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**MORPHOLOGICAL AND GENOTYPIC FEATURES
OF THE POPULATIONS OF FLEAS OF THE GENUS *XENOPSYLLA*
GLINKIEWICZ, 1907 (SIPHONAPTERA, PULICIDAE)
FROM THE AUTONOMOUS FOCI OF THE CENTRAL ASIAN
NATURAL DESERT PLAGUE FOCUS**

Abstract. The paper presents the results of the study of some morphological and genotypic features of fleas of the genus *Xenopsylla*: *X. gerbilli minax*, *X. skrjabini*, *X. hirtipes* - the main vectors in the Central Asian natural desert plague focus. In order to identify population characteristics, for the first time we attempted both morphological analysis using the ImageJ computer program and subsequent data processing in a free statistical environment R version 4.0.0 with R Studio graphic shell, as well as genetic analysis of a fragment of a nucleotide sequence of the *COII* gene of fleas sampled in Pre-Ustyurt, Betpakdala and Pre-Pre-Balkhash autonomous plague foci. As a result of the study of morphometric indices of the main plague vectors in Kazakhstan, independent populations of three species of fleas of the genus *Xenopsylla* have been identified: the Betpakdala and Pre-Pre-Balkhash populations of *X. gerbilli minax* and *X. hirtipes*, as well as the Pre-Pre-Balkhash, Betpakdala and Pre-Usturt populations of *X. skrjabini* fleas.

Keywords: fleas, *Xenopsylla*, plague, morphometry, DNA, genotyping, populations.

Introduction. Over the past 20 years, despite the absence of outbreaks, plague prevention in Kazakhstan is one of the important and priority tasks. Plague foci on the territory of the Republic remain active to this day, occupying about 40% of the territory and stretching from the north-western to south-eastern borders. A special position is occupied by the Central Asian natural desert plague focus, which is located in the desert zone, stretching from the eastern coast of the Caspian Sea to the south-east to the foothills of the Tien Shan [1]. The main host of plague in the Central Asian desert hearth is the great gerbil *Rhombomys opimus*, and its main vectors are fleas of the genus *Xenopsylla* [1, 2, 3]. The number and distribution of fleas depend on many different factors: natural and climatic, hydrological regime and soil structure, species, physiological state and gender-age characteristics of the host-feeder [4-7].

Fleas of the genus *Xenopsylla*, widely distributed in many countries of the world, are the most numerous, frequently occurring and play an important role in the circulation of zoonotic agents in natural foci [8, 9]. Fleas of *Xenopsylla* can feed on many species of animals, including synanthropic animals, and may participate in the circulation of zoonotic pathogens in domestic animals and humans [10].

Fleas of *Xenopsylla* belong to a family with a relatively wide range, typical for fauna of tropical and subtropical belts [8, 9]. They are found in Kazakhstan, Turkmenistan, Uzbekistan, as well as in the deserts of northern Mongolia, northwest China, eastern Iran, northern Afghanistan and Pakistan. *Xenopsylla*

gerbilli minax living in China are genotyped [11], while typing of the genome of fleas of the genus *Xenopsylla* in the Central Asian desert plague focus in Kazakhstan has not been previously conducted.

All of the above together served the purpose of our research - to study the genetic and phenotypic diversity of ecological populations of the plague pathogen transmission fleas from autonomous foci of the Central Asian natural desert plague focus.

Material and research methods. Studies in the foci were conducted in accordance with the Sanitary Regulations "Sanitary and epidemiological requirements for the organization and conduction of sanitary and epidemiological (preventive) measures to prevent infectious diseases (plague, cholera)," approved by the Government of the Republic of Kazakhstan on January 12, 2012 № 32 and the Resolution of the Chief State Sanitary Inspector of the Republic of Kazakhstan from 27. 11. 2015 № 20 "About the sanitary-epidemiological and prophylactic measures in the territory enzootic by plague of the Republic of Kazakhstan for 2016-2020.

The material for work served as fleas of genus *Xenopsylla* caught by employees of anti-plague stations in three autonomous foci of the Central Asian natural desert plague focus (Pre-Pre-Balkhash, Betpakdala and Pre-Ustyurt) at carrying out of annual planned epizootological inspection of territories of natural foci of Kazakhstan on plague according to Sanitary rules. Fleas from the collection of the zoonoparasitological museum of the Masgut Aikimbaev's National Scientific Center from the Pre-Pre-Balkhash, Pre-Ustyurt, Betpakdala and Tienshan plague foci were also studied.

For morphometric studies 681 specimens of fleas of three species of the genus *Xenopsylla* (*X. hirtipes*, *X. skrjabini*, *X. gerbilli minax*) were selected and studied from territorially isolated from each other in the Pre-Balkhash, Betpakdala, Pre-Ustyurt and Tienshan autonomous foci.

For species identification and morphological analysis preparations from fleas were done using standard methods [12]. Head and bristles of the head were measured using photos of flea preparations using the ImageJ program [13]. For correct interpretation of the results of the analysis, we used signs whose variability does not depend on body size [14-16]. In the preliminary stage meristic features that have a discrete variability were selected, and vary within certain limits, and represent one of the characteristic features of the external appearance of insects, allow to analyze the variability, do not undergo changes during the life of an imago: head length, distance between the eye and parietal bristles and distance between parietal and angular bristles.

Multiple linear regression was used to evaluate the impact on the listed phenotypic features of the trapping site (region) and sex of insects. Data processing was performed in a free statistical environment R version 4.0.0 with a graphical shell R Studio [17].

For genetic analysis, 22 specimens identified as *X. gerbilli minax* were selected from three isolated plague foci (table 1).

Table 1 - Flea samples taken for genetic analysis

Code	Coordinates	Autonomous focus
II-B1	10 II-1, ББ-225, Muyunkum N44°30'305" E77°25'802"	Pre-Pre-Balkhash
II-B2	10 II-1, ББ-225, Muyunkum N44°30'305" E77°25'802"	Pre-Pre-Balkhash
II-B3	11 II-4, ББ-226, Muyunkum N44°31'140" E77°26'368"	Pre-Pre-Balkhash
II-B4	11 II-4, ББ-227, Muyunkum N44° 31' 328" E77°25'928"	Pre-Pre-Balkhash
II-B5	13C-ББ-220, Malaisary N44° 31'328" E77°25'928"	Pre-Pre-Balkhash
II-B6	13C-ББ-220, Malaisary N44° 31'328" E77°25'928"	Pre-Pre-Balkhash
II-B7	13C-2, ББ-222, Malaisary N44°18'249" E77°08'137"	Pre-Pre-Balkhash
II-Sh1 - II-Sh15	N 45°12', E 67°25'; N 45°38', E 69°21'	Betpakdala

DNA was isolated from separate specimens using Qiamp DNA minikit for tissue protocol (Qiagen, Hilden, Germany). The samples were previously homogenized by shaking with a glass and steel ball and TissueLyser using the protocol.

Composition of the reaction mixture: Insect-A-tLEU and Insect-B-Tlys primers with 20 pmols each in the reaction; 75 mMTris-HCl (pH 8.8 at 25°C), 20 mM (NH₄)₂SO₄, 0.01% (volume/volume) Twin 20; DNTF in concentration of 200 nM each; 2 units of Taq DNA polymerase; magnesium ions 2.5 mM; *R. opimus* DNA - 3 ng. PCR amplification program: prolonged denaturation 95°C - 3 minutes; 35 cycles 95°C - 20 seconds, 59° - 30 seconds, 72°C - 1 minute; final elongation 7 minutes at 72°C.

Purification of PCR products was carried out by enzymatic method using Exonuclease I (Ferments) and alkaline phosphatase (Shrimp Alkaline Phosphatase, Ferments) (Werle et al. 1994). Sequencing was performed by using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the primers used for PCR amplification, according to the manufacturer's instructions. Fragment separation was carried out on a 3730xl DNA Analyzer (Applied Biosystems) automated genetic analyzer. Quality assessment and assembly in contigs was performed by using SeqScape 2.6.0 (Applied Biosystems).

Phylogenetic analysis was performed using MEGA 7.0 software. Using the maximum likelihood method, the Tamura 3-parameter model, discrete Gamma distribution, and Bootstrap 1000. Due to shorter gene fragments of other species of fleas from the NCBI database 651 b.p were used for phylogenetic analysis.

Results. For morphometric studies, 681 specimens of fleas of *Xenopsylla* genus were divided by species, each species was divided into groups at the place of sampling. Each species was considered separately.

X. gerbilli minax in the plague foci of Kazakhstan is distributed from the middle current of the Syr-Darya river to the east through the Betpakdala desert, Kyzylkum and Moyynkum sands, reaches the Karatal river, then interrupts and the most eastern part of the range lies between Alakol lake and the eastern border with the People's Republic of China (figure 1).

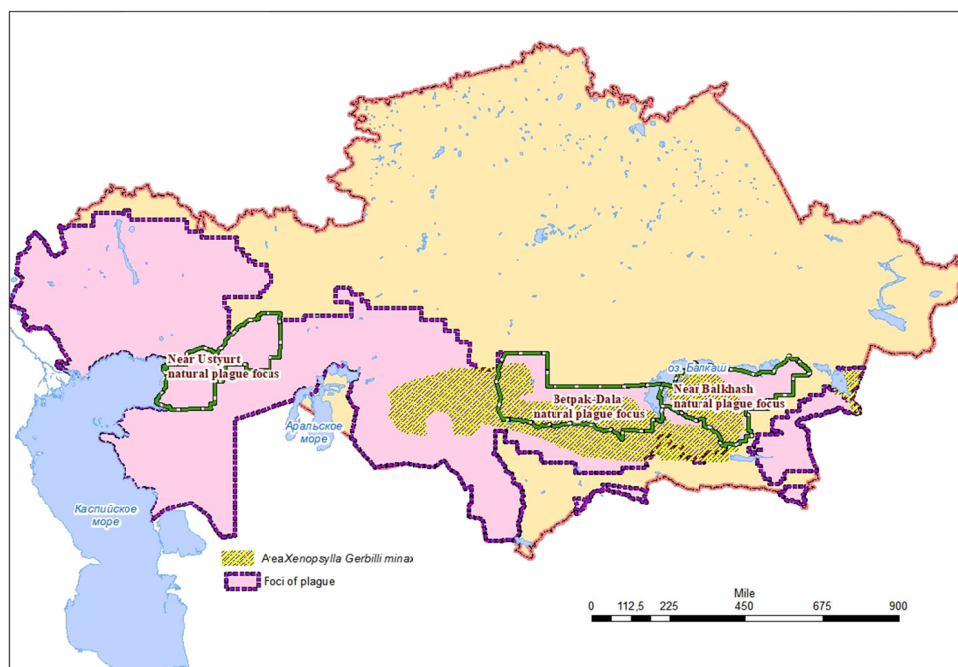


Figure 1 - Distribution of *X. gerbilli minax* in Kazakhstan

368 specimen fleas of this species were divided into 3 groups in their habitat - 147 specimen fleas collected from the Pre-Balkhash autonomous plague focus, 71 specimens - from the Betpakdala autonomous plague focus, 150 specimens - from the Tianshan autonomous plague focus, located on the territory of the People's Republic of China, kept in the zoo-parasitological museum of the Masgut Aikimbayev National Scientific Center of Especially Dangerous Infections.

X. gerbilli fleas sampled in Dzungarian autonomous plague focus, statistically significantly differed from the fleas of the same species from other foci by the distance between parietal and angular bristles ($p = 0.00149$), including after stratification by sex ($p = 0.00007$).

The area of *X. hirtipes* in plague foci of Kazakhstan occupies much smaller territory in comparison with *X. gerbilli minax* and is mainly connected with sandy areas (figure 2).

137 specimens of fleas of this species were divided into 3 groups in their habitat - 105 specimens of fleas collected from the Pre-Balkhash autonomous plague focus, 27 specimens - from the Betpakdala autonomous plague focus. For comparative assessment, we also studied 5 specimens of fleas from the

Tianshan plague focus located in the territory of the People's Republic of China, stored in the zoono-parasitological museum of the Masgut Aikimbayev National Scientific Center of Especially Dangerous Infections.

X. hirtipes fleas sampled in the Pre-Balkhash and Betpakdala foci statistically significantly differed from the fleas other foci by the distance between the ocular and parietal bristles ($p = 0,00126$ and $0,00025$ respectively), as well as by the distance between the parietal and angular bristles ($p = 0.00138$ and 0.00402 respectively), including after stratification by sex ($p = 0.0525$ and $p = 0.02127$).

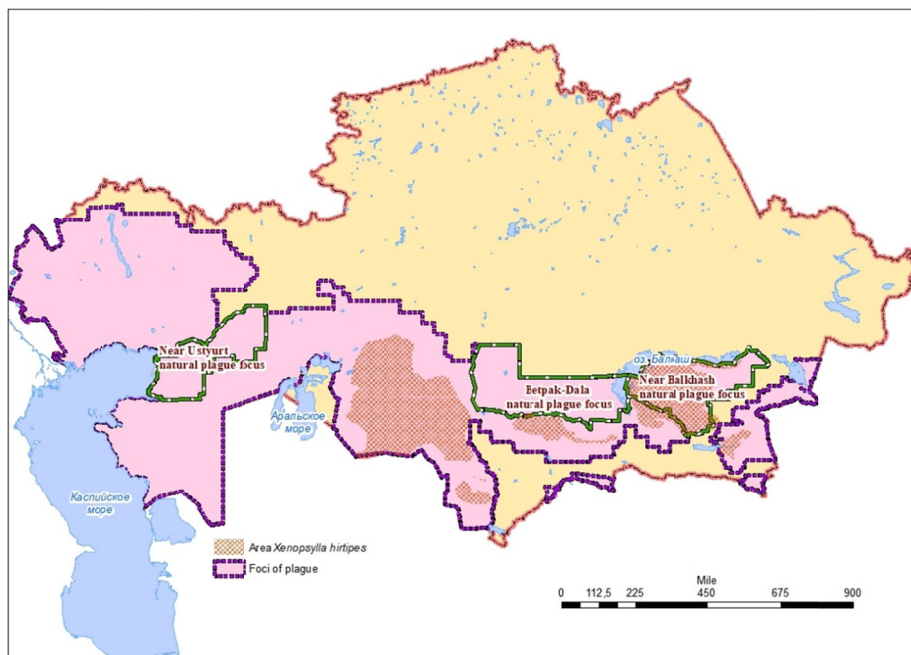


Figure 2 - Distribution of *X. hirtipes* in Kazakhstan

The area of *X. skrjabini* occupies a significant part of the plague foci of Kazakhstan and stretches from the north-western coast of the Caspian Sea to the east to the southeast border with China on the eastern border with the People's Republic of China (figure 3).

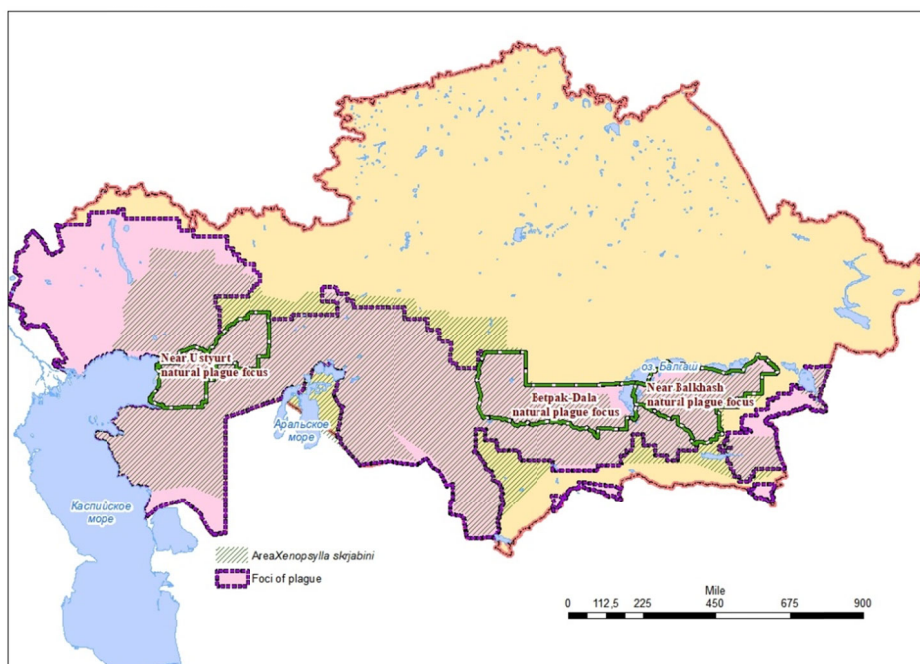


Figure 3 - Distribution of *X. skrjabini* in Kazakhstan

176 specimens of fleas of this species were divided into 4 groups in their habitat - 72 specimens of fleas collected from the Pre-Balkhash autonomous plague focus, 56 specimens - from Betpakdala and 13 specimens from the Pre-Ustyurt plague focus. For comparative assessment, we also studied 35 specimens of fleas from the Tienshan plague focus, located in the territory of the People's Republic of China, stored in the zoo-parasitological museum of the Masgut Aikimbayev National Scientific Center of Especially Dangerous Infections.

X. skrjabini fleas from Betpakdala, Pre-Balkhash and Pre-Ustyurt foci of Kazakhstan do not statistically significantly differ by the distance between the ocular and parietal bristles. Fleas caught in Betpakdala focus statistically significantly differ from other fleas in terms of head length ($p=0,0355$) and distance between parietal and angular bristles ($p=0,0181$).

Genotyping and search for population diversity and genome features of the genus *Xenopsylla* were performed by analyzing a fragment of the *COII* gene nucleotide sequence. Phylogenetic analysis clustered 22 samples from Kazakhstan into one treasure with *X. gerbilli minax* samples. Samples with code A4, A5, A2, A3 and A10 are located in the same branch. The remaining 17 sequences are completely identical to the samples from China.

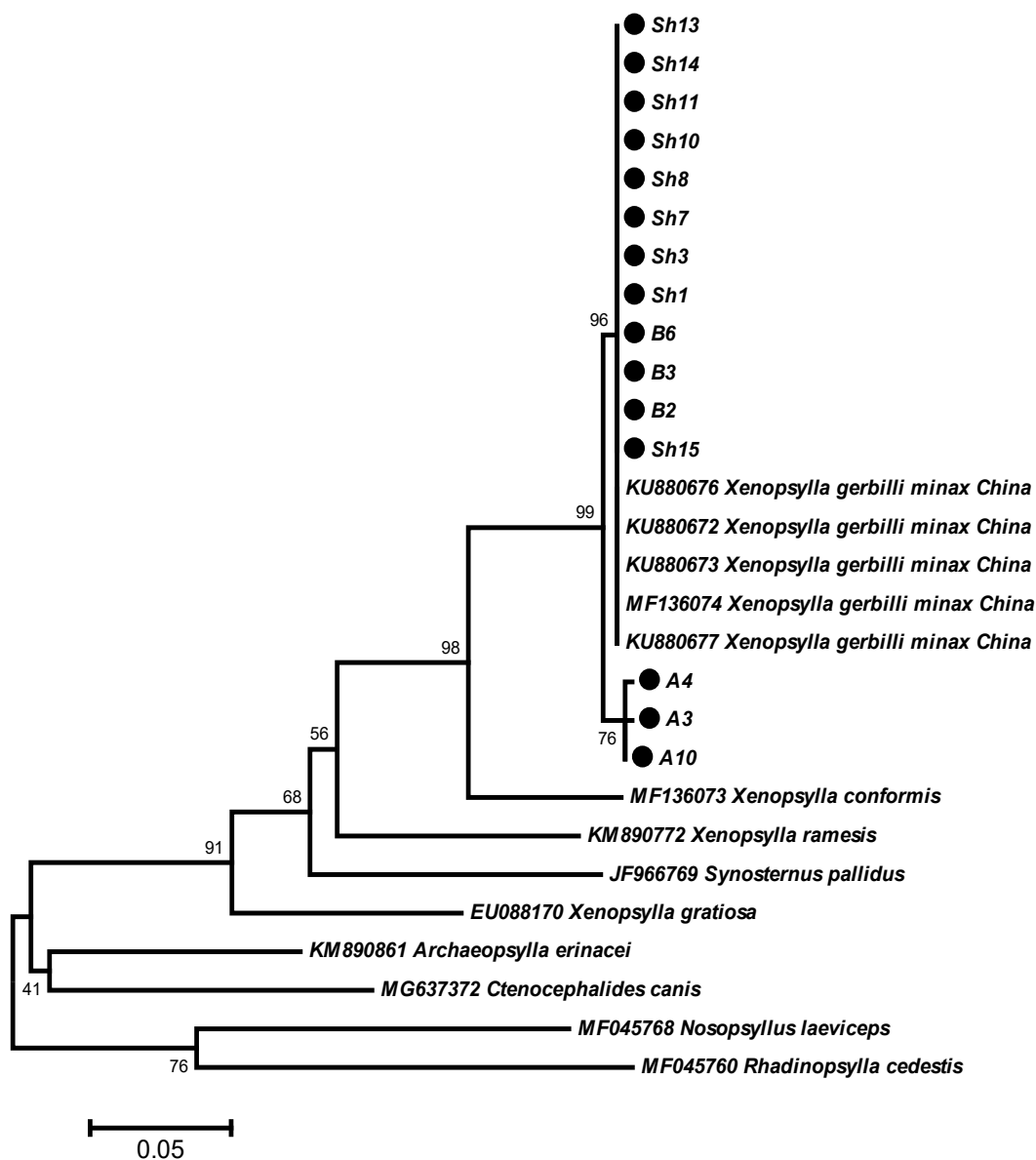


Figure 4 - Phylogenetic tree based on the analysis of the COXII nucleotide sequence of the genus *Xenopsylla*

Discussion. There is an opinion that *X. skrjabini*, *X. gerbilli minax* and *X. hirtipes* branched off in the second half of the Pleistocene from *X. gerbilli gerbilli*, which penetrated the territory of Kyzylkum with great gerbils from the Western Asia. With the penetration of great gerbils to the north distribution of fleas to new territories took a place and currently species of *Xenopsylla* genus within their ranges are unevenly distributed, which is probably due to the environmental, natural and climatic factors [18].

The study of morphological features of flea species sampled at geographically remote sites has revealed a number of significant differences in size of the head and distance between the parietal and ocular bristles, as well as between the parietal and angular bristles.

Based on the conducted studies, we can assume that studied flea species belong to different regional complexes – Pre-Balkhash, Pre-Ustyurt, Betpakdala and Tienshan foci.

Genotyping has shown that fleas *Xenopsylla gerbilli minax* from China, Western Betpakdala and Southern Pre-Balkhash are a separate autonomous group of populations.

Conclusions.

- *X. skrjabini* fleas caught in different autonomous foci do not statistically differ from each other in phenotypic features and probably belong to the same population group.

- *X. gerbilli minax* fleas caught in the Dzungarian autonomous focus are statistically significantly different from the fleas of their species from other foci by the distance between the parietal and angular bristles, which may indicate the formation of an independent population of *X. gerbilli* fleas in the Dzungarian autonomous plague focus.

- *X. hirtipes* fleas caught in the Pre-Balkhash and Betpakdala foci, statistically significantly different from the fleas of the same species from other foci on the basis of the distance between the eye and parietal bristles, as well as the distance between the parietal and angular bristles, which may indicate the formation of an independent population of fleas of the *X. hirtipes* in the Pre-Balkhash and Betpakdala plague foci.

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ОРТАЛЫҚ АЗИЯ ТАБИҒИ ШӨЛДІ ОБА ОШАҒЫ АВТОНОМДЫ ОШАҚТАРЫНЫҢ XENOPSYLLA GLINKIEWICZ, 1907 (SIPHONAPTERA, PULICIDAE) ТҰҚЫМДАС БҮРГЕ ПОПУЛЯЦИЯСЫНЫҢ МОРФОЛОГИЯЛЫҚ ЖӘНЕ ФЕНОТИПТІК ЕРЕКШЕЛІКТЕРІ

Аннотация. Жұмыста *Xenopsylla* тұқымдас бүргенің кейбір морфологиялық белгілері мен генотиптік ерекшеліктерін зерттеу нәтижелері келесідей келтірілген: *X. gerbilli minax*, *X. skrjabini*, *X. hirtipes* – обаның Орталық Азия табиғи шөлді ошағындағы негізгі тасымалдаушысы болып саналады. Популяциялық ерекшеліктерді анықтау үшін алғаш рет ImageJ компьютерлік бағдарламасы арқылы морфологиялық талдау және RStudio графикалық ортасымен R 4.0.0 нұсқасының еркін статистикалық ортасында деректерді өңдеу және Үстірталды, Бетпақдала және Балқаш маңы автономды ошақтарында ұсталған бүрге гені *COII* генінің нуклеотидтер тізбегінің фрагментіне генетикалық талдау жасадық. Қазақстанда обаның негізгі тасымалдау-

шысының морфометриялық көрсеткіштерін зерттеу нәтижесінде *Xenopsylla* тұқымдас бүргенің үш түрінің келесідей жеке популяциялары анықталды: Бетпақдала және Балқаш маңы популяциялары – *X. gerbilli minax* және *X. hirtipes*, сондай-ақ Балқаш маңы, Бетпақдала және Үстірталды популяциялары – *X. skrjabini*.

Түйін сөздер: бүрге, *Xenopsylla*, оба, морфометрия, ДНК, генотиптеу, популяция.

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МОРФОЛОГИЧЕСКИЕ И ГЕНОТИПИЧЕСКИЕ ОСОБЕННОСТИ ПОПУЛЯЦИЙ БЛОХ РОДА *XENOPSYLLA* GLINKIEWICZ, 1907 (SIPHONAPTERA, PULICIDAE) ИЗ АВТОНОМНЫХ ОЧАГОВ ЦЕНТРАЛЬНО-АЗИАТСКОГО ПРИРОДНОГО ПУСТЫННОГО ОЧАГА ЧУМЫ

Аннотация. В статье приведены результаты изучения некоторых морфологических признаков и генотипических особенностей блох рода *Xenopsylla*: *X. gerbilli minax*, *X. skrjabini*, *X. hirtipes* - основных переносчиков в Центральном-Азиатском природном пустынном очаге чумы. С целью выявления популяционных особенностей впервые нами были предприняты попытки морфологического анализа с помощью компьютерной программы ImageJ и обработки данных в свободной статистической среде R версии 4.0.0 с графической средой RStudio и генетического анализа фрагмента нуклеотидной последовательности *COII* гена блох, отловленных в Предустюртском, Бетпақдалинском и Прибалхашском автономных очагах чумы. В результате исследования морфометрических показателей основных переносчиков чумы в Казахстане были выделены самостоятельные популяции трех видов блох рода *Xenopsylla*: Бетпақдалинские и Прибалхашские популяции *X. gerbilli minax* и *X. hirtipes*, а также Прибалхашская, Бетпақдалинская и Предустюртская популяции блох *X. skrjabini*.

Ключевые слова: блохи, *Xenopsylla*, чума, морфометрия, ДНК, генотипирование, популяция.

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