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Candida steatolytica causing systemic candidiasis in a group of African spurred tortoises (Centrochelys sulcata)

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Keywords:	Centrochelys sulcata, Candida steatolytica, Systemic mycosis, Candidiasis, Fungaemia
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Abstract:	Three African spurred tortoises (Centrochelys sulcata) were referred for inappetence and lethargy of one week's duration. On presentation, one animal was confirmed dead and submitted for necropsy. The conspecifics were treated symptomatically for upper respiratory tract disease. The deceased individual tested negative for Herpes-, Rana- and Picornavirus but positive for Mycoplasma agassizii by PCR, however, without exhibiting pathological changes consistent with this infection. Instead, systemic mycosis was diagnosed, with isolation of Candida steatolytica. This allowed effective targeted treatment of the remaining animals. Candida steatolytica is commonly isolated from rotting fruits and has so far been rarely associated with clinical disease. In the present cases, the most likely source of infection were pruned vine shoots and leaves, which the tortoises had access to in their outdoor paddock. This case highlights the significance of rapid and comprehensive post-mortem diagnostics with swift interdisciplinary communication to allow effective handling of challenging cases.

TITLE OF CASE ***Do not include "a case report"***

Candida steatolytica **causing systemic candidiasis in a group of African spurred tortoises (*Centrochelys sulcata*)**

SUMMARY ***Up to 150 words summarising the case presentation and outcome (this will be freely available online)***

Three African spurred tortoises (*Centrochelys sulcata*) were referred for inappetence and lethargy of one week's duration. On presentation, one animal was confirmed dead and submitted for necropsy. The conspecifics were treated symptomatically for upper respiratory tract disease. The deceased individual tested negative for Herpes-, Rana- and Picornavirus but positive for *Mycoplasma agassizii* by PCR, however, without exhibiting pathological changes consistent with this infection. Instead, systemic mycosis was diagnosed, with isolation of *Candida steatolytica*. This allowed effective targeted treatment of the remaining animals. *Candida steatolytica* is commonly isolated from rotting fruits and has so far been rarely associated with clinical disease. In the present cases, the most likely source of infection were pruned vine shoots and leaves, which the tortoises had access to in their outdoor paddock. This case highlights the significance of rapid and comprehensive post-mortem diagnostics with swift interdisciplinary communication to allow effective handling of challenging cases.

BACKGROUND ***Why you think this case is important – why did you write it up?***

Fungi represent primary and/or opportunistic pathogens, with most medically important fungi belonging to the ascomycetes or zygomycetes (1). Primary fungal pathogens mainly include dimorphic fungi such as *Histoplasma sp.* or *Coccidioides sp*. and can lead to localised or systemic mycosis in immunocompetent hosts (2,3). On the other hand, opportunistic fungi are often found in the environment or are part of the commensal flora, causing disease in immunosuppressed individuals (2–4). In both human and veterinary medicine, fungi of the genus *Candida* are most relevant as pathogens (3).

In reptiles, fungal infections with *Candida sp.* commonly involve the gastrointestinal tract, especially the oropharyngeal mucosa as so-called trush, and less commonly the integumentary and respiratory tract (4–7). Systemic candidiasis is rarely reported, especially when compared to its occurrence in birds and mammals (5–7), although it is believed that fungal infections are underdiagnosed in reptiles (4). Furthermore, in many reports the fungi were not fully identified and conclusions were often drawn based on the fungal morphology in histological sections, without further identification by culture, thus leading to assumption and inconsistencies (4,8,9). On the other hand, histopathology is essential to confirm an invasive mycosis and distinguish it from post-mortem tissue colonisation (9).

Fungal pathogens are often not suspected at initial presentation in reptiles and diagnoses are only made after post-mortem or biopsy examination (4,5). This report highlights the importance to consider fungal infections as differential diagnosis already at an early stage, since they can be clinically silent or can present with clinical signs similar to those observed in other common chelonian diseases, are difficult to diagnose ante-mortem, and often show slow progression (4,5). The report also confirms the relevance of fast interdisciplinary communication between clinician, microbiologist and pathologist, especially in challenging cases, to allow rapid and effective treatment of other diseased animals.

CASE PRESENTATION ***Presenting features, clinical and environmental history***

A group of two male and one female 18-year-old captive-born, privately-owned African spurred tortoises were housed in a 12 m^2 conservatory with an average temperature of 25 °C (maintained by central heating). No artificial UV light was provided indoors, but the animals had access to the owner's garden from March to September when weather conditions permitted. Their diet consisted mainly of grass in the summer and hay or straw in the winter, without supplements. The animals had been housed together for the past 15 years, without changes to the husbandry or the group composition; the animals had no previous medical history.

In April 2016, the animals were presented to a private veterinary surgeon after they had shown lethargy and inappetence for one week. They were treated with unknown doses of enrofloxacin and meloxicam and referred to the Clinic for Zoo Animals, Exotic Pets and Wildlife on the following day. On presentation, the largest, male animal (#1) was confirmed to be dead by lack of vital signs and inability to auscultate the heart by Doppler. The carcass was promptly submitted for a full post-mortem examination at the Institute of Veterinary Pathology.

INVESTIGATIONS *If relevant*

At necropsy, the deceased animal (#1) was found to be in good body condition. Gross findings included a multifocal ulcerative glossitis and laryngitis (Fig. 1A), a multifocal ulcerative gastritis of the fundus (Fig. 1B) and a generalised mottling of the liver with multiple white, poorly demarcated, confluent foci of up to 5 mm in diameter (Fig. 1C-E). All other organs were grossly unremarkable. Nasal, choanal and conjunctival swabs as well as samples from tongue, liver and lung were submitted to a commercial laboratory and tested by PCR for Mycoplasma, Ranavirus, Picornavirus and chelonian Herpesvirus. Samples from liver, lung and oral mucosa were subjected to a bacteriological and fungal culture analysis at the Section of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich. Samples from all major organs were collected, fixed in 10% buffered formalin and routinely processed for histopathological examination.

The gross findings were immediately reported to the clinical veterinarians, who concurrently examined the remaining animals (#2 and #3). On clinical examination, both tortoises had a decreased muscle tone, interpreted as a sign of weakness, moderate bilateral serous to white mucous nasal discharge, moderate swelling of the ocular adnexa and sunken globes. The animals were placed under general anaesthesia induced with intravenous alfaxalone (Alfaxan, 10 mg/ml, Jurox Limited, United Kingdom) at a dose of 7 mg/kg (animal #2) and 5mg/kg (animal #3), and maintained after intubation with isoflurane 1-5% (Isofluran Baxter ad us. vet., Baxter AG, Switzerland) for full physical examination and placement of a gastric tube via oesophagostomy. Intubation revealed that animal #2 exhibited grey plaques on the tongue surface similar to the deceased animal, and thick white mucous discharge from the trachea.

Blood samples from the dorsal tail vein were obtained in both animals and subjected to routine haematological examination. This revealed moderate to severe leukopenia (0.4 x 10^3/µl) in animal #2, and a leukocyte count within the normal range (2.1 x 10^3/µl) in animal #3 (10). Both animals displayed a relative monocytosis (0.05 x 10^3/µl (12.5%) and 0.3 x 10^3/µl (14%)) with cytological evidence of monocyte and lymphocyte activation. It is worth noting that the absolute monocyte count was within reference ranges and the relative monocytosis may simply have been an artefact of the low leukocyte counts (10).

DIFFERENTIAL DIAGNOSIS *If relevant*

Concurrent illness with similar clinical signs in a group of captive chelonians is highly indicative of an infectious, toxic or husbandry-related disease process. Although lethargy and muscle weakness are relatively nonspecific, nasal discharge and eyelid oedema are typical clinical signs of upper respiratory tract disease (rhinitis, conjunctivitis) in chelonians. Nasal discharge can also occur due to lower respiratory tract disease, which is often accompanied by dyspnoea. Possible infectious agents that manifest as respiratory disease in chelonians are bacteria (e.g. *Mycoplasma agassizii*, *Aeromonas* spp., *Pasteurella* spp.), viruses (e.g. Herpesvirus, Adenovirus, Paramyxovirus, Ranavirus, Reovirus), fungi (*Aspergillus spp*, *Candida spp*, *Fusarium* spp.) and protozoan parasites (e.g. intranuclear coccidiosis) (11,12). Glossitis, identified in two of the three tortoises, is typically seen in chelonia with Herpesvirus infection but has also been reported with Ranavirus and *Mycobacterium* spp. infections or due to local irritants (13).

TREATMENT ***If relevant***

With upper respiratory disease of bacterial or viral aetiology as the main differential diagnosis, empiric treatment with danofloxacin 6mg/kg i.m. q48h (Advocid, 25mg/ml, Zoetis Schweiz GmbH, Switzerland) and meloxicam 0.2 mg/kg i.m. q48h (Metacam, 5mg/ml, Boehringer Ingelheim Schweiz GmbH, Switzerland) and rehydration by daily bathing in a commercial electrolyte mixture (Reptoboost, Vetark GmbH, United Kingdom) were initiated. After placement of a gastric tube, daily water (1% of bodyweight) and food every other day (0.5% of bodyweight; Critical Care, Oxbow PetProducts, USA) were administered.

OUTCOME AND FOLLOW-UP

The histopathological examination of animal #1 yielded results consistent with a disseminated systemic mycosis. There was a multifocal ulcerative glossitis and laryngitis with superficial fibrinonecrotic inflammation (Fig. 2). Fungal structures, represented by oval to round, 2-4 µm, single-celled and budding yeasts (blastospores) and pseudohyphae of up to 4 µm width formed a superficial layer and were present within the fibrin and the necrotic cell layers. Pseudohyphae were also present within the submucosa where they were accompanied by a mild macrophage dominated mononuclear infiltrate (Fig. 2B). They were also found to invade submucosal vessels (Fig. 2B). Additionally, the animal showed multifocal gastric erosions with fungal blastospores and pseudohyphae in both mucosa and submucosa; interestingly, this was not associated with an inflammatory reaction.

There were multifocal, variably sized areas of necrosis within the liver, with embedded fungal pseudohyphae and a moderate, macrophage dominated inflammatory infiltrate; occasional multinucleated cells were also observed (Fig. 3). The lungs exhibited extensive multifocal necrosis and oedema, with numerous fungal structures within alveolar spaces, vessels and the surrounding interstitial tissue, accompanied by a moderate pyogranulomatous inflammation comprising numerous heterophils, macrophages, and rare multinucleated giant cells. In addition, most other organs, including the brain (choroid plexus; Fig. 4), heart, spleen, kidney, and testicles exhibited intravascular pseudohyphae with thrombus formation indicative of fungal embolism. This was often seen in association with fungal structures extending from the lumen into the vessel wall, with focal necrosis of the wall and mild macrophage dominated inflammatory infiltration (Fig. 4).

Additional changes observed in the animal were mild submucosal granulocytic infiltrates and mild focal haemorrhage in the nasal mucosa and mild splenic lymphoid depletion.

Fungal culture of a pooled sample (lung, liver, oral mucosa) on Sabouraud dextrose agar with chloramphenicol (Thermo Fisher Scientific, Switzerland) yielded yeasts. Similarity search of the sequenced internal transcribed spacer (ITS) regions, ITS1 and ITS2, showed 100% homology to *Zygoascus hellenicus,* the teleomorph of *Candida steatolytica* in MycoBank and GenBank (14). The PCRs for Herpes-, Picorna- and Ranavirus yielded negative results, but the animal was found positive for *Mycoplasma agassizii.*

Based on the aetiological diagnosis in animal #1, treatment of the hospitalised conspecifics with a systemic antifungal (Itraconazol 5 mg/kg p.o. q48h, Sporanox®, Janssen-Cilag AG) was initiated on day 5 after admission to the clinic. The general health and fitness of both animals continuously improved during the next few days and the animals were discharged with antimycotic and antibiotic treatment and supplementary feeding via gastric tube on day 8. One month later, clinical signs had resolved and the gastric tubes were removed. A mild serous nasal discharge remained during the whole observation period. Approximately one month after the last medical follow-up animal #2 was found dead in its enclosure without ante-mortem clinical signs. It was not made available for post-mortem examination. Animal #3 remained in good general health to the time of writing this report (4 years).

DISCUSSION ***Include a very brief review of similar published cases***

Candida yeasts belong to the most common genera cultured from chelonians (6,7). They are ubiquitous in the environment and considered a commensal organism of the normal chelonian skin and gastrointestinal tract, but have been isolated from internal organs such as liver and lung without concurrent pathological changes (4,6,7,15). Systemic candidiasis is generally an opportunistic infection associated with poor husbandry or immunosuppression due to poor general health, other infections or diseases, iatrogenic manipulation (e.g. venous catheter) or various medical treatments (e.g. glucocorticoids, antibiotics) (4,16).

The *Candida* species most commonly isolated from tortoises are *C. albicans* and *C. tropicalis* (6,7,15) but reports of systemic chelonian candidiasis are generally rare (4,5,7). To the best of the authors' knowledge, this is the first report of an infection with *C. steatolytica*, the anamorph of *Zygoascus hellenicus*, in a reptile, or indeed of a systemic infection caused by this species in veterinary medicine in general (4,6,7,15,17). So far, infection with *C.*

steatolytica has only been reported twice, which includes a case of fungaemia in an immunocompromised stem cell transplantation recipient (18), and a localised infection of the udder in a cow (19). A closely related species (*Candida hellenica (*teleomorph *Zygoascus meyerae*) was reported as cause of fungaemia and pneumonia in a child suffering from myeloid leukaemia (20).

C. steatolytica was not identified as part of the normal mycobiota of tortoises in previous surveys (6,7,15) and thus the infection is more likely from an environmental source. *C. steatolytica* has repeatedly been isolated from fermenting fruits, particularly grapes (21). When discussing the pathological findings and culture results with the owner, he retrospectively recalled that the outdoor area to which the tortoises had access is a small vineyard that had recently been trimmed but not cleared. The owner further recalled that the tortoises had browsed on the pruned vine shoots and leaves on the ground approximately two to four weeks prior to the onset of anorexia. Therefore, uptake of a potentially high amount of yeast by the animals via ingestion or inhalation while browsing is considered the most likely route of exposure to the fungus in this case.

In reptile medicine, inadequate husbandry such as crowding, poor sanitation or suboptimal temperatures, predispose to mycotic infections (4,7,8,16). Such predisposing factors can only be speculated in the present case. Access to an outdoor space with suboptimal temperature in early spring and concurrent infection with *Mycoplasma agassizii*, as identified in the deceased animal, could have predisposed the animals. Primary active *M. agassizii* infection appears unlikely as the main cause of death because there was no evidence of the typical lesions of active mycoplasmosis in the nasal cavity of the deceased individual, such as basal cell hyperplasia of the respiratory and olfactory epithelium, erosion of the ciliated epithelium, submucosal lymphoid hyperplasia and infiltrates of heterophils and macrophages (22–24). However, since mycoplasmosis in tortoises by itself is a complex and multifactorial disease, and outbreaks are favoured by various co-factors similar to those leading to a candidiasis (22–24), the significance of this concurrent infection cannot be determined. Thus it remains unclear if the mild granulocytic infiltrates and haemorrhages observed in the nasal mucosa are related to the candidiasis, the mycoplasmosis or another unidentified agent. Furthermore, animals #2 and #3 displayed signs of upper respiratory disease (nasal discharge, eyelid oedema) which would be consistent with mycoplasmosis. It can therefore not be ruled out that the surviving conspecifics suffered from mycoplasmosis or another upper respiratory infection as no further tests were performed besides haematology due to the owner's financial constraints.

The site of initial infection in the present case could not be clearly identified but was either the lungs, oral cavity or stomach, as there was evidence of vascular invasion by the fungus secondary to tissue damage in all three locations. Both lungs and stomach have been reported to be affected in reptiles with systemic mycosis (4,7,8,15,24). Mycotic pneumonia consecutive to aspiration of oropharyngeal material, is often described in chelonians, with an overrepresentation of giant tortoises (4,7). Also, low environmental temperatures, as they likely occur in spring in Switzerland, are suggested to predispose chelonians to fungal pneumonia (16). Fungal infection will then either remain confined or disseminate to other organs haematogeneously or via direct spread (4). Given the clear evidence of vascular invasion and numerous fungal emboli, the former is more likely to have occurred in the fatal case.

In previous reports of systemic mycosis in reptiles, the causal relationship between lesions and putative agents was not always confirmed as histology or culture were not attempted (4,5,16). A histological examination is required to confirm an invasive mycosis, and the presence of necrosis and inflammation in direct association with fungal structures allows exclusion of fungal invasion of devitalised tissue (4,9). For definitive identification, fungal culture with subsequent molecular identification is needed (4,8,15). Even though the mycosis was not definitely verified in animals #2 and #3, the clinical presentation, history of browsing on pruned rotting vine shoots and leaves, the lesions in animal #2 and the relative monocytosis, are comparative to animal #1 and indicative of an active granulomatous inflammatory process (26).

Grapes and vine leaves are recommended as adequate food items for terrestrial chelonians (27) and although large quantities could interfere with protein metabolism due to the presence of tannins (28), the authors are not aware of any side effects being reported from feeding these items (29). However, given the presentation in the current manuscript, best practice would dictate avoiding the provision of discarded or potentially fouled vine leaves and grapes to chelonians.

Although fungal diseases are becoming more recognised in veterinary medicine, they are often not a top differential diagnosis and still diagnosed primarily at necropsy. This is especially true

in chelonians, which often exhibit nonspecific clinical signs and where a comprehensive clinical examination without sedation is hindered by the common lack of compliance in species such as the ones in this case and the specific anatomy of these animals (4,8)
This case confirms that if available, deceased animals can give valuable insight into the aetiology and pathogenesis of diseases in surviving conspecifics. Direct communication between pathologists, microbiologists and clinicians allowed for rapid changes (i.e. the addition of itraconazole, an antifungal) in the treatment regime of the two surviving animals in this case series, providing further evidence of the relevance of interdisciplinary diagnostic approaches.

LEARNING POINTS/TAKE HOME MESSAGES ***3 to 5 bullet points – this is a required field***

- Real-time collaboration between pathologists and clinicians can provide significant benefits towards a rapid diagnosis and positive patient outcome
- Best practice dictates avoiding the provision of discarded or fouled grapes and vine leaves to chelonians
- Fungal infections should be considered as a differential diagnosis for chelonians presenting with lethargy and signs of upper and lower respiratory tract disease

REFERENCES ***Vancouver style***

1. Howard DH. An introduction to the taxonomy of zoopathogenic fungi. In: Howard DH, eds. Pathogenic fungi in humans and animals. 2nd ed. Boca Raton, Florida: CRC Press, 2002:15–30.
2. van Burik JAH, Magee PT. Aspects of fungal pathogenesis in humans. *Annu Rev Microbiol.* 2001;55(**1**):743–72.
3. McAdam AJ, Milner DA, Sharpe AH. Infectious Diseases. In: Kumar V, Abbas AK, Aster JC, eds. Robbins and Contran Pathologic Basis of Disease. 9th ed. Philadelphia, Pennsylvania: Elsevier Saunders, 2015:341–402.
4. Paré JA, Conley KJ. Mycotic Diseases of Reptiles. In: Jacobson ER and Garner MM, eds. Volume 1 - Infectious Diseases and Pathology of Reptiles, Color Atlas and Text. 2nd ed.Boca Raton, Florida: CRC Press, 2021:795-857.
5. Jacobson ER, Cheatwood JL, Maxwell LK. Mycotic diseases of reptiles. *Sem Avian Exotic Pet Med* 2000;9(**2**):94–101.
6. Milde K, Kostka V, Kaleta EF et al. Multiplex-PCR-based differentiation and characterization of Candida- isolates derived from tortoises (Testudinidae). *Vet Microbiol*. 2000;76(**4**):395–402.
7. Kostka VM, Hoffmann L, Balks E et al. Review of the literature and investigations on the prevalence and consequences of yeasts in reptiles. *Vet Rec*. 1997;140(**11**):282–6.
8. Wellehan JFX, Divers SJ. Mycology. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St. Louis, Missouri: Elsevier Inc., 2019:270–80.
9. Jensen HE. Histopathology in the diagnosis of invasive fungal diseases. Curr Fungal Infect Rep. 2021;15:23–31.
10. Klaphake E, Gibbons PM, Sladky KK, Carpenter JW. Reptiles. In: Carpenter JW, ed. Exotic Animal Formulary. 5th ed. St. Louis, Missouri: Elsevier Saunders; 2018:132.
11. Boyer TH. Differential Diagnoses by Clinical Signs - Chelonians. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St. Louis, Missouri: Elsevier Inc.; 2019:1266–1275.
12. Kischinovsky M, Divers SJ, Wendland LD, Brown MB. Otorhinolaryngology. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St. Louis, Missouri: Elsevier Inc.; 2019:736–75.
13. De Voe R. Gastroenterology - Oral Cavity, Esophagus, and Stomach In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St. Louis, Missouri: Elsevier Inc.; 2019:752–60.
14. Robert V, Stegehuis G, Stalpers J. The MycoBank engine and related databases. www. mycobank. org. 2005.
15. Nardoni S, Papini R, Marcucci G, Mancianti F. Survey on the fungal flora of the cloaca of healthy pet reptiles. *Rev Med Vet*. 2008;3:159–65.
16. Reavill DR, Melloy M, Schmidt RE. Reptile mycotic infections from the literature and 55 cases. Proceedings of the Association of Reptilian and Amphibian Veterinarians. Naples, Florida; 2004.
17. Smith MT. *Zygoascus hellenicus* gen. nov., sp. nov., the teleomorph of *Candida hellenica* (= C. inositophila = C. steatolytica). Antonie van Leeuwenhoek.

1986;52(**1**):25–37.
18. Brandt ME, Kauffman CA, Pappas PG et al. Fungemia caused by *Zygoascus hellenicus* in an allogeneic stem cell transplant recipient. *J Clin Microbiol*. 2004;42(**7**):3363–5.
19. Robert V, Smith MT. Zygoascus M.Th. Smith (1986). In: Kurtzman CP, Fell JW, Boekhout T, eds. The Yeasts. 5th ed. London, UK: Elsevier, 2011:931–6.
20. Ioakimidou A, Vyzantiadis TA, Tragiannidis A et al. *Candida hellenica var. hellenica* as a possible cause of respiratory infection in a child with acute myeloid leukemia. *Med mycol. 2011;49*(**7**):771-774.
21. Smith MT, Robert V, Poot GA et al. Taxonomy and phylogeny of the ascomycetous yeast genus Zygoascus, with proposal of Zygoascus meyerae sp. nov. and related anamorphic varieties. *Int J Syst Evol Microbiol*. 2005;55(**3**):1353–63.
22. Jacobson ER, Brown MB, Wendland LD et al. Mycoplasmosis and upper respiratory tract disease of tortoises: A review and update. *Vet J*. 2014;201(**3**):257–64.
23. Jacobson ER. Bacterial Diseases of reptiles. In: Jacobson ER, ed. Infectious Diseases and Pathology of Reptiles, Color Atlas and Text. Boca Raton, Florida: CRC Press, 2007: 461–526.
24. Wendland LD, Brown MB. Tortoise Mycoplasmosis. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St. Louis, Missouri: Elsevier Inc., 2019:1353–4.
25. Hernandez-Divers ASJ. Pulmonary candidiasis caused by Candida albicans in a Greek tortoise (*Testudo graeca*) and treatment with intrapulmonary amphotericin B. *J Zoo Wildl Med*. 2001;32(**3**):352–9.
26. Knotek Z, Divers SJ. Pulmonology. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St. Louis, Missouri: Elsevier Inc., 2019:786–804.
27. Boyer TH, Scott PW. Nutrition. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St. Louis, Missouri: Elsevier Inc., 2019:201–23.
28. Rossi J V. General Husbandry and Management. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St. Louis, Missouri: Elsevier Inc., 2019:109-130.
29. Fitzgerald KT, Newquist KL. Poisonings in Reptiles. *Vet Clin North Am - Exot Anim Pract*. 2008; 11(**2**):327–57.

FIGURE/VIDEO CAPTIONS ***figures should NOT be embedded in this document***

Figure 1. Gross findings in the necropsied animal. A. Mandible, dorsal aspect, with tongue (T) and larynx (L) which both exhibit focal ulcerative lesions (arrows). B. Stomach with multifocal ulcerative inflammation in the fundus. C: cardia; P: pylorus. C. Coelomic cavity, ventral aspect. The liver (L) is mildly enlarged and exhibits a mottled beige-reddish colouration. H: heart. D. Liver after exenteration. Close view of the surface, with multiple white, poorly demarcated, partly confluent foci (arrows). E. Liver, cut surface.

Figures 2-4. Histological findings in the necropsied animal. Figure 2. Larynx with fibrinonecrotising inflammation. A. HE stain, showing the superficial layer of fungal pseudohyphae (arrowheads) and blastospores (short arrows) and the layer of fibrin (asterisk) and degenerate inflammatory cells (long arrow) replacing the epithelial layer. Pseudohyphae (arrowheads) are also seen in the submucosa, where they are accompanied by a minimal mononuclear inflammatory reaction. B. The PAS reaction highlights the presence and distribution of fungal structures. The left panel shows pseudohyphae (arrowheads) in the superficial layer, within fibrin and necrotic tissue, and in the submucosa where they are also present within the wall and lumen of submucosal vessels (inset). There are also aggregates of blastospores (short arrows). The right panel offers a closer view at submucosal vessels, where pseudohyphae (arrowheads) are found invading the vessels. The surrounding tissue exhibits a mild mononuclear, macrophage dominated infiltrate. Figure 3. Liver. A. HE stain, showing a focal area of coagulative necrosis, with moderate infiltration by macrophages (arrowheads) and occasional multinucleated cells (arrow), intermingled with a few heterophils. B. The PAS reaction highlights the presence of abundant pseudohyphae within the focal area of necrosis (arrows); these also grow into the adjacent intact tissue (long arrow). Figure 4. Brain, choroid plexus. A. HE stain showing a vein with thrombus formation (asterisks) and focal area of necrosis (long arrow). Fungal pseudohyphae are seen outside the vessel (arrowhead) where infiltrating macrophages are also present (short arrow). B. The PAS reaction highlights the presence of fungal pseudohyphae within the

lumen of a vessel (arrowheads), growing through the vessel wall (short arrow) and within the extravascular tissue (long arrow). Bars = 20 µm.

OWNER'S PERSPECTIVE *Optional*

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