloration of bearded dragon skin, which can make it difficult to observe abnormalities, including lesions, especially when gross lesions are < 1 cm in diameter. Because of the relatively small sizes of some lesions, it was difficult to divide samples and ensure that a portion of the lesion was analyzed both histologically and by culture. Moreover, capturing

the infection within any lesion histologically can be difficult because the thin sections viewed microscopically represent a very small portion of sampled tissue.

Nannizziopsis guarroi was able to be re-isolated even after the bearded dragons underwent ecdysis (shedding of the skin). In some animals shed remnants adhered to the new epidermis for as long as 14 days, and this time may have allowed the pathogen to establish into deeper tissues or enabled re-infection of the new epidermis. Epidermal abrasion does not appear to be a requirement for infection. Paré *et al.* (2006) demonstrated that breakage of the epidermis was an important variable for the development of infection using an isolate of a *Nannizziopsis sp.* on chameleons (*Chamaeleo calyptratus*). In our study infection also appeared to involve epidermal breakage as both visible gross lesions and histopathological evidence of disease were predominantly seen on abraded areas. However, there was also evidence of fungal elements in non-abraded areas, implying that an intact epidermis is not a completely effective deterrent to infection. Although epidermal breakage may facilitate infection and can serve as a primary route for invasion, fungal presence in non-abraded areas highlights the ability of *N. guarroi* to colonize even unabraded skin, similar to *O. ophiodiicola* (Lorch *et al.* 2015). Had the experiment continued, we hypothesize that disease would have appeared and progressed at all inoculated sites on every individual animal, abraded and unabraded. The dorsal side of the epidermis of bearded dragons is more heavily keratinized (it is thicker) than the ventral side, a feature which makes abrasions more likely to occur on the abdomen. The thickness of the dorsal side of the epidermis may slow the rate of fungal invasion which may explain why we found fungal elements within dorsal tissue but did not see visible clinical signs of infection in that area.

The ecology of *N.* guarroi is poorly understood. Apart from optimal growth temperatures little is known (Abarca *et al.* 2008a, 2009b). Closely related pathogenic fungi, including *O. ophiodiicola,* are thought to persist in environments outside of host animals, often establishing in soil which acts as a reservoir of infection (Guthrie *et al.* 2016). Reservoirs of potentially infectious material may facilitate consistent exposure to a host, continual reinfection, and potentially a more systemic and fatal disease. *Nannizziopsis guarroi* can generally tolerate warmer temperatures than such pathogens as *O. ophiodiicola* and *B. dendrobatidis* or *Batrachochytrium salamandrivorans* (Allender *et al.* 2015; Blooi *et al.* 2015), which may allow it to persist for longer periods of time and in a greater range of environments in the absence of a host. Research into *N. guarroi's* native geographic range, natural history, and ecology is critically needed to enable an understanding of the transmission and epidemiology of YFD.

Fungal pathogens often appear opportunistic in nature, primarily causing infections in hosts with pre-existing health problems and, in the case of *N. guarroi,* also in animals kept under poor husbandry conditions (Cabañes *et al.* 2014; Le Donne *et al.* 2016). However, although declining body conditions and suboptimal environments will certainly influence the onset of infection, the former CANV fungi (e.g. *O. ophiodiicola, N. guarroi*, *N. barbatae*) have increasingly been demonstrated to function as primary agents of diseases in various reptile species (Cabañes *et al.* 2014; Han *et al.* 2010). Furthermore, recent case studies of yellow fungus disease have eluded to the acceptance that *N. guarroi,* as well as other *Nannizziopsis* species, does not primarily act as an opportunistic fungus on its associated (now confirmed) hosts, identifying infection as aggressive

(Murillo *et al.* 2022; Paré *et al.* 2021). As we demonstrate here, *N. guarroi* also appears to be a primary agent of disease.

Nannizziopsis guarroi has never been discovered in a wild host and is only known from captive animals. Case reports of *N. guarroi* associated with fatal infections and implicating the fungus as a primary pathogen span 1997–2018, and disease has been described from multiple species of lizards in captivity across North America, Europe, Korea, and Australia, but without any discussion of how dispersal might occur or whether there is potential for the fungus to spillover and establish as an invasive pathogen in ecosystems (Fisher *et al.* 2020). If the fungus were to spillover into wild populations, it has the potential to cause significant morbidity and mortality to naïve populations of other reptile species. Although degrees of resistance have been documented for animals exposed to other fungal pathogens (e.g. Archey's frog, *Lepiopelma archeyi*, is less susceptible to *B. dendrobatidis* than other frog species, and the Big Brown Bat, *Eptesicus fuscus,* is resistant to infection by *P. destructans* (Frank *et al.* 2014; Savage and Zamudio 2011; Palmer *et al.* 2018), whether different reptile species vary in susceptibility to *N. guarroi* is unknown. However, the methods presented here could be used to establish differential susceptibility experimentally; the true risk that YFD poses to wild reptile populations requires further study.

Species known to be susceptible to *N. guarroi* are popular species commonly found in the pet trade (i.e. chameleons, green iguanas, and bearded dragons), and trade provides opportunities for the pathogen to be moved long distances and to jump to new hosts. Roughly 350 million live animals are imported and exported across the globe annually; many are poached from the wild (Warwick 2014). The potential spillover of YFD

into naïve populations is an imminent threat. Our findings are a first step toward illustrating the importance of *N. guarroi* as a primary pathogen and emphasize the need for additional research to better understand the ecology and impact of the fungus on reptile populations worldwide.

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Literature Cited

1. Abarca ML, Castellá G, Martorell J, Cabañes FJ. 2010. *Chrysosporium guarroi* sp. nov. a new emerging pathogen of pet green iguanas (*Iguana iguana*). Medical Mycology 48:365–72.

2. Abarca ML, Martorell J, Castellá G, Ramis A, Canañes FJ. 2009. Dermatomycosis in a pet inland bearded dragon (*Pogona vitticeps*) caused by a *Chrysosporium* species related to *Nannizziopsis vriesii*. Veterinary Dermatology 20:295–299.

3. Abarca ML, Martorell J, Castellá G, Ramis A, Canañes FJ. 2008. Cutaneous hyalohyphomycosis caused by a *Chrysosporium* species related to *Nannizziopsis vriesii* in two green iguanas (*Iguana iguana*). Medical Mycology 46:349–354.

4. Allender MC, Raudabaugh DB, Gleason FH, Miller AN. 2015. The natural history, ecology, and epidemiology of *Ophidiomyces ophiodiicola* and its potential impact on free-ranging snake populations. Fungal Ecology 17:187–196.

5. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

6. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2016. GenBank. Nucleic Acids Research 45:D37–D47.

7. Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, Buckles EL, Coleman JTH, Darling SR, Gargas A, Niver R, Okoniewski JC, Rudd RJ, Stone WB. 2009. Bat white-nose syndrome: an emerging fungal pathogen? Science 323:227.

8. Blooi M, Martel A, Haesebrouck F, Vercammen F, Bonte D, Pasmans F. 2015. Treatment of urodelans based on temperature dependent infection dynamics of *Batrachochytrium salamandrivorans*. Scientific Reports 5:1–4.

9. Bowman MR, Paré JA, Sigler L, Naeser JP, Sladky KK, Hanley CS, Helmer P, Phillips LA, Browers A & Porter R. 2007. Deep fungal dermatitis in three inland bearded dragons (*Pogona vitticeps*) caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii.* Medical Mycology 45:371–376.

10. Burbrink FT, Lorch JM, Lips KR. 2017. Host susceptibility to snake fungal disease is highly dispersed across phylogenetic and functional trait space. Science Advances 3:1–9.

11. Cabañes FJ, Sutton DA, Guarroi J. 2014. *Chrysosporium*-related fungi and reptiles: a fatal attraction. Plos Pathogens 10:1–4.

12. Fisher MC, Gurr SJ, Cuomo CA, Blehert DS, Jin H, Stukenbrock EH, Stajich JE, Kahmann R, Boone C, Denning DW, Grow NAR, Klein BS, Kronstad JW, Sheppard DC, Taylor JW, Wright GD, Heitman J, Casadevall A, Cowen LE. 2020. Threats posed by the fungal kingdom to humans, wildlife, and agriculture. American Society for Microbiology mBio 11:1–17.

13. Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ. 2012. Emerging fungal threats to animal, plant, and ecosystem health. Nature 484:186–194.

14. Frank CL, Michalski A, McDonough AA, Rahimian M, Rudd RJ, Herzog C. 2014. The resistance of a North America bat species (*Eptesicus fuscus*) to white-nose syndrome (WNS). Plos One 9:1–14.

15. Guthrie AL, Knowles S, Ballmann AE, Lorch JM. 2016 Detection of snake fungal disease due to *Ophidiomyces ophiodiicola* in Virginia, USA. Journal of Wildlife Diseases 52:143–149.

16. Han JI, Lee SJ, Na KJ. 2010. Necrotizing dermatomycosis caused by *Chrysosporium* spp. in three captive green iguanas (*Iguana iguana*) in South Korea. Journal of Exotic Pet Medicine 19:240–244.

17. Hedley J, Eatwell K, Hume L. 2010. Necrotising fungal dermatitis in a group of bearded dragons (*Pogona vitticeps*). Veterinary Record 166:464–465.

18. Hyde KD, Chaiwan N, Norphanphoun C, Boonmee S, Camporesi E, Chethana KWT, Dayarathne MV, de Silva NI, Dissanayake AJ, Ekanayaka AH, Hongsanan S, Huang SK, Jayasiri SC, Jayawardena RS, Jiang HB, Karunarathna A, Lin CG, Liu NG, Lu YZ, Luo ZL, Maharachchimbura SSN, Manawasinghe IS, Pem D, Perera RH, Phukhamsakda C, Samarakoon MC, Senwanna C, Shang QJ, Tennakoon DS, Thambugala KM, Tibpromma S, Wanasinghe DN, Xiao YP, Yang J, Zeng XY, Zhang JF, Zhang SN, Bulgakov TS, Bhat DJ, Cheewangkoon R, Goh TK, Jones EBG, Kang JC, Jeewon R, Liu ZY, Lumyong S, Kuo CH, McKenzie EHC, Wen TC, Yan JY, Zhao Q. 2018. Mycosphere notes. Mycosphere 9:169–224.

19. Isenberg HD. 1988. Pathogenicity and virulence: another view. Clinical Microbiology Reviews 1:40–53.

20. Johnson RSP, Sangster CR, Sigler L, Hambleton S, Paré JA. 2011. Deep fungal dermatitis caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* in captive coastal bearded dragons (*Pogona barbata*). Journal of the Australian Veterinary Association LTD 89:515–519.

21. Kandi V, Palange P, Vaish R, Bhatti AB, Kale V, Kandi MR, Bhoomagiri MR. 2016. Emerging bacterial infection: identification and clinical significance of *Kocuria* species. Cureus 8:1–6.

22. Karunarathna A, Papizadeh M, Senanayake IC, Jeewon R, Phookamsak R, Goonasekara ID, Wanasinghe DN, Wijayawardene NN, Amoozegar MA, Shahzadeh Fazeli SA, Camporesi E, Hyde KD, Weerahewa HLD, Lumyong S, McKenzie EHC. 2017. Novel fungal species of *Phaeosphaeriaceae* with an asexual/sexual morph connection. Mycosphere 8:1818–1834.

22. Koch R. 1884. Die aetiology der tuberkulosa. Mitt Kaiser Gesundh 2:1–88.

23. Le Donne V, Crossland N, Brandão J, Sokolova Y, Fowlkes N, Nevarez JG, Langohr IM, Gaunt SD. 2016. *Nannizziopsis guarroi* infection in 2 inland bearded dragons (*Pogona vitticeps*): clinical, cytologic, histologic, and ultrastructural aspects. Veterinary Clinical Pathology 45:368–375.

24. Longcore JE, Pessier AP, Nichols DK. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227.

25. Lorch JM, Knowles S, Lankton JS, Michell K, Edwards JL, Kapfer JM, Staffen RA, Wild ER, Schmidt KZ, Ballmann AE, Blodgett D, Farrell TM, Glorioso BM, Last LA, Price SJ, Schuler KL, Smith CE, Wellehan JFX, Blehert DS. 2016. Snake fungal disease: an emerging threat to wild snakes. Philosophical Transactions of The Royal Society Biological Sciences 371:1–8.

26. Lorch JM, Lankton J, Werner K, Falendysz EA, McCurley K, Blehert DS. 2015. Experimental infection of snakes with *Ophidiomyces ophiodiicola* causes pathological changes that typify snake fungal disease. American Society for Microbiology mBio 6:1–9.

27. Lorch JM, Meteyer CU, Behr MJ, Boyles JG, Cryan PM, Hicks AC, Ballmann AE, Coleman JTH, Redell DN, Reeder DM, Blehert DS. 2011. Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. Nature 480:376–378.

28. Manter DK and Vivanco JM. 2007. Use of the ITS primers, ITS1F and ITS4 to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. Journal of Microbiological Methods 71:7–14.

29. Mitchell KM, Churcher TS, Garner TWJ, Fisher MC. 2008. Persistence of the emerging pathogen *Batrachochytrium dendrobatidis* outside the amphibian host greatly increases the probability of host extinction. Proceedings of The Royal Society Biological Sciences 275:329–334.

30. Murillo DFB, Watanabe TTN, Perry S, Mitchell M, Del Perio F. 2022. Yellow fungus disease in a bearded dragon. Brazilian Journal of Veterinary Pathology 15:1 62–64.

31. Orélis-Ribeiro R, Boeger WA, Vicente VA, Chammas M, Ostrensky A. 2010. Fulfilling Koch's postulates confirms the mycotic origin of lethargic crab disease. Antonie van Leeuwenhoek Journal of Microbiology 99:601–608.

32. Palmer JM, Drees KP, Foster JT, Lindner DL. 2018. Extreme sensitivity to ultraviolet light in the fungal pathogen causing white-nose syndrome of bats. Nature Communications 9:1–10.

33. Paré JA, Wellehan J, Perry SM, Scheelings TF, Keller K, Boyer T. 2021. Onygenalean dermatomycoses (formerly yellow fungus disease, snake fungal disease) in reptiles. Journal of Herpetological Medicine and Surgery 30:4 198–209.

34. Paré JA and Sigler L. 2016. An overview of reptile fungal pathogens in genera *Nannizziopsis*, *Paranannizziopsis*, and *Ophidiomyces*. Journal of Herpetological Medicine and Surgery 26:46–53.

35. Paré JA and Jacobson ER. 2007. Infectious Diseases and Pathology of Reptiles. In: Jacobson ER, ed. Mycotic diseases of reptiles. Boca Raton, Florida: CRC Press. p. 533–534.

36. Paré JA, Coyle KA, Sigler L, Maas AK, III, Mitchell RL. 2006. Pathogenicity of the Chrysosporium anamorph of *Nannizziopsis vriesii* for veiled chameleons (*Chamaeleo calyptratus*). Medical Mycology 44:25–31.

37. Paré JA, Sigler L, Hunter B, Summerbell RC, Smith DA, Machin KL. 1997. Cutaneous mycoses in chameleons caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (apinis) currah. Journal of Zoo and Wildlife Medicine 28:443–53.

Savage AE and Zamudio KR. 2011. MHC genotypes associate with resistance to a frog-killing fungus. Proceedings of the National Academy of Sciences of the United States of America 108:16705–16710.

38. Schmidt-Ukaj S, Loncaric I, Spergser J, Richter B, Hochleithner M. 2016. Dermatomycosis in three central bearded dragons (*Pogona vitticeps*) associated with *Nannizziopsis chlamydospora*. Journal of Veterinary Diagnostic Investigation 28:319–322.

39. Schmidt-Ukaj S, Loncaric I, Klang A, Spergser J, Häbich AC, Knotek Z. 2014. Infection with *Devriesea agamarum* and *Chrysosporium guarroi* in an inland bearded dragon (*Pogona vitticeps*). Veterinary Dermatology 25:555–558.

40. Schneider J, Heydel T, Klasen L, Pees M, Schrödl W, Schmidt V. 2018. Characterization of *Nannizziopsis guarroi* with genomic and proteomic analysis in three lizard species. Medical Mycology 56:610–620.

41. Sigler L, Hambleton S, Paré JA. 2013. Molecular characterization of reptile pathogens currently known as members of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* complex and relationship with some human-associated isolates. Journal of Clinical Microbiology 51:3338–3357.

42. Skomorucha Ł, Marciniak M, Sobczak-Filipiak M. 2014. Pierwszy w polsce przypadek żółtej grzybicy u agamy brodatej. Życie Weterynaryjne 89:5 414–418

43. Thompson RCA, Kutz SJ, Smith A. 2009. Parasite zoonoses and wildlife: emerging issues. Environmental Research and Public Health 6:678–693.

44. Waeyenberghe LV, Baert K, Pasmans F, van Rooij P, Hellebuyck T, Beernaert L, de Backer P, Haesebrouck F, Martel A. 2010. Voriconazole, a safe alternative for treating infections caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* in bearded dragons (*Pogona vitticeps*). Medical Mycology 48:880–885.

45. Wang XW, Lombard L, Groenewald JZ, Li J, Videira SIR, Samson RA, Liu XZ, Crous PW. 2016. Phylogenetic reassessment of the *Chaetomium globosum* species complex. Persoonia 36:83–133.

46. Warwick C. 2014. The morality of the reptile “pet” trade. Biological Conservation 204:103–119.

47. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173:697–703.

FIGURES & TABLES

Figure 1. Animal enclosure.

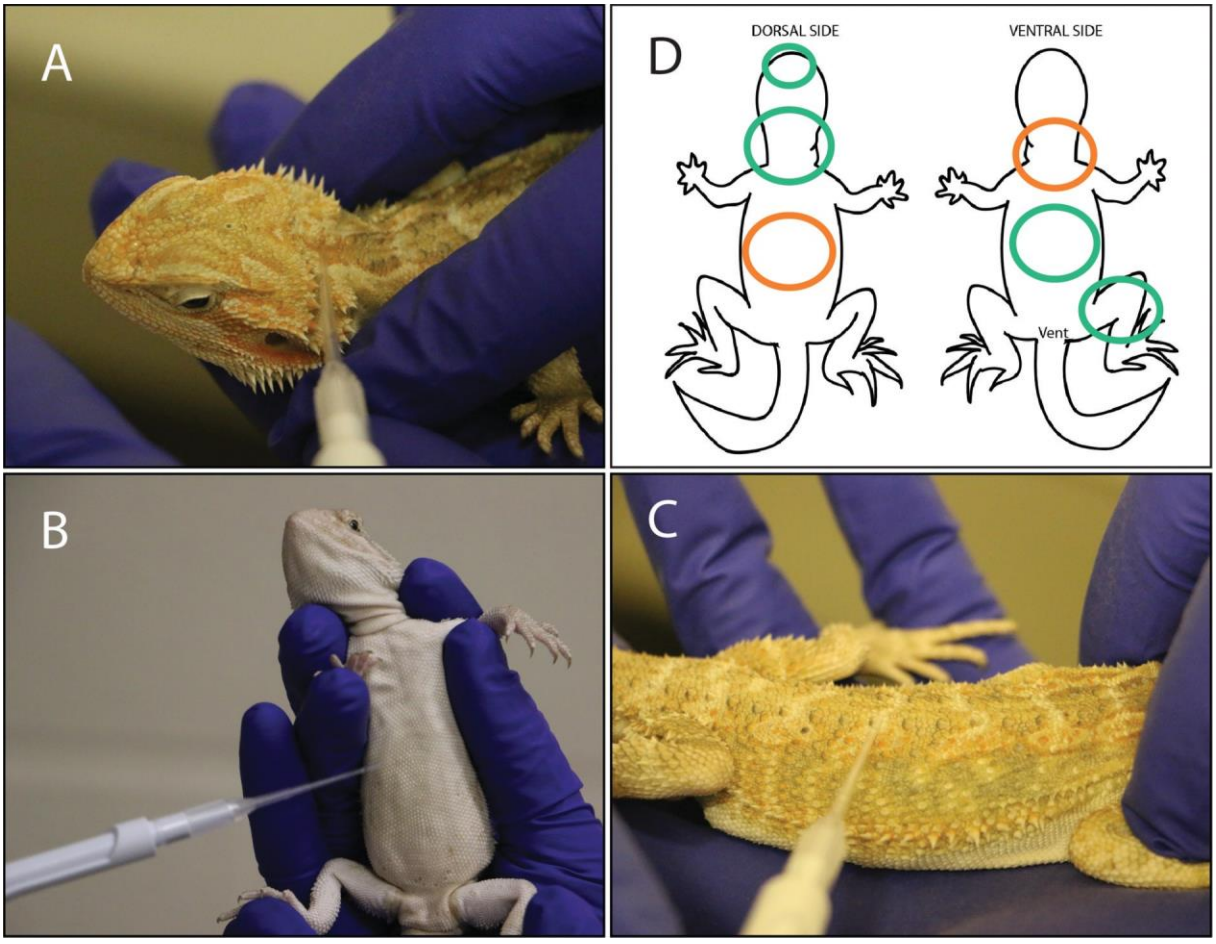

Figure 2. Inoculation of bearded dragons with *N. guarroi*. A – C. Photographs of the inoculation process using 4 µl of *N. guarroi* to inoculate sites on individual bearded dragons. D. Circles mark each of the 6 inoculation sites across the dorsal and ventral sides of the animal; green circles

represent abraded sites and orange circles represent sites left unabraded. The “vent” label distinguishes between the dorsal and ventral sides.

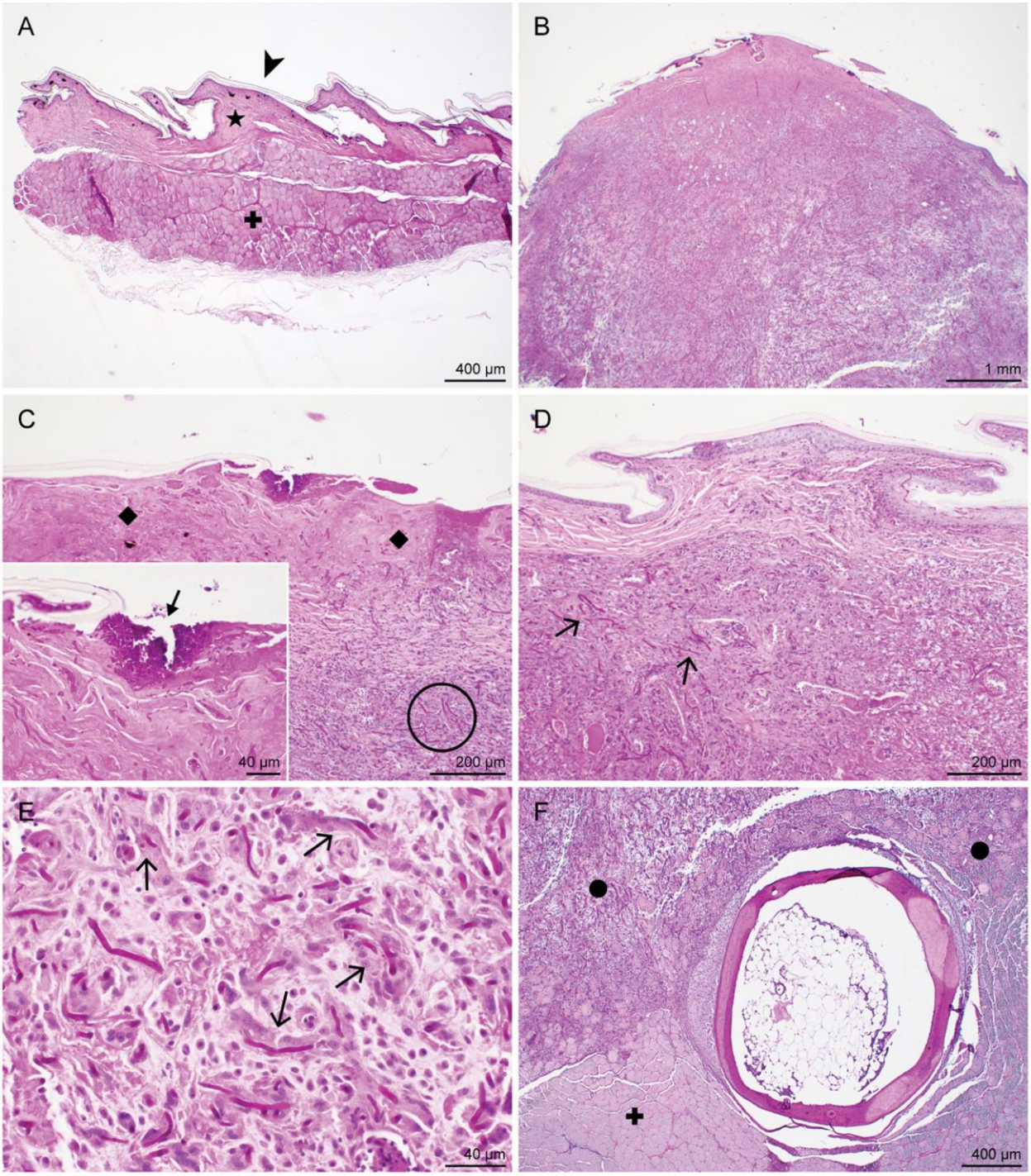

Figure 3. Histological images of tissue samples from control (A) and inoculated (B-F) sites stained with periodic acid-Schiff stain. (A) Dorsal neck, BD1NC, Negative control. A thin epidermis and stratum corneum (arrowhead) overlie a sparsely cellular dermis (star) and thick muscular layer (cross). (B) Leg, BD12N. The epidermis, dermis, and muscular layers are obscured and replaced by widespread necrosis and inflammation. (C) Leg, BD10N. There is marked epidermal and dermal necrosis (diamonds) and granulomatous dermatitis with intralesional fungal hyphae

(circle) and conidia; inset – a cluster of conidia (filled arrow) fills an ulcer. (D) Ventral body, BD12N. There is deep granulomatous dermatitis with intralesional fungal hyphae (lined arrows). (E) Ventral body, BD12N. Fungal hyphae within the dermis are surrounded by macrophages (lined arrows). (F) Leg, BD12N. There is marked necrotizing myositis (filled circles) extending to the femur. Only a small portion of unaffected muscle remains (cross).

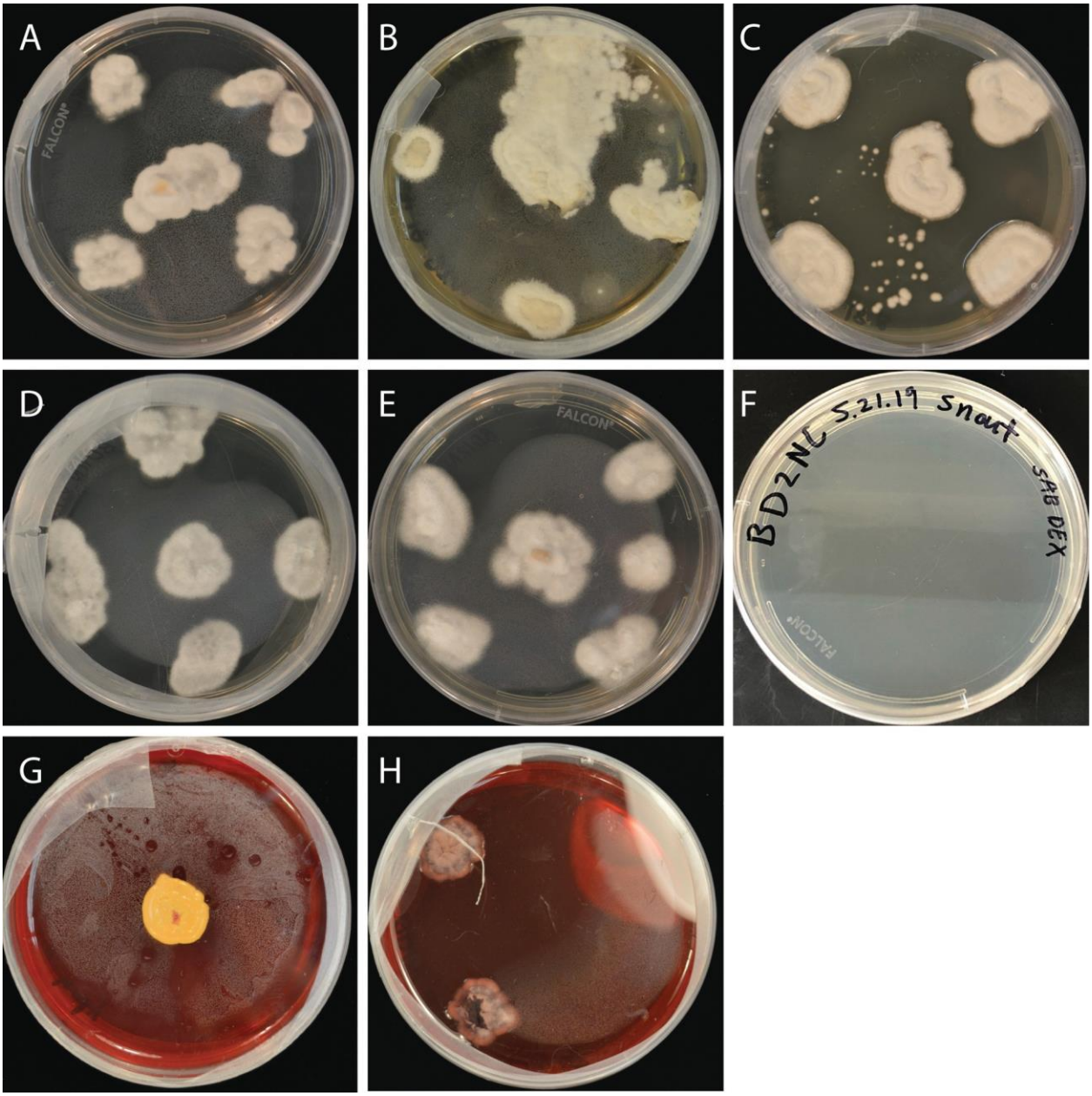

Figure 4. Culture results from each of the seven bearded dragons. A – E. Cultures of *N. guarroi* maintained on SDA isolated from diseased bearded dragons. F. Plate of SDA with no growth isolated from a negative control, BD2NC. G and H. Cultures of the *Kocuria sp.* and *C. globosum*, respectively, on DTM, isolated from the negative control, BD1NC.

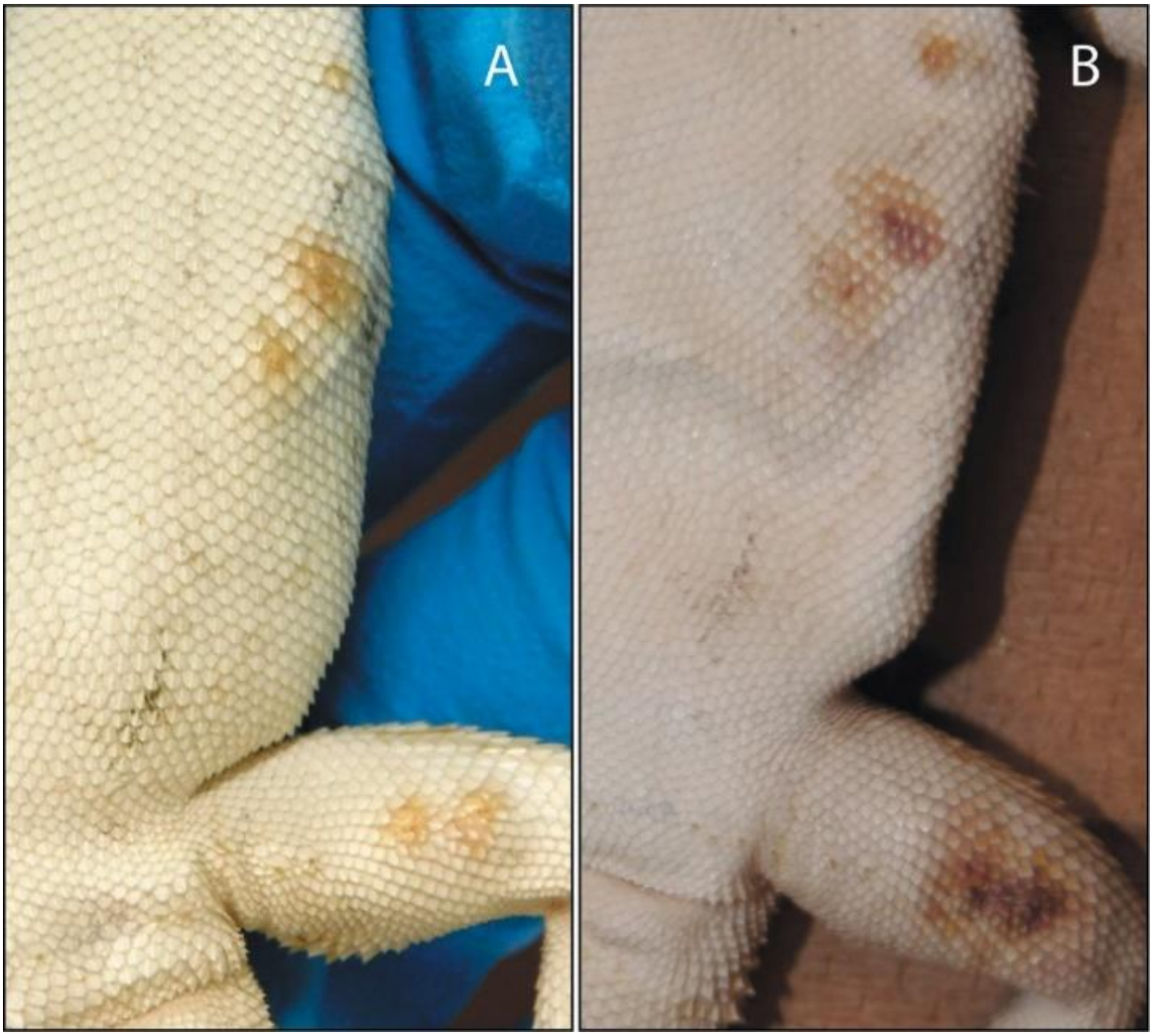

Figure 5. Disease progression on an individual bearded dragon photographed on different days. A and B. Manifestation of clinical signs of infection 20 days and 35 days post-inoculation, respectively.

Table 1. Clinical signs, histological, and re-isolation results of *Nannizziopsis guarroi*.

Animal ID	Treatment	Clinical Signs	Histopathology	Re-Isolation
Inoculated Sites				
Snout*				
BD1NC	Negative Control	-	-	-
BD2NC	Negative Control	-	-	-
BD8N	*N. guarroi*	-	-	+
BD9N	*N. guarroi*	-	-	+
BD10N	*N. guarroi*	-	-	+
BD11N	*N. guarroi*	+	-	+
BD12N	*N. guarroi*	-	-	-
Dorsal Neck*				
BD1NC	Negative Control	-	-	-
BD2NC	Negative Control	-	-	-
BD8N	*N. guarroi*	-	-	+
BD9N	*N. guarroi*	-	+	+
BD10N	*N. guarroi*	-	-	+
BD11N	*N. guarroi*	-	-	+
BD12N	*N. guarroi*	-	-	+
Ventral Neck				
BD1NC	Negative Control	-	-	-
BD2NC	Negative Control	-	-	-
BD8N	*N. guarroi*	+	-	+
BD9N	*N. guarroi*	+	+	+
BD10N	*N. guarroi*	-	-	+
BD11N	*N. guarroi*	-	-	+
BD12N	*N. guarroi*	-	-	-
Dorsal Midbody				
BD1NC	Negative Control	-	-	-
BD2NC	Negative Control	-	-	-
BD8N	*N. guarroi*	-	-	+
BD9N	*N. guarroi*	-	+	-
BD10N	*N. guarroi*	-	-	+
BD11N	*N. guarroi*	-	-	-
BD12N	*N. guarroi*	-	-	+
Ventral Midbody*				
BD1NC	Negative Control	-	-	-

*Inoculated site that was abraded before inoculation. (-) None observed. (+) Positive observation.

Table 2. *Simplified* - Cultured fungi and bacteria isolated from inoculated sites.

Inoculated Sites							
Animal ID	**Treatment**	**Snout**	**Dorsal Neck**	**Ventral Neck**	**Dorsal Midbody**	**Ventral Midbody**	**Ventral Hindleg**
BD1NC	Negative Control				*Chaetomium globosum*	*Kocuria* sp.	
BD2NC	Negative Control						
BD8N	*N. guarroi*	*Nannizziopsis guarroi*	*Neosetophoma guiyangensis* *Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	
BD9N	*N. guarroi*		*Nannizziopsis guarroi*			*Nannizziopsis guarroi*	
BD10N	*N. guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*
BD11N	*N. guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*		*Nannizziopsis guarroi*	*Nannizziopsis guarroi*
BD12N	*N. guarroi*	*Nannizzioppsis guarroi*	*Nannizziopsis guarroi*		*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*

*BD9N developed a lesion on its tail from which a culture was taken, resulting in a positive identification of *N. guarroi*. BD11N developed a lesion near the eye and a part was taken for culture and identified as *N. guarroi*. BD2NC had no microbes isolated from any site. Grey boxes indicate a location where no microbe was isolated. Refer to Gentry *et al.* 2021. Mycologia 113:1253-1263 for the non-simplified table.

Chapter II – A cross inoculation experiment reveals *Ophidiomyces ophiodiicola* and *Nannizziopsis guarroi* can each infect both snakes and lizards.

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Abstract

Host range and specificity are key concepts in the study of infectious diseases. However, both concepts remain largely undefined for many influential pathogens, including many fungi within the *Onygenales* order. This order encompasses reptile-infecting genera (*Nannizziopsis, Ophidiomyces*, and *Paranannizziopsis*) formerly classified as the *Chrysosporium* anamorph of *Nannizziopsis viresii* (CANV). Reported hosts for many of these fungi represent a narrow range of phylogenetically related animals, suggesting many of these disease-causing fungi are host-specific, but the true number of species affected by these pathogens is unknown. For example, to date, *Nannizziopsis guarroi* (the causative agent of yellow fungus disease) and *Ophidiomyces ophiodiicola* (the causative agent of snake fungal disease) have only been documented in lizards and snakes, respectively. In a 52-day reciprocal infection experiment, we tested the ability of these two pathogens to infect currently unreported hosts, inoculating central bearded dragons (*Pogona vitticeps*) with *O. ophiodiicola* and corn snakes (*Pantherophis guttatus*) with *N. guarroi*. We confirmed infection by documenting both clinical signs and histopathological evidence of fungal infection. Our reciprocity experiment resulted in 100% of corn snakes and 60% of bearded dragons developing infection with *N. guarroi* and *O. ophiodiicola*,

respectively, demonstrating that these fungal pathogens have a broader host range than previously thought, and that hosts with cryptic infections may play a role in pathogen translocation and transmission.

GENERAL AUDIENCE

When it comes to understanding fungal pathogens responsible for wildlife diseases, it is important to understand the number of hosts one pathogen can infect. Snake fungal disease (SFD), caused by the fungus *Ophidiomyces ophiodiicola*, and yellow fungus disease (YFD), caused by the fungus *Nannizziopsis guarroi*, affect snakes and lizards, respectively. The fungal infections of SFD and YFD can be fatal for wild and captive reptiles, and are known to be contagious. However, it is currently unknown whether either of these fungal pathogens can infect other reptile species. To test this, we conducted a cross infection experiment by inoculating groups of bearded dragons and groups of corn snakes with *O. ophiodiicola* and *N. guarroi*. Our results confirmed that both fungal pathogens can infect both corn snakes and bearded dragons, which has significant implications toward conservation and preventing disease outbreaks. Our main concern with our findings is the likelihood that an increase in the spread of either disease has gone unnoticed for a decade, thus becoming even more difficult to manage.

Keywords: Snake Fungal Disease, Yellow Fungus Disease, Emerging Infectious Disease, Spillover, Host Specificity, *Chrysosporium* anamorph of *Nannizziopsis vriesii*, Mycoses

Introduction

Host specificity is fundamental to understanding disease ecology and is often defined based on the ability of a pathogenic organism to infect singular or multiple host

species (1). However, host specificity is more complex than its simple definition as it involves a continuum of susceptibilities among individuals within a host species and evolves with host-pathogen interactions (2-5). Furthermore, pathogens may infect reservoir or intermediate hosts without causing overt disease. As such, host specificity emerges as a concept existing on a spectrum; there are rarely clear delineations of susceptibility based on host taxonomy alone. Nevertheless, identifying the range of potential hosts a fungus can persist on or infect is central to understanding the epidemiology of many important mycoses, especially for emerging diseases such as chytridiomycosis in amphibians, snake fungal disease (SFD or ophidiomycosis), and white-nose syndrome in bats.

Fungi within the Onygenales order, formerly classified within the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) complex, have been considered as generalist pathogens with a host range spanning multiple reptilian orders (Crocodilia [crocodilians], Rhynchocephalia [tuataras], Squamata [lizards and snakes]); CANV fungi have also been associated with opportunistic infections in humans (6-13). However, the former CANV fungi have since been separated into several genera (Ophidiomyces, Paranannizziopsis, Emydomyces, and Nannizziopsis) and multiple species (12, 14), leaving the host specificity of the newly described taxa ill-defined. Case reports of former CANV fungi demonstrate the complexity of host specificity; for example, N. crocodili has only been reported to infect crocodiles (12, 15); *N. barbatae*, *N. dermatitidis*, and *N. guarroi* are only known to infect lizards (9, 12); *O. ophiodiicola* has only been reported from snake hosts (12, 16, 17); and *N. hominis*, *N. infrequens*, and *N. obscura* have only been recovered from humans (9); *Paranannizziopsis* spp. have been recovered from multiple lizard,

snake, and tuatara species (18); *E. testavorans* has only been reported to infect freshwater aquatic turtles (19).

However, opportunistic reports of infected animals are unlikely to reveal the full host range of any pathogen, and two consequential fungal pathogens within the former CANV complex requiring further study with respect to host range are *O. ophiodiicola* and *N. guarroi*, responsible for two important emerging infectious diseases (EIDs) of reptiles, SFD, and yellow fungus disease (YFD), respectively (17, 20-24). The possibility of spillover from captive to wild populations exists for both SFD and YFD. Because spillover might negatively impact as-yet unreported wild hosts and allow these EIDs to further invade novel environments with unknown consequences, both pathogens are a concern for conservation (25, 26). Perhaps due to the underlying shared evolutionary history of *O. ophiodiicola* and *N. guarroi*, SFD and YFD elicit a similar disease process in their hosts, which includes necrotizing dermatitis and, in severe cases, death of the host (17, 27, 28). Yet, despite infecting phylogenetically related host taxa, current observations suggest both *O. ophiodiicola* and *N. guarroi* possess some level of host specificity, and each is reported from only snakes and lizards, respectively. Here we explore the host fidelity of *O. ophiodiicola* and *N. guarroi* in greater depth. Specifically, we conducted a 52-day challenge experiment inoculating groups of corn snakes (*Pantherophis guttatus*; a known host of *O. ophiodiicola*) and central bearded dragons (*Pogona vitticeps*; a known host of *N. guarroi*) with both fungi to test the ability of these pathogens to infect "nontarget" host species; our experiment also fulfilled Koch's postulates by confirming *N. guarroi* as a primary pathogen of YFD in bearded dragons (23).

Animal Husbandry

Our experimental protocols were approved by the University of Wisconsin Institutional Animal Care and Use Committee (IACUC, #V006070IACUC). We conducted live animal experiments at the Charmany Instructional Facility in Madison, Wisconsin, United States. We acquired 12 four- to ten-year-old (310 g – 930 g) captive-bred corn snakes and 12 two- to three-month-old (27 g to 48 g) captive-bred bearded dragons from a local vendor. Each animal was housed individually in a plastic tub with a ventilated cover and water available ad libitum; shelter and (for bearded dragons) enrichment in the form of climbable sticks were also provided. We randomly placed ten corn snakes and ten bearded dragons into four groups, separated by host species and treatment. Each group was assigned one pathogen treatment (*O. ophiodiicola* or *N. guarroi*) and contained five animals of one of the two host species (corn snakes or bearded dragons); each group had a its own individual room. The remaining four animals (two corn snakes and two bearded dragons) were designated as negative controls. We also grouped the negative controls by species and put the two groups into two separate rooms. In each room, temperatures were kept within an optimal range for fungi and a tolerable range for the animals; animals treated with *O. ophiodiicola* were maintained at 24° C – 27° C and animals treated with *N. guarroi* were maintained at 30°C – 35°C. No heat gradient was provided within the enclosures because of the relatively short (52 days) duration of our experiment. For corn snakes, humidity was kept within 40 – 50% and within 30 – 40% for bearded dragons; all rooms were set on a 12h light/dark cycle. All corn snakes were fed two adult mice per week, and bearded dragons were fed daily, alternating between fresh greens (e.g. collard greens, cabbage, etc.) and superworms (*Zophobas morio*). The research veterinary staff conducted initial physical and wellness checks of each animal

and determined all were physically healthy with no visible pre-existing conditions. We screened every animal for *O. ophiodiicola* and *N. guarroi* prior to the challenge study by swabbing the dorsal and ventral skin from snout to tail tip (23). Swabs were screened for the presence of *O. ophiodiicola* using real-time PCR (29). We also streaked all swabs onto dermatophyte test media (DTM) plates and incubated at 24 ºC for 20 days to screen for *N. guarroi*; there is currently no specific real-time PCR assay published for this organism. All animals were found to be free of both *N. guarroi* and *O. ophiodiicola*. We also tracked the behavior, body condition, ecdysis (shedding skin), and, after inoculation, infection progression, for each individual daily until the completion of the challenge experiment.

Inoculation and Re-inoculation

As inoculants, we used pure cultures of *O. ophiodiicola* (ATCC MYA-4974) isolated from a wild caught water snake (*Nerodia sipedon*) infected with SFD, and *N. guarroi* (UAMH-10352) isolated from a domestic bearded dragon infected with YFD; cultures were from the American Type Culture Collection (ATCC), Manassas, Virginia and the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, Alberta, Canada, respectively. Cultures were grown from single isolated colonies on Sabouraud's dextrose agar (SDA) at 24 ºC for 20 days. We prepared conidial suspensions from the cultures using phosphate-buffered saline with 0.5% Tween 20 solution (PBST)(17). Animals were inoculated with 168,500 or 325,580 conidia per inoculation site for *O. ophiodiicola* and *N. guarroi*, respectively. To facilitate infection and mimic the skin abrasions reptiles incur in nature, we gently abraded a 1 cm^2 area of skin at three or four sites, depending on the species, on each animal using sterile sandpaper, as has been

done in previous studies (14, 17, 23). We abraded the skin of corn snakes on the snout, dorsal neck, and ventral midbody; two additional sites to be inoculated (dorsal midbody and ventral neck) were left unabraded. The procedure for abrading the skin and inoculating bearded dragons is described in Gentry *et al.* (2021) (23). We inoculated the corn snakes with 2 µl of the designated spore suspension at each site and bearded dragons were inoculated with 4 µl of the spore suspension at each site; the difference in total volume applied between host species accommodates for differences in skin textures of the two species (snake skin does not retain water as easily as the skin of bearded dragons). Collectively, we inoculated the corn snake treatment groups at 25 sites and the bearded dragon treatment groups at 30 sites; we abraded 15 sites on corn snakes and abraded 20 sites on bearded dragons. We used the same protocol to abrade each negative control and inoculated the animals with a sham inoculant consisting of a sterile saline solution (17). We re-inoculated treated corn snakes with the same conidial concentration used in the first round of inoculation if ecdysis occurred before the completion of the experiment because ecdysis has been shown to clear infection in some cases (17). Three corn snakes within the *N. guarroi* treatment group and three within the *O. ophiodiicola* treatment group were re-inoculated 23 days after initial inoculation; we did not re-inoculate any bearded dragons.

Euthanasia and Necropsy

We euthanized any animal that developed lesions exceeding 1 cm in diameter or displayed a rapid decline in body condition; the remaining animals were euthanized on the final day (day 52) of the experiment. Prior to euthanasia, we anesthetized each animal in an induction chamber using isoflurane gas, and then we injected animals

intracoelomically with a fatal dosage of pentobarbital, with each dosage adjusted to accommodate animal body size. We used decapitation as a secondary method of euthanasia. The euthanasia chamber was disinfected between animals to prevent cross-contamination. At necropsy, we removed skin samples from each inoculated and sham-inoculated site. We divided the skin samples for use in both histopathological analyses and for fungal isolation. Samples were taken from each inoculated site, even if a site presented no visible gross lesions. In addition, we removed skin from areas that developed lesions at non-inoculated sites. Inoculated skin, viscera (including heart, lung, kidney, spleen, pancreas, esophagus, stomach, and small and large intestines), decapitated heads and (for bearded dragons) entire inoculated legs were fixed in 10% neutral buffered formalin. After fixation, heads and legs were decalcified in saturated ethylenediaminetetraacetic acid (EDTA) solution. Tissues for histopathology were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin-eosin (H&E) or periodic acid-Schiff (PAS) methods depending on tissue type: internal organs were stained with H&E and skin samples were stained with PAS. Sections were assessed for microscopic lesions of infection, including fungal elements (hyphae and/or conidia), by a board-certified veterinary pathologist.

Fungal Culture Analyses

We cultured inoculated and sham-inoculated skin samples on DTM at 30°C for 20 days. Fungal or bacterial colonies from the skin samples were isolated and subcultured on SDA. To extract fungal DNA from each subculture, we took a 0.5 cm diameter plug of fungal biomass from the subculture using a sterile scalpel and put in a two ml screw-cap tube with three mm diameter glass beads; we macerated the fungal material using a mini-

beadbeater (Product number: 112011, BioSpec Products, Bartlesville, Oklahoma, United States). We combined the macerated material with 600 µl of cetyltrimethylammonium bromide (CTAB) buffer and incubated the samples at 65°C for one hour. We then added 600 µl of chloroform isoamyl alcohol to each tube, mixed it with the CTAB, and centrifuged at 13,000 rpm for ten min. We placed the supernatants into new, sterile 2 ml Eppendorf tubes and added 600 µl chloroform isoamyl alcohol to each tube, gently rocking the tubes on a tipping tray for 10 min. We centrifuged the tubes a second time and added ice-cold 96% EtOH at 2x the total sample volume to wash the extracted DNA. We washed each DNA pellet twice and then dried the pellets in a DNA SpeedVac system for 30 min before re-dissolving the DNA in 400 µl of Tris-EDTA (TE) buffer; extracted DNA was stored at -20° C to be used for downstream PCR. We used the Qiagen DNEasy Powersoil kit (Germantown, Maryland) to extract bacterial DNA, following the manufacturer's protocol.

We conducted PCR for Sanger sequencing on all extracted DNA. For fungi, we amplified the ITS region using primers, ITS1-F and ITS4 (30), EconoTaq Plus 2X Master Mix, and distilled water. PCR reactions used the following cycling conditions: 2 min at 95° C; 30 cycles of: 15 s at 95° C, 15 s at 49° C, 1 min at 68° C; and 5 min at 68° C. For bacteria, we amplified the 16S ribosomal region using primers rp2 and fD1 (31) and PCR reactions used the following parameters: 5 min at 95°C; 25 cycles of: 1 min at 94°C, 1 min at 59°C, 2 min at 72°C; and 5 min at 72°C. We submitted the ITS and 16S amplicons to a third-party vendor (Functional Biosciences Madison, Wisconsin) for Sanger sequencing using the primers in one direction, receiving the ab1 files and then visually inspecting and trimming the chromatographs in Chromas to verify the quality of each of our samples. To determine the species of fungi and bacteria we cultured, we used BLAST

(Basic Local Alignment Search Tool)(32) to compare our sequences with sequences stored in GenBank (33). Subsequently, we deposited the sequences of the additionally isolated microbes into GenBank (accession nos. OP798776, OP798777, OP798778, OP798779, OP798780, OP801847, and OP801848). Because the re-isolation of *O. ophiodiicola* and *N. guarroi* were identical to the strains in our inoculation step, we did not deposit the duplicate sequences (ATCC MYA-4974 and UAMH-10352) in GenBank as they already exist.

Histopathological Analyses and Infection Status

We evaluated each inoculated and sham-inoculated site for gross signs of infection (e.g., swelling, discoloration of the skin), and for histopathological evidence of infection by identifying the presence of fungal elements (e.g., hyphae, conidia) with an associated tissue response such as inflammation or necrosis, indicative with SFD and YFD diagnostics (15, 34). We considered an animal infected if it developed clinical signs or histopathological evidence of fungal infection at any inoculated site.

Results

None of the negative controls developed clinical signs or histopathological evidence of infection at any of the abraded or non-abraded sites; neither *O. ophiodiicola* nor *N. guarroi* were cultured from any negative control animal.

Corn Snakes infected with Nannizziopsis guarroi.

All corn snakes inoculated with *N. guarroi* expressed clinical signs of infection and had histopathological evidence of infection, resulting in a 100% infection prevalence. Clinical signs of infection across the treatment animals were restricted to mild swelling

around the snout (FIG 1), first appearing nine days after inoculation. Despite the lack of obvious clinical signs at other sites of inoculation, there was histopathological evidence of infection in the form of associated fungal elements within tissues across multiple inoculation sites (Table 1). Microscopic lesions were characterized by superficial epidermal necrosis and mild granulocytic to lymphoplasmacytic dermatitis with occasional intra-epidermal fungal hyphae but no observed conidia. One corn snake (CS12N) lacked intralesional hyphae or conidia but did have a small area of epidermal necrosis and inflammation on the dorsal skin of the head, an abraded site.

Of the abraded inoculation sites, 33% of sites across all inoculated corn snakes demonstrated clinical signs of infection (all on the snout) and 26% presented histopathological evidence of infection. Although there were no clinical signs of infection at any of the non-abraded sites, 30% of the non-abraded sites presented histopathological evidence of infection. Based on our histopathological examination, infections were superficial and limited to the skin; there was no evidence that fungal invasion extended into the viscera. We re-isolated *N. guarroi* from 32% of the cultured skin samples irrespective of the presence of clinical or histopathological signs of infection (Table 1). Sequencing of DNA confirmed that each isolate of *N. guarroi* had an identical ITS sequence to the initial strain used for inoculation.

Corn Snakes infected with Ophidiomyces ophiodiicola

All corn snakes inoculated with *O. ophiodiicola* expressed clinical signs and histopathological evidence of infection typical of SFD (FIG 1; FIG 3) (17), resulting in a 100% infection prevalence; clinical signs of swelling and scale discoloration first appeared nine days after inoculation. All corn snakes developed microscopic cutaneous fungal

lesions with mild to moderate necrosis and granulocytic inflammation of the superficial epidermis and intralesional fungal hyphae. The underlying dermis exhibited mild granulocytic to lymphoplasmacytic inflammation; conidia were variably present on the skin surface. Many snakes were in the process of ecdysis; in these instances, lesions were confined to the layers of epidermis being shed, and the new underlying epidermis was microscopically unremarkable. Although each snake inoculated with *O. ophiodiicola* had at least two inoculated sites exhibit evidence of infection, not all of the 25 inoculated sites across the five corn snakes showed evidence of being infected (Table 1). Of the abraded inoculation sites, 66% demonstrated clinical signs of infection and 96% demonstrated histopathological evidence of infection; no clinical or histopathological signs of infection were seen at the non-abraded sites. Based on histopathological examination, there was no evidence of infection in the viscera. We re-isolated *O. ophiodiicola* from 84% of the inoculated skin samples taken at the time of necropsy, and growth occurred irrespective of the presence of clinical or histopathological signs of infection (Table 1). Sequencing of DNA confirmed that each isolate of *O. ophiodiicola* had an identical ITS sequence to the initial strain used for inoculation.

Bearded Dragons infected with Nannizziopsis guarroi

Results of the experiment in which bearded dragons were challenged with *N. guarro*i have been previously published (23). Briefly, all bearded dragons inoculated with *N. guarroi* developed clinical signs or histopathological evidence of infection, resulting in 100% incidence of infection. We euthanized one bearded dragon (BD11N) 22 days after our initial inoculation due to rapidly deteriorating body condition (23). Clinical signs first appeared 10 days after inoculation. All microscopic skin lesions were consistent with

those previously reported for YFD (FIG 2; FIG 3)(8, 35, 36), and detailed descriptions of clinical signs and histopathological lesions can be found in Gentry *et al.* (2021)(23). Of the abraded sites, 35% exhibited clinical signs of infection and histopathological evidence of infection. For non-abraded sites, 30% exhibited clinical signs and 20% contained histopathological evidence of infection. Infection did not extend into the viscera, although fungal invasion was noted deep into the dermis and underlying skeletal muscle. We re-isolated *N. guarroi* from 80% of the inoculated sites collected at the time of necropsy, and growth occurred irrespective of whether the inoculated site exhibited clinical signs or histopathological evidence of infection. Sequencing of DNA confirmed that each isolate of *N. guarroi* had an identical ITS sequence to the initial strain used for inoculation.

Bearded Dragons infected with Ophidiomyces ophiodiicola

Sixty percent of bearded dragons inoculated with *O. ophiodiicola* became infected, with 6% of sites exhibiting clinical signs of infection and 60% having histopathological evidence of infection. A single bearded dragon (BD3O) developed clinical signs of infection; these gross lesions occurred on the snout and dorsal neck skin (both abraded inoculation sites). The lesions appeared 20 days after inoculation and initially presented as mild swelling and a white discoloration of skin, progressing to brown discoloration and crusting of the skin (FIG 2). In two bearded dragons, histopathologic lesions were characterized by full-thickness epidermal necrosis and marked lymphoplasmacytic to histiocytic inflammation within the dermis. Early granulomas surrounding the fungal hyphae extended from the epidermis deep into the dermis. Conidia were present on the surface of the skin. In the third lizard that developed infection, similar dermal lesions were present, but epidermal necrosis was not observed, potentially due to the affected

epidermis being outside the portion of the sectioned skin. Despite the absence of clinical signs among four of the five bearded dragons, 25% of the abraded sites exhibited histopathological evidence of infection. Infections were histopathologically confirmed in 30% of the non-abraded sites. Based on histopathological examination, infection did not extend into the viscera. We re-isolated *O. ophiodiicola* from 76% of the inoculated skin samples collected at the time of necropsy and growth occurred irrespective of whether clinical signs or histopathological evidence of infection were observed within the samples. Sequencing of DNA confirmed that each isolate of *O. ophiodiicola* had an identical ITS sequence to the initial strain used for inoculation.

In addition to *O. ophiodiicola* and *N. guarroi*, other fungi were isolated from some inoculated skin samples, including *Neosetophoma guiyangensis*, *Kocuria sp*., *Chaetomium globosum*, *Purpureocillium lilacinum*, *Defltia sp*., *Achromobacter sp*., *Penicillium steckii*, *P. brevicompactum*, *Aspergillus terreus*, and *Beauveria bassiana* (STable 1; STable 2). These microbes were likely environmental contaminants.

Discussion

The results of our cross-inoculation experiment demonstrate *O. ophiodiicola* (previously understood as host specific to snakes) and *N. guarroi* (previously understood as host specific to lizards) infect a wider variety of hosts than previously suspected, and both may be better classified as host generalists – pathogens able to infect a diverse range of animals. Using a combination of gross examination, histopathologic analyses, and re-isolation of the pathogens, we confirmed that *O. ophiodiicola* and *N. guarroi* can each cause disease in both lizard and snake species. Clinical signs of infection in corn snakes inoculated with *O. ophiodiicola* and in bearded dragons inoculated with *N. guarroi*

(both “typical” hosts for the respective pathogens) followed the expected progression of disease detailed in the literature (9, 17, 21, 22, 27, 35, 36).

When animals were challenged with the pathogen known to infect them, 100% of the inoculated individuals developed clinical and/or histopathological signs of infection. However, when challenged with the pathogen not previously known to infect that host species, clinical signs of infection across the inoculated sites were more variable despite the histopathology confirming 100% infection prevalence in some treatment groups. Specifically, each of the five corn snakes challenged with *N. guarroi* developed infections while three of the five bearded dragons challenged with *O. ophiodiicola* exhibited clinical or histopathologic evidence of infection. Whether the lack of disease in the remaining two bearded dragons challenged with *O. ophiodiicola* was caused by a failure to establish (or ability to recover from) infection, or sampling technique, is unclear. Discrepancies between the presence of clinical signs, histopathologic findings, and re-isolation of the pathogen from the same skin lesion have been previously reported (23). The variation we observed across the animals may be explained by the relatively small sizes of some lesions, which made it difficult to divide samples and ensure a portion of the lesion was analyzed by culture and histopathology. Also, many of the inoculation sites on bearded dragons infected with *O. ophiodiicola* did not exhibit gross lesions, making it difficult to pinpoint the exact regions where fungal infection may have occurred. Infections in snakes caused by *O. ophiodiicola* were primarily seen at inoculation sites where skin had been lightly abraded to facilitate entry of the conidia into the skin (Table 1). By contrast, *N. guarroi* appeared capable of initiating infections in snakes at inoculation sites at which the stratum corneum was intact; this was also the case for bearded dragons infected with *O.*

ophiodiicola (Table 1). These findings indicate the possible differences in the infection mechanisms between *O. ophiodiicola* and *N. guarroi* and indicate that *O. ophiodiicola* may act differently in entirely new hosts (e.g., indicating different fungal roles between a primary and secondary pathogen).

Aside from the experimentally induced infections, the snakes and lizards appeared healthy throughout the experiment, suggesting that the ability to cross-infect the animals was not due to poor husbandry or other factors potentially affecting underlying host health. While a few other fungi and bacteria were isolated from the skin lesions of our animals, they represent common environmental flora (37-40). Some of these fungi (e.g., *B. bassiana* and *P. lilacinum*) have been previously reported from skin lesions in reptiles with *O. ophiodiicola* or *N. guarroi* infections and from healthy skin (17), suggesting they are likely commensals, “hitchhikers”, or secondary invaders.

While *O. ophiodiicola* and *N. guarroi* were able to infect both snakes and lizards, disease presentation differed by host species and pathogen. As expected, previously identified hosts of *O. ophiodiicola* and *N. guarroi* developed obvious gross lesions. However, infection by *O. ophiodiicola* or *N. guarroi* on newly identified hosts manifested more subtly (Table 1). Corn snakes infected with *N. guarroi* displayed little to no recognizable clinical signs of infection apart from slight swelling of the snout where minor distortions were easy to observe (FIG 1). In bearded dragons, only one individual challenged with *O. ophiodiicola* developed clinical signs of disease with lesions similar to, but more subtle than, lesions typically seen in SFD (FIG 2). Yet, infection was confirmed through histopathology in many inoculated sites lacking clinical signs, and the respective pathogens were re-isolated in culture from the grossly normal skin. Cryptic infections on

certain taxa may explain why *O. ophiodiicola* and *N. guarroi* were previously believed to be host specific and highlights the bias inherent in describing the host range of a pathogen from information compiled primarily through case reports in which animals exhibit clinical disease. Our results also highlight the variation in infection that exists within a single host species. For example, although each corn snake and bearded dragon inoculated with *O. ophiodiicola* and *N. guarroi*, respectively, showed signs of infection (clinical and/or histopathological) in at least one inoculated site, the proportion of inoculated sites that developed lesions varied among individual animals. The ability of snakes to harbor *O. ophiodiicola* in the absence of clinical signs of infection is supported by previous studies screening for the pathogen (10, 41), but such work has not been extended to other reptile taxa, *N. guarroi* or other fungi formerly classified as CANV.

Recovery of viable O. ophiodiicola and N. guarroi from inoculated skin generally occurred at a higher frequency than either the appearance of clinical signs or histopathologic evidence of infection. Although the caveats described above may explain at least some of these discrepancies, it is also possible that these fungi are capable of persisting for long periods of time (i.e. >50 days) on the host in the absence of infection. The ability for these fungi to infect or persist on a broad range of hosts has significant implications with respect to disease ecology, pathogen screening, and translocation, especially in light of the recent finding that O. ophiodiicola likely spilled over into wild snake populations in North America from captive reptile collections (42). Specifically, these pathogens may be capable of “hitch-hiking” on hosts not exhibiting signs of infection and persist beyond typical quarantine periods for animals. An expanded host range also emphasizes the

concern for disease spillover from captive into wild populations; if either pathogen escapes into new environments, it may put naïve hosts at risk.

Our challenge experiment lasted 52 days and, had we continued the experiment, we do not know if the infections in the newly identified hosts would have resolved or progressed to greater severity. Our observations of milder disease presentation in lizards and snakes infected by *O. ophiodiicola* and *N. guarroi*, respectively, cannot be interpreted as either host being more resistant or tolerant to the effects of the pathogens. Nonetheless, it is possible that the cryptic infections we observed are the result of a deep coevolutionary history between these fungi and their hosts. Specifically, *O. ophiodiicola* may have evolved with lizard hosts and *N. guarroi* with snake hosts. Many fungal pathogens do not cause significant disease in the host species they evolved with (e.g. Batrachochytrium in Asian amphibian species [43, 44]; *Pseudogymnoascus destructans* in Eurasian bat populations [45]). Future efforts to better elucidate the natural geographic distribution of *O. ophiodiicola* and *N. guarroi*, and determine preferred hosts within their native ranges, may enable better predictions of which reptile populations are at the greatest risk of developing severe disease should spillover occur.

Host specificity is a widely accepted concept, but it is not as simplistic as host susceptibility or resistance. Instead, host specificity functions on a continuum influenced by a range of variables shaped by the host, pathogen, environment, and their interactions. Phylogenetic specificity, a measure proposed by Poulin *et al.* (2011) (3), uses host species to distinguish between related pathogens. The shared evolutionary history of *O. ophiodiicola* and *N. guarroi* (reflected in their previous classification as a single taxon) was the reason we chose to compare the ability of these fungi to infect reciprocal,

previously undocumented hosts. Lizards and snakes are also phylogenetically related (they are both in the order Squamata) and the abilities of *O. ophiodiicola* and *N. guarroi* to infect both host classes are not overly surprising (46). However, additional work is necessary to explore the full range of hosts that *O. ophiodiicola* and *N. guarroi* (and other former CANV fungi) can infect, as well as how factors not accounted for in our study (e.g., host behavior, pathogen exposure, pathogen physiology) influence the likelihood of a host becoming infected in nature.

In summary, our results establish both *O. ophiodiicola* and *N. guarroi* as having a more generalized host range than previously thought. However, the clinical and histopathological signs of disease are expressed differently depending on which host the pathogen is infecting. Cryptic infections in a variety of host species may also be caused by other fungi formerly classified as part of the CANV complex. Thus, our findings emphasize a potential need to alter the screening approaches for these pathogens, opting to broaden surveillance efforts to include a greater diversity of fungi and hosts. The probability of spillover of former CANV fungi into additional naïve hosts, either across captive populations or from captive to wild populations, is a major concern. Reptile populations are experiencing worldwide declines and many species are in danger of extinction (47, 48). Additional research is needed to more holistically understand how fungal pathogens are affecting reptile populations so that conservation strategies can be developed to protect sensitive populations from the emerging threat of fungal diseases.

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References

1. Lymbery AJ. 1989. Host specificity, host range, and host preference. Parasitology Today 5:298.

2. Poulin R. 1992. Determinants of host-specificity in parasites of freshwater fishes. International Journal of Parasitology 22:6 753–758.

3. Poulin R, Krasnov BR, Mouillot D. 2011. Host specificity in phylogenetic and geographic space. Trends in Parasitology 27:8 355–361.

4. Isenberg HD. 1988. Pathogenicity and virulence: another view. Clinical Microbiology Reviews 1:1 40–53.

5. Goater TM, Goater CP, Esch GW. 2001. Effects of parasites on their hosts: from individuals to ecosystems, p 396–427. In Halliday K, Waddington M (ed), Parasitism: the diversity and ecology of animal parasites, 2nd edition. Cambridge University Press, New York, United State of America.

6. Abarca ML, Martorell J, Castellá G, Ramis A, Canañes FJ. 2008. Cutaneous hyalohyphomycosis caused by a *Chrysosporium* species related to *Nannizziopsis vriesii* in two green iguanas (*Iguana iguana*). Medical Mycology 46:349–354.

7. Abarca ML, Martorell J, Castellá G, Ramis A, Canañes FJ. 2009. Dermatomycosis in a pet inland bearded dragon (*Pogona vitticeps*) caused by a *Chrysosporium* species related to *Nannizziopsis vriesii*. Veterinary Dermatology 20: 295–299.

8. Bowman MR, Paré JA, Sigler L, Naeser JP, Sladky KK, Hanley CS, Helmer P, Phillips LA, Browers A, Porter R. 2007. Deep fungal dermatitis in three inland bearded dragons (*Pogona vitticeps*) caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii*. Medical Mycology 45:371–376.

9. Paré JA, Sigler L, Hunter B, Summerbell RC, Smith DA, Machin KL. 1997. Cutaneous mycoses in chameleons caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (apinis) currah. Journal of Zoo and Wildlife Medicine 28:443–453.

10. Paré JA, Sigler L, Rypien KL, Gibas CFC. 2003. Cutaneous mycobiota of captive squamate reptiles with notes on the scarcity of *Chrysosporium* anamorph of *Nannizziopsis vriesii*. Medicine 13:4 10–15.

11. Paré JA, Coyle KA, Sigler L, III Maas AK, Mitchell RL. 2006. Pathogenicity of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* for veiled chameleons (*Chamaeleo calyptratus*). Medical Mycology 44:1 25–31.

12. Sigler L, Hambleton S, Paré JA. 2013. Molecular characterization of reptile pathogens currently known as members of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* complex and relationship with some human-associated isolates. Journal of Clinical Microbiology 51:3338–3357.

13. Thomas AD, Sigler L, Peucker S, Norton JH, Nielan A. 2002. *Chrysosporium* anamorph of *Nannizziopsis vriesii* associated with fatal cutaneous mycoses in the salt-water crocodile (*Crocodylus porosus*). Medical Mycology 40:2 141–151.

14. Stchigel AM, Sutton DA, Cano-Lira JF, Cabañes FJ, Abarca L, Tintelnot K, Wickes BL, García D, Guarro J. 2013. Phylogeny of chrysosporia infecting reptiles: proposal of the new family Nannizziopsiaceae and five new species. Persoonia 31: 86–100.

15. Hill AG, Sandy JR, Begg A. 2019. Mycotic dermatitis in juvenile freshwater crocodiles (*Crocodylus johnstoni*) caused by *Nannizziopsis crocodile*. Journal of Zoo and Wildlife Medicine 50:1 225–230.

16. Franklinos LH, Lorch JM, Bohuski E, Rodriguez-Ramos J, Wright ON, Fitzpatrick L, Petrovan S, Durrant C, Linton C, Baláž V, Cunningham AA, Lawson B. 2017. Emerging fungal pathogen *Ophidiomyces ophiodiicola* in wild European snakes. Scientific Reports 7:3844.

17. Lorch JM, Lankton J, Werner K, Falendysz EA, McCurley K, Blehert DS. 2015. Experimental infection of snakes with *Ophidiomyces ophiodiicola* causes pathological changes that typify snake fungal disease. mBio 6:e010534–15.

18. Díaz-Delgado J, Marrow JC, Flanagan JP, Bauer KL, Zhang M, Rodrigues-Hoffmann A, Groch KR, Gomez G, Balamayooran G. 2020. Outbreak of *Paranannizziopsis australasiensis* infection in captive African bush vipers (*Atheris squamigera*). Journal of Comparative Pathology 181:97–102.

19. Woodburn DB, Miller AN, Allender MC, Maddox CW, Terio KA. 2019. *Emydomyces testavorans*, a new genus and species of Onygenalean fungus isolated from shell lesions of freshwater aquatic turtles. Journal of Clinical Microbiology 57:2 e00628–18.

20. Rajeev S, Sutton DA, Wickes BL, Miller DL, Giri D, Van Meter M, Thompson EH, Rinaldi MG, Romanelli AM, Cano JF, Guarro J. 2009. Isolation and characterization of a new fungal species, *Chrysosporium ophiodiicola*, from a mycotic granuloma of a black rat snake (*Elaphe obsolete obsoleta*). Journal of Clinical Microbiology 47:4 1264–1268.

21. Abarca ML, Martorell J, Castellá G, Ramis A, Canañes FJ. 2009. Dermatomycosis in a pet inland bearded dragon (*Pogona vitticeps*) caused by a *Chrysosporium* species related to *Nannizziopsis vriesii*. Veterinary Dermatology 20: 295–299.

22. Abarca ML, Castella G, Martorell J, Cabañes FJ. 2010. *Chrysosporium guarroi* sp. nov. a new emerging pathogen of pet green iguanas (*Iguana iguana*). Medical Mycology 48:2 365–372.

23. Gentry SL, Lorch JM, Lankton JS, Pringle A. 2021. Koch's postulates: confirming *Nannizziopsis guarroi* as the cause of yellow fungal disease in *Pogona vitticeps*. Mycologia 113:6 1253–1263.

24. Schmidt-Ukaj S, Loncaric I, Klang A, Spergser J, Häbich AC, Knotek Z. 2014. Infection with *Devriesea agamarum* and *Chrysosporium guarroi* in an inland bearded dragon (*Pogona vitticeps*). Veterinary Dermatology 25:555–e97.

25. Sun P-L, Yang C-K, Li W-T, Fan Y-C, Huang H-C, Yu P-H. 2021. Infection with *Nannizziopsis guarroi* and *Ophidiomyces ophiodiicola* in reptiles in Taiwan. Transboundary and Emerging Diseases 69:2 764–775.

26. Di Nicola MT, Coppari L, Notomista T, Marini D. 2022. *Ophidiomyces ophiodiicola* detection and infection: a global review on a potential threat to the world's snake populations. European Journal of Wildlife Research 68:64.

27. Allender MC, Raudabaugh DB, Gleason FH, Miller AN. 2015.The natural history, ecology, and epidemiology of *Ophidiomyces ophiodiicola* and its potential impact on free-ranging snake populations. Fungal Ecology 17:187–196.

28. Paré JA and Sigler L. 2016. An overview of reptile fungal pathogens in the genera Nannizziopsis, Paranannizziopsis, and Ophidiomyces. Journal of Herpetological Medicine and Surgery 26:1-2 46–53.

29. Bohuski E, Lorch JM, Griffin KM, Blehert DS. 2015. TaqMan real-time polymerase chain reaction for detection of *Ophidiomyces ophiodiicola*, the fungus associated with snake fungal disease. BMC Veterinary Research 11:95.

30. White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic p 315–322. In Innis MA, Gelfand DH, Sninsky JJ, White TJ (ed), PCR Protocols: A Guide to Methods and Applications. Academic Press, New York.

31. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S Ribosomal DNA Amplification for Phylogenetic Study. Journal of Bacteriology 173:2 697–703.

32. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:3 405–410.

33. Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2016. GenBank. Nucleic Acids Research 44:1 D67–72.

34. Le Donne V, Crossland N, Brandão J, Sokolova Y, Fowlkes N, Nevarez JG, Langohr IM, Gaunt SD. 2016. *Nannizziopsis guarroi* infection in 2 inland bearded dragons (Pogona vitticeps): clinical, cytologic, histologic, and ultrastructural aspects. Veterinary Clinical Pathology 45:2 368–375.

35. Paré JA and Jacobson ER. 2007. Infectious diseases and pathology of reptiles, p 527–547. In Jacobson ER (ed). Mycotic diseases of reptiles: color atlas and text. CRC Press, Boca Raton, Florida.

36. Hedley J, Eatwell K, Hume L. 2010. Necrotising fungal dermatitis in a group of bearded dragons (*Pogona vitticeps*). Veterinary Record 166:464–465.

37. Fogle MR, Douglas DR, Jumper CA, Straus DC. 2007. Growth and mycotoxin production by *Chaetomium globosum*. Mycopathologia 164:49–56.

38. Wang XW, Lombard L, Groenewald JZ, Li J, Videira SIR, Samson RA, Liu XZ, Crous PW. 2016. Phylogenetic reassessment of the *Chaetomium globosum* species complex. Persoonia 36:83–133.

39. Kandi V, Palange P, Vaish R, Bhatti AB, Kale V, Kandi MR, Bhoomagiri MR. 2016. Emerging bacterial infection: identification and clinical significance of *Kocuria* species. Cureus 8:8e731.

40. Hyde KD, Chaiwan N, Norphanphoun C, Boonmee S, Camporesi E, Chethana KWT, Dayarathne MV, de Silva NI, Dissanayake AJ, Ekanayaka AH, Hongsanan S, Huang SK, Jayasiri SC, Jayawardena RS, Jiang HB, Karunarathna A, Lin CG, Liu NG, Lu YZ, Luo ZL, Maharachchimbura SSN, Manawasinghe IS, Pem D, Perera RH, Phukhamsakda C,

Samarakoon MC, Senwanna C, Shang QJ, Tennakoon DS, Thambugala KM, Tibpromma S, Wanasinghe DN, Xiao YP, Yang J, Zeng XY, Zhang JF, Zhang SN, Bulgakov TS, Bhat DJ, Cheewangkoon R, Goh TK, Jones EBG, Kang JC, Jeewon R, Liu ZY, Lumyong S, Kuo CH, McKenzie EHC, Wen TC, Yan JY, Zhao Q. 2018. Mycosphere notes. Mycosphere 9:169–224.

41. McKenzie JM, Price SJ, Fleckenstein JL, Drayer AN, Connette GM, Bohuski E, Lorch JM. 2019. Field diagnostics and seasonality of *Ophidiomyces ophiodiicola* in wild snake populations. EcoHealth 16:141–150.

42. Ladner JT, Palmer JM, Ettinger CL, Stajich JE, Farrel TM, Glorioso BM, Lawson B, Price SJ, Stengle AG, Grear DA, Lorch JM. 2022. The population genetic of the causative agent of snake fungal disease indicate recent introductions to the USA. Plos Biology 20:6 e3001676.

43. Bai C, Liu X, Fisher MC, Garner TWJ, Li Y. 2012. Global and endemic Asian lineages of the emerging pathogenic fungus *Batrachochytrium dendrobatidis* widely infect amphibians in China. Diversity and Distributions 18:307–318.

44. Fong JJ, Cheng TL, Bataille A, Pessier AP, Waldman B, Vredenburg VT. 2015. Early 1900s detection of *Batrachochytrium dendrobatidis* in Korean amphibians. Plos One 10:3 e0115656.

45. Hoyt JR, Langwig KE, Sun K, Lu G, Parise KL, Jiang T, Frick WF, Foster JT, Feng J, Kilpatrick AM. 2016. Host persistence or extinction from emerging infectious disease: insights from white-nose syndrome in endemic and invading regions. Proceedings of the Royal Society B 283:1823 20152861.

46. Burbrink FT, Lorch JM, Lips KR. 2017. Host susceptibility to snake fungal disease is highly dispersed across phylogenetic and functional trait space. Science 3:12.

47. Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, Winne CT. 2000. The global decline of reptiles, déjà vu amphibians. Bioscience 50:8 653–666.

48. Böhm M, Collen B, Baillie JE, Bowles P, Chanson J, Cox N, Hammerson G, Hoffmann M, Livingstone SR, Ram M, Rhodin AGJ, Stuart SN, van Dijk PP, Young BE, Afuang LE, Aghasyan A, García A, et al. 2013. The conservation status of the world's reptiles. Biological Conservation 157:372–385.

FIGURES & TABLES

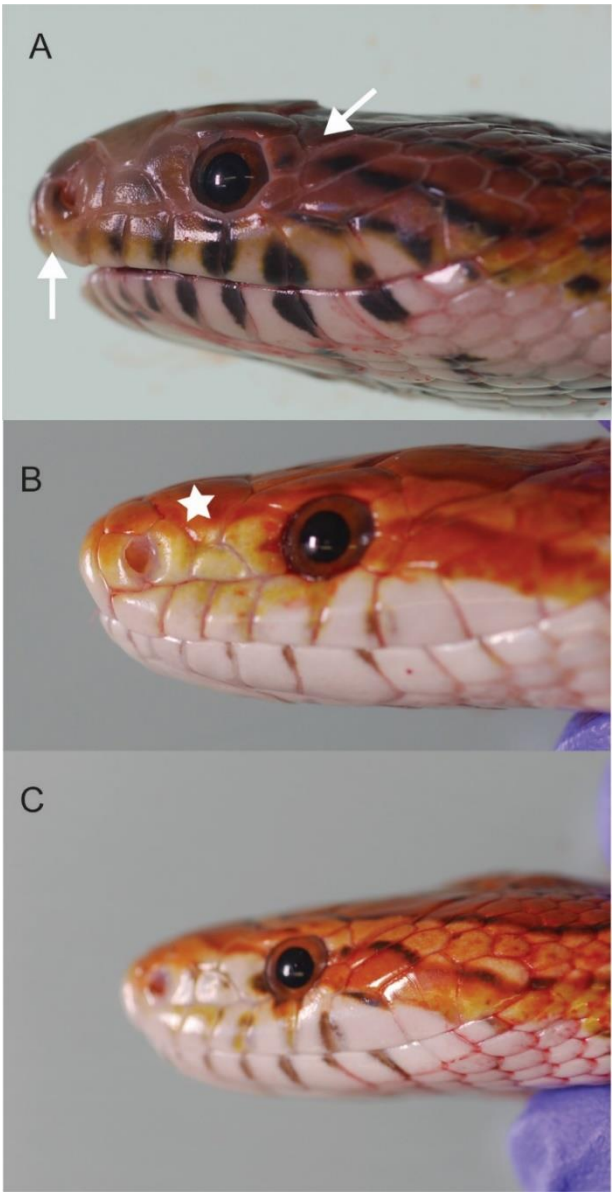

Figure 1. Comparison of clinical signs of *Ophidiomyces ophiodiicola* and *Nannizziopsis guarroi* infection in corn snakes. A. Corn snake infected with *O. ophiodiicola*. Note the severe swelling and thickening of skin on the snout (white arrows indicate the extent of the swelling). B. Corn snake infected with *N. guarroi*. Note the mild swelling and lack of apparent scale thickening (white star). C. Negative control corn snake with no swelling, thickening, or lesions.

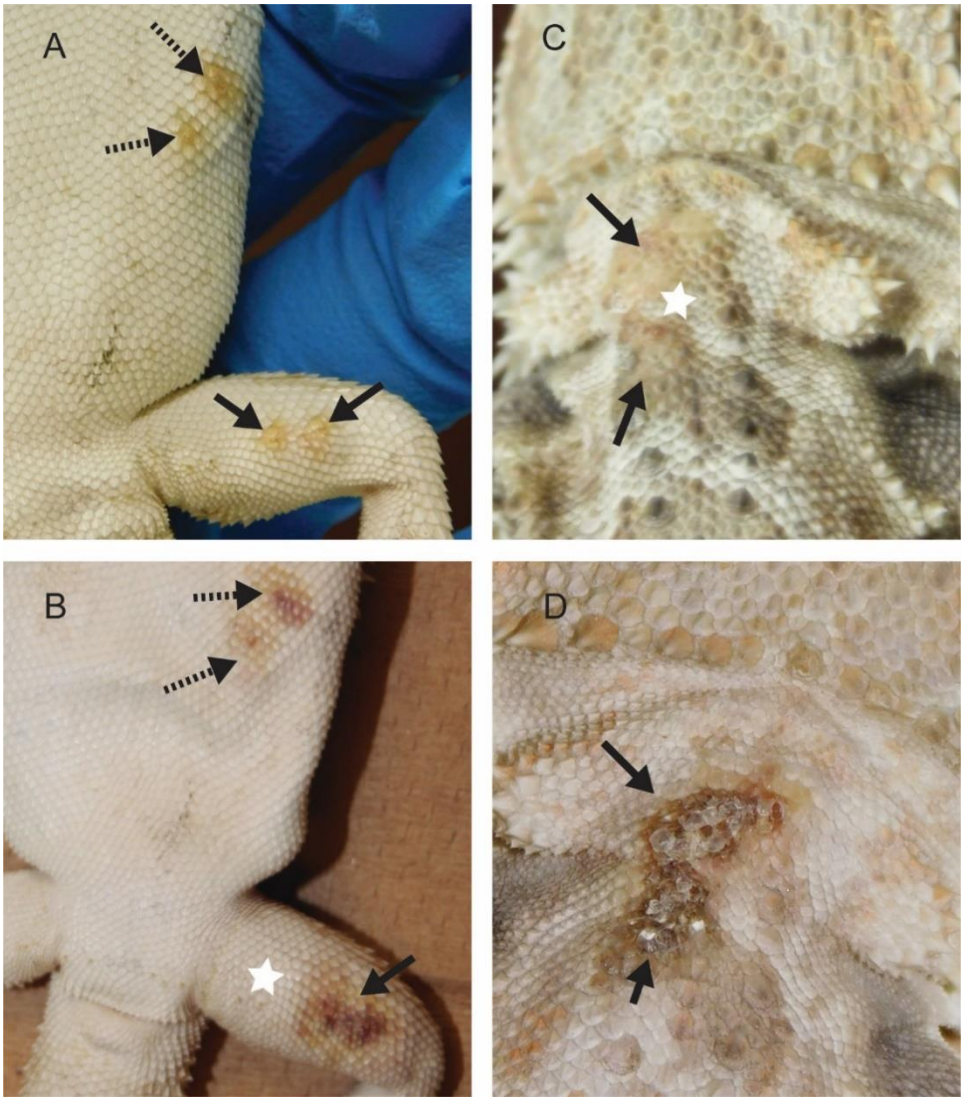

Figure 2. Clinical signs of disease on bearded dragons infected with *N. guarroi* and *O. ophiodiicola*. A and B. One bearded dragon inoculated with *N. guarroi* with clinical signs 20 days after inoculation (A) and 35 days after inoculation (B). C and D. One bearded dragon inoculated with *O. ophiodiicola* with clinical signs 20 days (C) and 41 days (D) after inoculation. Black solid arrows indicate areas of infection at abraded inoculated sites, black dashed arrows indicate areas of infection at non-abraded sites, and white stars indicates areas of swelling.

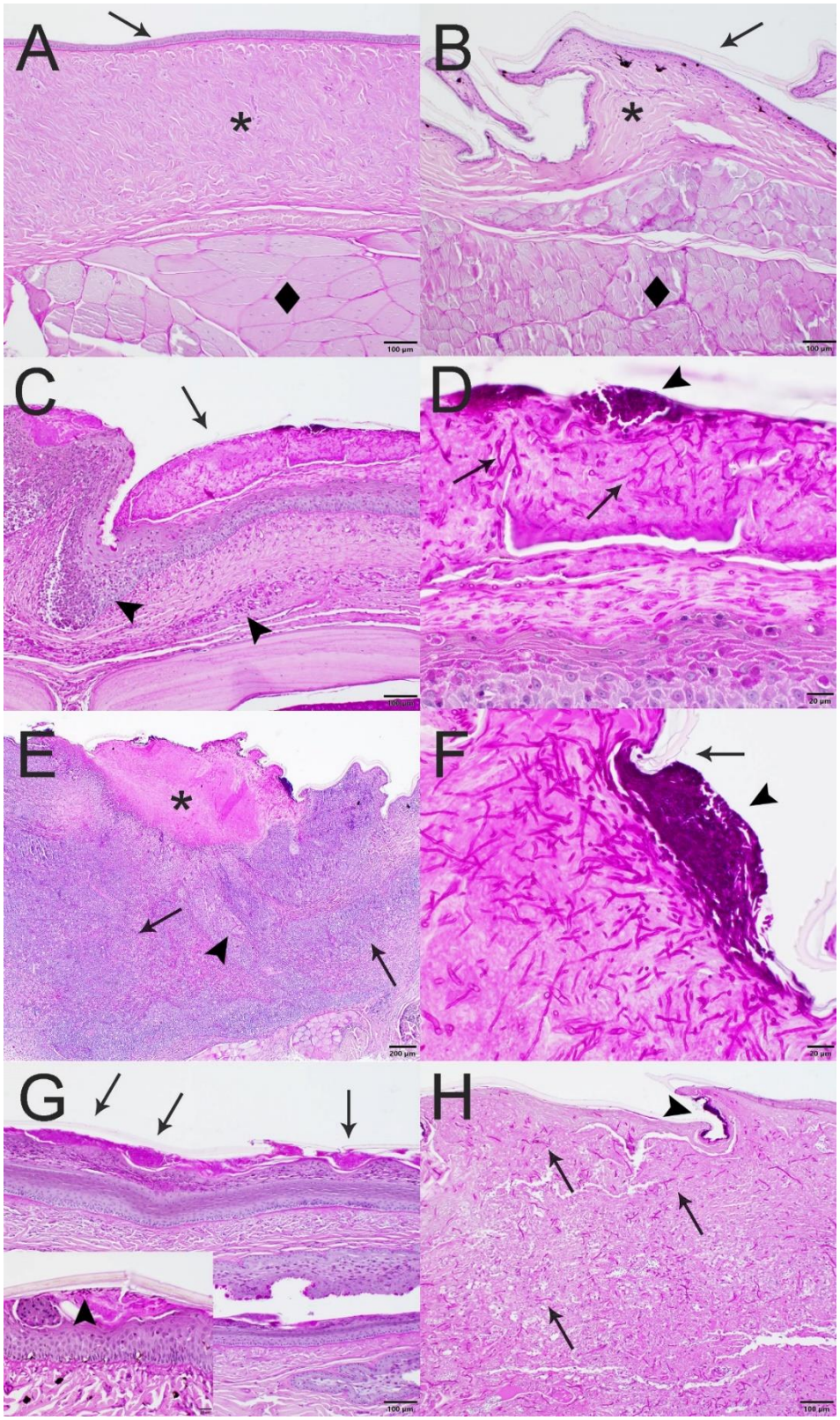

Figure 3. Photomicrographs of corn snakes (*Pantherophis guttatus*) and central bearded dragons (*Pogona vitticeps*) infected with *Ophidiomyces ophiodiicola* or *Nannizziopsis guarroi*. A. Skin from the neck of a corn snake negative control. A thin epidermis (arrow) overlies a sparsely cellular

dermis (asterisk) and deep muscular layer (diamond). Hematoxylin and eosin stain. B. Skin from the neck of a bearded dragon negative control. There is a thick stratum corneum (arrow), thin epidermis, sparsely cellular dermis (asterisk), and deep muscular layer (diamond). Hematoxylin and eosin stain. C. Skin from the head of a corn snake infected with *O. ophiodiicola*. The superficial epidermis is expanded by necrotic material (arrow) and the epidermis and dermis contain many granulocytic inflammatory cells (arrowheads). Periodic acid-Schiff stain. D. Detail of 2C, skin from the head of a corn snake infected with *O. ophiodiicola*. Many periodic acid-Schiff-positive fungal hyphae (arrows) and superficial conidia (arrowhead) are within the area of epidermal necrosis. Periodic acid-Schiff stain. E. Skin from the neck of a bearded dragon infected with *O. ophiodiicola*. There is a large area of epidermal necrosis (asterisk). The dermis is expanded by abundant lymphoplasmacytic to histiocytic inflammatory cells (arrows) and contains few fungal hyphae (arrowhead). Periodic acid-Schiff stain. F. Detail of 2E, skin from the neck of a bearded dragon infected with *O. ophiodiicola*. The epidermis contains many fungal hyphae. There is a large cluster of conidia in the superficial epidermis (arrowhead) which underlies an area of discontinuity in the stratum corneum (arrow). Periodic acid-Schiff stain. G. Skin from the head of a corn snake infected with *N. guarroi*. There is necrosis of the superficial epidermis (arrows). Inset: Detail of G. An area of epidermal necrosis underlies an area of discontinuity in the stratum corneum and contains low numbers of fungal hyphae (arrowhead). Periodic acid-Schiff stain. H. Skin from the mid-body of a bearded dragon infected with *N. guarroi*. The dermis is expanded by clear space (edema) and contains many fungal hyphae (arrows). There are conidia in the superficial epidermis within the crypt of a scale (arrowhead).

Table 1. Average percentages among inoculated sites (5 for corn snakes and 6 for bearded dragons per individual) that developed gross lesions of infection, histopathology consistent with infection, and from which the respective fungal pathogen was re-isolated.

Animal ID	Demonstrated Infection	Gross lesions of infection (per site)	Microscopic lesions of infection (per site)	Re-Isolation (per site)
Corn Snakes: *Ophidiomyces ophiodiicola*				
CS3O	Yes	20%	40%	40%
CS4O	Yes	60%	60%	100%
CS5O	Yes	60%	60%	100%
CS6O	Yes	20%	60%	80%
CS7O	Yes	40%	60%	100%
Average		**40%**	**56%**	**84%**
Corn Snakes: *Nannizziopsis guarroi*				
CS8N	Yes	20%	20%	20%
CS9N	Yes	20%	40%	60%
CS10N	Yes	20%	20%	0
CS11N	Yes	20%	40%	60%
CS12N	Yes	20%	20%	20%
Average		**20%**	**28%**	**32%**
Bearded Dragons: *Ophidiomyces ophiodiicola*				
BD3O	Yes	33%	83%	83%
BD4O	Yes	0	33%	66%
BD5O	Yes	0	16%	83%
BD6O	No	0	0	100%
BD7O	No	0	0	66%
Average		**6%**	**26%**	**80%**
Bearded Dragons: *Nannizziopsis guarroi*				
BD8N	Yes	16%	0	83%
*BD9N	Yes	33%	66%	66%
BD10N	Yes	33%	33%	100%
BD11N	Yes	50%	16%	83%
BD12N	Yes	33%	33%	66%
Average		**33%**	**30%**	**80%**

* BD9N developed a gross lesion on its tail, a location we did not inoculate. Colors of marked prevalence: 0-20% blue, 21-39% green, 40-60% orange, 61-80% red, 81-100% dark red.

Chapter III – Genome Assembly of *Nannizziopsis guarroi* and early-stage protein blasts of CANV-related fungi.

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Abstract

Nannizziopsis guarroi is a keratinophilic fungal pathogen of the Onygenales order and the primary agent of yellow fungus disease, reported in bearded dragons (*Pogona vitticeps*) and green iguana (*Igunana iguana*). With the surge in disease incidence in wild populations by other *Nannizziopsis* species, emphasizing concerns of disease spillover, we sequenced and assembled the genome of *N. guarroi.* Because of the pervasiveness of *N. guarroi* and other fungi once a part of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) complex, we additionally compared the absence and presence of specialized proteins associated with keratin degradation and pathogenicity. Our findings indicate differences in potential pathogenicity factors in *N. guarroi* compared to related fungi that may inform its virulence.

General Audience

Nannizziopsis guarroi is a fungal pathogen responsible for yellow fungus disease (YFD), a fatal skin infection in lizards. Because of the increasing spread of YFD from captive to wild lizard populations, we sequenced and assembled the genome of *N. guarroi.* Additionally, we compared the absence and presence of specialized proteins associated

with disease between *N. guarroi* to other fungi it shares an evolutionary history. Our findings lay the foundation for understanding the pathogenicity of *N. guarroi.*

Introduction

Historically, pathogenicity has been an ill-defined concept used across a multitude of disciplines (microbiology, epidemiology, medicine, etc.). It is often used as a synonym for virulence, further confusing attempts to understand how a pathogen, or disease-causing agent, infects and spreads disease within individuals, species, or across multiple species (Anderson & May 1982; Casadevall & Pirofski 1999; 2001; Thomas & Elkinton 2004). Despite the centuries long debate, in fields as diverse as ecology, physiology, and genetics, conceptualization of pathogenicity has always been intimately tied to investigating a pathogen's ability to incite infection. Here, we explicitly maintain that although pathogenicity and virulence are unavoidably linked, they are not synonymous, aligning with previous definitions attempting to maintain their separation (Blair 1965; Barr 1979; Tanada & Kaya 1993). We define pathogenicity as the inherent mechanisms, or molecular toolkit, a microbe possesses that can allow for infection, regardless of disease severity (virulence) or rate of infection.

With the advent of high throughput sequencing, pathogenicity factors (often secreted enzymes associated with the infection process) could finally be identified through the power of genomics and transcriptomics. However, in order to truly understand if putative, or potential, pathogenicity factors not only exist but are activated during an infection event, a series of requirements need to be met: 1) having a sequenced genome and transcriptome of the pathogen, 2) conducting gene knockouts to create a mutant, and 3) a re-inoculation of the mutated pathogen into the target host to determine if infection

establishes or not. Undeniably, just one of the steps requires a lot of time and resources further emphasizing the difficulty in quantifying pathogenicity at all.

To further complicate the meeting of these requirements, for the fungal kingdom, our ability to identify pathogenicity factors of fungal pathogens, particularly those responsible for emerging infectious diseases (EIDs), is limited by our understanding of fungal physiology and publicly available genomes. Currently, the estimation of fungal diversity remains uncertain at a conservative 3.8 million species, a modest 5.1 million species, or a projected ten million species or more (Hawksworth & Lücking 2016; Blackwell et al. 2018; Locey & Lennon 2016). No matter the true number, it is certain that only around 120,000 fungal species are currently described, leaving roughly 90% of fungi undiscovered (Hawksworth & Lücking 2016; Tripp et al. 2017). Despite the breadth of this diverse kingdom, the uncertainty around biodiversity makes it difficult to gain a holistic perspective of a fungal pathogen, requiring onerous preliminary work to understanding even the basic physiology of a fungus, especially in the context of wildlife disease.

For the last two decades, wildlife has seen an increase in EIDs caused by fungal pathogens (Baker et al. 2022; Stephen et al. 2022; Cardoso et al. 2022). Chytridiomycosis (caused by *Batrachochytrium dendrobatidis* and *B. salamandrivorans*), white nose syndrome (caused by *Pseudogymnoascus destructans*), and snake fungal disease (SFD; caused by *O. ophiodiicola*), are all significant diseases devastating animal populations. Additionally, *Nannizziopsis guarroi,* the causative agent of a necrotic and infectious skin disease (yellow fungus disease (YFD)), in green iguanas (*Iguana iguana*) and bearded dragons (*Pogona vitticeps*; Abarca et al. 2008; Gentry et al. 2021) is on the precipice of becoming a recognized EID. Despite the recognition of this disease in case reports since

the 1990s, only now has research started to focus on *N. guarroi* and other *Nannizziopsis* species from a conservation perspective. An EID can be defined as a newly appearing or existing infectious disease rapidly increasing in incidence in a population or rapidly expanding its geographic range (Ndow et al. 2019). By that definition, *N. guarroi* is considered an EID, especially bearing in mind its evolutionary history within the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) complex, the isolation of the fungus from reptiles around the globe, and the recent finding of a more generalized host range that includes snakes (Sigler et al. 2013; Gentry et al. 2021; Gentry et al. 2023 *in review*).

In Gentry *et al.* (2021) and Gentry *et al.* (2023), we focused on ecological questions regarding the physiology and host specificity of *N. guarroi* and *O. ophiodiicola* and found evidence of a more generalized host range for both pathogens than was previously suspected. However, a distinction between abraded skin and non-abraded skin emerged from the histopathological evidence of infection by *N. guarroi* and *O. ophiodiicola*. While *O. ophiodiicola* required a breakage in the outermost epidermal layer of its host for infection to establish, regardless of the animal species being infected, *N. guarroi* did not. Whether in bearded dragons or corn snakes, *N. guarroi* was able to establish infection without needing any breakage in the skin and could maintain infection even when the animals shed. Such a distinction could hypothesize that different pathogenicity factors are at play between the two fungi and suggests *N. guarroi* has the potential to cause systemic infection in a greater number of naive hosts because of its ability to bypass a host's first line of defense, its skin.

Like *O. ophiodiicola* and other Nannizziopsis species, *N. guarroi* has been described as a keratinophilic fungus (a keratin degrader; Cabañes et al. 2014). Although many fungi can break down keratin to a degree, complete degradation of keratin requires a specialized suite of endo- and exoproteases. Keratin is one of the most stable and difficult compounds to degrade in the environment. We aimed to investigate step 1) of the requirements needed to identify the pathogenicity factors of *N. guarroi* by conducting whole genome sequencing of *N. guarroi* and identifying putative genes involved in keratin degradation. Using a bioinformatic pipeline to assemble and annotate the genome of *N. guarroi*, we investigate the presence and absence of enzymatic families known to be involved with keratin degradation and compare the enzyme presence across other fungal pathogen genome assemblies. We are already witnessing the effects of disease spillover from captive to wild populations, specifically involving diseases caused by other Nannizziopsis species like *N. barbatae* (Peterson *et al.* 2020). That *N. guarroi* may have some advantage in its pathogenicity compared to *O. ophiodiicola*, and potentially other CANV genera, further highlights its future as an EID and raises significant concerns about disease spread and spillover into non-target hosts.

Methods

DNA extraction of N. guarroi

Cultures of *N. guarroi* were grown in glucose minimum media (GMM) with 5 g/L of yeast extract and incubated at 25◦C for three days. Mycelia was put into Eppendorf tubes and dried via liquid nitrogen and overnight lyophilization. The dried mycelia were ground into a fine powder, 700 µl of LETS buffer (20 mM EDTA (pH 8), 0.5% SDS, 10 mM Tris-HCL (pH 8), 0.1 M LiCl) was added, and we mixed by inversion, letting the samples settle for

five minutes at room temperature. We added 700 µl of Phenol:$CHCl_3$:Isoamyl alcohol (25:24:1), mixed by inversion, and let the samples sit for 5 minutes at room temperature. We used a microcentrifuge to spin the samples for 10 minutes at 4°C on maximum speed. We transferred the supernatants to new tubes and added 1 ml of 95% EtOH, gently mixed, and centrifuged the DNA pellets for 10 minutes at 4°C. We removed the supernatant, washed the DNA pellets with 70% EtOH, and centrifuged for 2 minutes at room temperature. We discarded the supernatant and dried the pellets at room temperature for 5 minutes. After, we resuspended the pellets with 50 µl 10 mM Tris buffer (pH 8) and added 2 µl RNase (10 mg/ml stock). We used heat inactivation of DNase and RNA digestion at 65°C for 30 minutes.

De Novo Genome assembly of N. guarroi

The DNA extracts were submitted to the Biotechnology Center at the University of Wisconsin—Madison for library preparation for Illumina (MiSeq) short reads platform with 250 bp paired-end reads and, additionally, for PacBio long reads platform. We used the Center for High Throughput Computing (CHTC) at the University of Wisconsin—Madison to assemble the genome of *N. guarroi* using the PacBio long reads, and we used the Illumina short reads for genome estimation via kmer analysis. We used Flye to assemble the PacBio reads, followed by assessing genome completeness using Busco, and genome annotation by Augustus (Fig. 1).

Protein BLAST

Using documented keratinases, specialized enzymes that degrade keratin, within the serine and metalloprotease enzymatic families, we identified the presence and absence of these enzymes across the assembled genomes of multiple CANV-related species; *N.*

guarroi, *N. barbatae* (PRJNA662660), *N. arthrosporioides* (PRJNA843904), and *O. ophiodiicola* (MWKM00000000) (Qiu *et al.* 2020; 2022; Stchigel *et al.* 2013; Sigler *et al.* 2013; Ohkura *et al.* 2017). Our comparison across the different fungi used the MEROPS database to choose specific keratinases identified from the literature as being involved in keratin degradation, S1, S8, S9, S10, S16, M3, M4, M14, M16, M28, M36, M38, and M55 (Table 2). Because some of the keratinase proteins denoted in the MEROPS database were often first isolated from bacteria, when it was available, we used homologs of those proteins identified in fungal species. Except for *N. guarroi*, the other publicly available assembled genomes were used from NCBI. Additionally, we investigated and compared the signal peptides and transmembrane proteins between *O. ophiodiicola* and *N. guarroi* using DeepTMHMM (STable 1).

Results

De Novo Genome assembly of N. guarroi

The de novo PacBio assembly created using Flye produced a high-quality assembly with a Busco completeness score of 99.4% (Table 1). With the Illumina reads used for kmer analysis, the genome was estimated to be toward 39.8 Mbp; we deposited the genome into the NCBI database (PRJNA1003134).

Protein BLAST and DeepTMHMM

Between the four fungal species, *N. guarroi* and *O. ophiodiicola* had all 13 serine and metalloproteases family proteins putatively appear in their assemblies while *N. arthrosporioides* and *N. barbatae* had 12 out of 13; chymotrypsin was absent from *N. arthrosporioides* and *N. barbatae*. Of the 13 proteins of interest, nine had an e-value of <0.05 and a percent identity over 60%, our chosen cut-off (Table 3); within the significant

nine, metalloproteases were the majority. Resulting from the DeepTMHMM interface, 1568 signal peptides between *N. guarroi* and *O. ophiodiicola* were identified either as transmembrane proteins (totaling to 1182) or as secreted proteins (totaling to 386; Fig. 3). Within our assembled *N. guarroi,* 1123 signal peptides were identified and split into 884 secreted proteins and 239 transmembrane proteins. Comparatively, within the 2015 Ohkura *et al.* assembled genome of *O. ophiodiicola,* 445 identified signal proteins split into 298 secreted proteins and 147 transmembrane proteins.

Discussion

Proteolytic activity has been investigated and implicated as being involved with pathogenicity for decades. Much of the research into genes behind the suite of endoproteases and exoproteases often used by pathogens have largely been within bacteria like *Bacillus subtilis* and other Bacillus species leaving a sizeable knowledge gap in fungi (Williams *et al.* 1990). However, within recent years many studies have dived into the secretomes of various fungal pathogens to identify specialized enzymes necessary for pathogenicity.

Our first steps into finding keratinases within CANV-related fungi revealed a variety of serine and metalloproteases previously associated with the infection process (Monod *et al.* 2002). Evident by our significant nine enzymes of interest (chymotrypsin, Map1, MepB, carboxypeptidase 1, pitrilysin, isoaspartyl dipeptidase, aminopeptidase, D-aminopeptidase, and fungalysin) our *N. guarroi* genome appears to contain the majority of the significant nine. Chymotrypsin, carboxypeptidase 1, and Map1 are all within the super-family of serine proteases known for hydrolytic cleaving of peptide bonds whereas MepB, pitrilysin, isoaspartyl dipeptidase, aminopeptidase, D-aminopeptisdase, and

fungalysin live within the diverse metalloprotease families known for microbial pathogenesis. Because we used publicly available genomes in our protein comparison, the quality of each assembly may vary affecting the blast results; underestimating the number of specialized proteins present. Furthermore, because the protein sequences used as a query were from different fungal species, we kept to a strict cut off using both the percent identity and e-value. Therefore, other genes that potentially code for more specialized enzymes may still be present despite their resulting low values from our blast pipeline (STable1-4).

Although blast hits of the Subtilisin Carlsberg, a serine protease, did not meet our strict cut off, its presence throughout all four fungi, especially within *N. guarroi*, is notable. Subtilisin proteases are ubiquitous in fungi and have been closely tied to host-pathogen interactions, especially with entomopathogenic fungi (Bagga *et al*. 2004). Specifically, the subtilisins are often secreted by fungal pathogens to digest protein components of their hosts (Segers *et al.* 1999). Even if Subtilisin Carlsberg may not be the specific protein being identified in our blast results, a putative subtilisin-like protein appears across all four fungi with multiple copies in *N. guarroi*, possibly alluding to why YFD progresses so severely.

Of our results, the comparison of signal peptides between *N. guarroi* and *O.ophiodiicola* is the most striking. Our analysis is the first to explicitly show that *N. guarroi* could have far more diverse and expansive pathogenicity factors evident by the quantity of secreted proteins compared to *O. ophiodiicola* (Fig. 3). That *O. ophiodiicola* requires a disturbance in the epidermis of reptiles for infection to establish is not an uncommon requirement among primary (infects healthy hosts without preexisting conditions) and secondary

(opportunistic) fungal pathogens (Lorch *et al*. 2015; Allender *et al.* 2015). However, *N. guarroi* can cause severe, and often, fatal infections within bearded dragons and green iguanas as a primary pathogen, and without requiring an entry point in the epidermis. Although putative, we believe our findings in addition to what is known about YFD support our initial hypothesis. *Nannizziopsis guarroi* is an EID and as we lay the groundwork for further research into the pathogenicity of the fungus, we may be on the precipice of creating a new model system with the Nannizziopsis genus.

Acknowledgements

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Literature Cited

1. Abarca ML, Martorell J, Castellá G, Ramis A, Canañes FJ. 2008. Cutaneous hyalohyphomycosis caused by a Chrysosporium species related to Nannizziopsis vriesii in two green iguanas (Iguana iguana). Medical Mycology 46:349–354.
2. Allender MC, Baker S, Loper D, Dreslik MJ, Phillips CA, Maddox C, Driskell EA. 2015. Development of snake fungal disease after experimental challenge with *Ophidiomyces ophiodiicola* in Cottonmouths (*Agkistrodon piscivorous*). Plos One 10:10 e0140193 https://doi.org/10.1371/journal.pone.0140193
3. Anderson RM and May RM. 1982. Coevolution of hosts and parasites. Parasitology 85:2 411–426 https://doi.org/10.1017/S0031182000055360
4. Bagga S, Hu G, Screen SE, St. Leger RJ. 2004. Reconstructing the diversification of subtilisins in the pathogenic fungus *Metarhizium anisopliae*. Gene 324: https://doi.org/10.1016/j.gene.2003.09.031

5. Baker RE, Mahmud AS, Miller IF, Rajeev M, Rasambainarivo F, Rice BL, Takahashi S, Tatem AJ, Wagner CE, Wang L-F, Wesolowski A, Metcalf JE. 2022. Infectious disease in an era of global change. Nature Reviews Microbiology 20:193–205 https://doi.org/10.1038/s41579-021-00639-z

6. Barr AR. 1979 Epidemiological concepts for entomologists. Bulletin of the Entomological Society of America 25:2 129–130 https://doi.org/10.1093/besa/25.2.129

7. Blackwell M and Vega FE. 2018. Lives within lives: hidden fungal biodiversity and the importance of conservation. Fungal Ecology 35: 127–134 https://doi.org/10.1016/j.funeco.2018.05.011

8. Blair JE. 1965. Host-parasite relationships: a summation. Annals of the New York Academy of Sciences 128:1 451–456 https://doi.org/10.1111/j.1749-6632.1965.tb11654.x

9. Cabañes FJ, Sutton DA, Guarroi J. 2014. *Chrysosporium*-related fungi and reptiles: a fatal attraction. Plos Pathogens 10:1–4.

10. Cardoso B, García-Bocanegra I, Acevedo P, Cáceres G, Alves PC, Gortázar C. 2022. Stepping up from wildlife disease surveillance to integrated wildlife monitoring in Europe. Veterinary Science 144:149–156 https://doi.org/10.1016/j.rvsc.2021.11.003

11. Casadevall A and Pirofski LA. 1999. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infection and Immunity 67:8 3703–3713 https://doi.org/10.1128/IAI.67.8.3703-3713.1999

12. Casadevall A and Pirofski LA. 2001. Host-pathogen interactions: the attributes of virulence. Journal of Infectious Disease 184:337–344

13. Gentry SL, Lorch JM, Lankton JS, Pringle A. 2021. Koch's postulates: confirming Nannizziopsis guarroi as the cause of yellow fungal disease in Pogona vitticeps. Mycologia 113:6 1253–1263.

14. Hawksworth DL and Lücking R. 2016. Fungal diversity revisited: 2.2 to 3.8 million species. Microbial Spectrum 5:4 https://doi.org/10.1128/microbiolspec.FUNK-0052-2016

15. Locey KJ and Lennon JT. 2016. Scaling laws predict global microbial diversity. Proceedings of the National Academy of Sciences 113:21 5970–5975 https://doi.org/10.1073/pnas.1521291113

16. Lorch JM, Lankton J, Werner K, Falendysz EA, McCurley K, Blehert DS. 2015. Experimental infection of snakes with *Ophidiomyces ophiodiicola* causes pathological changes that typify snake fungal disease. American Society for Microbiology mBio 6:1–9 https://doi.org/10.1128/mbio.01534-15

17. Monod M, Capoccia S, Léchenne B, Zaugg C, Holdom M, Jousson O. 2002. Secreted proteases from pathogenic fungi. International Journal of Medical Microbiology 292:405–419 https://doi.org/10.1078/1438-4221-00223

18. Ndow G, Ambe JR, Tomori O. 2019. Emerging infectious disease: a historical and scientific review. In: Tangwa G, Abayomi A, Ujewe S, Munung N (eds). Socio-cultural Dimensions of Emerging Infectious Diseases in Africa pp 31–40. Springer, Cham https://doi.org/10.1007/978-3-030-17474-3_3

19. Ohkura M, Fitak RR, Wisecaver JH, DeBlasio D, Niazi F, Egholm M, Rounsley SD, Kodira CD, Orbach MJ. 2015. Genome sequence of *Ophidiomyces ophiodiicola*, an emerging fungal pathogen of snakes. Genome Announcements 5:30 https://doi.org/10.1128/genomea.00677-17

20. Peterson NR, Rose K, Shaw S, Hyndman TH, Sigler L, Kurtböke Dİ, Llinas J, Littleford-Colquhoun BL, Cristescu R, Frère C. 2020. Cross-continental emergence of *Nannizziopsis barbatae* disease may threaten wild Australian lizards. Scientific Reports 10:20976 https://doi.org/10.1038/s41598-020-77865-7

21. Segers R, Butt TM, Carder JH, Keen JN, Kerry BR, Peberdy JF. 1999. The subtilisins of fungal pathogens of insects, nematodes and plants: distribution and variation. Mycological Research 103:4 395–402 https://doi.org/10.1017/S0953756298007345

22. Sigler L, Hambleton S, Paré JA. 2013. Molecular characterization of reptile pathogens currently known as members of the Chrysosporium anamorph of Nannizziopsis vriesii complex and relationship with some human-associated isolates. Journal of Clinical Microbiology 51:3338–3357.

23. Souto XM, Branquinha MH, Santos AL. 2019. Chymotrypsin- and trypsin-like activities secreted by the multidrug-resistant yeasts forming the *Candida haemulonii* complex. Anais da Academia Brasileira de Ciências 91:3 e20180735 https://doi.org/10.1590/0001-3765201920180735

24. Stephen C, Carmo LP, de las Nieves Montano Valle D, Friker B, Sousa FM, Vidondo B, Berezowski J. 2022. The implementation gap in emerging disease risk management in the wildlife trade. Journal of Wildlife Disease 58:4 705–715 https://doi.org/10.7589/JWD-D-21-00199

25. Tanada Y and Kaya HK. 1993. Insect pathology. Academic Press, Inc. 318–387

26. Thomas SR and Elkinton JS. 2004. Pathogenicity and virulence. Journal of Invertebrate Pathology 85:3 146–151 https://doi.org/10.1016/j.jip.2004.01.006

27. Tripp EA, Zhang N, Schneider H, Huang Y, Mueller GM, Hu Z, Häggblom M, Bhattacharya D. 2017. Reshaping Darwin's tree: impact of the symbiome. Trends in Ecology & Evolution 32:8 552–555 https://doi.org/10.1016/j.tree.2017.05.002

28. Tsuboi R, Sanada T, Takamori K, Ogawa H. 1987. Isolation and properties of extracellular proteinases from *Sporothrix schenckii*. Journal of Bacteriology 169:9 4104–4109 https://doi.org/10.1128/jb.169.9.4104-4109.1987

29. Williams CM, Richter CS, MacKenzie Jr. JM, Shih JCH. 1990. Isolation, identification, and characterization of a feather-degrading bacterium. Applied and Environmental Microbiology 56:6 https://doi.org/10.1128/aem.56.6.1509-1515.1990

FIGURES & TABLES

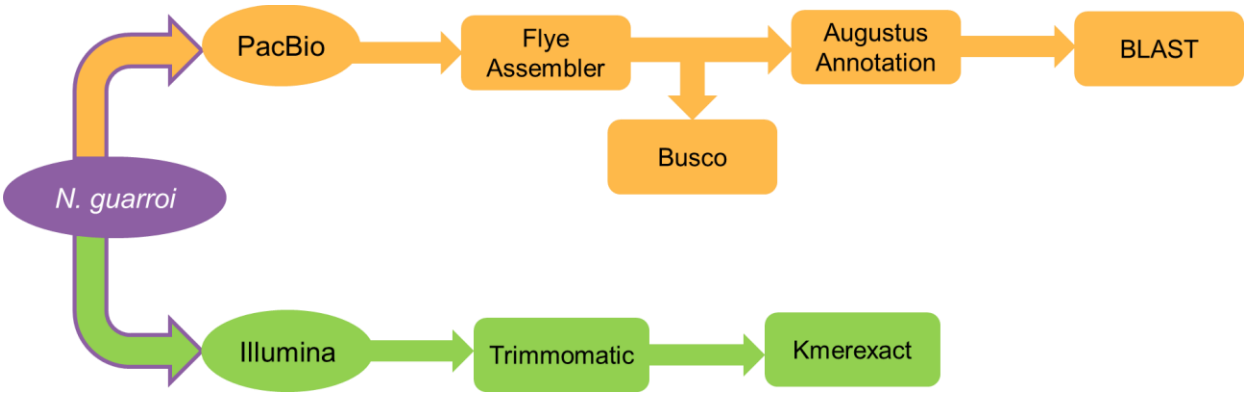

Figure 1. Bioinformatic Workflow. The yellow path illustrates the programs used to assemble PacBio data of *N. guarroi.* The green path illustrates programs used on the Illumina data of *N. guarroi.*

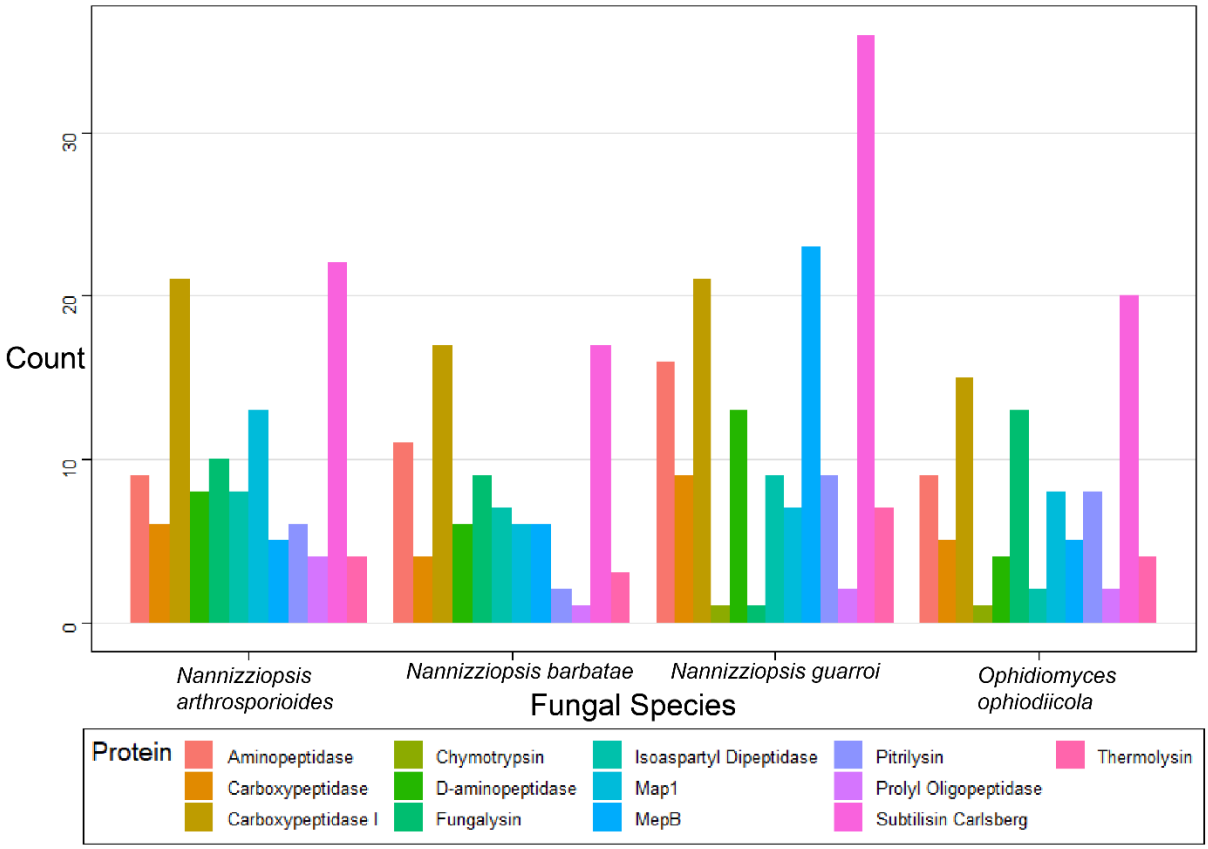

Figure 2. Protein blast comparisons across four fungal species. The X axis denotes fungal species, and the Y axis denotes the number of times a sequence within the assembled genomes potentially codes for a protein of interest.

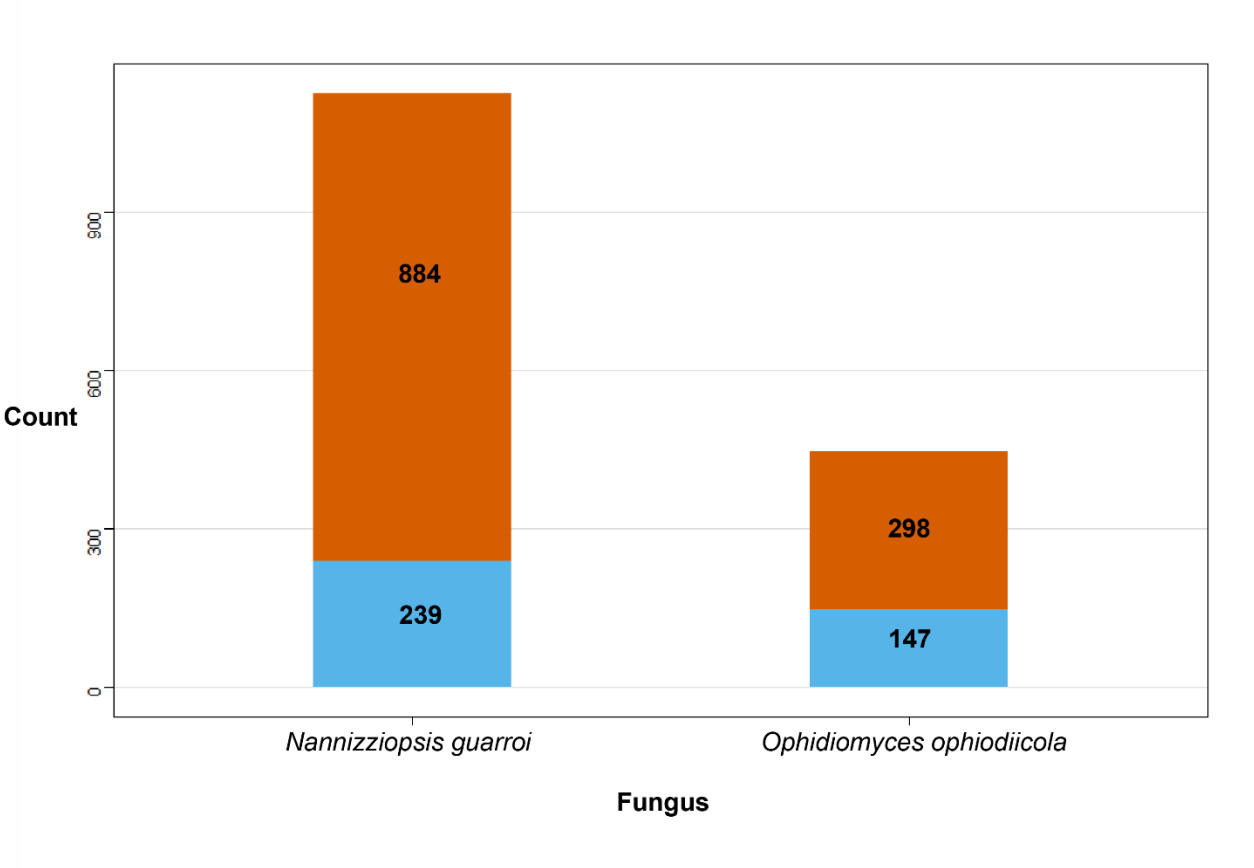

Figure 3. Putative secreted and transmembrane proteins from DeepTMHMM. The X axis denotes fungal species, and the Y axis denotes the number of genes identified as signal peptides. The orange represents secreted proteins, and the blue represents transmembrane proteins.

Table 1. PacBio genome assembly statistics.

Total Length	37647880 bp
Contigs	44
N50	4331355 bp
Mean Coverage	929
Busco Completeness	99.4%

Table 2. MEROPS protein identifiers.

Protein MEROPS ID	Proteins We Used	Our NCBI Accessions
S1	Chymotrypsin	CAB60729.1
S8	Subtilisin Carlsberg	XP_049137919.1
S9	Prolyl Oligopeptidase	QKM76211.1
S10	Carboxypeptidase I	AAR91697.1
S16	Map1	XP_003867298.1
M3	MepB	AAB66656.1
M4	Thermolysin	XP_025455915.1
M14	Carboxypeptidase	GBF59608.1
M16	Pitrilysin	KAG8205452.1
M28	Aminopeptidase	CAC38353.1
M36	Fungalysin	KMQ48706.1
M38	Isoaspartyl Dipeptidase	KAF5563136.1
M55	D-aminopeptidase	KGQ03962.1

Table 3. Protein blast e-value and percent identity.

	N. arthrosporioides	***N. barbatae***	***N. guarroi***	***O. ophiodiicola***
Protein	BLAST e-value | percent identity			
Aminopeptidase	0 | 67%	0 | 69%	0 | 62%	0 | 67%
Carboxypeptidase I	0 | 62%	0 | 62%	6.30e-1 | 74%	0 |66%
Chymotrypsin			4.30e-153 | 71%	
D-aminopeptidase			0 | 99%	
Fungalysin	5.54e-84 | 80%	0 | 74%	0 | 75%	0 |75%
Isoamyl Dipeptidase	4.35e-169 | 81%	1.28e-164 | 83%		
Map I	1.28e-114 | 71%	4.20e-121 | 72%	1.83e-108 | 93%	1.83E-116 | 73%
Mep B	0 | 79%	0 | 80%	0 | 78%	0 | 78%
Pitrilysin	0 | 65%	0 | 67%	0 | 66%	0 | 65%

The shaded boxes represent the absence of a protein at the chosen cut off.

APPENDIX I – Supplementary Material

Supplementary Table 1. Fungi and bacteria cultured from individual inoculation sites on each corn snake used in this study. ant

Fungal and bacterial species cultured from each animal					
Animal ID/ Genbank Accession No.	**Snout**	**Dorsal Neck**	**Ventral Neck**	**Dorsal Midbody**	**Ventral Midbody**
Corn snake negative controls					
CS1NC OP798776 OP798777 OP798778 OP801847		*Penicillium brevicompactum*	*Aspergillus terreus*	*Penicillium steckii* *Archromobacter sp.*	*Archromobacter sp.*
CS2NC					*Penicillium steckii*
Corn snakes inoculated with *Ophidiomyces ophiodiicola*					
CS3O		*Ophidiomyces ophiodiicola*		*Ophidiomyces ophiodiicola*	
CS4O	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*
CS5O	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*
CS6O	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*		*Ophidiomyces ophiodiicola*
CS7O	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*
Corn snakes inoculated with *Nannizziopsis guarroi*					
CS8N				*Nannizziopsis guarroi*	
CS9N OP798779		*Nannizziopsis guarroi*	*Beauveria bassiana*		
CS10N					
CS11N		*Nannizziopsis guarroi*		*Nannizziopsis guarroi*	*Nannizziopsis guarroi*

Grey boxes indicate no microbe isolated from that area.

Supplementary Table 2. Fungi and bacteria cultured from individual inoculation sites on each bearded dragon used in this study.

Fungal and bacterial species cultured from each animal						
Animal ID/ Genbank Accession No.	**Snout**	**Dorsal Neck**	**Ventral Neck**	**Dorsal Midbody**	**Ventral Midbody**	**Hindleg**
Sham-inoculated						
**BD1NC MT503285 MT503223				*Chaetomium globosum*	*Kocuria sp.*	
BD2NC						
Bearded dragons inoculated with ***Ophidiomyces ophiodiicola***						
BD3O	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	
BD4O	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*		*Ophidiomyces ophiodiicola*	*Purpureocillium lilacinum*	
BD5O	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	
BD6O	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*	*Ophidiomyces ophiodiicola*
BD7O OP798780 OP801848	*Ophidiomyces ophiodiicola*	*Purpureocillium lilacinum*	*Deftia sp.*			
Bearded dragons inoculated with ***Nannizziopsis guarroi***						
**BD8N MT503284	*Nannizziopsis guarroi*	*Nannizziopsis guarroi* *Neosetophoma guiyangensis*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	
BD9N	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*		*Nannizziopsis guarroi*	
BD10N	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*
BD11N	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*		*Nannizziopsis guarroi*	*Nannizziopsis guarroi*
BD12N		*Nannizziopsis guarroi*		*Nannizziopsis guarroi*	*Nannizziopsis guarroi*	*Nannizziopsis guarroi*

This table includes data from Gentry *et al.* 2021. * BD8N developed a lesion on its tail from which a culture was taken, resulting in a positive identification of N. guarroi. BD11N developed a lesion near the eye and a part was taken for culture, also resulting in a positive identification of N. guarroi. Grey boxes indicate no microbe isolated from that area. **Genbank accession no. included from our previously published manuscript (Gentry *et al.* 2021).

APPENDIX II – Supplementary Material

Supplementary Table 1. Protein blast results of *Nannizziopsis arthrosporioides*.

Nannizziopsis arthrosporioides

qseqid	sseqid	identity	length	mismatch	gapopen	qstart	qend	sstart	send	evalue	bitscore
XP_003867298.1	CP098259.1	71.362	213	60	1	159	371	1079304	1079939	1.28E-114	330
XP_003867298.1	CP098259.1	43.796	137	65	2	27	163	1078885	1079259	1.28E-114	101
XP_003867298.1	CP098259.1	44.737	38	20	1	9	46	1078670	1078780	0.003	39.3
XP_003867298.1	CP098259.1	50	34	15	2	31	63	3933008	3933106	5.7	28.9
XP_003867298.1	CP098259.1	48.128	27	14	0	263	289	2973761	2973841	9.1	28.1
XP_003867298.1	CP098263.1	60.337	227	74	1	162	372	2319590	2320270	3.37E-109	292
XP_003867298.1	CP098263.1	46.316	136	65	3	32	163	2319150	2319545	3.37E-109	121
XP_003867298.1	CP098263.1	44.186	43	22	2	5	46	2319008	2319133	0.001	40.4
XP_003867298.1	CP098262.1	28.155	103	52	4	60	145	2026798	2027091	2.7	29.6
XP_003867298.1	CP098261.1	33.336	63	39	2	306	367	4376 79	4374 97	4.7	28.9
XP_003867298.1	CP098261.1	41.176	17	10	0	268	284	4723951	4724001	9.3	22.7
XP_003867298.1	CP098261.1	26.332	38	28	0	284	321	4723982	4724095	9.3	20.8
XP_003867298.1	CP098260.1	25.324	63	41	3	187	246	6532226	6532411	9.2	28.1
KMQ48706.1	CP098259.1	60.244	342	118	2	124	463	4721448	4720423	1.11E-141	409
KMQ48706.1	CP098259.1	51.412	103	49	1	22	123	4721815	4721507	1.11E-141	114
KMQ48706.1	CP098263.1	66.663	219	54	1	436	654	4720397	4719741	9.07E-92	311
KMQ48706.1	CP098263.1	58.144	354	119	4	122	469	2208 17	2197 65	6.83E-137	406
KMQ48706.1	CP098263.1	51.712	87	42	0	36	122	2211 30	2208 70	6.83E-137	100

KMQ48706.1	CP098263.1	73.381	139	37	0	436	574	21975135	21933588	5.54E-84	222
KMQ48706.1	CP098263.1	80.328	61	12	0	575	5	21927088	21908484	5.54E-84	108
KMQ48706.1	CP098259.1	36.735	49	31	0	28	7676	33071771	33057163	0.63	32.7
KMQ48706.1	CP098259.1	28.846	52	35	1	583	632	53846621	53864704	4.7	30
KMQ48706.1	CP098260.1	25	72	54	0	304	3705	98221702	98209807	7	29.3
KGQ03962.1	CP098260.1	23.958	192	117	5	23	205	48998825	48993670	1.18E-06	51.2
KGQ03962.1	CP098260.1	33.721	86	48	3	344	4232	31181002	31178490	0.39	33.1
KGQ03962.1	CP098260.1	36.538	52	28	2	449	498	13870971	13869518	0.86	32
KGQ03962.1	CP098260.1	27.027	37	27	0	160	196	72004252	72015202	2.5	30.4
KGQ03962.1	CP098260.1	25.294	170	99	9	385	528	21680408	21685435	5.5	29.3
KGQ03962.1	CP098263.1	25.601	164	92	6	346	502	85318132	85268991	5.2	29.6
KGQ03962.1	CP098263.1	25.556	90	61	2	143	226	36108096	36113078	9.2	28.9
KGQ03962.1	CP098262.1	38.462	39	24	0	277	315	49189074	49178348	8.3	28.9
AAB66656.1	CP098261.1	74.83	441	109	1	1	439	54685029	54693511	0	644
AAB66656.1	CP098261.1	78.713	202	43	0	440	641	54694043	54700090	0	342
AAB66656.1	CP098261.1	78.125	64	14	0	653	716	54700996	54702902	0	108
AAB66656.1	CP098263.1	22.407	723	451	22	27	700	38357120	38337277	2.55E-37	150
AAB66656.1	CP098262.1	26.263	99	57	3	566	654	22834932	22832153	2.6	30.8
XP_025455915.1	CP098261.1	44.444	36	18	1	282	317	53389357	53390360	0.29	32.7
XP_025455915.1	CP098262.1	24.194	62	43	1	255	316	87484922	87508750	1.6	30.4
XP_025455915.1	CP098263.1	37.931	29	18	0	301	329	22459144	22450580	6.8	28.5
XP_025455915.1	CP098263.1	32.727	55	30	1	130	177	77437612	77447676	6.4	28.5
AAR91697.1	CP098261.1	61.967	610	208	4	21	607	10767457	10785710	0	748
AAR91697.1	CP098263.1	27.273	506	254	16	57	539	50469811	50482250	6.13E-35	141

AAR91697.1	CP098261.1	25.241	519	274	15	41	53 6	2837 426	2836 143	9.60E-31	128
AAR91697.1	CP098261.1	48.462	130	62	3	393	52 1	6120 755	6120 378	3.67E-29	114
AAR91697.1	CP098261.1	33.333	48	30	1	523	57 0	6120 312	6120 175	3.67E-29	32.7
AAR91697.1	CP098261.1	41.509	159	56	6	21	15 7	6122 337	6121 906	2.03E-25	112
AAR91697.1	CP098261.1	29.06	117	38	5	215	29 0	6121 652	6121 314	2.43E-07	37
AAR91697.1	CP098261.1	41.379	29	17	0	308	33 6	6121 147	6121 061	2.43E-07	30.8
AAR91697.1	CP098261.1	50	22	11	0	288	30 9	6121 263	6121 198	2.43E-07	23.9
AAR91697.1	CP098261.1	48.276	29	13	1	32	58	1310 527	1310 613	6.6	29.3
AAR91697.1	CP098260.1	39.626	588	323	14	41	60 9	2977 934	2979 658	7.60E-119	389
AAR91697.1	CP098260.1	31.551	187	73	7	101	28 5	1812 770	1813 171	2.02E-13	73.6
AAR91697.1	CP098260.1	24.59	61	39	1	414	47 4	3635 761	3635 600	1.6	31.6
AAR91697.1	CP098260.1	30.769	78	48	2	226	30 2	2675 610	2675 392	2.8	30.8
AAR91697.1	CP098262.1	27.826	460	243	15	88	53 6	5072 27	5083 72	2.57E-33	135
AAR91697.1	CP098262.1	31.915	47	21	2	41	87	5070 57	5071 64	2.57E-33	25.4
AAR91697.1	CP098262.1	42.222	135	61	3	26	15 8	8026 80	8023 21	8.59E-22	100
AAR91697.1	CP098259.1	43.529	85	47	1	74	15 8	2864 678	2864 427	8.11E-13	71.6
AAR91697.1	CP098259.1	30.159	63	43	1	471	53 2	1493 519	1493 331	2.4	30.8
AAR91697.1	CP098263.1	42.424	66	38	0	85	15 0	3112 005	3112 202	6.36E-10	62
AAR91697.1	CP098263.1	26.667	150	96	3	403	54 0	3113 041	3113 484	2.07E-05	47.4
KAG820545.2.1	CP098259.1	64.689	914	292	6	1	89 6	2149 669	2146 967	0	1179
KAG820545.2.1	CP098259.1	69.753	162	48	1	894	10 55	2146 976	2146 494	1.28E-66	246
KAG820545.2.1	CP098262.1	27.184	206	119	5	110	31 1	6204 54	6209 90	2.39E-11	68.2
KAG820545.2.1	CP098263.1	46.667	30	16	0	447	47 6	8627 57	8626 68	3.3	31.2
KAG820545.2.1	CP098263.1	23.944	71	54	0	154	22 4	3590 999	3591 211	8.4	30

KAG820545	CP0982						19	1371	1370		
2.1	61.1	40	40	23	1	152	1	012	896	6.5	30.4
CAC38353.	CP0982	66.6					84	5593	5590		
1	61.1	67	870	261	2	1	1	263	654	0	1192
CAC38353.	CP0982						88	5590	5590	9.40E-	
1	61.1	70	40	12	0	841	0	601	482	11	65.5
CAC38353.	CP0982	28.5					74	1182	1181		
1	61.1	71	84	54	3	661	0	097	852	2.6	31.2
CAC38353.	CP0982						61	5074	5073	4.46E-	
1	60.1	39.2	375	134	8	320	0	819	725	135	228
CAC38353.	CP0982	36.4					31	5075	5074	4.46E-	
1	60.1	58	288	124	8	87	9	723	872	135	165
CAC38353.	CP0982	25.8					87	5073	5072	4.46E-	
1	60.1	74	286	192	3	611	7	675	821	135	129
CAC38353.	CP0982						79	6642	6641		
1	60.1	40	35	21	0	756	0	005	901	4.9	30.4
CAC38353.	CP0982	26.6					34	5290	5288	1.48E-	
1	62.1	67	105	64	2	247	8	90	06	04	45.4
CAC38353.	CP0982						86	2135	2135		
1	63.1	25	84	53	2	783	0	052	291	0.29	34.7
KAF556313	CP0982	62.0					37	1182	1181	4.35E-	
6.1	62.1	97	248	57	3	162	3	282	542	169	298
KAF556313	CP0982	80.6					46	1181	1181	4.35E-	
6.1	62.1	45	93	18	0	374	6	488	210	169	162
KAF556313	CP0982	65.5					16	1182	1182	4.35E-	
6.1	62.1	17	87	27	1	85	8	574	314	169	123
KAF556313	CP0982	54.8						1182	1182	4.35E-	
6.1	62.1	39	62	28	0	30	91	797	612	169	73.9
KAF556313	CP0982						19	2451	2450		
6.1	59.1	50	26	13	0	167	2	047	970	3.3	30
KAF556313	CP0982	46.1					40	3297	3297		
6.1	63.1	54	26	14	0	382	7	277	200	5	29.3
KAF556313	CP0982	29.6					27	6715	6715		
6.1	60.1	3	54	38	0	226	9	128	289	5.3	29.3
KAF556313	CP0982	28.7					11	3515	3516		
6.1	60.1	67	73	42	1	52	4	998	216	7.4	28.9
XP_049137	CP0982	31.0					77	2168	2166	4.34E-	
919.1	63.1	39	770	439	23	52	1	194	011	85	300
XP_049137	CP0982	33.3					25	1148	1148		
919.1	63.1	33	75	30	3	185	9	701	865	0.3	34.7
XP_049137	CP0982	27.4					34	2810	2810		
919.1	63.1	39	164	82	7	185	1	480	881	4.4	30.8
XP_049137	CP0982	29.1					92	4428	4430	2.02E-	
919.1	60.1	87	627	395	19	315	5	856	637	82	191
XP_049137	CP0982	35.1					24	4428	4428	2.02E-	
919.1	60.1	65	182	97	5	68	8	052	537	82	90.1
XP_049137	CP0982	50.6					31	4428	4428	2.02E-	
919.1	60.1	49	77	31	2	246	7	579	803	82	64.7

XP_049137	CP0982	57.1						4427	4427	2.02E-	
919.1	60.1	43	14	6	0	50	63	939	980	82	22.3
XP_049137	CP0982	28.8					35	6015	6016		
919.1	60.1	89	225	124	10	143	5	628	230	0.008	39.7
XP_049137	CP0982	22.6					29	6087	6093		
919.1	60.1	77	269	142	9	47	1	17	97	0.51	33.9
XP_049137	CP0982	30.0					29	4162	4183		
919.1	60.1	88	113	38	6	185	7	0	5	0.82	33.1
XP_049137	CP0982	36.9					59	4232	4245		
919.1	60.1	57	46	29	0	553	8	2	9	1.7	32
XP_049137	CP0982	30.3					29	1615	1615		
919.1	60.1	57	112	52	6	185	6	729	472	2	32
XP_049137	CP0982	33.9					67	5854	5854		
919.1	60.1	62	53	31	1	625	7	572	426	9.8	29.6
XP_049137	CP0982	29.4					92	1288	1291	3.54E-	
919.1	59.1	87	780	466	19	202	9	871	114	77	276
XP_049137	CP0982	34.2					21	1288	1288	3.28E-	
919.1	59.1	54	181	87	6	55	3	395	907	19	93.6
XP_049137	CP0982	26.4					34	3189	3189		
919.1	59.1	71	170	88	8	185	7	976	557	1.5	32.3
XP_049137	CP0982	25.7					34	4065	4065		
919.1	59.1	67	163	86	7	185	1	205	606	4.5	30.8
XP_049137	CP0982	27.3					34	1467	1467		
919.1	62.1	29	161	85	6	185	1	719	321	0.015	38.9
XP_049137	CP0982	27.8					34	4724	4724		
919.1	61.1	79	165	78	6	185	0	713	315	0.051	37
XP_049137	CP0982	33.3					25	3202	3202		
919.1	61.1	33	75	31	4	185	9	668	501	1.1	32.7
XP_049137	CP0982	36.1					59	5168	5168		
919.1	61.1	11	72	32	3	541	8	229	014	1.5	32.3
XP_049137	CP0982	35.4					47	3253	3252		
919.1	61.1	17	48	25	2	432	3	000	857	8	29.6
QKM76211	CP0982	33.3					42	1778	1777		
.1	60.1	33	51	30	1	373	3	055	915	0.22	34.7
QKM76211	CP0982	35.8					14	3196	3197		
.1	61.1	49	53	31	1	94	3	906	064	1.2	32
QKM76211	CP0982	30.1					45	7414	7417		
.1	59.1	89	106	45	4	354	4	97	42	1.9	31.6
QKM76211	CP0982	23.8					45	2161	2160		
.1	62.1	1	168	91	6	326	6	243	740	2.5	31.2
GBF59608.	CP0982	59.2					45	2435	2436	1.09E-	
1	63.1	07	429	139	7	47	7	220	452	138	439
GBF59608.	CP0982	27.8					34	1228	1227	2.78E-	
1	61.1	01	241	142	10	131	5	093	389	06	49.7
GBF59608.	CP0982						21	3892	3892		
1	60.1	38	50	29	1	170	7	665	814	1.2	31.2
GBF59608.	CP0982						17	8132	8133		
1	60.1	42.5	40	22	1	139	7	47	66	1.9	30.8

GBF59608.1	CP098262.1	34	50	29	1	120	16 9	1751 313	1751 450	1.6	30.8
GBF59608.1	CP098259.1	29.412	68	42	1	128	18 9	2093 381	2093 178	9.5	28.5

Supplementary Table 2. Protein blast results of *Nannizziopsis barbatae*.

		Nannizziopsis barbatae									
qseqid	sseqid	identity	length	mismatch	gapopen	qstart	qend	sstart	send	evalue	bitscore
XP_007298.1	JACVYH010000002.1	72.22	216	59	1	159	37 4	2199 343	2199 987	4.20E-121	337
XP_007298.1	JACVYH010000002.1	33.503	197	87	3	9	16 2	2198 712	2199 299	4.20E-121	116
XP_007298.1	JACVYH010000004.1	58.952	229	74	1	164	37 2	1431 633	1432 319	1.11E-112	290
XP_007298.1	JACVYH010000004.1	50.4	125	57	2	43	16 3	1431 211	1431 582	1.11E-112	123
XP_007298.1	JACVYH010000004.1	34.043	47	29	2	1	1431 46	1431 033	1431 170	1.11E-112	34.3
XP_007298.1	JACVYH010000003.1	22.321	112	77	3	96	20 6	9645 02	9648 10	2	30.4
KMQ48706.1	JACVYH010000005.1	60.182	329	116	1	122	43 5	4133 64	4123 78	0	395
KMQ48706.1	JACVYH010000005.1	68.323	161	48	2	433	59 0	4123 33	4118 51	0	226
KMQ48706.1	JACVYH010000005.1	51.724	87	42	0	36	12 2	4136 75	4134 15	0	103
KMQ48706.1	JACVYH010000005.1	77.778	63	14	0	573	63 5	4118 46	4116 58	2.71E-24	108
KMQ48706.1	JACVYH010000006.1	67.582	182	59	0	254	43 5	6584 63	6579 18	0	254
KMQ48706.1	JACVYH010000006.1	50.199	251	104	3	22	25 1	6592 74	6585 22	0	248
KMQ48706.1	JACVYH010000006.1	70.548	146	43	0	436	58 1	6578 59	6574 22	0	220
KMQ48706.1	JACVYH010000006.1	73.77	61	16	0	575	63 5	6573 84	6572 02	0	105
KMQ48706.1	JACVYH010000002.1	22	250	137	10	56	26 7	3007 120	3006 431	2	31.6
KGQ03962.1	JACVYH010000001.1	24.457	184	128	7	23	20 5	7894 899	7894 378	9.04E-06	48.5
KGQ03962.1	JACVYH010000001.1	39.394	33	20	0	108	14 0	2272 727	2272 629	0.55	33.1
KGQ03962.1	JACVYH010000002.1	26.316	57	42	0	202	25 8	3851 800	3851 970	1.1	25.8
KGQ03962.1	JACVYH010000002.1	81.818	11	2	0	153	16 3	3851 714	3851 746	1.1	23.1

KGQ03962.1	JACVYH010000004.1	38.76	49	26	1	473	51	1203	1203	2.7	30.8
KGQ03962.1	JACVYH010000003.1	26.923	52	38	0	306	35	4050	4050	5.2	30
AAB66656.1	JACVYH010000007.1	75.283	441	107	1	1	43	4135	4122	0	646
AAB66656.1	JACVYH010000007.1	79.703	202	41	0	440	64	4121	4115	0	346
AAB66656.1	JACVYH010000007.1	78.125	64	14	0	653	71	4114	4112	0	107
AAB66656.1	JACVYH010000001.1	23.974	609	362	20	136	70	3097	3099	1.95E-36	148
AAB66656.1	JACVYH010000004.1	28	75	47	2	433	50	3733	3733	1.9	32
AAB66656.1	JACVYH010000003.1	22.388	134	88	5	461	59	6732	6736	5.2	30.4
XP_025455915.1	JACVYH010000001.1	25.287	87	47	1	44	13	3419	3420	4.3	29.6
XP_025455915.1	JACVYH010000001.1	40	35	20	1	110	14	9225	9225	7.1	28.9
XP_025455915.1	JACVYH010000001.1	33.333	57	32	2	163	21	2146	2147	9.2	28.5
AAR91697.1	JACVYH010000004.1	62.193	611	207	4	21	60	3314	3312	0	761
AAR91697.1	JACVYH010000001.1	40.582	584	315	14	41	60	5120	5119	9.51E-123	400
AAR91697.1	JACVYH010000001.1	28.261	460	241	16	88	53	6274	6285	2.91E-34	139
AAR91697.1	JACVYH010000001.1	31.183	186	75	7	101	28	6352	6352	2.62E-13	73.6
AAR91697.1	JACVYH010000001.1	43.939	66	37	0	85	15	3915	3915	6.25E-10	62.4
AAR91697.1	JACVYH010000001.1	25.333	150	97	4	403	54	3914	3914	3.49E-04	43.9
AAR91697.1	JACVYH010000006.1	27.536	483	257	17	84	55	1083	1084	2.72E-32	134
AAR91697.1	JACVYH010000005.1	50	130	60	3	393	52	2347	2347	3.31E-31	119
AAR91697.1	JACVYH010000005.1	37.5	48	28	1	523	57	2347	2347	3.31E-31	35.4
AAR91697.1	JACVYH010000005.1	51.02	98	31	4	24	11	2345	2345	7.44E-23	92
AAR91697.1	JACVYH010000005.1	39.474	38	22	1	119	15	2345	2346	7.44E-23	34.7
AAR91697.1	JACVYH010000005.1	36.765	68	25	1	287	33	2346	2346	4.17E-09	41.6
AAR91697.1	JACVYH010000005.1	35.714	42	25	1	250	29	2346	2346	4.17E-09	30

AAR91697.1	JACVYH010000005.1	36.11	36	21	1	215	24	2346	2346	4.17E-09	26.6
AAR91697.1	JACVYH010000003.1	26.535	456	247	15	88	53	4638	4636	1.42E-29	125
AAR91697.1	JACVYH010000003.1	41.304	138	65	2	24	15	5159	5159	1.17E-23	106
AAR91697.1	JACVYH010000002.1	23.542	480	266	14	74	52	4389	4388	2.04E-15	80.5
KAG8205452.1	JACVYH010000002.1	66.542	107	327	6	1	10	3589	3586	0	1427
KAG8205452.1	JACVYH010000001.1	27.67	206	118	6	110	31	7433	7438	3.58E-11	67.8
CAC38353.1	JACVYH010000007.1	68.844	796	245	1	49	84	2742	2766	0	1140
CAC38353.1	JACVYH010000007.1	70	40	12	0	841	88	2767	2768	1.53E-10	65.5
CAC38353.1	JACVYH010000007.1	50.98	51	25	0	1	51	2740	2741	4.80E-09	60.5
CAC38353.1	JACVYH010000003.1	38.79	281	129	6	369	61	2493	2494	3.23E-74	161
CAC38353.1	JACVYH010000003.1	26.923	286	189	2	611	87	2494	2495	3.23E-74	137
CAC38353.1	JACVYH010000003.1	40.526	190	69	4	212	35	2492	2493	2.80E-55	139
CAC38353.1	JACVYH010000003.1	40.602	133	64	4	97	21	2492	2492	2.80E-55	92
CAC38353.1	JACVYH010000003.1	25.532	47	30	1	357	39	2493	2493	2.80E-55	24.6
CAC38353.1	JACVYH010000003.1	46.667	30	16	0	432	46	2493	2493	1.3	32.7
CAC38353.1	JACVYH010000001.1	50	34	17	0	315	34	6497	6496	4.96E-04	43.9
CAC38353.1	JACVYH010000004.1	43.75	32	18	0	162	19	3033	3033	8.3	30
KAF55631.1	JACVYH010000001.1	60.811	148	39	1	245	37	1441	1440	1.28E-164	186
KAF55631.1	JACVYH010000001.1	82.796	93	16	0	371	46	1440	1440	1.28E-164	166
KAF55631.1	JACVYH010000001.1	74.118	85	21	1	162	24	1441	1441	1.28E-164	125
KAF55631.1	JACVYH010000001.1	63.529	85	31	0	79	16	1441	1441	1.28E-164	117
KAF55631.1	JACVYH010000001.1	58.929	56	23	0	30	85	1441	1441	1.28E-164	69.7
KAF55631.1	JACVYH010000001.1	30.952	42	28	1	370	41	6941	6941	4.1	30
KAF55631.1	JACVYH010000003.1	26.087	46	34	0	296	34	3080	3080	2.7	30.8

XP_04913	JACVYH0100	28.9					91	2473	2475	7.82E-	
7919.1	00002.1	64	618	393	14	318	9	522	285	94	229
XP_04913	JACVYH0100	39.6					22	2472	2473	7.82E-	
7919.1	00002.1	1	154	82	4	70	2	706	137	94	102
XP_04913	JACVYH0100	39.2					32	2473	2473	7.82E-	
7919.1	00002.1	86	84	40	2	246	0	227	472	94	55.1
XP_04913	JACVYH0100	30.3					28	1878	1877		
7919.1	00002.1	03	99	45	5	185	3	159	935	1.9	32.3
XP_04913	JACVYH0100	25.1					36	4869	4868		
7919.1	00002.1	23	203	100	8	185	5	492	974	3.5	31.2
XP_04913	JACVYH0100	26.3					34	6282	6282		
7919.1	00002.1	8	163	85	7	185	1	554	955	4.7	30.8
XP_04913	JACVYH0100	31.1					77	1284	1282	1.36E-	
7919.1	00004.1	14	781	450	22	37	1	347	131	85	302
XP_04913	JACVYH0100	36.3					31	1421	1420	6.36E-	
7919.1	00006.1	64	275	127	9	68	7	580	825	80	142
XP_04913	JACVYH0100	29.4					92	1419	1419	6.36E-	
7919.1	00006.1	62	353	208	10	590	5	987	001	80	119
XP_04913	JACVYH0100	27.2					58	1420	1419	6.36E-	
7919.1	00006.1	73	275	187	6	315	9	778	993	80	75.1
XP_04913	JACVYH0100	26.4						1421	1421	6.36E-	
7919.1	00006.1	15	53	39	0	11	63	810	652	80	23.5
XP_04913	JACVYH0100	26.2					35	6622	6618		
7919.1	00006.1	57	179	85	7	185	4	32	10	0.3	35
XP_04913	JACVYH0100	34.7					59	1204	1204		
7919.1	00006.1	22	72	33	3	541	8	667	452	7	30.4
XP_04913	JACVYH0100	24.3					34	1960	1961	4.29E-	
7919.1	00005.1	75	320	174	13	47	1	869	699	04	44.3
XP_04913	JACVYH0100	27.8					35	1428	1428		
7919.1	00003.1	26	230	131	9	138	5	901	281	0.009	39.7
XP_04913	JACVYH0100	30.8					27	5067	5067		
7919.1	00003.1	51	94	42	5	185	8	668	456	0.16	35.8
XP_04913	JACVYH0100	26.7					34	1729	1729		
7919.1	00001.1	08	161	86	6	185	1	654	256	0.22	35.4
QKM7621	JACVYH0100	34.4					42	4141	4140		
1.1	00006.1	83	58	35	2	373	7	89	16	8	29.6
GBF59608.	JACVYH0100	60.1					45	1550	1551	3.33E-	
1	00004.1	9	422	138	6	52	8	646	866	143	452
GBF59608.	JACVYH0100	27.5					15	3449	3449		
1	00004.1	51	98	56	3	66	6	257	526	0.37	33.5
GBF59608.	JACVYH0100	37.7					28	5165	5166		
1	00004.1	78	45	26	2	238	1	29	60	8.6	28.9
GBF59608.	JACVYH0100	28.1					28	3686	3686		
1	00003.1	25	128	79	5	170	6	653	276	1.1	31.6

Supplementary Table 3. Protein blast results of *Nannizziopsis guarroi*.

Nannizziopsis guarroi

qseqid	sseqid	identity	length	mismatch	gapopen	qstart	qend	sstart	send	evalue	bitscore
XP_003867298.1	contig_12	70.053	187	56	0	164	35 0	2943 36	2948 96	1.82E-108	287
XP_003867298.1	contig_12	37.41	139	71	1	41	16 3	2938 58	2942 74	1.82E-108	93.2
XP_003867298.1	contig_12	92.593	27	2	0	346	37 2	2949 37	2950 17	1.82E-108	53.5
XP_003867298.1	contig_12	46.875	32	16	1	22	52	2937 33	2938 28	0.29	33.5
XP_003867298.1	contig_15	34	50	33	0	191	24 0	1789 564	1789 415	1.9	30.8
XP_003867298.1	contig_15	37.838	37	23	0	94	13 0	4172 665	4172 775	8.5	28.9
XP_003867298.1	contig_11	28.358	67	44	2	1	65	4313 407	4313 601	2.6	30.4
KMQ48706.1	contig_19	30.12	83	43	4	68	14 5	1291 643	1291 425	0.094	36.2
CAB60729.1	contig_19	70.886	395	93	5	1	37 3	6975 0	7093 4	4.30E-153	477
KGQ03962.1	contig_43	98.516	539	8	0	1	53 9	6352 4	6514 0	0	1103
KGQ03962.1	contig_15	70.513	156	46	0	384	53 9	3837 457	3836 990	1.74E-91	237
KGQ03962.1	contig_15	47.788	113	59	0	277	38 9	3837 777	3837 439	1.74E-91	118
KGQ03962.1	contig_15	22.12	217	138	7	163	35 2	2506 245	2505 607	5.79E-04	43.1
KGQ03962.1	contig_15	31.111	45	31	0	405	44 9	9003 44	9002 10	8.7	29.3
KGQ03962.1	contig_26	24.074	324	204	11	49	34 6	1279 129	1280 052	1.04E-07	55.1
KGQ03962.1	contig_12	22.458	236	142	7	143	35 4	6132 897	6133 553	2.07E-06	50.8
KGQ03962.1	contig_12	22.34	188	121	4	160	32 8	2940 131	2939 586	2.21E-05	47.8
KGQ03962.1	contig_12	22.927	205	131	5	163	34 6	1596 07	1590 11	0.001	42.4
KGQ03962.1	contig_12	19.697	66	53	0	235	30 0	4888 238	4888 041	2.8	31.2
KGQ03962.1	contig_12	45	40	21	1	2	41	3031 468	3031 352	8.1	29.6
KGQ03962.1	contig_11	24.103	195	125	7	22	20 6	6057 941	6058 486	0.35	33.9
KGQ03962.1	contig_30	30.952	42	29	0	161	20 2	6185 55	6184 30	2.6	31.2
AAB66656.1	contig_12	45.893	767	344	7	1	71 6	5783 889	5781 649	0	634

AAB66656.1	contig_21	50.9	389	167	4	240	62 7	1890 114	1889 017	2.12E-126	380
AAB66656.1	contig_21	63.2 18	87	32	0	630	71 6	1888 949	1888 689	2.12E-126	92.4
AAB66656.1	contig_21	32.1 43	504	307	7	1	48 4	1374 607	1373 141	1.73E-97	215
AAB66656.1	contig_21	35.3 85	130	79	2	585	71 3	1372 738	1372 361	1.73E-97	91.7
AAB66656.1	contig_21	45.0 98	102	56	0	481	58 2	1373 102	1372 797	1.73E-97	91.3
AAB66656.1	contig_21	40.4 67	257	149	3	1	25 3	1890 911	1890 141	3.93E-46	178
AAB66656.1	contig_27	29.3 85	439	289	6	10	43 8	1610 985	1609 702	1.97E-91	169
AAB66656.1	contig_27	40.8 38	191	90	2	481	65 2	1609 452	1608 892	1.97E-91	149
AAB66656.1	contig_27	36.5 08	63	39	1	652	71 3	1608 814	1608 626	1.97E-91	43.9
AAB66656.1	contig_27	34.8 84	43	28	0	440	48 2	1609 640	1609 512	1.97E-91	36.2
AAB66656.1	contig_27	40.9 84	61	33	2	639	69 6	1060 823	1060 641	0.001	42.7
AAB66656.1	contig_27	27.1 19	59	35	1	551	60 1	3772 35	3774 11	4.9	30.8
AAB66656.1	contig_11	27.3 28	494	322	8	10	48 0	5537 102	5535 663	1.77E-73	168
AAB66656.1	contig_11	30.2 08	192	107	4	481	65 2	5535 601	5535 047	1.77E-73	95.5
AAB66656.1	contig_11	35.9 38	64	41	0	652	71 5	5534 984	5534 793	1.77E-73	53.9
AAB66656.1	contig_19	26.0 59	472	252	18	276	70 0	2447 705	2446 440	1.67E-27	120
AAB66656.1	contig_19	27.5 13	189	123	3	221	40 4	3745 541	3746 080	6.89E-13	72.8
AAB66656.1	contig_19	29.0 91	55	39	0	652	70 6	3746 251	3746 415	6.10E-06	35.8
AAB66656.1	contig_19	61.9 05	21	8	0	632	65 2	3746 136	3746 198	6.10E-06	33.5
AAB66656.1	contig_30	35.6 32	87	56	0	106	19 2	2366 876	2367 136	5.44E-11	66.6
AAB66656.1	contig_18	28.0 37	107	73	2	12	11 8	5665 58	5668 66	4.68E-05	47
AAB66656.1	contig_15	34.2 11	38	25	0	9	46	2529 098	2529 211	3.5	31.2
XP_025455915.1	contig_30	33.4 88	215	106	6	64	25 3	1043 811	1043 203	6.64E-53	118
XP_025455915.1	contig_30	50	104	50	1	256	35 9	1043 140	1042 835	6.64E-53	108

XP_025455915.1	contig_19	35.065	77	39	3	144	220	1620711	1620908	0.25	33.5
XP_025455915.1	contig_27	38.235	34	21	0	53	86	7516058	7517059	1.4	31.2
XP_025455915.1	contig_27	35.714	42	27	0	53	94	8094016	8092091	4.3	29.6
XP_025455915.1	contig_11	38.636	44	23	1	44	83	1211050	1210019	6.3	29.3
XP_025455915.1	contig_15	30.357	56	36	1	308	360	3375476	3375309	9.2	28.5
AAR91697.1	contig_11	56.94	281	92	5	32	286	2363888	2363055	2.93E-137	294
AAR91697.1	contig_11	41.72	314	171	7	299	608	2362945	2362028	2.93E-137	214
AAR91697.1	contig_11	35.036	548	303	16	41	578	2851328	2852842	6.77E-74	258
AAR91697.1	contig_11	73.684	19	5	0	99	117	2364384	2364328	0.63	33.5
AAR91697.1	contig_11	52	25	12	0	23	47	2363977	2363903	7.1	30
AAR91697.1	contig_15	44.048	504	268	5	23	519	2233516	2232026	1.14E-125	397
AAR91697.1	contig_15	39.326	89	53	1	523	610	2231939	2231673	1.14E-125	72.8
AAR91697.1	contig_15	33.804	263	98	7	53	286	1321088	1315041	9.60E-32	132
AAR91697.1	contig_15	54.015	74	33	1	85	157	2945629	2945850	4.10E-17	85.9
AAR91697.1	contig_15	29.154	96	56	2	452	543	1308076	1306013	5.13E-11	47
AAR91697.1	contig_15	33.345	129	73	4	342	488	1313048	1309021	5.13E-11	39.3
AAR91697.1	contig_15	45	40	16	2	40	73	1322049	1321030	0.89	32.7
AAR91697.1	contig_12	37.561	605	331	16	30	600	5931125	5932870	1.18E-109	362
AAR91697.1	contig_12	33.353	42	28	0	493	544	1775480	1775605	0.098	35.8
AAR91697.1	contig_12	37.227	51	27	3	228	274	1242054	1241905	3.1	31.2
AAR91697.1	contig_29	28.053	449	243	12	88	534	5362404	5373016	4.30E-36	145
AAR91697.1	contig_29	28.254	495	255	16	57	541	2921648	2920434	2.34E-34	140
AAR91697.1	contig_30	27.953	487	251	16	57	534	1826062	1827249	1.85E-34	140
AAR91697.1	contig_27	40.417	89	53	0	83	171	1607296	1607030	2.34E-13	73.9

AAR91697.1	contig_26	27.5	240	105	7	48	259	3661	3655	2.91E-11	67
AAR91697.1	contig_19	38	50	31	0	108	157	1038	1038	0.001	42.4
KAG820545.2.1	contig_30	48.28	930	452	9	128	1054	2439	2442	0	826
KAG820545.2.1	contig_30	65.934	91	31	0	38	128	2439	2439	0	136
KAG820545.2.1	contig_30	25.792	221	137	3	102	318	2423	2423	3.34E-13	74.7
KAG820545.2.1	contig_30	28.75	80	54	1	720	799	5224	5226	5.9	31.2
KAG820545.2.1	contig_30	30	70	40	1	705	774	2432	2432	9.8	30.4
KAG820545.2.1	contig_34	37.255	51	29	1	666	716	5075	5061	0.95	33.5
KAG820545.2.1	contig_11	29.167	96	59	4	129	220	2212	2212	3.4	32
KAG820545.2.1	contig_11	27.778	54	37	1	218	269	3437	3437	5.6	31.2
KAG820545.2.1	contig_71	30	40	28	0	93	132	1675	1687	5.3	31.2
CAC38353.1	contig_15	62.295	793	296	2	85	875	4003	4005	0	1028
CAC38353.1	contig_15	37.931	348	209	4	336	681	3708	3709	5.27E-77	239
CAC38353.1	contig_15	23.858	197	141	4	689	884	3709	3710	5.27E-77	68.9
CAC38353.1	contig_15	41.071	336	156	7	50	384	3707	3708	3.59E-62	221
CAC38353.1	contig_15	53.125	32	14	1	19	49	3707	3707	3.59E-62	38.1
CAC38353.1	contig_15	55.405	74	31	1	1	72	4003	4003	6.59E-17	86.3
CAC38353.1	contig_19	55.312	640	280	2	88	727	8439	8459	0	728
CAC38353.1	contig_19	41.873	153	88	1	726	877	8460	8464	0	133
CAC38353.1	contig_19	37.268	397	231	7	298	693	1996	1995	1.78E-126	251
CAC38353.1	contig_19	41.264	269	131	6	50	317	1997	1996	1.78E-126	189
CAC38353.1	contig_19	19.892	186	142	2	698	881	1994	1994	1.78E-126	55.1
CAC38353.1	contig_19	51.429	70	31	1	1	70	8436	8438	1.06E-11	69.3
CAC38353.1	contig_19	43.333	30	15	1	342	371	1783	1783	2.3	32

CAC38353.1	contig_11	23.265	245	129	11	146	348	8810	8817	0.003	41.6
CAC38353.1	contig_11	27.869	61	42	1	268	326	3541	3541	9.4	30
CAC38353.1	contig_29	30.357	112	66	5	592	702	2031	2032	2	32.3
KAF556313.1	contig_29	26.11	383	239	12	116	471	3142	3143	1.12E-18	90.1
KAF556313.1	contig_29	24.494	445	231	17	57	442	1695	1696	9.00E-07	52
KAF556313.1	contig_27	42.222	45	26	0	397	441	1790	1790	0.013	38.5
KAF556313.1	contig_27	38.889	54	31	1	73	126	1791	1791	3.6	30.4
KAF556313.1	contig_11	34.783	69	36	3	61	126	4679	4681	0.33	33.9
KAF556313.1	contig_15	32.71	107	64	4	364	469	2768	2768	0.65	33.1
KAF556313.1	contig_15	30	50	35	0	123	172	2353	2353	1.5	32
KAF556313.1	contig_12	30.864	81	46	2	113	185	5799	5799	6.8	29.6
KAF556313.1	contig_19	28.125	64	41	2	243	302	3167	3167	6.9	29.6
XP_049137919.1	contig_14	37.333	750	423	17	192	919	1602	1624	3.88E-138	449
XP_049137919.1	contig_14	31.967	122	72	4	80	197	1598	1602	3.88E-138	63.9
XP_049137919.1	contig_26	34.748	754	425	20	197	918	1282	1280	5.34E-105	344
XP_049137919.1	contig_26	33.043	115	58	4	92	197	1283	1282	5.34E-105	57.8
XP_049137919.1	contig_26	29.579	808	444	26	197	923	4418	4395	2.77E-87	283
XP_049137919.1	contig_26	28.221	163	96	7	48	197	4423	4418	2.77E-87	59.3
XP_049137919.1	contig_26	42.262	168	73	6	197	358	4485	4481	9.89E-38	120
XP_049137919.1	contig_26	26.974	152	77	7	55	199	4489	4486	9.89E-38	57.4
XP_049137919.1	contig_26	29.484	407	230	12	541	917	4480	4468	3.44E-34	142
XP_049137919.1	contig_26	45.652	46	21	2	734	777	2406	2407	9.8	30
XP_049137919.1	contig_27	26.402	731	411	21	281	923	1866	1868	1.48E-69	191
XP_049137919.1	contig_27	46.914	81	31	3	197	277	1866	1866	1.48E-69	67.4

XP_049137	contig	56.7					19	1866	1866	1.48E-	
919.1	_27	57	37	14	1	165	9	451	561	69	46.6
XP_049137	contig						26	1797	1797		
919.1	_27	42.5	40	19	1	224	3	318	425	2.7	32
XP_049137	contig	28.8					27	1797	1797		
919.1	_27	29	111	56	5	185	6	150	470	4.9	31.2
XP_049137	contig	34.1					91	2280	2281	7.12E-	
919.1	_19	09	387	230	9	541	9	866	975	55	208
XP_049137	contig	49.6					35	2280	2280	7.72E-	
919.1	_19	77	155	73	4	197	0	314	766	48	139
XP_049137	contig	38.3					20	2279	2280	7.72E-	
919.1	_19	33	120	58	3	84	3	967	278	48	68.2
XP_049137	contig	53.3						2279	2279	7.72E-	
919.1	_19	33	15	7	0	50	64	818	862	48	24.3
XP_049137	contig	27.6					28	3493	3493		
919.1	_19	12	134	61	6	152	5	367	660	0.076	37
XP_049137	contig	30.6					25	4392	4392		
919.1	_19	67	75	32	3	185	9	790	954	3.4	31.6
XP_049137	contig	44.7					35	3124	3124	3.95E-	
919.1	_12	85	163	75	4	197	8	066	512	43	124
XP_049137	contig	32.6					19	3123	3123	3.95E-	
919.1	_12	39	144	73	6	54	7	639	998	43	70.9
XP_049137	contig	29.8					91	3124	3125	1.80E-	
919.1	_12	47	392	236	10	541	6	600	706	32	137
XP_049137	contig	33.1					35	1292	1291	3.67E-	
919.1	_30	4	344	159	13	48	8	606	689	39	159
XP_049137	contig	29.9					91	1291	1290	5.19E-	
919.1	_30	49	394	233	10	541	4	601	489	30	129
XP_049137	contig	28.3					35	7236	7230	3.68E-	
919.1	_11	9	236	134	11	133	9	37	08	06	51.2
XP_049137	contig						34	4621	4622		
919.1	_11	25	164	85	6	185	1	650	048	7.8	29.6
XP_049137	contig						38	4622	4622		
919.1	_11	36	25	14	1	357	1	122	190	7.8	16.9
XP_049137	contig	27.6					34	1691	1697	1.66E-	
919.1	_29	79	224	104	10	126	2	90	08	04	45.8
XP_049137	contig	31.8					35	4481	4435	2.84E-	
919.1	_72	18	176	89	9	185	2	4	6	04	45.1
XP_049137	contig	27.5					35	6964	6968		
919.1	_23	28	178	100	7	185	5	03	70	0.003	41.6
XP_049137	contig	34.7					60	6968	6971		
919.1	_23	22	72	39	2	541	6	95	04	0.11	36.6
XP_049137	contig	29.4					28	2727	2730		
919.1	_21	74	95	48	3	192	6	92	19	0.9	33.5
XP_049137	contig	31.7					62	2354	2354		
919.1	_15	65	85	54	2	547	7	634	380	7	30.4
XP_049137	contig	57.8					70	3846	3852		
919.1	_43	95	19	8	0	688	6	5	1	7.5	30.4

QKM76211.1	contig_23	32.609	46	31	0	639	68 4	7652 7	7639 0	3	31.6
QKM76211.1	contig_30	23.711	97	70	3	282	37 8	1303 22	1300 44	8	30
GBF59608.1	contig_12	38.15	346	175	8	66	40 3	5481 325	5480 381	1.74E-52	191
GBF59608.1	contig_12	38.994	318	137	12	64	34 3	5722 463	5721 567	1.41E-43	164
GBF59608.1	contig_12	56.061	66	26	1	393	45 8	5480 363	5480 175	1.75E-13	73.2
GBF59608.1	contig_12	46.97	66	32	1	393	45 8	5721 399	5721 211	8.11E-10	61.6
GBF59608.1	contig_19	26.496	234	147	7	61	28 6	2820 832	2820 182	4.36E-05	46.2
GBF59608.1	contig_19	31.132	106	58	5	170	26 2	1863 522	1863 833	0.28	33.9
GBF59608.1	contig_26	26.966	89	47	2	243	31 3	1346 997	1346 731	2.7	30.8
GBF59608.1	contig_15	50	32	16	0	428	45 9	2529 442	2529 347	5	30
GBF59608.1	contig_15	43.243	37	19	1	420	45 6	2112 521	2112 417	9	28.9

Supplementary Table 4. Protein blast results of *Ophidiomyces ophiodiicola*.

Ophidiomyces ophiodiicola

qseqid	sseqid	identity	length	mismatch	gapopen	qstart	qend	sstart	send	evalue	bitscore
XP_007298.1	NW_026054650.1	72.727	209	57	0	164	37 2	2485 42	2479 16	1.83E-116	333
XP_007298.1	NW_026054650.1	52.427	103	49	0	61	16 3	2489 07	2485 99	1.83E-116	104
XP_007298.1	NW_026054650.1	43.478	46	25	1	1	46	2491 99	2490 65	3.80E-04	42
XP_007298.1	NW_026054650.1	35.802	81	41	5	76	15 3	5014 57	5016 75	0.9	31.2
XP_007298.1	NW_026054643.1	20.455	88	70	0	100	18 7	4840 39	4843 02	1.6	30.4
XP_007298.1	NW_026054657.1	29.487	78	48	1	76	15 3	1697 14	1699 26	1.6	30.4
XP_007298.1	NW_026054681.1	20.155	258	151	11	101	31 6	3776 4	3703 0	3.7	29.3
XP_007298.1	NW_026054659.1	25.581	86	54	3	285	36 4	2452 3	2476 8	8.6	28.1
KMQ48706.1	NW_026054644.1	65.789	342	96	1	254	57 4	8511 66	8501 41	0	471
KMQ48706.1	NW_026054644.1	53.012	249	92	5	22	25 1	8519 53	8512 25	0	251

KMQ4870	NW_02605	75.4					63	8500	8498		
6.1	4644.1	1	61	14	1	575	5	63	84	0	96.7
KMQ4870	NW_02605	72.7					63	1725	1731		
6.1	4648.1	27	220	39	2	436	5	14	70	0	303
KMQ4870	NW_02605	73.9					44	1719	1724		
6.1	4648.1	36	188	46	1	254	1	13	67	0	279
KMQ4870	NW_02605	66.1					25	1714	1718		
6.1	4648.1	54	130	44	0	122	1	46	35	0	187
KMQ4870	NW_02605	56.3					12	1711	1713		
6.1	4648.1	22	87	37	1	36	2	29	86	0	102
KMQ4870	NW_02605	28.0					15	1800	1798		
6.1	4669.1	7	57	40	1	99	4	28	58	0.32	33.5
KMQ4870	NW_02605	21.5					25	5659	5662		
6.1	4649.1	69	102	69	1	151	2	63	35	1.4	31.6
KMQ4870	NW_02605						44	8615	8627		
6.1	4645.1	37.5	40	25	0	410	9	2	1	2.3	30.8
KMQ4870	NW_02605	45.8					43				
6.1	4643.1	33	24	13	0	411	4	6067	5996	2.9	30.4
KMQ4870	NW_02605	24.2					49				
6.1	4659.1	99	107	68	3	393	1	2792	2487	7.7	29.3
KMQ4870	NW_02605	30.5					28	7776	7777		
6.1	4647.1	56	36	23	1	252	7	32	33	9	28.9
CAB60729.	NW_02605	39.2					27	2203	2204		
1	4643.1	86	28	17	0	243	0	49	32	1.2	30.8
KGQ03962	NW_02605	30.6					52	5507	5488		
.1	4656.1	45	62	30	2	472	0	1	6	5.4	29.3
KGQ03962	NW_02605	26.6					20	9750	9748		
.1	4641.1	67	75	47	3	136	4	52	34	5.4	29.3
KGQ03962	NW_02605	28.5					20	1086	1087		
.1	4641.1	71	84	51	2	130	4	777	028	7.7	28.9
KGQ03962	NW_02605	30.1					50	1035	1035		
.1	4643.1	89	53	31	1	460	6	786	628	6	29.3
AAB66656.	NW_02605	76.4					43	4599	4586		
1	4655.1	17	441	102	1	1	9	42	20	0	659
AAB66656.	NW_02605	77.7					64	4585	4579		
1	4655.1	23	202	45	0	440	1	66	61	0	337
AAB66656.	NW_02605	77.7					71	4578	4576		
1	4655.1	78	63	14	0	653	5	68	80	0	102
AAB66656.	NW_02605	24.0					70	7404	7591	8.73E-	
1	4661.1	17	687	409	22	72	7	1	5	40	158
AAB66656.	NW_02605	23.8						1853	1855		
1	4642.1	81	67	51	0	9	75	96	96	1.6	31.6
XP_02545	NW_02605	39.5					20	7469	7483		
5915.1	4657.1	83	48	26	3	162	9	9	3	1.1	30.8
XP_02545	NW_02605	32.5					35	3204	3205		
5915.1	4647.1	58	43	29	0	311	3	49	77	4.8	28.9
XP_02545	NW_02605						32	5457	5439		
5915.1	4672.1	30	60	38	2	266	1	4	5	4.9	28.9

XP_02545	NW_02605	33.3					32	1091	1091			
5915.1	4644.1	33	36	24	0	286	1	645	752	8.8	28.1	
AAR91697.	NW_02605	66.0					60	1601	1618			
1	4662.1	75	563	185	4	50	8	32	14	0	768	
AAR91697.	NW_02605	74.0						1599	1600	1.80E-		
1	4662.1	74	27	7	0	21	47	87	67	04	44.3	
AAR91697.	NW_02605	39.6					60	6443	6426	6.68E-		
1	4648.1	64	595	331	12	32	9	61	10	121	394	
AAR91697.	NW_02605	38.7					43	5437	5436			
1	4648.1	1	31	19	0	409	9	85	93	4.1	30	
AAR91697.	NW_02605	33.3					52	9026	9036	2.51E-		
1	4643.1	33	348	141	10	250	2	93	88	42	159	
AAR91697.	NW_02605	35.4					57	9037	9038	2.51E-		
1	4643.1	17	48	29	1	523	0	56	93	42	32.3	
AAR91697.	NW_02605	48.4					11	9017	9020	1.32E-		
1	4643.1	85	99	34	3	23	8	99	53	22	89.7	
AAR91697.	NW_02605	43.2					15	9021	9022	1.32E-		
1	4643.1	43	37	20	1	119	5	07	14	22	35.4	
AAR91697.	NW_02605	27.8					53	7013	7001	3.98E-		
1	4644.1	87	459	244	13	88	6	02	57	35	142	
AAR91697.	NW_02605	25.4					53	1690	1691	1.52E-		
1	4641.1	75	526	267	17	41	6	402	694	31	130	
AAR91697.	NW_02605	37.3					15	4059	4029	1.09E-		
1	4641.1	74	99	61	1	60	8	0	7	11	67.8	
AAR91697.	NW_02605	30.4					28	1911	1907	3.52E-		
1	4672.1	35	184	76	7	102	5	05	10	12	69.3	
AAR91697.	NW_02605	35.0					16	3716	3713	2.99E-		
1	4658.1	52	97	59	2	74	8	54	70	05	46.6	
AAR91697.	NW_02605	30.6					16	2342	2344			
1	4665.1	45	62	34	2	109	1	96	81	4.5	29.6	
AAR91697.	NW_02605	30.7					44					
1	4668.1	69	52	35	1	399	9	2808	2963	5.7	29.6	
KAG82054	NW_02605	64.8					10	2672	2700			
52.1	4654.1	62	942	310	6	116	48	40	29	0	1187	
KAG82054	NW_02605	57.8					12	2668	2672	2.48E-		
52.1	4654.1	12	128	49	1	1	8	60	28	37	152	
KAG82054	NW_02605	28.0					31	3126	3181	2.64E-		
52.1	4644.1	37	214	121	7	110	9	0	4	11	67.8	
KAG82054	NW_02605	24.7					31	6190	6160			
52.1	4642.1	52	101	67	3	220	1	6	4	2.3	31.6	
KAG82054	NW_02605	36.1					77	1883	1884			
52.1	4673.1	7	47	26	1	727	3	35	63	3.8	30.8	
KAG82054	NW_02605	29.2					71	3326	3338			
52.1	4652.1	68	41	29	0	672	2	4	6	6	30.4	
KAG82054	NW_02605	29.7					54	4213	4215			
52.1	4653.1	62	84	52	4	462	4	03	36	8.7	29.6	
KAG82054	NW_02605	38.0					92	6104	6105			
52.1	4645.1	95	42	26	0	879	0	47	72	8.8	29.6	

CAC38353.1	NW_026054655.1	66.918	795	258	1	52	84 1	3176 05	3152 21	0	1121
CAC38353.1	NW_026054655.1	62.162	37	14	0	19	55	3177 68	3176 58	6.13E-10	62.8
CAC38353.1	NW_026054655.1	65.854	41	13	1	842	88 1	3151 62	3150 40	2.05E-08	57.8
CAC38353.1	NW_026054642.1	32.984	667	332	14	320	87 7	8862 35	8882 17	1.03E-97	333
CAC38353.1	NW_026054642.1	48.837	43	22	0	281	32 3	8860 56	8861 84	1.03E-97	43.5
CAC38353.1	NW_026054642.1	41.007	139	65	4	87	21 2	8852 98	8857 02	1.42E-20	97.8
CAC38353.1	NW_026054642.1	58.333	24	10	0	438	46 1	8867 47	8868 18	2.3	31.2
CAC38353.1	NW_026054695.1	50	34	17	0	315	34 8	1373 5	1363 4	3.10E-04	43.9
CAC38353.1	NW_026054650.1	44.828	29	16	0	509	53 7	1232 93	1232 07	3.7	30.8
KAF5563136.1	NW_026054659.1	25.974	77	53	1	160	23 2	3348 16	3345 86	1.9	30.8
KAF5563136.1	NW_026054644.1	37.5	40	25	0	203	24 2	7482 4	7470 5	2.3	30.4
XP_047917919.1	NW_026054644.1	31.015	719	421	21	246	92 0	9895 29	9915 92	3.00E-91	255
XP_047917919.1	NW_026054644.1	34.946	186	98	7	68	25 2	9890 05	9894 96	3.00E-91	100
XP_047917919.1	NW_026054657.1	33.85	387	220	10	554	92 3	3751 30	3740 27	1.98E-90	182
XP_047917919.1	NW_026054657.1	33.61	241	111	9	35	24 9	3767 95	3761 42	1.98E-90	108
XP_047917919.1	NW_026054657.1	27.907	344	194	11	246	55 5	3760 97	3751 26	1.98E-90	83.2
XP_047917919.1	NW_026054641.1	35.315	286	154	8	47	31 4	7371 94	7380 12	2.16E-78	164
XP_047917919.1	NW_026054641.1	27.391	617	409	17	321	92 5	7380 87	7398 56	2.16E-78	148
XP_047917919.1	NW_026054641.1	26.22	164	84	8	185	34 1	3622 00	3617 99	0.85	32.7
XP_047917919.1	NW_026054661.1	28.639	632	398	18	322	92 6	2036	3853	2.14E-55	209
XP_047917919.1	NW_026054661.1	30.267	337	155	12	52	33 1	1068	2009	6.34E-31	131
XP_047917919.1	NW_026054671.1	30.137	219	116	11	150	35 5	5037 7	5096 1	0.001	42
XP_047917919.1	NW_026054663.1	31.897	116	52	5	185	30 0	1653 80	1656 46	0.009	39.3
XP_047917919.1	NW_026054690.1	25.397	126	67	4	175	30 0	9006	9302	0.01	39.3

XP_04913	NW_02605	29.8					28	1345	1343		
7919.1	4667.1	08	104	38	4	181	0	83	65	0.023	38.1
XP_04913	NW_02605	28.6					34	9442	9402		
7919.1	4666.1	59	164	80	8	185	1	9	8	0.095	36.2
XP_04913	NW_02605	32.8					25	1420	1421		
7919.1	4646.1	95	76	30	3	185	9	11	78	0.54	33.5
XP_04913	NW_02605	25.5					27	1708	1711		
7919.1	4647.1	32	94	47	2	185	8	90	02	4.5	30.4
XP_04913	NW_02605	38.5					39	2662	2664		
7919.1	4649.1	96	57	29	2	341	1	54	24	7.2	29.6
XP_04913	NW_02605	34.2					59	4968	4966		
7919.1	4642.1	47	73	33	2	541	8	63	45	7.7	29.6
XP_04913	NW_02605	30.6					18	2659	2657		
7919.1	4656.1	67	75	43	1	107	1	75	78	9.4	29.3
QKM7621	NW_02605	29.3					43	2446	2444		
1.1	4647.1	1	58	41	0	378	5	58	85	3	30.8
QKM7621	NW_02605	29.0					32				
1.1	4738.1	32	62	34	2	267	3	1011	841	4.2	30
GBF59608.	NW_02605	56.6					45	1889	2009	1.26E-	
1	4680.1	67	420	148	5	54	9	9	8	134	427
GBF59608.	NW_02605	29.3					28	1330	1293		
1	4708.1	23	133	71	6	170	6	9	2	1.5	30.8
GBF59608.	NW_02605	39.3					19	5967	5968		
1	4647.1	94	33	20	0	165	7	33	31	2.7	30
GBF59608.	NW_02605	27.2					30	5854	5856		
1	4649.1	73	66	47	1	238	2	62	59	4.3	29.3
GBF59608.	NW_02605						45	3589	3588		
1	4645.1	35	40	26	0	420	9	66	47	9.9	28.1