Streszczenie rozprawy doktorskiej:

Understanding the innate fire of life – the metabolic rate, with its many sinks and drivers, has been the focus of researchers for many decades, both in basic and application research. Better understanding the underpinnings of metabolic relationships, such as the one tying the rate at which organisms metabolize resources and consume oxygen, with their body mass, is of fundamental interest in the fields of physiological and evolutionary ecology. This thesis undertakes to shed light on the many questions still at hand regarding metabolic scaling, using males and females of 19 species of ground beetles (*Carabidae*) as a convenient study system for their known phylogeny, a wide span of body masses and similar body plan and lifestyle. The studied beetles originate from a natural environment, and they were studied in the laboratory to measure their body mass, body composition, cell size and metabolic rates at rest.

What prevails in nature is a sub-linear increase of metabolic rates with body mass, which leads to negative allometry (a mass-scaling exponent $b < 1$). While studied extensively across many different taxa this pattern remains a hotly debated subject, with a range of theories on its origin. Among them is the idea that organisms match their cellularity in the whole body to meet metabolic demand and supply. Existing theories consider that having body made up of smaller cells increases the metabolic cost of maintaining large surface area of plasma membranes, but this cost can be counterbalanced by increased capacity of small cells to transport crucial molecules and exchange them with cellular environment. On the other hand, tissues built from large cells with the high-volume-to-area ratios, can benefit organisms as it pays to cut down metabolic costs of body maintenance. Unfortunately, data venturing into the mechanisms of this interrelation remains sparse. One of the reasons for this is the labore intensive nature of cell number and size assessment, which neatly introduces the first study in my thesis (Chapter I), where I have undertaken to develop, validate and distribute an algorithm for automated image analysis, based on which a pipeline was created, that served in collecting data in my next works in this thesis. My focus was on a fast and easy but still reliable method of measuring the area of ommatidia in the eye of beetles, which is regarded as a satisfactory cell size proxy of cells that form the ommatidia. After taking transparent replicas of the entire eye and flattening them on a microscopic slide, I took their images. Next, the images from different beetle species were assessed to create a robust automated binary image segmentation macro. This pipeline has been tested against manual measurements by trained human counterpart, achieving a 70x decrease in time expenditure, while not sacrificing reliability.

In chapter II I shifted my focus back to the sub-linear allometric relationship between metabolic rates and body mass, showing that it also exists in the studied beetles. I succussed in evaluating whether the decrease in mass-specific metabolic rates in larger species of beetles can be affected by disproportionately fast increases in body components with low or no metabolic activity, such as fat stores and skeletal material. Contrary to my initial expectations, across 15 species of Carabidae beetles involved in this analysis, the relative contribution of fat and the exoskeleton to body mass decreased, rather than increased in larger species. This indicates that, in beetles, the relative content of metabolically inert and active components does not stay invariant across species and larger species systematically become richer in body components that are metabolically active and poorer in body components that have low (fat) or no (skeleton) metabolic activity. Having said that, the findings of Chapter II do not support hypotheses that body composition is the main driver of the body mass to metabolic rate relationship, at least in the studied case of beetles. In other words, my results mean that after removing all metabolically inert components from the body mass, the negative allometry of

metabolic rate would not only not disappear but would become even greater. These findings further magnified the weight of the question of where the sublinear increase of metabolic rate with body mass originates from.

In Chapter III, I explore whether the energetic maintenance costs of tissue may explain this enigma. Based on the theory of optimal cell size, I hypothesized that with higher cost of maintaining the cross-membrane gradient and general cell membrane upkeep and remodeling, small cells will build more energetically wasteful individuals, due to the high ratio of cell surface area to cell volume/mass. And in reverse, I considered that larger cells should aid in forming a more frugal organism. So, if smaller species evolved smaller cells in a body compared to larger species (whatever was the reason for this evolution), the metabolic effects of cell size could account for the negative allometry of metabolic rates. Utilizing the tools created in Chapter I, as well as self-taught machine learning algorithms, I measured cell size in ommatidia and Malpighian tubules in different species of carabids, relating this variance to body mass and metabolic rates. The findings of Chapter III indicate that larger species and larger sex were characterized by larger cells in both organs. This shows the systemic orchestration of cell size and its contribution to the origin of evolutionary and sex differences in body mass. Beetles from larger species or larger sex had not only larger cells but also lower mass-specific metabolic rates, indicating that having larger cells in a unit of body mass coincides with less costly tissue. It is important to note such characteristics evolved in beetles representing both larger species and larger sex. Given theories used here, the low surface-to-volume ratio of large cells helps decrease the costs of maintaining ionic gradients across cell membranes, but why such strategy evolves in large and not small species is still an open question.

In summary, while my work does not support the hypothesis that metabolically less active body components explain the sublinear increases of metabolic rates with body mass in Carabidae beetles, I was able to show a clear indication of interspecific and intersexual drivers of this relationship in the form of potential metabolic effects of cell size variance. It is telling that all my analyses were performed with and without phylogenetic information, and both analyses were yielding similar results, indicating that the patterns I have studied here, and my conclusions, are free from bias caused by shared ancestry. It certainly remains to be seen and tested how universal are the patterns in cell size, body mass, body composition and metabolic rates in other taxonomic groups and levels. Finding definitive evidence tying cell size optimization with final developmental and evolutionary outcomes is an essential piece of the puzzle to influence future energetic research in both basic physiology and pre-clinical settings, and ultimately shed more light on the natural history of life on Earth.