

Article

New Records of *Simulium murmanum* Enderlein, 1935 and *Simulium reptans* (Linnaeus, 1758) (Diptera: Simuliidae) in North-Eastern Kazakhstan: Bionomics and Habitat Range

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Abstract

This study investigates the species composition and distribution of blackflies (Diptera: Simuliidae) in Kazakhstan, with a focus on two species newly recorded for the country: *Simulium murmanum* (Enderlein, 1935) and *Simulium reptans* (Linnaeus, 1758). The presence of *S. murmanum* in Kazakhstan is reported for the first time, supported by morphological and molecular genetic analyses. Diagnostic features of the larva, pupa, and adult stages are described in detail, including the structure and coloration of the larval head capsule, pupal cocoon, and genitalia of both sexes. Habitat preferences and pupal substrate attachment patterns are illustrated, with observations on variations in cocoon branching across different flow regimes. Species identification was conducted using the morphological keys of Rubtsov and Yankovsky, and taxonomic classification was confirmed using the framework proposed by Adler. Molecular confirmation of *S. murmanum* was performed via DNA analysis. The species was found to be restricted to the foothill regions of East Kazakhstan, suggesting a distribution closely associated with the Altai mountain systems and adjacent regions in Mongolia and China. Unlike its status as a dominant hematophagous species in parts of Russia, *S. murmanum* has not demonstrated biting activity in Kazakhstan, Mongolia, or China. Additionally, the study provides the first records of *S. reptans* within the fauna of Kazakhstan, initially identified in the Irtysh River (Pavlodar Region). Subsequent sampling conducted in June 2024 revealed a continuous distribution of *S. reptans* along the Irtysh River through to the mountain streams of East Kazakhstan. The species was found in mountainous, foothill, and lowland environments, highlighting its wide ecological plasticity.

Keywords: new country record; biogeography; bionomics; Irtysh River; ecological plasticity; molecular identification



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1. Introduction

The global fauna of blackflies (Diptera: Simuliidae) comprises approximately 2415 species. In Kazakhstan, 89 species have been recorded to date, with 15 identified as endemic to the region [1].

The species *Simulium murmanum* (Enderlein, 1935) has historically been cited under various taxonomic names in the literature, owing to its morphological resemblance to other species within the *Simulium* (s. str.) complex. In northern Europe and Siberia, it has been referred to as *S. relictum* Stukolkina, 1939 (in the Palearctic range) and *S. rostratum* (Lundström, 1911) (not according to Lundström); in Canada and the USA, as *S. corbis* Twinn, 1936; and in Sweden, as *Gnus forsi* (Carlsson, 1962). In Scandinavia, it was also listed as *Simulium* sp. II based on Puri's classification (1926). A comprehensive morphological and karyological revision conducted by Raastad and Adler (2001) consolidated these forms under the valid name *S. murmanum*, with the type locality designated along the Murmansk coast (Aleksandrovsk, Reindeer Island) [2].

Thus, *S. murmanum* represents a boreal-distributed species with a complex taxonomic history, reflecting a pattern of intercontinental synonymy across its Holarctic range. This species is associated with boreal and subarctic landscapes throughout northern Europe (including Scandinavia and Russia's Murmansk region), Siberia, and the northern parts of North America [3]. It is well adapted to cold climates, short growing seasons, and habitats characterized by clean, oxygen-rich water bodies.

According to biogeographical analysis, *S. murmanum* is classified within the Pan-Holarctic boreal chorotype, indicating its broad distribution in taiga and adjacent cold ecosystems across continents. This chorotype group species adapted to cool environments and is regarded as an important ecological indicator, particularly in the context of global climate change [4].

Cytogenetic data from NIFA/USDA reports support the chromosomal identity (B cytotype) of *S. murmanum* populations in both Sweden and Canada. This provides further evidence of its Holarctic distribution and faunogenetic links between Palearctic and Nearctic Simuliidae, suggesting a long-standing evolutionary history and ecological stability of the species in high latitudes [5].

In North America, *S. murmanum* is considered a common and stable species within the boreal forests of Canada, particularly in ecozones such as the Northwest Territories. The NWT Species Search database indicates that its distribution is strictly limited to northern ecosystems, with no penetration into temperate zones [6]. Regional faunistic reviews further confirm its northern habitat preference. In eastern Transbaikalia (bordering northern Mongolia), *S. murmanum* is documented among species confined to high latitudes and montane taiga landscapes, suggesting the possible presence of ecologically similar forms in adjacent regions.

In the Murmansk region and Karelia, *S. murmanum* is recognized as one of the characteristic species of the northern blackfly fauna, along with other members of the tribe Simuliini that are adapted to cold, oxygen-rich river systems [7]. In these regions of Russia, including the Murmansk area, Karelia, and other taiga zones, *S. murmanum* is classified as an active hematophagous species, with documented negative impacts on local economic activities, particularly in areas related to agriculture and outdoor labor [8]. However, in Kazakhstan, this species has not yet been recorded as hematophagous, and the literature review shows no confirmed presence in neighboring countries such as China or Mongolia. It is likely that its distribution is confined to more northerly boreal zones and that its presence in mountainous areas of Eastern Kazakhstan is limited to isolated populations that do not exhibit blood-feeding behavior.

By contrast, *S. reptans* (Linnaeus, 1758) has been recorded in north-eastern Kazakhstan (Pavlodar region, Irtysh River) as an active blood-feeding species [9]. In Russia and neighboring regions, it is similarly recognized as hematophagous [3,8]. The *S. reptans* (L.) complex is known for its cryptic structure, which complicates taxonomic resolution and assessment of its epidemiological significance [10].

The aim of this study is to investigate the diversity of blackfly species along the Irtysh River in North-Eastern Kazakhstan, with a particular focus on identifying previously unrecorded species, analyzing their distribution patterns, bionomics, and assessing their potential ecological and epidemiological significance. Certain blackfly species may pose threats to agricultural productivity, public health, and the quality of life in river-side settlements. Therefore, defining the habitat ranges of these species and establishing baseline data for future monitoring of their distribution and hematophagous activity is of significant importance.

2. Materials and Methods

2.1. Sample Collection and Analysis

The material for this study was collected during fieldwork in the upper reaches of the Irtysh River, conducted in the second ten-day period of June 2024 in the East Kazakhstan region (Figure 1).



Figure 1. Distribution map of *Simulium murmanum* and *Simulium reptans*. Sampling points are marked by numbers: *S. reptans* was recorded at locations 1–8, while *S. murmanum* was detected at sites 1 and 2.

At the studied sites, the presence of several blackfly species previously recorded for the fauna of Kazakhstan was confirmed. In addition, two species not previously reported from these locations—*S. murmanum* (86 adults reared from pupae, 112 mature larvae, and 17 medium-aged larvae) and *S. reptans* (275 pupae, 93 mature larvae, and 36 medium-aged larvae)—were identified. Other species identified based on morphological examination include *S. noelleri* (Friederichs, 1920)—1 mature larva and 2 medium-aged larvae; *S. ornatum* (Meigen, 1818)—5 mature larvae and 1 medium-aged larva; *Wilhelmia equinum* (Linnaeus,

1758)—26 pupae, 18 mature larvae, and 14 medium-aged larvae; *Byssodon maculatum* (Meigen, 1804)—12 pupae and 5 mature larvae; and *Boophthora erythrocephalum* (De Geer, 1776)—4 pupae and 8 mature larvae.

Sampling locations, including coordinates and site characteristics, are presented in Table 1. The classification of mountain stream communities followed the Illies system [11].

Table 1. Landscape and ecological characteristics of midge collection sites in 2024, North-Eastern Kazakhstan.

No.	Region	Vegetation Zone	Coordinates	Altitude (m a.s.l.)	Water Temperature (t °C)	Number of Samples Examined
1	East Kazakhstan region, Katon-Karagay village	Foothill and low mountain–plain belt; small mountain river with a rocky substrate	49°10′48″ N 85°33′37″ E	955	±14	<i>S. murmanum</i> : 73 pupae, 113 larvae; <i>S. noelleri</i> : 3 larvae.
2	East Kazakhstan region, Bukhtarma river	Lowland–plain belt, river with a rocky pebble bottom	49°46′35.2″ N 84°02′39.4″ E	398	±17	<i>S. murmanum</i> : 13 pupae, 16 larvae; <i>S. reptans</i> : 13 pupae, 2 larvae.
3	East Kazakhstan region, Ust-Kamenogorsk city	Lowland–plain belt of the Irtysh River; main channel with a rocky–sandy bottom	49°53′27″ N 82°41′01″ E	332	±17	<i>S. reptans</i> : 1 pupa; <i>W. equinum</i> : 2 pupae.
4	Abay region, Semey city	Lowland–plain belt of the Irtysh River; main channel with a rocky bottom	50°21′56″ N 80°21′30″ E	192	±18	<i>S. reptan</i> : 2 larvae; <i>B. maculatum</i> : 5 pupae, 3 larvae.
5	Pavlodar region, Beskaragay village	Flat steppe zone; Irtysh River main channel with a sandy bottom	51°23′16″ N 77°50′41″ E	170	±20	<i>S. reptans</i> : 111 pupae, 57 larvae; <i>W. equinum</i> : 3 pupae; <i>B. maculatum</i> : 2 larvae; <i>Odagmia ornatum</i> : 6 larvae.
6	Pavlodar region, Pavlodar city	Plain forest-steppe zone; Irtysh River with a sandy bottom	52°16′37″ N 76°55′35″ E	112	±20	<i>S. reptans</i> : 1 pupa.
7	Pavlodar region, Terenkol village	Low mountain–plain belt of the Irtysh River; stratified sandy loam and loam substrate	53°02′33″ N 76°03′06″ E	87	±20	<i>S. reptans</i> : 56 pupae, 47 larvae; <i>W. equinum</i> : 11 pupae, 9 larvae.
8	Pavlodar region, Zhelezinka village	Low mountain–plain belt of the Irtysh River; stratified sandy loam and loam substrate	53°32′24″ N 75°14′50″ E	83	±20	<i>S. reptans</i> : 93 pupae, 21 larvae; <i>W. equinum</i> : 10 pupae, 23 larvae; <i>B. maculatum</i> : 7 pupae; <i>B. erythrocephalum</i> : 4 pupae, 8 larvae.

For genetic analysis, part of the material was preserved in 70% ethanol. For morphological studies, both temporary and permanent microscope slides were prepared. A 60% aqueous glycerol solution was used for temporary mounts, while permanent slides were mounted in Euparal. Slide preparation followed the methods described by Rubtsov (1956) and Khalin et al. (2023) [12,13]. Morphological identification was performed using the taxonomic keys of Rubtsov and Yankovsky [14,15].

Microscopic analysis was performed using an MBS-10 binocular magnifying glass (Lytkarinsky Optical Glass Plant, Lytkarino, Russia) and an Altami BIO-1 light microscope equipped with a UCMOS03100KPA digital camera (Altami LLC, Moscow, Russia). Landscape and midge substrate photographs were taken using a Samsung Galaxy S23 Ultra camera (Samsung, Ho Chi Minh, Thai Nguyen, Vietnam).

2.2. Entomological Studies

Material collection for entomological analysis was carried out using standard methods [13]. Larvae and pupae of blackflies were collected from substrates submerged in water, such as stones, branches, and aquatic vegetation. The specimens were preserved in test tubes containing 90% ethanol and stored at a temperature of approximately ± 15 °C.

To obtain adult specimens (imagines), some mature pupae were placed in dry test tubes sealed with a cotton pad moistened with water. After adult emergence, 90% ethanol was added to the same tube to preserve the imago along with the pupal case and exuviae containing respiratory filaments. The tubes were then hermetically sealed for further analysis.

A collection of blood-feeding adults from a host was conducted to estimate the species composition of anthropophilic forms. The host was fanned with an aerial entomological net for three minutes. The net was then sealed by twisting the opening, and the captured insects were anesthetized. This was performed by placing the twisted portion of the net into a plastic bag containing a cotton ball soaked in chloroform. The material was subsequently sorted into test tubes and preserved as described above, depending on the study objectives.

Water current velocity at collection sites was measured using the standard float method.

2.3. DNA Extraction Method

Genomic DNA was extracted by homogenizing a portion of a larva ($n = 5$) and imago ($n = 5$) in an Eppendorf centrifuge tube using the standard phenol–chloroform method with proteinase K digestion, followed by ethanol precipitation [16]. The concentration and purity of the extracted DNA were determined by measuring absorbance at 260 nm and 280 nm using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Carlsbad, CA, USA). The resulting DNA was dissolved in double-distilled water (ddH₂O) and stored at -20 °C until further use.

2.4. PCR and Sequencing

Polymerase chain reaction (PCR) was employed to assess the genetic diversity of different blackfly species. To amplify a 710 bp *COX1* segment from the mitochondrial DNA, PCR was performed using the primers *LCO1490* (F: 5'-GGTCAACAAACAAATCATCATAAAGATATATTGG-3') and *HCO2198* (R: 5'-TAAAACTTCAGGGTGACCAACCAAAAAAATCA-3') [17]. PCR were performed in a 25 μ L mixture containing 2 $\times$ DreamTaq PCR Master Mix (Thermo Fisher Scientific, Carlsbad, CA, USA), nuclease-free water, 10 pmol of each primer, and 20 ng of template genomic DNA. The PCR program included an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 40 s, 49 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. Amplified products were separated by electrophoresis on a 1.5% agarose gel prepared with 1 $\times$ TBE buffer, stained with ethidium bromide (8 ng/ μ L), and visualized under UV light.

All PCR-positive products were purified using the Quick PCR Purification Kit (QIAGEN, Germantown, MD, USA) according to the manufacturer's instructions, then subjected to sequencing and genotyping. Sequencing was performed on a 3730xl DNA Analyzer 96-Capillary Array (Thermo Fisher Scientific, Applied Biosystems, Foster City, CA, USA). The resulting nucleotide sequences were manually edited and compared with reference sequences from the GenBank database using the BLAST algorithm (<https://www.ncbi.nlm.nih.gov/>).

2.5. Phylogenetic Analysis

Nucleotide sequences were aligned using the MUSCLE algorithm for multiple sequence alignment, applied to partial sequences of the *cox1* mitochondrial gene, and sequence similarity searches were performed using the BLAST algorithm (<https://blast.ncbi.nlm.nih.gov>) to compare them with reference sequences from GenBank. The nucleotide sequences obtained in this study have been deposited in the GenBank database. The accession numbers are as follows: *S. murmanum*—PV269838 and PV269839. *Musca domestica* (OK065531) was used as the outgroup. A maximum-likelihood phylogenetic tree was constructed based on the Tamura–Nei model using MEGA version 11 software [18].

3. Results

3.1. Entomological Studies

Morphological diagnostics *Simulium* spp. were performed using standard identification guides. Species determination was based on the examination of larvae, pupae (including cocoon shape), and imagos, either hatched or extracted from pupae. At all surveyed locations (Table 1), the presence of *S. reptans* was confirmed. The morphological characteristics of the collected specimens fully corresponded to those previously described for this species, showing no notable variation [9]. This allowed us to confidently attribute the blackflies to *S. reptans* and to extend the known distribution range of the species within Kazakhstan (Figure 1). In addition to *S. reptans*, we also identified specimens belonging to *S. murmanum*. This species exhibits distinct structural traits that clearly differentiate it from *S. reptans*, thus representing a separate taxonomic entity within the collected material.

The following is a description of the morphological features of *S. murmanum* relevant for its identification.

Larva. The larval body measured 6.0–6.5 mm in length and exhibited a dirty gray coloration. A key diagnostic feature was the frontal sclerite, which displayed a characteristic dark pyramidal spot along the lower dorsal edge of the head capsule (Figure 2A). In some individuals, additional small dark spots were observed above the apex of the pyramid. The ventral notch was arched, broad at the base, and tapered toward the lower margin of the submentum (Figure 2B). Each larval fan consisted of 40 to 48 rays.

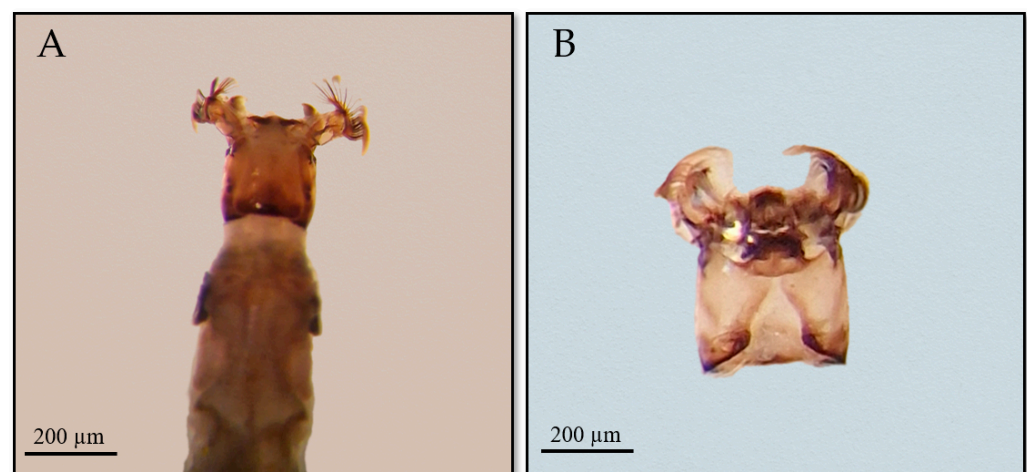


Figure 2. Morphological features of the larva of *Simulium murmanum*: (A) head capsule with characteristic dark pyramidal marking on the frontal sclerite; (B) shape of the ventral notch reaching the submentum.

Pupae. Depending on the velocity of the water flow, we observed morphological variations in pupal structures. In the first type, the pupal body measured approximately 4.0

to 6.5 mm in length. The cocoon was shoe-shaped, featuring a collar with 10 to 12 square openings along its margin. From these openings, loops extended upward in an arched formation, forming a tent-like structure above the developing imago (Figure 3A). The central opening of the cocoon was relatively deep, extending forward to nearly half the length of the pupal body from its base (“sole”). The distinctive weaving pattern along the collar margin is a key taxonomic feature and leaves little ambiguity regarding species identification. The second pupal type was distinguished by cocoons with sharply curved loops directed vertically upward, which resulted in the head of the pupa remaining fully exposed (Figure 3B). There are ten respiratory filaments: two are single, unbranched threads, while the remaining four branches bifurcate dichotomously into two threads each, extending from the base (Figure 3C). Their length corresponds to the height of the loops. When the imago is present inside the pupae, the respiratory filaments do not extend beyond the height of the collar. All threads are approximately equal in shape and thickness.

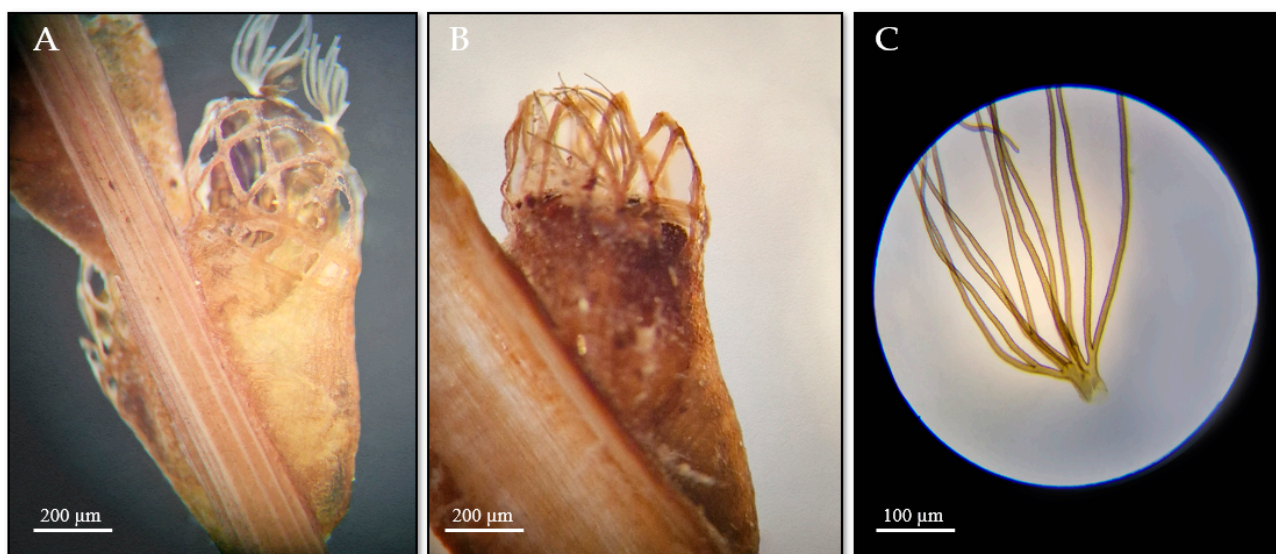


Figure 3. Morphological characteristics of the pupae of *Simulium murmanum*: (A,B) Shoe-shaped cocoon with a collar bearing 10–12 square openings and looped filaments forming a tent-like structure above the head of the imago; (C) Respiratory filaments: two single unbranched threads and four dichotomously branched pairs extending from the base.

Female. Body length ranges from 3 to 4 mm. The anal plates have a deep notch in the upper part, narrowing toward the bottom, and are square-shaped with slightly rounded edges. The cerci are approximately twice as small as the anal plates and are sparsely covered with hairs (Figure 4A). The genital plates are broad at the base, sparsely haired, and nearly equal in length to the basisternum. Their upper margin is slightly rounded. The branches of the genital fork have anterolateral sclerotized dark projections directed upward in the middle section (Figure 4B), which are widely spaced. The stem of the genital fork is dark, thin, and slightly thickens from the middle. The length of the stem is approximately 1.5 times the length of the fork.



Figure 4. Female morphology of *Simulium murmanum*: (A) Anal plates with deep upper notch and cerci showing sparse hairs. (B) Genital plates and genital fork with sclerotized anterolateral projections and stem.

3.2. Molecular Genetics Identification

Molecular identification of *Simulium* spp. was carried out using primers specific to partial regions of the mitochondrial *cox1* gene, producing an amplicon of approximately 710 bp. The resulting sequences of *S. murmanum* were submitted to GenBank and subsequently included in the phylogenetic analysis (Figure 5).

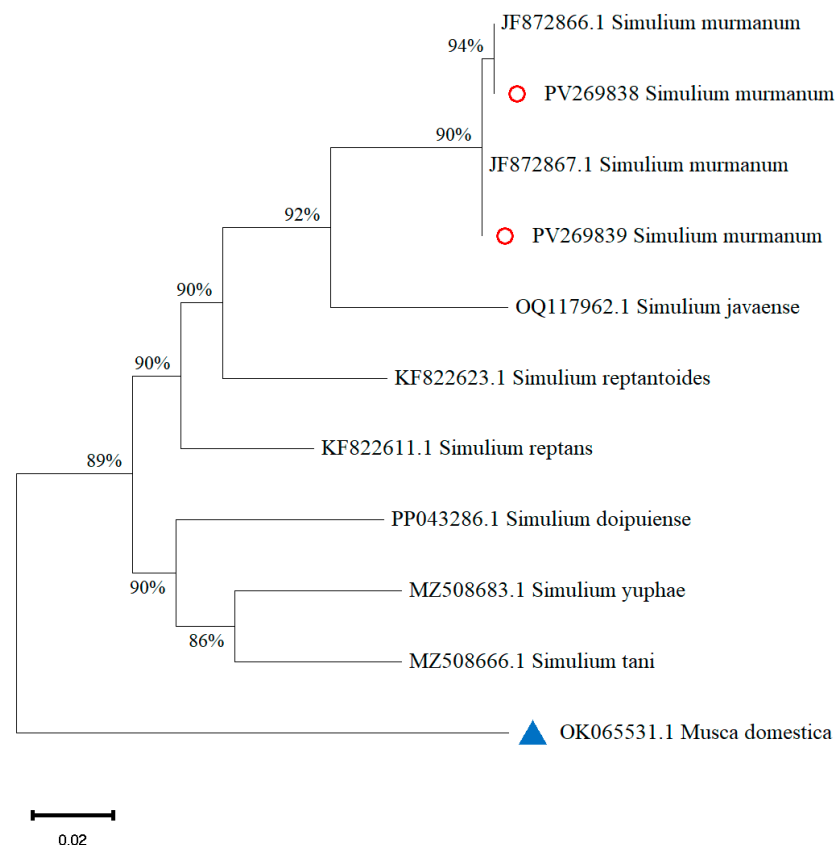


Figure 5. A phylogenetic analysis of the maximum likelihood tree of the *Simulium* species. (Red circle—isolates from this study, blue triangle—outgroup).

The evolutionary history was meticulously reconstructed utilizing the Maximum Likelihood method alongside the Tamura–Nei model [19]. The phylogenetic tree presented here, characterized by the highest log likelihood score of -2331.36 , showcases the most statistically robust representation of the data. To initiate the heuristic search process, preliminary trees were generated automatically through the application of Neighbor-Joining and BioNJ algorithms. These algorithms operated on a matrix of pairwise distances that were estimated using the Tamura–Nei model. Subsequently, the topology with the most favorable log likelihood value was selected for further analysis. This comprehensive examination involved a total of 11 nucleotide sequences, encompassing 672 aligned positions in the final dataset. All evolutionary analyses were performed using MEGA11 software [20], enabling a sophisticated evaluation of the genetic relationships among the sequences.

4. Discussion

Orographically, Kazakhstan can be divided into two main geographic zones: the western part, consisting mainly of flat, semi-desert steppes with a dry climate and low annual precipitation, and the eastern part, characterized by cereal steppes, foothills, and the mountain systems of the Tien Shan and Altai [21]. While the mountainous part of the Tien Shan within Kazakhstan has been relatively well studied, the presence of *S. murmanum* in this region has not been previously recorded. The Northern and Eastern regions of Kazakhstan border the taiga forests of Siberia, whose blackfly fauna influences the species composition in adjacent areas [22].

Foothill and mountainous environments significantly affect the morphology and biology of blackflies. These areas are typically inhabited by monocyctic species [21,23]. The vegetation period in the mountains is short, lasting only 2–3 months. In this zone, *S. murmanum* breeds in the first ten days of July. Night temperatures average $17\text{--}18\text{ }^{\circ}\text{C}$, while daytime temperatures reach $32\text{--}35\text{ }^{\circ}\text{C}$. In mountainous areas, water temperatures do not exceed $13\text{ }^{\circ}\text{C}$, but in the flatlands, the river warms up to $19\text{ }^{\circ}\text{C}$.

S. murmanum was found in the north-eastern part of Kazakhstan, specifically in the foothill and low-mountain belts. During the fieldwork in late June 2024, material was collected at eight sites along the Irtysh River in the East Kazakhstan Region, extending downstream toward the Russian border (Figure 1).

Pupation of larvae was observed during the second ten-day period of June, at a water temperature ranging from $17\text{ to }19\text{ }^{\circ}\text{C}$ and a flow velocity of approximately $1.5\text{--}2.0\text{ m/s}$. The collection site was a shallow mountain river with a rocky bottom, 3 to 4 m wide (Figure 6). Low shrubs and trees such as *Populus alba* and *Betula alba* grew along the riverbanks.

As previously noted by Rubtsov [12], the pupae of this species tend to cluster on submerged substrates (Figure 7A). On the branches of aquatic plants and fallen woody debris, where there is limited space for individual cocoon attachment, larvae construct cocoons in tight groups (Figure 7B). The pupal cocoons are positioned closely together, often in direct contact with one another.

At the second sampling point (Figure 1), where larvae and pupae of this species were also found, the river current was relatively slow, ranging from $0.5\text{ to }1\text{ m/s}$. The structure of the cocoons of this species shows notable differences depending on flow conditions. In the fast-flowing mountain river (Point 1), the loops of the pupal cocoon collar are more rounded, directed upward, and toward the center. They appear to cover the head of the pupa and the respiratory filaments, forming a tent-like structure (Figure 3A). At the second sampling point, located in a flatter section of the river, the cocoons have sharply curved loops directed strictly upward, leaving the head of the pupa exposed (Figure 3B). It is also worth noting that in the mountain river, the collar of the pupal cocoon lies close to the substrate, whereas in the slower river section, the collar protrudes noticeably above

the substrate. This difference in cocoon structure may serve an adaptive function: in fast-flowing waters, the downward orientation and tent-like shape of the loops likely help protect the head and respiratory filaments from the force of the current. In contrast, in low-flow environments, such protective adaptations are unnecessary, and the loops remain upright and open.



Figure 6. Mountain river habitat of *Simulium murmanum* in the East Kazakhstan region: shallow rocky-bottomed stream with surrounding riparian vegetation.

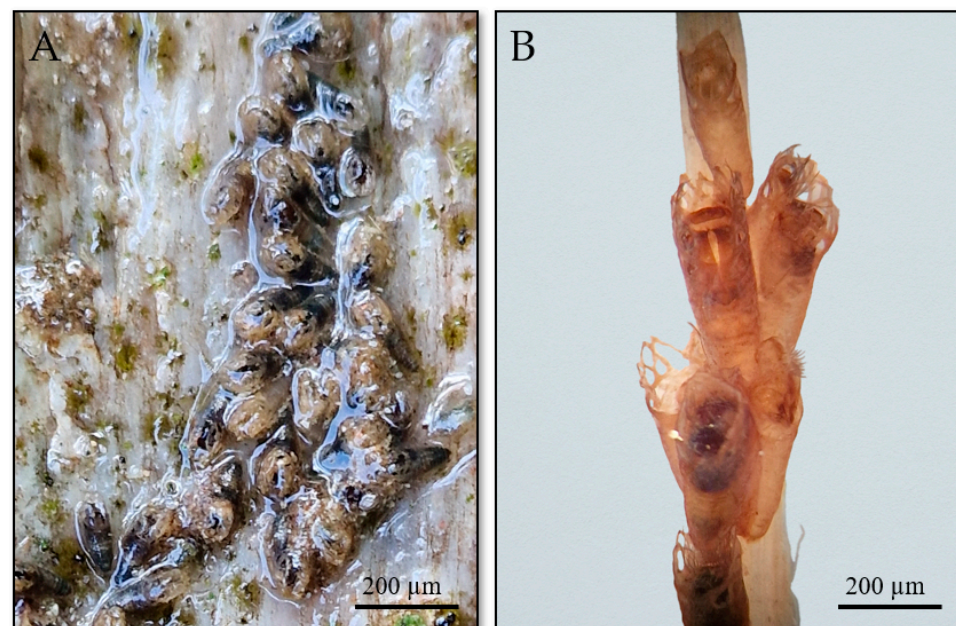


Figure 7. Clustering behavior of *Simulium murmanum* pupae on submerged substrates: (A) pupae grouped on aquatic vegetation; (B) densely packed cocoons on submerged woody debris.

Our observations are generally consistent with previously described characteristics of *S. murmanum* [12,15]. However, several notable morphological distinctions were identified in the female genital structures of specimens collected in Katon-Karagay (Figure 8A), when compared to classical descriptions provided in standard identification keys. In our material, the genital furcula is 2–2.5 times longer than the genital stem. In contrast, according to Rubtsov’s identification guide, the genital stem is reported to be 1.3–1.5 times longer than the furcula. Further discrepancies were observed in the anterolateral sclerotized areas on the branches of the genital furcula. In our specimens, each branch bears a well-defined

single outgrowth directed upward. In the identification guides, these outgrowths are less pronounced, whereas in Rubtsov's original illustrations (Figure 8B), they are robust, jagged, and form prominent projections that effectively divide each branch of the furcula.

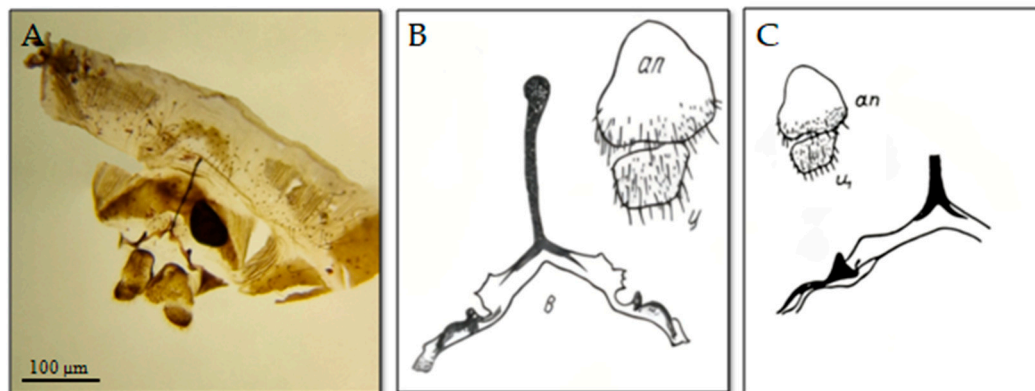


Figure 8. Structure of the female genitalia of *Simulium murmanum*: (A) photograph of a specimen collected in the mountainous region of East Kazakhstan; (B) illustration from I.A. Rubtsov [12]; (C) illustration from A.V. Yankovsky [15].

Additionally, the cerci of the females in our material appear more rounded at the base, whereas in descriptions provided by Rubtsov and Yankovsky, the cerci exhibit a beveled inner side and a square-shaped base (Figure 8B,C). Based on these findings, we propose that the female genital morphology of *S. murmanum* from the mountainous region of East Kazakhstan demonstrates notable structural differences from the populations previously described in European Siberia and the Russian Far East. These variations may reflect geographic differentiation, ecological adaptation, or cryptic speciation within the *S. murmanum* complex and merit further investigation using both morphological and molecular methods.

S. murmanum exhibits significant intraspecific variability, which is reflected in its repeated descriptions under different taxonomic names, including *S. corbis*, *S. relictum*, and *Gnus forsi*. This variability has contributed to historical misidentifications by Rubtsov and others, highlighting the species' morphological plasticity and the challenges it poses for accurate taxonomic delimitation [2].

The two new isolates, *S. murmanum* from this study (PV269838 and PV269839), cluster strongly with existing sequences of *S. murmanum* (JF872866.1 and JF872867.1). Their grouping is well-supported by a high bootstrap value of 94%, indicating a confident phylogenetic placement. This suggests that the new isolates belong to the same species and share a recent common ancestor with the previously known *S. murmanum* sequences. Other *Simulium* species (*S. reptantoides* Carlsson, 1962, *S. reptans*, *S. doipuiense* Takaoka & Choochote, 2005, *S. yuphae* Takaoka & Choochote, 2005, and *S. tani* Takaoka & Davies, 1995) form distinct subgroups, with varying levels of relatedness. *Musca domestica* Linnaeus, 1758 (OK065531.1) is used as the outgroup, a reference point for rooting the tree (Figure 5). It is consistent with findings in studies of *Simulium* and related genera. For example, in the Non-*Simulium* damnosum complex in Cameroon, *ITS2* and *Cox1* data revealed that individuals of the same species cluster tightly with bootstrap > 70% across ecological zones [24]. Similarly, barcoding work on Palearctic *Wilhelmia* blackflies showed clear monophyletic groups with high bootstrap values [25]. These parallels reinforce the robustness of our identification of *S. murmanum* and support that the new isolates are indeed conspecific with previously known entries.

Based on both morphological and genetic analyses, we report *S. murmanum* as a newly recorded species for the fauna of Kazakhstan. Specimens were collected from two

distinct locations. The first site (49°10′48″ N, 85°33′37″ E) was a shallow mountain stream approximately 1.5–2 m wide, characterized by clear, fast-flowing water. Pupae were found attached to submerged stones, while larvae were collected from aquatic vegetation. The second site (49°46′35.2″ N, 84°02′39.4″ E) was located on the Bukhtarma River, a large river approximately 400–500 m wide at the collection point. The water here was turbid, and pupae were found on aquatic plants along the riverbank.

In Russia (Murmansk region, Karelia, and the European part), as well as in the USA, Finland, Sweden, Norway, and other European countries, *S. murmanum* has been documented as an active blood-feeding species on both humans and animals [2,26,27]. However, in the north-eastern regions of Kazakhstan, our observations did not confirm any hematophagous activity of this species. Furthermore, a review of the existing literature revealed no reports of either the presence or blood-feeding behavior of *S. murmanum* in neighboring countries such as China or Mongolia, regions that share borders with Kazakhstan. This suggests a potential geographical or ecological limitation of hematophagous behavior within the species' range, or the presence of ecotypes with reduced or absent anthropophilic tendencies in Central Asian populations.

Related species within the *Simulium* genus are established vectors of several pathogens. Most notably, *S. damnosum* and *S. neavei* transmit *Onchocerca volvulus*, the causative agent of onchocerciasis (river blindness), which affects millions in sub-Saharan Africa [28,29]. In addition to filarial nematodes, blackflies have been implicated in the transmission of arboviruses such as vesicular stomatitis virus, Rift Valley fever virus, and other agents of veterinary concern [30,31]. Furthermore, species like *S. ornatum* have been associated with *Onchocerca lienalis*, a parasite of cattle in Europe [32]. These data indicate that members of the *Simuliidae* family can serve as vectors for a broad range of pathogens affecting both humans and animals. Given the discovery of *S. murmanum* in East Kazakhstan and its confirmed hematophagy in other regions, further monitoring is warranted to assess its vector potential in local ecosystems.

As some authors note, *S. murmanum* is associated with high-mountain, low-mountain, and foothill taiga landscapes [7,33]. This species was detected in habitats that match this description, as indicated at points 1 and 2 in Figure 1. The remaining course of the Irtysh River is characterized by flat, forest-steppe terrain, making the presence of this species in those areas unlikely. We assume that the habitat of *S. murmanum* in north-eastern Kazakhstan is limited to the foothill and mountain-forest massifs of the Southern Altai spurs, with small mountain streams. No data on the presence of this species in other parts of Kazakhstan or further downstream along the Irtysh River have been reported to date. The Tien Shan Mountains could potentially serve as a habitat for this species of midge, but this natural zone belongs to the alpine type, characterized by high mountain systems with alpine meadows and a snow belt. All of this data support the hypothesis that the presence of *S. murmanum* in the southern highlands of Kazakhstan is unlikely. We cannot state with certainty that this species of midge inhabits only the mountainous regions of the East Kazakhstan area, as our study sites were located along the Irtysh River, which flows from south to north through eastern Kazakhstan. The fauna of Western Kazakhstan remains insufficiently studied. However, it is highly likely that this species may also occur in adjacent regions, including the mountainous areas of the Russian Altai, China (Xinjiang Uyghur Autonomous Region), and western Mongolia. *S. murmanum* has not been previously recorded in these regions [1].

This hypothesis may apply to individual non-plastic blackfly species, as the blackfly fauna of the East Kazakhstan region also includes species that exhibit a high degree of ecological plasticity and resilience to changes in abiotic factors, such as *S. reptans*. In our earlier work, we reported the discovery, in 2023, of a new blackfly species for the fauna

of Kazakhstan—*S. reptans* in the Irtysh River (Pavlodar Region). This species was first found in the main channel of the Irtysh River near the border with Russia. In 2024, we expanded our survey upstream along the Irtysh River, reaching the border with China, where the river originates. The study confirmed the presence of *S. reptans* along the entire length of the Irtysh River from its origin in the East Kazakhstan region (near the Chinese border) to its outflow into Russia. *S. reptans* was also found in small numbers in mountain streams, at the foothill outlets (e.g., Bukhtarma River), and throughout the entire river course. As previously established, *S. reptans* is the dominant active bloodsucking species in the Pavlodar Region. However, during the collection period in the East Kazakhstan region, the species did not exhibit hematophagous behavior. We have thus identified the habitat of *S. reptans* as extending throughout the entire Irtysh River, covering the whole of north-eastern Kazakhstan.

Our findings regarding the ecological plasticity of *S. reptans* along the Irtysh River corridor are consistent with patterns observed in other blackfly species across various biogeographic regions. For instance, Figueiró et al. (2015) demonstrated significant phenotypic plasticity in *S. subpallidum* Lutz, 1910 populations in response to local environmental conditions in the Brazilian Cerrado, with morphological traits such as labral fans varying according to flow velocity and substrate type [31]. Similarly, studies of *S. noelleri* Friederichs, 1920 have shown that blackfly larvae adaptively modulate morphological and life-history traits depending on food availability and hydrodynamic constraints [32]. McCreddie et al. (2024) also documented latitude-dependent shifts in blood-feeding behavior and voltinism across North American Simuliidae, emphasizing the influence of climatic and geographic gradients on ecological strategies [33]. Furthermore, vector studies in Nigeria have shown that species such as *S. squamosum* (Enderlein, 1921) may shift host preferences based on local host availability [34], indicating behavioral flexibility. These examples support our observations that *S. reptans*, while actively hematophagous in the Pavlodar Region, did not exhibit such behavior during the collection period in East Kazakhstan. This behavioral variation, combined with the species' widespread distribution across diverse habitats, from highland streams to large river channels, highlights its ecological adaptability and suggests that abiotic and biotic factors, such as temperature, hydrology, and host presence, may modulate its feeding behavior and population dynamics within different parts of its range.

The climate and landscapes of Kazakhstan are highly diverse, ranging from deserts and arid regions to mountainous areas and forest-steppe plains. This diversity creates favorable conditions for the existence and development of various species of midges, including some that are endemic to Kazakhstan. In this context, further study of the *Simuliidae* fauna is necessary, as well as determining the boundaries of their distribution within the country.

5. Conclusions

S. murmanum (Enderlein, 1935) was recorded for the first time in north-eastern Kazakhstan. The species was identified by both morphological characteristics and molecular genetic methods. No blood-feeding activity of females has been observed within the studied territory. The species appears to be non-anthropophilic under current ecological conditions. This midge develops in mountain and low-mountain streams of the Irtysh River basin, particularly in fast- and slow-flowing waters of the Altai foothills. A notable feature of *S. murmanum* is the variation in the pupal cocoon structure depending on water flow velocity. Its distribution is associated with low water temperatures and specific bottom substrate types.

S. reptans (Linnaeus, 1758) is widespread throughout the Irtysh River in Kazakhstan, from mountainous regions to steppe plains. Its larvae demonstrate high ecological plasticity, thriving in areas with elevated water temperatures and diverse bottom substrates. This

adaptability contributes to its dominance in various aquatic habitats. Females of *S. reptans* are active blood feeders and have been recorded attacking humans and livestock in northern Kazakhstan. Their role as potential vectors makes the species of epidemiological interest, particularly in agricultural zones and rural communities near river systems.

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Abbreviations

The following abbreviations are used in this manuscript:

bp	base pairs
cox1	cytochrome c oxidase subunit 1
DNA	deoxyribonucleic acid
F	Forward
ML	Maximum Likelihood
R	Revers
TBE	Tris-borate-EDTA buffer
USA	United States of America

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