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Project title: **The role of cell size in evolutionary and thermally induced changes in body size of ectothermic vertebrates**

Short visit to: Professor Jan Kozłowski
Institute of Environmental Sciences
Jagiellonian University,
Gronostajowa 7, 30-387 Krakow, Poland

Purpose of the visit and background

Change in body size - either evolutionary or as a consequence of phenotypic plasticity caused by environmental factors including temperature is one of the key topics of last decades. Studies of proximate mechanisms of body size change show that ectotherms living in different temperatures or latitudes often differ in cell size with or without effect on cell number. In vertebrate ectotherms for example fish reared in colder temperatures have larger erythrocytes than those from warmer temperatures (Van Voorhies 1996: *Evolution* 50: 1259-1264). Similarly, correlation between erythrocyte size and body size was found also at the interspecific level in the closely related lizards differing in body size (Starostová et al. 2005: *Funct. Ecol.* 19: 744- 749).

Therefore I would like to determine if evolutionary and thermally induced changes in cell size of ectothermic vertebrates (lizards) follow patterns documented for erythrocytes and to verify using erythrocytes as a proxy for cell size in general. The main goal of my visit to Professor Jan Kozłowski at the Institute of Environmental Sciences at Jagiellonian University was to develop together with him and experienced histologist Professor Wincenty Kilarski (Department of Cytology and Histology, Institute of Zoology, Jagiellonian University Krakow) methods of proper slides preparation for cell size and nucleus size measurements and also to determine suitable candidate cell types and tissues which will be suitable for the measurements of cell size.

Description of the work carried out during the visit

During my visit in Krakow we had at our disposal slides of different tissues (as many as we were able to sample) of two gecko species of different size – *Coleonyx elegans* and *Eublepharis macularius*. In the first days I learned how to recognize different structures typical for given tissues. We have selected tissue types suitable for our purpose of cell size measurements such as chondrocytes in trachea, cells of proximal kidney tubules, skin and hepatocytes. Later we developed and standardized technique of slides preparation and measurement of different cell types – i.e. measuring single cells or groups of cells. Standardization of the protocol is very important because it is planned to measure cell size of different tissues also in other ectotherms – other reptiles, fish or molluscs, which will enable us to compare results among many organismal groups. For visualization of cells we have tried fluorescence and confocal microscopy (staining for example with DAPI, fibronectin or phaloidin) as well as classical histological staining for

light microscopy (hematoxylin and eosin). Our results show that using classical staining techniques and light microscope followed by subsequent image analysis is more suitable for our purpose because it gives similar and sometimes even better result than techniques applying fluorescent staining and there is indisputable benefit in lower costs of light microscopy and methods of classical histology.

Besides standardizing technique of slides preparation I was also learning anatomy and practicing dissections of geckos (using preserved animals from our collections at the Charles University, Prague), because it is very important to sample always the same part of the tissue (exact part of intestine or trachea).

Description of the main results obtained

As mentioned above the main result of my visit was developing of standardized protocol of histological slides preparation, selection of suitable tissues and most importantly we suggested and examined methods for cell size measurements with respect to tissue type. Significant advantage of my visit in Krakow was an opportunity to learn from long experience of Professor Kilarski, who is working in the field of histology many years and also fruitful discussions with Professor Kozłowski and other colleagues from his research group.

Future collaboration with host institution

It turned out that we share with Prof. Kozłowski common interest in studying cell size. We agreed on intensive collaboration between his research group and me and my colleagues at the "gecko research group" at the Faculty of Science, Charles University in Prague.

Projected publications/articles

Now, after coming back to Prague I will start with slides preparation and with measurements of selected cell types in 10 species of eyelid geckos (family Eublepharidae) where I have previously measured erythrocytes. Subsequently I will focus on difference in cell size in gecko *Paroedura picta* reared in different temperatures. I hope that these experiments will bring interesting results which will be published in suitable scientific journals.

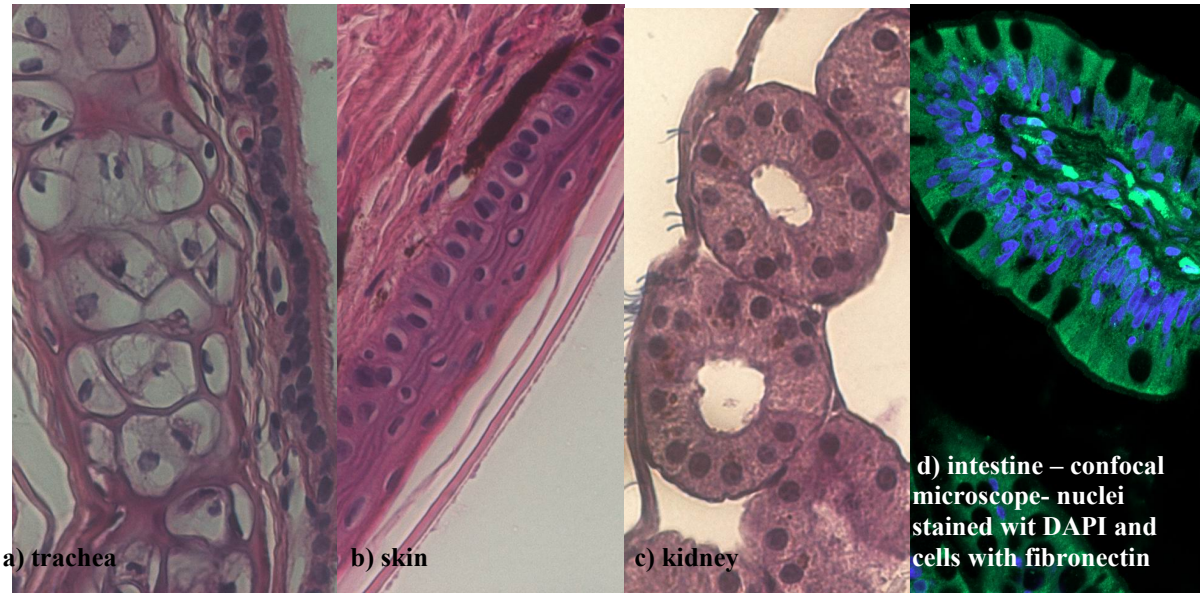

Fig: examples of selected tissues, different magnifications – just illustrative – not in scale