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REVIEW

for the selection of an Associate Professor in the field
“Biological Sciences”, scientific domain “Genetics”, for the needs of the research group “Molecular Evolutionary Studies”, section “Biodiversity and Ecology of Parasites”, Department “Animal Diversity and Resources” of IBER-BAS, announced in issue No. 48 of the State Gazette of 7.6.2024
Candidate: Ch. Assistant Prof. Dr. Boyko Stoykov Neov
Reviewer: Prof. Dr. Aneta Kostadinova, IBEI-BAN

At the announced competition (SG, No. 48/7.6.2024) for Associate Professor inGenetics at the Section “Biodiversity and Ecology of Parasites”, Department “Animal Diversity and Resources”(IBER-BAS), Dr. Boyko Stoykov Neov, currently Chief Assistant Professor in the same section, is the only candidate.

1. Overview of the candidate’s career development

Boyko Neov graduated from the University of Sofia “St. Kliment Ohridski” with a MSc degree in the field “Animal and Human Physiology” in 2010. He worked as a technical associate at the Institute for Nuclear Research and Nuclear Energy (BAS) (2004-2006) and as a biologist at the Institute of Biophysics and Biomedical Engineering (BAS) (2008-2012). From January 2012 to 2021, he was appointed to IBER-BAN initially as a biologist, and later as a Chief Assistant Professor in the department“Animal Diversity and Resources”. In 2021, he defended a dissertation on the topic “*Evolutionary processes in the formation of the diversity of hymenolepidid cestodes in insectivorous mammals of the family Soricidae*”and was awarded the scientific and educational degree “doctor”(PhD) in parasitology and helminthology.

2. Publications submitted in connection with the competition

For participation in the current competition, Dr.Neov has submitted a list of 25 publications (outside of 2 related to the dissertation, which are not reviewed, but are taken into account in the general evaluation of the candidate’s scientific activity) as follows: 1 book chapterpublished by an international academic publishing house; 20 articles published in international (17) and foreign journals (3) with an impact factor; 1 article published in a foreign journal without impact factor (with impact rank); and 3 articles published in Bulgarian journals without impact factor (with impact rank). The candidate is the first author of 5 publications, second – of 4, third – of 6, fourth –

of 4, fifth – of 4, sixth – of 1, and eighth – of 1. Eight (32%) of the publications are literature reviews. The individual contributions of the co-authors were published in only 12 articles. From these, it is clear that Dr.Neov prepared the first version of 7 papers (58%), performed the experiments for 5 papers (42%), and performed the analyzes (marked as “software”) for 2 papers (17%). These data are an indication that the candidate is likely to have made similar substantial contributions to the studies published without indicating the individual contributions of co-authors. In general, the publications submitted for the competition illustrate research and (co)authorship approach characteristic of the candidate’s research group, which, although leading to fulfilling the criteria for the academic position of Associate Professor, does not contribute to the development and differentiation of the candidate’s scientific profile. However, molecular genetic research on the origin and evolution of domestic animals and their relatives predominates quantitatively and qualitatively (12 articles, contributions 1-9, 16), and in my opinion can be considered the main direction of the candidate’s research activity shaping his scientific profile. Although another major part of the publications is related to pathogens and symbionts of the honeybee (11 articles, contributions 10-14, 16), only 5 of them contain results of experimental research.

As his habilitation work, the candidate has chosen 6 publications - 5 in journals with an impact factor (Q1: 1 article [7]; Q2: 3 articles [4, 5, 8] and Q3: 1 article [3]) and 1 in journals with impact rank (SJR) [6]). Four of the publications included in the habilitation work present results of genetic studies of microsporidia (*Nosema* spp.) and viruses of *Apis mellifera* [4, 5, 6, 7] and one article [3] on a fish trematode; a literature review on the phylogeography of bat lyssaviruses in Europe is also included [8]. Although the data are incomplete, I believe that Dr.Neov has a significant, albeit not unambiguously leading, role in these studies: second author on 3 articles [3, 5, 6]; drafted the first version of 1 [4], performed the experiments for 3 [3, 4, 5] and performed the analyses for 1 [7] of these publications.

3. Main directions in the candidate’s research work and most important scientific contributions

I accept the report on the scientific contributions presented by Dr.Neov in principle, but I will allow myself a different formulation of the scientific contributions based on the content of the publications presented for the competition.

(i) Molecular-genetic studies on the origin and evolution of domestic animals and their wild relatives

1. The first pilot genetic study of mitochondrial DNA (mtDNA) was conducted for two local breeds (long-horned grey cattle and short-horned Rhodope cattle) on materials from Bulgaria. Based on a total of 20 sequences of a partial region of the so-called hypervariable region of mtDNA (D-loop), 5 haplogroups/sub-haplogroups (T1, T1a, T2, T3a and T3b) were established with a predominance in both breeds of the European-specific haplogroup T3. A new sub-haplogroup (T3c) found only in short-horned Rhodope cattle was defined [11, 12]. The contribution is original, but still preliminary, given the small number of sequences.

2. Studies of the allelic diversity of genes for milk proteins related to the quantity and qualities of milk in local breeds of cattle were conducted. The studied allelic forms of the kappa-casein (CSN3) and alpha-S1 casein (CSN1S1) genes showed a much higher allelic diversity in the local cattle breeds, compared to other local cattle breeds in Europe, and that the Bulgarian local breeds are similar to populations from South-East Europe [9, 10]. The contribution is original, but still preliminary, given the limited number of samples.

3. Sequences of a partial region of the D-loop mtDNA in two Bulgarian plain horse breeds (Plevenski and East Bulgarian) were generatedfor the first time. Specific mitochondrial profiles were discovered: the European haplogroup L was found to dominate in the Pleven breed, followed by the Central Asian haplogroup C), and the Central Asian haplogroup Q and the European haplogroup L was found to dominate in the Eastern Bulgarian horse with a similar frequency [18]. The contribution is original.

4. The mitochondrial profile (D-loop region) of three breeds of Eastern European horses, Hungarian Nonius, Danube horse and Serbian Nonius, was studied. Nine of the 17 haplogroups described in modern horses were identified. Specific genetic profiles were found: in the Danube horse, the Anatolian haplogroup G dominated (45%), followed by an almost equal percentage of the West Eurasian haplogroups L and M (≈ 21% each); in the Hungarian Nonius, haplogroup M (44%) was the most common, followed by Middle Eastern O'P (26%) and Central Asian E and G (≈ 13% each); the relatively rare European haplogroup D (67%), followed by G (17%), strongly dominated the Serbian Nonius. The high percentage of shared haplogroups between the Danube horse and the Hungarian Nonius confirms the common origin of the two breeds, while the specific genetic profile of the Serbian Nonius suggests a different, independent origin [22]. The contribution is original.

5. A study was conducted using 15 microsatellite markers of the genetic profile of the existing six paternal lines of the Danube horse (Zdravko, Nonius XVII-30, Torpedo, Leader, Kalif and Hrabar) for the first time. The grouping of the paternal lines into 3 separate clusters suggested a different origin of the Zdravko and Hrabar lines and the common origin of the Nonius XVII-30, Torpedo, Leader and Kalif lines. Despite the established low level of inbreeding in the breed, the small number of remaining animals is a risk for the future of the breed; the information obtained during this study can be used as a basis for future breeding programs [24]. The contribution is original.

6. Genetic studieswere carried out using bone remains of dogs from the Early Neolithic (8000 BC) to Antiquity (1st-3rd centuries AD), found during archaeological research in Bulgaria. The analysis of 16 sequences of a partial region of D-loop mtDNA revealed a genetic profile similar to that of modern dogs and characterized by high frequencies of haplogroups A and B, absence of haplogroup C, as well as a weak representation of haplogroup D. The finding in the studied material of haplogroup B, which is found at high frequency in modern grey wolf and dog populations in the Balkans, is evidence of its presence in ancient Eurasia and is related to the hypothesis of the Balkans as a centre of domestication of the dog. The spread of haplogroups A and B in Europe in later historical times may be related to the spread of new breeds from Ancient Greece, Thrace and the Roman Empire [14]. The contribution is original, but still preliminary, given the small number of sequences.

7. Examination of 50 sequences of a partial region of D-loop mtDNA from the Eastern Balkan pig, considered an ancient autochthonous breed, led to identification of 4 haplotypes: 3 (H1, H2 and H3) specific to Asia and 1 (E1a1) specific to Europe. Haplotypes H2 and H3 were first described [15]. The contribution is mainly confirmatory.

8. Sequences of a partial region of D-loop mtDNA from 7 samples of subfossil bone remains of Holocene bison from Bulgaria were generated for the first time. A phylogenetic analysis including the available GenBank sequences for the genus *Bison* demonstrated a clustering of the extinct Holocene bison from the Bulgarian lands with those of the ancient populations from France, Austria and Switzerland, but not with those from Central Europe and the extinct Caucasian population. The results are indicating that the Balkan bison was a relict and possibly isolated population [21]. The contribution is original.

9. A pilot study was conducted for the presence of a defective hexosaminidase A gene leading to Tay-Sachs disease in Jacob sheep. The pathological mutation in the studied specific fragment of the gene (between exon 11 and intron 11) was not found in the 20 animals studied [23]. The contribution is original but preliminary in nature given the limited number of samples.

(ii) Molecular genetic studies of pathogens and symbionts of *Apis mellifera*

10. In genetic analysis of 16S rDNA sequences for *Nosema* spp. of *Apis mellifera* from three localities in Bulgaria, only the microsporidian parasite *Nosema ceranae* was found [4]. The contribution is preliminary given the small number of regions studied.

11. The first genetic study in Bulgaria was conducted on the presence of 6 viruses in *Apis mellifera* individuals from 50 colonies. Four viruses were identified: DWV (deformed wing virus); acute bee paralysis virus (ABPV); sacbrood virus (SBV); and black queen cell virus (BQCV). One of these, ABPV was found for the first time in Bulgaria. A phylogenetic analysis was carried out to clarify the biogeographical features, and hypotheses were proposed for the ways of spread of the established viruses [5]. The contribution is original.

12. The possibility of using “environmental DNA” for the detection of bee parasites and pathogens *Varroa destructor*, *Nosema apis* and *Nosema ceranae* has been demonstrated. *Nosema ceranae* DNA was detected in only 6 out of 20 honey samples examined [7]. The contribution is confirmatory.

13. Molecular screening for *Apis mellifera* pathogens (two *Nosema* spp. and 6 viruses) was performed. DNA of *Nosema ceranae* and 4 viruses (DWV, ABPV, SBV and BQCV) was found in samples of honey from apiaries in three regions of Bulgaria (two plain regions and one mountainous region). The results revealed a significantly higher incidence of positive samples for *N. ceranae* from the plain regions (Ruse: 77.2%; Sofia: 64.2%) compared to the mountainous region (Smolyan: 5.2%) as well as a low incidence of positive samples for the four viruses (1.2–5.9%) [6]. The contribution is original.

14. The first study in Bulgaria of the composition and structure of the microbiome of bees from 2 hives, one from a plain area (town of Byala) and one from a mountain area (town of Smolyan) in Bulgaria, was conducted, based on sequencing of the hypervariable regions V3-V4 of the bacterial

16S rRNA gene. A total of 66 taxa were found; bacteria of 7 genera (*Lactobacillus*, *Gilliamella*, *Bifidobacterium*, *Commensalibacter*, *Bartonella*, *Snodgrassella*, and *Frischella*) were found to dominate (92-100%) the bacterial communities of bees. Analyzes of the alpha and beta diversity of these communities revealed significant differences between mountain and lowland bees, the microbiome of the latter being characterized by a higher diversity due to the greater representation of non-dominant taxa [26]. The contribution is original.

(iii) Other

15. The trematode *Aphalloides coelomic*ola, a parasite of *Knipowitschia caucasica* in Lake Atanasovo, was redescribed. Molecular genetic characterization (fragment of the 28S rRNA gene) was also carried out. Based on a phylogenetic analysis, the place of *A. coelomicola* in the monophyletic family Cryptogonimidae and the non-monophyletic status of the family Heterophyidae were confirmed [3]. The contribution is confirmatory.

16. A series of literature reviews was published on methods used in bee genotyping in Bulgaria [27], the role of bees as pollinators, biotic and abiotic factors affecting bee mortality and the death of entire hives [13, 16, 17, 19, 20], the phylogeography of bat lyssaviruses in Europe [8], and hypotheses about the origin and centers of domestication of horses [25].

4. Significance of the research conducted by the candidate

The candidate submitted a list of 277 citations, the majority of which (209.75%) refer to the published literature reviews. The citations of experimental studies with contributions assigned above to group (i) are 24 (9%), and those with contributions assigned to group (ii) are 28 (10%). These data support the relevance of the scientific contributions of the candidate's experimental research in both areas of research (i.e.> 20 citations for only one area). It should also be noted that the preparation of literature reviews is an activity that has a significant impact on the evaluation of IBER at BAS due to the typically large number of citations these publications carry.

Conclusion: the citation data presented by the candidate exceed many-fold the minimum national requirements and those of the BAS for the academic position Associate Professor, as well as the Regulations for the terms and conditions for acquiring scientific degrees and for occupying academic positions at IBER-BAS.

5. Most significant applied science contributions

Dr.Neov's experimental studies on native breeds of domestic animals (group i) and bee pathogens (group ii) in Bulgaria may be significant for both conservation biology and veterinary practice.

6. Project activities

The applicant's curriculum vitae does not contain data, but the compliance report shows that Dr.Neov engages effectively in team research; he participated in 5 national scientific projects (of which 1 is ongoing) and in 1 international project.

7. Candidate's research profile

Dr.Neov is a specialist with a still vaguely defined scientific research profile related to the application of molecular genetic methods in solving a diverse range of problems and using different model systems. Based on the documents submitted for the competition, molecular genetic research on domestic animals can be considered as the main direction of the candidate's scientific research activity shaping his scientific profile. I believe that after his habilitation he should make an effort to develop his research in a focused manner. I am confident that he can contribute to the enrichment and development of the lines of research of his research group. In this respect, an upgrade to the approach and model systems of his PhD thesis would be fruitful.

8. Role of the candidate in training of young scientific personnel

In the submitted documents, there are no data about Dr.Neov's participation in training young scientific personnel.

9. Critical notes

(i) Paper 3: Although the paper does not provide information on the length of the alignment used for the phylogenetic hypothesis, I consider it to be quite short, as the new published 28S rDNA sequence for *A. coelomicola* is 747 bp (i.e. domains D2 -D3 instead of D1-D3), and that for *Centrovarium* sp. VT-2007a is even shorter (574 bp). The article states that "All positions containing gaps and missing data were eliminated", which is an indication of sequence overlap within a very small segment of the gene.

(ii) I should note that despite the availability of data on the individual contributions of the authors in 12 of the articles, some of them are filled in formally and quite generally and do not allow to highlight the contributions of the candidate in these studies. For example, the text in article [7] "software, B.N., visualization, B.N" means that the applicant created new software or tested existing code (does not match the content of the article), and prepared Fig. 1 (map of Bulgaria with an indication of the locations of the samples - too insignificant a contribution to justify authorship). This is just a recommendation to pay more attention to this part in future publications.

(iii) In Table 3 of the compliance report, the applicant has indicated that all citations of his publications are in international publications with an impact factor – this does not correspond to reality.

Conclusion

The documents for participation in this competition submitted by the candidate meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Rules of the BAS for the implementation of this law and the criteria for occupying scientific positions of the Scientific Council of IBER-BAS. That is why I recommend the Scientific Jury to support and propose to the scientific council of IBER-BAS the election of Dr. Boyko Neov as an Associate Professor at the section "Biodiversity and Ecology of Parasites" – IBER-BAS.

October 2, 2024

Reviewer:

(Prof. Dr. Aneta Kostadinova)