JAGIELLONIAN
UNIVERSITY
IN KRAKÓW

**PhD Thesis Acceptance Report**
**Research Discipline Council of Biological Sciences University of Gdańsk**

**Candidate's name and surname: Roya Adavoudi Jolfaei**
**PhD Thesis Title: The Role of Hybridizaton in the Evolutionary Response to Environmental Change in the Genus *Canis***
**Thesis Supervisor: Dr. hab. Małogorzata Pilot**
**Assistant Supervisor / Second Supervisor/ Co-supervisor (if applicable): n/a**
**Reviewer: Prof. UJ dr hab. Izabela Wierzbowska**

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**1.The topic of the dissertation**

The topic of the dissertation is highly relevant to the advancement of biological sciences and represents a significant contribution to the current state of knowledge, particularly in the genomic analysis of the genus *Canis*. The dissertation addresses critical issues related to ongoing changes in the natural world. With increasing anthropogenic pressure and climate change, the dynamics of dispersion and interspecific interactions have intensified. Among others, there is a growing frequency of interspecific associations. The dissertation focuses on a detailed analysis of hybridisation within the genus *Canis*, particularly concerning two wild species: the grey wolf (*Canis lupus*) and the golden jackal (*Canis aureus*), as well as the domestic dog (*Canis lupus familiaris*). The work consists of four chapters, along with a general introduction and a discussion that includes conclusions from all four parts.

In the first chapter, the reader is introduced to the phenomenon of hybridisation, with a particular emphasis on mammals. This section provides a systematic literature review, where the authors focus on 105 scientific articles after an in-depth analysis. Additionally, the dissertation offers explanations and definitions of phenomena associated with hybridisation. The dissertation provides information regarding the types of consequences of hybridisation, which are most often commonly described in this literature review as negative, and less frequently as positive or neutral. The authors suggest that this may be related to the specific method employed, utilising microsatellite genetic markers, which do not allow for the identification of hybridisation beyond the first generation and backcrosses. They propose that to conduct a thorough analysis of hybridisation and its effects, it is essential to also consider the analysis of neutral loci and markers located in coding regiones.

In the second chapter, a comparison of methods for analysing introgressive hybridisation using genome-wide SNP data of the aforementioned *Canis* species at both global and local levels is conducted. The analysed material, comprising various tissue samples and saliva, comes from different regions worldwide, primarily India and selected European countries. The authors assert that the results of this analysis indicate two main factors that may significantly disrupt drawn conclusions. Methods such as ADMIXTURE may overestimate the level of hybridisation compared to regional methods like LAMP-LD, Ghap or ELAI.

Therefore, both method types, i.e. regional and global, should be used together to achieve greater reliability and precision in results. The quality of genotypes and the genetic structure of populations are of paramount importance.

The next chapter is dedicated to studying the impact of genetic variability within the genus *Canis* on adaptive introgression. Results showed that hybridisation is a relatively common phenomenon and was more frequently recorded in areas where interspecific interactions occur due to various environmental factors. A higher rate of introgression was confirmed in wolves from dogs than in jackals, and it was demonstrated that wolf ancestry occurred in dogs and golden jackals, while no golden jackal ancestry was detected neither in wolves nor in domestic dogs. This was explained by the closer evolutionary similarity of wolves and dogs. Genetic introgression may have positive effects on the further adaptive process for both domesticated and wild canids, including increased immune system resistance in wild species and improved morphological, physiological, and behavioural traits in domestic dogs. Evidence of harmful selection was also confirmed in chromosomal blocks in dogs and jackals.

The final chapter aims to identify the key factors influencing the frequency of introgressive genetic variants derived from domestic dogs. The results indicate selected environmental, topographical, and anthropogenic factors that may be directly related to hybridisation in canids and, consequently, the adaptation especially of wild species to changing environment.

In conclusion, the dissertation is pertinent to the development of the biological sciences discipline.

## 2. The Candidate's Knowledge

The doctoral dissertation demonstrates the candidate's profound knowledge of topics mainly related to hybridisation, as well as genome evolution and ecology. The complexity of the work, encompassing both laboratory and statistical analyses, indicates familiarity with the subject matter and the meaningful use of appropriate skills and theoretical understanding. The candidate clearly demonstrates knowledge of advanced analytical methods and their appropriate application for testing research hypotheses. The dissertation reflects a broad general theoretical knowledge in the biological sciences discipline.

## 3. Independence of the Candidate

The dissertation consists of four chapters, of which only one has been published, with MSc Adavoudi as the first author. In the following chapters, the use of the term "we" suggests that the work was not solely written by one individual. This is rather evident, which is common and appropriate in contemporary scientific research, considering the scope of material sourced from various regions of the world, as well as the list of individuals to whom the author expresses gratitude. In today's context, it would be impossible to independently gather and compile such a diverse database. I believe that the work demonstrates M.Sc. Adavoudi's ability to conduct scientific research independently.

## 4. Originality of the Dissertation

The dissertation constitutes a significant contribution to the understanding of genetic introgression in *Canis* species. I found the information regarding adaptive introgression and the

utilisation of environmental factors that enable species to evolve and adapt to dramatically changing environments particularly interesting. Accompanying climate changes, the expansion of the golden jackal represents a new phenomenon that was previously unknown in Northern European countries. Similarly, the dispersion and increasing global population of wolves increasingly result in interspecific interactions and potential hybridisation with other canids from *Canis* genus. The dissertation indicates that hybridisation does not necessarily lead to negative effects but can also enhance adaptation to environmental changes. Therefore, it is crucial to employ effective and efficient methods for monitoring ongoing changes or population status, which will consequently allow for the implementation of appropriate conservation measures or management of both wild and domesticated species.

5. Questions to which the Reviewer expects the candidate to respond during the defence

During the defence, please provide basic information about each of the three species, particularly regarding their range of occurrence and potential dispersion (concerning wild species), estimated population size, population trends, and conservation status, especially concerning the locations from which the samples were analysed.

During the defence, please provide a detailed tabular information regarding the origin of the samples (Chapter 2), including not only the number and type of samples but also the country of origin and the period during which they were collected.

Please explain/extend "…**help mitigate some negative consequences of domestication…**" in the statement on page 116 of Chapter 3: "Therefore, this difference in population size likely accounts for the greater number of overrepresented introgressed chromosomal blocks in dogs, which may contain genetic variants that help mitigate some negative consequences of domestication, such as decreased genetic diversity and heightened tameness."

Chapter 3, page 119, sentence: "This would be particularly beneficial in environments where jackals encounter dogs frequently and where pathogens are constantly evolving…" Please elaborate on this thought and specify what pathogens are being referred to.

Chapter: General Discussion, p. 156: "Therefore, identification of these factors and their direct and indirect effects on the hybridization frequency is important from the perspective of management of cross-breeding canids." Please elaborate on the proposed "management" during the defence.

6. Other Observations on the Content or Form of the Dissertation

Aside from the first chapter, the dissertation is written rather carelessly. There are numerous citations missing in the bibliography that are referenced in the text, and there are frequent errors in citation formatting, e.g., where two authors should be cited, only one is listed, or the publication year is incorrect.

There is a lack of citations for methodologies in Chapter 2, subsections: SNP genotyping, a two-stage approach in Axiom Analysis Power Tools (p. 40), global ancestry analysis (p. 42), local ancestry inference (LAI) (p. 43), in Chapter 3, 3.2 Methods – 4th paragraph (p. 82), detection of chromosomal blocks… (p. 83).

Figures and tables should appear immediately after the text in which they are cited, rather than several pages later, as this greatly complicates text analysis and referencing cited datasets. For example, the cited figure 2.8 (p. 53) appears only on page 56.

Abbreviations related to species and their associated origins should be explained at the beginning of the dissertation and not changed; for instance, on page 57, we have WJD, then IWJD – explained as once Indian and once Himalayan wolves.

A glossary explaining abbreviations related to both the studied species and methods should be included at the beginning of the dissertation.

Consistent spelling should be maintained; both "gray" and "grey" are used in the text and figures.

Page 113 – Latin names for red wolf and coyote should be provided.

Figures – font and overall resolution should be improved so that characters are legible (sometimes it is impossible to read a figure, e.g., Fig. 3.2 – legend).

Tables require better (more precise) descriptions; they should be self-explanatory, and all abbreviations should be clarified.

Supplementary material should be included in the first chapter.

**7. Concluding remarks**

I, hereby, declare that the reviewed PhD thesis by Roya Adavoudi meets the criteria pursuant to art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended) and request that the Research Discipline Council of Biological Sciences of University of Gdańsk accepts Roya Adavoudi for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences

YES/~~NO~~

I, hereby, request that the thesis is accepted with distinctions

~~YES~~/NO

Date: 25.09.2025

Reviewer's signature