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# First morphological and molecular isolation of *Talaromyces marneffei* in beech marten (*Martes foina*) in Portugal

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ABSTRACT

*Talaromyces marneffei* is a zoonotic fungus that mostly infects immunocompromised individuals. For the first time, this fungus was isolated in an adult beech marten (*Martes foina*) hit by a car, found dead in Penamacor, Portugal. During the necropsy, different samples (skin, fur, lymph nodes, lung, spleen, kidneys, and brain) were collected and processed for microbiology (including mycology) and molecular biology. *T. marneffei* was identified through its mycological characteristics and confirmed by PCR in hair samples. No other lesions or alterations were reported, except a concomitant presence of *M. avium* subsp. *paratuberculosis* in lung, kidney and brain samples. To the authors' knowledge, this is the first description of this fungus beech marten, as well as the first case of co-infection with *M. avium* subsp. *paratuberculosis* in wildlife fauna. These results suggest a sylvatic life-cycle of *T. marneffei*, involving beech martens, in Portugal.

*Talaromyces marneffei* is a fungus species, recently transferred from the *Penicillium* genus to *Talaromyces* (Samson et al., 2011). Cases of talaromycosis (or, more commonly, penicilliosis) are disseminated infections, similar to histoplasmosis, cryptococcosis, or even tuberculosis (Chaiwun et al., 2011; Su et al., 2019). Untreated cases can cause fatal systemic mycosis in immunocompetent and immunocompromised patients (Qiu et al., 2015).

*T. marneffei* is originally endemic in Southeast Asia and Southern China. Due to human migration and travel, this fungal infection has been diagnosed in Europeans (Gladieux et al., 2011). However, it has only been reported once in wild animals on this continent (Matos et al., 2019).

Beech marten (*Martes foina*) is a small carnivore mostly present in the North of Portugal, although its distribution data is scarce (Bencatel et al., 2019). Infectious diseases (some of them with zoonotic potential) are usually cause of morbidity and mortality in martens, such as canine distemper, sarcoptic mange and rabies (Akdesir et al., 2018). In Portugal, the number of pathogens reported in *M. foina* is scarce. Occasional reports refer to nematodes (*Eucoleus aerophilus*, *Crenosoma vulpis, Angiostrongylus* sp., *Toxocara* sp., *Toxascaris leonina, Ancylostomatidae, Strongyloides* sp., and *Thelazia callipaeda*) (di Cesare et al., 2014; Figueiredo et al., 2018; Seixas et al., 2018), protozoan (*Toxoplasma gondii*) (Waap et al., 2016), bacterium (*Mycobacterium avium* subsp. *paratuberculosis* (Matos et al., 2014), and parvovirus (Duarte et al., 2013; Santos et al., 2009). The absence of previous reports of *T. marneffei* in beech marten represents the opportunity for this communication.

An adult male beech marten was found dead due to a car collision in Penamacor, east-centre Portugal (40°10′8″North, 7°10′14″West) and submitted to the Histopathology Service of the Department of Animal Science, Polytechnic Institute of Castelo Branco, for a postmortem

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examination. The animal showed middling body condition 3 (on a common scale of 1 to 5). No other clinical lesions were visually detected during the necropsy. Samples from fur, skin and internal organs (lymph nodes, lung, spleen, kidneys, and brain) were collected and processed for microbiological and molecular examination at the Laboratory of Medical Microbiology and at the Laboratory of Applied Molecular Genetics, respectively, of the University of Trás-os-Montes and Alto Douro. Considering that the animal was already found dead before arriving at the laboratory, no ethical concerns are applied to this work.

Fur and skin were submitted for mycological culture. Samples were incubated in Potato Dextrose Agar medium (Oxoid®, Hampshire, United Kingdom) and Sabouraud Dextrose Agar medium (Oxoid®) and incubated under 25 °C and 37 °C for 3–7 days. Petri dishes were checked daily. Positive cultures for *T. marneffei* were obtained as a mould at 25 °C, and as yeast at 37 °C in marten's fur. Moreover, this sample revealed red-wine coloured diffusible pigment on Sabouraud dextrose agar and Potato Dextrose Agar medium **(**Fig. 1**).** Microscopic examination was then confirmed with the lactophenol cotton blue stain that revealed highly branched hyphae (Vanittanakom et al., 2002).

Polymerase chain reaction (PCR) was used for the molecular confirmation of this fungus in pure cultures. Deoxyribonucleic acid was extracted from a single culture using a commercially available kit (Plant/ Fungi DNA Isolation Norgen Biotek®, Bad Friedrichshall, Germany). DNA was tested by using primers designed to identify *T. marneffei* 18S rDNA gene sequence, as described previously by Vanittanakom et al. (2002). In the first PCR, 3 μl of the extracted DNA were used in a 20 μl reaction mix with 10 μl of Mastermix (Bioron®, Ludwigshafen, Germany), 1 μl of RRF1 primer (5′ATCTA AATCCCTTAACGAGGAACA3′) and 1 μl of RRH1 primer (5′CCGTCAATTTCTTTAAGTTTCAGCCTT3′. The PCR conditions were 95 °C for 5 min, then 35 cycles at 95 °C for 30 s; 55 °C for 30 s, 72 °C for 2 min, followed by 72 °C for 10 min. One microliter of the first PCR product (previously subjected to a 1:1000 dilution) was used in a second PCR with the specific primers, Pm1 (5′ATGGGCCTTTCTTTCTGGG3′) and Pm2 (5′GCGGGTCATCATAGAAACC). The nested PCR mixture and conditions were the same as those described in the first PCR, except for the annealing temperature (65 °C). Fourteen microliters of PCR products were analyzed in 1% agarose gels **(**Fig. 2**).** The Nested PCR results showed positive identification of the isolates with the amplification of an approximately 400-bp fragment (Fig. 2), confirming the presence of *T. marneffei.* The sequencing of the 380-base pair PCR product was performed using the Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, USA). The NCBI BLAST program (http://www.ncbi.nlm.nih.gov) was used and showed a 98.98% identity to T. marneffei.

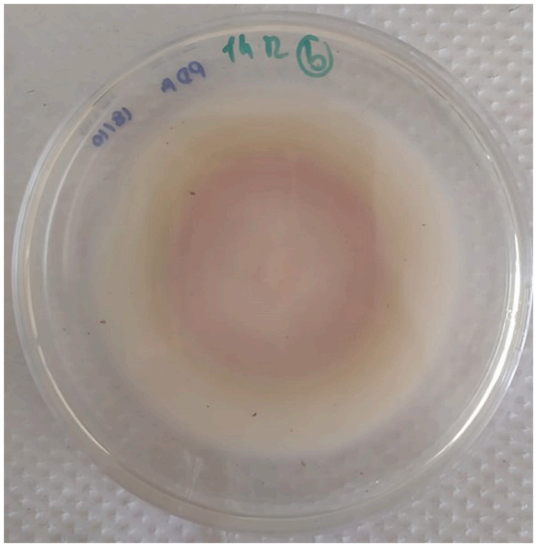

**Fig. 1.** *Talaromyces marneffei* with a characteristic red pigment in PDA medium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

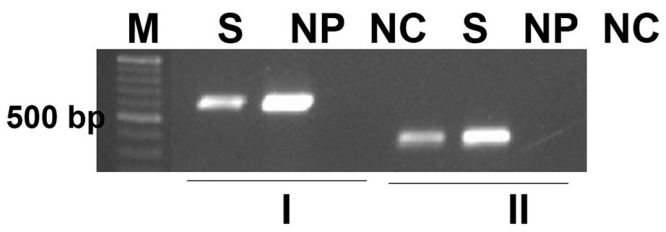


**Fig. 2.** Nested-PCR specific for *T. marneffei.*: An approximately 600 base pair product (I) was amplified with the of RRF1 and RRH1 primers and an approximately 400 base pair product (II) was amplified with the of RRF1 and RRH1 primers (S1: sample; PC: Positive control; NC: negative control; M: Molecular Weight Marker (Gene RulerTM DNA Ladder Mix (Thermo Scientific®)).

Detection of *T. marneffei* is well documented among HIV human patients, which illustrates the higher susceptibility of immunocompromised individuals to this fungus (Zheng et al., 2015). In domestic animals, *T. marneffei* has already been isolated in nasal swabs, collected from outdoor dogs in Thailand (Chaiwun et al., 2011). Moreover, a case study reported pulmonary penicilliosis with concomitant Canine Distemper Virus Infection in a dog in Brazil (Headley et al., 2017). Regarding wild hosts, the occurrence of this agent is scarcely reported. However, it was already reported in some rodents (*Rhizomys sinensis*, *Rhizomys pruinosus*, *Rhizomys sumatrensis and* and *Cannomys badius*) in China, which are mentioned as reservoirs to humans (Cao et al., 2011). In Portugal, this fungus has been previously detected in wild mammals; particularly on the skin of another carnivore, the Egyptian Mongoose (*Herpestes ichneumon*) (Matos et al., 2019). These findings in very distant countries (Asia, South America and Europe) and species show a wide geographical distribution, as well as a high adaptation of this pathogen to different hosts and environments, which reinforces a public health concern. People in close contact with wild carnivores (as martens or mongooses), as veterinarians or other nature conservation and health professionals, have a high risk of being infected when handling infected samples and tissues (Garland-Lewis et al., 2017).

The detection of *T. marneffei* in this wild carnivore suggests a sylvatic life cycle of this pathogen that may contribute to the maintenance of this disease in Portugal. Nevertheless, the importance of beech marten as a reservoir of *T. marneffei* and the possibility of future disease outbreaks with this origin requires further research and disease surveillance. To the present date, human penicilliosis has not been reported in Portugal, even in immunocompromised patients.

Lung, kidney and brain samples from this animal were positive for *Mycobacterium avium* subspecies *paratuberculosis,* in both culture and PCR, as it was previously published by these authors (Matos et al., 2014). Coinfections of *T. marneffei* with another opportunistic infection are possible. A dog with concomitant infection with Canine Distemper Virus was described in China (Headley et al., 2017). In human medicine, a disseminated coinfection of *T.marneffei* and *M. avium* has also been reported (Su et al., 2019).

To the authors' knowledge, this is the first microbiological and molecular evidence of *T. marneffei* isolated in beech marten worldwide. Adding this new wild host to the list contributes to a better knowledge regarding this fungus epidemiology. This work also represents the first case of *M. avium* subsp. *paratuberculosis* and *T. marneffei* concomitant infection in wildlife worldwide.

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## Authors' contributions

ACM and CJB both equally contributed to this work. All authors have read and agreed to the published version of the manuscript.

## Declaration of Competing Interest

The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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