# Pathogenic *Chrysosporium*-Related Fungi in Reptiles and Other Animals

3

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**Abstract**

Pathogenic *Chrysosporium*-related fungi (PCRF) have manifested themselves in recent decades as the serious causative agents of mycoses in captive and free-living reptiles. The anamorphic (asexual) genus *Chrysosporium* Corda comprises a number of species including *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV), which is considered as a main fungal pathogen in reptiles in many countries of the world. Due to increased popularity of exotic reptiles as pets, these infections have become widespread around the world in recent decades. Taxonomy and nomenclature of *Chrysosporium*-related fungi have been revised radically. The present chapter puts together the recent advances in classification, physiology, etiological significance, epidemiology, and occurrence of PCRF-induced mycoses in different species of reptiles. Mycoses in mammals including humans associated with PCRF are also colligated together with our published and unpublished experiences in clinical and laboratory diagnosis, antifungal susceptibility, therapy, and prevention of reptile mycoses caused by *Chrysosporium*-related fungi. The data demonstrate that PCRF-associated mycoses are important aspects of veterinary mycology and herpetology and thus should be explored further.

**Keywords**

*Chrysosporium* · *Nannizziopsis* spp. · Pathogenic fungi · Mycosis · Dermatomycosis · Reptile

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## 3.1 The Current Significance of the Fungal Infections in Reptiles

Exotic reptiles took up a niche of popular companion animals in recent decades. However, inadequate maintenance conditions predispose animals to various infectious diseases including mycoses. Due to their biological features (exothermy, etc.), reptiles are considered to be naturally predisposed for fungal infections [1].

However, the importance of fungal infections in reptiles is still underestimated in many cases. Fungi tend to occupy the lowest position in the differential diagnosis list of veterinary clinicians. The primary reason for this is that fungi are generally considered to be opportunistic pathogens in reptiles, rather than obligate pathogens such as viruses, parasites, and bacteria [2]. The anamorphic (asexual) genus *Chrysosporium* Corda includes mostly keratinophilic species that live on the remains of hair and feathers in soil. Except reptiles, fungi are rarely reported as animal pathogens. Pathogenic *Chrysosporium*-related fungi (PCRF) are able to cause superficial and deep mycoses that affect both captive and wild reptiles [3].

In cold-blooded animals including reptiles, fungal infections can be caused by a variety of fungal species. But in last 15 years, PCRF gained etiological importance, and now they are evaluated as emerging pathogens in reptiles [4]. In many publications, the most important *Chrysosporium*-related pathogen is designated as *Chrysosporium* anamorph of *Nannizziopsis vriesii* (abbreviated as CANV in many reports). However, there is some inconsistency in the traditional and modern nomenclature of *Chrysosporium*-related fungal pathogens.

*Nannizziopsis* spp. was isolated for the first time in the 1990s from sick captive day geckos imported from Madagascar to Germany and from chameleons in Canada [5, 6]. Outbreaks of CANV in Australia occurred on two separate occasions in 1994 and 1997 in crocodiles sourced from the same crocodile farm [7]. The disease was known as “yellow fungus” because of the characteristic color of skin lesions of affected animals. The most demonstrative yellow fungus manifestations can be seen in bearded dragons (*Pogona* spp.)

The reptile trade, which occurs on a worldwide scale, has obscured the provenance of CANV isolates recovered from sick captive reptiles [8]. Abarca et al. described the first isolation of CANV in Spain from green iguana in the year 2008 and in bearded dragon (*Pogona vitticeps*) in 2009 [9, 10]. In 2010, Hellebuyck et al. diagnosed CANV in girdled lizard (*Cordylus giganteus*) in Belgium [11], whereas in Australia, it was detected in *Pogona barbata* by Johnson et al. (2011) [12]. In Russia, CANV was mycologically detected for the first time in green iguana [13]. In subsequent years, a trend has been observed toward the spread of CANV among captive reptiles. Three cases of CANV in pet reptiles were detected in the year 2008 [14], and up to 2014, CANV became the dominant pathogen of reptilian fungal infections in Russia with a share of 37% [15]. Till date, CANV has been isolated from captive reptiles of Asia, Australia, Europe, and North America [2].

## 3.2 *Chrysosporium*-Related Fungal Species and Their Nomenclature and Host Specificity

The genus *Chrysosporium* is polyphyletic, having affiliation with at least two orders of the *Ascomycota*. About 65 *Chrysosporium* species are currently accepted, and their sexual morphs (teleomorphs) are found in a variety of genera such as *Aphanoascus*, *Arthroderma*, or *Nannizziopsis* [16].

As mentioned earlier, the main *Chrysosporium*-related pathogen in reptiles is identified in most reports as *Chrysosporium* anamorph *of Nannizziopsis vriesii* (CANV). The species *Nannizziopsis vriesii* (Apinis) Currah (*Ascomycota*, *Onygenales*, *Onygenaceae*) has white ascomata, asperulate peridial hyphae constricted at septa, hyaline and globose ascospores, and a *Chrysosporium* asexual morph.

In routine laboratory practice, the identification of *Chrysosporium*-like fungal isolates is performed on the basis of morphological features. However, several years ago from preliminary molecular phylogenetic analysis, it was suggested that the *Chrysosporium* anamorph of *N. vriesii* actually represented a species complex rather than a single species, containing members that could be allied to specific hosts [17]. Recently, Sigler et al. [8] and Stchigel et al. [18] published independently the latest taxonomic revisions of *Chrysosporium*-related fungi together with the relationships between specific fungal species with their hosts [8, 18]. According to Sigler et al. (2013), one lineage of *Chrysosporium*-related fungi represents the genus *Nannizziopsis* and comprises *N. vriesii*, *N. guarroi*, and six newly described species *N. dermatitidis*, *N. crocodili*, *N. barbata*, *N. infrequens*, *N. hominis*, and *N. obscura* isolated from chameleons and geckos, crocodiles, agamid and iguanid lizards, and humans.

*N. guarroi* and *N. dermatitidis* were found to be major pathogens of lizards. *N. guarroi* was described originally from captive green iguanas in Spain. They possibly acquire the fungus from pet trade. Interestingly, this case is the first case of *N. guarroi* in green iguanas coincided temporally with the first documented European cases of yellow fungus disease in bearded dragons [10, 19]. Till then, it has been isolated repeatedly from pet inland bearded dragons with yellow fungus disease in North America.

Human-related species of *Nannizziopsis* are *N. infrequens*, *N. hominis*, and *N. obscura*. Other two lineages comprise the genus *Ophidiomyces*, with the species *O. ophiodiicola* (occurring only in snakes), and *Paranannizziopsis* gen. nov., with three new species *P. australasiensis*, *P. californiensis*, and *P. crustacea* infecting squamates and tuataras [8].

Moreover, based on ribosomal ITS region, actin and β-tubulin gene sequence four new species of *Nannizziopsis*, viz., *N. chlamydospora*, *N. draconii*, *N. arthrosporioides*, and *N. pluriseptata* were described by Stchigel et al. (2013) [18], which differ from those described by Sigler et al. (2013) [18]. They also described *Chrysosporium longisporum*, which was renamed *Paranannizziopsis* by Sigler et al. (2013). However, Stchigel and coauthors were unable to define clear-cut host specificity in newly described *Chrysosporium*-related fungi. Thus, the modern nomenclature of *Chrysosporium*-related fungi is not completely established and requires further study.

Table 3.1 summarizes the current data on the nomenclature and host specificity of 15 *Chrysosporium*-related fungi pathogenic for reptiles, as well as their specific morphological features.

**Table 3.1** Current nomenclature of *Chrysosporium*-related fungi pathogenic for reptiles

| Fungus species | Reptile species (source of isolation) | Morphological features |
|---|---|---|
| Genus *Nannizziopsis* Currah (1985) | | |
| *N. arthrosporioides* [18] | Water dragon (*Physignathus* sp.) | Colonies on peptone yeast extract (PYE) at 30 °C attaining a diameter of 34.0–37.0 mm after 14 days, yellowish white, zonate, felted, slightly cottony at center, with lobate margins; reverse yellowish white. Hyphae hyaline, septate, smooth walled, 1.0–4.0 µm wide, straight or twisted. Conidia 1(−2) celled, mostly sessile, also produced short protrusions or terminal, hyaline, thin and smooth walled, subglobose, pyriform, obovate, or claviform to cylindrical, 2.5–7.0 × 1.5–3.0 µm; intercalary conidia present, similar to the arthroconidia in shape and size; arthroconidia arranged in short terminal and intercalary chains, doliiform to cylindrical or irregularly shaped, 5.0–15.0 × 1.5–4.0 µm. Chlamydospores absent. Sexual morph not observed. Fetid (skunk-like) odor present on all the culture media tested |
| *N. barbata* [18] | Coastal bearded dragon (*Pogona barbata*) | Colonies on potato dextrose agar (PDA) were 5.5–6.0 cm in diameter, powdery, flat to slightly raised and cottony at the center, but otherwise zonate after 21 days. There was no growth at 35 °C. Aleurioconidia pyriform to clavate, measured 3.0–6.5 µm long and 1.8–2.5 µm wide, and sessile or borne on slightly swollen cells. Fission arthroconidia measuring 4.4–8.5 µm long and 1.7–3.5 µm wide, as well as undulate hyphae, are commonly produced. Moist colonies on PDA demonstrated budding |
| *N. chlamydospora* [18] | Inland bearded dragon (*Pogona vitticeps*) | Colonies on peptone yeast extract (PYE) at 30 °C attaining a diameter of 41.0–48.0 mm after 14 days, yellowish white elevated at the center and radially folded, compact, with an irregular margin; reverse yellowish white. Hyphae hyaline, septate, smooth walled, straight or twisted, 1–3(−4) µm wide. Conidia unicellular, sessile, on short protrusions or on side branches, less frequently terminal, hyaline, thin and smooth walled, pyriform, claviform, or cylindrical, 3.0–9.0 × 1.5–2.0 µm; intercalary conidia, cylindrical to doliiform, 6.0–10.0 × 1.5–2.0 µm; arthroconidia catenate, cylindrical to doliiform, 4.0–10.0 × 2.0–4.0 µm. Chlamydospores globose, broadly ellipsoidal or irregular, smooth and thick walled, 5–15(−20) µm in diameter. Sexual morph not observed. Fetid (skunk-like) odor produced on all the culture media tested |

(continued)

**Table 3.1** (continued)

| Fungus species | Reptile species (source of isolation) | Morphological features |
|---|---|---|
| *N. crocodili* [8] | Saltwater crocodile (*Crocodylus porosus*) | Colonies on PDA were 4.0–5.5 cm in diameter, velvety to powdery, slightly to strongly zonate, and sometimes with exudate droplets after 21 days. Growth was slow at 35 °C (1.0–2.7 cm in diameter after 21 days). Aleurioconidia subglobose, measuring 1.5–2.5 µm long and 1.3–2.4 µm wide, and sessile or borne on swollen cells either on the vegetative mycelium or within ascomata-like structures (pseudogymnothecia). Arthroconidia measuring 3.7–7.5 µm long and 2.0–3.0 µm wide are produced at low frequency and often show germination. Undulate hyphae are formed |
| *N. dermatitidis* [8] | Chameleons, geckos | Colonies on PDA attained 3.8–4.7 cm in diameter after 21 days and were strongly zonate and powdery with a thin margin. Most isolates failed to grow at 35 °C. Aleurioconidia were clavate to pyriform and measured 2.8–7.5 µm long if single celled, up to 9.0 µm long if two celled, and 1.2–3.0 µm wide. Undulate branches and cylindrical to slightly barrel-shaped fission arthroconidia were formed with arthroconidia measuring 2.8–9.0 µm long and 1.5–3.0 µm wide. Transitory yeast-like colonies grown on PDA at 30 °C were composed of ovoid to cylindrical yeast-like cells and arthroconidia |
| *N. draconii* [18] | Inland bearded dragon | Colonies on PYE at 30 °C attaining a diameter of 32.0–38.0 mm after 14 days, yellowish white, felted, slightly elevated at center, with regular margin; reverse yellowish white to pale yellow at center. Hyphae hyaline, septate, smooth walled, 1–3(−5) µm wide. Conidia unicellular, mostly sessile, also produced on short protrusions or on side branches, or terminal, hyaline, thin and smooth walled, claviform or cylindrical, 4.0–7.0 × 1.5–2.0(−2.5) µm; intercalary conidia scarce, cylindrical, 4.0–9.0 × 1.5–2.0 µm; arthroconidia catenate, mostly cylindrical or doliiform, scarcely produced, 5.0–9.0 × 1.5–2.5 µm. Chlamydospores absent. Sexual morph not observed. Fetid (skunk-like) odor produced on all the culture media tested |

(continued)

**Table 3.1** (continued)

| Fungus species | Reptile species (source of isolation) | Morphological features |
|---|---|---|
| *N. guarroi* [8] | Green iguana (*Iguana iguana*), inland bearded dragon, lizard (*Agama agama*) | Colonies on PDA 2.7–4.7 cm in diameter, powdery, sometimes sectoring to cottony, often strongly zonate, sometimes with exudate droplets. Growth at 35 °C was similar, with colonies attaining 2.3–4.0 cm in diameter. Aleurioconidia clavate to pyriform and measured 3.2–6.5 μm long and 1.5–2.5 μm wide. Undulate hyphae were common. Arthroconidia in chains measured 2.8–7.0 μm long and 2.0–3.7 μm wide and sometimes showed budding in young cultures. *N. guarroi* is distinguished from other reptile-associated *Nannizziopsis* species by its slightly lower growth rate at 30 °C and good growth at 35 °C. This species is considered the etiologic agent of yellow fungus disease in inland bearded dragons, a contagious and progressive necrogranulomatous dermatomycosis first observed about 15 years ago |
| *N. pluriseptata* [18] | Skink lizard (*Eumeces inexpectatus*) | Colonies on PYE at 30 °C attaining a diameter of 38.0–40.0 mm after 14 days, white to orange white, zonate, felted, slightly cottony at the center, with regular margins; reverse orange white. Hyphae hyaline, septate, smooth walled, 1.0–5.0 μm wide, straight. Conidia 1(−5) celled, mostly sessile, also produced on short protrusions or on side branches, or terminal, hyaline, thin and smooth walled, pyriform, obovate, claviform to cylindrical, 2.5–8.0(−15.0) × 1.5–2.5 μm; intercalary conidia occasionally present, cylindrical to doliiform or irregularly shaped, 2.5–5.0 × 2.0–2.5 μm; arthroconidia, disposed in lateral or terminal short chains, cylindrical to doliiform, 4.0–7.0 × 2.5–3.5 μm, usually bearing sessile conidia. Chlamydospores and sexual morph absent. Fetid (skunk-like) odor present on all culture media tested |
| *N. vriesii* Currah (1985) [8] | Lizard (*Ameiva* sp.) | *Nannizziopsis vriesii* is distinguished from all the other *Nannizziopsis* species described here by the production of ascomata (gymnothecia) produced on oatmeal salts agar (OAT) at 30 °C. Colonies on PDA were 4.5–5.5 cm in diameter after 21 days, velvety to slightly cottony, and furrowed. Growth was inhibited at 35 °C, with colonies attaining 2.5 cm in diameter. Aleurioconidia 2.5–6.0 μm long (in rare cases up to 8 μm long) and 1.5–2.7 μm wide. Isolates produced undulate hyphae and cylindrical fission arthroconidia measuring 2.7–7.3 μm long and 1.7–2.7 μm wide and sometimes showing yeast-like budding |

(continued)

**Table 3.1** (continued)

| Fungus species | Reptile species (source of isolation) | Morphological features |
|---|---|---|
| Genus *Paranannizziopsis* [8] | | Colonies were pale and moderately fast growing. Vegetative hyphae were narrow, branched, and septate, sometimes with racquet mycelia. Conidia (aleurioconidia) sessile or produced on slightly swollen cells or on short stalks and released by rhexolytic dehiscence. They are hyaline, smooth, pyriform, and clavate to obovate. Arthroconidia absent, intercalary, or produced in adjacent chains. Undulate lateral branches were produced. No teleomorph was produced. *Paranannizziopsis* species are distinguished from *Nannizziopsis* and *Ophidiomyces* species by the uncommon occurrence or absence of fission arthroconidia |
| *P. australiensis* [8] | Northern tuatara (*Sphenodon punctatus punctatus*), coastal bearded dragon, aquatic file snake (*Acrochordus* sp.) | Colonies on PDA attained 4.5–5.0 cm in diameter and were powdery or sometimes cottony, flat, or faintly zonate. There was no growth at 35 °C. Aleurioconidia sessile or subtended by slightly swollen cells from which one or two conidia were produced. Pyriform to clavate conidia of 3.5–8.0 μm long and 1.5–2.7 μm wide. Occasional intercalary arthroconidia and undulate hyphae were produced. Ascomatal initials occurred in cottony sectors and appeared as inflated cells with secondary proliferations |
| *P. californiensis* [8] | Tentacled snake (*Erpeton tentaculatum*) | Colonies on PDA attained 4.5–5.2 cm in diameter and were powdery and flat to slightly zonate. Growth at 35 °C was strongly inhibited. Aleurioconidia were clavate to pyriform or obovate, measured 4.0–8.5 μm long and 1.8–2.6 μm wide, and were sessile or borne on a slightly swollen cell. Undulate hyphae were uncommon, and arthroconidia were not observed. Ascomatal initials occurred in cottony sectors and were associated with large irregularly shaped cells. The latter measured 10.0–36.0 μm long and 3.5–9.5 μm wide |
| *P. crustacea* [8] | Tentacled snake | Colonies on PDA attained 5.8–6.5 cm in diameter and were powdery, flat, and occasionally with dense downy overgrowth. There was no growth at 35 °C. Aleurioconidia were clavate to pyriform or obovate, sessile or formed on short stalks, and measured 4.0–7.5 μm long and 2.0–3.5 μm wide. Undulate hyphae, fission arthroconidia, and occasional intercalary arthroconidia were produced. Arthroconidia measured 3.8–9.2 μm long and 1.9–2.7 μm wide |

(continued)

**Table 3.1** (continued)

| Fungus species | Reptile species (source of isolation) | Morphological features |
|---|---|---|
| *P. longispora* [8] | Tentacled snake | The sequence of *C. longisporum* groups closest to *P. crustacea* but differs at 9 positions in the ITS region. This level of sequence difference, combined with morphological differences, including the absence of growth at 30 °C, absence of fission arthroconidia in chains, and longer conidia (3.0–13.0 μm long), provides support for the retention of both species |
| Genus *Ophidiomyces* [8] | | Colonies were yellowish white and moderately fast growing. Vegetative hyphae were narrow, branched, and septate, occasionally with racquet mycelia. Conidia sessile or borne on short stalks and released by rhexolytic dehiscence (aleurioconidia). Aleurioconidia hyaline, smooth, and cylindrical to clavate. Arthroconidia were formed in chains by schizolytic fragmentation of hyphae or were sometimes intercalary. Short, undulate, and sparsely septate lateral branches were common. No teleomorph is known |
| *O. ophiodiicola* [8] | Captive and wild snakes (black rat snake, brown tree snakes, garter snake, green anacondas, broad-headed snake, carpet snakes, *Boa constrictor*, *Nerodia* species, timber rattlesnakes, eastern massasauga rattlesnakes) | Colonies on PDA were 4–6 cm in diameter and were velvety to powdery, dense, flat, frequently zonate, and sometimes with cottony sectors. Clear exudate droplets were often present. Most isolates failed to grow at 35 °C. Aleurioconidia were sessile or borne at the ends of short stalks, cylindrical to clavate, and 2.5–7.5 μm long and 1.5–2.5 μm wide. Arthroconidia were 3.0–12.5 μm long (in rare cases up to 15.0 μm long) by 1.5–3.5 μm wide. In young cultures on PDA, arthroconidia sometimes showed budding or germination. Undulate hyphae were commonly produced. Some isolates produced ascomatal initials in cottony sectors. Most isolates produced a strong to weak mercaptan-like odor |
| Genus *Chrysosporium* Corda (1833) | | |
| *C. longisporum* [18] | Tentacled snake | Colonies on PYE at 25 °C attaining a diameter of 40.0–46.0 mm after 14 days, white to pale orange (M. 6A3), zonate, felted, slightly cottony at center, with regular margins; reverse pale orange (M. 5A2). Hyphae hyaline, septate, smooth walled, 1.0–5.0 μm wide, straight. Conidia 1(−2) celled, mostly sessile, or produced on short protrusions or on side branches or terminal, hyaline thin and smooth walled, pyriform, obovate, claviform to cylindrical, 3.0–13.0 × 2.0–3.5 μm; intercalary conidia present, cylindrical to doliiform, 3.0–6.0 × 2.0–3.0 μm, usually bearing sessile conidia; arthroconidia in chains absent. Chlamydospores and sexual morph absent. Fetid (skunk-like) odor present on all the culture media tested |

## 3.3 Physiological and Morphological Features of Pathogenic *Chrysosporium*-Related Fungi

The morphological differences between genera and species of *Chrysosporium*-related fungi are not so clear as to differentiate these species without DNA sequencing. All isolates are moderately fast growing on PDA (potato dextrose agar) at 30 °C and have yellowish white, velvety to powdery, dense, and sometimes zonate colonies with uncolored to yellowish reverse [8]. Similar colonial morphology can be observed on SDA (Sabouraud's dextrose agar) (Fig. 3.1). In some cases, the heterogenous colonial morphology can be seen (Fig. 3.2). It is noteworthy that PCRF can actively grow on media enriched with the sheep blood (Fig. 3.3). In a number of isolates of *N. guarroi*, we observed the presence of incomplete (partial) hemolysis (Fig. 3.4). This indicates that some of PCRF fungi are able to synthesize the hemolysins.

All isolates of PCRF produce aleurioconidia which are solitary conidia released by lytic dehiscence. The aleurioconidia are commonly sessile, sometimes subtended by slightly swollen cells, or formed at the ends of short stalks. They are clavate or pyriform with truncate bases, occasionally subglobose or obovate, mostly single celled, and occasionally two celled (Fig. 3.5). *Nannizziopsis* and *Ophidiomyces* species commonly have chains of adjacent cylindrical arthroconidia that are produced by schizolytic fragmentation of the hyphae. Arthroconidia sometimes demonstrate budding and are found especially in the moist yeast-like colonies. In tissues, arthroconidia occur in the stratum corneum or deeper in the epidermis or in characteristic aggregates or tufts at the surface of lesions (Fig. 3.6). Aleurioconidia also can be seen in affected tissues. According to Sigler et al. (2013), the notable characteristic, occurring in members of all three genera (*Nannizziopsis*, *Paranannizziopsis*, and *Ophidiomyces*) but not in *Chrysosporium* species or dermatophytes, was the formation of short, solitary, undulate, lateral branches that were occasionally sparsely septate [8]. They may play a role in pathogenicity, at least in reptiles, by possibly

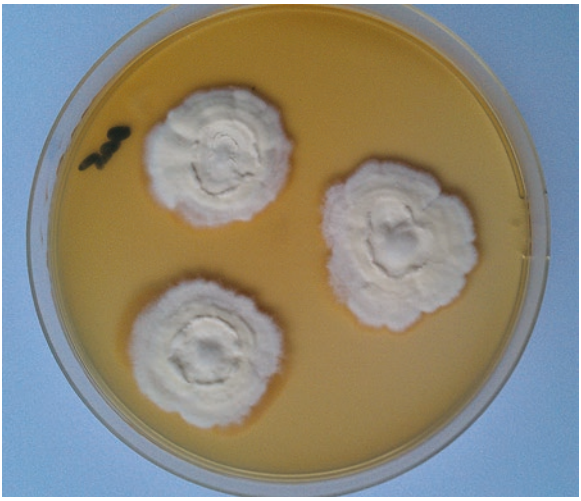


**Fig. 3.1** Colonies of *Nannizziopsis guarroi* on SDA

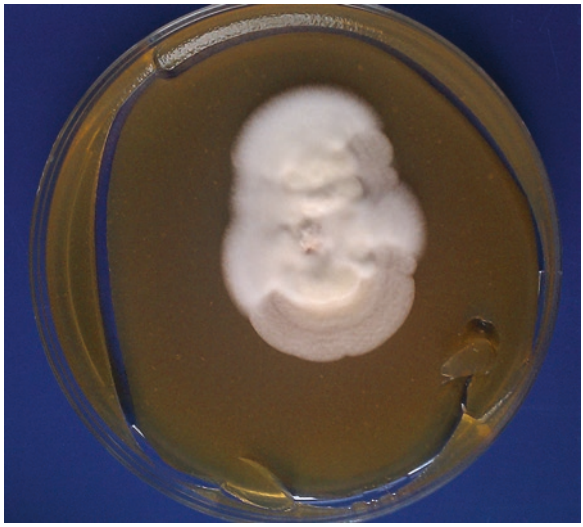


**Fig. 3.2** Heterogenous colonial morphology of *N. guarroi*

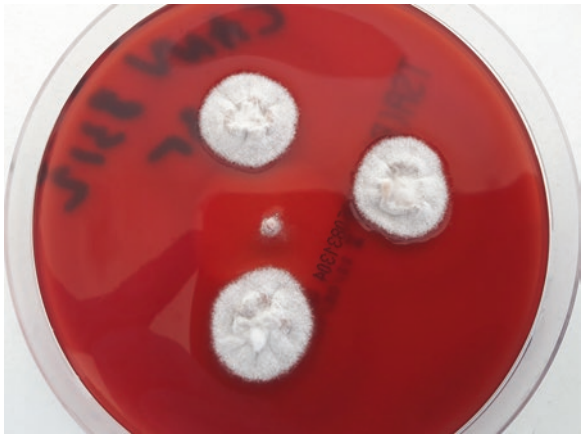


**Fig. 3.3** Colonies of *N. guarroi* on agar with sheep blood

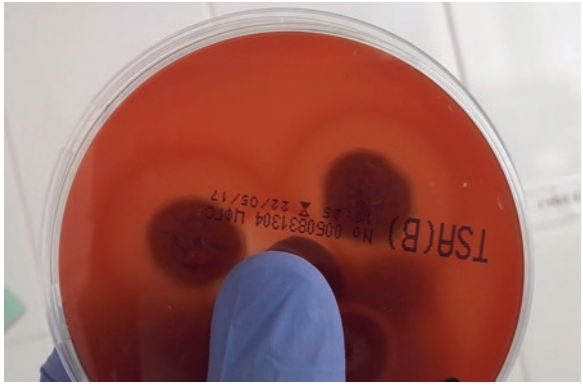



**Fig. 3.4** Partial hemolysis caused by *N. guarroi* on agar with sheep blood

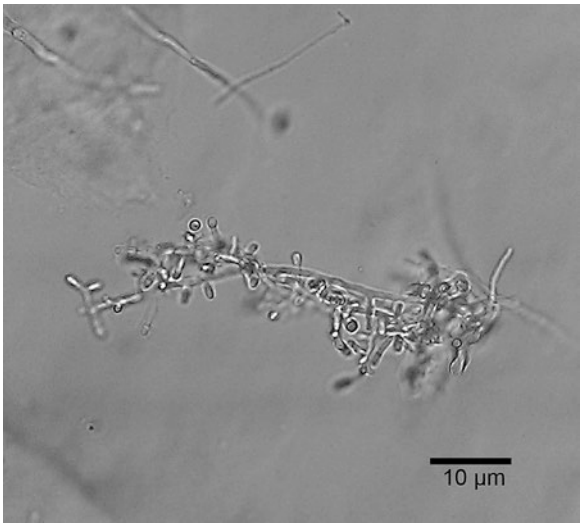



**Fig. 3.5** Sessile aleurioconidia and hyphae of *N. guarroi* (400×)

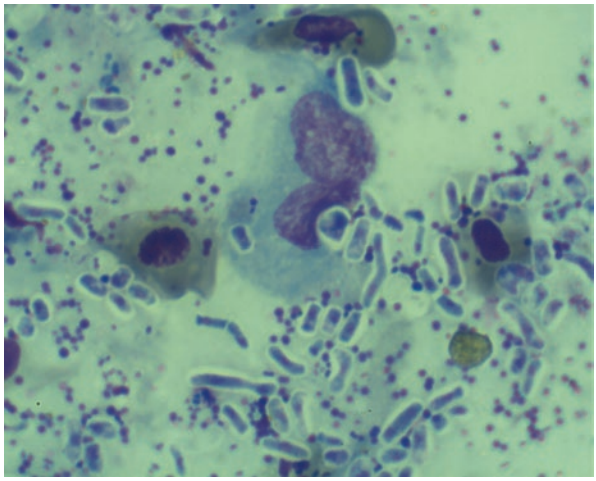


**Fig. 3.6** Arthroconidia of *N. guarroi* in affected reptile skin. (Diff-Quik-stained cytological smear, 1000×)

aiding in attachment. PCRF isolates failed to produce ascospores with the exception of *N. vriesii* isolates, that were incubated on culture media for several months. Cultures often have a specific unpleasant odor which is compared with the smell of a skunk or the smell of a mercaptan.

The keratinolytic activity of PCRF is attributed to the keratinase which relates them with dermatophytic fungi. Keratinases are the most important virulence factors of dermatotropic fungi (*Microsporum* spp., *Trichophyton* spp.), ensuring the penetration of pathogen into the stratum corneum. These enzymes stipulate the positive results in in vitro hair perforation test for both dermatophytes and PCRF. The presence of keratinolytic activity indirectly confirms that PCRF are primary pathogens not opportunists. The temperature optimum for PCRF growth is 28–30°C. The

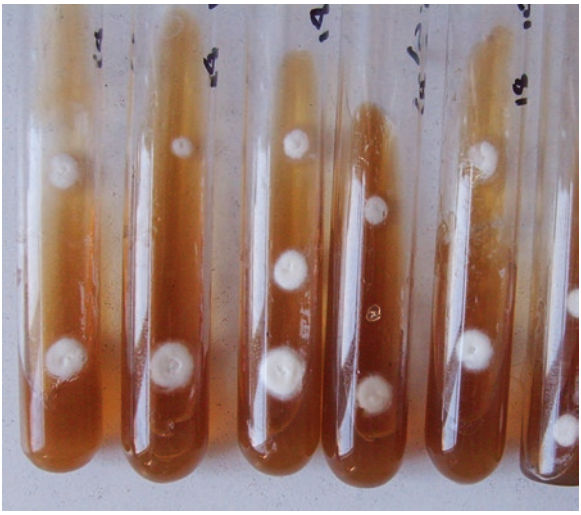


**Fig. 3.7** Colonies of *N. guarroi* isolated from clinical samples on SDA supplemented with chloramphenicol and cycloheximide

**Fig. 3.8** Colonies of *N. guarroi* do not turn DTM medium red

temperature of 37 °C is unfavorable, and the growth of fungal cultures was found to be restricted. Thus, these fungi, unlike dermatophytes, are not thermotolerant and affect warm-blooded animals rarely except *Nannizziopsis guarroi*, which shows optimal growth at 35 °C [20].

This feature can be used to treat reptilian mycosis, and exposure of high temperature (37–39 °C) may be helpful for the treatment; however, it should be tolerable to the patient. The PCRF fungi are naturally resistant to cycloheximides, which are protein biosynthesis inhibitors in eukaryotes. Due to this feature, culture media with cycloheximide can be successfully used for the selective isolation of these fungi in the laboratory (Fig. 3.7).

In our practice, for PCRF isolation, we use media with selective supplement for dermatophytes containing cycloheximide and an antibiotic. PCRF fungi can also be isolated on DTM (dermatophyte test medium). Unlike dermatophytes, these fungi do not cause the alkalinization and redness of the medium (Fig. 3.8).

## 3.4 Clinical Manifestations of PCRF-Associated Mycoses in a Range of Reptile Species

PCRF mycoses are diagnosed in various representatives of the class Reptilia, including order Squamata which comprise lizards (Lacertilia), chameleons (Chameleontes), snakes (Ophidia), and order Crocodilia. In tortoises (Chelonia), there are no cases of PCRF mycoses reported in literature. But in the author's practice, rare cases of these infections have been found. Reptile species susceptible to PCRF mycoses are listed in Table 3.2.

Reptiles infected with PCRF can present with a range of clinical signs, from focal skin lesions to systemic disease. The most common clinical signs are associated with the integument. Crust formation, color change, and necrosis are commonly seen. Like any other cutaneous fungal lesions, PCRF infections tend to start as focal lesions that spread from a central point. Because of the invasive nature of this fungus, it is common to observe pyogranulomatous disease as it invades through the epidermis and dermis. Once the fungus invades through the integument, it can spread locally or systemically. However, the fungus is reported to be locally invasive [2].

**Table 3.2** Species of reptiles found to be infected with pathogenic *Chrysosporium*-related fungi [2]

| Common name | Scientific name | References |
|---|---|---|
| **Lizards** | | |
| Ameiva | *Ameiva chaitzarni* | [21] |
| Day geckos | *Phelsuma* spp. | [22] |
| Green iguanas | *Iguana iguana* | [9] |
| Central inland bearded dragon | *Pogona vitticeps* | [23] |
| Coastal bearded dragon | *Pogona barbata* | [12] |
| Panther chameleon | *Furcifer pardalis* | [6] |
| Jackson's chameleon | *Chamaeleo jacksoni* | [6] |
| Jeweled chameleon | *Chamaeleo lateralis* | [6] |
| Parson's chameleon | *Chamaeleo parsonii* | [6] |
| Veiled chameleon | *Chamaeleo calyptratus* | [24] |
| Girdled lizard | *Cordylus giganteus* | [11] |
| Leopard geckos | *Eublepharis macularius* | [28] |
| **Snakes** | | |
| Boa constrictor | *Boa constrictor* | [29] |
| Ball pythons | *Python regius* | [22] |
| Garter snakes | *Thamnophis* spp. | [22] |
| Brown tree snake | *Boiga irregularis* | [26] |
| Milk snake | *Lampropeltis triangulum* | [22] |
| Corn snake | *Pantherophis guttatus* | [22] |
| Tentacle snakes | *Erpeton tentaculatum* | [27] |
| Eastern massasauga rattlesnakes | *Sistrurus catenatus catenatus* | [3] |
| File snakes | *Acrochordus* spp. | [30] |
| **Crocodilians** | | |
| Saltwater crocodiles | *Crocodylus porosus* | [7] |

The clinical manifestations of PCRF-associated mycoses are supposed to vary among different species of reptiles. Thus, bearded dragons affected with PCRF (CANV) typically present with dermatitis characterized by crusts, ulcers, and pyogranulomatous disease [12, 23]. Originally described as yellow fungus disease, the crusts found on bearded dragons tend to have a yellow coloration. Lesions in bearded dragons are often multifocal and may include the head, oral cavity, limbs, ventrum, or dorsum. Infection tends to be aggressive and disseminate into the subcutaneous tissues which is usually followed by necrosis, sloughing, and ulceration involving muscle and bone. The infection can disseminate with a fatal outcome [19, 23, 30].

Chameleons are the other group of lizards that seem to be highly susceptible to PCRF (CANV) infections. Additionally, these infections are unlikely to be limited to a group from a single native origin. Affected chameleons often present with focal to multifocal necrotic (black) areas of skin surrounded by crusts. The lesions may be found on the body, limbs, and tail [6]. Other species of lizards that have been reported to develop PCRF (CANV) infections in captivity include day geckos (*Phelsuma* spp.) [22], a wild-caught girdled lizard (*Cordylus giganteus*) [11], green iguanas (*Iguana iguana*) [9, 14], and an ameiva (*Ameiva chaitzarni*) [21]. The lesions in these animals were similar to those described for bearded dragons and chameleons and included crusting and ulcerative dermatitis lesions on the head and body. An outbreak of CANV mycosis in colony of leopard geckos (*Eublepharis macularius*) was reported by Toplon et al. (2013) [28]. Histopathology of the affected animal revealed multifocal to coalescing dermal and subcutaneous heterophilic granulomas that contained septate fungal hyphae. The multifocal epidermal hyperplasia with hyperkeratosis was also observed. Moreover, hyphae of causative fungus, occasionally with terminal chains of arthroconidia found within the stratum corneum, were consistent with the CANV. In one case, focal extension of granulomatous inflammation into the underlying masseter muscle was also seen, and *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) was identified by sequencing of the internal transcribed spacer region of the rRNA gene.

According to our observations, sloughing yellowish scales and decolorized skin foci are often observed in green iguanas affected by PCRF (Figs. 3.9 and 3.10). In chameleon (*Chamaeleo calyptratus*), the mycosis was characterized by necrosis and parakeratosis of the epidermis, edema and infiltration of subepidermal tissues, and the formation of granulomas and severe scabby crusts (Figs. 3.11 and 3.12), whereas in monitor lizard (*Varanus exanthematicus*), a severe peripheral edema with muscle infiltration was manifested along with depigmentation and desquamation of the epidermis (Fig. 3.13). In above mentioned cases, CANV was culturally diagnosed as etiologic agent. The authors noticed that superficial infections in reptiles are clinically different from bacterial dermatoses and characterized by pronounced hyper- and parakeratose reactions accompanied by huge scabby crusts with hydrophobic properties resembling the structure of the squama (Fig. 3.14). The thickness of the crust correlates with the aggressiveness of the infection (Fig. 3.15).

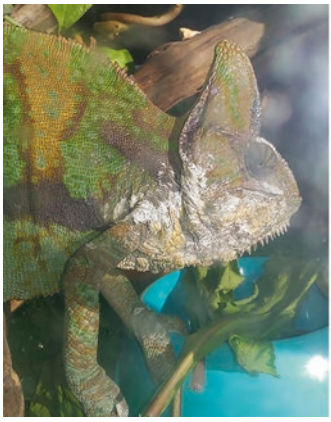
In our opinion, the signs of muscle infiltration are unique for PCRF infection. The fungus is especially actively disseminating before molting, during the accumulation of lymph in the zona intermedia, where there is a demarcation of new and old epidermal skin generation. Therefore, the use of multivitamin preparations and

**Fig. 3.9** Yellowish skin scales in green iguana infected by PCRF

**Fig. 3.10** Decolorization of the skin infected by PCRF in green iguana

retinol for stimulation of molting in the case of fungal infections is not recommended. In other cases, molting usually leads to a reduction of lesions (in case of the concomitant etiotropic therapy).

According to the published data, bearded dragons and chameleons tend to be the most common case presentations of PCRF mycoses in reptiles. Based on the data obtained from Moscow Zoo, the predisposed species are green iguana (*Iguana*



**Figs. 3.11 and 3.12** PCRF-associated mycosis in chameleon (*Chamaeleo calyptratus*). Formation of granulomas and severe scabby crusts, parakeratosis, edema, and subepidermal infiltration

**Fig. 3.13** PCRF-associated mycosis in monitor lizard (*Varanus exanthematicus*). Depigmentation and desquamation of the epidermis and severe peripheral edema along with muscle infiltration

*iguana*) and bearded dragons (*Pogona vitticeps*). *Chrysosporium*-related fungal elements were cytologically detected in the following:

Blue-tongued skink (*Tiliqua scincoides*) – periorbital infection together with finger infection.

**Fig. 3.14** Huge scabby crusts resembling the structure of the squama in green iguana affected by PCRF

**Fig. 3.15** The thickness of the crusts correlates with the aggressiveness of the PCRF infection in green iguana

Ocellated lizard (*Lacerta lepida*) – local lesions of dark color on lateral and abdominal scales (3–5 mm in size), with the formation of rugged scabs and local infiltration of subcutaneous tissue.

Armenian rock lizard (*Lacerta rudis*) – similar lesions as found in *Lacerta lepida.* Probably the same strain of pathogenic fungus, because animals were kept in closely spaced terrariums and common tools for cleaning were used.

Western fence lizard (*Sceloporus occidentalis*) – merging parakeratosis foci on the skin of the neck and sides, with the formation of scabs and local infiltration of muscles.

Parrot-beaked tortoise (*Homopus areolatus*) – vesicular merging foci on the skin of the thighs and tail, differing in color, but without the formation of crusts. Perhaps due to an early stage of the infection.

Chinese softshell turtle (*Pelodiscus sinensis*) – local ulcerative-necrotic lesions on the skin of the zygomatic area of the head, covered with a strong scab of yellow color.

The determined cases of PCRF infections in these animal species are not described in the literature. Although mycological culturing of these samples was not carried out, characteristic arthrospores and aleurioconidia were observed in direct microscopical examination. It makes possible to presume a preliminary diagnosis of PCRF by cytological examination of skin lesions. Along with the lacertian, both captive and wild snakes can be affected by PCRF. At least 9 species of snakes have been diagnosed with CANV till 2011 (Table 3.2). Although the distribution of CANV lesions in snakes seems to be primarily associated with the head [3, 25, 27, 29], in some cases, it can also be found on ventrum [26]. The active burrowing by the snakes may stir up the fungus from the substrate. Lesions are similar to those described with necrotizing dermatitis and may include erythema, plaque and crust formation, and the presence of vesicles. Among PCRF, the most important pathogen for wild snakes is *Ophidiomyces ophiodiicola*. The mycosis caused by *O. ophiodiicola* can be identified by skin lesions and thick blisters that can distort the face of a snake and even prevent it from feeding which often leads to starvation. The outcome of the disease varies between species, but the mortality rate is especially high in rattlesnakes, including the eastern massasauga rattlesnake. According to the latest data, about 30 snake species are infected in the United States (15 states), and 3000 snake species of rest of the world are vulnerable for the disease. The disease has also been reported in captive snakes from England, Germany, and Australia [25].

In Moscow Zoo, CANV was observed in snakes common boa (*Boa constrictor*), woma python (*Aspidites ramsayi*), and coastal taipan (*Oxyuranus scutellatus*) (authors' unpublished data). In *Boa constrictor*, lesions were expressed as deformation, depigmentation, and desquamation of squama. The absence of a scab indicated a subacute course of the disease (Fig. 3.16). In coastal taipan, lesions were located on the ventral and lateral sides of the body and were manifested in the form of local and confluent foci of necrosis penetrating to the intercostal muscles. Peripheral local edema and the formation of scabby crusts were also seen (Fig. 3.17). There is a single report of PCRF (CANV) infection in captive saltwater crocodiles (*Crocodylus porosus*) [7]. The lesions were associated with the skin and mortalities ($n = 548$) were recorded. Infections were recorded twice on the same farm in a span of 3 years. High-density production facilities, such as those used for the crocodilian leather industry, probably made crocodiles highly susceptible to PCRF infections. Moreover, a case of PCRF mycosis was also diagnosed in *Caiman crocodilus* in Moscow Zoo by the authors.

In general, there are few reports on the epidemiology and prevalence of PCRF infections among reptiles in the literature. Most publications represent the descriptions of individual clinical cases or outbreaks. To clarify the situation, we conducted

**Fig. 3.16** PCRF-associated mycosis in common boa (*Boa constrictor*). Deformation, depigmentation, and desquamation of squama

**Fig. 3.17** PCRF-associated mycosis in coastal taipan (*Oxyuranus scutellatus*). Lesions on ventral and lateral side of the body are manifested as necrotic foci penetrating to the intercostal muscles. Edema and scabby crusts are also seen

an etiological study of skin lesions of reptiles kept in captivity in the Moscow region [15]. The mycological examination of clinical samples from 109 reptiles having skin lesions was performed. Seventeen reptile species were presented including green iguana (*Iguana iguana*) (35 animals), central bearded dragon (*Pogona vitticeps*) (9 animals), red-eared slider (*Trachemys scripta elegans*) (14 animals), Chinese soft-shell turtle (*Pelodiscus sinensis*) (10 animals), monitor lizard (*Varanus* spp.) (5 animals), frill-necked lizard (*Chlamydosaurus kingii*) (4 animals), spiny-tailed lizards (*Uromastyx* spp.) (4 animals), geckos (*Gekko* spp.) (9 animals), chameleons (*Chameleo* spp.) (8 animals), skinks (*Scincidae* spp.) (7 animals), python (*Python regius*) (3 animals), and caiman (*Caiman crocodilus*) (1 animal). Fungal infections

were diagnosed in 86 reptiles, which accounted for 79% of the total number of animals examined. Eighteen fungal species were isolated, among which the *Chrysosporium* anamorph *of Nannizziopsis vriesii* (CANV) dominated (37%). The most susceptible reptile species was found to be green iguana (89% of CANV cases).

Identification of fungal species in the aforementioned study was performed on the basis of conventional mycological techniques (morphological characteristics, etc.). Three most typical CANV cultures isolated from captive green iguanas were identified by sequencing of ITS region. In the NCBI GeneBank database, the best match was found with the sequence KX755439 belonging to the species *Nannizziopsis guarroi* (unpublished data 2017).

Although reptiles are the main targets of PCRF, several cases have also been described in warm-blooded animal species. *Chrysosporium pannicola* (formerly *C. evolceanui*) was isolated from the skin of a dog [31] and from a horse [32]. Similarly, the probable cases of mycosis caused by *C. tropicum* were reported in two breeds of chickens [33] and in a dog [34]. Recently, Cook et al. (2015) reported the disseminated infection in a German shepherd dog caused by *Chrysosporium* spp. The diagnosis was based on a positive fungal culture and cytological investigations of intralesional fungi associated with granulomatous splenitis and neutrophilic lymphadenitis. The patient showed rapid clinical improvement on oral posaconazole. Based on colonial and microscopic features, the fungus was identified as *Chrysosporium* spp. Unfortunately, further speciation of the isolate and antifungal susceptibility testing could not be performed [35].

Several cases of mycoses caused by genus *Nannizziopsis* have been described in humans. Diagnosed fungal species apparently are specific for humans [8]. Human cases of *Nannizziopsis* mycoses are summarized in Table 3.3.

Most of these human cases occurred as opportunistic infections in immunocompromised patients. Suchonwanit et al. (2015) reported a case of primary cutaneous *Chrysosporium* infection following ear piercing in an immunocompetent patient [40]. A 25-year-old healthy woman presented with a 2-year history of an itchy erythematous plaque on the right ear pinna. PCR was performed on the colony sample using the gene fragment, and BLASTN search against the GeneBank database revealed a 98% nucleotide sequence identity to *Chrysosporium* spp.

Apparently, the probability of transmission of PCRF infection from domestic reptiles in humans is very low. *N. guarroi*, the main causative agent of mycoses in bearded dragons and iguanas, has not yet been isolated from humans [8]. The environment and wild animals seem to be a more likely source of infection for humans [4]. However, precautions when dealing with reptiles are still advisable.

## 3.5 Ecology of PCRF and Predisposing Factors

The source of the etiologic agents of contagious PCRF-associated mycoses is not well defined as CANV is not a member of the resident or transient microbiota of reptile skin. Pare et al. [22] evaluated the mycobiota of skin in different reptiles and found the rare presence of CANV in comparison to *Aspergillus* spp., *Paecilomyces*

**Table 3.3** Cases of human mycoses caused by the species of genus *Nannizziopsis* [37]

| Species | Disease, dissemination | Immune status | References |
|---|---|---|---|
| *N. infrequens* | Localized, bronchial wash specimen, M, 40 years old, USA, IA, 2004 | HIV positive | Sigler et al. (2013) [8] (elucidated further from Brandt et al. 2005) [36], who named isolate as *Nannizziopsis vriesii*) |
| *N. hominis* | Disseminated disease. Right thigh mass with lung lesion, M, USA, CA, 1994 | HIV positive | [8] |
| *N. hominis* | Disseminated inguinal node, Nigerian, M, 32 years old, with disseminated adenopathy, USA, MA, 2000 | Immunocompetent | [8] |
| *N. obscura* | Localized disease. Abscess right ankle, African, M, 24 years old (isolated twice), USA, NY, 1984 | Immunocompetent | Sigler et al. (2013) (elucidated further from Stillwell et al. [1984] [38] who named isolate as *Chrysosporium* spp. [8] |
| *N. vriesii* | Disseminated disease. Lung infiltration and a brain abscess in a Nigerian, M, 38 years old, Germany, 2005 | HIV positive | Steininger et al. (2005) [39] |
| *N. obscura* | Disseminated disease. Thoracic collection, lymphadenopathy, and skin rash in Gambian, M, 34 years old, UK. 2015 | Immunosuppressed for renal transplant | [37] |

spp., and *Penicillium* spp. In total, 127 reptile (36 lizards, 91 snakes) sheds were evaluated, and CANV was isolated only from an African rock python (*Python sebae*). Thus, the rarity of CANV suggested that it is not an opportunistic fungal but an obligate pathogen that infects reptiles after exposure. In order to confirm its status, veiled chameleons (*Chamaeleo calyptratus*) experimentally infected with the fungus *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV). Chameleons were inoculated by direct application of a conidial suspension on intact and abraded skin [24]. The CANV induced lesions in all experimental groups and was recovered from infected animals, thus fulfilling Koch's postulates. A breach in cutaneous integrity, as simulated by mild scarification, increased the risk of infection. CANV dermatomycosis was shown to be contagious and could readily spread within a reptile collection, either directly through contact with infective arthroconidia or indirectly via fomites. Dense tufts of arthroconidiating hyphae were demonstrated histologically on the skin surface of many animals that developed dermatomycosis, and these arthroconidia may act as infective propagules. The infection was similar to that described for clinical cases.

Recently, the pathogenicity of *Ophidiomyces ophiodiicola* (a member of CANV complex) was experimentally studied by Lorch et al. (2015) [41]. They experimentally infected captive-bred corn snakes (*Pantherophis guttatus*) in the laboratory

with pure cultures of *O. ophiodiicola*. All snakes in the infected group ($n = 8$) developed gross and microscopic lesions identical to those observed in wild snakes with snake fungal disease (SFD). Furthermore, the same strain of *O. ophiodiicola* was recovered from lesions of all animals in the infected group. The host response to the infection included marked recruitment of granulocytes to sites of fungal invasion; increased frequency of molting and abnormal behaviors, such as anorexia; and resting in conspicuous areas of enclosures. While these responses may help snakes to fight infection, they could also impact host fitness and may contribute to mortality in wild snakes with chronic *O. ophiodiicola* infection. This experiment demonstrates that *O. ophiodiicola* is the causative agent of SFD and can elicit pathological changes and affects the fitness of wild snakes.

Since reptile owners usually have many animals in their collection, hence awareness regarding good hygiene practices is essential to prevent the dissemination of the disease. Although inadequate diet and husbandry, environmental stresses, trauma, and existing dermatitis are likely contributors, however, the circumstances under which mycotic diseases in reptile species occur are still unknown [24].

In Moscow Zoo, fungal infections caused by PCRF are very rare. In general, the disease is prevalent in captive animals of private owners or in the animals having been recently imported from dealers. PCRF mycoses occur more often during group maintenance of reptiles. It is important to determine more specific risk factors associated with this fungal pathogen. Substrate also plays an important role in disease dissemination; therefore, it is important to examine substrates under different conditions (e.g., temperature and humidity) for the presence of this organism. Pet owners should be advised to maintain the cleanliness of pet's habitat. Moreover, PCRF being keratophilic, it is possible that the food sources (e.g., crickets) can also be the source of infection for reptiles. Therefore, its association with foodstuffs must be evaluated and discussed [2]. Since little is known about dissemination of PCRF, thus further research is needed on the epidemiology of this infection.

## 3.6 Diagnosis of PCRF-Associated Mycoses

In case of superficial lesions (dermatitis) in reptiles, veterinary practitioners are inclined to predict the bacterial etiology of the disease. Although bacteria can be isolated from most skin lesions, they do not always possess the clinical significance. The primary etiological role may belong to pathogenic fungi. The clinical signs usually do not differentiate the bacterial infection from the fungal one. Thus, mycological diagnostic testing considering PCRF in cases presenting with dermatitis or necrosis is recommended [2].

Wearing medical clothes during examination and treatment of infected reptiles can help in reducing transmission to other uninfected reptiles, as transmission occurs by direct contact or indirectly via fomites. Primary mycological diagnosis can be carried out directly in the doctor's office. It consists of direct microscopy (cytology) of the samples from the affected areas. The samples (crusts, scabs, scales, etc.) are taken from the periphery of the lesion focus by sterilized forceps and (or) scalpel.

Direct microscopy is done in 10–15% solution of potassium hydroxide with subsequent moderate heating of the slide. In samples positive for PCRF, the fungal elements can be detected occasionally segmenting apart the arthrospores (Figs. 3.18 and 3.19). If numerous fungal fragments are found in affected tissues, the mycotic etiology of the infection can be confirmed. Full-thickness biopsy samples can be submitted for culture, histopathology, and/or polymerase chain reaction (PCR) testing. Fungal elements can be detected in histological sections (Fig. 3.20), but due to presence of inflammatory cells (macrophages and heterophils), identification of PCRF seemed difficult.

Granuloma formation is a common defense reaction in reptiles which consists of central fibrin, cell detritus, and fungal elements surrounded by heterophils, macrophages, and connective tissue. The infected epidermis is often ulcerated, with fibrin deposition, fungal hyphae, and conidia [20]. However, mycological examination should not be limited to microscopy of the samples. Identification of fungal species and determination of antifungal susceptibility can be obtained only by cultural study (inoculation of the samples on mycological nutrient media). PCRF cultures (tested in our laboratory) do not cause reddening of DTM (dermatophyte test media) in contrast to dermatophytes (*Trichophyton* spp., *Microsporum* spp.) (Fig. 3.8). Incubation of the inoculated media is carried out at 28–30 °C. The beginning of growth of PCRF colonies can be seen on the 4th to 6th days. The colonies on SDA or Malt Extract Agar (MEA) media are usually white, velvety, or powdery, with diameter up to 1 cm on the 7th day (Fig. 3.7). From the 7th to 10th days, fungal cultures begin to sporulate which is necessary for proper identification of fungus. In case of slow growth, the cultures are incubated for 14–21 days. A fragment of mycelium can be taken for microscopy from mature colonies. PCRF usually forms numerous unicellular aleurioconidia, located directly on unspecialized hyphae

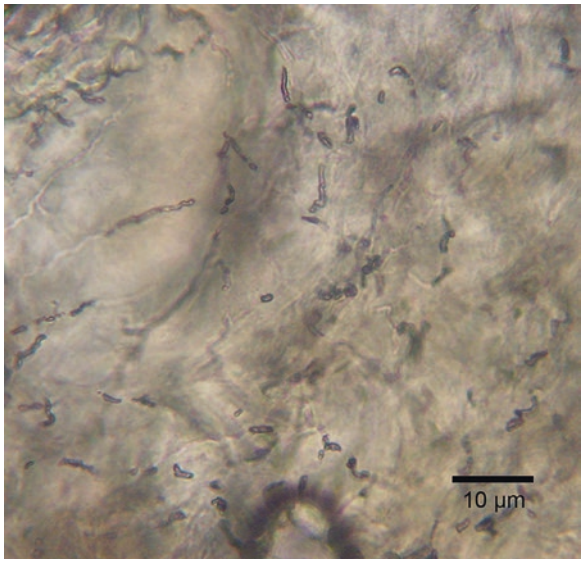



**Fig. 3.18** Microscopic detection of branching hyaline hyphae in skin scales affected by PCRF. (Smear in 15% solution of potassium hydroxide, 400×)

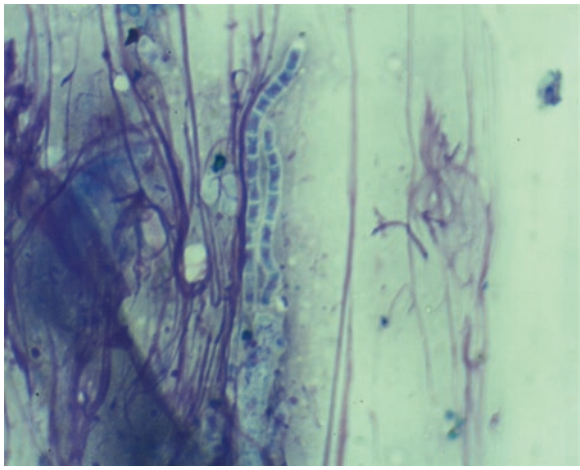


**Fig. 3.19** Hyphae forming arthrospores in skin scales affected by PCRF. (Diff-Quik-stained cytological smear, 1000×)

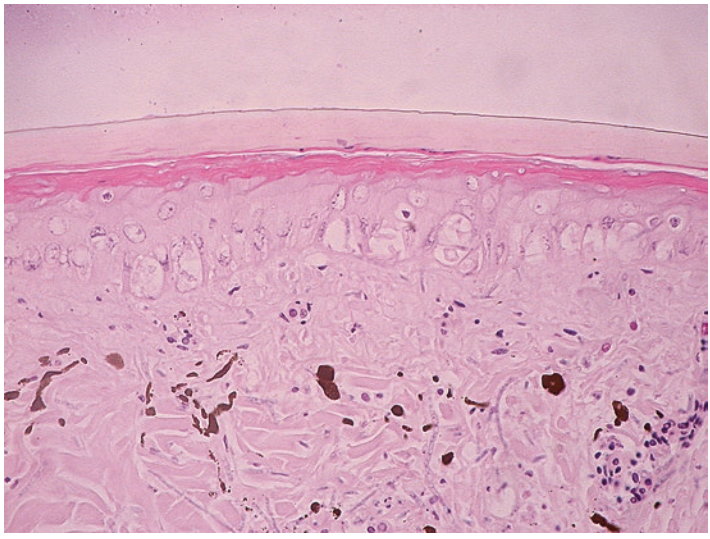


**Fig. 3.20** Fungal elements in histological section of affected skin. (Hematoxylin and eosin 1000×)

(Fig. 3.5). Morphological features intrinsic for different species of PCRF have been described in Table 3.1.

When the culture of PCRF is isolated and identified, the antifungal susceptibility testing can be performed [20]. In our laboratory, we use the discs with clotrimazole (10 μg, HiMedia), nystatin (100 U, HiMedia), miconazole (10 μg, HiMedia), fluconazole (25 μg, HiMedia), ketoconazole (10 μg, HiMedia), itraconazole (10 μg, HiMedia), and voriconazole (1 μg, HiMedia) (Fig. 3.21). Moreover, E-test strips which quantitatively determine MIC (minimal inhibitory concentrations) for some antifungals have also been used (Fig. 3.22). The appropriateness of this test in a particular case should be discussed with the attending veterinarian. Considering

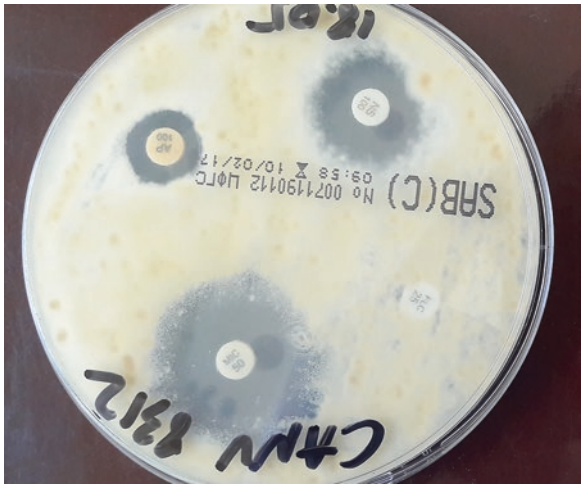


**Fig. 3.21** Disc diffusion method for antifungal susceptibility testing of PCRF


**Fig. 3.22** Quantitative susceptibility testing of PCRF to ketoconazole using E-test strips. Minimal inhibitory concentration (MIC) value is 4.0 μg/ml for tested culture

osteomyelitis which is common in affected reptiles, radiographs can be used to assess the integrity of the bone while planning medical and surgical options. Advanced imaging such as computed tomography can also be used for better characterization of extent of lesions (especially bony involvement) [2].

Few publications indicate the applicability of PCR (polymerase chain reaction) for the diagnosis of PCRF mycoses [9]. For instance, Schmidt-Ukaj et al. (2016) applied PCR for identification of *Nannizziopsis chlamydospora* in 3 central bearded dragons (*Pogona vitticeps*) [42]. PCR kits that are useful for routine diagnosis of these mycoses are currently being developed. TaqMan real-time PCR has also been used to detect *Ophidiomyces ophiodiicola* (a member of PCRF group) in clinical samples [43]. One assay targets the internal transcribed spacer region (ITS) of the fungal genome while the other targets the more variable intergenic spacer region (IGS). Both assays

performed equivalently and proved to be more sensitive than traditional culture methods, detecting *O. ophiodiicola* in 98% of the culture-positive samples and in 40% of the culture-negative snakes having clinical signs of the disease.

At present, MALDI-TOF MS (matrix-assisted laser desorption-ionization-time-of-flight mass spectrometry) is being introduced to identify pathogenic fungi. As obligate reptile pathogenic fungi (PCRF, etc.) are not included in the commercially available MALDI-TOF MS databases, thus, identification of reptile-associated fungi using this method has not been reported. However, MALDI-TOF MS is a rapid and reliable alternative to multilocus sequencing for the differentiation of reptile pathogenic fungi, and it is most likely that future databases would be expanded to cover reptile isolates in the near future. To date, the gold standard of fungal differentiation for fungal organisms that infect reptile species is multilocus sequencing of the large or small subunit and the internal transcribed spacer (ITS) region of the nuclear ribosomal gene [20].

As the above mentioned molecular methods are unavailable for identification of fungi in most veterinary laboratories, therefore, in routine practice, the identification of fungi is based on morphological features and cannot exactly correspond to the modern nomenclature. In such circumstances, it seems acceptable to use the traditional name of the pathogen (e.g., CANV *complex* or PCRF group) in the clinical context.

## 3.7 Therapy and Prevention of PCRF-Associated Mycoses in Reptiles

Treatment of fungal infections in reptiles includes the administration of effective antifungal agents (both topical and systemic) for a long term, along with maintenance of optimal environmental conditions. Debridement and surgical removal of crusts is the first step for successful treatment of deep fungal dermatitis (Fig. 3.23). Disinfection of skin lesions with a 0.125% chlorhexidine solution is also

**Fig. 3.23** Debridement and surgical removal of crusts in green iguana affected by PCRF

**Table 3.4** Antifungals employed for therapy of PCRF (CANV) mycoses in reptiles

| Animals and clinical manifestation | Treatment | Outcome | References |
|---|---|---|---|
| Two green iguanas (*Iguana iguana*) – cutaneous hyalohyphomycosis | Oral ketoconazole and topical 2% chlorhexidine solution and terbinafine | Clinical cure | [9] |
| Bearded dragon (*Pogona vitticeps*) – dermatomycosis | Oral ketoconazole (20 mg/kg 24 h PO) and topical chlorhexidine and terbinafine | Lesions regressed; lost for follow-up | [10] |
| Bearded dragon (*Pogona vitticeps*) – focal maxillary swelling involving the skin and gingiva | Itraconazole and topical miconazole therapy | Failure (fatal) | [23] |
| Bearded dragon (*Pogona vitticeps*) – focally extensive discoloration and thickening of the skin | Itraconazole | Failure (euthanized after 10 weeks of therapy) | [23] |
| Bearded dragon (*Pogona vitticeps*) – hyperkeratotic exudative dermatitis on a swollen forelimb | Amputation and itraconazole | Clinical cure | [23] |
| Fourteen naturally infected bearded dragons (*Pogona vitticeps*) | Itraconazole (5 mg/kg q24h) or voriconazole (10 mg/kg q24h) until complete clearance of the fungus | 2 out of 7 survived after itraconazole treatment. Only a single animal died in the voriconazole-treated group | [45] |
| Girdled lizard (*Cordylus giganteus*) – cutaneous hyalohyphomycosis | Voriconazole 10 mg/kg of body weight once daily for 10 weeks | Clinical cure | [11] |

recommended [20]. There are relatively few reports that discuss effective dosages and dosage intervals of antifungal agents. Most of them are summarized in Table 3.4.

Most treatment regimens use systemic azole antifungals such as ketoconazole, itraconazole, or voriconazole. Treatment typically consists of both topical and systemic application of antifungals. In the past, ketoconazole was used as the drug of choice for treating fungal diseases in vertebrates; however, newer drugs with fewer side effects have been developed (e.g., itraconazole and voriconazole) to replace it. Itraconazole is often used for therapy of CANV infections, but treatment is not always successful [23]. Voriconazole is a second-generation triazole that is being used more frequently in human and veterinary medicine because it seems to have a lower incidence of side effects compared with other antifungals [44].

The oral treatment of CANV dermatomycosis in bearded dragons (*Pogona vitticeps*) with itraconazole (5 mg/kg, once a day) versus voriconazole (10 mg/kg orally, once a day) at an ambient temperature of 28–30 °C was compared [45]. Both drugs were found to treat the animals successfully (in 27 and 47 days for itraconazole and

voriconazole, respectively); however, voriconazole appeared to be safer for the animals with 6 of 7 survivors as compared to 2 of 7 in the itraconazole group. Notably, hepatocellular injury may have occurred in approximately 50% of the animals in both groups as there were significant elevations of aspartate transaminase levels, while plasma concentrations of voriconazole in the bearded dragons showed more interindividual variation than itraconazole plasma concentrations. To minimize the risk of side effects with itraconazole, lower doses at less frequent intervals are recommended. Pulse therapy is another potential consideration for reducing toxic side effects associated with itraconazole, as it was demonstrated in mammalian hosts [46].

Side effects and toxicity of triazole antifungals in reptiles represent an important issue in veterinary medicine. The most common clinical signs associated with triazole toxicity are anorexia and depression. Affected animals tend to develop mild hepatitis as a result of the toxicity; therefore, liver functioning tests should be performed or are recommended before, during, and after treatment regimens. Behavioral observation is the best method for monitoring animals. Further, serial measurement of clinical chemistries and determination of level of drug (by performing liver biopsies) should be undertaken. Reptiles with hepatic disease secondary to drug toxicity are often depressed and anorexic. The clinical chemistries commonly used to asses sliver disease include aspartate aminotransferase (AST), gamma glutyltransaminase (GGT), and bile acids. Although AST and GGT are not liver specific, they may be helpful in combination with other parameters. Bile acid testing is the most useful for evaluating liver function and should be performed if secondary liver disease is suspected. Results of bile acid testing should be interpreted carefully taking into account the species of the animal. Liver biopsies can be used to assess liver disease and check for the accumulation of drugs beyond safe levels [2].

An increase in the level of AST (120–420 mmol/l) in 57.1% of cases ($n = 7$) was observed when ketoconazole therapy course in green iguanas lasted over 2 weeks. However, supportive therapy with heptral, B complex, and LRS (lactated Ringer's solution) usually allowed withstanding course duration up to 4–5 weeks with the dosages of ketoconazole 20 mg/kg daily. The toxicity of voriconazole for snakes was reported by Allender (2017) [47]. In his practice, 4 of 7 snakes died in 12 h after the commencement of voriconazole. However, voriconazole was successfully used for therapy of CANV in 9 green iguanas by us (Fig. 3.23, 3.24 and 3.25). The choice of the drug was determined by the susceptibility of the CANV cultures in vitro. Voriconazole was administered orally at doses of 5 mg/kg/day (4 cases) and 10 mg/kg/day (5 cases). The duration of the course was 3–7 weeks, usually until removal of the scabs and epithelialization on the periphery of the dermatitis foci. Therapy for two additional weeks is also recommended. Topical antifungals (ointment or emulsion) were applied along with the systemic therapy. The complete cure was observed in 3 cases. In other cases, the persistent remission was achieved.

In three cases, despite complete epithelialization, granulomas remained in the subepidermal layers, which were surgically removed (Fig. 3.25). Relapses of the mycosis occurred not only in animals having granulomas but also in completely cured reptiles. New lesions appeared both in the zone of primary infection and in

**Fig. 3.24** Subepidermal darkly pigmented granuloma remained after antifungal therapy

**Fig. 3.25** Epithelialization of lesions after 3 weeks of combined antifungal therapy in green iguana

completely different areas of the body. In some cases, the antifungal susceptibility of CANV isolates also differed from the initial one.

Among systemic azoles, posaconazole could be a promising drug for the treatment of mycoses in reptiles. It was already used for successful treatment of fusariomycosis in marine turtles *Caretta caretta* [48]. Posaconazole has also been successfully used in wild and exotic animals for treatment of resistant mycoses, e.g., coccidioidomycosis in dolphins [49]. Recently, a successful treatment of *Nannizziopsis obscura* infection by posaconazole in immunocompromised human patient was reported [37]. Moreover, oral posaconazole therapy demonstrated rapid clinical improvement in disseminated *Chrysosporium* spp. infection in a German shepherd dog [35]. But as far as PCRF in reptiles is concerned, there is no report on posaconazole therapy. In some cases of PCRF mycoses in reptiles, voriconazole-resistant fungal isolates showed susceptibility against ketoconazole therapy. This indicates that the therapy should be based on laboratory data on the antifungal susceptibility of a particular fungal isolate.

In addition to azoles, terbinafine can also be effective in the treatment of mycoses caused by PCRF. The pharmacokinetics of terbinafine with the use of a subcutaneous implant and for nebulization therapy in the case of CANV in snakes was investigated by Allender [47]. The topical application of terbinafine in green iguanas in the Moscow Zoo with confirmed CANV mycoses showed efficiency in 44.4% of cases ($n$ = 9). However, in comparison to azoles, terbinafine showed more potent dermato-toxicity. Usually pathological changes resembling a chemical burn appear on the skin of green iguanas as early as 1–2 weeks of therapy.

Terbinafine was successfully used by us for the systemic treatment of disseminated mycoses caused by *Paecilomyces lilacinus* in the group of green iguanas and the *Fusarium moniliforme* mycosis in the group of frilled-neck lizard (*Chlamydosaurus kingii*). Terbinafine was administered at a dose of 5 mg/kg by pulse therapy: daily, for 5 days, followed by a break for 7 days, etc. [50]. However, in all cases of oral administration of terbinafine, lizards developed severe depression, temporary paresis, vomiting attempts, and polyuria. But, no increase in AST, ALT (alanine aminotransferase), and GGT levels in the blood was observed.

In two cases of CANV treatment with terbinafine in green iguanas, by the 5th day of therapy, there was an increase in the level of uric acid up to 867 and 617 μmol/l, respectively. After the acute episode of renal failure, the animals were relieved with allopurinol (20 mg/kg/daily) and polyionic crystalloid solutions intravenously drip (30 ml/kg/daily). The animals remained stabilized, but therapy was not found effective. Therefore, the use of terbinafine for the systemic therapy in lizards should be prescribed only in accordance with laboratory indications.

Caspofungin, the most modern antifungal belonging to echinocandins, is of interest for the therapy of PCRF mycoses. In our practice, it was used for the treatment of CANV dermatomycosis in green iguana, but the clinical improvement was not significant. The drug was used at a dose of 1 mg/kg every 48 h by slow intravenous bolus administration for 14 days, and no distinct side effects were found associated with the use of caspofungin.

Chitin synthesis inhibitors (lufenuron and nikkomycin) have been used for the therapy of human and animal mycoses [51]. In our practice, we used the 5% emulsion of lufenuron (manufactured by Syngenta) to treat CANV dermatomycosis in two green iguanas. Despite the declared low toxicity of the drug for humans and bees, the topical application of emulsion diluted at 1:10 caused a severe toxic reaction (salivation, apnea, stupor) in lizards. Apparently, the reactions were associated with inhalation of drug vapors. Then the drug was used topically at a dilution of 1:1000, daily. Within 2 weeks, significant clinical improvements such as spontaneous removal of the scrotal crusts and partial epithelialization at the periphery of the dermatitis foci were noticed. However, further use of lufenuron as monotherapy failed to prevent the recurrence of the disease. Oral form of the drug (Program tablets for dogs, 67.8 mg) at doses of 10 mg/kg once a week for 4 weeks was not found effective.

As tools of adjunctive topical treatment of PCRF mycoses, nystatin, terbinafine, clotrimazole, and enilconazole were also used (authors' unpublished data). Unfortunately, we have no sufficient data to objectively compare the clinical efficacy of these drugs. However, based on limited observations, clinical improvement

with nystatin was observed in 41% of reptiles ($n$ = 7), terbinafine 44% ($n$ = 9), clotrimazole 58% ($n$ = 12), and enilconazole 66% ($n$ = 6) (Fig. 3.25). These data need to be verified in the future, taking into account the background systemic therapy.

As mentioned earlier, adequate light and heat are essential for reptilian health and largely influence clinical recovery because metabolism of drugs, use of fluid therapy, and immune system of reptiles are heat dependent [52].

Taking into account the contagious nature of the disease, during the treatment of PCRF infections in reptiles, it is necessary to employ hygienic measures. If affected reptile is detected, the cage mate needs to be observed closely for signs of disease. It is desirable to isolate a sick animal to reduce the risk of cross-infection. Other exposed reptiles should have thorough veterinary examinations, and any skin lesions should be assessed to rule out fungal infection as the underlying cause. Reptile that comes with a skin lesion or that develops a skin lesion while in quarantine should not be released until mycosis is eliminated.

In the prevention of fungal infections, the quarantine of animals destined for sale, especially those imported, plays a crucial role. The standard veterinary quarantine at 30 days may not be sufficient to detect infected animals. The quarantine in the Moscow Zoo lasts up to 60 days, during which the latent PCRF infection usually turns into a clinically manifested form. Reptiles imported illegally can possess a dangerous source of fungal pathogens.

Proper sanitation and hygiene are key risk reducers. Disinfectants possessing antifungal activity should be used for decontamination of reptilian cages, animal care equipment, and house environment. Future studies on fungicidal activity of available disinfectants against *Chrysosporium*-related fungi are highly needed. In-depth studies on the ecology of the fungal pathogens, pathways of infection, and its transmission are also required.

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