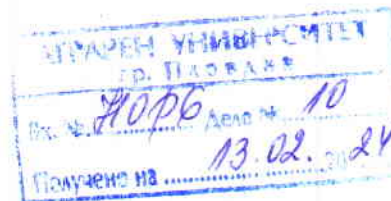


Opinion



on a dissertation for obtaining the educational and scientific degree "PhD" in: field of higher education 6. Agricultural sciences and veterinary medicine, professional direction 6.3. Stock-breeding, the scientific specialty "Breeding of agricultural animals, biology and biotechnology of reproduction"

Author of the dissertation: Georgi Todorov Yordanov, part-time doctoral student, at the Department of Animal Husbandry 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 an overall concept for the development of the breed"

Reviewer: prof. Zhivka Ilieva Gergovska DSc...

Academic position: professor, scientific degree: Doctor of Sciences. Field of higher education: 6. "Agrarian sciences and veterinary medicine", professional direction: 6.3 "Stock-breeding", scientific specialty: Doctor of Sciences in scientific specialty "Breeding of agricultural animals, biology and biotechnology of reproduction", professor in scientific specialty "Cattle and Buffalo Breeding." Place of employment: Trakia University, Stara Zagora - retired.

Appointed as a member of the scientific jury by order No. RD-16-1299/ 18.12.2023 by the Rector of AU.

1. Relevance of the problem.

The greatly reduced, to a critical, number of animals of the Danube horse breed faces the breed with serious breeding and organizational problems. The dissertation presents a detailed analysis of the breed at the moment, provides a basis for laying the foundations of a general strategy to preserve its unique genetic diversity and guidelines for development so that it is not swallowed up by the general mass of the European Nonius. In addition, the dissertation also provides a very good analysis of the condition of the breed in exterior terms, which is also a basis for possible selection decisions for its improvement in the future.

The results of the DNA analyzes carried out are particularly relevant, on the one hand, proving the uniqueness of the Danube horse breed compared to our other horse breeds, and on the other hand, shedding light on the origin of domesticated horses on our lands. The very fact that the published results were cited 21 times shows the relevance of the study.

2. Purpose, tasks, hypotheses and research methods.

The aim of the dissertation is based on phylogenetic, genealogical, molecular and population genetic analyses, to assess the current state of the Danube horse breed, its genealogical structures and their relationship with the structure of the Nonius breed, as a prerequisite for the development of a concept for breeding work and future development of the breed. To achieve the goal, three main tasks have been set with a total of 10 subtasks to them.

In order to fulfill the tasks set in this way, various methods were used, mainly breeding ones, such as processing of exterior signs, calculation of physique indices, genealogical analysis of the lines and families of the breed, inbreeding according to Wright, genetic similarity, consanguinity, generation interval and a number of others were calculated DNA analysis - microsatellite analysis of nuclear DNA, sequencing analysis of HVR, D-loop.

Almost all animals of the Danube horse breed - 118 stallions and 231 mares - were covered in the study, and a significant number of animals - 166 from the breed and 115 from three Nonius horse breeds - were sampled in the various genetic analyses. Modern

methods of information processing have been applied, enabling better evaluation and analysis.

During the development of the dissertation work, the dissertation student has mastered a significant number of methodologies and research methods, which is a reason to consider that he has acquired the necessary methodological experience for conducting a scientific experiment, and also that the educational task of this scientific work has been fulfilled degree.

3. Transparency and presentation of the obtained results.

The dissertation is properly structured and includes all the required sections in an adequate volume. It was developed in 289 standard typewritten pages. The obtained results are very well illustrated and analyzed, being presented in 34 tables, 93 figures and also attached are 43 photos of stallions of the breed. Tables and figures fully reflect the research carried out and are a good basis for analysis.

The dissertation meets all the requirements and criteria according to the rules of AU, Plovdiv.

4. Discussion of the results and used literature.

427 literary sources were used, of which 87 in Cyrillic and 340 in Latin, of which 128 or 30% are from the last 5 years. This shows a very good awareness of the doctoral student, especially in the field of the latest scientific achievements in the field of horse breeding, both in our country and in other countries. The obtained results were skilfully compared with what was established by other researchers, an indicator of the good awareness and analytical thinking of the doctoral student.

The "Results and Discussion" section has been developed in detail in accordance with the purpose and the tasks set. A detailed analysis of the obtained results was made, and the good literary awareness of the doctoral student made it possible, not only for the adequate analysis, but also for appropriate comparisons with results obtained by other authors. The interpretation of the results is at a high professional level and meets the set goals and objectives. The section ends with a very good summary summarizing the overall study and specific conclusions from the results obtained.

Based on the conducted research, 10 conclusions and three recommendations for practice were made, which correspond to the obtained results and are adequate for our conditions. The doctoral student has achieved the set goal of the work and has fulfilled the set tasks to the maximum extent. The applied and mastered methods and methodologies are, in addition to the classical ones applied in the breeding of domestic animals and horses in particular, and a number of modern methods related to DNA analyses.

Dissertation student **Georgi Yordanov** has mastered various methods for research and processing of the received information, has shown skills for proper planning and conducting experiments, as well as skills for professional analysis of the obtained results and formulation of logical conclusions and recommendations.

5. Contributions of the dissertation work.

The more important scientific and scientific-applied contributions of the dissertation work are:

Scientific contributions

On the basis of microsatellite analysis of 15 marker loci, the relationship of the Danube horse breed with the populations that participated in its creation - Serbian and Hungarian Nonius - was analyzed for the first time at the genetic level.

On the basis of mtDNA analysis, it was established that the Danube horse is a unique national breed, with a high haplotype diversity and a specific mitochondrial profile, demonstrating its relationship both with the modern populations of the Serbian and Hungarian Nonius, and with the gene pool of the local national populations.

On the basis of mtDNA analysis, it has been established that the newly created Bulgarian breeds - Danube, Plevan and Eastern Bulgarian horses - 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 obtained results contribute to clarifying the origin, domestication and biogeography of the species *Equus ferus caballus*. The 120 sequences obtained for the first time during sequence analysis of populations of modern Bulgarian horse breeds and data on the genetic profile of Nonius and Serbian Nonius have been published in the genetic database (GenBank).

Scientific and applied contributions

A genealogical structure and the genetic status of the Danube horse breed at the modern stage have been established, as prerequisites for developing a strategy for the development of the breed in the short-term and long-term aspects.

On the basis of a complete genealogical analysis of the lineal and family structures of the Danube horse and an analysis of the generational dynamics of the exterior signs, the trends in the change of the exterior of the breed were demonstrated for the first time, in a phylogenetic aspect, and recommendations were made for future work.

The Danube breed has been found to have high genetic diversity and is not at risk of inbreeding depression at this stage. The lines of the Danube breed have a high intralineal diversity, but with an extremely high genetic similarity to each other, which requires maximum precision when developing the selection schemes.

6. Critical Notes and Questions.

I have no notes on the dissertation.

7. Published articles and citations.

The doctoral student presented 9 scientific publications on the dissertation work, two of which were reported at scientific conferences. In 5 of them he is the first author, and in 4 - the second. Five of the publications are in indexed journals with a total SJR = 1.767 and IF=5.84. Four of the cited publications were cited 21 times. 71.31 points of publications are presented, which significantly exceeds the scientometric indicators of 30 points required to acquire the Ph Doctor.

The number and quality of the publications meet the requirements of the Regulations on the terms and conditions for acquiring scientific degrees and holding academic positions, and their content reflects basic results that are sufficiently presented to a specialized scientific audience.

The presented abstract reflects objectively the structure and content of the dissertation work.

CONCLUSION:

Based on the various research methods learned and applied by the doctoral student, the correctly conducted experiments, the generalizations and conclusions made, I consider that the presented dissertation meets the requirements of the Law on the Growth of the Academic Staff in the Republic of Bulgaria and the Regulations of the Agrarian University for its application, which gives me grounds to evaluate it **POSITIVE**.

I take the liberty of proposing to the honorable Scientific Jury to also vote positively and award **Georgi Todorov Yordanov**, the educational and scientific degree "Doctor" in field of higher education 6. Agricultural sciences and veterinary medicine, professional direction 6.3. Stock-breeding, the scientific specialty "Breeding of agricultural animals, biology and biotechnology of reproduction".

Date: 08.02.2024 r
Plovdiv

PREPARED THE OPINION:.....
(prof. Zh. Gergovska)