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To the question of current species composition of blackflies (Diptera, Simuliidae) of the Irtysh river in Pavlodar region

The article presents a list of species diversity of the family Simuliidae identified in the Pavlodar Priirtyshye during the period from 2022 to 2024. In the basin of the middle reaches of the Irtysh River (from the border of the Maysky district to the Zhelezinsky district, and the border with Russia), the presence of 5 species of blackflies was noted. The identified blackflies belong to the Palaearctic and Holarctic species of plastic blackflies capable of living in polluted water bodies: *Wilhelmia equine* (Linnaeus, 1758), *Boophthora erythrocephala* (De Geer, 1776), *Odagmia ornata* (Meigen, 1818), *Argentisimulium noelleri* (Friederichs, 1920), *Simulium reptans* (Linnaeus, 1758). The history of the study of blackflies in Kazakhstan and the studied area is presented. The frequency of occurrence and the biology of the species in the Pavlodar region are described. Dominant species are identified.

Keywords: blackflies, Simuliidae, species composition, habitat area, Irtysh.

Introduction

Over the past 10 years, active work has been carried out to reduce the participation of blood-sucking blackflies not only in the North-Eastern part of Kazakhstan, but also in many southern regions (Syrdariya River). The establishment and current species structure allow us to develop more effective methods of combating blackflies. In addition, knowledge of international species and changes in their dynamics allows us to control the level of pollution of water bodies.

The last inventory of species composition in the North-Eastern part of Kazakhstan took place about 20 years ago. During this period, it is customary to consider individual species in the relationship, as well as the emergence of new, more plastic to pollution species of blackflies.

The global blackfly fauna (family Simuliidae) currently includes 2,424 recognized species [1].

Members of this family are distributed across all continents, except the Arctic and Antarctic regions [2]. Many blackfly species are considered important bioindicators of freshwater quality and can be effectively used to monitor the ecological status of aquatic ecosystems. Beyond their ecological relevance, blackflies are of considerable interest due to their significant veterinary and public health importance. Certain species act as vectors of serious invasive and infectious diseases affecting both humans and domestic animals, including onchocerciasis, anaplasmosis, and tularemia [3].

In this context, comprehensive studies of the faunistic diversity and species composition of blackflies are essential across all regions of Kazakhstan. Accurate information on species distribution is a prerequisite for the implementation of effective vector control, preventive, and public health strategies. The present study aims to identify the species composition of blackflies inhabiting the Pavlodar Region along the Irtysh River and to provide a concise overview of the biometric characteristics of the species recorded.

Studies of blackflies in Kazakhstan have been carried out by Russian and Kazakh scientists in different years. The southern and eastern regions of Kazakhstan are considered the most researched [4]. In the 50s–80s, the researches are reflected in the works of Shakirzyanova M.S. [5], Rubtsov I.A. [6]. Zoogeographic species composition of midges of Kazakhstan was summarized in the work of Kenzhebaev Zh.K. [7], of Konurbayev E.O [8].

The Pavlodar region, through which the blackfly course of the Irtysh River flows, is not fully studied [9]. The first records on the species composition and population of blackflies in this area were made by Sinelshchikov V.A. [10]. Further study of blackflies was carried out in the context of finding measures to control nuisance gnats. The mass extirpation of gnats harmed the agricultural and economic activity of the region. In subsequent years, data on the study of the faunistic composition of the region were conducted by Gabdulin E.S. [11], Alshin A.R. [12]. On the territory of the Bayanaulsky mountain-forest range, the study of blackflies was also conducted by Alikhanov Sh.A. [13].

It is worth noting that the study of blackflies in the Pavlodar region has been conducted for many years as part of the annual fight against blood-sucking insects. The study includes studies of the fauna, distribution, dynamics, and density of blackflies, as well as their impact on the economic part of our region.

This article presents data on the study of the species composition of blackflies in the Pavlodar Irtysh region from May 2023 to September 2024.

Materials and methods

The studied material was collected during field research on the Irtysh River, from early May 2023 to September 2024 in the Pavlodar region using standard methods described by Rubtsov I.A. [14; 56]. 413 blackfly imagoes were studied and identified, including 91 males and 322 females. 82 larvae and more than 110 pupae were collected.

The larvae and pupae were collected from a substrate immersed in water: fallen tree branches, plastic debris floating in the water. After collection, the research material was placed in test tubes with 90 % ethanol at a temperature of $\pm 20^{\circ}\text{C}$. Total preparations were made using a binocular magnifying glass MBS-10 and a biological light microscope with a UCMOS03100KPA Altami camera, type BIO-1. Pictures of blackflies' habitat landscapes were taken with a Samsung Galaxy S23Ultra camera. Permanent preparations of the head, limbs, and genitals were made using the method of Rubtsov I.A. [14] and fixed in Euparal. Pictures were taken from temporary preparations, and then the preparations were disposed of.

The method of hatching imagoes from pupae was also used. Collection and hatching of imagoes were carried out according to the methods of Khalin A.V. [15]. For these purposes, the pupa was placed in a clean test tube with a moistened wet swab and sealed. Then, a few hours after hatching, the empty pupa (exuvium) and the midge imago were fixed with 90 % ethanol in the same test tube for further identification.

The studied material was identified to the species level by the modern system of the *Simuliidae* family [1]. Morphological diagnostics to the species level was carried out using the identifiers of Rubtsov A.I. [14], Yankovsky A.V. [18]. The main features in the diagnosis of imago forms were: the color of the legs, mouth apparatus, and genitals; in larvae, the color of the head capsule, the respiratory threads of the larvae, and the posterior attachment organ. Pupae were diagnosed by the following main features: the shape of the cocoon, the nature of the branching (weaving) of the cocoon.

The assessment of blackfly species biodiversity was carried out based on the Shannon index (H'), calculated using the formula:

$$H' = -\sum(p_i \times \log_2(p_i))$$

where p_i is the proportion of individuals of species i relative to the total number of individuals in the district.

The evenness index (E) was calculated as:

$$E = H' / H'_{\max}, \text{ где } H'_{\max} = \log_2(S)$$

where S is the number of distinct species in the districts (with non-zero abundance). The calculation results and their interpretation are presented in the "Results and Discussion" section.

To ensure comprehensive coverage of the potential habitats of blackflies (*Simuliidae*), sampling sites were selected along the entire course of the Irtysh River within the Pavlodar region.

In each administrative district (Table), at least four sampling points were established, representing various types of watercourses (main river channel, floodplain areas, and river branches), flow velocities, and riparian biotopes characteristic of the region. The sampling points within each district were placed at approximately equal distances, with an average distance of about 100 km between districts.

Blackfly collection was carried out during peak periods of daily activity before noon and in the evening from 4:00 PM to 9:00 PM. Standardized methods were employed, including sweep netting, host-baited collection, and sampling of larvae and pupae from submerged substrates.

Species identification was based on the analysis of a combination of diagnostic morphological features. For accurate identification, larvae were collected, and adult flies (imago) were reared from mature pupae to enable species-level determination not only from the adult specimens but also based on the structure and branching pattern of the pupal cocoon filaments.

The number of samples and their spatial distribution adhered to established zoological standards for faunal surveys of the family *Simuliidae* [6, 14, 15, 16].

Potential biases are associated with the limited seasonal coverage of the sampling period. For example, non-bloodsucking species of blackflies may not have been captured using host-baited collection methods. For this reason, material was also collected directly from aquatic substrates. However, several authors have noted [6, 8] that in large and medium-sized rivers, predominantly bloodsucking blackfly species emerge. To increase sampling diversity, sweep-netting of riparian vegetation was also conducted.

The species *Argentisimulium noelleri* was represented in the collection by two female specimens. It was not detected in aquatic substrates; the two individuals were collected from vegetation. This may be because the emergence period for this species had already passed by the time of sampling, and the species is generally rare in the Pavlodar region. It is also noteworthy that no male specimens of this species were found. Males typically emerge 5–7 days earlier than females, which may explain their absence. Therefore, in the following season, we plan to begin sampling for this species 10–14 days earlier.

Sampling was not conducted during windy or rainy weather. The primary focus was on collecting from aquatic vegetation, which allowed for the identification of multiple developmental stages (larva, pupa, imago). This approach also enabled us to estimate the timing of emergence and assess the seasonal activity of blackfly species based on the developmental stage and maturity of larvae and pupae.

The permanent preparations made are stored in the collection of Orazbekova A.

Results and Discussion

As a result of the conducted study of the species composition of blackflies in the Pavlodar Irtysh region, the species composition of blackflies was clarified and supplemented, including 5 genera and 5 species of blackflies. The collection was carried out from the main channel of the Irtysh River.

Subfamily SIMULIINAE Newman, 1834

Tribe WILHELMIINI Baranov, 1926

Genus *Wilhelmia* Enderlein, 1921

Wilhelmia equine (Linnaeus, 1758)

The studied material. Imago: 162 females, 32 males collected. Date of collection: May, July 2023, June 2024.

Palaearctic, European-Asian species.

A plastic, widespread European species, including in the European part of Russia.

In the Pavlodar region, during the period of mass flight, it was noted in the coastal river zone in various biotopes: forest-steppe, forest zones, and coastal shrub thickets. The larvae of this species actively populate coastal vegetation. An active bloodsucker, attacks humans and animals, the dominance index is 47 %.

Morphological identification was carried out based on the genitalia of both the female and male, as well as the respiratory filaments of the pupa. A distinctive feature of the female genital structure is the presence of spines: the bases of the genital plates are curved inward and bear spines. The anterolateral sclerotized areas of the genital fork are weakly developed (Fig. 1a). The pupa possesses eight respiratory filaments, two of which are adjacent to the pupal collar, forming a semicircle. In the central region, six smaller filaments are arranged laterally to the main filaments and directed anteriorly (Fig. 1b). In the male, a small apical spine is present at the base of the gonostylus. The gonostylus is approximately 4.5 times narrower than the gonocoxite and is slender. The base of the gonosternum is triangular and curved inward (Fig. 1c).

Subfamily SIMULIINAE Newman, 1834

Tribe NEVERMANNIINI Enderlein, 1921

Genus *Boophthora* Enderlein, 1921

Boophthora erythrocephala (De Geer, 1776)

The studied material. Imago: 28 females, 3 males collected. Date of collection: June 2023.

Palaearctic, European-Asian-North American species.

Widespread throughout most of Europe and Russia. It is also found in North Africa and the East Asian region. In the territory of the Pavlodar region in 2016, it was one of the dominant species according to collections [17]. In the 2023 collections, the species was not abundant, with a dominance index of 8 %; in 2024, it was not recorded in the collections.

Species identification was based on the morphology of the male and female genitalia, as well as the pupae. The pupa possesses six respiratory filaments. All filaments are nearly identical in diameter and size. Each group originates from a common base, splitting into three branches, with two filaments arranged dichotomously on each branch (Fig. 1d). The male genitalia exhibit distinctive characteristics — the

gonostyles are short with a square-shaped base. On the inner surface of the gonostyle base, there are five small spines (Fig. 1e).

Subfamily SIMULIINAE Newman, 1834
 Tribe SIMULIINI Newman, 1834
 Genus *Odagmia* Enderlein, 1921
Odagmia ornata (Meigen, 1818)

The studied material. Imago: 12 females collected. Date of collection: June 2023.

Palaearctic, European-Asian-North American species.

This species of blackflies is a polyzonal and widespread European species. It also robs in the European part of Russia. According to scientists [18], it is distributed from the countries of northwestern Africa and Western Europe to Eastern Siberia and the Far East. The species is characteristic of forest-steppe landscapes. In the Pavlodar region, it is found in the coastal part of the Irtysh River and the adjoining forest-steppe zone. It attacked the feeder mainly in open biotopes. The species is not numerous in terms of dominance, 3 %.

Species identification was conducted based on the genitalia of females reared from pupae. The cocoon is simple, with an indistinct or absent pupal collar. The anterolateral angles are widely spaced, projecting laterally and dorsally. There are six respiratory filaments, originating as three main filaments, each of which branches dichotomously into two filaments (Fig. 1f).

Genus *Argentisimulium* Rubzov et Yankovsky, 1982
Argentisimulium noelleri (Friederichs, 1920)

The studied material. Imago: 2 females collected. Date of collection: May 2023.

Holarctic, European-Asian-North American species.

The habitat is mainly in most of the countries of foreign Europe and Russia. On the territory of the Pavlodar region, the species is very scarce. The presence of this species was not noted in the 2024 collections.

Species identification was based on the morphology of the female genitalia and legs. The stem of the furcal stalk is slender and approximately 1.5 times longer than the fork itself. The gonapophyses exhibit a deep, straight median incision. On the branches of the fork, the anterolateral projections are prominently developed and directed upward. The anal plates are rounded, and the cerci are also rounded and relatively small (Fig. 1g).

Genus *Simulium* Latreille, 1802
Simulium reptans (Linnaeus, 1758)

The studied material. Imago: 118 females, 56 males collected. Date of collection: May 2023, August 2024.

Palaearctic, European-Asian species.

On the territory of Kazakhstan, the presence of this species is noted for the first time. The species is widely distributed in most of Europe, including the European part of Russia. On the territory of Pavlodar region, it is an active bloodsucker with a high dominance index in the spring period — 42 %. It is found in the coastal part, it is mainly observed in open biotopes: under growths, tree and shrub thickets.

Morphological identification was carried out based on the genitalia of males (Fig. 1i) and females. A distinctive feature of the species is the structure of the pupal cocoon. Along the edge of the cocoon collar, there are large square fenestrae (windows) (Fig. 1h). Imago specimens were reared from mature pupae for morphological examination. In addition, molecular genetic analyses were conducted to confirm the species identification. The corresponding sequence data have been deposited in GenBank under accession numbers

PP396815 — <https://www.ncbi.nlm.nih.gov/nuccore/PP396815.1>,
 PP396749 — <https://www.ncbi.nlm.nih.gov/nuccore/PP396749.1>,
 PP400833 — <https://www.ncbi.nlm.nih.gov/nuccore/PP400833.1>,
 PP400933 — <https://www.ncbi.nlm.nih.gov/nuccore/PP400933.1>,
 PP400977 — <https://www.ncbi.nlm.nih.gov/nuccore/PP400977.1>.



Figure 1. Morphological features of black flies: (a) Female genitalia of *Wilhelmia equine*; (b) Pupal respiratory filaments of *W. equine*; (c) Male genitalia of *W. equine*; (d) Pupal respiratory filaments of *Boophthora erythrocephala*; (e) Male genitalia of *B. erythrocephala*; (f) Pupa of *Odagmia ornate* on substrate (plastic bag); (g) Female genitalia of *Argentisimulium noelleri*; (h) Cocoon collar of *Simulium reptans*; (i) Male genitalia of *S. reptans*

Based on the results of the conducted research, it can be concluded that the species diversity of gnats in the Pavlodar Irtysh region is quite poor. The Shannon diversity index in the Pavlodar Irtysh region was 1.042, with an evenness of 0.647. The total number of blackfly species was 5, and the average number of individuals was 82. Based on the species composition data for each district (Table), the index was calculated separately for each district (Fig. 2).

Table

Species composition of blackflies in the Pavlodar Irtysh region by districts (2023-2024)

Species name	Total imagos	Maysky District	Pavlodar City	Zhelezinsky District
<i>Wilhelmia equine</i>	194	101	19	74
<i>Boophthora erythrocephala</i>	31	18	0	13
<i>Odagmia ornate</i>	12	9	1	2
<i>Argentisimulium noelleri</i>	2	0	0	2
<i>Simulium reptans</i>	174	63	29	82

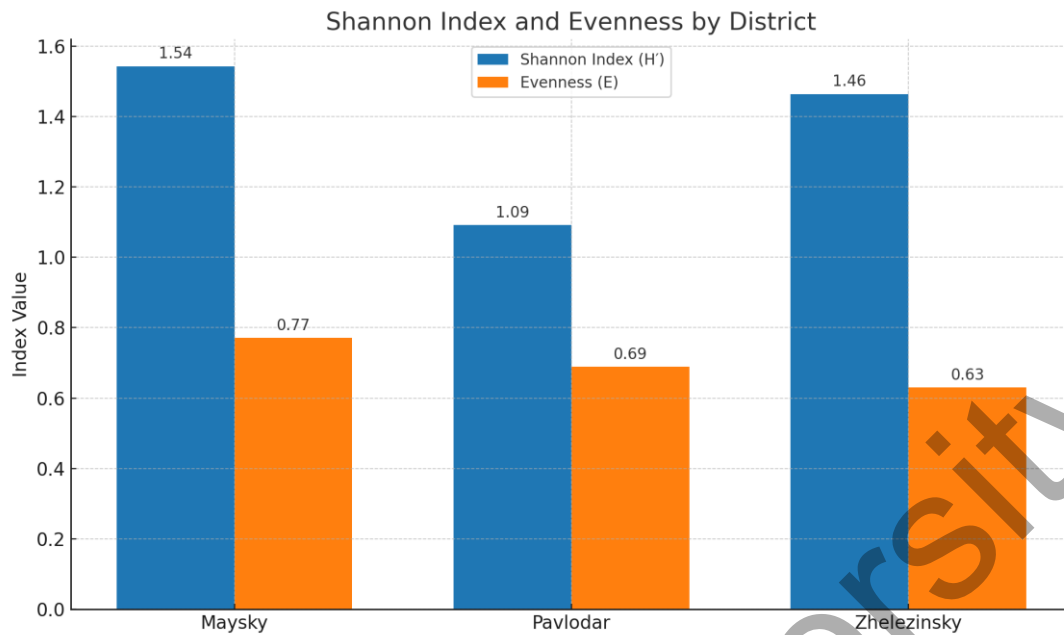


Figure 2. Shannon index and evenness values by district in the Pavlodar Irtysh region — from the border of Maysky District downstream to Zhelezinsky District (border with Russia) during the period from May 2023 to September 2024

As we can see from Table, among the five recorded species, the main dominant ones are *W. equine* and *S. reptans*. Figure 2 demonstrates that the blackfly community is generally balanced; however, despite this, the level of evenness remains approximately the same within each community (ranging from 0.6 to 0.7). The Shannon index increases both with the number of species and with greater evenness. However, the level of dominance (evenness) is not consistent. This indicates that in all three regions, one or more species (*W. equine* and *S. reptans*) dominate. In the Maysky District, species are distributed almost evenly (0.77); in the area near Pavlodar, there are dominant species, but not a complete skew (0.69); and in the Zhelezinsky District, clear dominance is observed along with a lower evenness value (0.63).

Such an uneven distribution indicates the dominance of certain taxa, which may suggest an ecological imbalance in the studied environment. However, many authors have noted that the low species diversity of blackflies is characteristic of lowland rivers [8], [6].

Active bloodsuckers attacking humans and animals in mid-May are midges — *W. equinum* and *S. reptans*. They are also absolute dominants. In the 2023 collections, blackflies of the species *Arg. noelleri* and *B. erythrocephala* were present in small quantities, but these species were not found in the 2024 collections. Although *B. erythrocephala* is a widespread eurybiont and synanthropic species that also develops in large rivers, withstanding significant temperature fluctuations, it is noticeable that its numbers in the Pavlodar region are declining.

In the Pavlodar Irtysh region, the widespread European species of blackflies, *S. reptans*, was identified for the first time. The presence of this species in Kazakhstan was noted for the first time. The data are confirmed by molecular genetic studies [19].

In the studies of Makatov T.K. [20], the fauna of the Pavlodar Irtysh region consisted of 6 genera and 9 species of midges. According to the data of 2016 [17], based on the results of our collections in 2023-2024, there are 5 species of blackflies in the fauna of the Pavlodar Irtysh region, belonging to 5 genera. A significant reduction in species diversity can be noted, and the presence today is of only more plastic species of blackflies. Since midges are amphibious animals, it can be assumed that less plastic species either disappeared from this range or make up a small percentage of the total number. Also, some abiotic factors could have had an impact: a decrease in oxygen in the water, high turbidity. It is not worth excluding the fact that, since 2015, every year, there has been carried out an active work in the region to reduce the number of midges. And there is a possibility that in the Pavlodar Irtysh region, species that are less flexible in processing will decrease in number over time.

Conclusion

The tribe *Simuliini* consists of genera widespread in the Northern Palearctic. In the fauna of the Pavlodar Irtysh region, the presence of 5 species of blackflies was noted. Including *S. reptans*, which is a new species for the fauna of Kazakhstan.

The most common species are *W. equinum* and *S. reptans*.

The plain and forest-steppe landscapes contribute to a relatively low level of blackfly species diversity, as well as the predominance of bloodsucking species. In the Pavlodar Irtysh region, the dominant species are those tolerant to water pollution. It is difficult to definitively attribute the reduction in blackfly species richness to specific anthropogenic factors, as large and medium-sized plain rivers are typically characterized by low species diversity. Moreover, rivers such as the Irtysh mainly support the emergence of hematophagous blackfly species, while non-bloodsucking blackflies are generally not dominant in such communities [6, 8, 14].

The species diversity of blackflies in the Pavlodar Irtysh region corresponds to its geo-hydrological regime. The lowest level of blackfly biodiversity was recorded in Pavlodar city; however, the evenness index here did not indicate strong dominance by individual species. A more balanced species distribution was observed in the Maysky District.

The results of the study on the blackfly fauna (Simuliidae) in the Irtysh River basin within the Pavlodar region may serve as a foundation for the development of an integrated ecological monitoring system for the region's aquatic ecosystems.

Large and medium-sized lowland rivers such as the Irtysh provide favorable conditions for the mass emergence of hematophagous blackfly species. The surveyed areas were dominated by *Wilhelmia equina*, *Boophthora erythrocephala*, and *Simulium reptans* species, characterized by a high degree of ecological plasticity. Their abundance can serve as an indicator of persistent pollution, eutrophication, and other forms of anthropogenic impact on aquatic environments. Therefore, the species composition of blackflies may be used as a sensitive indicator for assessing the ecological status of freshwater bodies.

Given these characteristics, it is recommended to implement regular monitoring of blackfly communities in strategically important sections of the Irtysh basin, particularly within populated areas, floodplain zones, and downstream of hydraulic structures. An optimal sampling frequency would be at least twice per season: once in the spring-summer period and once in the late summer-autumn period. This approach would allow for the detection of changes in species composition, early signs of ecological disturbances, and the timely development of measures for the management and protection of regional aquatic ecosystems.

In the future, monitoring activities are needed to track changes in species dynamics. The identification of a new species for the fauna of Kazakhstan confirms the necessity of continuing faunal surveys in the Irtysh region.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Orazbekova A.A.**: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing; **Akhmetov K.K.**: Formal analysis, Validation, Writing – review & editing; **Burkitbayeva U.D.**: Resources, Investigation, Project administration; **Aubakir A.S.**: Supervision, Writing – review & editing, Funding acquisition.

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Павлодар өңіріндегі Ертіс өзенінің шыбын-шіркейлерінің (Diptera, Simuliidae) қазіргі түрлік құрамы туралы мәселеге

Мақалада 2022-2024 жылдар аралығында Павлодар өңіріндегі Ертіс өзенінен тіркелген *Simuliidae* тұқымдасының түрлік әртүрлілігінің тізімі келтірілген. Ертіс өзенінің орта ағысы бассейнінде (Май ауданының шекарасынан Ресеймен шекаралас Железин ауданына дейін) шыбын-шіркейдің 5 түрінің бар екені анықталды. Анықталған шыбын-шіркейлер ластанған су айдындарында тіршілік ете алатын палеарктикалық және голарктикалық пластикалық түрлерге жатады, олар: *Wilhelmia equina* (Linnaeus, 1758), *Boopthora erythrocephala* (De Geer, 1776), *Odagmia ornata* (Meigen, 1818), *Argentisimulium noelleri* (Friederichs, 1920), *Simulium reptans* (Linnaeus, 1758). Қазақстандағы және зерттелген аумақтағы шыбын-шіркейлерді зерттеу тарихы қарастырылған. Түрлердің таралу жиілігі көрсетіліп, Павлодар өңіріндегі олардың биономикасы сипатталған. Басым түрлер анықталған.

Кілт сөздер: шыбын-шіркейлер, *Simuliidae*, түрлік құрамы, таралу аймағы, Ертіс.

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К вопросу о современном видовом составе мошек (Diptera, Simuliidae) на реке Иртыш в Павлодарской области

В статье приведен перечень видового разнообразия семейства Simuliidae, выявленного в Павлодарском Прииртышье в период с 2022 по 2024 год. В бассейне среднего течения реки Иртыш (от границы Майского района до Железинского района и границы с Россией) отмечено присутствие 5 видов мошек. Выявленные мошки принадлежат к палеарктическим и голактическим видам пластиковых черных мух, способных обитать в загрязненных водоемах: *Wilhelmia equine* (Linnaeus, 1758), *Boopthora erythrocephala* (De Geer, 1776), *Odagmia ornata* (Meigen, 1818), *Argentisimulium noelleri* (Friederichs, 1920), *Simulium reptans* (Linnaeus, 1758). Приведена история изучения черных мух в Казахстане и на изучаемой территории. Указана частота встречаемости и описана биономика видов для Павлодарской области. Определены доминирующие виды.

Ключевые слова: мошки, Simuliidae, видовой состав, ареал обитания, Иртыш.

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