

Ye.A. Sheruova<sup>1</sup>, Y.V. Andreeva<sup>2</sup>, U.D. Andreeva<sup>3</sup>, A.K. Sibataev<sup>4\*</sup>

<sup>1,4</sup>L.N. Gumilyov Eurasian National University, Astana, Kazakhstan;

<sup>2,4</sup>Tomsk State University, Tomsk, Russia;

<sup>3</sup>Municipal Autonomic General Educational Institution School "Perspectiva", Tomsk, Russia;

<sup>4</sup>Kazakh Agrotechnical Research University named after S. Seifullin, Astana, Kazakhstan

\*Corresponding author: [asibataev@gmail.com](mailto:asibataev@gmail.com)

## Species Composition of Blood-Sucking Mosquitoes of the Genus *Aedes* (Diptera: Culicidae) in the Karaganda Region

This study examines the species composition of blood-sucking mosquitoes in the Karaganda Region of Central Kazakhstan — a region where faunal and ecological data have been scarce. Through field surveys and morphological identification, we documented at least ten species belonging to the genus *Aedes* Meigen, 1818. Notably, several specimens exhibited atypical morphological traits, suggesting the presence of previously unrecognized or cryptic forms within local populations. These findings underscore the importance of integrative taxonomic approaches — particularly molecular methods — for clarifying the status of morphologically distinct variants and improving our understanding of their epidemiological relevance. Community-level analyses of species diversity and evenness revealed pronounced spatial differences between localities: The species *Ae. (Och.) dorsalis* Meigen, 1830 was dominant in and around Karaganda City, whereas *Ae. (Rus.) subdiversus* Martini, 1926 was more prevalent in the Nura District. The detection of species not previously reported from the region further suggests a potential range expansion of *Aedes* mosquitoes. Collectively, these results provide the first systematic baseline for *Aedes* biodiversity in Central Kazakhstan and underscore its importance for vector ecology, arbovirus transmission risk, and public health surveillance in the region.

**Keywords:** *Aedes*, *Culicidae*, insecta, species, Shannon index, Pielou's evenness index, Jaccard index, fauna.

### Introduction

*Aedes* (Diptera: Culicidae) is a genus of mosquitoes that includes the largest number of species of significant medical importance, as they serve as vectors for a variety of viral pathogens. Notably, they are known to transmit the deadly viruses of Dengue, Zika, Chikungunya, and West Nile fever [1]. Mosquitoes of the genus *Aedes* Meigen, 1818 are found in various regions of the Republic of Kazakhstan, with their abundance and species composition varying significantly depending on the local climate and landscape. One of the least studied regions of Kazakhstan in terms of the species diversity of blood-sucking mosquitoes of this genus is the Karaganda Region, located in Central Kazakhstan.

In recent decades, certain mosquito species have expanded their range into parts of Europe, Russia, and the North America due to climatic and environmental changes. These include *Ae. (Stegomyia) albopictus* Skuse, 1894, *Ae. koreicus* Edwards, 1917, and *Ae. japonicus* Theobald, 1901. Intercontinental spread is primarily the result of egg transportation attached to various plant products and tires shipped via cargo vessels, which are of particular relevance [2]. Air travel also plays a significant role in their dispersal [3]. However, on national and regional scales, ground transportation — particularly by cars and trains — is considered the most important route of spread [4]. For instance, the invasive mosquito *Ae. koreicus*, a Far Eastern species, has been detected within the territory of the Republic of Kazakhstan [5]. Reports of this species have become increasingly common in Europe, Russia Far East, and the North Americas [6]. As the ranges of mosquito vectors expand, so too do the distributions of the pathogens they transmit. While the potential for future arbovirus transmission by invasive mosquito species in these regions is concerning, circulation of several arboviruses has already been observed among native species. Notably, three such viruses include Sindbis virus, Usutu virus (USUV), and West Nile virus (WNV) [7].

The aim of our study is to clarify the species composition of g. *Aedes* mosquitoes in the Karaganda Region of Kazakhstan.

*Experimental*

To study the species composition of g. *Aedes* mosquitoes, larval samples of blood-sucking mosquitoes were collected in the Karaganda Region (Fig. 1). Larvae were collected (Table 1) using the methods described by Monchadskij (1952) [8], employing a specialized dipping tray and a pear-shaped pipette. The collected larvae were preserved in 5 ml tubes containing 96 % ethanol or Carnoy's solution for cytogenetic analysis. Species identification was carried out using morphological methods based on the works of Gucevich et al. (1970) [9], Dubitsky [10], and Becker (2010) [11].

Samples were divided according to geographic origin. The first zone included specimens collected in Karaganda city and adjacent areas. The second zone comprised samples from the Nura District, located in the southwest of the Karaganda Region, approximately 200 km from the regional center (Table 3). Larvae were collected from water bodies formed by melting snow.

Table 1

*Aedes* Larvae Sampling Sites within the Karaganda Region

No.	Location of Temporary and Permanent Water Bodies (Flood Areas)	Sampling Date	Number of Larvae	Coordinates
<b>Zone I</b>				
1	Karaganda, Yugo-Vostok Microdistrict	24.04.24	65	49.77816 N, 73.1631 E
2	Karaganda, Yugo-Vostok Microdistrict	24.04.24	50	49.7577 N, 73.15977 E
3	Karaganda, Kungey Microdistrict	24.04.24	82	49.77806 N, 73.16301 E
4	Karaganda, Kazbek Bi District	24.04.24	21	49.74874 N, 73.1669 E
5	Karaganda, Kazbek Bi District	24.04.24	17	49.80769 N, 73.14454 E
6	Karaganda, Karaganda–Almaty Highway	24.04.24	15	49.74887 N, 73.167 E
7	Karaganda, Maikuduk Microdistrict	24.04.24	30	49.83559 N, 73.17974 E
8	Karaganda, Spassk Highway	24.04.24	5	49.75345 N, 73.16607 E
9	Karaganda Region, Prishakhtinsk Residential Area	25.04.24	2	49.92204 N, 73.06991 E
10	Karaganda, A. Bokeikhan District, E018	24.04.24	50	49.82488 N, 73.13184 E
11	Karaganda, A. Bokeikhan District, E018	24.04.24	35	49.83606 N, 73.11525 E
<b>Zone II</b>				
12	Karaganda Region, Nura District, Karkaraly Highway	22.04.24	20	50.66456 N, 71.46498 E
13	Karaganda Region, Nura District, Karkaraly Highway	22.04.24	14	50.61068 N, 71.46704 E
14	Karaganda Region, Nura District, Karkaraly Highway	22.04.24	36	50.3233 N, 71.52544 E
15	Karaganda Region, Nura District	22.04.24	5	50.25673 N, 71.56424 E
16	Karaganda Region, Nura District, Karatal Forest Plantation	23.04.24	2	50.30295 N, 71.61795 E

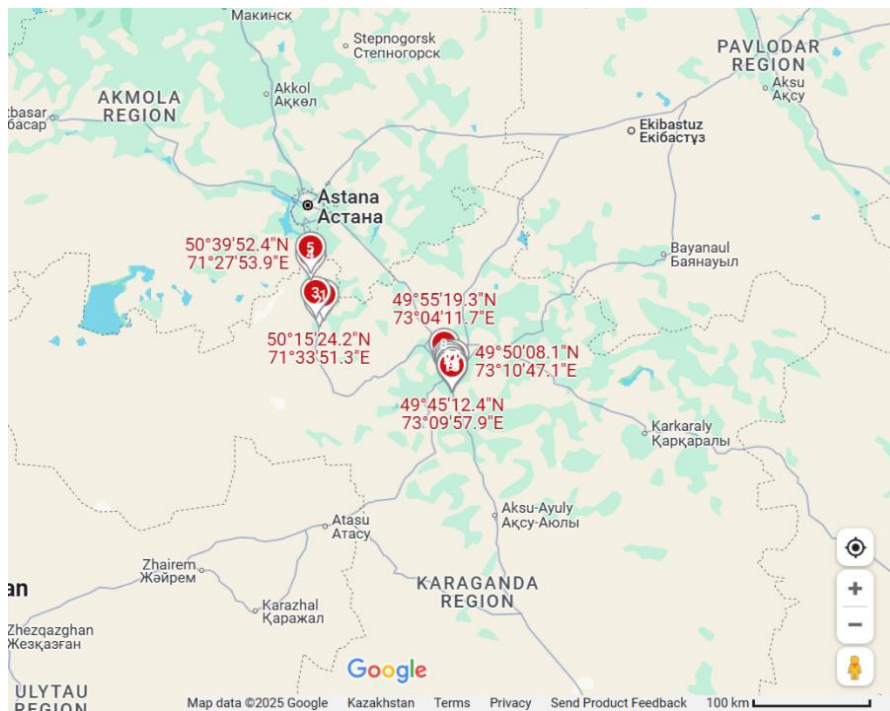


Figure 1. Geographic Distribution of Sampling Sites in the Karaganda Region

The initial mosquito generation develops in small water bodies formed by snowmelt. These shallow, sun-warmed pools provide a warm and favorable environment for larval development.

In Zone I, the water bodies were predominantly shallow and small puddles. In contrast, Zone II was characterized by water bodies with abundant green vegetation (Fig. 2).



a.



b.

Figure 2. Water Bodies of the Karaganda Region a. In the vicinity of Karaganda city; b. Nura District

The division into zones was based on geographical, climatic, and precipitation factors to examine the variation in mosquito species distribution across the studied region.

The climate is sharply continental and arid, characterized by significant temperature fluctuations throughout the year and diurnally, as well as variability in weather conditions [12].

Maximum precipitation values (350–400 mm) were recorded in the Nura District, attributed to its location on the western slope of the low mountain range. The central part of the region exhibits a more uniform

distribution of precipitation, averaging around 300 mm. The spring period accounts for approximately 25 % of the annual precipitation volume [13]. Several soil districts, zones, and subzones are distinguished within the region. The areas adjacent to the city of Karaganda belong to the subzone of Humic Kastanozems, while the Nura District is located within the kastanozems subzone.

Humic Kastanozems are characterized by a clayey, heavy and medium loamy granulometric composition, which slows down water infiltration into the ground and leads to water retention on the surface.

Kastanozems differ from Humic Kastanozems by having lower humus content, a tendency toward compaction, and the presence of readily soluble salts. These soils are formed under non-leaching water regimes typical of dry steppe and semi-desert environments, where surface water is rapidly absorbed into the soil [14].

To analyze species diversity, the Shannon index was used. This index allows for the comparison of species diversity across different communities. Pielou's evenness index (E), which is calculated based on the Shannon index, provides a measure of the uniformity of species distribution in terms of their abundance within a community [15].

To assess the similarity between the two zones, the Jaccard index was used. In the context of ecology and species diversity, this index allows for the evaluation of the degree of similarity in species composition between two different habitats [16].

To assess the similarity in species composition and structure between the two geographic zones, hierarchical cluster analysis was performed. The analysis was based on the relative abundance of g. *Aedes* mosquito larvae across all sampled habitats. We applied hierarchical agglomerative clustering using the complete-linkage method, in which inter-cluster distance is calculated as the maximum pairwise distance between observations belonging to different clusters. The analysis was performed using the Euclidean distance metric. The Bray-Curtis [17] dissimilarity index was used as the measure of distance, as it is appropriate for ecological data and accounts for differences in species abundances. The resulting dendrograms visually represent the degree of similarity between mosquito species in each zone, highlighting dominant species and indicating patterns of co-occurrence and species clustering.

### Results

The samples contained representatives of the genera *Culex* and *Anopheles*, with *Aedes* larvae being predominant. In total, ten mosquito species belonging to the genus *Aedes* were identified: *Ae. (Ochlerotatus) caspius* Pallas, 1771; *Ae. (Och.) dorsalis* Meigen, 1830; *Ae. (Och.) flavescens* Müller, 1764; *Ae. (Rusticoides) subdiversus* Martini, 1926; *Ae. (Och.) euedes* Howard, Dyar & Knab, 1913; *Ae. (Och.) cataphylla* Dyar, 1916; *Ae. (Och.) cyprius* Ludlow, 1920; *Ae. (Och.) annulipes* Meigen; *Ae. (Och.) cantans* Meigen, 1818; and *Ae. (Och.) communis* De Geer, 1776 (Table 2).

Table 2

Species Composition of *Aedes* Mosquitoes Collected in the Karaganda Region

Types/ Locations of samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Ae. (Och.) caspius</i> Pallas, 1771	+	+	+		+	+	+		+	+	+				+	
<i>Ae. (Och.) dorsalis</i> Meigen, 1830	+	+			+	+	+			+	+				+	
<i>Ae. (Och.) flavescens</i> Muller, 1764				+		+		+								+
<i>Ae. (Rus.) subdiversus</i> Martini, 1926												+		+		
<i>Ae. (Och.) euedes</i> Howard, Dyar et Knab, 1913		+					+					+	+			
<i>Ae. (Och.) cataphylla</i> Dyar, 1916				+				+					+	+		
<i>Ae. (Och.) cyprius</i> Ludlow, 1920													+	+		
<i>Ae. (Och.) annulipes</i> Meigen,				+				+								

Continuation of Table 2

Types/ Locations of samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Ae. (Och.) cantans</i> Meigen, 1818,				+						+	+					
<i>Ae. (Och.) communis</i> De Geer, 1776										+	+				+	
Note. The column number corresponds to the sample number from Table 1. Samples 1–11 belong to Zone I, and samples 12–16 belong to Zone II.																

The division into zones helped reveal differences in the distribution of mosquito species across the studied region.

It is worth noting that among the *Ae. subdiversus* specimens collected in the study area, individuals were found with a branched siphonal tuft 1-S (Fig. 3), whereas the species is described as having single siphonal tuft [9, 11]. In all other morphological characteristics, these individuals fully corresponded to the typical description of the species, leaving no doubt regarding the accuracy of their identification.

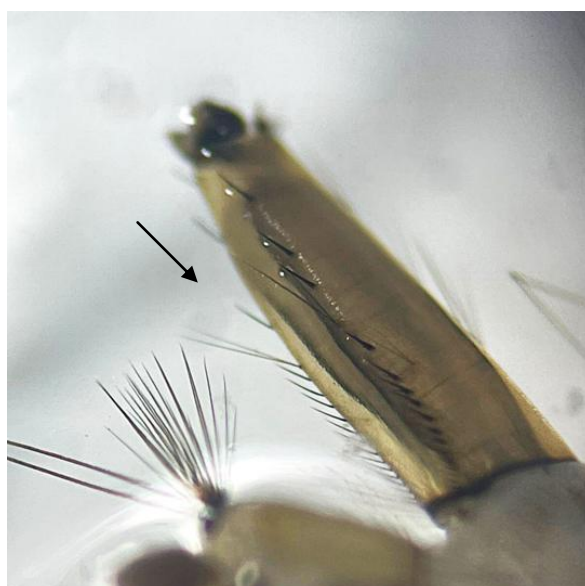


Figure 3. Siphonal tuft 1-S with two setae in *Ae. subdiversus*

The Shannon index ( $H$ ) in the first geographic zone was 1.23, indicating relatively low species diversity within the mosquito community. Since the index value is below 1.5, this suggests that a few species are dominant.

The evenness ( $E$ ) value of 0.59 indicates an uneven distribution of species abundance among the samples from Zone I (Table 1). A value below 0.7 also points to the dominance of several species.

According to the study, eight *Aedes* species were identified in the territories classified as Zone I, with a significant numerical dominance of *Ae. caspius* and *Ae. dorsalis* (Fig. 4).

The results for Zone II (Table 1) differ slightly. The Shannon index for this area was lower ( $H = 0.78$ ) than in Zone I, indicating a lower level of species diversity. Seven *Aedes* species were recorded in this zone. The evenness value ( $E = 0.40$ ) was also lower than in Zone I, suggesting a less even distribution of species abundance — i.e., a stronger dominance of a single species. In this region, *Ae. subdiversus* was the dominant species (Fig. 4).



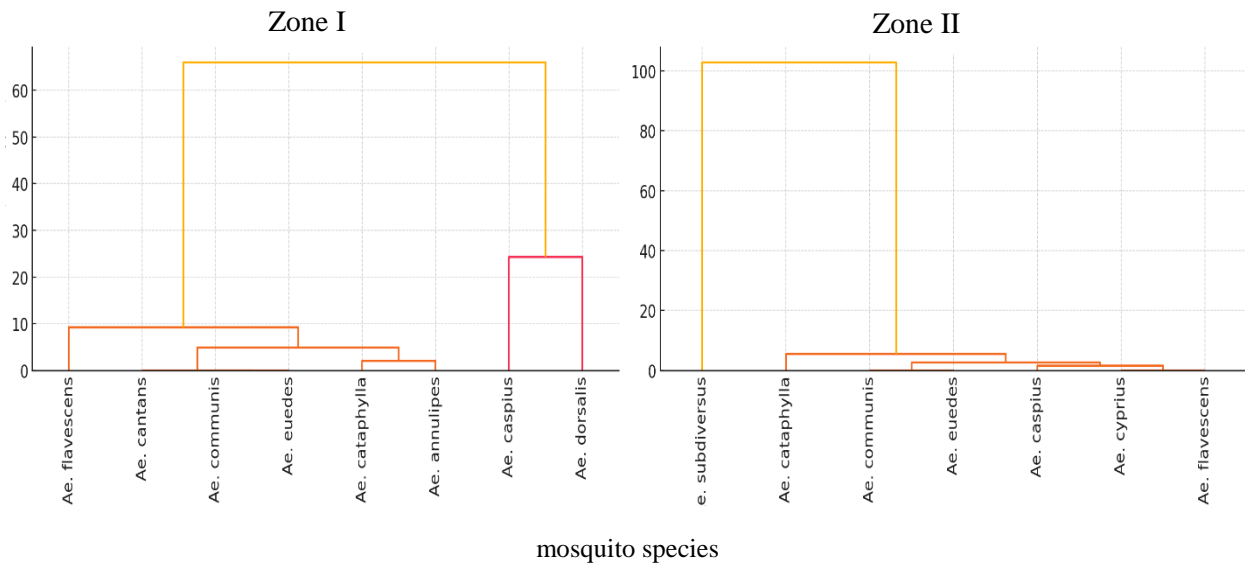


Figure 4. Cluster dendrograms based on the relative abundance of mosquito larvae

In both zones, a nearly equal number of g. *Aedes* species was recorded — eight in Zone I and seven in Zone II — but the species composition differed (Table 2). For instance, larvae of *Ae. cyprius*, *Ae. annulipes*, *Ae. cantans*, and *Ae. subdiversus* were not found in the water bodies of Zone I, whereas the latter species was dominant in Zone II. Conversely, *Ae. dorsalis* larvae were not found in Zone II, and *Ae. caspius* larvae were detected at very low abundance (3.9 %). In Zone I, however, these two species were predominant in terms of abundance. To show the similarities in the species composition of *Aedes* mosquitoes across two geographic zones, a cluster analysis was conducted based on the relative abundance of larvae. The dendrograms (Fig. 4) present the results of hierarchical clustering using the complete linkage method, where the distance between clusters is defined as the maximum distance between individual elements in each cluster. Euclidean distance was applied as the dissimilarity measure. The resulting dendrograms allowed for visualization of species associations based on their dominance within each zone. In Zone I, the most similar species in terms of abundance were *Ae. dorsalis* and *Ae. caspius*, forming a distinct cluster, which confirms their dominance in this area. The remaining species, which were less numerous, grouped into separate branches, indicating greater differences. In Zone II, *Ae. subdiversus* is clearly separated from the other species, reflecting its numerical dominance. The rest of the species form a compact cluster with minor differences in abundance (Fig. 4).

The Jaccard index was approximately 0.545, indicating a moderate level of similarity in mosquito species composition between Zone I and Zone II. About 54.5 % of the species recorded across both zones were found in both areas.

### Discussion

We identified at least ten species of *Aedes* mosquitoes in the Karaganda Region, exhibiting heterogeneous patterns of distribution across the area. Notably, the presence of *Ae. annulipes*, *Ae. cantans*, *Ae. cataphylla*, *Ae. euedes*, *Ae. cyprius*, *Ae. dorsalis*, and *Ae. communis* has not been previously recorded in the studied territory.

Previously, the following *Aedes* species have been reported in the Karaganda Region: *Ae. (Rusticoides) subdiversus* Martini, 1926; *Ae. (Ochlerotatus) rossicus* Dolbeskin, Gorickaja & Mitrofanova, 1930; *Ae. (Ochlerotatus) caspius* Pallas, 1771; *Ae. (Ochlerotatus) flavescens* Müller, 1764; *Ae. (Aedes) cinereus* Meigen, 1818; and *Ae. (Aedimorphus) vexans* Meigen, 1830 [10]. In our samples, larvae of *Ae. rossicus*, *Ae. cinereus*, and *Ae. vexans* were not detected. The spring generation of the first two species typically develops later than that of other g. *Aedes* species. *Ae. vexans* is also considered a thermophilic species [9, 10]. Since our sampling was conducted at the end of April, it can be assumed that the larvae of these species may appear in water bodies at a later time.

The Jaccard index indicated considerable similarity in species composition (six shared species), although each zone also supported distinct species not found elsewhere. In Zone II, a smaller number of water bodies was surveyed, which may lead to some adjustments in future studies. However, it is unlikely that the

species ratio between the zones will change significantly, as the numerical dominance of certain species is particularly pronounced. Zone I is characterized by moderate diversity, dominated by two species. A distinct cluster emerges comprising *Ae. dorsalis* and *Ae. caspius* (53.2 % and 28.9 %, respectively), the most abundantly represented species in the study area. Other species, including *Ae. communis*, *Ae. euedes*, and *Ae. cantans*, form separate clusters, indicating their low and similar encounter rates (Fig. 3).

Zone II exhibits extremely low diversity, with the absolute dominance of a single species. *Ae. subdiversus* stands out as a clear dominant with a substantial prevalence of 81.6 %, positioned distinctly apart from other species on the dendrogram. The remaining species are more evenly distributed, though their proportions are minimal (ranging from 1.3 % to 6.6 %) (Fig. 3).

The differences in species composition across the surveyed areas can primarily be attributed to ecological factors, such as the predominant types of water bodies, aquatic vegetation (Fig. 2), and soil types. These factors influence the mineral composition of the water and the persistence of temporary water bodies [16].

### Conclusion

The species composition of blood-sucking mosquitoes of the genus *Aedes* was studied in the Nura District of the Karaganda Region, the city of Karaganda, and adjacent areas. The analysis revealed the presence of 10 g. *Aedes* species in the surveyed areas, with an uneven distribution. Dominant species were identified: in Zone I, *Ae. dorsalis* and *Ae. caspius* (53.2 % and 28.9 %, respectively), and in Zone II, *Ae. subdiversus* (81.6 %).

The identification of seven species (*Ae. annulipes*, *Ae. cantans*, *Ae. cataphylla*, *Ae. euedes*, *Ae. cyprius*, *Ae. dorsalis*, *Ae. communis*) not previously recorded in the Karaganda Region suggests a range expansion of *Aedes* mosquitoes, with important epidemiological implications for arbovirus transmission.

Additionally, the study identified a previously undescribed morphological variation in *Ae. subdiversus*, not reflected in existing identification keys.

The findings underscore the species diversity and the necessity for further studies on the fauna of bloodsucking mosquitoes, utilizing molecular methods, monitoring their abundance, and mapping their distribution in central Kazakhstan.

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Sheruova Ye.A.** – Sample Collection, Investigation, Writing Draft, Editing, Preparation of figures; **Sibataev A.K.** – Supervision, Conceptualization, Data curation, Methodology; **Andreeva Yu.V.** – Data curation, Formal analysis, Editing; **Andreeva U.D.** – Sample collection.

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Е.А. Шеруова, Ю.В. Андреева, У.Д. Андреева, А.К. Сибатаев

### Қарағанды облысының қансорғыш *Aedes* (Diptera: Culicidae) тұқымдасының масаларының түрлік құрамы

Жұмыста Қарағанды облысындағы (Қазақстан) масалардың түрлік құрамын зерттеу нәтижелері негізінде жасалған түрлердің тізбесі ұсынылған. Біздің нәтижелеріміз *Aedes* тұқымдасына жататын қансорғыш масалардың кем дегенде 10 түрінің бар екендігін көрсетті. Сонымен қатар, зерттеу атипті морфологиялық сипаттамаларды көрсететін үлгілерді анықтады, бұл жаңа вариация мүмкіндігін көрсетеді. Бұл нәтижелер осы морфологиялық тұрғыдан ерекшеленетін үлгілердің таксономиялық мәртебесін нақтылау үшін, әсіресе молекулалық әдістерді қолдана отырып, қосымша зерттеулер жүргізу қажеттілігін анықтайды. Сондай-ақ, жұмыста түрлердің әртүрлілігін және түрлердің популяциядағы көптігі бойынша біркелкі таралуының талдау нәтижелері келтірілген. Қарағанды қаласы мен оның маңайындағы *Aedes* туысының масаларының түрлік құрамы Қарағанды облысы Нұра ауданының масалар фаунасынан ерекшеленетіні көрсетілген. Облыс орталығының маңындағы ең көп кездесетін түрі *Ae. (Och.) dorsalis*, ал Нұра ауданында *Ae. (Rus.) subdiversus* түрі басым. Бұл зерттеу *Aedes* тұқымдасы масаларының биоәртүрлілігін және оның Орталық Қазақстандағы қоғамдық денсаулық сақтау үшін маңыздылығын түсінуге бағытталған болашақ жұмыстарға негіз қалайды.

*Кілт сөздер:* *Aedes*, *Culicidae*, жәндіктер, түр, Шеннон индексі, Пиелу индексі, Жаккар индексі, фауна.

Е.А. Шеруова, Ю.В. Андреева, У.Д. Андреева, А.К. Сибатаев

### Видовой состав кровососущих комаров рода *Aedes* (Diptera: Culicidae) Карагандинской области

В настоящей работе представлен перечень видов, составленный на основе результатов изучения видового состава комаров в Карагандинской области (Казахстан). Наши результаты свидетельствуют о наличии не менее 10 видов кровососущих комаров рода *Aedes*. Кроме того, в ходе исследования были выявлены экземпляры, демонстрирующие атипичные морфологические характеристики, что указывает на возможность новых вариаций. Эти результаты подчеркивают необходимость дальнейших исследований, особенно с использованием молекулярных методов, для уточнения таксономического статуса этих морфологически отличных экземпляров. В работе также приведены результаты анализа видового разнообразия и равномерности распределения видов по их обилию в сообществе. Показано, что видовой состав комаров р. *Aedes* г. Караганды и его окрестностей отличается от фауны комаров Нуринского района Карагандинской области. Доминирующим по численности видом в окрестностях областного центра является *Ae. (Och.) dorsalis*, тогда как в Нуринском районе численно преобладает



*Ae. (Rus.) subdiversus*. Данное исследование закладывает основу для будущих работ, направленных на понимание биоразнообразия комаров рода *Aedes* и его значения для общественного здравоохранения в центральном Казахстане.

**Ключевые слова:** *Aedes*, *Culicidae*, насекомые, виды, индекс Шеннона, индекс Пиелу, индекс Жаккара, фауна.

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## Information about the authors

**Sheruova Yerkezhan Aitbaykyzy** — PhD-student, Department of General Biology and Genomics, L.N. Gumilyov Eurasian National University, Astana, Kazakhstan; e-mail: [erkezhan.sheruova@mail.ru](mailto:erkezhan.sheruova@mail.ru), <https://orcid.org/0000-0002-0982-7202>

**Andreeva Yuliya Victorovna** — PhD of Biological Sciences, Associate Professor of the Department of Agricultural Biology, Tomsk State University, Tomsk, Russia; e-mail: [andreeva\\_y@mail2000.ru](mailto:andreeva_y@mail2000.ru), <https://orcid.org/0000-0003-2238-8766>

**Andreeva Ulyana Dmitrievna** — Learner in Municipal Autonomic General Educational Institution School “Perspectiva”, Tomsk, Russia

**Sibataev Anuarbek Karimovich** — Doctor of Biological Sciences, Professor of the Department of General Biology and Genomics, Kazakh Agrotechnical Research University named after S. Seifullin, Astana, Kazakhstan; e-mail: [asibataev@gmail.com](mailto:asibataev@gmail.com), <https://orcid.org/0000-0002-3434-6590>