Ophidiomycosis affecting Southern smooth snake (*Coronella girondica*) in Pyrenees (North Spain)

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Fecha de aceptación: 3 de diciembre de 2024.
Key words: *Ophidiomyces ophidiicola*, Snake Fungal Disease, Catalonia, *Coronella*.

Resumen: Recientemente se ha descrito en el Pirineo Español la enfermedad fúngica de las serpientes causada por el hongo *Ophidiomyces ophidiicola*. En esta nota se describe un nuevo caso de la misma enfermedad que afecta a una nueva especie de serpiente, la culebra lisa meridional (*Coronella girondica*). La presencia de este patógeno en diferentes especies de los Pirineos alerta de su amplia distribución y afectación a distintos hospedadores en lugar de una distribución focalizada, lo que podría convertirse en un importante problema de conservación.

The fungal pathogen *Ophidiomyces ophidiicola* (Oo), the aetiologic agent of Snake Fungal Disease (SFD) or Ophidiomycosis, has raised a growing interest in European scientific communities, in particular toward conservation. To date, the negative impacts of *Ophidiomyces* are controversial (Di Nicola *et al.* 2022). For some species it appears to be negatively impacting the conservation of their populations (Allender *et al.*, 2015) but in other cases ophidiomycosis may have sublethal effects and no direct effects on survival, ovipositioning, or viability of the studied populations (Dillon *et al.* 2024). This pathogen is suspected to be associated with the declines of some snake populations in North America and recently has been detected in European wild snakes (Blanvillaine *et al.* 2022; Joudrier *et al.* 2024). The first description of this disease in Spain was detected affecting one Aesculapian snake, *Zamenis longissimus*, in Catalonia in 2023 (Martínez-Silvestre *et al.* 2024). Next, we describe a new case detected in 2024 in the Pyrenees (Catalonia, North-East Spain) and affecting a new host belonging to the genus *Coronella*.

The animal was an adult female of the Southern smooth snake, *Coronella girondica*, and was found on May 27, 2024 in Porcingles (42º49'33.1"N / 0º46'29.9"E), a little town at 2 km from the border with France. She was a little active on a rainy day among some rocks in a clearing next to a paved road, with a certain slope. It was near a mountain stream with a strong flow. The main vegetation was ferns and deciduous trees such as beech and birch.

The snake had a general body condition index of 2/5 and the body section was triangulated due to advanced thinning. She was kept alive in an animal carrier for supportive care and was brought to the Catalonian Reptiles and Amphibians Rescue Centre (CRARC). The animal had external lesions affecting the skin and forming scabs that exfoliate during handling. The snake was immediately hospitalized, with fluid administration in a humidity and temperature controlled terrarium, but died 12 hours after getting.

At necropsy, skin lesions measuring between 1 and 3 cm in length were observed, which altered the skin structure, causing wounds and crusts. Some of the crusts were

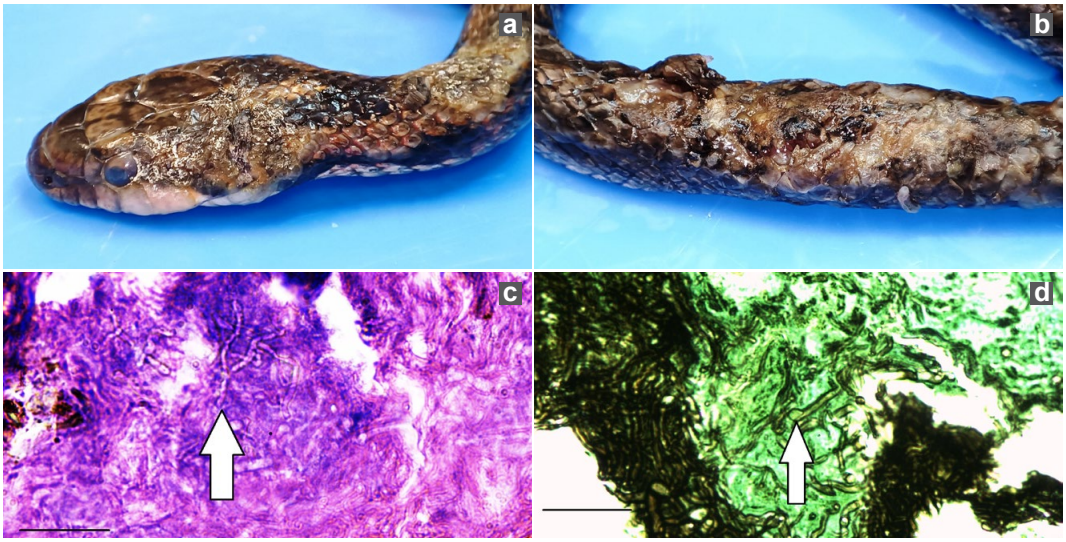

Figure 1: Macroscopic (a & b) and microscopic (c & d) appearance of the snake affected by Ophidiomycosis. The arrows indicate the hyphae stained with Hematoxylin/Eosin (c) and Grocott (d). x 600 magnification.
Figura 1: Aspecto macroscópico (a y b) y microscópico (c y d) de la serpiente afectada por Ophidiomycosis. Las flechas indican las hifas teñidas con Hematoxylina/Eosina (c) y Grocott (d). Ampliación x 600.

soft, fragile and fell off very easily. Near these lesions there were ulcers that revealed the subcutaneous space and, occasionally, the underlying muscle tissue. These lesions occupied the entire length of the snake, and especially the dorsal and lateral areas. In the head, the lateral, supralabial and mandibular areas were especially affected (Figure 1a,b).

Samples of the skin, bone, tongue, intestine, stomach, liver, kidney, lung and gonads were collected for histopathological analysis. 4 µm sections of formalin-fixed paraffin-embedded tissue specimens were stained by haematoxylin and eosin (H&E) and Grocott's methenamine silver (GMS) stains.

Histologically, fungal structures (hyphae) were observed infiltrating the different dermal layers and causing an inflammatory reaction around them (Figure 1c). The most affected areas had necrotic tissue with abundant bacterial contamination. The fungal hyphae were confirmed by GMS staining (Figure 1d) which were septated, with parallel walls of black colour and measured 3,0 to 3,4 µm width. No lesions were observed in the other tissues analyzed.

The lesions observed on the skin were consistent with fungal lesions, but were not specific to any particular fungus, so a specific PCR was performed to rule out the causal agent. One skin sample was sent to *Laboklin Laboratories* (Bad Kissingen, Germany) for genetic analysis using an *O. ophidiicola* specific PCR methodology to target the internal transcribed spacer 2 (ITS2) within the ribosomal RNA gene as described in Bohuski *et al.* (2015). Results were positive to presence of *Ophidiomyces ophidiicola*.

This note adds one more species of snake (*Coronella girondica*) to those affected by ophidiomycosis in the Pyrenees in particular and in Europe in general. Fungal dermatopathies in snakes might have a very similar appearance both macroscopically and microscopically. Hence, skin lesions are not pathognomonic of ophidiomycosis. In *Coronella*

austriaca from Switzerland, vulnerability to opportunistic fungus (*Alternaria* sp or *Rhodotorula* sp) causing dermatopathies has been noted in particularly cold and humid springs (Dubei *et al.*, 2022).

Consequently, once the lesion has been diagnosed, mycological culture or PCR are the essential techniques to confirm the involved fungus in that particular lesion.

In the Pyrenees and surrounding areas, from 2018 to date, a total of 104 *Natrix maura*, 3 *Natrix astreptophora*, 6 *Coronella girondica*, 1 *Coronella austriaca*, 10 *Malpolon monspessulanus*, 7 *Zamenis sacalaris*, 2 *Zamenis longissimus*, 1 *Vipera aspis* and 1 *Hierophis viridiflavus* have been analysed by PCR for Oo detection within active surveillance monitoring programs. Except for the positive case of *Zamenis longissimus* (Martínez Silvestre *et al.*, 2024) all the other results were negative (Blanvillain *et al.*, 2023). Then, this is the first time that infection by Oo related with mortality has been confirmed in a wild *Coronella girondica* specimen. In both cases, the two snakes were very weak and the stress of handling and captivity were decisive in triggering their death shortly after being captured.

The positive individual presented here was located very close (just 1 km) to the area where the first case in Spain was described (Martínez-Silvestre *et al.*, 2024). This suggests that the first case might not be just an isolated one and that the pathogen may be present in the area and may already affecting several species. Consequently, a detailed survey of the area is recommended to determine the real impact of this pathogen in the Pyrenees.

Acknowledgments: R. Marschang (*Laboklin Laboratories*), G. Blanvillaine (Virginia University), J. Bosch, B. Thumsovà (AHE), N. Collado (CRARC), F. Loras, J. Melero, M. Nadal, M. Carreras (SCH) and F. Amat (Museu de Granollers), for their collaboration during sampling or reviewing the manuscript.

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