

## REVIEW

of the doctoral dissertation by **Francelly Martínez Sosa**, Msc., entitled. „**The effect of environmental factors on adaptive genetic variation in grey wolves (*Canis lupus*) and free-ranging dogs (*Canis lupus familiaris*)**”, prepared at the Museum and Institute of Zoology, Polish Academy of Sciences Warsaw, Poland (BioPlanet Doctoral School) under the supervision of Prof. Małgorzata Pilot (University of Gdańsk)

The doctoral dissertation presented for review, including supplements, consists of 409 pages and is structured as a collection of four papers: one published scientific article and three manuscripts. The first article included in the dissertation is a literature review, providing a solid foundation and justification for the research, and is thematically linked to chapters 2, 3, and 4. The dissertation includes abstracts in both Polish and English. The doctoral candidate provided a 'General introduction and summary of results', followed by an analysis in the 'Discussion' section. The work also features conclusions and a comprehensive, up-to-date bibliography. Furthermore, the dissertation contains the co-author statements required by Polish law. The entire thesis is written in a correct scientific style and language, making it an engaging read. I have found no significant linguistic or grammatical errors, let alone substantive ones.

The doctoral dissertation of Francelly Martínez Sosa, MSc, addresses a highly significant aspect of adaptive genomic variation in free-ranging dogs (FRDs) and wolves, linking this diversity to several environmental factors. In a systematic review (Chapter 1), the doctoral candidate investigates the molecular mechanisms underlying vertebrate adaptive evolution and in Chapters 2 and 3 performs a comparative analysis of population structure and diversifying selection patterns between grey wolves and free-ranging dogs, as well as comparative genomics. Finally, in Chapter 4, she identifies a decline in adaptive potential in free-ranging dogs along the south-north axis and points to dog domestication in southern Eurasia. The title of the dissertation accurately reflects its content.

**I rate the scientific quality of this thesis very highly and am deeply impressed by its substantive value.** It is one of the best doctoral dissertations I have had the opportunity to review to date. The justification for my opinion is presented below. I also address the individual sections, including three manuscripts included in the dissertation, and formulate a few comments and questions for the candidate.

**Chapter 1** consists of a published review article (Martínez Sosa, F., & Pilot, M. (2023). Molecular mechanisms underlying vertebrate adaptive evolution: a systematic review. *Genes*, 14(2), 416., cited 21 times to date). It is a highly mature literature review that excellently presents this important research area. The candidate indicates that regulatory elements and regulatory proteins involved in gene expression and/or cellular pathways play key roles in adaptive evolution. Interestingly, gene losses could also be associated with an adaptive response. While in the General Introduction of her PhD thesis she claims: '*...adaptive evolution is not only driven by natural selection but also gene flow, genetic drift, ...*', I did not find in the abovementioned paper published in *Genes* any sentence concerning the role of gene flow and admixture in adaptive evolution. Extensive gene flow can break down local adaptations; however, genetic admixture due to limited dispersal could be an effective way of gaining new evolutionary adaptations/characters in recipient populations. Thus, admixture could have been at least mentioned in this very valuable review. I am aware that the surveys undertaken in Chapters 1-4 were not directly focused on admixture, as was clearly stated on page 142 of this PhD thesis: '*...that genomic offset calculations do not account for gene flow between populations [and I do believe admixture], which can impact adaptive potential (Reilstab et al., 2021).*' Despite this technical comment, I rate this review article very highly and consider it a necessary and valuable part of this doctoral dissertation."

**Chapter 2** is a manuscript titled: Comparative analysis of population structure and diversifying selection patterns between Eurasian grey wolves (*Canis lupus*) and free-ranging dogs (*Canis lupus familiaris*). In this work, the doctoral candidate convincingly demonstrated that both canids from peninsular populations were genetically distinct, with a more pronounced population structure in wolves. She concluded that evolutionary trajectories for FRDs and grey wolves were similar and that diversifying selection favored mostly the same genes. Similar patterns of genetic differentiation for FRDs and wolves could be explained by the Last Glacial Maximum. This could be the case since '*...dogs are predicted to have been domesticated i.e., 16,000-40,000 years ago.*', as stated on page 143 of this PhD thesis. If so, domesticated dogs, as a species that arose due to artificial selection, were experiencing the same evolutionary forces as closely related wolves in response to climate change. This is truly interesting and influential, as it proves that humans are also affected by climate change, despite some politicians denying such a scenario. The results are sound, and the conclusions are strongly supported by the findings. I am impressed by the figures, especially Figure 2 for wolves and Figure 3 (is it for FRDs?). These figures clearly show the most distinct, usually peninsular and presumably isolated populations of the studied canids, as well as substantial admixture among others (Figure 4). It would be very interesting in future surveys to compare genetic divergence and admixture using neutral SNP markers *versus* those SNPs located in genomic regions under selection,

taking also into account admixture. Other valuable diagrams include Figure 6, which shows candidate SNPs and proteins identified under diversifying selection using PCAdapt and Bayescan, as well as Figure 7 with the gProfiler gene ontology enrichment bubble plot. I rate this chapter very highly; it is a complete manuscript of an extremely valuable scientific work concerning genomic divergence, admixture, and diversifying selection in FRDs and wolves.

**Chapter 3** is a manuscript of a publication titled: Comparative Genomics of Local Adaptive Evolution in Eurasian Grey Wolves and Free-Ranging-Dogs. The doctoral candidate demonstrated that precipitation and temperature gradients drive local adaptation in wolves and FRDs in different ways. She detected over a thousand genes in both wolves and FRDs that displayed signatures of local adaptation, predominantly linked to metabolism, immune response, morphology, and sensory perception. Once again, the figures and tables are highly valuable and informative. The discussion is extensive, mature, and very valuable, providing *'deeper insights into the interplay between natural selection and anthropogenic factors in shaping the genomes of closely related taxa,'* illustrating the significant dependence of commensal dogs on humans. I also agree that a novel finding is the identification of molecular adaptations (e.g., cardiovascular, prey detection, and metabolism) in both canids associated with mammalian species richness. These factors, combined with the human impact on the adaptive evolution of both canids, make this manuscript (Chapter 3) very good and sound."

**Chapter 4** is a manuscript entitled: South-north decline in adaptive potential of free-ranging dogs points to dog domestication in southern Eurasia. The doctoral candidate showed that adaptive potential is highest in the southernmost parts of Eurasia and declines along a latitudinal gradient. I found the species distribution modelling analyses and figures to be very valuable. She also provides strong genetic evidence that early dogs originated from the southern parts of Eurasia. The discussion is sound and carries several important implications regarding climate change, as FRD populations may grow unless controlled by humans. I would like to emphasize the practical aspect of this doctoral dissertation in the context of managing both wolf and FRD populations. I am curious about the candidate's opinion on whether it is possible that dog domestication occurred independently in several different locations in southern Eurasia?

I would like to congratulate the Doctoral Candidate and her Supervisor on an interesting, important, and, both in terms of laboratory and analytical work, demanding doctoral thesis addressing the topic of adaptive variation. I have read it with great interest and believe that the research contained therein brings new value to the knowledge of the genetics and adaptive genomics of feral dogs and wolves.

Taking the above arguments and comments into account, I state that **the doctoral dissertation of Ms. Francelly Martínez Sosa, MSc, entitled 'The effect of environmental factors on adaptive genetic variation in grey wolves (*Canis lupus*) and free-ranging dogs (*Canis lupus familiaris*)', meets the requirements set out in Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws 2023, item 742, as amended).**

Consequently, I submit to the Scientific Council for Biological Sciences of the Museum and Institute of Zoology, Polish Academy of Sciences in Warsaw, Poland, my motion to admit the candidate to the further stages of the procedure for the award of the doctoral degree in the field of Natural and Exact Sciences, in the discipline of Biological Sciences.

At the same time, I apply to the Scientific Council for Biological Sciences at MiIZ PAS in Warsaw for this doctoral dissertation to be granted a distinction (honors), as I consider it an exceptionally valuable piece of work."



Miroslaw  
Ratkiewicz