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3 FACTORS INFLUENCING THE AMPHIBIAN MYCOBIOME, WITH A
4 FOCUS ON KNOWN AND UNKNOWN CHYTRIDS

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MRES PROJECT PROPOSAL

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SUPERVISOR:

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PROF MATTHEW FISHER

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IMPERIAL COLLEGE LONDON

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matthew.fisher@imperial.ac.uk

12 **Keywords:** Chytrid; Amphibian; Mycobiome; DNA barcoding; Fungus; Novel lineages

13 **1 Introduction and Proposed Questions**

14 Chytridiomycosis, caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), has
15 been linked to a presumed 90 extinctions and the decline of over 500 more species in the past
16 decades (Scheele et al. 2019). Given the discovery of *Batrachochytrium salamandrivorans* (*Bsal*), a
17 more recently emerged, highly virulent pathogen that is the sister taxon to *Bd* (Martel et al. 2013), and
18 the prediction that more than 92% of fungal species are yet to be described (Hawksworth and Lück-
19 ing 2017), it is likely that there are other undiscovered chytrids that parasitise amphibians. These
20 are likely to be phylogeographically constrained endemic species that become tomorrow's emerg-
21 ing infections. Conversely, evolutionarily-distinct lineages with superior competitiveness may provide
22 protection against virulent strains, such as *Bd* and *Bsal*, through competitive exclusion (Hardin 1960).

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24 In the past few years, there has been an increase in research on the amphibian skin microbiome, for
25 example that by (Bates et al. 2018) and (Bates et al. 2019) but little work specialising on the fungal
26 communities present on amphibian skin, the mycobiome, and there has been no study on the factors
27 influencing the diversity of the amphibian mycobiome globally. A global fungal composition analy-
28 sis of the fungi that have evolved the ability to colonise, or even infect, amphibian skin would allow
29 the association between different commensal fungi and *Bd* and *Bsal* to be determined for the first
30 time. Negatively associated species may be evidence of niche exclusion (Hardin 1960), and posi-
31 tively associated species may represent secondary infections as a consequence of chytridiomycosis-
32 associated dysbioses. This evidence could provide important groundwork for future conservation and
33 novel promycotic treatments if strong negative associations are found.

- 34 • *Which factors influence the amphibian mycobiome on a global scale?*
- 35 • *What are the associations between *Bd*, *Bsal* and other fungal species in the amphibian myco-*
36 *biome?*
- 37 • *Are novel chytrid species a key link to providing a defence against virulent evolutionarily-distinct*
38 *lineages or do they themselves pose a threat of becoming virulent in the future?*

39 **2 Proposed Methods**

40 Over the past decade, amphibian skin swabs and environmental abiotic data have been collected
41 for a variety of species and life stages from all over the world, allowing an analysis of the amphibian
42 mycobiome to be done on a global scale for the first time. Using these processed data, it can be
43 determined which commensal fungal species are present on large numbers of amphibian species
44 from different populations and whether we can also detect unknown fungal species that may be
45 pathogens. After accounting for potential autocorrelation and multiple comparisons, patterns can be
46 identified and tested using statistical analyses in terms of possible influencing biogeographic factors,
47 such as altitude, temperature, longitude and latitude etc. to deduce which factors better influence
48 the composition of fungal species in an amphibian's mycobiome. These models will then be used to

generate plots and GIS maps of locations of mycobiome similarity. The spread of *Bd*, focusing on the novel lineages, will also be looked at using GIS. The R package *cooccur* (Charles J. Marsh Daniel M. Griffith 2016) will be used to analyse cooccurrence between fungal species and *Bd* to evaluate which fungi are positively and negatively associated with *Bd*. Some of the unidentified fungal species could be unknown species of chytrid. Novel chytrid lineages will be identified through sequence alignment against pre-existing datasets in NCBI and by building phylogenetic trees in order to compare the candidate sequences with those of other chytrids.

3 Anticipated Outputs and Outcomes

Statistically and biologically significant patterns in the mycobiome data will be determined in order to confidently predict which factors most influence the amphibian mycobiome. This will give an overview of the global amphibian mycobiome and help to understand why certain amphibians are affected by *Bd* and *Bsal*, both in terms of abiotic factors and in terms of the other fungal species that are present. Identification of previously unknown chytrid species that may act as promycotics, causing niche exclusion of a competing virulent species/lineage through prior occupancy, could contribute to pre-emptive conservation work against unknown virulent chytrids.

4 Project Feasibility

All data has already been collected using MiSeq 2x300bp v3 chemistry sequencing and almost all has already been processed and is ready for analysis. The time period for analysis and write up should be adequate to generate possible answers to all three of the above research questions.

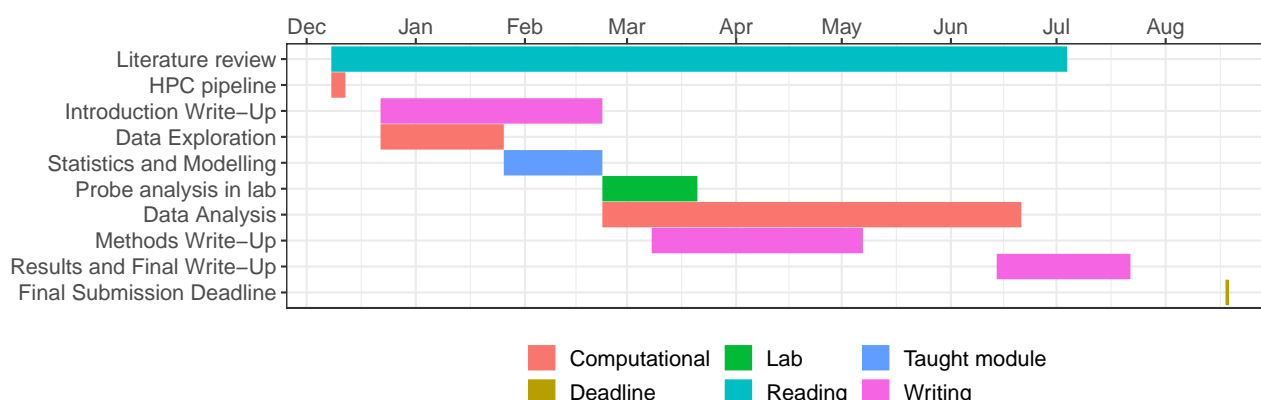


Figure 1: Proposed Project Timeline

| Budgeted Item | Cost | Reasoning |
|-----------------------------------|-------------|--|
| Herpetofauna Workers Meeting 2020 | £250 | Herpetological conference cost and accommodation |
| qPCR Reagents | £175 | To perform <i>Bd</i> qPCR in the laboratory |
| Laptop Adaptor | £75 | To enable me to use USB sticks, HDMI etc. with my laptop |
| Total | £500 | - |

Table 1: Proposed Project Budget

68 **References**

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86 **5 Approval**

87 I have seen and approved the proposal and the budget.

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