1. Research project objectives/ Research hypothesis

Most of the insects show the highest activity in moderate temperature, however some show full activity in temperatures close to freezing point. On the other hand certain insects are capable of withstanding extremely high temperatures. In the course of evolution, insects exposed to low temperatures developed number of adaptations which allow to survive in unfavorable thermal conditions. For example, they synthesize cryoprotectants, such as polyols and amino acids, proteins inoculating ice, so called ice nucleating agents (INAs) or antifreeze proteins. Moreover, other adaptations may include changes in metabolic pathways and differences in protein expression such as heat shock proteins (HSP) or aquaporins (AQP). On the cellular level these mechanisms protect cells from rapid environmental changes. At present, relatively much is known about cryoprotective mechanisms in insects from temperate zones or subpolar, while little is known about cryoprotective mechanisms in insects from tropical zone. The proposed project aims to examine the effect of low temperatures on physiology of tropical cockroach *Gromphadorinha coquereliana* and define cryoprotective mechanisms occurring in this insect. In the proposed project we hypothesise that:

1. Cold stress induces changes in protein expression, including heat shock proteins (HSP) and aquaporins (AQP) level.

2. Cold stress modify enzymes activity of key metabolic pathways and affects the respiration rate (RQ) and energy production by mitochondria.

3. Cold stress generates oxidative stress.

2. Research project methodology

In the project it is planned to study the effect of low temperature (4°C) on physiological processes of tropical insect *G. coquereliana*. This species living in tropical climate of Madagascar is periodically exposed to low temperatures (2-3°C for 2-3 h/day). The effect of cold will be studied on adult males and the research will be conducted on fat body (physiological equivalent of fat tissue and liver of mammals), gut (involved in regulation of water and electrolyte homeostasis) and leg muscle. The effect of low temperature on metabolic activity will be determined spectrophotometrically by measurements of key metabolic pathways enzymes such as phosphofructokinase, citrate synthase and β-hydroxyacyl-CoA dehydrogenase. Moreover, studies aiming to specify the respiratory rates (respiratory quotient, RQ) and mitochondrial activity to determine the utilization of energetic substrates as energy source during thermal stress are planned. In the tissues tested the total protein profile will be analyzed using 2-D electrophoresis combined with mass spectrometry to identify potential cryoprotective factors. The negative influence of environmental factors on organism is usually related to oxidative stress and for this reason it is planned to examine the level of reactive oxygen species and lipid peroxidation in tissues in the response to cold stress. Additionally, the analysis of activity of antioxidant enzymes as superoxide dismutase and catalase will be conducted. Proteins which have an important role in response to cold stress are aquaporins (AQP) and heat shock proteins (HSP). Thus, in the project we plan to determine the changes in expression of particular isoforms of these proteins at mRNA and protein level using qPCR and Western Blot methods, respectively.

3. Expected impact of the research project on the development of science, civilization and society

The results of proposed project will allow to determine the biochemical and physiological changes occurring in tropical insect in response to cold stress. Such studies were not performed so far, thus they will have significant impact on basic knowledge about cold resistance. The obtained results will allow to understand better the cryoprotective mechanisms in insects as such. The knowledge about antifreeze mechanisms obtained in this project may be in the future applied as method of food preservation or treatment of hypothermia. Nowadays, studies on use of substances produced by organisms resistant to cold regarding food preservation, organ transport for transplantation and storage of stem cells are already performed