



# OPINION

on a dissertation for acquiring of the educational and scientific degree of "doctor" in field of higher educational 6. Agrarian sciences and veterinary medicine; professional field 6.3. "Stock-breeding"; scientific specialty "Breeding of agricultural animals, biology and biotechnology of reproduction"

**Author of the PhD thesis:** **Georgi Todorov Yordanov**, part-time doctoral student at the Department of Animal Husbandry, University of Agriculture, Plovdiv

**Title of the PhD thesis :** "Genealogical structure of the Danubian horse breed, its place in the Nonius structure and developmental guidelines in the context of the wholesome concept of the breed development"

**Reviewer:** **Assoc. prof. Stefka Nikolova Stoyanova, PhD**, Trakia University, Stara Zagora, Faculty of Agriculture, 6.3. Animal Husbandry, scientific specialty "Breeding of agricultural animals, biology and biotechnology of reproduction"

Assigned a member of the Academic Jury by Order № RD-16-1299/ 18.12.2023 by the Rector of the University of Agriculture.

## 1. Actuality of the research problem.

The topic of the dissertation is exceptionally relevant and has a high practical value. It tackles the assessment of the current state of the Danubian horse breed in a study of its genealogical structures and their relation to the Nonius breed. This comprehensive research work was done on the basis of a phylogenetic, genealogical, molecular-genetic and population genetic analysis. The obtained results can serve to develop a concept of reproduction and future development of the breed.

## 2. Aim, tasks, hypotheses and research methods.

The aim of the dissertation has been clearly and precisely formulated and reflects the essence of the conducted study. The author has presented his analyses sufficient for the completion of the main task set for meeting the goal of the dissertation work. The accurate presentation of the applied analyses signals the depth of the investigations, which also reasons the objectivity of the results from the scientific study and outlines trends for future development.

## 3. Exemplification and presentation of the obtained results.

The presented dissertation covers 300 pages and has a standard structure typical of similar scientific works. The main part of the study is very well illustrated with 36 tables and 38 figures reflecting the research. The list of used literature includes 427 titles - 417 authors (87 in Cyrillic), strategic documents and electronic sources of information. The structure, volume, correct use of research methods and illustrated presentation of the obtained results are an important prerequisite for objective analysis, correct formulation of conclusions and accurate scientific contributions applicable to animal husbandry practice made by the author of the dissertation.

#### **4. Discussion of the results and literature used.**

The thorough literature review indicates Georgi Yordanov's excellent knowledge of the various aspects of the topic. The obtained results comply with the set task for meeting the research aim. The Results and Discussion section is the most voluminous, and is illustrated with a sufficient number of tables and figures accompanying the highly competent scientific interpretation of the results. The set task has been fulfilled through rigorously performed, actual analyses characterizing the work of Georgi Yordanov. This allowed him to formulate specific inferences and recommendations for the improvement of the reproduction and future development of the Danubian horse breed.

#### **5. Contributions of the dissertation.**

The dissertation on "Genealogical structure of the Danubian horse breed, its place in the Nonius structure and developmental guidelines in the context of the wholesome concept of the breed development", contains eleven scientific theoretical, two applied scientific and two applied contributions. They reflect the results from the research activity of the doctoral candidate.

##### **Research contributions:**

1. For the first time in Bulgaria the study presents a complex genealogical, microsatellite and mitochondrial analysis on the characterization of the genetic structure, state and phylogenetic aspects of population development in livestock farming.
2. The author has established that the data from the genealogic and DNA analyses used independently do not provide precise information about the state and the genetic processes in the population during its phylogenesis and they must therefore be used together with the development of breeding strategies.
3. On the basis of microsatellite analysis of 15 marker loci for the first time at a genetic level the candidate has analyzed the relationship between the Danubian horse breed and the populations participating in its formation – Serbian Nonius and Hungarian Nonius. It was found that the three populations have a specific genetic structure, and that the Danubian horse is closer to the Hungarian Nonius.
4. To explain the phylogenetic relations of the Danubian horse with breeds participating in its creation, the candidate has performed a large scale study of the D-loop region of mtDNA of prehistoric wild horses which inhabited our lands, current representatives of local indigenous horse populations from Stara Planina, Rila-Rhodopes mountains, and the Karakachan horse, of the newly created Bulgarian breeds Danubian horse, Pleven horse, and East Bulgarian horse, of the Serbian and Hungarian population of the Nonius.
5. On the basis of mtDNA analysis he established that the Danubian horse is a unique national breed with high haplotype diversity and specific mitochondrial profile, demonstrating its relationship both with the current populations of the Serbian and Hungarian Nonius, as well as with the genetic fund of local native populations.
6. On the basis of mtDNA analysis he found that the newly created Bulgarian breeds – Danubian horse, Pleven horse and East Bulgarian horse are genetically close to each other, to the current local populations and the prehistoric wild horses, which inhabited today's territory of the country.
7. On the basis of mtDNA analysis for the first time the candidate has demonstrated the loss of genetic diversity of the local populations in creating cultural breeds through crossbreeding with introduced ones.

8. The obtained results from the performed study contribute to explaining the origin, domestication and biogeography of *Equus ferus caballus*.
9. On the basis of mtDNA analysis of prehistoric wild horses and the current native breeds and populations, and also according to phylogenetic and historical analysis the candidate has formulated a supposition that today's territory of Bulgaria was the site of the horse's domestication, as part of the western periphery of the Pontian-Caspian centre.
10. The obtained for the first time 120 sequences during a sequential analysis of populations of current Bulgarian horse breeds have been published in a genetic database (GenBank) under the numbers: Danubian horse (GenBank Acc. no. MG420898-MG420955); Pleven horse (GenBank Acc. no. MK465427-MK465437) and East Bulgarian horse (GenBank Acc. no. MK465388-MK465426).
11. The obtained for the first time molecular data on the genetic profile of Nonius and Serbian Nonius were published in a genetic database (GenBank) under the numbers: Nonius (GenBank Acc. no. MG420956-MG420978) and Serbian Nonius (GenBank Acc. no. MG420979-MG420990).

#### **Applied scientific contributions:**

1. On the basis of a conducted microsatellite analysis of 15 marker loci the candidate found that the Danubian breed had a high genetic diversity and at this stage it was not threatened by inbred depression.
2. On the basis of a performed microsatellite analysis of 15 marker loci he found that the lines of the Danubian breed had a high intralinear diversity with exceptionally high genetic similarity, which requires maximality precision in the development of selection schemes. The distances between the six lines of the breed were established.

#### **Applied contributions:**

1. The current genealogic structure and state of the Danubian horse breed were established as pre-conditions for developing a strategy for the short-term and long-term development of the breed. It was also shown that despite having a smaller population, the Danubian breed has a well-built genealogical structure, which guarantees the sustainable development of the breed.
2. On the basis of a genealogical analysis of the line and family structure of the Danube horse and an analysis of the generational dynamics of the exterior parameters, the trends in the exterior change of the breed, in a phylogenetic aspect were demonstrated for the first time and proposed recommendations for future work. It was found that selection was aimed at creating animals of the "medium" type, intermediate between the "small" and "large" vernier, but closer to the latter. As a result, a breed consolidated by its exterior was created.

#### **6. Critical notes and questions.**

I have no significant critical remarks to the presented dissertation, as well as questions to the doctoral student.

#### **7. Published articles and citations.**

The candidate has presented nine relevant to the dissertation publications in renowned scientific journals, five of which were peer-reviewed and indexed in international databases with scientific information (two with Q2; two with Q3; one with Q4). In five of the publications

Georgi Yordanov was the leading author, and in the rest - a second author, which indicates his personal involvement and contribution to the research work. The publications of Georgi Todorov Yordanov related to the dissertation have received 21 citations. The abstract paper reflects truthfully and precisely the sections of the dissertation.

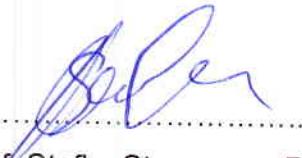
## **CONCLUSION:**

On the basis of the scientific and applied by the candidate various research methods, the correctly conducted experiments, the generalizations and inferences, I believe that the presented dissertation meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Rules of the University of Agriculture for its application, which allows me to offer my **POSITIVE** evaluation.

I allow myself to suggest to the honorable Scientific Jury to also vote in favour of conferring the educational and scientific degree "**doctor**" to Georgi Todorov Yordanov in scientific speciality 6. Agricultural sciences and veterinary medicine; professional field 6.3. "Stock-breeding"; scientific specialty "Breeding of agricultural animals, biology and biotechnology of reproduction".

**Date:** 06.02.2024

Stara Zagora

**PREPARED THE OPINION:** ..... 

(Assoc. Prof. Stefka Stoyanova, PhD)