

Symbioses, defined as close, long-term relationships between unrelated organisms, have played a crucial role in the evolution of life on Earth. Since their origins, Eukaryotes formed a variety of associations with other organisms, which often significantly influenced their biology. Symbioses involving microorganisms, especially bacteria, have left a particularly deep trace in the history of our planet. However, current knowledge about the diversity, dynamics, evolution, and function of symbioses remains quite limited.

The proposed project focuses on the symbioses of Auchenorrhyncha, a diverse group of true bugs that includes cicadas, spittlebugs, leafhoppers, treehoppers, and planthoppers, including species of high economic importance. Auchenorrhyncha host some of the oldest and most specialized symbiotic organisms known to science. It is thought that approximately 300 million years ago, **the common ancestor of all Auchenorrhyncha was colonized by two different bacteria**. By producing amino acids and vitamins that were lacking in plant sap (which contains little nutrients other than sugars), these bacteria enabled the adaptation of host insects to feeding only on this unbalanced food. That way, symbiotic bacteria became indispensable for their hosts, but at the same time, they lost their ability to live outside host tissues. The resulting symbiotic association became obligatory for all partners.

However, in the long run, this relationship was often unstable. As shown by microscopic studies and older works based on sequencing, **in many groups of Auchenorrhyncha, one or both of these ancient symbionts have been supplemented or replaced by other microorganisms**. Many of these substituting bacteria and fungi are related to the known reproductive manipulators of insects as well as to the pathogens of insects, plants, and even humans. It seems, however, that in Auchenorrhyncha, these different microorganisms have converged towards providing the hosts with essential nutrients. **This raises many questions about the causes and mechanisms of symbiont replacements by other microbes, as well as the biology and evolution of substituting microbes**. Among others,

- (a) In which groups of Auchenorrhyncha, how many times, and how long ago did symbiont replacements happen?
- (b) What is the relationship between the microorganisms forming new symbioses with Auchenorrhyncha and the known pathogens of plants and animals?
- (c) How did the symbiont swapping influence the biology and genomic evolution of insects and the new symbionts?

The goal of the proposed project is to answer these and similar questions about the biology of Auchenorrhyncha and their symbionts, and reconstruct the patterns and mechanisms relevant to the broad spectrum of symbioses. **The project plan includes the acquisition of a large number of Auchenorrhyncha specimens from around the world**, from existing collections, through collaboration with other projects, as well as field sampling in Poland, Sweden, the U.S.A., and Madagascar. **Thousands of representative individuals for different populations will be characterized using next-generation sequencing**, an efficient and quickly evolving technology that can provide vast amounts of information about genomes at a relatively low price. Detailed data analysis will ensure precise reconstruction of the relationships among the collected specimens. These analyzes will also explain in which branches of the phylogenetic tree of Auchenorrhyncha the symbiont replacements occurred, and where the replacing microorganisms originated from. Also, the project will provide information on the functions and evolution of Auchenorrhyncha symbionts and their impact on the host biology.

The implementation of the project will result **in a comprehensive picture of the relationships between the biology of Auchenorrhyncha and the properties of their symbionts**. The results of these studies will allow a **detailed description of the processes and mechanisms that determine relationships between partners in symbiotic relationships, with relevance for a wide spectrum of symbioses**. Because many Auchenorrhyncha play significant roles in agriculture and the ecosystem function, the results can affect the biological control and natural resources management strategies. In addition, many microorganisms inhabiting the tissues of Auchenorrhyncha belong to groups that influence the health of plants, insects and other organisms, including humans. The proposed research will provide comprehensive information on the biological and evolutionary potential of these groups. At the same time, **this project will enable the creation of a new research group by an experienced scientist interested in returning to Poland after more than ten years of conducting research abroad**. His expertise and the project resources would allow comprehensive training of young scientists in the cutting-edge genomics techniques and will contribute to the development of robotic infrastructure for high-throughput next-generation sequencing at Jagiellonian University.