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Low-level Detection of SFD-causing *Ophidiomyces* on Burmese Pythons in Southwest Florida, with Confirmation of the Pathogen on Co-occurring Native Snakes

Snake fungal disease (SFD), or ophidiomycosis, is caused by the fungus *Ophidiomyces ophiodiicola* (Allender et al. 2015; Lorch et al. 2015). SFD is widespread across wild populations in the eastern United States (Lorch et al. 2016) and is known to infect more than 30 species of snake in North America and Europe (Lorch et al. 2016; Franklinos et al. 2017). No known phylogenetic or ecological patterns have been observed in susceptibility among snake taxa, and it is presumed that all species are likely susceptible (Burbrink et al. 2017).

Burmese Pythons (*Python bivittatus*) are native to Southeast Asia and have been established in southern Florida since at least the early 2000s (Meshaka et al. 2000; Snow et al. 2007; Willson et al. 2011). It is unknown if Burmese Pythons carry the *Ophidiomyces* fungus or if they exhibit clinical signs consistent with SFD. From Dec 2017 to Nov 2018, we captured 32 Burmese Pythons in southwest Florida, USA. Before humane euthanasia of these 32 animals, we swabbed them to screen for the presence of *Ophidiomyces*. In early 2018, we swabbed an additional 13 Burmese Pythons that are part of a long-term radio telemetry project. We also opportunistically swabbed any native snakes captured during searches for Burmese Pythons.

We wore disposable gloves and changed gloves between each snake. To swab snakes, we used MW113 Dry Swabs with Sterile Fine Tips in a Peel Pouch (Medical Wire & Equipment, Corsham, England), using firm pressure on each side of the dorsum as well as the dorsal midline, and along the ventral surface, while rotating the swab continuously. We placed swabs into sterile vials and stored them in the freezer until we shipped them to the U.S. Geological Survey National Wildlife Health Center (NWHC).

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At the NWHC, we extracted DNA from swabs as described in Franklinos et al. (2017) and screened for the presence of *Ophidiomyces ophiodiicola* using a specific real-time PCR assay targeting the internal transcribed spacer region (Bohuski et al. 2015). Samples were considered to contain DNA from *Ophidiomyces* if they crossed the cycle threshold (Ct) at ≤ 40 cycles.

We captured and removed pythons from the wild during two time periods and sent off swabs after each period. In the first period (Jan 2018–Jul 2018) we were able to maintain sterile protocols and are confident in the results from 31 removed pythons. In the second period (Oct 2018–Mar 2019) we swabbed only one python in the field under sterile protocols. All native snakes opportunistically captured (Nov 2017–Mar 2019) were swabbed using sterile protocols at the site of capture and promptly released after swabbing. We took standard measurements of snout–vent length (SVL), total length (TotL), and body mass of removed pythons after humane euthanasia. No measurements were taken of native snakes.

In addition to removed pythons, we swabbed an additional 13 individual radio tracked pythons from 25 Jan 2018 to 2 Mar 2018 using sterile protocols. All individuals were adults with one female and 12 males. No *Ophidiomyces* DNA was detected on any of the tracked pythons. In addition, *Ophidiomyces* was not detected on the first batch of swabs from 31 pythons removed from Dec 2017 to July 2018 under normal sterile protocols. *Ophidiomyces* DNA was detected on one field-swabbed juvenile python in the second batch (Nov 2018), but the fungal load was very low (Ct = 37.75) and no clinical signs of SFD were observed. Examining size class of the 32 total removed pythons with field swabs, DNA of *Ophidiomyces* was detected on 0% (0 of 3), 100% (1 of 1), and 0% (0 of 28) of hatchlings, juveniles, and adults, respectively (Table 1). No python had any evidence of clinical signs of SFD.

Of the 26 individual snakes of 11 indigenous species that were opportunistically swabbed between Nov 2017 and Mar 2019 for the presence of *Ophidiomyces* during python searches, only three had *Ophidiomyces* DNA present (Table 2). Two of the three were *Lampropeltis floridana* (Florida Kingsnake) and the other was *Crotalus adamanteus* (Eastern Diamond-backed Rattlesnake). The rattlesnake was a radio tracked individual that was part of a separate study and swabbed opportunistically. One kingsnake

TABLE 1. Descriptive data on Burmese Pythons (*Python bivittatus*) swabbed for SFD-causing *Ophidiomyces* in southwest Florida, USA. Tracked pythons were released at the site of capture whereas all others were removed and euthanized. Reported lengths and mass below are reported in cm and kg, respectively. SVL = snout–vent length; TotL = total length. No. snakes with *Ophidiomyces* DNA is indicated.

	N	Mean SVL (range)	Mean TotL (range)	Mass (range)	DNA detected
Tracked Pythons (N = 13)					
Males (N = 12)					
Adults	12	284 (200–350)	325 (267–400)	17.0 (10.2–24.5)	0
Females (N = 1)					
Adults	1	291	330	28.1	0
Removed Pythons (N = 32)					
Males (N = 16)					
Hatchlings	2	116 (106–125)	132 (121–143)	1.0 (0.6–1.4)	0
Juveniles	1	178	155	2.6	1
Adults	13	235 (190–299)	270 (218–343)	8.9 (3.9–18.8)	0
Females (N = 16)					
Hatchlings	1	143	163	1.5	0
Adults	15	340 (261–408)	385 (297–462)	33.3 (9.2–56.5)	0

TABLE 2. Descriptive data on native snakes that were swabbed opportunistically for SFD-causing *Ophidiomyces* during Burmese Python (*Python bivittatus*) removals in southwest Florida, USA. "Ct Value" refers to the cycle threshold value of each sample for the real-time PCR assay which is inversely proportional to the amount of DNA in a sample; thus lower Ct values indicate that a higher amount of target DNA is present in the swab sample whereas Ct values closer to 40 (the upper threshold for the PCR assay) indicate a relatively small amount of target DNA in the swab sample.

Species	Capture/ Swab date	Ct value	*Ophidiomyces* detected
Cemophora coccinea	6-Jul-18	–	No
Crotalus adamanteus	8-Mar-19	–	No
Crotalus adamanteus	18-Mar-19	25.890	Yes
Diadophis punctatus	31-May-18	–	No
Farancia abacura	29-May-18	–	No
Lampropeltis floridana	22-Mar-18	30.143	Yes
Lampropeltis floridana	16-Jan-19	33.495	Yes
Liodytes pygaea	30-May-18	–	No
Nerodia clarkii	23-May-18	–	No
Nerodia fasciata	9-Nov-17	–	No
Nerodia fasciata	9-Nov-17	–	No
Nerodia fasciata	23-May-18	–	No
Nerodia fasciata	29-May-18	–	No
Nerodia fasciata	30-May-18	–	No
Nerodia fasciata	20-Jun-18	–	No
Nerodia floridana	23-May-18	–	No
Nerodia floridana	23-May-18	–	No
Nerodia floridana	30-May-18	–	No
Nerodia floridana	15-Jun-18	–	No
Pantherophis guttatus	24-May-18	–	No
Pantherophis guttatus	29-May-18	–	No
Pantherophis guttatus	30-May-18	–	No
Pantherophis guttatus	26-Nov-18	–	No
Thamnophis saurita	24-May-18	–	No
Thamnophis saurita	31-May-18	–	No
Thamnophis saurita	15-Jun-18	–	No

had low levels of fungus present (Ct = 33.495), whereas the other kingsnake had a considerably higher fungal load (Ct = 30.143), though neither exhibited clinical signs of SFD. The rattlesnake had a very high pathogen load (Ct = 25.890), but gross lesions were not observed. The prevalence of *Ophidiomyces* in these native snakes is similar to what has been reported previously in Pygmy Rattlesnakes (*Sistrurus miliarius*) in Florida without clinical signs of SFD, although the prevalence of individuals with clinical signs appeared to be lower in our study (Lind et al. 2019). The difference may be due to geographic, temporal, or host-related factors as has been documented elsewhere (McKenzie et al. 2018).

Only one python, a juvenile, had any evidence of *Ophidiomyces* on its skin, with no gross lesions. The amount of *Ophidiomyces* DNA detected on that individual was miniscule and we consider the sample to be "equivocal." *Ophidiomyces* DNA was not detected on the tracked pythons, which had surgically implanted transmitters that possibly could be an entryway for the pathogen. Given the real-time PCR detections of *Ophidiomyces* in this study, the pathogen is present in the habitats where pythons have established in southwest Florida but may not be readily detectable on pythons themselves. It is possible that Burmese Pythons either rarely carry the fungus or harbor such low amounts that it is not easily detected by qPCR. However, pythons are known to be susceptible to SFD (Sigler et al. 2013). Since January 2013, over 700 pythons between 55 cm and 466 cm SVL have been observed, none of which showed clinical signs of the pathogen (IB pers. obs.). More research is needed to understand the susceptibility of pythons to infection from *Ophidiomyces* and their possible role as reservoir hosts for the fungus.

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First Evidence of the Amphibian Chytrid Fungus Likely Driving Dramatic Frog Community Changes on the New England Tablelands of Eastern Australia

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The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), responsible for the potentially fatal disease chytridiomycosis, is implicated in population declines and extirpations of many frog species around the world, including Australia (Skerratt et al. 2007; Scheele et al. 2019). Within Australia, almost 20% of frog species are estimated to have declined or become extinct due to *Bd* (Scheele et al. 2017a), yet data on *Bd* infection prevalence in most frog species and over most of the continent remains unknown.

Frogs in eastern Australia have undergone some of the greatest declines in population, many of which are attributed to *Bd* (Scheele et al. 2017a; Newell 2018). Of these, frog population declines in the New England Tablelands, a stepped plateau with elevations between 600 and 1500 m, have been some of the most dramatic and enigmatic. The area is home to two missing frog species—the Peppered Tree Frog (*Litoria piperata*) and the Yellow-Spotted Bell Frog (*L. castanea*)—and another species that has only recently been rediscovered from the area, the Booroolong Frog (*L. booroolongensis*; Rowley and Cutajar 2018). In addition, the Tusked Frog (*Adelotus brevis*), despite remaining common in other parts of its range, has declined on the Tablelands, resulting in the population being listed as Endangered (Gillespie and Hines 1999). Several other frog species have also experienced population declines on the Tablelands (Mahony 1999; Mahony et al 2001). Most of these species were once common on the New England Tablelands (Heatwole et al. 1995), and *Bd* has been