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A review of the Ph.D. thesis written and submitted by Kristina Kverkova, MSc., for defending her Ph.D. degree in the Study program of Zoology

The submitted dissertation of Kristina Kverkova, MSc., was written at the Faculty of Science of Charles University under the supervision of Dr. Pavel Nemeč. The thesis is entitled "Evolution of brain complexity and processing and cognitive capacity in selected vertebrates". I have been appointed as one of the two reviewers of this thesis. The review has been written for the Doctoral Committee of Zoology at the Faculty of Science of Charles University. I have prepared the assessment based on my knowledge, expertise, and best conscience, and I am not in a conflict of interest in the sense of being personally involved in defense of this thesis.

The work is highly topical regarding the choice of topic; it is a very studied topic in contemporary zoology, neuroscience, and biology. I will first comment on the professional level of the dissertation. The thesis focused on an important research topic and studied evolutionary processes leading to varying brain complexity, brain size, and numbers of neuronal cells in the brains of various taxons of vertebrates.

The thesis is a so-called "compact dissertation", i.e., reprints of four top-tier papers completed with an introduction, reprinted documents, a summary of the findings, and a scientific commentary discussing the results and outlining perspectives. The four included studies represent an excellent contribution to biology and neuroscience. They made great use, especially of advanced volumetry and isotropic fractionation methods.

The first study, printed in the *Biology Letters* published by the Royal Society, assessed the numbers of neuronal and other brain cells in a squamate reptile, the Madagascar ground gecko (*Paroedura picta*). The paper tested two captive populations and three age groups. It found increasing numbers of neurons with age. It suggested that adult neurogenesis in the brain of this reptile adds significant numbers of neural cells to the telencephalon of this animal during adulthood, contrasted with mammals. The second study, published in the journal *Evolution* by Wiley Periodicals, Inc., tested the hypothesis on a relation between the brain size and number of neurons in artificially-selected lines of female guppies (*Poecilia reticulata*) exhibiting different cognitive capacities and brain masses. The results supported the hypothesis, showing that selection for brain mass also leads to increased numbers of neurons. The neuronal densities were similar. The third study tested the social brain hypothesis, positing that living in cohesive social groups favors the

evolution of bigger brains and more elaborated cognitive abilities. It used the African mole rats (*Bathyergidae*). No positive correlation between sociality and neuroanatomical correlates of mental capacity was found. Therefore, the results of this study did not support the social brain hypothesis. The paper was published in Scientific Reports. Finally, the fourth excellent study published in the Proceeding of the National Academy of Sciences of the U.S.A. provided comprehensive insights into amniote brain evolution. It showed a dramatic increase in neuronal numbers in birds and mammals compared to reptiles in the telencephalon and cerebellum. The study also showed four significant changes in neuron-brain scaling. All four studies included in this thesis bear substantial contributions by the author. Scientifically, all studies underwent a demanding review process in scientific journals and represented one of the best contributions I have recently seen in this scientific field, interfacing zoology, evolutionary biology, and neuroscience.

As to the formal aspect of the dissertation, I have to say that it has almost 100 pages and contains abstracts in English and Czech, a list of attached papers with the denoted contribution of the authors signed by the supervisor, an introduction section, then reprinted publications, and is closed by a scientific commentary on them, general discussion, also outlining the perspectives and list of references containing dozens of citations. The thesis is written in solid English and formally correct. The printing font of the main text was less legible for me than traditional fonts, but the size of the letters was bigger, which compensated for that.

I found the thesis reading intriguing and evaluated its scientific quality as excellent. In my qualified opinion, the dissertation showed the author's capacity to think scientifically, design experiments, test hypotheses, and interpret the obtained data.

In conclusion, given the evaluation mentioned above, it can be stated that the thesis meets the criteria for dissertations submitted to the Zoology Council. It presents new and original findings that advance knowledge in the evolution of neuronal numbers and brain size. Despite its recentness, publications from the thesis have been cited more than 40 times. Therefore, I recommend to the Departmental Council that this thesis be defended and the candidate is awarded the degree of Ph upon successful completion.

I have a couple of questions that came to my mind during this review:

1. While neuronal numbers are crucial for estimating cognitive capacities, what other factors could play a role? What do you think about the functional contribution of glial cells, functional differentiations of brain areas for specific functions, laminations, gyrification, and forming of delineated nuclei) and especially about the numbers of synaptic connections as even more basic computational elements of the nervous systems.
2. Regarding the synapses, the mammalian cerebellum contains billions of granular cells forming about one-half of total neuronal numbers. It seems that these cells bear as few as four synapses. So does anyone in the world aims to test the relation of mental abilities to the number of synapses, or are you planning to focus on them?

The other two questions are more general:

3. What was the most difficult challenge you faced on your way to this dissertation, what have you learned on it, and what most important things you learned besides the scientific knowledge?
4. What do you subjectively perceive as the most significant and original discovery she has made in her work, and what future directions in this field are you planning?

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