Biodiversity is not always accessible to naked eyes, even in the tropics, and lichens (often called lichenized fungi) belong to this group of organism, which in many cases can be easily neglected. Lichens are ecological group of fungi, which evolved a symbiotic life with eukaryotic (mainly green algae) and/or prokaryotic (cyanobacteria) photosynthetic organisms (photobionts). They play an important role in ecosystems serving as food for animals or a living place for invertebrates. Lichens are also used by humans as a source of biologically active molecules. Only the fungal partner reproduces sexually, by forming meiotic spores, while the photobionts reproduce only asexually through cell division or mitotic spores. Since the middle of XIXth century, the type of spores of the fungal partner and other anatomical characters such as type of sporocarps are used to distinguish genera and families in many of fungi, however numerous homoplasic characters are among them, therefore different molecular approaches are recently used for the separation of taxa of different rank. Most lichens belong to Pezizomycotina, phylum Ascomycota, where also truffles are classified. These lichens are characterized by meiotic spores (ascospores) produced in protective ascomata (apothecia, perithecia or pseudothecia), or more rarely directly in the thallus. Only few lichens belong to the phylum Basidiomycota where fungus forms meiotic spores (basidiospores) on basidiomata of different shape and structure (e.g., mushroom-like structures similar to edible fungi). In both cases, sporocarps, and even meiotic spores, may be missing and then the thalli are said ‘sterile’, but it usually occurs when the lichen species produce asexual diaspores, very often associating the fungus and the photobiont and thus propagating the symbiosis. The most common are soredia and isidia, but also other types of asexual structures exist (e.g. conidia, blastidia). In numerous lichens the production of asexual propagules is constant, while meiotic spores are only occasionally produced, e.g. in Lecanora expallens, Fuscidea lightfootii, Ropalospora viridis. On the other hand, some lichens only rarely form soralia and isidia, probably as a response to environmental factors: however, the reason for asexual propagules initiation remains poorly studied.

In some lichens meiotic spores and the sexual processes completely disappeared during the evolution and they are exclusively sterile. The best known examples are the worldwide widespread and common genera Botryolepraria, Lepraria and Leprocaulon, which have never been found with apothecia. It is often considered that their adaption to special habitats (usually places sheltered from rain and direct water flow) may encompass the loss of sexual reproduction and spore dispersal. Similarly, most members of the strictly tropical genus Herpothallon reproduce exclusively by asexual propagules.

The absence of ascomata or basidiomata entails the loss of useful identification criteria and often difficulties in the proper evolutionary placement of sterile lichen species in the fungal tree of life. In fruticose and foliose lichens, several morphological and anatomical characters of the thalli that enable identification remain available. However, in sterile crustose lichens, which thalli are much poorer in easily definable characters, morphological tools reach their limits. In such cases, the secondary chemistry was of great taxonomic help, since some of such species differ in the combination of their secondary metabolites (called ‘lichen substances’). However, some species are not determinable without sporocarps, as they contain the same lichen substances (or no such metabolites at all) and their thalli do not differ from each other. Thus, in sterile crustose lichens, identity in morphology and lichen substances does not necessary entail taxonomic identity.

Sterile lichens with crustose thalli were always considered as difficult group in all type of lichenological studies and were very often omitted or neglected by lichenologists. They were mostly studied in Europe, and numerous species new for science were discovered in several lichen genera, based on differences in secondary chemistry and/or in molecular markers used in phylogenetic analyses. North American lichens also received much attention recently, resulting in the discovery of many new taxa as well.

Little is known, however, about sterile crustose lichens in the tropics, including Central and South America, and despite several recent revisions the knowledge of their distribution remains fragmentary. The genus Lepraria is the best studied and several taxa were recognized, new to science or to South America or Southern Hemisphere. The genus Herpothallon (order Arthoniales, class Arthoniomycetes) also received much attention recently, although it may comprise several phylogenetically unrelated lineages, as recently proved for some species. Some other papers also contain reports of these lichens, but usually single records have been provided.

Sterile lichens usually produce asexual diaspores jointly propagating fungal and algal or cyanobacterial bionts, that is an usual feature nowhere else found in the entire fungal world. In the majority of the associations, the phycobiont belongs to the genera Trebouxia or Trentepohlia (green algae) or Nostoc (cyanobacteria). About 85% of lichen-forming fungi associate with green algae, however, these associations are still poorly understood and numerous groups of photobionts have not been identified or thoroughly studied. For example, photobionts have been identified in only a few lichens and in even fewer lichens, the whole range of compatible photobiont taxa has been investigated. Tropical lichens are poorly studied with respect to their photobionts, so that a limited number of publications is available. Nevertheless, previous studies suggest that lichens with trentepohlialean algae are common in humid and shaded undisturbed tropical forests.

Bolivia is one of the countries with the highest biodiversity in the world mainly due to presence of the Andes, which possess an amazing variety of suitable habitats for lichens, including epiphytes in the different types of forests. Amazonia also has great importance as it offers still rather undisturbed and continuous forest areas. Bolivian lichen biota was rather poorly studied, until the record of 150 species in 1998, and at present only ca. 1300 species are known (http://bio.botany.pl/lichens-bolivia/english.html), although up to 4000 taxa are expected in this area. Numerous new species were recognized during the last years, emphasizing the uniqueness of Bolivia to investigate tropical lichen diversity.

A large part of Bolivia is still untouched by human activities, but many areas of Bolivia are disturbed by agricultural management, and several forests are disturbed by grazing or plantations. In all of these areas, lichens were found, including sterile crustose species. However, only few could have been hitherto determined due to insufficient morphological characters, so molecular methods will be essential for further study of this difficult group. Accurate species delimitation is crucial for ecological and conservation studies, and to identify factors driving diversification. Thus we plan to conduct our research in Bolivia as its biodiversity is among the greatest and most poorly known in the world and we want to explore the hidden diversity of sterile crustose lichens in Bolivia The goal of the project is to use Bolivia as a first model to unravel tropical diversity of sterile lichens, with the target of protection of biodiversity in general.

We combine different approaches to study this difficult group of organisms. Morphological and chemical analyses of specimens will be conducted in order to develop essential ITS nu-rDNA barcodes to allow rapid determination of morphologically similar or indistinguishable species, as well as cryptic species. The use of ITS, a widely accepted DNA barcoding marker effective for fungal species identification, is promising. The obtained sequences can be incorporated into similar inventories in other Neotropical regions and stimulate further studies of these difficult and neglected, but widespread, ecologically important and rich in secondary metabolites organisms. Considering the symbiotic nature of lichens, we also want to document the photobiont diversity in selected neotropical sterile crustose lichens.

Concluding, it is assumed, based on our previous studies, that a large lichen biodiversity in Neotropical forests implies a large variety of sterile crustose lichens. The project intends to verify this hypothesis by answering the following questions:

1. What is the level of the biodiversity of sterile crustose lichens (within and between species and different ecosystems in the Neotropics?
2. What is their diversity in disturbed forests as compared to natural, undisturbed forests?
3. Is ITS barcoding suitable and effective for rapid identification of tropical sterile crustose lichens?
4. Which of the morphological and chemical characters are homoplasic versus apomorphic, and thus sufficient for species identification of sterile crustose lichens?
5. How often did cryptic speciation take place in sterile crustose lichens, and do the cryptic lineages differ in ecology?
6. Do the photobionts of sterile crustose lichens genetically differ between different ecologies (e.g., elevation above sea level)?
7. Are species with wide ecological amplitude homogeneous in photobiont or do they undergo local adaptation by way of partner change?