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Spring fauna of blackflies *(Diptera, Simuliidae)* of Syrdaria river middle reaches

Abstract: Starting from summer 2012, in the valley of the middle reaches of the Syrdaria river, from the Shardara reservoir to the level of the village of Shaulder, South Kazakhstan region, in some years to the railway station of the Tomenaryk, Kyzylorda region, as well as on the territories adjoining the valley massive attacks of blackflies (*Diptera, Simuliidae*) on people and agricultural animals are observed. This led to a number of economic, sanitary, epidemiological and social problems: it became difficult to conduct field agricultural work, grazing; cases of seeking medical assistance from persons who have been bitten by blackflies become more frequent; part of the population expresses the intention to move to another, more prosperous places. From March 31 to April 4, 2015 we conducted route studies from Shardara to the village of Shaulder in order to get familiar with the natural conditions of area being studied and obtain preliminary data on the species of attacking insects. From dry stems of plants growing in shallow water, from sunken snags, tree trunks, and other objects accidentally caught in water, about 800 cocoons and pupal exuvial pelts of the genus *Boophthora* were collected with about 200 mature pupae, more than 1000 cocoons and exuvia, one mature larva of the genus *Wilhelmia* and 1♂ of the same genus. Processing of the material made it possible to define them as *Boophthora erythrocephala De Geer* and *Wilhelmia turgaica Rubzov*. Probably, females of the same species attack in summer. Collection and processing of the material was carried out according to the well-known methods developed by I.A Rubcov (1956) and others. The insects were dissected using a stereomicroscope DM 143 with the Motic Images Plus 2.0 software, with x10 and x20 magnifications. Identification was carried out under a Leica DML B2 microscope with a digital camera Leica DFC 320 with magnifications x60 and x90. The above-mentioned species of *Simuliidae* were known from the valley of the middle reaches of the Syrdaria кiver in the 1970s and 1980s, but their numbers were low and the attacks were not of a mass nature. Current mass breeding of blackflies is linked with the changed, after the commissioning of the Koksarai water reservoir-counter regulator in 2011, hydrological regime of Syrdaria. Studies do not answer the question of specific factors favoring the mass reproduction of blackflies in the middle reaches of Syrdaria and point to the need for further detailed studies on the biology and ecology of blackflies in the Syrdaria Valley in order to develop measures to reduce the number of blackflies in this region.

Key words: Syrdaria river, Koksarai reservoir, *Diptera*, *Simuliidae*, spring fauna, massive attacks of blackflies.

Introduction

Blackflies (*Simuliidae*) are included in the group of bloodsucking dipterous insects, in the russian-language scientific literature united under the name "gnus". Preimaginal phases of blackflies develop in flowing, oxygen-rich water – from shallow streams to large rivers. Representatives of this family occur on all continents, with the exception of Antarctica, and most of the oceanic islands. According to the latest data, there are more than 2,200 species of blackflies in the world [1]. Females for ripening eggs need additional protein nutrition in the form of blood, which is obtained by attacking large vertebrates, including

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humans. They are specific or mechanical carriers of pathogens of a number of vector-borne diseases. Besides, injected saliva into the wound during blood-sucking, cause allergic reactions, with numerous bites develops a specific toxic-allergic disease known as "simulidotoksikoz" [2]. In connection with the above circumstances, representatives of the family are examined from a variety of positions and for different purposes: inventorying and studying the bioremediation of blackflies of a certain territory [3-5] spatial and biotopic species distribution [6], life cycles of mass economically important species [7], the impact of economic development of territories, changes in the hydrological regime of water courses and pollution on biodiversity and the habitat of blackflies [8]. Sometimes in the course of such studies, new species of blackflies are found for science [9].

In the past in Kazakhstan, blackflies had significance as ectoparasites of humans and agricultural animals mainly in mountainous areas. However, from the 60-70s of the XX century, after regulation of the runoff most rivers through the construction of reservoirs, they act as an important component of the "gnus" in the valleys of lowland rivers. This phenomenon was particularly clearly manifested in the Irtysh valley, where a cascade of reservoirs was built. In connection with the mass attacks of blackflies on people in the area of Pavlodar, measures were taken to reduce the number of blackflies in the Irtysh valley [10-12].

In spring of 2015, in connection with numerous complaints from the population of the Shardara and Otyrar districts, South Kazakhstan, on attack of *Simuliidae*, with an initiative of the regional administration the research group was established to study the situation on the place. The study carried reconnaissance character. In this paper, we present the results of the processing of materials collected during a routine survey conducted in 2015.

Materials and methods

Collection of material was conducted from March 31 to April 4, 2015 in the Syrdaria valley from the dam of the Shardara reservoir to the administrative boundary of the Kyzylorda region. The distance between the extreme points of selection sampling was, in a straight line, about 300 km. Study of the preimaginal phases of blackflies development was conducted at 13 points (Figure 1).

The geographical coordinates of selection sampling points were determined using the GPS navigator GARMIN GPSMAP 62s (Taiwan).

Point 1. Filtration streams in the floodplain of the river at the foot of the dam of the Shardara reservoir (Figure 2, A). Geographical coordinates: 41o14'15"/67o52'30". Water seeping through the body of the dam forms streams with a depth up to 20 cm, width up to 1 m. Before entering the river, the streams merge into one channel with a width of 2.0-2.5 meters. In streams are developed the vegetation. The aquatic phases of the development of blackflies were not detected, the attacks of imago were not observed.

Point 2. Syrdaria river in the vicinity of. Kosseit (Sunrise), left coast (Figure 2, B). Coordinates: 41°25'56"/68°05'09". Collected 549 cocoons with exuviae of departed blackflies and empty cocoons attached to dry, submerged stem of reed in water. Attacks of imago were not observed.

Point 3. Syrdaria river at the crossing of the main gas pipeline across the river, the left coast. Coordinates: 41°39'37"/68°02'20". Collected 592 cocoons with exuviae of departed blackflies and empty cocoons, 74 pupae attached to dry, submerged stem of reed in water. Attacks of imago were not observed.

Point 4. Syrdaria river in the vicinity of. Sutkent, at the confluence of the drainage collector into the river. Coordinates: 41°59'13.7"/68°09'56.2". Collected 569 cocoons with exuviae of departed blackflies and empty cocoons, 141 pupae, 1 mature larva of the 4th age with dry, submerged stem of reed and sunken branches. Attacks of imago were not observed.

Point 5. The floodplain of Syrdaria river in the vicinity of Sutkent, drainage collector (Figure 2, C). Coordinates: 41°59'42.5"/68°03'44.3". The aquatic phases of development of blackflies were not detected, attacks of imago were not observed.

Point 6. Syrdaria river, at the beginning of Koksaray, the right coast. Coordinates: 42°06'12.3"/68°12'52.7". The stones in the spillway were examined. The aquatic phases of blackflies development were not detected, attacks of imago were not observed.

Point 7. Syrdaria river, the channel 2 km below Koksaray, left coast. Coordinates: 42°07'36.5"/68°12'45.8". Various objects were examined immersed in water. The aquatic phases of blackflies development were not detected, attacks of imago were not observed.

Point 8. Syrdaria river in the vicinity of. Akkum, the left coast. The water level is high. Coordinates: 42°23'33.3"/68°14'41.5". Wrecked branches and stems of herbaceous plants, brought by the current, branches of willows were examined in shallow water. The aquatic phases of blackflies development were not detected, attacks of imago were not observed.

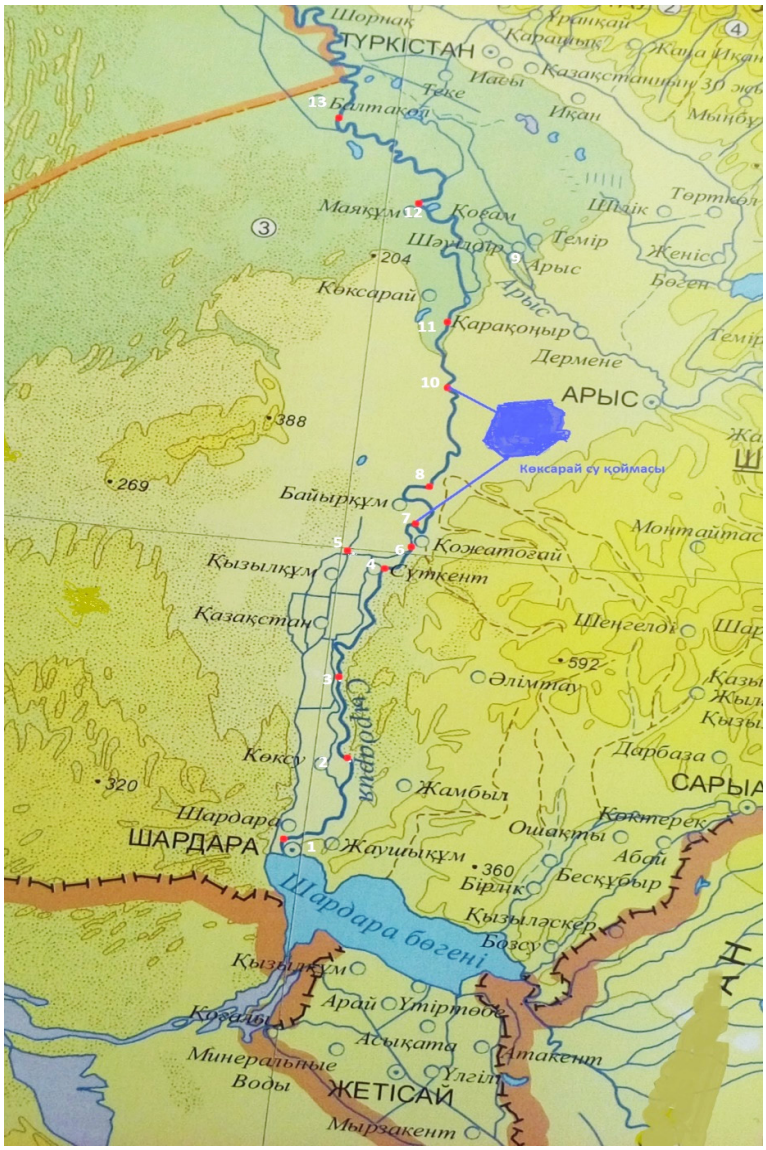


Figure 1 – Map of the valley of Syrdaria river within the South-Kazakhstan region with the designation of sampling points.
The names and coordinates of the points are indicated in the text

Point 9. Arys river, the right tributary of the Syrdaria, near the village of Shaulder. Coordinates: 42°46’45.5”/68°22’20.5”. The stems of coastal bushes were examined at the time of survey flooded with high water. The aquatic phases of blackflies development were not detected, attacks of imago were not observed.

Point 10. Syrdaria river at the confluence of the diverting channel from Koksarai. The water level is high. Coordinates: 42°24’21.3”/68°22’10.3”. The branches of willows were examined in shallow water, plant stems brought by the current. The aquatic phases of blackflies development were not detected, attacks of imago were not observed.

Point 11. Syrdaria river at the bridge in the area of Koksaray. The water level is high. Coordinates: 42^{0}35’15.3”/68^{0}14’30.5”. Wrecked branches and snags were examined, plant stems brought by the current. The aquatic phases of blackflies development were not detected, attacks of imago were not observed.

Point 12. Syrdaria river in the vicinity of Mayakum. The water level is high. Coordinates: 42°52’23.6”/68°05’24.2”. Sunken snags and plant stems were examined, brought by the current. The aquatic phases of blackflies development were not detected, attacks of imago were not observed.

a b c

Figure 2 – Some of the sampling points: filtration stream at the foot of the dam of Shardara reservoir (A), shore near Kosseit village (B) and drainage collector near Sutkent village (C)

Point 13. Syrdaria at the bridge in the village of Baltakol. The water level is high. Coordinates: 43º09'32.0"/67º51'65.8". Sunken snags and plant stems were examined, brought by the current. The aquatic phases of blackflies development were not detected. In the air, one adult male blackfly is caught.

In total, 1710 cocoons, left after the gab of adult, 215 mature, close-to-fly pupae, one mature larva of IV age were collected, and one male is caught in the air. The processing of the material showed that in the collection 2 species of blackflies are present: *B. erythrocephala* (779 empty cocoons) and *W. turgaica* (931 empty cocoons, 215 pupae, 1 larva, 1 imago).

Collection and processing of the material were conducted according to well-known methods [13]. To study the aquatic phases of blackflies, we collected dry stems of herbaceous plants, sunken branches, stones in shallow water, as well as other objects found in the water. Most part of collected material was placed in 96% ethanol for fixation. Part of the pupae was placed in large chemical tubes with filter paper to grow to the adult stage. Primary cameral processing of the collected material: purification from debris, counting of insects in each sample, clarification of accompanying labels was conducted in the laboratory of epizootology and prevention of especially dangerous infections of the Chimkent anti-plague station. After the cameral processing, the material was transferred to 70% ethanol. Production of microscopic preparations and identification of the species was conducted at the Department of Biophysics and Biomedicine, al-Farabi Kazakh National University.

Species belonging to insects were determined by the available determinants of blackflies of the fauna of the CIS and Russia [14]. All material collected during the expedition is stored at the Department of Biophysics and Biomedicine, al-Farabi Kazakh National University. Primary cameral processing of the material was conducted using a binocular magnifier MBS-10 manufactured by JSC "LOMO" with magnifications x6 and x10.

Dissection of insects and production of temporary micro-preparations was conducted using a stereomicroscope DM 143 (Motic, China), with the software Motic, Images Plus 2.0, with x2, x6 and x10 magnifications; the determination was conducted under a Leica DML B2 microscope (Germany) with a digital camera Leica DFC 320 with magnifications x60 and x90.

To determine the species temporary micro-preparations were prepared. Cocoons and pupae exuvia after 3-fold washing in 70% ethanol were consequently transferred to Petri dishes in a mixture of glycerin with alcohol, to a glass with drop of glycerin, covered with a slide glass, considered under magnifications of x2, x6, x20. Mature pupae with formed chitin structures of imago were fixed with 96% ethanol, dried and placed in a solution of potassium hydroxide for 24 hours. After that, they were washed in distilled water and transferred to glass with a drop of glycerin and cut according to a known scheme. The prepared parts of the head, wings, and sexual appendages were placed, in a drop of clove oil, and then transferred to glass in a drop of glycerin, covered with a slide glass. In this form, the prepared parts of the insect body were studied under a light microscope.

Results and discussion

A part of the middle and lower reaches of Syrdaria, one of the largest rivers of Central Asia, runs through Kazakhstan. In the valley of the Syrdaria,

as well as other rivers of Central Asia, bloodsucking dipterous insects have been numerous at all times.

In 1967, the construction of Shardarinskaya HPP was completed in the middle reaches of Syrdaria, in 1968 have finished the filling of the same reservoir with a capacity of 5 km^3. Before the construction of a reservoir in this part of the Syrdaria Valley, bloodsucking dipterous insects have not been studied. According to conducted studies in the mid-1970s in the lower reaches of the river in the Kyzylorda region, utmost importance among the components of "gnus" had mosquitoes (*Culicidae*) and horseflies (*Tabanidae*), single attacks of *Simuliidae* on domestic animals were observed [16]. At that time, from this part of the valley a new bloodsucking species of blackflies *Sulcicnephya syrdarienzis Rubzov* was described [17]. These data presents a certain value, to study the *Simuliidae* fauna of Syrdaria in general, but they do not fully reflect the situation in the middle reaches of the river.

In 1986 and 1987, we conducted faunistic studies in the middle reaches of the river from the Shardara reservoir to the village of Shaulder. 6 blackfly species were identified, including one new for science *Psilocnetha almae Yankovsky et Koshkymbaev* [18; 19]. By number *Wilhelmia turgaica Rubz.* dominated by the preimaginal phases of development, collected in the river and by imago. Females of this species attack mainly large domestic animals (horses, donkeys, cattle) getting to suck the blood in their ears [20]. They do not generally attack humans, although, within large numbers they can circle around people. The aquatic phases of *Boophthora erythrocephala De Geer* accounted for 3 to 5% of all collected larvae and pupae, and no imago was caught around human. At the same time, the aquatic phases of *Psilocnetha almae Yankovsky et Koshkymbaev*, accounted for 6-8% of the collected larvae and pupae and up to 10% of the imago was caught around human. Preimaginal phases of development of other species were found only in small streams below the dam of the Shardara reservoir and the overall pattern of the attack of *Simuliidae* in the Syrdaria valley did not exert any significant influence. Thus, the picture was very different from the one we observed in the spring of 2015.

The current mass reproduction and attacks of blackflies on the local population is associated with the construction and operation of the Koksarai reservoir-counter regulator. The reservoir is located on the right coast of the river, in the Otyrar district of the region in South Kazakhstan region, designed to solve a number of important economic and environmental problems, such as: protection of locality and economic objects located below the Shardara reservoir, including the regional center of Kyzylorda, from winter floods; uniform water supply in summer on irrigated fields of South Kazakhstan and Kyzylorda region; increase entrance of water into the lower reaches of the river and into the Small Aral. However, such a massive breeding of blackflies in the valley was not expected and it is not yet clear how the erection of the Koksarai reservoir contributed to the reproduction of representatives of this family of dipterous insects. The reservoir was partially filled in the winter of 2009-2010 and 2010-2011, and in the winter of 2011-2012 it was filled till the project capacity of 3 km^3.

For the first time, massive attacks of *Simuliidae* on people and domestic animals were noted in the river valley and adjacent territories in the summer of 2012. In subsequent years, attacks were repeated with some increase or decrease the intensity of attacks. This led to a number of economic, sanitary and epidemiological, social problems: it became difficult to conduct field agricultural work, grazing; cases of seeking medical assistance from persons who have been bitten by *Simuliidae* have become more frequent; a part of the population expresses the intention to move to another, more prosperous places [21].

We previously planned to study 5 types of potential habitats of breeding blackflies: the main channel and the major channels of the Syrdaria river; filtration streams below the dam of the Shardara reservoir; The Kyzylkum main canal and channels of the second-third order supply water directly to the fields; drainage collectors taking away ground and return-irrigation water from the fields of Kyzylkum irrigation array. In the filtration streams, despite the most thorough searches, the aquatic phases of blackflies were not found. The water began to flow into the main canal and channels of the second-third order 4-5 days before the beginning of our investigations, before that they remained dry, respectively, in them the aquatic phases of the blackflies could not be detected. The drainage collector functions throughout the year, but the water level in it is subject to considerable fluctuations. During our research, the level of water was high, the stems of large waterbirds and aquatic plants (cane, cattail, reed lake) that could serve as a substrate for the aquatic phases of *Simuliidae* were found at great depths and were practically inaccessible for survey. Thus, full-scale collections of larvae and pupae were made only in the river channel.

The Koksarai reservoir itself, as well as the inflow and outflow canals, cannot serve as biotopes for

the reproduction of blackflies: the reservoir is due to the lack of directional flow, the conditions necessary for the habitat of preimaginal phases of the blackflies, the canals, due to short-term functioning.

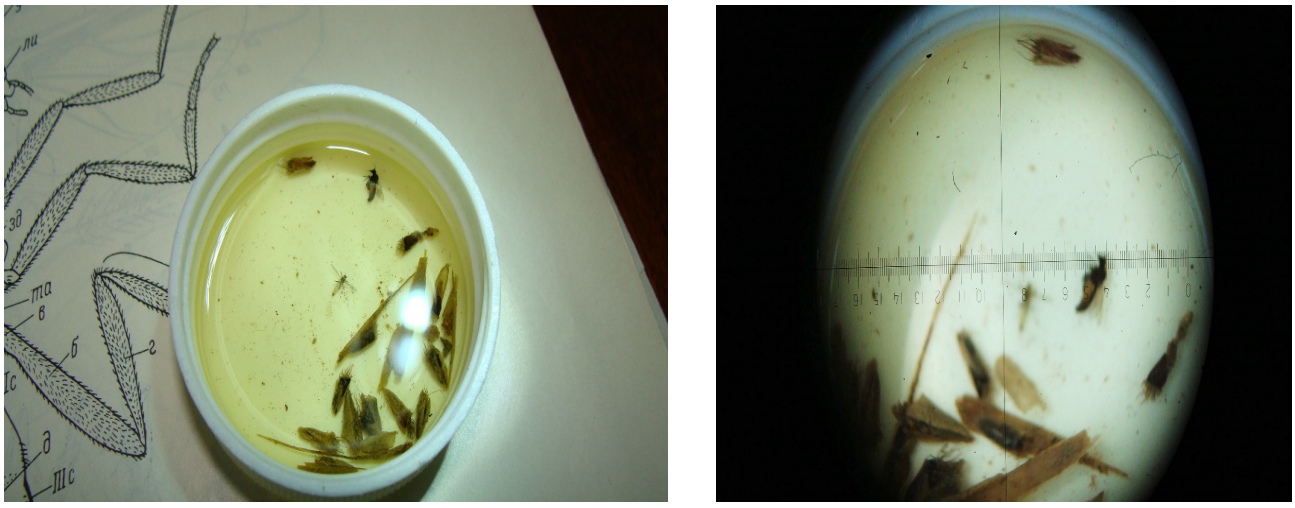
The inflow canal functions during the winter-spring period when the reservoir is filled, the outflow channel functions in the summer time for 2.5-3 months.

In autumn the reservoir is completely emptied and is preparing for re-filling. Consequently, the main mass of blackflies is melted in the Syrdaria river and its ducts. Some number of blackflies can produce artificial channels supplying water to the fields of the Kyzylkum irrigation array and channels of the drainage-collector system, but their significance as biotopes of the reproduction of the blackflies is difficult to estimate. The channel of Syrdaria can be divided into three segments, differing in the hydrological regime and according to their suitability for settling the aquatic phases of blackflies: the first section – from the Shardara reservoir to the water intake dam near the village Bayırkum; the second section – from the Bayyrkum dam to the mouth of the outflow channel when it flows into Syrdaria; the third is the river below the confluence of the outflow channel.

As can be seen from the study materials, the aquatic phases of blackflies were found only on the first stretch of the river – from Shardara to Bayırkum (points No. 2, 3, 4). In this case, in point No. 2 are collected only empty cocoons and pupae exuvia, in point No. 3 in 11.1% of the collected cocoons were mature pupae, in point No. 4 – in 19.8% – mature pupae, and was also found one larva of IV age. All found pupae and larvae belonged to *W. turgaica Rubz*., another species – *B.erythrocephala De Geer* had time to fly completely.

In spring of 2015 in the middle reaches of Syrdaria the found species of blackflies are defined as *B.erythrocephala De Geer* and *W. turgaica Rubz*. These species have been noted here and before [16; 18]. *B.erythrocephala* females actively attack both humans and domestic animals, while *W. turgaica* attack mainly large domestic animals (horses, donkeys, cattle) getting to suck the blood into their ears [20].

The collected material shows that local populations of these species hibernate in the phase of the larva, their pupation and emergence take place in the II – III decade of March. By this time *B. erythrocephala* managed to fly out completely – left empty cocoons and puffed skins- exuvia. Gab of *W. turgaica* finished in the southern, adjoining the reservoir are part of river channel (point No. 2) and continued in the more northern part of the length (points No. 3 and 4).



a b

Figure 3 – Pupae of *W.turgaica* in general view (A) and under x6 magnification (B)

During our research, there was not any attack of blackflies because of the unusually cold weather for this season of the year: the unstable rainy weather that has been standing since the beginning of the III decade of March has passed into a snowfall from 27 to 29 March, 2015. Then frosts followed up to -12°C [22]. This led to the death of those who had time to fly out of the adult. The imago of spring generation

of these species under more favorable weather conditions can attack people and domestic animals, but due to low numbers should not cause much concern. In our opinion, the contribution of insects of spring generation to the settling of aquatic phase of blackflies by existing waterways are more important, including artificial channels, into which water began to flow in the end of March-beginning of April. By the beginning of May in canals, as well as in the river itself, near-water and water vegetation are rapidly developing serving as a substratum for laying eggs and attaching larvae of blackflies, which greatly extends the area of biotopes of reproduction of blackflies. The presence of other bloodsucking species of *Simuliidae*, whose outbreaks of quantities have been observed in the past in the valley of Syrdaria should also be kept in mind. This years of their high numbers that the new species *Sulcicnephya syrdarienzis Rubzov* [17] and *Psilocnetha almae Yankovsky et Koshkymbaev* [19] were found and described.

Conclusion

Our study showed that in the middle reaches of the Syrdaria river, two species of blackflies – *Wilhelmia turgaica* and *Boophthora erythrocephala* – hibernate in the phase of the larva. By the beginning of April all the wintering individuals of *Boophthora erythrocephala* had time to fly out, flight of *Wilhelmia turgaica* still continued. These species were observed in valley of Syrdaria and before, but their numbers were insignificant and they did not show high aggression towards humans. It is known that female of *Wilhelmia turgaica* usually do not attack a person, although they can circle around people. Another species – *Boophthora erythrocephala* is known as a malicious bloodsucker attacking both animals and humans. Probably females of this species make main part of the insects attacking humans in summer periods. The spring of 2015 was anomalously cold for this season of year – the temperature decreased to -12.8^{0}C. In such conditions, all adults of blackflies, flying before cold snap are died. This can be explaint the absence of attacks of blackflies during research period. But, part of the flight females had time to lay eggs. Thus, the populations of these species plummeted into numbers, but were not completely destroyed. According to data received from settlements located in the valley of Syrdaria. in summer of 2015 there were no attacks of blackflies, in summer of 2016 there were isolated single attacks, and in summer of 2017 the number of attacking blackflies was again high, populations of these species regained their numbers. The absence of specialist-entomologists, who conduct account attacking blackflies to people does not allow us to judge about the number of insects more objectively. Judging by our collections, the highest density of overwintered blackfly was recorded in the channel of the Syrdaria, at a distance from a little below the dam of the Shardara reservoir to the water intake dam of the Koksarai reservoir. This does not exclude the reproduction of blackflies in other parts of the river, as well as in artificial channels: the Kyzylkum main irrigation canal and canals of II-III orders, drainage reservoirs. At the same time, the inflow and outflow canals of the Koksarai reservoir are of little use to inhabit the aquatic phases of blackflies, because of the short duration of their functioning. In order to clarify all the conditions that facilitate the mass reproduction of blackflies in the middle reaches of Syrdaria Valley, detailed studies on the ecology and biology of blackflies should be carried out, taking into account the hydrological regime of the river and the climatic features of each year.

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