REVIEW

on a dissertation for acquiring the educational and scientific degree "PhD" in the field of higher education: 6 "Agricultural Sciences and Veterinary Medicine", professional direction: 6.3. "Livestock Breeding" and scientific specialty: "Breeding of Farm Animals, Biology and Biotechnology of Reproduction".

Author of the dissertation: Georgi Todorov Yordanov, part-time PhD student at the Department of Animal Sciences at the Agricultural University - Plovdiv.

Dissertation topic: "Genealogical structure of the Danube horse breed, its place in the Nonius structure and direction of development, in the context of the overall concept of development of the breed".

Reviewer: Prof. Radoslav Ivanov Slavov DSc, from the Faculty of Agriculture at Trakia University, Stara Zagora - retired, field of higher education: 6. "Agricultural Sciences and Veterinary Medicine", professional direction: 6.3. "Livestock Breeding" and scientific specialty: "Breeding of Farm Animals, Biology and Biotechnology of Reproduction". Nominated as a member of the Scientific Jury by Order No RD-16-1299 dated 18.12.2023 of the Rector of the Agricultural University - Plovdiv.

1. Brief introduction of the PhD student

The PhD student was born on 16.05.1966. In 1996 he graduated from the Faculty of Agriculture at Trakia University, being awarded the educational and qualification degree "Bachelor", with the professional qualification "Zooengineer". In 2006 he completed, again at the Faculty of Agriculture at Trakia University, his studies on a master's program, being awarded the educational and qualification degree "Master", with an acquired qualification in "Horse Breeding". Since 1987 until 1998 he worked in the KZ "Khan Asparuh", going through the positions of jockey, Chief Specialist and Deputy Director. Since 1998 until 2000 he was an expert, and since 2000 to 2009 he has been a Head of Department at the Executive agency for selection and reproduction in livestock breeding (EASRLB). Since 2009 until 2017 he was the Executive Director of the National Horse Breeding Association. In 2016 he was the Chairman of the National Union of Zoo Engineers in Bulgaria. Since 2017, and currently, he is the Executive Director of the EASRLB.

On 28.03.2017, he was enrolled in a part-time doctoral program in the scientific specialty "Breeding of Farm Animals, Biology and Biotechnology of Reproduction" in the Department of "Animal Science" at the Agricultural University - Plovdiv, with a scientific supervisor Prof. Vasil Nikolov PhD. On 01.03.2021 his doctoral studies were suspended, with the right of defending a dissertation

2. Relevance of the studied problem

Through the development of the current dissertation, the PhD student gives answers to a number of important questions related to the creation, current state, as well as the strategy for the future development of the Danube horse breed. Through the use of standard zootechnical methods and modern DNA technologies, the uniqueness of the breed, the development of its genealogical structures, the genetic diversity in the population, enabling the maintenance of low levels of inbreeding, its genetic similarity to the breeds that participated in its creation, and to other national breeds as well were proven. Light has also been shed on the questions of the origin and domestication of horses in our lands.

Given the issues under consideration, the goals and tasks set by the PhD student, the large volume of research, the applied modern methods of processing the obtained results, the analyzes performed, the conclusions drawn and the recommendations addressed to horse breeding science and practice, I believe that the dissertation developed by Georgi Yordanov has high degree of relevance

3. Aim, tasks, hypotheses and research methods.

The aim and tasks are correctly formulated and implemented, and the summaries, conclusions and recommendations made fully reflect the results obtained.

A phylogenetic analysis of the Danube horse breed and its genealogical structure since the end of the 80^{s} of the 20th century was carried out. The genetic structure and genetic diversity of the breed and its genealogical lines, by microsatellite loci, were studied, as well as the genetic similarity and distances of the Danube horse breed with populations of the Nonius breed, Bulgarian breeds and horse populations. Studies on mtDNA polymorphism and neutrality tests were carried out in the Danube breed, Hungarian Nonius and Serbian Nonius, as well as comparative genetic and phylogenetic analysis of the breeds based on mtDNA sequences. Based on the results

of the research, the PhD student provides an in-depth analysis and presents his views on the directions for the future breeding activity of the Danube horse breed.

I believe that through the development of this dissertation, the PhD student has acquired the necessary theoretical training, knowledge and skills for planning and conducting experiments, for professional analysis and interpretation of the obtained results, for using modern methods and equipment for conducting research, as well as for using modern software products and models for data processing, skills for formulating conclusions and recommendations for practice

4. Visualization and presentation of the obtained results.

The dissertation is written on 295 pages, incl. content - 2 pages, introduction - 2 pages, literature review - 43 pages, aim and tasks - 2 pages, material and methods - 7 pages, results and discussion - 176 pages /including summary of 5 pages/, conclusions and recommendations - 2 pages, references - 26 pages, contributions - 3 pages, publications related to the dissertation - 1 page, citations of publications related to the dissertation - 3 pages. In terms of structure and ratio between the sections, the dissertation has been developed according to the requirements. The obtained results are very well presented – by text /analytically/, in 34 tables, 93 figures and 43 photos. It is written in a very good style and language.

5. Discussion of the results and literature used.

The analyzes of the many systematically and thoroughly conducted research are aimed at fulfilling the main goal of the dissertation. The obtained results have a high degree of significance, given the importance of the Danube breed for Bulgarian horse breeding. They are convincing and based on methodologically correctly conducted experiments, studies and analyses, carried out with sufficient numbers of animals and biological samples, modern apparatus and modern processing methods. The interpretation of the obtained results was carried out professionally, which is indicative of the very good scientific preparation of the PhD student. I also highly appreciate the summary presented after the Results and Discussion section. The ten conclusions and three recommendations derived from the obtained results are of interest to horse breeding science and practice.

A total of 427 literary sources are cited in the dissertation, of which 87 are in Cyrillic and 340 are in Latin. The very good literary awareness of the PhD student, the excellent knowledge and handling of the literary sources on the studied topic, both in the development of the literature review and in the analysis of the obtained results, are impressive.

6. Dissertation Contribution.

Based on the results of the dissertation, the doctoral student presents a reference on the contributions. Contributions of an original scientific and scientific-applied character have been formulated. I express my agreement with the contributions thus presented. I would like to emphasize some of the results of the scientific research, which have particular contributions in the field of horse breeding science and practice:

▶ For the first time in our country, a complex genealogical, genetic, microsatellite and mitochondrial analysis was carried out to characterize the genetic structure, condition and phylogenetic aspects of the development of the Danube horse population, which could serve in the development of a strategy for the future development of the breed. ***Original scientific contribution.***

▶ Through microsatellite analysis of 15 marker loci, the relationship between the Danube horse breed and the populations involved in its creation - Serbian Nonius and Hungarian Nonius has been analyzed for the first time at the genetic level. It has been established that the Danube horse is closer to the Hungarian Nonius. ***An original contribution of a scientific character.***

▶ A large-scale study was conducted to clarify the phylogenetic relationships of the Danube horse with breeds that participated in its creation, through the study of mtDNA in prehistoric wild horses that inhabited our lands, modern representatives of local autochthonous populations of horses from the Stara Planina, the Rilo-Rhodope massif and the Karakachan horse, of the newly created Bulgarian breeds - Pleven horse and Eastern Bulgarian horse, of the Serbian and Hungarian Nonius. It has been established that the newly created Bulgarian breeds - the Danube horse, the Pleven horse and the Eastern Bulgarian horse are genetically closely related to each other, to the modern local populations and to prehistoric wild horses that inhabited today's territories of the country. The results obtained from the conducted research also

contribute to clarifying the origin, domestication and biogeography of the species *Equus ferus caballus*. ***Original scientific contribution.***

► On the basis of mtDNA analysis, it was established that the Danube horse is a unique Bulgarian breed, possessing a high haplotype diversity and a specific mitochondrial profile, demonstrating its relationship both with the modern populations of the Serbian and Hungarian Nonius, as well as with the gene pool of the local national populations. ***Original contribution with scientific character.***

►Through research and analysis of mtDNA of prehistoric wild horses and modern national breeds and populations, phylogenetic and historical analysis, it has been suggested that the present-day territories of Bulgaria were a place of domestication of the horse, as part of the western periphery of the Pontic-Caspian center. ***Original scientific contribution.***

► Based on a sequencing analysis of 120 sequences, the data obtained for the first time on the genetic profiles of the Danube horse, Pleven horse, Eastern Bulgarian horse, Nonius and Serbian Nonius breeds have been published with corresponding unique numbers in the genetic database (GenBank). An original contribution of a scientific nature. ***Original contribution with scientific character.***

► On the basis of a complete genealogical analysis of the lineal and family structures of the Danube horse, as well as on the basis of an analysis of the generational dynamics of the exterior parameters, the tendencies in the change of the exterior of the breed were established for the first time and recommendations were made for the future selection. ***An original contribution of a scientific and applied character.***

► Through microsatellite analysis on 15 marker loci, it was established that the Danube breed has a high genetic diversity, which at this stage does not make it threatened by inbreeding depression. The genetic distances between the six lines in the breed were determined. It was established that the lines have a high in-line diversity, but with an extremely high genetic similarity between them, which requires maximum precision when developing the selection schemes. ***An original contribution of a scientific and applied character.***

7. Published articles and citations:

In relation to the dissertation the PhD student presents nine scientific publications, of which he is an independant author in one, in four he is the first author and in another four he is the second author. Five of them were published in journals with SJR, IF and Q /from Q2 to Q4/. The total SJR of the publications is 1.764 and the total IF is 5.841. The total number of points formed by the publications is 71.31, with requirements according to the scientometric indicators of 30 points. Four of the publications are cited in 21 scientific works by other authors, printed in Bulgarian and foreign refereed scientific publications. I give a high positive assessment for the quality of the scientific publications reflecting the results of the dissertation of the PhD student Georgi Yordanov.

Conclusion:

Based on the research methods learned and applied by the doctoral student, the correctly conducted experiments, the summaries and conclusions made, I consider that the presented dissertation meets the requirements of the LDASRB and the Regulations of the Agricultural University for its application, which gives me grounds to evaluate it POSSITIVELY.

I would propose to the honorable Scientific Jury to also vote positively and award Georgi Todorov Yordanov the educational and scientific degree "PhD" in the field of higher education "Agricultural Sciences and Veterinary Medicine", professional direction "Livestock Breeding" and scientific specialty "Breeding of Farm Animals, Biology and Biotechnology of Reproduction".

22.01.2024

Stara Zagora

Prepared by: .....

/Prof. Radoslav Slavov DSc/