Report Checklist accompanying the application for Derogation Under the European Communities

1. General context:

The fungus *Pseudogymnoascus destructans (Pd)*, a psychrophilic fungus, is the causative agent of the White-Nose Disease (WND; associated with the White-Nose Syndrome in North America). WND has caused of one the most extensive and sudden morality in bats in North America. In Europe, Pd does not seem to cause mass mortality but it causes the disease, diagnosed with histopathology, which can have negative consequences on the bats. Pd has been introduced from Europe where the species is native, to North America. So far, Pd has been identified in 26 countries in Europe. The presence of the species across Europe, including in the UK, and the high climatic suitability of Ireland to the development of the disease (Blomberg et al. 2023) suggest the fungus is present in Ireland. We herein propose to search for the fungal pathogen in Irish bats, and characterise its link with other European Pd populations (via full genome sequencing). This work will bring information on the presence (or absence of evidence) in Irish bats and if the fungus is identified, it will allow the population to be genetically characterised to investigate potential movements of the fungus (via bats/humans) with neighbouring countries (UK, France) from where we have already collected samples. Based on our current sampling across more than 260 sites across 26 countries in Europe, the fungus has a very strong population structure with nearly no movements at scales greater than a few tens of kilometres (even on land). Such strong population structure means that the fungal populations are not mixing too much. However, large phenotypic leaps leading to host jumps and increased virulence can be precipitated by anthropogenic mixing of previously allopatric fungal populations; i.e., when two different populations within the same species, are brought into contact and start exchanging gene (Fisher et al. 2020; Stulenbrock 2016; Voelz et al. 2013).

Hence our work would be a key element to avoid transporting fungal pathogens between countries and hence, if our study confirms the presence of Pd in Ireland, it would permit the proactive setup of regulations to avoid such movements of pathogens. Therefore, our application falls under the reason (d) listed in Regulation 54, more specifically, with an objective "***For the purpose of research and education, of re-populating and re-introducing these species and for the breeding operations necessary for these purposes, including artificial propagation of plants***". We aim to investigate the presence of a fungal pathogen in Irish bats, which are listed in Annex IV of the Directive Habitat. Besides investigating its presence, we will also investigate how much genetic exchange there is with neighbouring countries, a key element to decide on preventive measures to put in place to avoid cross-border pathogen movements, and its potential negative consequences (cf. general context above).

2. No satisfactory alternatives exist

We do not see satisfactory alternatives to achieve our goal. Alternatives considered are:

1-Alternative 1: bats could be sampled during the summer time when they are active. However, the fungus is psychrophilic and does not grow on bats during their active season as their body temperature is above what the fungus can tolerate (ca.18-20°C). Out of thousands of bats tested in the summer (in North America and Europe), only a few of them carried traces of DNA identified as Pd (identified via qPCR), meaning that it is extremely challenging to work with active bats (see also alternative 4 below).

2-Alternative 2: bats could be sampled during the autumn time when they are active and when they get infected by the fungus at their swarming/hibernacula site. However, many teams have attempted to cultivate the fungus from samples taken at this period and have failed (including our team). This is probably either because bats get infected later in the season or because there are so few spores on the bats that we cannot recover them. Indeed,

bats also have many other fungi and bacteria on them and because Pd grows slowly, other fungi/bacteria often grow first and unless the sample has good quantities of Pd (as during the late winter), it becomes very challenging to detect it.

3- Alternative 3: walls from bat hibernacula could be sampled to try to isolate Pd. This work is extremely challenging given that there are several hundreds if not thousands of fungi species growing in underground sites (Vanderwolf et al. 2013) and these are often faster growing compared to Pd (and also orders of magnitude more numerous). For this reason, unless there is a high density of bats at hibernacula (which is not the case in Ireland [besides *R. hipposideros*]), this alternative is bound to fail. Ourselves and several teams have learned this the hard way.

4- Alternative 4: because of the difficulty in cultivating Pd from the environment, we could work with genetic identification (including qPCR). Although several qPCR or LAMP assays have been published to discriminate Pd from its close relatives (including one designed by us; Niessen et al. 2022), there are tens of yet undescribed species of *Pseudogymnoascus* in underground environment and it is not yet possible to differentiate a false positive from a true positive in such case. Only the sequencing of several genes (or the full genome) of a live isolate can be affirmative (as we propose to do in our work). Besides, only knowing that Pd is present, as would be obtained via qPCR, would not allow us to elucidate potential gene flow with neighbouring populations.

Therefore, altogether, we conclude that no satisfactory alternatives exist to achieve our goal.

3. The Derogation would not be detrimental to the maintenance of bat populations.

The first step of the method is to search for hibernating bats. This is carried out exactly as per the classic hibernacula counts where a limited number of people (classically 2-3 depending on the hibernacula size) search for hibernating bats. This is done swiftly as quietly as possible and lighting of the bats is reduced to the strict minimum (to identify the species and count individuals). When done by experienced people, this step does not negatively affect hibernating bats. The applicant has >20 years of experience with counting hibernating bats in several countries (e.g. Germany, France, Poland), going from small to large hibernacula (>30,000 bats), in a variety of environment such as cellars, mines, caves.

Once a bat is located and reachable, it is left free hanging (no handling of the bat is needed) and the sampling simply consists in gently and lightly swabbing the ears/wings with a sterile swab (a new swab is used for each bat). This step takes a few seconds for a trained person. The applicant has extensive sampling experience with more than 2,000 samples collected since 15 years with this technique. This technique has been successfully implemented in over 26 European countries as part of an ongoing project to study White-Nose Disease across the continent. This initiative was developed following Resolution 6.6 of the 6th Session of the Meeting of Parties of EUROBATS (EUROBATS, 2010). We have extensively evaluated the potential impact of this method on bats at one hibernacula in Germany whereby we compared acoustic activity inside the hibernacula, in and out of the hibernacula (with light barrier), and activity of sampled bats (with camera traps and thermal cameras) and have not identified any measurable effect. The swabbing is swift and gentle and seem to mimic the contact between bats as it if often happen during hibernation. Therefore, the hibernation patterns of bats do not seem to be affected by the sampling.

Altogether, there is substantial evidence that the proposed project would not be detrimental to the maintenance of bat populations.

4. Biosecurity measures.

To avoid contaminations and transporting microorganisms, only sterile swabs and tubes will be used for sampling. Once collected, swabs are placed in a sterile tube that is closed with a cap lock system. Tubes are then placed in a zip-bloc bag until analysed in the lab. Other

equipment used during the sampling (e.g. clothes, shoes, etc.) will be cleaned to avoid transporting microorganisms. With respect to Covid-19, recommendations in place at the time of sampling will be strictly followed.

References:

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