The Journal of Veterinary Medical Science

FULL PAPER

Wildlife Science

First report of ophidiomycosis in Asia caused by *Ophidiomyces ophiodiicola* in captive snakes in Japan

Yoshinori TAKAMI[1)#], Kyung-Ok NAM[2)], Youki TAKAKI[1)], Sho KADEKARU[3)], Chizuka HEMMI[3)], Tsuyoshi HOSOYA[2)] and Yumi UNE[3)#*]

[1)]Verts Animal Hospital, 4-3-1 Morooka, Hakata-ku, Fukuoka-shi, Fukuoka 812-0894, Japan
[2)]Department of Botany, National Museum of Nature and Science, Tsukuba-shi, Ibaraki 305-0005, Japan
[3)]Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Ehime 794-8555, Japan

ABSTRACT. Ophidiomycosis is an emerging infectious disease caused by the fungus *Ophidiomyces ophiodiicola,* which has been affecting wild and captive snakes in North America, Europe, and Australia. We report 12 cases of suspected ophidiomycosis in captive colubrid snakes in Japan. Pathological and microbiological examinations were performed, and the results confirmed the diagnosis of ophidiomycosis in two snakes, which indicated that the remaining sympatrically raised snakes also had ophidiomycosis since they exhibited similar lesions. This is the first report of ophidiomycosis in Asia caused by *O. ophiodiicola*. To prevent the expansion of ophidiomycosis in the natural environment in Japan, there is a need to evaluate the ophidiomycosis carrier status of imported snakes, the pathogenicity of the infection in native snakes, and the prevalence and distribution of *O. ophiodiicola* in wild and captive snakes. Measures also must be taken to prevent endemicity globally.

KEY WORDS: Asia, *Ophidiomyces ophiodiicola*, ophidiomycosis, snake fungal disease

J. Vet. Med. Sci.
83(8): 1234–1239, 2021
doi: 10.1292/jvms.21-0177

Received: 23 March 2021
Accepted: 10 June 2021
Advanced Epub:
25 June 2021

Chytridiomycosis in amphibians, white-nose syndrome in bats, and ophidiomycosis are serious fungal diseases that harm ecosystems [4, 6, 7, 17]. Ophidiomycosis is caused by the fungus *Ophidiomyces ophiodiicola*, which belongs to a monotypic, asexual genus in the order Onygenales [14]. Since 2006, severe skin infections in the timber rattlesnake (*Crotalus horridus*) have been observed in the northeastern United States [6]. In 2008, a similar infection involving a fungal pathogen occurred in endangered massasaugas (*Sistrurus catenatus*) in Illinois [1]. The infection became known as ophidiomycosis, and by 2015, ophidiomycosis had been documented in wild snakes throughout most of the eastern US [11], probably causing the decline of natural populations.

Examinations of isolates revealed that *O. ophiodiicola* has been present in captive snakes in the eastern United States since 1986 [14]. In contrast, isolates from wild snakes were not reported until 2008 [13]. Therefore, *O. ophiodiicola* is thought to have been spread from the captive to the wild snake population [11]. Moreover, several studies support the idea that the pathogen is endemic [3, 8].

Currently, the known geographical distribution of *O. ophiodiicola* is wider in captive snakes than in wild snakes [11]. In the US, isolates have been obtained from captive snakes in California, Georgia, Maryland, New Mexico, New York, and Wisconsin [14]. *O. ophiodiicola* has also been isolated from lesions in captive snakes in the United Kingdom, Germany, and Australia [14]. In Europe, the fungus has been shown to have a wider range in wild snakes than in captive snakes [8]. Currently, ophidiomycosis is recognized as an emerging infectious disease observed in more than 30 snake species [8], affecting wild and captive snakes throughout North America, Europe, and Australia [8]. However, so far, there have been no reports from Asia.

In this study, we report the first epidemic cases in Asia caused by *O. ophiodiicola* in captive snakes.

MATERIALS AND METHODS

History

The owner of 12 colubrid snakes (one hognose snake [*Heterodon* sp.], one California kingsnake [*Lampropeltis getula californiae*], two Mexican black kingsnakes [*L. getula nigrita*], one green vine snake [*Oxybelis fulgidus*], three corn snakes

*Correspondence to: Une, Y.: y-une@vet.ous.ac.jp
#These authors contributed equally to this work.

Table 1. Colubrid snake species, date of introduction, date of first consultation, birth origin, degree of appetite, and clinical outcome

No.	Species	Introduced	Onset	Birth origin	Degree of appetite	Clinical outcome
1	Green vine snake (*Oxybelis fulgidus*)	2019.04.21	2019.04.22	WC	Anorexia	Death
2	Texas rat snake (*Pantherophis obsoleta lindheimeri*)	2018.10.07	2019.06.07	CB	Anorexia	Death
3	Texas rat snake (*P. obsoleta lindheimeri*)	2018.08.02	2019.06.07	CB	Anorexia	Death
4	Black rat snake (*P. obsoletus*)	2018.10.07	2019.07.19	CB	Anorexia	Death
5	Corn snake (*P. guttatus*)	2018.07.17	2019.07.19	CB	Anorexia	Death
6	California kingsnake (*Lampropeltis getula californiae*)	2017.06.04	2019.06.16	CB	Normal appetite	Survival
7	Mexican black kingsnake (*L. getula nigrita*)	2016.11.20	2019.07.19	CB	Normal appetite	Survival
8	Black rat snake (*P. obsoletus*)	2018.06.03	2019.10.11	CB	Normal appetite	Survival
9	Mexican black kingsnake (*L. getula nigrita*)	2017.11.01	2019.07.19	CB	Normal appetite	Survival
10	Hognose snakes (*Heterodon* spp.)	2018.11.03	2019.07.19	CB	Normal appetite	Survival
11	Corn snake (*P. guttatus*)	2016.09.22	2019.07.19	CB	Normal appetite	Survival
12	Corn snake (*P. guttatus*)	2018.11.01	2019.07.19	CB	Normal appetite	Survival

CB, Captive-born; WC, Wild-caught.

[*Pantherophis guttatus*], two black rat snakes [*P. obsoletus*], and two Texas rat snakes [*P. obsoletus lindheimeri*]) noticed blisters, pustules, and crusts on the snakes' skins and took them to a veterinary hospital from April to October 2019 (snakes are numbered 1 to 12 in Table 1). They were kept in separate plastic cages with paper on the floor in a room but were not disinfected. The first snake with suspected ophidiomycosis was a wild-caught green vine snake (No. 1) that was purchased on April 21, 2019. Subsequently, the remaining 11 domestic captive-born snakes successively exhibited skin lesions resembling ophidiomycosis between June and October of 2019. Eventually, 5 of the 12 snakes (No. 1–5) died; among them, three (No. 2, 4, 5) were frozen immediately after death, thawed 12 (No. 2) and 44 (No. 4, 5) days later, and pathologically and microbiologically examined. The tissue samples collected from dead snakes were brought to a veterinary hospital, and therefore, ethical approval was not required from the Institutional Review Board.

Pathological examination

Three (No. 2, 4, 5) of the five dead snakes in this study were submitted for full necropsy. The snakes were photographed, and their lengths and weights were measured. The distribution and character of skin lesions were observed over the whole body. The snakes were incised along the ventral midline and the subcutaneous lesions and internal organs were examined. After the necropsy, the whole body with skin and organs was placed in 10% neutral buffered formalin for histopathologic examination. Paraffin blocks were produced from at least five skin lesions (the head, tail, and three parts of body) and internal organs (heart, lung, liver, kidney, gastrointestinal tract, pancreas, spleen, genital tract, and adrenal glands) by routine processing. Paraffin sections (3–4 µm thick) were stained with hematoxylin and eosin, periodic acid Schiff, and Fungi-Fluor Y (Cosmo Bio, Tokyo, Japan).

Isolation of the pathogen

Several severe (typical) lesions from three (No. 2, 4, 5) of the five dead snakes in this study were collected for fungal examination and culture. For fungal isolation, three pieces of 10 × 5 × 5 mm tissue samples were collected from snakes 2, 4, and 5 and were inoculated on a cornmeal agar plate (CMA, Nissui, Tokyo, Japan), and incubated at room temperature for approximately 2 weeks to allow fungal colonization. White, compact, powdery fungal colonies appeared on two samples (No. 2 and 4), which were associated with bacteria and faster-growing fungi. To isolate the target fungus, single conidia produced in powdery parts of the colony were isolated using Skerman's micromanipulator [16], which allows for single-spore isolation. For microscopic observation of the morphology, potato dextrose agar plates (PDA, Nissui) were inoculated with the isolate in the center and incubated at 25°C for 10 days. The isolates were deposited at the Biological Resource Centre, National Institute of Technology and Evaluation (NITE BRC, Tokyo, Japan).

Extraction of fungal DNA and internal transcribed spacers (ITS) region sequence analysis

Following the protocol from Itagaki *et al*. [10], approximately 2.0 ml of 2% malt extract (Difco, BD Biosciences, San Jose, CA, USA) broth (MEB) was inoculated with mycelia of each isolate and incubated for 2 weeks at 20°C in the dark. After incubation, the mycelia were picked and frozen at −80°C for 24 hr in 2.0-m*l* round-bottom tubes. Mycelia were lysed by a Qiagen Tissuelyser (Hilden, Germany) using zirconia beads. DNA was extracted by incubation in 800 µl of hexadecyltrimethylammonium bromide buffer (CTAB buffer; 2% CTAB, 100 mM Tris pH 8.0, 20 mM EDTA, 1.4 M NaCl) at 65°C for 60 min, and proteins were removed using chloroform/isoamylalcohol mixture (24:1). The supernatant was further treated with 1,000 µl of 6 M sodium iodine buffer (6 M NaI, 50 mM Tris pH 8.0, 10 mM EDTA, 0.1 M Na_2SO_3) and glass milk and stirred by a rotator for more than 1 hr. After centrifugation at 12,000 rpm for 15 min, the precipitate was washed with 1,000 µl of ethanol/buffer solution (80% EtOH, 10 mM Tris pH 8.0, 1 mM EDTA), and finally DNA was eluted in 120 µl of TE at pH 8.0 (Tris-EDTA buffer, Wako, Tokyo, Japan). The extracted DNA samples were preserved in the Center for Molecular Biodiversity Research in the National Museum of Nature and Science and are available for collaborative studies. Polymerase chain reaction (PCR) was conducted to amplify internal

transcribed spacers (ITS1 and ITS2) and 5.8S ribosomal regions (ITS-5.8S rDNA) with the ITS1F and ITS4 primer pairs [18]. The PCR cocktail contained the following reagents: 1.0 µl of extracted DNA, 3.5 µl of DNA-free water, 5.0 µl of EmeraldAmp PCR MasterMix (Takara Bio, Kusatsu, Japan), 0.25 µl of forward primer, and 0.25 µl reverse primer. The following protocol was applied: initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 35 sec, 51°C for 30 sec, and 72°C for 60 sec; final extension at 72°C for 10 min. The amplified PCR products were purified with ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol. Sequencing reactions were carried out using an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Norwalk, CT, USA). The obtained sequences were assembled using ATGC software, version 7.0.3 (Genetyx, Tokyo, Japan).

The newly obtained sequences were deposited with the DNA Data Bank of Japan (DDBJ).

RESULTS

Macroscopic findings

Skin changes were uniform in all snakes; specifically, all had skin necrosis. They were the predominant lesions observed in the affected snakes; the extent of necrosis ranged from small, individual scales to large patches or bands of skin. The necrotic skin was discolored (yellow, light brown, or red), cloudy, and fragile, and the necrotic scales were thickened throughout the trunks of the affected snakes. They peeled off easily, and blister formation and fluid accumulation could be observed between the skin layers. In addition to necrosis, multiple foci of skin crusting were evident. The lesions were a few millimeters in size, reddish, with raised surfaces. These lesions had varying degrees of similarity among the three snakes. Crusting was more prominent on the dorsal and lateral sides of the snakes. In case 2, large necrotic skin lesions were evident throughout the body, especially on the head. There were a few multifocal crusting lesions on the skin. In case 4, the whole body was covered in red patches and necrotic scales were widely distributed. In case 5, skin crusting was observed throughout the body. Severe skin necrosis was observed predominantly on the neck, with exfoliated skin adhering to the surface. There were no lesions in the oral cavity as they were limited to the skin. No significant changes were observed in the internal organs, and there was no evident atrophy of the spleen.

Microscopic findings

Histopathological examination of the skin lesion samples of snakes 2, 4, and 5 revealed severe degeneration and necrosis of scales, accompanied by crusting, with fungal infiltration (Fig. 1A–G). In mild lesions, the crusts consisted of keratin, fibrin, and cellular debris with fungi accumulated at the base of the scales. In advanced lesions, epidermal necrosis extended into the dermis, and the entire scale was necrotic. In other parts of some of these snakes, the affected skin was moderately hyperplastic and covered with a serocellular crust composed of fragmented or layered keratin, fibrin, and cellular debris. Severe vacuolar degeneration was observed in the stratified squamous epithelial cells just below the stratum corneum. Separation of the stratum corneum was observed. The dermis and underlying subcutis were enlarged due to edema. Bacterial colonies were found on the crust surfaces. The inflammation was very mild and comprised a few heterophils. Fungal hyphae were widespread within the necrotic debris (crust), and rod-shaped 2 × 5-µm arthroconidia were often found on the lesion surfaces. The fungal hyphae had parallel walls of widths of up to 5 µm and exhibited occasional septa branching at acute angles. The fungal hyphae and arthroconidia showed pale, basophilic staining with hematoxylin & eosin stain, red staining with PAS stain, and fluorescent green staining with Fungi-Fluor Y (Cosmo Bio). The fungus was present primarily in the stratum corneum and crust, and it occasionally invaded the underlying dermis. The spleens of the three snakes were atrophied. Snakes 2 and 4 had moderate pulmonary edema. Snakes 4 and 5 had small amounts of fat accumulation in their hepatocytes, and snake 4 had a slight brown pigmentation (hemosiderin). No fungus was found in their organs.

Mycological feature of the isolates

In the three subjects (No. 2, 4, and 5), fungal isolates were detected in only No. 2 and 4; isolates were collected from the most severe lesions of crusting or skin necrosis. No isolates were detected during pathological examination of No. 5. After single-spore isolation, we confirmed that all colonies had identical morphology and one representative colony was selected from each sample (NBRC 114438 from *P. obsoletus lindheimeri* [No. 2] and NBRC 114439 from *P. obsoletus* [No. 4]). We confirmed that the micromorphology of the conidia-producing structure was consistent with previous descriptions [14, 15] (Fig. 2). The mycological features of the isolates were as follows (Fig. 2): slow growth on PDA, colony attaining a diameter of 40 mm (25°C, 3 weeks), white to pale yellow, powdery to floccose at the center, clear exudate produced on the surface, part of mycelia immersed in the agar, forming ring-like zonation. Conidia were of two types: aleurioconidia produced terminally, intercalary, or laterally from the conidia producing hyphae 2–3 µm wide, forming a system of repeated branches, often separated by the rhexolytic dehiscence of separating cell produced between the hyphae and the conidia; clavate with truncated base to elliptical, 3−7 × 2 (5.05 ± 1.10 × 2 ± 0 average ± standard deviation) µm, some clavate conidia equipped with a membranous fringe at the base showing vestige of the separating cells, often separated by leaving protrusions on conidia producing hyphae. Arthroconidia are produced by the fission of hyphae, are rectangular, and 6–14 × 1.5 (8.70 ± 1.94 × 1.5 ± 0) µm in size. Both types of conidia smooth, hyaline. Sterile undulating hyphae, 2–3 µm wide with fewer septa are also produced. No growth was observed at 35°C.

The above description was consistent with the study by Sigler *et al*. (2013) [14].

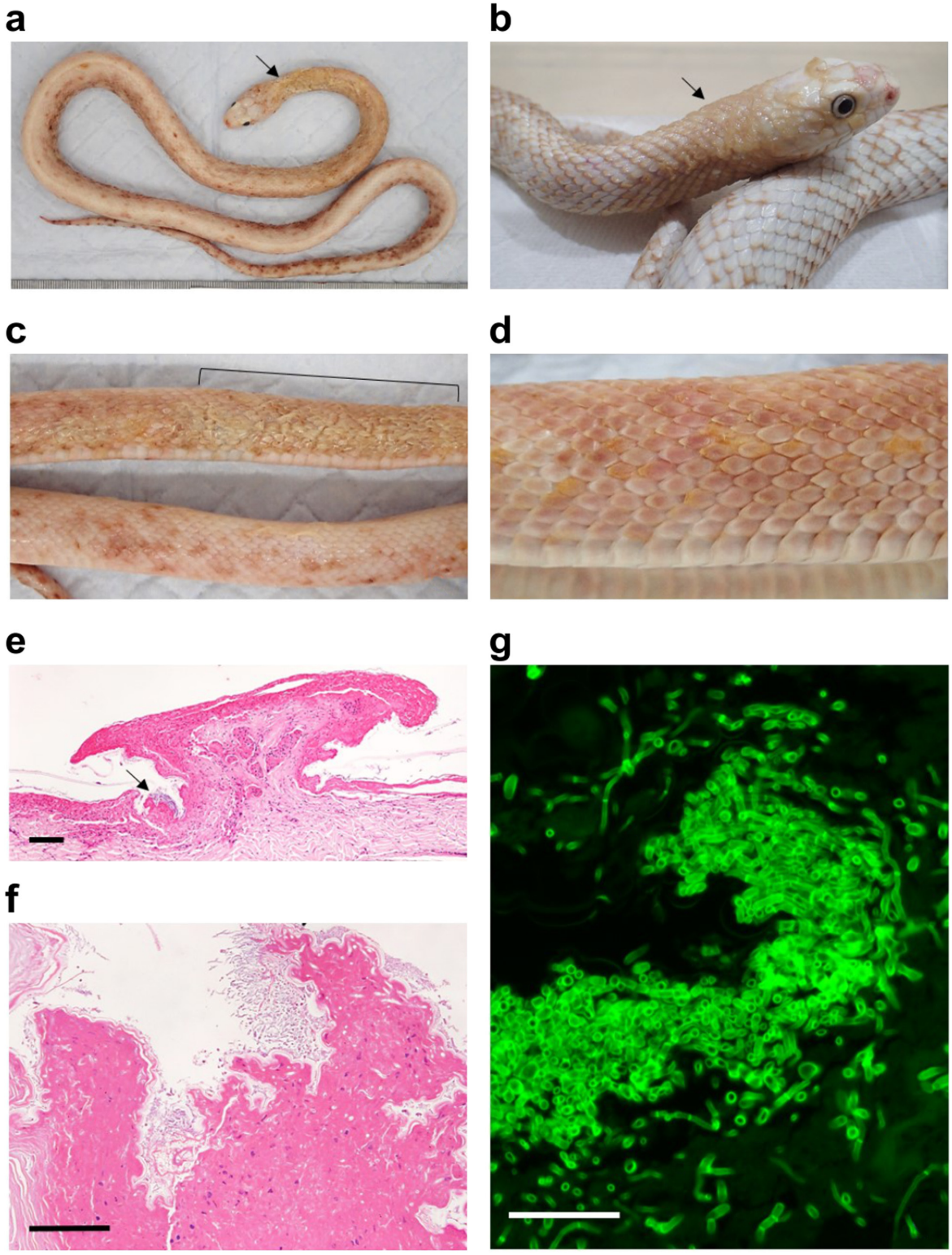

Fig. 1. Pathological features of ophidiomycosis. **a.** No. 4 (*Pantherophis obsoletus*). The skin of the cranial body is extensively necrotic (arrow), and the skin of the caudal body has multifocal bleeding and crusting lesions. They are severe at the tail. **b.** Exterior view prior to death of No. 2 (*P. obsoletus lindheimeri*). Discoloration of scale (browning); at first, edges of the individual scales change color, then gradually the whole scale is involved. As the discoloration progresses further, it becomes more diffuse in the skin on the neck (arrow). **c.** The skin lesions of No. 4 (*P. obsoletus*). Upper: extensive necrosis of skin (brackets); Lower: multifocal hyperemia and crusting. **d.** No. 5 (*P. guttatus*). Necrotic scales are yellow. **e.** The skin lesions (Hematoxylin and eosin stain) of No. 5 (*P. guttatus*). The entire scale is necrotic; fungal growth foci are seen at the base of the scale (arrows). Scale bar: 100 µm. **f.** The skin lesions (hematoxylin and eosin stain) of No. 5 (*P. guttatus*). High magnification of fungal growth foci with severe necrosis of the scales (arrows). Scale bar: 100 µm. **g.** Fungi in tissue (Fungi-Fluor Y stain) of No. 4 (*P. obsoletus*). Crust and fungal growth are present; Fungi-Fluor Y stains the fungal cell membrane green. The fungus penetrates deep into the surface and crust. The fungus is rather thin and has a septum. Scale bar: 100 µm.

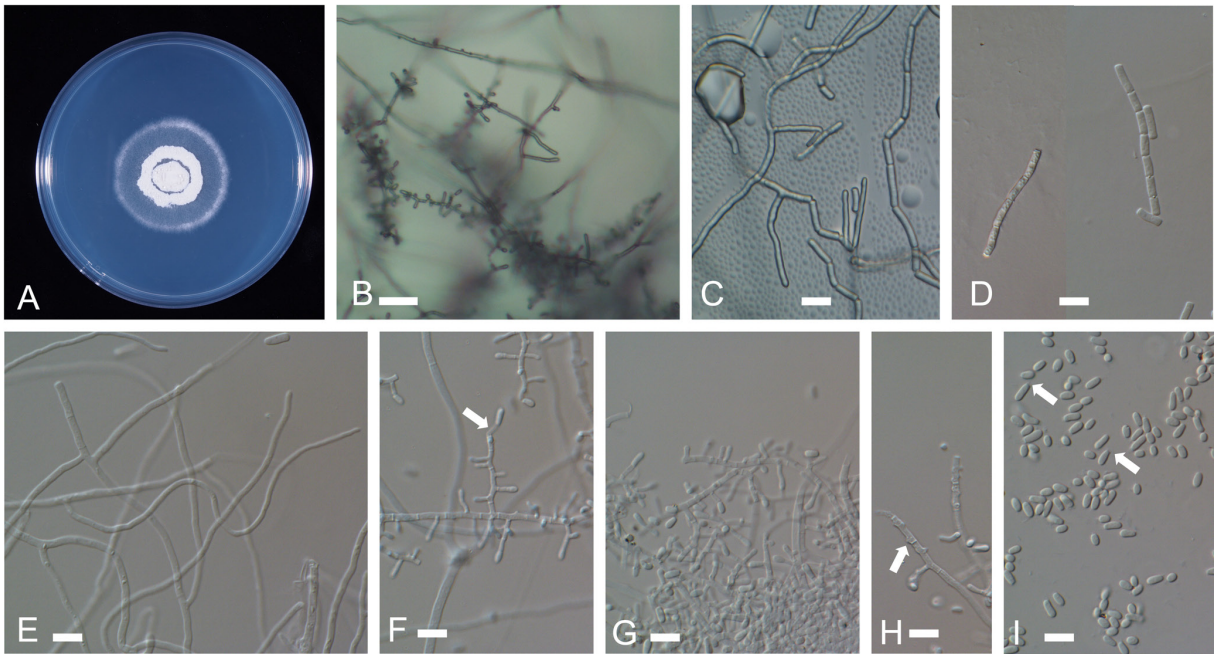

Fig. 2. Micrograph of *Ophidiomyces ophiodiicola.* **A.** Colony on PDA (25°C, 2 weeks) showing a typical ring-like zonation. **B.** Part of colony showing branching conidia-producing structure. **C.** Arthroconidia observed in the air bubble in preparation. **D.** Arthroconidia. **E.** Undulating hyphae. **F.** Conidia producing structure showing aleurioconidia produced laterally or terminally, borne on a separating cell. Arrow shows the collapsing separating cells. **G.** Complex conidia producing structure bearing aleuroconidia laterally. **H.** Vestige of separation of conidia. Arrow shows one representative structure. **I.** Conidia. Arrow shows two representatives of vestige (membranous fringe) of separating cell. Scales B: 50 µm. C–I: 10 µm.

BLAST search results of sequences obtained from the isolates

The obtained sequences (LC521891 from NBRC 114438 and LC521892 from NBRC 114439) were identical and were analyzed using Basic Local Alignment Search Tool (BLAST) and compared to the sequence database in The National Centre for Biotechnology Information (NCBI). The most similar sequences with E-value=0.0 were all of *O. ophiodiicola*, except for EU715819 (*Chrysosporium* sp.=*O. ophiodiicola*). The sequence with the highest similarity was KF225599 (*O. ophiodiicola* voucher MYCO-ARIZ AN0400001), with 99.52% identity and 100% query coverage, while the rest showed a 98.13–100% identity. These analyses confirmed that the isolated fungus was *O. ophiodiicola,* consistent with previous literature [14, 15].

DISCUSSION

Our examination confirmed ophidiomycosis diagnoses in two snakes, and the remaining sympatrically raised snakes had exhibited similar lesions. *O. ophiodiicola* has different degrees of pathogenicity depending on the strain, host species, and environmental conditions, with a mortality rate of ≥90% in some snake species such as massasaugas in the eastern US [2]. There are many questions raised about this disease, particularly regarding its effects on wild and captive snakes, infection vectors, and the change in prevalence over time [5].

According to the owner, the present outbreak among snakes began after the introduction of a wild-caught green vine snake (*Oxybelis fulgidus*) indigenous to South America. The ITS region in fungi is known to be highly variable at the isolate level, and all snakes were housed together in an enclosed space; the identical sequences suggest that a single isolate was the source of this outbreak. Based on these results, *O. fulgidus* was the suspected infection source, and the snakes may have acquired the infection while being housed at an exporter's or importer's facility. Since no case of ophidiomycosis has been reported previously from *O. fulgidus*, this snake seems to be a new host and the fungus may have a wider host range than previously suspected.

The snakes had only moderate pulmonary edema and mild fatty degeneration of hepatocytes, suggesting that these lesions were not associated with their deaths. In contrast, the skin lesions were widespread and severe, suggesting that the snakes had died under debilitating conditions. Bacterial infections on the skin were secondary in nature and had exacerbated the poor prognosis of snakes.

There are 51 wild snake species in Japan, including 23 threatened species, with high diversity in the mid-latitude areas [9, 12]. Those snakes occupy an important position as predators in the food chain. To prevent expansion of ophidiomycosis into the natural environment in Japan, there is a need to evaluate the ophidiomycosis carrier status of imported snakes, the pathogenicity of the infection in native snakes, and the clarification of prevalence and distribution of ophidiomycosis in wild and captive snakes.

The present isolates did not grow at 35°C, so there is little risk of infection in humans or other mammals.

POTENTIAL CONFLICTS OF INTEREST. The authors declare no conflict of interest.

REFERENCES

1. Allender, M. C., Dreslik, M., Wylie, S., Phillips, C., Wylie, D. B., Maddox, C., Delaney, M. A. and Kinsel, M. J. 2011. *Chrysosporium* sp. infection in eastern massasauga rattlesnakes. *Emerg. Infect. Dis.* **17**: 2383–2384. [Medline] [CrossRef]
2. Allender, M. C., Baker, S., Britton, M. and Kent, A. D. 2018. Snake fungal disease alters skin bacterial and fungal diversity in an endangered rattlesnake. *Sci. Rep.* **8**: 12147. [Medline] [CrossRef]
3. Allender, M. C., Ravesi, M. J., Haynes, E., Ospina, E., Petersen, C., Phillips, C. A. and Lovich, R. 2020. Ophidiomycosis, an emerging fungal disease of snakes: targeted surveillance on military lands and detection in the western US and Puerto Rico. *PLoS One* **15**: e0240415. [Medline] [CrossRef]
4. Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G. and Parkes, H. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc. Natl. Acad. Sci. USA* **95**: 9031–9036. [Medline] [CrossRef]
5. Chandler, H. C., Allender, M. C., Stegenga, B. S., Haynes, E., Ospina, E. and Stevenson, D. J. 2019. Ophidiomycosis prevalence in Georgia's Eastern Indigo Snake (Drymarchon couperi) populations. *PLoS One* **14**: e0218351. [Medline] [CrossRef]
6. Clark, R. W., Marchand, M. N., Clifford, B. J., Stechert, R. and Stephens, S. 2011. Decline of an isolated timber rattlesnake (*Crotalus horridus*) population: Interactions between climate change, disease, and loss of genetic diversity. *Biol. Conserv.* **144**: 886–891. [CrossRef]
7. Frank, C. L., Davis, A. D. and Herzog, C. 2019. The evolution of a bat population with white-nose syndrome (WNS) reveals a shift from an epizootic to an enzootic phase. *Front. Zool.* **16**: 40. [Medline] [CrossRef]
8. Franklinos, L. H. V., Lorch, J. M., Bohuski, E., Rodriguez-Ramos Fernandez, J., Wright, O. N., Fitzpatrick, L., Petrovan, S., Durrant, C., Linton, C., Baláž, V., Cunningham, A. A. and Lawson, B. 2017. Emerging fungal pathogen *Ophidiomyces ophiodiicola* in wild European snakes. *Sci. Rep.* **7**: 3844. [Medline] [CrossRef]
9. Herpetological Society of Japan. http://herpetology.jp/wamei/index_j.php [accessed on March 22, 2021].
10. Itagaki, H., Nakamura, Y. and Hosoya, T. 2019. Two new records of ascomycetes from Japan, *Pyrenopeziza protrusa* and *P. nervicola* (Helotiales, Dermateaceae sensu lato). *Mycoscience* **60**: 189–196. [CrossRef]
11. Lorch, J. M., Knowles, S., Lankton, J. S., Michell, K., Edwards, J. L., Kapfer, J. M., Staffen, R. A., Wild, E. R., Schmidt, K. Z., Ballmann, A. E., Blodgett, D., Farrell, T. M., Glorioso, B. M., Last, L. A., Price, S. J., Schuler, K. L., Smith, C. E., Wellehan, J. F. Jr. and Blehert, D. S. 2016. Snake fungal disease: an emerging threat to wild snakes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **371**: 20150457. [Medline] [CrossRef]
12. Ministry of the Environment Government of Japan. 2020. Red list. https://www.env.go.jp/press/files/jp/114457.pdf [accessed on March 22, 2021].
13. Rajeev, S., Sutton, D. A., Wickes, B. L., Miller, D. L., Giri, D., Van Meter, M., Thompson, E. H., Rinaldi, M. G., Romanelli, A. M., Cano, J. F. and Guarro, J. 2009. Isolation and characterization of a new fungal species, *Chrysosporium ophiodiicola*, from a mycotic granuloma of a black rat snake (*Elaphe obsoleta obsoleta*). *J. Clin. Microbiol.* **47**: 1264–1268. [Medline] [CrossRef]
14. Sigler, L., Hambleton, S. and Paré, J. A. 2013. Molecular characterization of reptile pathogens currently known as members of the *chrysosporium* anamorph of *Nannizziopsis vriesii* complex and relationship with some human-associated isolates. *J. Clin. Microbiol.* **51**: 3338–3357. [Medline] [CrossRef]
15. Sigler, L. 2013. Ophidiomyces Sigler, Hamleton. and Paré, gen. nov. IF550166 and IF550167 in nomenclatural novelties. Index Fungorum no. http://www.indexfungorum.org/Publications/Index%20Fungorum%20no.19.pdf [accessed on April 20, 2021].
16. Skerman, V. B. D. 1968. A new type of micromanipulator and microforge. *J. Gen. Microbiol.* **54**: 287–297. [Medline] [CrossRef]
17. Tamukai, K., Une, Y., Tominaga, A., Suzuki, K. and Goka, K. 2014. *Batrachochytrium dendrobatidis* prevalence and haplotypes in domestic and imported pet amphibians in Japan. *Dis. Aquat. Organ.* **109**: 165–175. [Medline] [CrossRef]
18. White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322. In: PCR Protocols: A Guide to Methods and Applications (Innis M. A., Gelfand D. H., Sninsky J. J. and White T. J. eds.), Academic Press, San Diego.