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Detection of *Ophidiomyces ophidiicola* in a Wild Burmese Python (*Python bivittatus*) in Hong Kong SAR, China

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Abstract

Ophidiomycosis (also referred to as snake fungal disease) is an emerging infectious disease caused by *Ophidiomyces ophidiicola* (*Oo*). PCR was used to detect *Oo* in a Burmese python (*Python bivittatus*) with skin lesions submitted to a rescue center in Hong Kong. This is the first report of this disease in this species. More research is needed in Asia to determine the prevalence of this fungus, its relationship with other species, and its ecological importance. These findings also highlight the significant role wildlife rescue centers play in monitoring wildlife diseases and ecosystem health.

Key Words: Mycosis, microbiota, environment, conservation, emerging infectious disease, *Ophidiomyces ophidiicola*

Introduction

In recent years, the threat to conservation posed by emergent wildlife diseases has become increasingly apparent. Factors include anthropogenic-driven environmental destruction, climate change, and emerging infectious diseases (EIDs) (Fisher *et al*., 2012; Allender *et al*., 2016b; Franklinos *et al*., 2017; Chandler *et al*., 2019; Long *et al*., 2019; Walker *et al*., 2019). Fungal EIDs have been widely recognized as one of the causes of population declines and, in some cases, species extinction. Mycotic diseases affect multiple taxa including plants, mammals, fish, corals, and amphibians, among others (Sutherland *et al*., 2014). For instance, *Pseudogymnoascus destructans* causes white-nose syndrome (WNS) in bats (Blehert *et al*., 2009; Gargas *et al*., 2009), *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans (Bsal)* cause chytridiomycosis in frogs, salamanders, newts, and caecilians (Allender *et al*., 2018; Chandler *et al*., 2019; Lastra Gonzalez *et al*., 2019), *Aspergillus sydowii* has been associated with disease in soft corals (Fisher *et al*., 2012), and *Nosema* spp. affect bees (Fisher *et al*., 2012). While these pathogens can cause clinical disease, subclinical cases may also threaten fitness, reproduction, and ultimately species survival (Lind *et al*., 2019a; Lind *et al*., 2019b).

Ophidiomyces ophidiicola (*Oo*), the pathogen causing ophidiomycosis (also referred to as snake fungal disease, SFD), is a keratinophilic fungus that can affect wild and captive snakes (Franklinos *et al*., 2017; Ohkura *et al*., 2017; Long *et al*., 2019; Paré, 2019). Molecular studies suggest that the fungus has been circulating since 1985, when it was classified as part of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* complex (CANV) and later assigned to the genus *Ophidiomyces* (Lorch *et al*., 2016; Ohkura *et al*., 2016; Paré and Sigler, 2016). The geographical distribution of *Oo* has been reported to be broader in captive snakes than in wild ones (Lorch *et al*., 2016), and it includes the countries and host species listed in Table 1.

Ophidiomyces ophidiicola has been detected in different Serpentes families. Within the genus *Python*, cases have been reported in ball pythons (*Python regius*) (Lorch *et al*., 2016; Franklinos *et al*., 2017; Picquet *et al*., 2018) and African rock pythons (*Python sebae*) (Lorch *et al*., 2016; Ohkura *et al*., 2016; Paré and Sigler, 2016).

Some recent studies have focused on the role of individual and environmental factors regarding survival

Table 1. Known host range of *Ophidiomyces ophidiicola* by continent.

Continent	Host species	References
North America	Eastern massasauga, *Sistrurus catenatus*	(Allender *et al*., 2011; Allender *et al*., 2013; Allender *et al*., 2016b; Robertson *et al.,* 2016; Allender *et al*., 2018; Hileman *et al.,* 2018)
	Eastern indigo snake, *Drymarchon couperi*	(Chandler *et al*., 2019)
	Plains garter snake, *Thamnophis radix*	(Dolinski *et al*., 2014)
	Brown water snake, *Nerodia taxispilota*	(Guthrie *et al*., 2016)
	Rainbow snake, *Farancia erytrogramma*	(Guthrie *et al*., 2016)
	Northern water snake, *Nerodia sipedon*	(Sleeman, 2013; Guthrie *et al*., 2016; Lorch *et al*., 2016; McKenzie *et al*., 2019)
	Eastern racer, *Coluber constrictor*	(Guthrie *et al*., 2016; Ohkura *et al*., 2016; Hill *et al*., 2018; McKenzie *et al*., 2019)
	Mud snake, *Farancia abacura*	(Last *et al*., 2016; Lorch *et al*., 2016)
	Dekay's brownsnake, *Storeria dekayi*	(Licitra *et al*., 2019)
	Eastern ratsnake, *Pantherophis alleghaniensis*	(Licitra *et al*., 2019)
	Eastern garter snake, *Thamnophis sirtalis sirtalis*	(Lorch *et al*., 2016; Ohkura *et al*., 2016; Licitra *et al*., 2019; Long *et al*., 2019; McKenzie *et al*., 2019)
	Northern water snake, *Nerodia sipedon sipedon*	(Licitra *et al*., 2019)
	Northern black racer, *Coluber constrictor constrictor*	(Lorch *et al*., 2016; Ohkura *et al*., 2017; Licitra *et al*., 2019; Long *et al*., 2019)
	Grey rat snake, *Pantherophis spiloides*	(Long *et al*., 2019; McKenzie *et al*., 2019)
	Eastern black kingsnake, *Lampropeltis nigra*	(Lorch *et al*., 2016)
	Eastern milk snake, *Lampropeltis triangulum*	(Lorch *et al*., 2016; McKenzie *et al*., 2019; Stengle *et al*., 2019)
	Eastern foxsnake, *Pantherophis vulpinus*	(Lorch *et al*., 2016)
	Foxsnake sp., *Pantherophis* sp.	(Lorch *et al*., 2016)
	Bullsnake, *Pituophis catenifer sayi*	(Lorch *et al*., 2016)
	Louisiana pinesnake, *Pituophis ruthveni*	(Lorch *et al*., 2016)
	Queensnake, *Regina septemvittata*	(Lorch *et al*., 2016; McKenzie *et al*., 2019; Stengle *et al*., 2019)
	Western ribbonsnake, *Thamnophis proximus*	(Lorch *et al*., 2016)
	Smooth earth snake, *Virginia valeriae*	(Lorch *et al*., 2016; McKenzie *et al*., 2019)
	Copperhead, *Agkistrodon contortrix*	(Lorch *et al*., 2016; McKenzie *et al*., 2019)
	Cottonmouth, *Agkistrodon piscivorus*	(Latney and Wellehan, 2013; Lorch *et al*., 2016)
	Timber rattlesnake, *Crotalus horridus*	(Clark *et al.,* 2011; Smith *et al*., 2013; McBride *et al*., 2015; Lorch *et al*., 2016; Hill *et al*., 2018; McKenzie *et al*., 2019)
	Dusky pygmy rattlesnake, *Sistrurus miliarius barbouri*	(Lorch *et al*., 2016)
	Eastern worm snake, *Carphophis amoenus*	(McKenzie *et al*., 2019)
	Ring-necked snake, *Diadophis punctatus*	(McKenzie *et al*., 2019)
	Common kingsnake, *Lampropeltis getula*	(McKenzie *et al*., 2019; Stengle *et al*., 2019)
	Plain-bellied water snake, *Nerodia erythrogaster*	(McKenzie *et al*., 2019)
	Red-bellied snake, *Storeria occipitomaculata*	(McKenzie *et al*., 2019)
	Pygmy rattlesnake, *Sistrurus miliarius*	(Cheatwood *et al*., 2003; McCoy *et al*., 2017; Lind *et al*., 2018a; Stengle *et al*., 2019)
	Black rat snake, *Pantherophis obsoletus*	(Rajeev *et al*., 2009)
	Northern ring-necked snake, *Diadophis punctatus edwardsii*	(Sleeman, 2013)
	Corn snake, *Pantherophis guttatus*	(Sigler *et al*., 2013; Lorch *et al*., 2015)
	Broad-banded water snake, *Nerodia fasciata confluens*	(Glorioso *et al*., 2016)
	Brown treesnake, *Boiga irregularis*	(Nichols *et al*., 1999; Sigler *et al*., 2013)
	Garter snake, *Thamnophis* sp.	(Vissiennon *et al*., 1999; Sigler *et al*., 2013)
	Java wart snake sp., *Acrochordus* sp.	(Sigler *et al*., 2013)
	Green anaconda, *Eunectes murinus*	(Sigler *et al*., 2013)
	Milksnake sp., *Lampropeltis* sp.	(Sigler *et al*., 2013)
	Atlantic saltmarsh water snake, *Nerodia clarkii taeniata*	(Sigler *et al*., 2013)
	Broad-headed snake, *Hoplocephalus bungaroides*	(Sigler *et al*., 2013)
	Ball python, *Python regius*	(Sigler *et al*., 2013)
	African rock python, *Python sebae*	(Sigler *et al*., 2013)
	Eastern diamondback rattlesnake, *Crotalus adamanteus*	(Sigler *et al*., 2013; Steeil *et al*., 2018)

Table 1. Continued.

Continent	Host species	References
Europe	Grass snake, *Natrix natrix*	(Franklinos *et al*., 2017)
	Adder, *Vipera berus*	(Franklinos *et al*., 2017)
	Dice snake, *Natrix tessellata*	(Franklinos *et al*., 2017)
	Bocourt's water snake, S*ubsessor bocourti*	(Picquet *et al*., 2018)
	Pueblan milk snake, *Lampropeltis triangulum campbelli*	(Picquet *et al*., 2018)
	Garter snake, *Thamnophis* sp.	(Paré and Sigler, 2016)
Australia	Java wart snake, *Acrochordus* sp.	(Paré and Sigler, 2016)
	Broad-headed snake, *Hoplocephalus bungaroides*	(Paré and Sigler, 2016)
Asia	Black rat snakes, *Pantherophis obsoletus*	(Takami *et al*., 2021)
	Texas rat snakes, *Pantherophis obsoletus lindheimeri*	(Takami *et al*., 2021)
	Red-banded snake, *Dinodon rufozonatum*	(Sun *et al*., 2021)
	Chinese cobra, *Naja atra*	(Sun *et al*., 2021)

and spread of the fungus (McCoy *et al*., 2017; Lind *et al*., 2019b; Long *et al*., 2019). Walker *et al*. (2019) found that snake skin bacterial taxa not only differ from the environmental bacterial assemblage but also vary throughout wider geographical spaces and seasons. They concluded that *Oo* infection is predictive of the bacterial taxa on the snake skin that associate with *Oo*. Allender *et al*. (2018) evaluated the snake–host microbiota relationship in eastern massasauga rattlesnakes (*Sistrurus catenatus*) as a predictor of the emergence of pathology, variability in snake health, therapeutic intervention, and reduction of disease impact. Although *Oo* infection was associated with shifts in microbiome composition, the authors did not find a quantitative correlation between the two; this contrasts with *Bd* in frogs where a quantitative correlation exists. This difference was attributed to difficulties in measuring *Oo* abundance and to the fact that *Oo* spreads deep to the skin–microbiome interface.

Skin microbiota is not only altered by the presence of *Oo* because some bacteria may also act as probiotics against *Oo*. Hill *et al*. (2018) hypothesized that skin microbiota might manifest differently in the presence of *Oo* and that some bacteria may produce an anti-fungal effect. The authors identified 16 different bacterial strains that demonstrated antifungal effects. Among them, *Morganella morganii*, a snake skin commensal, elicited a potent anti-*Oo* effect.

Clinical signs of ophidiomycosis have been linked to circulating corticosteroid concentrations, which can vary depending on body condition, environmental temperature, and metabolic functions (e.g., pregnancy and vitellogenesis). Poor body condition, due to starvation and other diseases, as well as low environmental temperatures, negatively affect circulating stress hormone concentrations. It also appears that pregnancy not only influences the ability to feed but could also lead to clinically significant disease and eventually death (Lind *et al*., 2018b; Licitra *et al*., 2019). Moreover, concerning the pregnancy status, Lind *et al*. (2018a) suggested that the postgravid energetic state predisposes a snake to the infection.

Apart from an individual's predisposition to the disease, environmental components may also influence the pathogenicity of this fungus. Knowing the ecology of *Oo* in relation to the environment and time (season) is essential when setting mitigation and conservation actions against this EID. All published studies support the theory that environmental temperature and humidity play significant roles in the expression of this disease. According to Long *et al*. (2019), *Oo* exhibits optimal growth at temperatures around 25°C (77°F), which corresponds to spring temperatures in the eastern United States. Temperature-driven pathogenicity has also been observed in other fungal diseases such as *Bd* in amphibians (Latney and Klaphake, 2013). Identifying the effects of temperature on pathogenicity would help in narrowing the surveillance period for this EID. Snakes are believed to be predisposed to the development of ophidiomycosis during brumation (Steeil *et al*., 2018). The most severe pathological changes could coincide with the winter months when the physiological capability of an individual to fight a disease is low. When environmental conditions are not suitable, ectotherms may have to tradeoff an effective metabolic rate to downregulation of immune functions to promote a positive energy balance (McCoy *et al*., 2017; Lind *et al*., 2019b). Lind *et al*. (2019b) suggest that seasonal tradeoffs between host defense, reproduction, and behavior may affect population-level responses to disease.

In addition to temperature and humidity, other specific environmental conditions may also affect the persistence or transmission of the fungus. Some snake species share environmental niches with other species of reptiles (e.g., gopher tortoises [*Gopherus polyphemus*]) (Chandler *et al*., 2019). Gopher tortoise burrows present a stable temperature and high humidity, which are favorable conditions for fungal growth; however, the relationship between this mycosis and other species requires further study. It is, however, fair to hypothesize that the concentration of the pathogen, either due to multiple snakes sharing the same environmental niche or because of the use of another species' shelter, may serve in maintaining and propagating the disease (Steeil *et al*., 2018; Long *et al*., 2019).

Figure 1. Lesions on the left side of the head of a wild-caught Burmese python (*Python bivitattus*) that tested positive for *Ophidiomyces ophidiicola* (Photo credit Kadoorie Farm and Botanic Garden/Chung Pui Ue).

Figure 2. Necrotic lesions along the body of a wild-caught Burmese python (*Python bivitattus*) that tested positive for *Ophidiomyces ophidiicola* (Photo credit Kadoorie Farm and Botanic Garden/Chung Pui Ue).

Kadoorie Farm and Botanic Garden (KFBG), in collaboration with the Agriculture, Fisheries and Conservation Department (AFCD) of the Government of the Hong Kong Special Administrative Region, initiated the Wild Snake Rescue Project in 1999 to mitigate human–snake conflict by providing a resource for snakes captured following police call outs in Hong Kong. Snakes received by the KFBG generally strayed into human habitation and were subsequently captured by local snake catchers under the authority of the police. Once at KFBG, snakes are identified to the species level and subject to a full physical examination; native species are then released back to the wild in suitable habitats. To date, over 14,000 snakes have been received *via* the project, with the majority (88%) being released. As part of this project, KFBG and AFCD started an *Oo* surveillance study in 2018. About 170 snakes have been sampled since the study was initiated. Clinically healthy snakes and snakes that present with any skin alterations are included in the survey, which uses single swabs rubbed along the body length or in the oral cavity for screening. In snakes with skin lesions, the lesions are swabbed. This brief communication represents the first report of *Oo* in a Burmese python (*Python bivittatus*).

Case Report

A 1.7 kg, 1.75-m female wild caught Burmese python was admitted to the KFBG Wild Animal Rescue Center in November 2019. The snake appeared dull, but was alert and responsive when stimulated. Her body condition score was rated as "moderately underconditioned" and a physical examination revealed areas of necrosis on the left side of the head and mid-body (Figs. 1–3). The head lesions were compatible with focal ulcerative dermatitis around the left eye and upper lip, and facial disfiguration was apparent. There was necrotic oral mucosa lateral to the upper row of teeth. Some scales of the lower lip appeared to have become thickened and yellow-brown, but no ulcers were visible. The left eye had a diffuse opacity, possibly a sign of ophthalmitis, and the globe rim displayed an irregular shape. The body lesions, with an irregular pattern and color, extended about 20 cm in length and were around the whole girth of the snake. Some areas had prominent caseous material and necrosis. A sterile cotton-tipped applicator (Medical Wire & Equipment, Corsham, Wiltshire, United Kingdom) was used to collect DNA from the affected areas on the head and body. Due to the severity of the lesions, the snake was not deemed suitable for release

Figure 3. Close-up of the necrotic lesions along the body of a wild-caught Burmese python (*Python bivitattus*) that tested positive for *Ophidiomyces ophidiicola* (Photo credit Kadoorie Farm and Botanic Garden/Chung Pui Ue).

and was subsequently euthanized. A postmortem was not performed.

Nucleic Acid Extraction: A bacterial genomic DNA extraction kit (TianLong, Xian, China) was used to extract DNA from the swab using a modified protocol. The swab was immersed in 250 µl of bacterial digestion buffer (TianLong) and pretreated with 250 U lyticase (Sigma-Aldrich, St. Louis, MO, USA) at 37°C (98.6°F) for 30–60 min with occasional vortexing. After a brief centrifugation at 18,800 g, 200 µl of supernatant was transferred to the sample well for extraction. Automatic nucleic acid extraction was performed using a TianLong nucleic acid extractor (TianLong). Nucleic acids were eluted in elution buffer (TianLong) at the end of the extraction.

Real-time Polymerase Chain Reaction (PCR): TaqMan™ real-time PCR was used to detect *Oo* from the swab. Three sets of primer pairs and probes were used to target the internal transcribed spacers (ITS) 1 and 2 and intergenic spacer (IGS) loci of *Oo* (i.e., OphioITS-F, -R, -P; Oo-rt-ITS-F, -R, -P; and Oo-rt-IGS-F, -R, -P, respectively) (Table 2). For the OphioITS PCR, a hot-start Taq polymerase and probe with conjugated minor groove binder (MGB) was used to enhance the specificity of the PCR. For the Oo-rt-ITS and Oo-rt-IGS PCRs, hot-start Taq polymerase and probes with conjugated FAM/BHQ1 were used. In addition, a snake endogenous gene (the β-actin gene) real-time PCR was performed to ensure the presence of enough genetic material and the absence of PCR inhibition. Real-time PCR was performed using the Bio-Rad CFX96 thermal cycler (Bio-Rad, Hercules, CA, USA), and data were analyzed using Bio-Rad CFX Maestro™ Software. The OphioITS, Oo-rt-ITS, and Oo-rt-IGS PCRs were performed in a total volume of 20 µl, which was comprised of 5 µl DNA template, 10 µl Bio-Rad SSo Advanced Universal Probe Supermix, and a final concentration of 400 nM for primers and 200 nM for probes. The thermal profile consisted of 3 min at 95°C (203°F), followed by 45 cycles of denaturation at 95°C (203°F) for 10 sec, and annealing/elongation at 60°C (140°F) for 30 sec. The real-time PCR for the detection of the β-actin gene was also performed in a total volume of 20 µl (2 µl DNA template, 10 µl Bio-Rad SSo Advanced Universal Probe Supermix, and a final concentration of 300 nM for primers and 100 nM for the probe). The thermal profile consisted of 2 min at 95°C (203°F), followed by 50 cycles of denaturation at 95°C (203°F) for 10 sec, and annealing/elongation at 60°C (140°F) for 60 sec.

Synthetic Positive Control: A synthetic DNA template of the target loci was used for the generation of standard curves to evaluate the detection limit and amplification efficiency of the real-time PCR assays. Two plasmids containing the IGS and ITS sequences were used for the generation of standard curves (GenScript, Piscataway, NJ, USA). The ITS and IGS fragments were selected with reference to accession numbers KF225599 and KP691510.1 in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Both IGS and ITS plasmids carried vectors 2,752 base pairs (bp) and inserts 290 bp long. Therefore, 1 fg of plasmid DNA yielded 305 copies of *Oo* IGS/ITS (i.e., 3.27 pg plasmid DNA = 1.0×10^6copies of *Oo* IGS/ITS). The plasmid DNA (both ITS and IGS) at a concentration of 1.0×10^6copies/µl was prepared using Tris-EDTA (TE) buffer (Thermo Fisher Scientific Waltham, MA, USA). Concentrations at ten-fold dilutions ranging from 1.0×10^6copies/µl to 1.0×10^1copies/µl were used for the generation of the standard curve.

Sequencing: The Oo-rt-IGS PCR was performed on the positive sample using the Oo-rt-IGS primer set and PCR conditions listed above. The amplified PCR product was sequenced using the Oo-rt-IGS forward and reverse primers. Sequencing data was compared to sequences deposited in GenBank using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi). The swab from the Burmese python was positive in the OphioITS, Oo-rt-ITS, and Oo-rt-IGS PCRs, with Ct values of 26.24, 26.83, and 29.22, respectively. Oo-rt-IGS (133 bp) was sequenced and the result was 100% identical to the corresponding *O. ophidiicola*-IGS sequence (GenBank ac-

Table 2. Information on sets of primers and probes used for the detection of *Ophidiomyces ophidiicola* and endogenous β-actin genes.[a]

Primer ID	Target	Types	Primer/probe sequences (5′→3′)	5′ Modified	3′ Modified	Reference
OphioITS-F	ITS1 of *Ophidiomyces ophidiicola*	Forward	TGTTTCTGTCTCGCTCGAAGAC	None	None	(Allender *et al*., 2015)
OphioITS-R	ITS1 of *O. ophidiicola*	Reverse	AGGTCAAACCGGAAAGAATGG	None	None	(Allender *et al*., 2015)
OphioITS-Probe-FAM	ITS1 of *O. ophidiicola*	Probe	CGATCGGCGCCCGTCGTC	FAM	MGBNFQ	(Allender *et al*., 2015)
Oo-rt-ITS-F	ITS2 of *O. ophidiicola*	Forward	GAGTGTATGGGAATCTGTTTC	None	None	(Bohuski *et al*., 2015)
Oo-rt-ITS-R	ITS2 of *O. ophidiicola*	Reverse	GGTCAAACCGGAAAGAATG	None	None	(Bohuski *et al*., 2015)
Oo-rt-ITS-probe	ITS2 of *O. ophidiicola*	Probe	TCTCGCTCGAAGACCCGATCG	FAM	BHQ1	(Bohuski *et al*., 2015)
Oo-rt-IGS-F	IGS of *O. ophidiicola*	Forward	CGGGTGAATTACCCAGTT	None	None	(Bohuski *et al*., 2015)
Oo-rt-IGS-R	IGS of *O. ophidiicola*	Reverse	AGCCATCCTTCCCTACAT	None	None	(Bohuski *et al*., 2015)
Oo-rt-IGS-probe	IGS of *O. ophidiicola*	Probe	ATACTCTCCGGGCGCTTGTCTTCC	FAM	BHQ1	(Bohuski *et al*., 2015)
ACTB-F	β-actin-encoding seq	Forward	GTSTGGATYGGHGGHTCBATC	None	None	(Piorkowski *et al*., 2014)
ACTB-R	β-actin-encoding seq	Reverse	GAYTCRTCRTAYTCCTSCTTG	None	None	(Piorkowski *et al*., 2014)
ACTB-P	β-actin-encoding seq	Probe	ACCTTCCAGCAGATGTGGATC	FAM	BHQ1	(Piorkowski *et al*., 2014)

[a]ITS = internal transcribed spacer; IGS = intergenic spacer; seq = sequence.

cession no. KP691514.1). The sequence has been submitted to GenBank (accession no. MT459829).

Discussion

This article describes a case of *Oo* in a Burmese python. Since the Wild Snake Rescue Project commenced in 1999, very few snakes have shown clinical signs of sickness, and sampling for *Oo* only started in 2018. The results of this case suggest more testing is needed to measure the prevalence of *Oo* in Hong Kong.

Seasonality has been implicated as a contributing factor in ophidiomycosis outbreaks, and low environmental temperatures may negatively influence the immune system of the infected snakes (Lind *et al*., 2019b; Long *et al*., 2019; Walker *et al*., 2019). Hong Kong is located closer to the equator than other countries in which *Oo* has been previously detected (e.g., United States, Europe, Australia, and Asia). The climate of Hong Kong is considered subtropical, and with climate change the average temperatures have been increasing in recent years. Winters have become milder and the number of very cold days has decreased in the past decades; 2019 was found to be the warmest year in Hong Kong since recording began in 1884, with 11/12 months warmer than average and characterized by an annual mean temperature of 24.5°C (76.1°F), which is 1.2°C (2.2°F) above the average. In the same period of time, the average rainfall and number of tropical cyclones were also found to increase (Hong Kong Observatory, 2020). These changes in climate could influence the epidemiology of this disease in Hong Kong and warrant further study.

The PCR tests used in this study targeted the multicopy ITS or IGS of *Oo* and are extremely sensitive and highly specific. These assays can be used to confirm a diagnosis of ophidiomycosis in both wild and captive snakes, in addition to providing an important research tool for better understanding the biology of the fungus and ecology of this disease (Bohuski *et al*., 2015).

The case described represents the first *Oo*-positive in a group of sampled snakes. Only one snake was found to be *Oo*-positive out of 170 (0.5%) snakes sampled, suggesting a low prevalence of disease. Asymptomatic and clinically sick snakes were sampled in random numbers, and a single swab was collected for every snake. Recent literature reports that the presence of lesions is the best predictor of *Oo* infection, and failure in sampling infected tissues in clinically healthy snakes is possible because of the nature of the disease affecting the deeper layers of the skin (Chandler *et al*., 2019; McKenzie *et al*., 2019). Unfortunately, no necropsy or histopathology was performed for this case; thus, a direct link could not be proven between the skin lesions and the pathogen, and other possible causes of the lesions could not be ruled out.

Future sampling efforts should aim to 1) increase the sensitivity of the screening protocol, and 2) evaluate the connection between clinical signs and pathogen presence. Sensitivity would likely be increased by modifying the swabbing technique to include larger body areas and by performing PCR on skin biopsies as well as swabs. Skin biopsies can, *via* histopathology, also be used to confirm lesion etiology (Allender *et al*., 2016a; Hileman *et al*., 2018; Chandler *et al*., 2019; Long *et al*., 2019). The KFBG's Wild Animal Rescue Center receives many Burmese python hatchlings in September and October and, in view of the possibility of vertical transmission (Stengle *et al*., 2019), samples could also be taken from hatchlings. Including this age range would contribute to the expansion of the sample size and potentially provide data on whether hatchlings may be more predisposed to infection due to their immature immune system.

The recent appearance of *Oo* infection in snakes in various countries suggests that this mycosis is becoming a global conservation concern (Lorch *et al*., 2016; Franklinos *et al*., 2017; Takami *et al*., 2021). Its emergence could be caused by individual and/or environmental variables that could be linked (Franklinos *et al*., 2017; Steeil *et al*., 2018).

Climate change (Paré *et al*., 2019), other pathogens (Allender *et al*., 2018), and population dynamics (Chandler *et al*., 2019) may all contribute to various degrees in propagating the disease. Detection techniques are also becoming more precise and sophisticated, which may lead to earlier detection. Snakes are regarded as important models for evolutionary and ecological investigations; they are indicators of ecosystem health and may yield potential benefit to humans as agents of pest control (Lind *et al*., 2005; Walker *et al*., 2019).

Conclusions

The findings reported here highlight the significant role wildlife rescue centers serve for monitoring wildlife diseases and ecosystem health. Considerable information can be gained by analyzing the data collected from rescue center admissions and collaborating with different stakeholders regarding disease surveillance (Trocini *et al*., 2008). It is important that veterinarians, conservationist biologists, and governments jointly participate in the collection and analysis of data and develop conservation strategies and mitigation actions that are aimed at minimizing disease in populations.

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