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Fungal dermatitis, glossitis and disseminated visceral mycosis caused by different *Metarhizium granulomatis* genotypes in veiled chameleons (*Chamaeleo calyptratus*) and first isolation in healthy lizards

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ABSTRACT

Metarhizium (*M.*) *granulomatis* (formerly *Chamaeleomyces granulomatis*) invariably causes fatal fungal glossitis and systemic mycosis in veiled chameleons (*Chamaeleo calyptratus*). Isolation of *M. granulomatis* in other lizards thus far has not been described. The aim of this study therefore was to obtain information on the presence of *M. granulomatis* in reptiles kept as pets, and to examine whether there was an association between specific genotypes and clinical/pathological outcomes. Besides 18S ribosomal (r) DNA (SSU) and internal transcribed spacer1-5.8S (ITS1-5.8S) rDNA, a fragment of the large subunit of the 28S rDNA (LSU), including the domains 1 (D1) and D2, were sequenced for identification of the fungus and phylogenetic analysis. *Metarhizium granulomatis* was isolated from 23 veiled chameleons, two panther chameleons (*Furcifer pardalis*) and one central bearded dragon (*Pogona vitticeps*). Only the veiled chameleons revealed corresponding pathological findings in the form of glossal hemorrhage, granulomatous glossitis, pharyngitis, dermatitis and/or visceral mycosis. The infection site correlated to survival times of infected veiled chameleons. Combined long-term treatment with terbinafine and nystatin based on susceptibility testing may be helpful for prevention of disease and visceral spreading of the fungus, but elimination of the fungal pathogen or successful treatment of diseased veiled chameleons have not been achieved yet. Sequencing of the ribosomal genes yielded five different genotypes, with genotype A being strongly correlated with dermatitis, and remaining genotypes with pharyngitis and glossitis. However, disseminated visceral mycosis developed irrespective of the genotypes.

1. Introduction

Reports on fungal infections in captive as well as in free-living reptiles have increased during the last decade (Mitchell and Walden, 2013; Schmidt, 2015). Among the most common fungal diseases in reptiles is yellow fungus disease in lizards and snake fungal disease in snakes, caused by keratinophilic ascomycetous fungi of the family *Onygenaceae* (Eurotiomycetes: Onygenales), formerly described as *Chrysosporium* anamorph of *Nannizziopsis* (*N.*) *vriesii* complex (CANV) (Sigler et al., 2013; Stchigel et al., 2013; Cabañes et al., 2014). *Nannizziopsis guarroi* has emerged predominantly in central bearded dragons (*Pogona vitticeps*) and green iguanas (*Iguana iguana*) with a worldwide distribution, causing the so called ‘yellow fungus disease’ (Bowman et al., 2007; Abarca et al., 2010; Le Donne et al., 2016). Koch's postulates have been fulfilled in a study with experimentally infected veiled chameleons (*Chamaeleo calyptratus*) which developed dermatomycosis, thus confirming that *N. guarroi* is capable of acting as a primary fungal pathogen in this species (Paré et al., 2006). *Nannizziopsis chlamydospora* and *N. draconii* have recently been described in central bearded dragons with dermatomycosis (Schmidt-Ukaj et al., 2016). Besides skin lesions, rare cases of systemic infection exist (Abarca et al., 2009; Schmidt-Ukaj et al., 2014). Another experimentally proven primary pathogenic species of the Onygenales order is *Ophidiomyces ophiodiicola* inducing snake fungal disease (Allender et al., 2015; Lorch et al., 2015). While snake fungal disease is well-known in captive snakes all over the world, it is also an emerging disease in free-ranging snake populations across the USA (Cheatwood et al., 2003; Allender et al., 2011; Guthrie et al., 2016).

Metarhizium (*M.*) *granulomatis* is an ascomycetous fungus of the family *Clavicipitaceae* (Sordariomycetes: Hypocreales), formerly described as *Chamaeleomyces* (*C.*) *granulomatis* in captive veiled chameleons kept as pets as well as in a zoological enclosure (Sigler et al., 2010; Schmidt et al., 2012). *Metarhizium viride*, formerly described as *Paecilomyces viridis* and as *Chamaeleomyces* (*C.*) *viridis*, is phylogenetically closely related to *M. granulomatis* (Kepler et al., 2014). Both fungi are known to elicit glossitis, pharyngitis and disseminated visceral mycosis, including

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Table 1
Summary of *Metarhizium granulomatis* genotypes (GT) and isolates identified by sequence analysis of ribosomal DNA, origin of isolates, pathological findings associated with oral lesions (OL), dermatitis (D), disseminated visceral mycosis (DVM), antifungal treatment (AT) and survival time (ST).

GT	Isolate (GenBank accession numbers)	Host species	Origin of isolation	OL	D	DVM	AT	ST in weeks (median)
A	VS9614 (KY563209, KY565260)	Central bearded dragon (*Pogona vitticeps*) n = 1	cloaca	n.a.	n.a.	n.d.	n.a.	n.d.
	VS6223, VS3308[a], VS6016[a], VS5706 (KY563210, KY565261), VS2301*[b] (KY563211, KY565262)	Veiled chameleon (*Chamaeleo calyptratus*) n = 5	skin (n = 4); tongue (n = 3); cloaca (n = 2); pharynx, visceral organs (n = 1)	n = 3	n = 4	n = 1	n = 4	128–160 (140)
B	VS10222 (KY563213.1, KY565264.1)	Panther chameleon (*Furcifer pardalis*) n = 1	cloaca	n.a.	n.a.	n.d.	n.a.	n.d.
	VS6017, VS9407[b] (KY563212.1, KY565263.1), VS7520 (KU342060.1, KU342061.1)	Veiled chameleon n = 3	tongue (n = 3); skin, cloaca, visceral organs (n = 1)	n = 3	n = 1	n = 1	n.a.	0–4 (1)
C	VS10612[c] (KY563215.1, KY565266.1)	Panther chameleon n = 1	cloaca	n.a.	n.a.	n.d.	n.a.	n.d.
	VS2913*[c] (KY563214.1, KY565265.1), VS5713 (KU342062.1, KU342063.1)	Veiled chameleon n = 2	tongue, pharynx, visceral organs (n = 1)	n = 1	n.a.	n = 1	n.a.	0–4 (2)
D	VSP04, VS3206*, VS2002*, VS7518, VS3305, VS4301, VS3702, VS3509, VS7811 (KY563216.1, KY565267.1), VS3202* (KU342064.1, KU342065.1)	Veiled chameleon n = 10	tongue (n = 6); visceral organs (n = 5); cloaca (n = 4); pharynx (n = 3); skin (n = 2)	n = 7	n = 3	n = 5	n = 3	4–120 (100)
E	VS2202*, VS2504*, VSP02 (KY563217.1, KY565268.1)	Veiled chameleon n = 3	tongue, visceral organs (n = 3); pharynx (n = 2); cloaca (n = 1)	n = 3	n.a.	n = 3	n.a.	4–28 (16)

* re-cultured, –sequenced and –analysed; formerly described (Schmidt et al., 2012).
[a,b,c] kept together in one household.
n = number of animals with applicable findings.
n.a. = not applicable.
n.d. = not determined.

granulomas in lung, liver, kidney, ovary and other visceral organs (Sigler et al., 2010; Schmidt et al., 2012, 2017; Pfaff et al., 2015). *Metarhizium viride* displays a similar pathologic profile in carpet chameleons (*Furcifer lateralis*), panther chameleons (*Furcifer pardalis*) and central bearded dragons (Segretain et al., 1964; Pfaff et al., 2015; Schmidt et al., 2017). In contrast, *Metarhizium granulomatis* has only been described as a primary fungal pathogen of veiled chameleons in captivity. Ulcerative dermatitis, especially of the digits, and fungal pneumonia without oral lesions or visceral dissemination have been recorded solely for *M. granulomatis*-induced disease (Sigler et al., 2010; Schmidt et al., 2012). Susceptibility testing revealed potential treatment options in both mycoses, but treatment success has yet to be described (Schmidt et al., 2012, 2017).

Phylogenetic studies of fungal pathogens within the order Hypocreales have employed fragments of the small subunit ribosomal (r) DNA (SSU), the nuclear ribosome internal transcribed spacer (ITS)1-5.8S- ITS2 and domains (D)1 and D2 of the large subunit rDNA (LSU) (Sigler et al., 2010; Schmidt et al., 2017). Protein-coding genes BTUB, RPB1, RPB2, TEF and actin were also found applicable in the determination of species boundaries (Kepler et al., 2014). According to different genotypes of *M. viride*, the aim of this investigation was to assess whether different genotypes of *M. granulomatis* are present in lizards kept as pets. In comparison to the analysis formerly described in *M. viride*-associated mycoses, the clinical and pathological findings as well as antifungal susceptibility were evaluated in order to interpret the relevance of genomic differences for *M. granulomatis*-associated mycoses (Schmidt et al., 2017).

2. Animals and methods

2.1. Animals and sampling sites

Isolates of *M. granulomatis* were recovered from 26 lizards representing three different species including veiled chameleons (n = 23), panther chameleons (n = 2) and one central bearded dragon. Seven cases of *M. granulomatis*-mycosis in veiled chameleons, which were included in this study for further evaluation, were described previously (Schmidt et al., 2012). Veiled chameleons (15 males, 8 females) were between 6 and 70 months of age (mean 29 months ± 18 months standard deviation). Both panther chameleons were adult males (30 and 33 months of age, respectively). The central bearded dragon was female and 36 months old. Except for five veiled chameleons and one panther chameleon kept as pairs in one household each, lizards were housed individually. Panther chameleons, the central bearded dragon and one veiled chameleon presented for routine health checks, while the remaining veiled chameleons presented for a variety of symptoms. Owners agreed to complete a questionnaire, including detailed queries regarding gender, age, origin (bred in Germany/imported), previous diseases as well as precise husbandry and feeding conditions. Based on this information, husbandry quality was classified into two categories: unremarkable (all climatic, feeding and technical parameters within the required ranges) and remarkable (deficits). All lizards were captive-bred in Germany and in possession of the current owners for more than four months. For four of the lizards, including the central bearded dragon and three veiled chameleons from different owners, husbandry deficits – such as a lack of mineral supplementation and/or UV-B light or inadequate airflow – were documented. Clinical examination of each individual included determination of body mass, body and overall condition and inspection of the oral cavity including