Lichenized fungi, known as lichens, consist of two main partners creating a lichen thallus. Each thallus forms a separate ecosystem. The mycobiont (heterotrophic fungi) is the partner that shapes the thallus, while the autotrophic partner is called the photobiont. Mycobionts are usually fungi of the *Ascomycota* group. The photobiont can be green algae or cyanobacteria. The thallus can also be inhabited by other groups of fungi, including endolichenic and lichenicolous fungi, known as the mycobiome. In addition, the thallus of lichens contains populations of bacteria that form the so-called microbiome. Therefore, current research on lichen symbiosis considers the impact and functioning of a complete holobiome, also known as a "mini ecosystem".

The main reproductive strategies of lichens are sexual reproduction and propagation via vegetative propagules – isidia or soredia. In the first case, only the mycobiont is capable of sexual reproduction, producing characteristic structures called apothecia. They consist, among others, of the hymenial layer, in which we distinguish spore-producing sacs (asci). The dissemination of spores then results in the need to select a new photobiont. The process of establishing a new relationship and forming a new thallus is called relichenization. Whereas propagation via vegetative propagules ensures the continuity of the symbiotic relationship, as it allows the dispersal of both symbiotic partners. This is because soredia are photobiont cells intertwined with mycobiont hyphae, and isidia are special elongated structures placed on the thallus containing both partners.

So far, it has been proven that soredia allows the dispersion of mycobiome - additional fungi, along with mycobiont and photobiont, i.e. the basic components of the lichen symbiosis. However, these studies were conducted under strictly controlled laboratory conditions (*in vitro* studies). Furthermore, there are reports indicating that the surfaces of apothecia can be colonized by lichenicolous fungi (part of the mycobiome). This report is based solely on the examination of external structure (morphology) using a stereoscopic microscope. **Therefore, the project aims to compare the mycobiome of the entire lichen thallus with the mycobiome of soredia and the hymenium.**

The study will focus on two model species from the genus *Ramalina*, which exhibit different reproductive strategies. *Ramalina farinacea* primarily reproduces through soredia (asexual strategy), while *Ramalina fraxinea* mainly develops structures of sexual reproduction - apothecia (sexual strategy).

Current studies on lichen mycobiome present many uncertainties. There is a lack of a synthetic approach addressing the mycobiome structure of two coexisting lichen species in a broad environmental context. **Therefore, another aim of the project is to examine the variability of the lichen mycobiome in relation to reproductive strategies and the anthropogenic and latitudinal gradient of *R. farinacea* and *R. fraxinea* in Poland, considering local environmental conditions.**

Another important aspect of the project will be studying the dependence between the communities of mycobiome and photobionts. A frequently observed phenomenon in lichens is the replacement of the photosynthetic partner (photobiont) with a better-adapted one to local environmental conditions. It is known that a change in the photobiont within a lichen may be accompanied by a shift in the population of bacteria associated with the lichen thallus. However, the context of the mycobiome in this case is still unclear. Therefore, in this study, the community of lichen photobionts of *R. farinacea* and *R. fraxinea* will be evaluated, to be able to compare the ascending changes in both the composition of green algae and mycobiome. A sampling design that comprises an anthropogenic and latitudinal gradient will allow us to delimit if, in case of photobiont shifts depending on different environmental conditions, the mycobiome composition also undergoes modification.

The above goals will be achieved by the application of a metabarcoding approach with the high-throughput amplicon sequencing. In this case, a fragment of the ITS rDNA region, commonly used to identify fungi and green algae. This approach will allow simultaneous amplification and identification of different fungi and algae present in the lichen thalli.

The above research proposal will contribute to the general knowledge of the lichen mycobiome and their photobionts' diversity in the context of two different species of *Ramalina* and changes in its composition under the influence of ecological conditions, including anthropogenization. Most importantly, it will indicate whether the studied reproductive strategies allow for the spread of mycobiome parts associated with the lichen thallus. The project will provide insight into the dependence of lichen mycobiome on photobiont present in the lichen thallus, which is important from the perspective of complete lichen mini-ecosystem functioning. The estimation of the general diversity of specimens in the *Ramalina* population, their mycobiome, and photobionts under different ecological conditions will be suitable for further predicting the species' threats. Also, it will significantly impact the knowledge about habitat success and the welfare of the species.