N.V Mit , **G.S. Zhunussova** , **A.S. Amirgaliyeva** ,

K.B. Bespalova , **A.V. Perfilyeva** , **Zh.M. Seisenova**

Institute of Genetics and Physiology of the Ministry of Education
and Science of the Republic of Kazakhstan, Almaty, Kazakhstan
*e-mail: nata-mit@yandex.ru

REVIEW OF GENETIC STUDIES
OF MASTIF-LIKE DOG BREEDS

Currently the genetic studies of dogs are carried out quite widely. However, there are more than 400 varieties and breeds of dogs in the world, and not all of them are equally well studied. Thus, the genetical characteristics of mastiff dogs, which include the Kazakh national breed of herding dogs Tobet, have not been sufficiently studied. The purpose of this work is to review the genetic studies available in the scientific literature in varieties of mastiff-like dogs. The review examines the genetic diversity of mastiff dogs, the mechanisms of adaptation of breeds to living conditions, genetic factors of predisposition to diseases, as well as phylogenetic relationships between different breeds. Revealing phylogenetic connections of aboriginal dog breeds allows us to trace the correlation with the history and phylogeography of the peoples who inhabited the areas where these breeds were formed. Analysis of the parameters of genetic diversity and the level of inbreeding will allow us to apply science-based approaches to preserve and improve national dog breeds. Genetic research will improve breeding programs, reduce the number of hereditary diseases in dogs, help preserve the gene pool of the breed, as well as restore local breeds, including Tobets, which are on the verge of extinction.

Key words: mastiff dog breeds, Tobet, genetic diversity, phylogeny.

Н.В. Мить, Г.С. Жүнісова, А.С. Әмірғалиева,
К.Б. Беспалова, А.В. Перфильева, Ж.М. Сейсенова
ҚР БҒМ ҒК «Генетика және физиология институты» РМК, Алматы, Қазақстан
*e-mail: nata-mit@yandex.ru

Мастиф тәрізді ит тұқымдарының
генетикалық зерттеулеріне шолу

Қазіргі уақытта иттерге генетикалық зерттеулер кеңінен жүргізілуде. Дегенмен, әлемде 400-ден астам түрлері мен тұқымдары бар, және олардың бәрі бірдей жақсы зерттелмеген. Мәселен, қазақтың ұлттық төбет иттеріне жататын мастиф тәрізді иттер генетикалық тұрғыдан жеткілікті зерттелмеген. Бұл жұмыстың мақсаты – ғылыми әдебиеттердегі мастиф тәрізді иттердің әртүрлі түрлері бойынша генетикалық зерттеулерге шолу жасау. Шолуда мастиф тәрізді иттердің генетикалық алуантүрлілігі, тұқымдардың тіршілік ету жағдайларына бейімделу механизмдері, ауруларға бейімділіктің генетикалық факторлары, сондай-ақ әртүрлі тұқымдар арасындағы филогенетикалық байланыстар қарастырылған. Жергілікті ит тұқымдарының филогенетикалық байланыстарын анықтау, осы тұқымдар қалыптасқан аймақтарды мекендеген халықтардың тарихымен және филогеографиясымен байланысын қадағалауға мүмкіндік береді. Генетикалық әртүрлілік параметрлерін және инбридинг деңгейін талдау ұлттық ит тұқымдарын сақтау және жетілдіру үшін ғылыми негізделген тәсілдерді қолдануға, оларды өсіру жөніндегі бағдарламаларды жақсартуға, иттердегі тұқым қуалайтын аурулардың санын азайтуға, тұқымның генофондын сақтауға, сондай-ақ, жойылу шегінде тұрған жергілікті тұқымдарды, соның ішінде Төбеттерді қалпына келтіруге мүмкіндік береді.

Түйін сөздер: мастиф тәрізді ит тұқымдары, Төбет, генетикалық әртүрлілік, филогения.

Н.В. Мить*, Г.С. Жунусова, А.С. Амиргалиева,
К.Б. Беспалова, А.В. Перфильева, Ж.М. Сейсенова

Институт генетики и физиологии МНВО РК, Алматы, Казахстан
*e-mail: nata-mit@yandex.ru

Обзор генетических исследований мастифообразных пород собак

Генетические исследования собак в настоящее время проводятся достаточно широко. Однако, в мире насчитывается более 400 разновидностей и пород собак, и не все они изучены одинаково хорошо. Так, мастифообразные собаки, к которым относится казахская национальная порода пастушьих собак Тобет, в генетическом плане исследованы недостаточно. Целью данной работы является обзор имеющихся в научной литературе генетических исследований у различных разновидностей мастифообразных собак. В обзоре рассмотрены генетическое разнообразие мастифообразных собак, механизмы приспособленности пород к условиям обитания, генетические факторы предрасположенности к заболеваниям, а также филогенетические связи между различными породами. Установление филогенетических связей аборигенных пород собак позволяет проследить корреляцию с историей и филогеографией народов, населявших районы формирования данных пород. Анализ параметров генетического разнообразия и уровня инбридинга позволит применить научно-обоснованные подходы для сохранения и совершенствования национальных пород собак, улучшить программы по их разведению, уменьшить число наследственных заболеваний у собак, способствовать сохранению генофонда, а также восстановлению местных пород, в том числе и Тобетов, находящихся на грани исчезновения.

Ключевые слова: мастифообразные породы собак, Тобет, генетическое разнообразие, филогения.

Introduction

Mastiff-type dogs have been faithful companions of man since ancient times and were used mainly as guard and hunting dogs. Images of mastiff-type dogs have been found on Egyptian monuments dating back to 3000 BC. During archaeological excavations in the city of Nineveh, a vase dating back to 612 BC was discovered; the vase depicts a massive dog with a large square head and armor [1]. Julius Caesar mentioned the huge dogs of the Britons who fought with their masters against the Roman legions in 55 BC. [2]. These dogs came to Great Britain together with the Celtic tribes who came from Asia Minor in the 4th-3rd centuries BC. There are different points of view on the origin of the breed, but the exact origin is unknown. According to many researchers, Tibetan mastiffs, which lived in the mountains of Central Asia two thousand years ago, are the most likely ancestors of modern mastiffs. Considering that in ancient times the most important trade routes passed through Asia, Asian dog breeds could have spread throughout the world.

The beginning of purebred breeding of mastiffs dates back to the 15th century. The breed underwent various changes several times, as well as periods of decline in numbers up to almost complete extinction, and periods of recovery. Thanks to thoughtful selection, it was possible to recreate and significantly improve the breed. Now mastiffs are widespread throughout the world, the breed is gaining popularity, and all new varieties are achieving official recognition.

In modern cynological science, there is a tendency to reorient research of various dog breeds to the genetic level of analysis, which contributes to a deeper understanding of the genetic mechanisms that determine the health, behavioral characteristics and evolutionary development of dogs. And in general, it should be noted that genetic research of dogs is carried out quite widely. However, genetic studies of mastiffs are few and far between, and many of their varieties, including the Kazakh Tobet, have still been virtually unstudied at the molecular genetic level. At the same time, the results of genetic analysis can influence such important aspects as reducing the genetic load and decreasing hereditary diseases, improving the health and well-being of breeds, and improving breeding and restoration programs.

In this paper, we reviewed literature on genetic studies of mastiff-like dog varieties common in different countries of the world.

Literature review

Currently, the mastiff-like group of dogs includes 24 breeds, 14 of which are internationally recognized and another 10 are not officially recognized yet. All mastiffs are large, well-proportioned and powerful animals, with an average weight of

50 kg and an average height of at least 60 cm. All varieties have a muscular build, a large head and a flat, wrinkled forehead, a square muzzle with pronounced flews, a powerful neck, a wide mouth, a thick tail, slightly outwardly turned paws, and V-shaped hanging ears. The length of the coat, color, eye and nose color depend on the breed variety.

The Kazakh Tobet [3] also belongs to the mastiff-like group of dogs. This is an ancient breed of large and strong shepherd dogs, which was known even before our era. Tobets are described in the books of Marco Polo as large, donkey-sized, assistant dogs for nomads. Tobets are strong, hardy, unpretentious dogs, adapted to harsh climatic conditions and a nomadic way of life. The coat is thick, coarse, with a well-developed undercoat, the color is black, brown, red, gray, their combinations are possible. Height at the withers is 66-76 cm, weight is 45-65 kg. The average life expectancy is 11-14

years. Tobets were faithful assistants of nomads, they have long been used for grazing and guarding livestock and protecting them from wolf attacks. These are calm, balanced dogs with a pronounced territorial instinct, natural guard dogs. They bark little. One of the characteristic differences of the Tobets from other wolfhounds is friendliness to a peacefully minded person and a child. In an unfamiliar environment, the behavior is calm, confident. It has a high intelligence, is able to assess the situation and make decisions.

Unfortunately, in recent years the population of Tobets has significantly decreased. Today, the breed is on the verge of extinction. To preserve the breed, it is necessary to study it thoroughly, including genetic research to study the genetic structure of the population.

The appearance of some breeds from the Mastiff-like dog group is shown in Figure 1.

Figure 1 – Appearance of some mastiff-type dog breeds.
A – Turkish Mastiff (Akbash), B – English Mastiff, C – Neapolitan Mastiff,
D – Kazakh Tobet, E – Bullmastiff, F – Tibetan Mastiff.

One of the most studied genetically among mastiff-like breeds is the Tibetan Mastiff (TM), which is considered to be the ancestor of all mastiffs.

The TM is a breed of domestic Chinese dogs well adapted to the high-altitude conditions of Tibet, considered one of the most archaic, ferocious and largest dogs in the world. There are a number of studies, mainly by Chinese scientists, devoted to the genetic mechanisms of adaptability of this breed to the extreme environmental conditions of the Qinghai-Tibet Plateau, such as low oxygen concentration, very cold and dry climate, strong UV radiation. At high altitudes, high levels of UV radiation can cause direct DNA damage. In addition, hypoxia increases the production of reactive oxygen species, which increase DNA damage. A number of studies have revealed that the mechanisms of TM adaptation to high altitude are determined by several genes associated with adaptation to hypoxia, in particular EPAS1 and HBB [4-7]. Based on genome-wide data analysis, Cai C et al. identified, that families of DNA repair and damage response genes are expanded in TM [8]. Contrariwise, the authors revealed a limitation of the gene families associated with ATPase activity. This allows cells to reduce ATP production and oxidative phosphorylation of mitochondria, and thus reduce ATP consumption under hypoxic conditions. Genes involved in the regulation of RNA polymerase II transcription (p65, ATF3, EHF, Loc4981817, ZNF280D, MYPOP, ATF5, and FOXI2), as well as some genes involved in the hypoxia response and α/β-oxidation of fatty acids, were shown to be under positive selection in the Tibetan Mastiff [8].

Based on the SNP polymorphism studies, Li Y. et al. conducted a comparative analysis of TM, aboriginal Chinese dogs and gray wolves to identify candidate genes for high-altitude adaptation [9]. The investigation also confirmed the important role of genes associated with the response to hypoxia in adaptation to the Tibetan Plateau invironment in different groups of organisms. Of the 16 genes that showed positive selection signals, 12 genes were associated with the body's response to hypoxia, including the genes EPAS1, SIRT7, PLXNA4 and MAFG .

Studies of altitude adaptation in TM are mainly related to genes localized in autosomes. However, Hong Wu et al. focused on the X chromosome [10]. The advantage of this approach is the reduced population size and the ability to analyze the hemizygous state in males. Based on publicly available genome-wide data from five TM and five dog populations living at altitudes from 300 to 4000 m, the authors identified five genes localized in the X chromosome and showing signs of selection. One of these genes was angiomotin (AMOT), which is associated with the process of angiogenesis.

A number of investigations are also devoted to the study of genetic diversity and structure of TM populations. In the work of Ren D.R. et al., using 10 microsatellite loci, an assessment of the genetic diversity of TM from 4 populations (Tibet, Gansu, Qinghai and Beijing) was carried out [11]. The results of microsatellite analysis showed high genetic diversity in all studied TM populations. Thus, the number of alleles per locus (Na) ranged from 6 to 13, the observed heterozygosity Ho = 0.69, the expected heterozygosity He = 0.79, the average PIC value was 0.758. Interestingly, within the populations there was a significant deficit of heterozygotes (the average FIS value = 0.11,), as well as fairly high inbreeding rates (the average FIT value = 0.12, bootstrap 95% CI (0.06, 0.18)). Thus, inbreeding has occurred quite frequently in TM populations, so the breed needs effective breeding projects based on genetic research.

In the work of Miao B. et al. the origin of genes of high-altitude adaptation in TM was studied [12]. Comparative analysis of canine genomes showed that the differences in haplotypes of the EPAS1 and HBB loci in high-mountain dogs and dogs from lowland areas are much greater than in high-mountain dogs and wolves. Based on the calculation of the recombination rate, the authors excluded the influence of ancestral polymorphisms in the EPAS1 and HBB loci. Lower sequence divergence compared to the genomic background indicates a relatively recent splitting of alleles and confirms the introgression hypothesis. Thus, introgression of the EPAS1 and HBB loci in mountain dogs helped them adapt to a hypoxic environment in a relatively short period of time.

Research is underway to study the genetic polymorphism of STR loci to create a gene polymorphism database for the Tibetan Mastiff. Using fluorescent multiplex amplification of STR loci, Xiong X et al. analyzed the polymorphism of 16 STR loci in 449 TM [13]. As a result, it was shown that all the studied loci are highly polymorphic. Thus, the FH2010 locus had 10 alleles, the PEZ21 locus had 12 alleles, and the PEZ05 locus had 13 alleles. The remaining 13 loci had more than 15 alleles. Thus, the analysis of polymorphic STR loci provides the opportunity for individual identification and can be used to determine paternity in TM. The high degree of polymorphism of the studied loci provides the basis for the creation of a TM gene polymorphism database.

The mitochondrial genome of Tibetan Mastiffs has been extensively studied. Since mitochondrial DNA is not highly conserved and has a fairly high mutation rate, the mitochondrial genome sequence can be used to identify and reveal phylogenetic relationships between different groups of organisms, and in particular, between dog breeds.

Deng LX and He S. revealed the complete sequence of the TM mitochondrial genome [14]. The authors showed that the TM mitochondrial genome consists of 16,729 bp and has a typical structure: 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and a non-coding region. The non-coding region (D-loop region) has common features with the mitochondrial genomes of other mammals, and the largest number of variable sequences is located in this region.

The complete mitochondrial genome sequence of TM was also characterized by Guo X. et al. in another study using Illumina high-throughput sequencing technology [15]. The authors showed that the total length of the circular mitochondrial DNA included 16,730 bp. Similar to the previous study the mitogenome structure was characterized as typi- cal for vertebrates and included the same genomic components. The authors identified transcription initiation codons, as well as 3 types of stop codons for mitochondrial genes. According to the nucleotide composition, the mitogenome of TM is distributed as follows: 31.6% A, 25.5% C, 14.2% G and 28.7% T. Thus, TM mtDNA is significantly enriched in A-T pairs, which make up 60.3%.

In a study of mtDNA of purebred Chinese Tibetan Mastiffs by Li YX et al., the mtDNA control region (D-loop region), which is non-coding but regulates the processes of mitochondrial replication and transcription, was analyzed [16]. The authors found that this region in TM contained blocks of conserved sequences, a central domain, and sequences associated with termination, and the length of the sequence varied due to a different number of 10-nucleotide repeating units. In the control region of TM mtDNA, the authors identified 75 polymorphic sites that constitute 37 haplotypes; the diversity of the haplotype was 0.990, and the diversity of nucleotides was 1.201. Based on the sequences of the control regions, a phylogenetic tree was constructed in which Chinese TM were divided into three categories according to the origin and geographical classification of TM. Phylogenetic analysis of the hypervariable sequence HVR-I showed a close relationship between TM and Labrador retrievers.

Cai Z.F. et al. analyzed the mtDNA genomes of TM using long-range sequencing technology [17].

Since this technology increases the accuracy of reads, the authors were able to analyze high-quality reads from the mitochondrial genomes of 79 TMs. As a result, it was found that Tibetan Mastiffs living outside the Tibetan Plateau have hybridized with other dogs.

To preserve the population of TM, studies on their genetic diversity are of great importance. Ren Z. et al. conducted a comparative analysis of mtDNA of individuals from Gansu Province and the Tibetan region [18]. As a result of sequencing the hypervariable region I of mtDNA, the authors identified twelve polymorphic sites and eight haplotypes. Haplotypes H4 and H8 were found only in the Tibetan population. In Gansu Province, the genetic diversity of the TM population was higher than in the Tibetan region. Genetic variability in the general population was low, which indicates the need for measures to protect the TM gene pool. Based on the results of sequencing the hypervariable region I of mtDNA, the authors constructed a phylogenetic tree and found that TM originated from gray wolves and had different maternal lines.

The work of Zhang S. and co-authors is devoted to the study of biological characteristics of mesenchymal stem cells of TM bone marrow [19]. As is known, pluripotent stem cells can differentiate into various cell types and have great potential for use in therapy and restoration of tissues and organs. The authors cultured TM bone marrow stem cells for 23 passages and then analyzed their karyotype. As a result, it was shown that the mesenchymal stem cells were diploid, genetically stable and had the potential to differentiate into adipocytes, hepatocytes, osteoblasts, cartilage cells and insulin-secreting cells. Using semi-quantitative PCR and ELISA, it was shown that TM stem cells express cluster of differentiation (CD) 73, CD90, CD105, CD166 and vimentin. It was revealed that these cells can self-renew, but their proliferation rate decreases with age. The study showed the potential of TM stem cells for therapy and regeneration processes.

There are far fewer published studies on other mastiff varieties, even for common breeds such as the English Mastiff and Bullmastiff. The available studies focus primarily on health issues related to longevity and mortality. Health issues may be the result of complex interactions between many genes, or may be due to limited genetic variation within a breed, which favors the inheritance of defective genes.

A study by Bell L et al. reported morbidity and mortality data for mastiffs based on a survey of dog owners [20]. According to the survey results, the av-

erage age at death was 8 years, with the average age at death in Europe being 7.72 years and in North America 8.17 years. The most common causes of death for all dogs were cancer (47%), old age (16%), heart problems (8%), and stomach problems (7%). The average age at death from cancer in dogs was 7.85 years, with 23 types of cancer identified, the most common being osteosarcoma, especially in neutered animals.

Familial cerebellar ataxia with hydrocephalus in Bullmastiffs has been previously described as a monogenic autosomal recessive trait [21]. Christen M. et al. examined two bullmastiffs with disease-onset traits at a young age [22]. They sequenced the affected bullmastiff's genome and compared it with genomes from different dog breeds, as well as with archival samples from two other affected bullmastiffs. They found that the affected dogs had a pathogenic homozygous frameshift variant in the MFF gene that shortens the wild-type open reading frame and is the most likely cause of the disease. Because the phenotype of this disease in bullmastiffs is similar to that in humans, the authors propose renaming it "mitochondrial fission encephalopathy (MFE)".

Lymphoma is a fairly common disease among bullmastiffs. Lymphoma is a disease with a hereditary predisposition. The study by Mortlock S.A. et al. was devoted to identifying risk loci predisposing to the disease in this breed [23]. Based on a genome-wide association analysis (GWAS) of 49 sick mastiffs and 281 control cases, the authors identified genomic regions associated with the risk of lymphoma, localized to CFA13 and CFA33. SNPs with both genome-wide and chromosomal significance were identified in these regions. The authors identified potential candidate genes associated with lymphoma, including the MYC proto-oncogene, the bHLH (MYC) transcription factor, the Pvt1 lncRNA oncogene (PVT1), and a region syntenic with human and mouse lncRNA Pvt1. The authors suggest that the increased risk of developing lymphoma may also be due to a predisposing genetic background.

Among other tumors in mastiff-like dogs, cases of sarcoma have been described, the incidence rate is also quite high [24-25]. Genetic factors are also involved in the development of this disease, since familial cases of the disease have been described. Based on the expression of the CD28, abca5, CCDC3 and SMOC2 genes, three molecular subgroups of lymphoma were identified and demonstrated the possibility of predicting the development of the disease and a correlation with survival. Prognostic factors also include the expression of the p53 and p16 genes, as well as the expression of the VH1-44 gene, encoding the variable region of the immunoglobulin heavy chain [26-28].

There are a number of works in the literature devoted to the study of hereditary ophthalmological diseases. This is a rather heterogeneous group of eye diseases described in various dog breeds. Ki- jas J.W. and co-authors studied the inheritance of progressive retinal atrophy (PRA) in the English Mastiff [29-30]. The disease has a dominant type of inheritance. Analysis of candidate genes allowed to characterize two synonymous and one non-synonymous mutation in the canine rhodopsin gene. The authors found that the non-synonymous mutation (T4R) causes progressive retinal atrophy in the English Mastiff. At the same time, this mutation was not detected among dogs suffering from PRA and belonging to other studied breeds. Thus, testing of bullmastiffs for the presence of T4R revealed that one of them was heterozygous for this mutation, and the second was homozygous for the wild type. This fact allowed the researchers to conclude that the T4R mutation is specific to the English Mastiff breed. The authors described the disease phenotype in the English Mastiff as similar to the phenotype of people with mutations in the rhodopsin gene. Thus, studying this disease on a canine model will allow us to better understand the mechanisms of disease development and open up new prospects for the development of therapeutic methods.

Caduff M. et al. studied the mechanisms of oculocutaneous albinism development in bullmastiffs [31]. It is known that this disease in humans and many animal species is caused by variants of the SLC45A2 gene. Caduff M et al. analyzed the genome sequence of a highly inbred bullmastiff with oculocutaneous albinism. As a result of the genomic data analysis, the authors were able to identify a single nucleotide deletion in exon 6 of the SLC45A2 gene (NM_001037947.1:c.1287delC), previously not registered in known databases. According to the predictors, this deletion will lead to an early premature stop codon. The authors validated the obtained result using the Sanger sequencing method, the results of which coincided with the phenotypes of the family members of the affected bullmastiff. Genotyping results from 174 unrelated dogs of different breeds showed that they were homozygous for the wild type. This allowed the authors to conclude that SLC45A2:c.1287delC is the cause of oculocutaneous albinism in the affected bullmastiff.

Overall, it should be noted that studies of inherited ophthalmologic diseases in dogs are important for the search for candidate genes responsible for the development of the disease, as well as for the

development of animal models that mimic human diseases.

Among other inherited diseases, familial juvenile glomerulonephropathy has been described in French Mastiffs [32]. In addition to analyzing clinical and histological data, the authors also studied the pedigrees of the affected mastiffs. It was found that individuals of both sexes were affected by glomerulopathy with the same frequency, therefore, the genes associated with the development of the disease are not linked to the X chromosome, but are inherited autosomal. Analysis of the inheritance of glomerulonephropathy in a number of generations allowed the authors to assume an autosomal recessive type of inheritance, although more complex variants cannot be ruled out, for example, autosomal dominant with incomplete penetrance or polygenic.

Since mastiffs are large dogs, they are susceptible to various joint diseases. Cases of elbow and hip dysplasia have been reported in various dog breeds [33-35]. These diseases often occur together with osteoarthritis. Ginja M. et al. searched for candidate SNPs to identify associations with various joint diseases and assess genetic risk, but did not identify significant polymorphisms [35]. Presumably, this is due to the complex multifactorial nature of the diseases under study, which may include polygenic inheritance and significant influence of environmental factors. Research to identify the molecular genetic basis of joint dysplasia continues, but progress in this direction is slow.

A number of studies have been devoted to the investigation of the genetic diversity of different dog breeds. Thus, in the study by Mortlock S.A. et al., the genomic diversity of Bullmastiffs was stud- ied using genealogical and molecular analysis. [36]. The genealogical analysis took into account the data on the pedigrees of Bullmastiffs for more than 30 years. The inbreeding coefficient based on the genealogical analysis averaged 0.044, the number of common founders (142) was greater than the effective number of founders (79). The assessment of genetic diversity and population structure was based on the analysis of genome-wide genotyping data of 170,000 single nucleotide polymorphisms in 185 Bullmastiffs. Main indicators were calculated on the basis of the molecular analysis of SNPs. The average inbreeding coefficient was 0.033, multilocus heterozygosity (MLH) – 0.206, the effective population size (Ne) – 29.1. In general, it can be noted that the molecular and genealogical estimates of the inbreeding coefficients for genotyped dogs show a positive correlation. Given the relatively small effective population size, reducing the level of in-

breeding may be beneficial to the overall welfare of the breed. To determine the phylogenetic relationships of the Bullmastiff, the authors constructed a neighbor joining tree, which showed a close relationship with the English Bulldog, one of the ancestors of the Bullmastiff breed.

In another study by the same authors [37], genetic variants specific to the Bullmastiff breed were identified using high-density signal intensity data and whole genome sequencing. The genome-wide analysis identified approximately 3000 copy number variants (CNVs) in Bullmastiffs. After sequence alignment, 82 CNV regions were identified, of which 50 regions had not been previously identified in other dog breeds and are potentially breed-specific. Finding specific genetic markers may be important for future studies of breed-specific characteristics or diseases.

In recent years, the number of studies devoted to the phylogenetic relationships and the evolution of various dog breeds has increased.

In the study by Cai C et al., a comparative analysis of the genomes of Tibetan Mastiff, Mongolian Mastiff and Canis Lupus was carried out [8]. The authors performed de novo sequencing of the Tibetan and Mongolian mastiffs and Canis Lupus genomes and conducted a comparative analysis of repetitive sequences in their genomes. Based on the genomic sequence data, the authors constructed a maximum likelihood phylogenetic tree and determined the taxonomic status of the studied dog breeds. As a result, a close relationship was revealed between the Tibetan and Mongolian mastiffs, which turned out to be very close in phylogenetic terms. In addition, TM and Mongolian mastiff formed a common clade with Canis Lupus, despite the diversity of their habitats. This suggests that the Tibetan and Mongolian mastiffs originated from Canis Lupus.

Similar results were obtained when analyzing the results of mtDNA sequencing of TM. The work of Li Q. et al., based on the analysis of mitochondrial DNA sequences, is devoted to the study of the origin of Tibetan mastiffs [38]. The authors determined the complete mitochondrial genome (mtDNA) sequence of the Tibetan mastiff and analyzed the phylogenetic relationships between the Tibetan mastiff and other Canidae species using the coyote (Canis latrans) and The gray wolves (Canis lupus) as the outgroups. As a result, the authors showed that TM and other dog breeds are grouped together with the gray wolf, while coyotes are grouped separately. This proves the origin of TM and other dog breeds from the gray wolf. At the same time, TM is grouped with large dog breeds, while smaller ones are grouped sepa-

rately. Therefore, the origin of large breeds such as the Old English Sheepdog, Leonberger, and Saint Bernard from TM seems quite likely. The authors also showed that the approximate time of TM divergence from the gray wolf turned out to be 16,000 years earlier than in other breeds.

Similar data on the origin of the Tibetan Mastiff were obtained using DNA barcoding, which is also an effective method for species identification and analysis of phylogeny and evolutionary relationships [39]. For the first time, the authors applied the method of DNA encoding of the cytochrome oxidase C gene subunit I in various representatives of the canine family. The authors constructed a phylogenetic tree and identified 4 different maternal lineages in dogs (A-D). They revealed, that TM is of East Asian origin, as it belongs to the A line, and is probably the ancestor of a number of large dog breeds.

The study by Australian scientists Hsu WT, Williamson P, Khatkar M is devoted to the analy- sis of genomic differences and similarities between groups of individuals of many breeds. [40]. The authors combined the available genetic resources of their own laboratory biobank with publicly available data on the genotypes and phylogenetic structure of 23 breed clades to detect genomic regions specific to bullmastiffs. In total, more than 8,000 dogs belonging to 250 breeds were examined. As a result, significant genomic regions specific to bullmastiffs were identified on 15 chromosomes and the similarity of bullmastiffs with the ancestors of the breed: mastiffs and bulldogs was shown. The most specific regions were found on chromosomes CFA1, CFA9 and CFA18. Significant regions were identified on 15 chromosomes, with the most differentiated regions found on CFA1, CFA9 and CFA18. The study contributes to the understanding of the processes of genetic drift in the formation of a breed, as well as the relationship of genetic determinants with its characteristic features and morphological traits.

The population structure of Chinese dog breeds was studied in the work of Yang Qiu et al. [41]. To analyze genetic diversity and determine family relationships, the authors genotyped 157 unrelated dogs belonging to 15 different Chinese breeds. For comparison with some Western breeds (Rottweiler, Papillon) and the Asian wolf, data from open sources (LUPA database) were used. As a result, it was found that the indigenous breeds of Chinese dogs had a lower LD index, as well as a lower level of heterozygosity. When constructing a phylogenetic tree, a clear division of Chinese and Western dog breeds into two clades was found. The authors included Ka-

zakh Shepherd Dogs and Mongolian Si Dogs among the indigenous Chinese breeds. Evidence of introgression of Western dogs into the genomes of Chinese and Kazakh Shepherd Dogs and Mongolian Si Dogs was obtained. It is noteworthy that the largest number of polymorphic SNPs were found in Kazakh Shepherd Dogs. The authors identified candidate genes that may be responsible for certain phenotypic traits of Chinese indigenous dog breeds. All the dog breeds studied that inhabit the Qinghai-Tibet Plateau, including the TM, were grouped together and showed signs of genetic adaptation to high altitude. At the same time, breeds belonging to the moun- tain hound group had the genes KRT9, CAMTA1, NOL8 and RORB, which are supposedly candidate genes that affect running speed. The authors also calculated the genetic distances between the breeds, which turned out to be greater between Chinese breeds than between Western breeds. This suggests greater differences between Chinese breeds, which is likely due to earlier domestication and less strict breeding programs for Chinese dogs. Based on the results of the phylogenetic analysis, the authors suggested that Western breeds such as the Papillon, Eurasier and Greenland Sled Dog may have Chinese origins, since these dogs were grouped with Chinese populations.

Genetic studies of the Kazakh Tobet breed of dogs were initiated by Perfilieva A. et al. To date, studies of the genetic structure of the Tobet population from southern Kazakhstan have been conducted using minisatellite markers. Based on the analysis of allele frequencies of 19 microsatellite loci recommended by the International Society of Animal Genetics for dogs, the authors determined the main indicators of genetic variability in the studied sample [42]. The obtained data were analyzed in a comparative aspect with other dog breeds. The authors revealed a high level of heterozygosity and polymorphism in the studied population. The percentage of polymorphic loci was 100%, with 5-10 alleles per locus. The Hardy-Weinberg equilibrium assessment showed a deviation for two loci AHT137 and AHTh260. The average number of alleles and the average effective number of alleles in Tobets were higher than in other molossoid dog breeds. The level of PIC polymorphism exceeded 60% for all loci. The high level of genetic diversity and the overall low level of inbreeding, quantitatively assessed using microsatellite loci, were comparable with similar indicators characteristic of mongrel dogs. According to the authors, the high observed heterozygosity indicates the presence of crossbreeding processes in the studied sample [42].

These data were confirmed by the authors on a larger sample, including dogs from different regions of Kazakhstan and Mongolia [43]. In addition to the microsatellite analysis data, this work analyzed the results of whole-genome sequencing of two Tobet dogs. The authors confirmed the high level of heterozygosity and polymorphism characteristic of the Tobet breed. The analysis of genomic data showed a complex genetic structure of Tobets, characterized by seven different clusters found in all studied populations from three regions of Kazakhstan and Mongolia. Based on the genomic data of 45 dogs from 25 breeds, including two Tobets, the authors constructed a phylogenetic tree that showed a genetic relationship between the Kazakh Tobets, Central Asian Shepherd Dog, and Turkish Akbash. The presence of the A18 haplotype in Kazakh Tobets confirms the hypothesis of the ancient origin of the breed, which was previously suggested based on archaeological finds and written sources.

To summarize, it can be noted that the Tibetan Mastiff is the most studied genetically among mastiff-like dog breeds. It is followed by the Bullmastiff, English Mastiff, Kazakh Tobets, Neapolitan Mastiff, and French Mastiff, with a fairly large gap (Figure 2). For many other mastiff-like breeds, there is no information on genetic studies in the scientific literature.
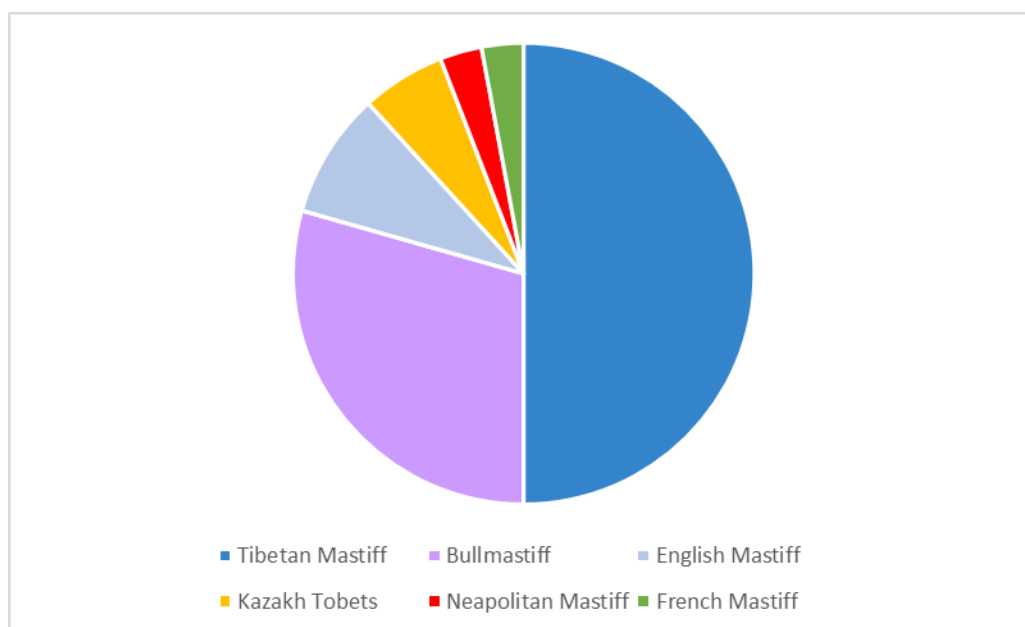

Figure 2 – Number of genetic studies for different mastiff-type dog breeds

The largest number of studies are devoted to the study of diseases among different dog breeds, since hereditary pathology can play an important role in selection work to improve breed characteristics. Researchers are also interested in the origin and phylogenetic relationships with other breeds, since these aspects help to better understand the processes of ancient migrations. Many works are devoted to the study of genetic diversity and population structure, as well as adaptation resources (Figure 3).
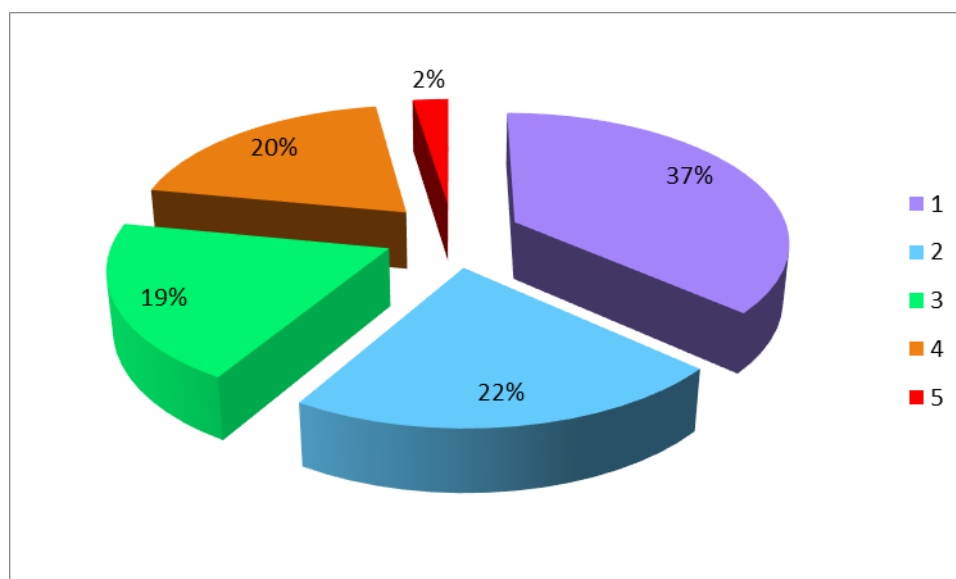
1 – diseases; 2 – phylogenetic relationships; 3 – genetic diversity and population structure;
4 – adaptive capabilities; 5 – stem cell research.

Figure 3 – Share of research on different topics.

Conclusion

Thus, genetic studies of dogs allow us to identify genetic markers specific to a particular breed, characterize the population structure, assess genetic diversity and trace phylogenetic relationships between different breeds, and assess the contribution of genetic variants to the development of diseases. In most cases, known dog breeds have limited genetic diversity, which is a consequence of a small founder population, selection for exterior appearance, and minimal geographic locations for breeding. In addition, inbreeding used in dog breeding to preserve breed characteristics increases the genetic load of the population, contributing to the accumulation of mutations and the spread of hereditary diseases. Genetic studies contribute to an increase in knowledge about mastiff diseases and determine future prospects for the treatment of pets. The results of genetic studies can also be used to improve the welfare of the breed. Assessing the genetic diversity and identifying phylogenetic relationships of dog breeds can significantly complement and improve breeding and restoration programs.

In conclusion, it should be noted that the aboriginal dog breeds in Kazakhstan, which are a unique historical heritage, have not yet been studied at the molecular genetic level. Meanwhile, knowledge of the genetic structure and identification of genetic markers of a particular breed are important for the preservation and improvement of the breed, as well as for the conservation of the biodiversity of the region. The results of genetic studies can be used in drawing up programs for the preservation and breeding of dog breeds adapted to local conditions and the characteristics of human economic activity.

Funding

Conflict of interest

The authors of the article confirm the absence of a conflict of interest.

Reference

1. Flaim D. Mastiff History: The Huge & Noble Ancient Dog Breed // American Kennel Club – 2024. – [Electronic resource] // – URL: https://www.akc.org/expert-advice/dog-breeds/mastiff–history–huge–noble–ancient–dog–breed (date of access: 02.03.2024).

2. Chelnokova V.N., Dorogan M. Everything about the dog // Vladis. – Rostov-on-Don, 2005. – 1-640 p. – [Electronic resource]. // – URL: https://www.maxima-library.com/year/b/441605 (date of access 28.02.2024).

3. Shmonina T. Tobet (Kazakh wolfhound) // Usatiki.ru – 2024. – [Electronic resource] // URL: https://usatiki.ru/tobet/ (date of access 02.03.2024).

4. van Patot MC, Gassmann M. Hypoxia: adapting to high altitude by mutating EPAS-1, the gene encoding HIF-2α. High Alt Med Biol. 2011 Summer;12(2):157-67. doi: 10.1089/ham.2010.1099. PMID: 21718164.

5. Li Y, Wu DD, Boyko AR, Wang GD, Wu SF, Irwin DM, Zhang YP. Population variation revealed high-altitude adaptation of Tibetan mastiffs. Mol Biol Evol. 2014 May;31(5):1200-5. doi: 10.1093/molbev/msu070. Epub 2014 Feb 11. PMID: 24520091.

6. Signore AV, Yang YZ, Yang QY, Qin G, Moriyama H, Ge RL, Storz JF. Adaptive Changes in Hemoglobin Function in High-Altitude Tibetan Canids Were Derived via Gene Conversion and Introgression. Mol Biol Evol. 2019 Oct 1;36(10):2227-2237. doi: 10.1093/molbev/msz097. PMID: 31362306; PMCID: PMC6759075.

7. von Holdt B, Fan Z, Ortega-Del Vecchyo D, Wayne RK. EPAS1 variants in high altitude Tibetan wolves were selectively introgressed into highland dogs. PeerJ. 2017 Jul 12;5:e3522. doi: 10.7717/peerj.3522. PMID: 28717592; PMCID: PMC5510585.

8. Cai C., Yang Y., Ga Q., Xu G., Ge R., Tang F. Comparative genomic analysis of high-altitude adaptation for Mongolia Mastiff, Tibetan Mastiff, and Canis Lupus // Genomics. – 2022. – Vol.114. – №3. – DOI: 10.1016/j.ygeno.2022.110359.

9. Li Y., Wu D.D., Boyko A.R., Wang G.D., Wu S.F., Irwin D.M., Zhang Y.P. Population variation revealed high-altitude adaptation of Tibetan mastiffs // Mol Biol Evol. – 2014. – Vol.31. – №5. – DOI: 10.1093/molbev/msu070.

10. Wu H., Liu Y.H., Wang G.D., Yang C.T., Otecko N.O., Liu F., Wu S.F., Wang L., Yu L., Zhang Y.P. Identifying molecular signatures of hypoxia adaptation from sex chromosomes: A case for Tibetan Mastiff based on analyses of X chromosome // Sci Rep. – 2016. – Vol. 6. – DOI: 10.1038/srep35004.

11. Ren D.R., Yang Q.Y., Ye J.H., Xu L., Zhao H.A., Wu X.P. Strong heterozygote deficit in Tibetan Mastiff of China based on microsatellite loci // Animal. – 2009. – Vol.3. – №9. – DOI: 10.1017/S1751731109004704.

12. Miao B., Wang Z., Li Y. Genomic Analysis Reveals Hypoxia Adaptation in the Tibetan Mastiff by Introgression of the Gray Wolf from the Tibetan Plateau // Mol Biol Evol. – 2017. Vol.34. – №3. – DOI: 10.1093/molbev/msw274.

13. Xiong X., Zhang X.F., Mu L.W., Wang M.L., Yang J.L., Zhang B., Mang J.W., Deng Y.J. Genetic polymorphisms of 16 STR loci in Tibetan Mastiff // Fa Yi Xue Za Zhi. – 2013. – Vol.29. – №4.

14. Deng L.X., He C. The complete mitochondrial genome of a purebred Tibetan Mastiff (Canis lupus familiaris breed Tibetan Mastiff) from Lijiang, China, and comparison of genome-wide sequence variations // Mitochondrial DNA A DNA Mapp Seq Anal. – 2016. – Vol.27. – №1. – DOI: 10.3109/19401736.2013.873897.

15. Guo X., Pei J., Bao P., Yan P., Lu D. The complete mitochondrial genome of Hequ Tibetan Mastiff Canis lupus familiaris (Carnivora: Canidae) // Mitochondrial DNA A DNA Mapp Seq Anal. – 2016. – Vol.27. – №6. – DOI: 10.3109/19401736.2015.1106490.

16. Li Y.X., Gao Y.L., He X.L., Cao S.X. Exploration of mtDNA control region sequences in Chinese Tibetan Mastiffs // Mitochondrial DNA A DNA Mapp Seq Anal. – 2018. – Vol.29. – №5. – DOI: 10.1080/24701394.2017.1357714.

17. Cai Z.F., Hu J.Y., Yin T.T., Wang D., Shen Q.K., Ma C., Ou D.Q., Xu M.M., Shi X., Li Q.L., Wu R.N., Ajuma L., Adeola A.C., Zhang Y.P., Peng M.S. Long amplicon HiFi sequencing for mitochondrial DNA genomes // Mol Ecol Resour. – 2023. – Vol.23. – №5. – DOI: 10.1111/1755-0998.13765.

18. Ren Z., Chen H., Yang X., Zhang C. Phylogenetic analysis of Tibetan mastiffs based on mitochondrial hypervariable region I // J Genet. – 2017. – Vol.96. – №1. – DOI: 10.1007/s12041-017-0753-3.

19. Zhang S., Zhao C., Liu S., Wang Y., Zhao Y., Guan W., Zhu Z. Characteristics and multi-lineage differentiation of bone marrow mesenchymal stem cells derived from the Tibetan mastiff // Mol Med Rep. – 2018. – Vol.18. – №2. – DOI: 10.3892/mmr.2018.9172.

20. Bell L., Hesketh S. Mastiff breed mortality: A study of owner experience, dog age and longevity // Vet Anim Sci. – 2021. – Vol.13. – DOI: 10.1016/j.vas.2021.100194.

21. Johnson R.P., Neer T.M., Partington B.P., Cho D.Y., Partington C.R. Familial cerebellar ataxia with hydrocephalus in bull mastiffs // Vet Radiol Ultrasound. – 2001. – Vol.42. – №3. – DOI: 10.1111/j.1740-8261.2001.tb00934.x.

22. Christen M., Gutierrez-Quintana R., Vandenberghe H., Kaczmarska A., Penderis J., José-López R., Rupp A., Griffiths I.R., Jagannathan V., Leeb T. Mitochondrial fission factor (MFF) frameshift variant in Bullmastiffs with mitochondrial fission encephalopathy // Anim Genet. – 2022. – Vol.53. – №6. – DOI: 10.1111/age.13263.

23. Mortlock S.A., Asada M.C., Soh P.X.Y., Hsu W.T., Lee C., Bennett P.F., Taylor R.M., Khatkar M.S., Williamson P. Genomic Analysis of Lymphoma Risk in Bullmastiff Dogs // Vet Sci. – 2023. – Vol.10. – №12. – DOI: 10.3390/vetsci10120703.

24. Onions D.E. A prospective survey of familial canine lymphosarcoma // J Natl Cancer Inst. – 1984. – Vol.72. – №4.

25. Sanders N.A., Kerlin R.L., Dambach D.M. Aggressive, undifferentiated sarcoma with widespread metastasis in a six-month-old Neopolitan mastiff // J Am Anim Hosp Assoc – 1996. – Vol.32. – №2. – DOI: 10.5326/15473317-32-2-97.

26. Dhaliwal RS, Kitchell BE, Ehrhart E, Valli VE, Dervisis NG. Clinicopathologic significance of histologic grade, pgp, and p53 expression in canine lymphoma. J Am Anim Hosp Assoc. 2013 May-Jun;49(3):175-84. doi: 10.5326/JAAHA-MS-5843. Epub 2013 Mar 27. PMID: 23535752.

27. Chen HW, Small GW, Motsinger-Reif A, Suter SE, Richards KL. VH1-44 gene usage defines a subset of canine B-cell lymphomas associated with better patient survival. Vet Immunol Immunopathol. 2014 Feb 15;157(3-4):125-30. doi: 10.1016/j.vetimm.2013.10.020. Epub 2013 Nov 21. PMID: 24332568; PMCID: PMC3923267.

28. Fujiwara-Igarashi A, Goto-Koshino Y, Mochizuki H, Maeda S, Fujino Y, Ohno K, Tsujimoto H. Simultaneous inactivation of the p16, p15 and p14 genes encoding cyclin-dependent kinase inhibitors in canine T-lymphoid tumor cells. J Vet Med Sci. 2013;75(6):733-42. doi: 10.1292/jvms.12-0351. Epub 2013 Jan 24. PMID: 23343657.

29. Kijas J.W., Miller B.J., Pearce-Kelling S.E., Aguirre G.D., Acland G.M. Canine models of ocular disease: outcross breedings define a dominant disorder present in the English mastiff and bull mastiff dog breeds // J Hered – 2003. – Vol.94. – №1. – DOI: 10.1093/jhered/esg007.

30. Kijas J.W., Cideciyan A.V., Aleman T.S., Pianta M.J., Pearce-Kelling S.E., Miller B.J., Jacobson S.G., Aguirre G.D., Acland G.M. Naturally occurring rhodopsin mutation in the dog causes retinal dysfunction and degeneration mimicking human dominant retinitis pigmentosa // Proc Natl Acad Sci U S A – 2002. – Vol.99. – №9. – DOI: 10.1073/pnas.082714499.

31. Caduff M., Bauer A., Jagannathan V., Leeb T. A single base deletion in the SLC45A2 gene in a Bullmastiff with oculocutaneous albinism // Anim Genet. – 2017. – Vol.48. – №5. – DOI: 10.1111/age.12582.

32. Lavoué R., van der Lugt J.J., Day M.J., Georges M., Busoni V., Merveille A.C., Poujade A., Peeters D. Progressive juvenile glomerulonephropathy in 16 related French Mastiff (Bordeaux) dogs // J Vet Intern Med. – 2010. – Vol.24. – №2. – DOI: 10.1111/j.1939-1676.2010.0474.x.

33. Janutta V, Distl O. Review on canine elbow dysplasia: pathogenesis, diagnosis, prevalence and genetic aspects. Dtsch Tierarztl Wochenschr. 2008 May;115(5):172-81. PMID: 18547017.

34. Clements DN, Short AD, Barnes A, Kennedy LJ, Ferguson JF, Butterworth SJ, Fitzpatrick N, Pead M, Bennett D, Innes JF, Carter SD, Ollier WE. A candidate gene study of canine joint diseases. J Hered. 2010 Jan-Feb;101(1):54-60. doi: 10.1093/jhered/esp088. PMID: 19965910

35. Ginja M, Gaspar AR, Ginja C. Emerging insights into the genetic basis of canine hip dysplasia. Vet Med (Auckl). 2015 May 20;6:193-202. doi: 10.2147/VMRR.S63536. PMID: 30101106; PMCID: PMC6070022.

36. Mortlock S.A., Khatkar M.S., Williamson P. Comparative Analysis of Genome Diversity in Bullmastiff Dogs // PLoS One. – 2016. – Vol.11. – №1. – DOI: 10.1371/journal.pone.0147941.

37. Mortlock S.A., Williamson P., Khatkar M.S. Copy number variation and variant discovery in Bullmastiff dogs // Anim Genet. – 2019. – Vol.50. – №2. – DOI: 10.1111/age.12754.

38. Li, Q., Liu, Z., Li, Y., Zhao, X., Dong, L., Pan, Z., Sun, Y., Li, N., Xu, Y., Xie, Z. Origin and phylogenetic analysis of Tibetan Mastiff based on the mitochondrial DNA sequence // J Genet Genomics. – 2008. – Vol.35. – №6. – DOI: 10.1016/S1673-8527(08)60049-1.

39. Li Y., Zhao X., Pan Z., Xie Z., Liu H., Xu Y., Li Q. The origin of the Tibetan Mastiff and species identification of Canis based on mitochondrial cytochrome c oxidase subunit I (COI) gene and COI barcoding // Animal. – 2011. – Vol.5. – №12. – DOI:10.1017/S1751731111001042.

40. Hsu W.T., Williamson P., Khatkar M.S. Identification of Genomic Signatures in Bullmastiff Dogs Using Composite Selection Signals Analysis of 23 Purebred Clades // Animals. – 2023. – Vol.13. –№7. – DOI: 10.3390/ani13071149.

41. Yang Q., Chen H., Ye J., Liu C., Wei R., Chen C., Huang L. Genetic Diversity and Signatures of Selection in 15 Chinese Indigenous Dog Breeds Revealed by Genome-Wide SNPs // Front Genet. – 2019. – Vol.10. – DOI: 10.3389/fgene.2019.01174.

42. Perfil'eva A.V., Bespalova K.B., Begmanova M.O., Kuzovleva E.B. Assessment of genetic diversity of dogs of the Kazakh national breed Tobet in the southern region of Kazakhstan // 3i: intellect, idea, innovation – intelligence, idea, innovation, 2024, No. 1, pp. 58-67. https :// doi . org /10.52269/22266070_2024_1_58

43. Perfilyeva, A., Bespalova, K., Kuzovleva, Y. Mussabayev, R., Begmanova, M., Amirgalyeva, A., Vishnyakova, O., Nazarenko, I., Zhaxsylykova, A., Yerzhan, A., Perfilyeva, Y., Dzhaembaeva, T., Khamchukova, A., Plakhov, K., Torekhanov, A., Djansugurova, L., Zhunussova, G., Bekmanov, B. Genetic diversity and origin of Kazakh Tobet Dogs. Sci Rep 14, 23137 (2024). https://doi.org/10.1038/s41598-024-74061-9

Сведения об авторах:

Мить Наталья Викторовна – к. б. н., заведующая лабораторией молекулярной генетики, РГП на ПХВ «Институт генетики и физиологии» КН МНВО РК, г. Алматы, Казахстан, e-mail: nata-mit@yandex.ru.

Жунусова Гульнур Сагиндыковна – PhD, ассоц. профессор, ведущий научный сотрудник лаборатории молекулярной генетики, РГП на ПХВ «Институт генетики и физиологии» КН МНВО РК, г. Алматы, Казахстан, e-mail: g.zhunussova@ igph.kz.

Амиргалиева Алмира Смаиловна – старший научный сотрудник лаборатории молекулярной генетики, РГП на ПХВ «Институт генетики и физиологии» КН МНВО РК, г. Алматы, Казахстан, e-mail: almira-71@mail.ru.

Беспалова Кира Булатовна – научный сотрудник лаборатории молекулярной генетики, РГП на ПХВ «Институт генетики и физиологии» КН МНВО РК, г. Алматы, Казахстан, e-mail: kiradzzz@gmail.com.

Перфильева Анастасия Викторовна – PhD, ассоц. профессор, ведущий научный сотрудник лаборатории молекулярной генетики, РГП на ПХВ «Институт генетики и физиологии» КН МНВО РК, г. Алматы, Казахстан, e-mail: nastyaper2009@ mail.ru.

Сейсенова Жанель Муратбековна – старший лаборант лаборатории молекулярной генетики, РГП на ПХВ «Институт генетики и физиологии» КН МНВО РК, г. Алматы, Казахстан, e-mail: s_seisenova@mail.ru.

About the Authors

Mit Natalia Viktorovna – candidate of biological sciences, Head of the Laboratory of Molecular Genetics, RSE REM "Institute of Genetics and Physiology" SC MSHE RK, Almaty, Kazakhstan, e-mail: nata-mit@yandex.ru

Zhunussova Gulnur Sagindykovna – PhD, Associate Professor, Leading Researcher, Laboratory of Molecular Genetics, RSE REM "Institute of Genetics and Physiology" SC MSHE RK, Almaty, Kazakhstan, e-mail: g.zhunussova@igph.kz

Amirgalieva Almira Smailovna – Senior Researcher, Laboratory of Molecular Genetics, RSE REM "Institute of Genetics and Physiology" SC MSHE RK, Almaty, Kazakhstan, e-mail: almira-71@mail.ru

Bespalova Kira Bulatovna – Researcher, Laboratory of Molecular Genetics, RSE REM "Institute of Genetics and Physiology" SC MSHE RK, Almaty, Kazakhstan, e-mail: kiradzzz@gmail.com

Perfilieva Anastasia Viktorovna – PhD, Associate Professor, Leading Researcher, Laboratory of Molecular Genetics, RSE REM "Institute of Genetics and Physiology" SC MSHE RK, Almaty, Kazakhstan, e-mail: nastyaper2009@mail.ru

Seisenova Zhanel Muratbekovna – Senior Laboratory Assistant, Laboratory of Molecular Genetics, RSE REM "Institute of Genetics and Physiology" SC MSHE RK, Almaty, Kazakhstan, e-mail: s_seisenova@mail.ru