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Review of the doctoral dissertation of
Roya Adavoudi Jolfaei, M.Sc., entitled:
" The Role of Hybridization in the Evolutionary Response to Environmental Change in the Genus *Canis* "

The doctoral dissertation of Roya Adavoudi Jolfaei, entitled "The Role of Hybridization in the Evolutionary Response to Environmental Change in the Genus *Canis*" submitted to me for review describes the results of ecological and evolutionary studies, obtained after analyzing the genome-wide single nucleotide polymorphism. The study involved free-ranging dogs (*Canis lupus familiaris*), Eurasian gray wolves (*Canis lupus*), and golden jackals (*Canis aureus*), across Eurasia. The novelty of the study was the inclusion of golden jackal. That would allow to obtain additional information on the impact of evolutionary distance between on hybridization and introgression.

The study carries out five main tasks: (1) to investigate the possible consequences of hybridization and its impact on the parental species, (2) to find the best way to estimate the proportion of variants derived from hybridization in wolves, jackals and free-living dogs, (3) to estimate the frequency of hybridization between the studied taxa using the selected method, (4) to assess the role of hybridization in species adaptation by identifying chromosomal fragments subject to adaptive introgression and analyzing the functions of the genes contained therein, (5) to examine the influence of environmental variables on the proportion of variants derived from hybridization and the frequency of adaptive introgression in canid populations. These objectives are accomplished in four chapters and the results were obtained by combining genomic data environmental varibles (spatial data).

The dissertation itself (224 pages) contains mentioned four chapters:

1. Consequences of Hybridization in Mammals: A Systematic Review;
2. Evaluation of Global and local Ancestry Reconstruction Method for Admixture Detection Using Genome-Wide SNP Data in Genus *Canis*;
3. The Evolutionary Consequences of Hybridization in Grey Wolves, Golden Jackals, and Domestic Dogs;
4. The Role of Hybridization Between Wild Canids and Domestic Dogs in Adaptation to Environmental Change;

preceded by Acknowledgement, Summary, List of Figures, List of Tables, General Introduction and Bibiography. The 1-4 Chapters are followed by General Discussion, Bibliography, Supplementary Information and Appendix.

Chapter 1 **Consequences of Hybridization in Mammals: A Systematic Review** is a review with the PhD Candidate as a first author, published in MDPI journal Genes in 2021. The authors reviewed 115 original studies published in 2010-2021 that concerned various aspects of mammalian hybridization, based on different combinations of genetic information. In most of the reviewed papers, the hybridization outcomes were regarded as a negative in 49%, while positive in 13%. The authors underline the complexity and difficulty of estimation of hybridization potential, as well as the necessity of application both: neutral and functional markers combined with fitness effects to achieve an unbiased assessment of different hybridization outcomes.

Chapters 2-4 are extensive descriptions of performed analysis. Each chapter consists of its own Introduction, Methods, Results, Conclusion and Bibliography.

Chapter 2 **Evaluation of Global and local Ancestry Reconstruction Method for Admixture Detection Using Genome-Wide SNP Data in Genus *Canis*** presents approaches for estimating global and local ancestry analyses in estimating introgressive hybridization.
The analyses performed aimed to test different methods to infer the proportions of introgression from both global (genome-wide) and local (chromosome-level) perspectives and evaluate the consistency of results among these methods in a dataset composed of three canids, gray wolves, golden jackals, and domestic dogs, known from earlier studies to interbreed. The samples came from India (100 individuals) and Balkans (327 individuals). The analyses were performed on both, the main and regional datasets.
Extensive and comprehensive description of the performed analyses show deep knowledge of the Author of the applied methodological approaches as well as awareness of the factors that can limit the effectiveness of the studies.

Chapter 3 **The Evolutionary Consequences of Hybridization in Grey Wolves, Golden Jackals, and Domestic Dogs** addresses to the 3rd and 4th tasks.
The analyses show that hybridization is common in Canidae, with higher frequency of dog introgression found in wolves (6,4%) when compared to golden jackal (1,2%).
The Author finds out that increased hybridization in certain regions may be related to certain factors like: human activity/habitat loss or low conservation status.
The results of Gene Ontology analyses of the introgressed regions show that hybridization might be beneficial to either domestic or wild *Canidae*.

Chapter 4 **The Role of Hybridization Between Wild Canids and Domestic Dogs in Adaptation to Environmental Change** describes the key environmental factors, that possibly contribute to the frequency of dog-derived genetic variants in wild Canids. The Author's findings support the hypothesis, that dogs' genes might help wolves adapt to human related environments – there is a significant association between introgressed adaptive loci from dogs in wolves and environmental factors.

I would like the Author to refer to these inquiries:

1. The Author showed that although admixed dogs acquired a larger pool of beneficial genetic variants of wolf ancestry compared to admixed wolves, none of these CAI loci (3,228 loci) was associated with environmental variables.
   What might be then the potential effects and consequences of hybridization between wolf and dogs on dogs?
2. *What is the Author's opinion on (dog or general) hybridization? Opinions among scientists differ, from "let's keep them pure" or "we have to preserve this world as it is to our children" to "it is the essence of evolution".*
3. *What is the most important, original value (or values) of this dissertation, in the Candidate's opinion?*

The author achieved her planned research goals.
The presented topic falls within the scope of basic research.
The dissertation had been prepared carefully, edited clearly, written in a readable and understandable manner. The amount of data presented is immense.
It is divided into neatly designed tables and figures. However the presence of direct references in the text to tables and figures, could make reading this this epic and full of details work easier.
The study is methodically correct. The Candidate thoroughly analyzes subsequent stages of the analyses and navigates the presented issues with ease and confidence.

The scientific achievements of the Candidate applying for a doctoral degree meet the requirements specified in Article 187 paragraphs 1 and 2 of the Act of 20 July 2018 - The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended).

The doctoral dissertation submitted to me for review meets the requirements for doctoral dissertations and is exceptionally comprehensive. For its excessiveness and complexity I recommend it to be distinguished.

With respect

Małgorzata Tokarska

dr hab. Inż. Małgorzata Tokarska