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Letter to the Editor

Response to Pr L. Mendoza and coll.

We would like to thank Mendoza and colleagues for their constructive remarks on our article (Bernheim et al., 2019). We provide responses to their comments below.

"The diagnosis was made through molecular methodologies, but no DNA sequences were deposited at GenBank". Indeed, considering the paper format (medical image), there was no specific query about accession numbers. Since it is important to prove the lack of ambiguity in the molecular identification, we deposited the sequences in GenBank: accession numbers *Pythium* MN148708 and *Pythium* MN148707. These numbers have also been added to the article.

" . . . the isolate was not deposited at a culture collection". We do not completely understand this point, because depositing a strain in a culture collection is not required for any infectious agent when publishing an infectious disease case report. Nevertheless, we have kept the strains in our private strain collection.

" . . . there was not a description on how the isolate recovered from BHI was finally identified as *P. insidiosum*". Again, the paper format did not allow us to give a detailed description of all identification procedures. However, molecular identification was performed directly on the corneal biopsy and then on the strain isolated from brain–heart infusion medium (BHI). Both molecular results provided non-ambiguous identification of *Pythium insidiosum*. The sentence was modified to "The corneal biopsy specimen and the mycelium extracted from BHI were, therefore, sent for panfungal PCR."

Mendoza et al. cite two recent articles on *P. insidiosum* and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), (Krajaejun et al. 2018; Mani et al., 2019). We admit that we missed this most recent literature on the subject. We added these two articles to the references list and provide a short sentence to cite and discuss them.

The study by Krajaejun et al. assessed the utility of MALDI-TOF MS for the identification of *Pythium* with an in-house database from 10 strains (Krajaejun et al., 2018). They applied the exact same protocol as used by us for our strain, which corresponds to the strict procedure for creating a main spectral profile Main Spectra profiles (MSP) provided by Bruker for the MALDI Biotyper. They concluded that "any clinical microbiology laboratories equipped with a MALDI-TOF MS can supplement the mass spectra of *P. insidiosum* into their databases for the identification of this pathogen . . . " Using the same MSP protocol, we did this with our strain, but considering that the in-house database is not available for others laboratories, we also implemented the spectra in the MSI database, which is a freely available, regularly updated database (https://msi.happy-dev.fr/). Indeed, these 24 spectra might not be fully effective for optimal identification, because they correspond to only one strain, as discussed by Mendoza et al.

Mendoza et al. have recently published a large work involving another in-house database from nine isolates (Mani et al., 2019) . They tested this with 43 strains of different origins and showed that there was no cross-reaction with other *Pythium* species. When comparing the reference spectra to one another, seven strains cross-reacted correctly with other strains but two did not, which suggests that if these two MSP had been the only ones in the database, the other strains would not have been identified properly. We agree with the statement that the higher the number of strains in a database, the better the correct identification of the microorganism achieved. However, we believe that in microbiology laboratories, even one strain (24 spectra) is better than nothing; microbiologists are well aware of the risk of false-negative results when a database is poorly implemented and they deal with this limitation every day. Even the Bruker Filamentous Fungi database and the National Institutes of Health (NIH) database propose some species identification with the spectra of only one reference strain. For example, there is only the spectra from one strain in the NIH database for *Fonsecaea pedrosoi*, the agent of chromoblastomycosis. A sentence was added to the article to clarify this point for the users of MSI: "Of note, because the 24 added spectra correspond to one strain only, and because of geographical strain diversity, the users of the MSI platform must be aware that it will not provide a 100% sensitivity for *P. insidiosum* identification."

Also, if no identification is given with the spectra of another *Pythium* strain on MSI, this negative result does not stop or delay the process for identifying the agent, because a negative result on MALDI-TOF MS usually leads to sequencing. So we truly think that although not optimal, providing free reference spectra on the MSI platform (achieved with the same protocol as used by Bruker for MSP creation), can be of help in some cases, even if not in all cases. We added a sentence suggesting the limitation of the reference spectra (see above). Of note, none of the spectra generated by Krajaejun et al. and Mendoza et al. have been made freely available for any laboratory throughout the world, but we are open to internal spectra exchange between our laboratories to test our respective databases.

Finally, this case report aimed to inform readers of pythiosis, in particular its atypical clinical and microbiological presentations, and to highlight new treatments and the approach to identification; the focus was not on MALDI-TOF MS technology alone.

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Ethical approval

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Conflict of interest

No conflict of interest to declare.

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