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RESEARCH ARTICLE

Morphological and molecular identification of *Anopheles* (Diptera: Culicidae) mosquitoes in Surkhandarya region, Uzbekistan

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Abstract

Mosquitoes of the genus *Anopheles* Meigen, 1818 are representatives of blood-sucking insects from the family Culicidae. Some representatives of this genus carry malaria and distribute through humans. In Uzbekistan six species of malaria distributors have been recorded so far. Thus, investigating such kind of malaria distributors and determining the exact number of *Anopheles* species in order to prevent malaria epidemy in the republic is crucial. In this study we tried to study the species composition and ecology of mosquitoes of the genus *Anopheles* in Surkhandarya region and described the species characteristics of mosquitoes, both in terms of morphological and molecular genetic features. To reach our aim we collected samples of the genus *Anopheles* and conducted morphological, molecular-genetic and phylogenetic studies. According to results in the sampled collections of *Anopheles* mosquitoes, the ratio of males and females was 1:1. The nucleotide sequence data from the mtDNA of our sampled mosquitos were 99.8-100% similar with those of *An. hyrcanus* Pallas, 1771; *An. pulcherrimus* Theobald, 1902 and *An. superpictus* Grassi, 1899 derived from Genbank. Also, molecular genetic studies of adult mosquitoes from Surkhandarya region confirmed the data of their identification as identified by morphological characters. The sequences of their nucleotides were submitted to the database of the Genbank (NCBI). As a result of morphological studies of imago mosquitoes, it was found that 4 species of mosquitoes of the genus *Anopheles* occur in natural landscapes of Surkhandarya region: *An. claviger* Meigen, 1804; *An. hyrcanus*, *An. pulcherrimus* and *An. superpictus*.

Keywords
Anopheles, adult, COI, DNA, identification, malarial mosquito, morphology, molecular genetics

Introduction

Mosquitoes of the genus *Anopheles* Meigen, 1818 are representatives of blood-sucking insects from the family Culicidae. Currently, there are about 500 species of mosquitoes of the genus *Anopheles* in the world, of which about 100 species are potential carriers of malarial plasmodium in humans in natural conditions (Djakhongirov et al. 2016). In the CIS countries, 22 species of *Anopheles* mosquitoes were found, of which 7 occur in Uzbekistan (Harbach 2013; Djakhongirov et al. 2014, 2016; Fang et al. 2016). *Anopheles* mosquitoes are common in the eastern and Palearctic regions (Alam and Khan 2010; Paredes-Esquivel et al. 2011).

The two main approaches used in species identification are morphological characters and DNA barcoding. The correct identification of mosquito species is important for a better understanding of the relevant bionomic characteristics that influence the composition and distribution of mosquitoes in local settings. Morphological identification is currently the most common and generally effective tool, but can be outdated, controversial, and difficult to explain key points [Lobo, 2015; Laurent et al., 2016; Laurent and Supratman, 2016]. In addition, comprehensive and meticulous preparation is required to achieve proper morphological identification. A modern taxonomy system called "DNA barcode coding" based on molecular methods is becoming more and more popular as it gives very high accuracy and detailed results on unknown and similar species in a relatively short time compared to traditional morphological based taxonomy (Stresman 2010; Sinka 2011; Davidson 2020). Currently, specific genes of the mitochondrial genome are used to analyze differences intra- or inter species. For example, the mitochondrial cytochrome oxidase I (COI) gene sequence has been used as a DNA barcode to distinguish mosquito species (Wang, Li 2012; Gao and Fang 2017). To determine the genetic population structure of *An. hyrcanus* COI (Feng 2017), COII (Yang et al. 2011), and ND5 (Junget al. 2007; Makhawi 2013) regions were used. Mitochondrial genome of *An. sinensis* has a length of 15076 bp and 15138 bp and consists of 13 protein-coding genes, 22 transfer RNA (тRNA) genes, two ribosomal RNA (pRNA) genes, and control regions (Demari-Silva et al. 2015; Chu, Li 2016; Chen, Wang 2017).

In settlements of Uzbekistan according to Sh.M. Djakhongirova, A.B. Zvantsova, I.I. Goryacheva et al. (2014c) 6 species of adults of malarial mosquitoes were recorded: *Anopheles artemievi* Gordeev et al., 2005; *An. claviger* Meigen, 1804; *An. hyrcanus* Pallas, 1771; *An. martinius* Shingarev, 1926; *Cellia pulcherrimus* Theobald, 1902 and *C. superpictus* Grassi, 1899. Of these, *An. superpictus* is the dominant species (49.4±0.5%), *An. artemievi* is subdominant (37.7±0.3%); *An. pulcherrimus* is less numerous; *An. martinius*, *An. hyrcanus* and *An. claviger* is rare. Active vectors

of malaria in Uzbekistan in the XX century were *An. superpictus*, *An. pulcherrimus* and *An. maculipennis* (now called *An. artemievi* Gordeev et al., 2005).

An. superpictus in the 1950s was recorded only in the southwestern regions of Kyrgyzstan, and their larvae developed in groundwater rich in calcium salts (Monchadsky 1951). Later and today, this species has become widespread in all mountainous and foothill regions of Uzbekistan as dominant in the flat territories of the landscape of Surkhandarya region (Djakhongirov et al. 2014a). The maximum number of mosquitoes is observed in August and early September. *An. superpictus* is widespread in all mountainous and foothill areas. In Surkhandarya and Kashkadarya valleys, it inhabits not only the foothills and mountains, but also the plains. The maximum number is observed in August – early September (Zhakhongirov et al. 2014b).

An. pulcherrimus is distributed in flat river valleys. It occurs in the floodplain of the lower reaches of Sirdarya, Zarafshan, Surkhandarya and Amudarya rivers (Djakhongirov et al. 2014v). This species has not been recorded only in Kashkadarya physiographic region. The number of this species of mosquitoes is growing very slowly. They are characterized by the fact that the first mosquitoes are found at the end of June.

An. hyrcanus was previously found in Central Asia and the Far East (Monchadsky 1951; Gutsevich 1976). Now the species is widely distributed in Kazakhstan, Kyrgyzstan, Tajikistan and Uzbekistan. In recent years, due to the risk of flight of *Anopheles* mosquitoes from Afghanistan, a border state with unfavorable environmental conditions, the risk of malaria spread among the population in the south of our country remains high. Accordingly, malaria as a disease still remains one of the serious problems among the population, especially in the south of the republic (Ezhov et al. 2004; Gordeev et al. 2004b; Djakhongirov 2016).

On the territory of Surkhandarya region, the study of the ranges and prevalence of mosquitoes of the genus *Anopheles* and their significance in the spread of malaria is of great practical importance. Naturally, an adequate study of bioecology, identification of species by morphological and molecular genetic methods is of theoretical and practical importance.

The purpose of this work is to study the species composition and ecology of mosquitoes of the genus *Anopheles* in Surkhandarya region, to describe the species characteristics of mosquitoes, both in terms of morphological and molecular genetic features.

Materials and methods

Collection of entomological material

Mosquito samples for research were collected in the settlements of Surkhandarya region located near rice fields and in livestock farms during May–October, 2019–

2022 with setting the coordinates of collection sites (Fig. 1, 2, 3; Table 1). The total number of *Anopheles* mosquitoes collected was 301 individuals, of which 158 were males and 143 were females.

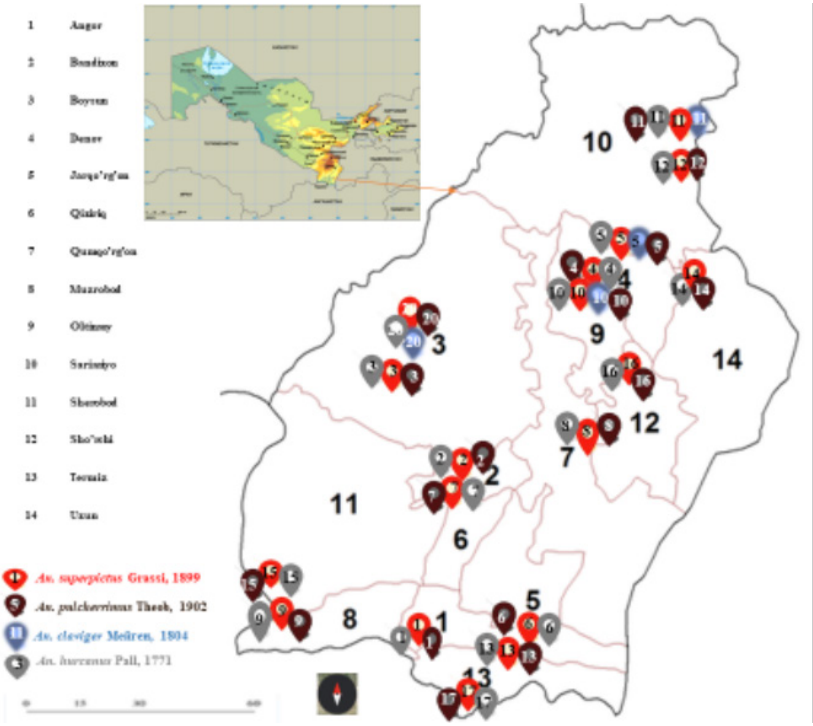

Figure 1. Map of distribution and collection sites of mosquitoes of the genus *Anopheles* in Surkhandarya region of Uzbekistan (2019–2022).

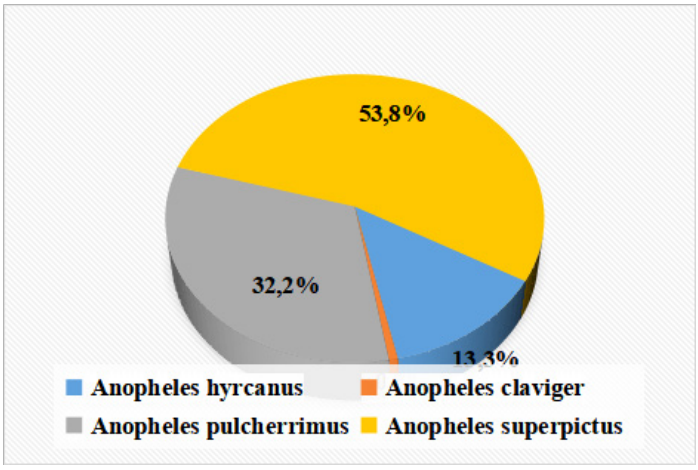

Figure 2. The ratio of species of mosquitoes of the genus *Anopheles* in Surkhandarya region (2019–2022).

Table 1. Species composition of mosquitoes of the genus *Anopheles* and coordinates of their collection sites in Surkhandarya region (2019–2022)

№	Coordinates, height, date of collection point	Total, ind.	%	Including by type, %			
				An. hyrcanus	*An. claviger*	*An. pulcherrimus*	*An. superpictus*
1	Navshakhar, Angor district; 37°39'06"N, 67°07'54"E; 195 m, 05.26.2019	15	5.0	13.3	-	40.0	46.7
2	Kaldirgach, Bandykhan district; 37°79'43"N, 67°43'16"E; 531 m, 06.04.2020	20	6.6	15.0		25.0	60.0
3	Sairob, Baysun district; 38°07'21"N, 66°97'26"E; 74 m, 06.11.2020	18	6.0	16.7		38.9	44.4
4	Dunyatepa, Denau district; 38°01'41"N, 67°86'23"E; 374 m, 07.26.2021	16	5.3	12.5		37.5	50.0
5	Burizharsay, Denau district; 38°17'34"N, 67°84'40"E; 133 m, 07.27.2021	16	5.3	6.7	6.7	3.7	86.7
6	Three lake, Dzharkurgan district; 37°34'17"N, 67°46'18"E; 96 m, 05.30.2019	17	5.7	5.9		29.4	64.7
7	Bustan, Kizirik district; 37°74'22"N, 67°13'79"E; 212 m, 06.05.2019	18	6.0	5.6		11.1	83.3
8	Gulistan, Kumkurgan district;37°73'60"N, 67°62'34"E; 213 m, 21.08.2022	22	7.3	9.1		31.8	59.1
9	Shurab, Muzrabad district; 37°40'04"N, 67°04'72"E; 90 m, 08.15.2019	20	6.6	15.0		40.0	45.0
10	Dagrez, Altynsay district; 38°19'36"N, 67°77'92"E; 374 m, 07.14.2022	20	6.6	10.0		35.0	55.0
11	Chakar, Sariasia district; 38°38'80"N, 68°08'86"E; 140 m, 06.18.2022	14	4.7	14.3		35.7	50.0
12	Forestry, Sariasia district; 38°40'10"N, 67°95'74"E; 218 m, 06.19.2022	12	4.0	27.3	9.1	25.0	45.5
13	Manguzar, Termez district; 37°24'67"N, 67°33'20"E; 162 m, 07.20.2020	24	8.0	20.8		37.5	41.7
14	Serharakat, Uzun district; 38°27'76"N, 67°99'41"E; 535 m, 06.17.2022	15	5.0	20.0		33.3	46.7
15	Istiklal, Sherabad district; 37°49'39"N, 66°77'97"E; 1429 m, 10.16.2022	14	4.6	7.2		57.1	35.7
16	Azad, Shurchinsky district; 38°01'41"N, 67°86'23"E; 374 m, 07.12.2022	17	5.7	17.7		35.3	47.1
17	Alpamysh, city of Termez; 37°23'84"N, 67°28'97"E; 85 m, 06.10.2020	23	7.6	13.1		30.4	56.5
	Total	301	100.0	13.3	0.7	32.2	53.8

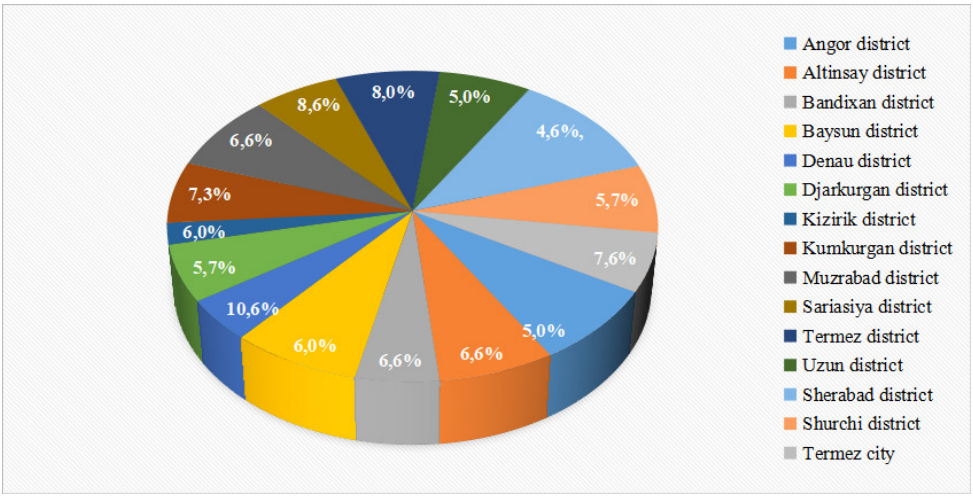

Figure 3. Diagram of the occurrence of species of mosquitoes of the genus *Anopheles* in the districts of the Surkhandarya region (2019–2022).

For mitochondrial DNA belonging to subunit 1 amplification F-LCO-1460: GGYCAACAAAAATCATAAGATATTGG and R-HCO-2198: TAAACTCAGGGG-GACCAAAAAAATCA primers were used.

The PCR was carried out using a programmable automatic chain reaction amplifier (Touchgene Gradient, UK) (PCR). PCR was carried out according to the following scheme: stage 1 – DNA denaturation at 95°C for 3 minutes, stage 2 – DNA denaturation at 93°C for 20 seconds, stage 3 - adhesion of primers in DNA at 55°C for 30 seconds, stage 4 – elongation at 72°C for 2 minutes, stage 5 – elongation of the chain at 72°C for 10 minutes. From the second to the fourth stage, the process was repeated up to 35 times in a cycle.

To determine the presence of DNA in the samples, electrophoresis was performed at a voltage of 100 volts in a 2% agarose gel; after 40–45 minutes, the gel was examined and photographed in the beams of a transilluminator with recording of the results.

To purify the DNA samples, the desired DNA fragments obtained by electrophoresis were excised from the gel with a scalpel and placed in a 1.5 ml eppindorph tube. When extracting DNA from the gel, a set of reagents manufactured by “Sileks M” (Moscow, Russia) was used in accordance with the manufacturer's instructions.

The DNA content of the purified PCR products was measured and sent to sequencing. Sequencing was carried out at the center of the Central Collective Use Center "Genome" ("Gentotex", Moscow).

Sequence data were obtained in "ab1" format and analyzed using "Chromas version 1.45" (McCarthy, 1996–1998). In order to correct errors in the data obtained from sequencing, the results processed using the correct and reverse primers

were converted to the FASTA format. It was then implemented using the program "Clustal X version 1.81" (Thompson, Gibson 2000) to combine the results of two chromatographs. Unnecessary nucleotides are removed using the Gendoc version 2.5.000 program (Nicholas 1999). To convert to the Nexus format, "ForCon version 1.0 for Windows" (Raes, Van de Peer 1996) was used.

To build a phylogenetic tree, DNA sequences were obtained from *Anopheles* samples, which were compared with the nucleotide sequences of NCBI database https://www.ncbi.nlm.nih.gov/), edited using the program Generous prime, while the consensus sequences were calculated using the Mega X computational program.

The obtained nucleotide sequences of the ITS and COI domains were used to build a phylogenetic tree utilizing IQ-TREE version 1.6.12 by maximum likelihood (ML) method (1000 iterations), and analyzed in CIPRES Science Gateway V3.3. *Armigeres subalbatus* (KJ768190) was used as an outgroup for consensus tree. The resulting phylogenetic tree was analyzed and edited using the iTOL v6.6 program.

Results and discussion

Morphological studies

In the collections and studies of mosquitoes in Surkhandarya region for 2019-2022, distribution sites of 4 species of *Anopheles* mosquitoes have been identified: *An. hyrcanus* Pallas, 1771, *An. claviger* Meigen, 1804, *An. pulcherrimus* Theobald, 1902 and *An. superpictus* Grassi, 1899.

An. hyrcanus and *An. claviger* belong to the subgenus *Anopheles*, the main distinguishing features of their adults are the color of the costal vein along the anterior margin of the wing. The wings of these species have up to two light spots, sometimes the spots are dark or not visible. Hypopygium, first valvular segment at base with one or three large setae, one seta situated on tubercle.

An. superpictus and *An. pulcherrimus* belong to the subgenus *Cellia* and the main distinguishing features of their adults are the color of the costal vein along the leading edge of the wing. The presence of four white spots on the front of the wing for these two species is a distinguishing feature from other mosquitoes.

Below, brief descriptions of the distinctive morphological features of the species are provided.

1. An. hyrcanus

The "reed malarial mosquito" is distinguished by white spots along the costal vein, on the anterior margin of the wing (Fig. 4a).

Gonotrophic development. Egg (embryo) 3-6 days. Larva 5-7 days. Pupa 2-10 days. Imago 1-2 months. A full cycle takes 10-23 days. Fertility up to 500 eggs.

Size. Eggs 0.6-0.8 mm, larvae 1.0 mm, adults 6-8 mm, wings 2.4-2.5 mm, proboscis 2.5-2.6 mm, antennae 1.1-1.3 mm.

Morphology. Mesoscutum brown, divided in middle part by dark stripes into two or four or more narrow gray stripes (Fig. 4b). Wings are mostly covered with dark scales; along the anterior margin of the wing on the costal vein, they have two large white spots, the first of which occupies the veins: costal, first radial, the second - from costal to second radial (Fig. 4c). The proboscis and tentacles are dark brown, the tentacles of females have three white rings and a white apex. Antennae of females are dark brown, covered with white fluff, with long brown hairs at the base; first 5-7 segments of antennae bear some amount of white scales. The tarsi are dark brown, with white rings at the apex of the first three or four segments (Fig. 4d). Hypopygium: 1st segment with 2 setae at base. The tenth tergite has long narrow outgrowths. For the class pettah wide plate. For *An. hyrcanus*, a number of forms are described, which are mainly seasonal aberrations (Fig. 4).

Location of two hairs on the first segment of the valves of the hypopygium in *An. hyrcanus* confirms the morphological identity of this species.

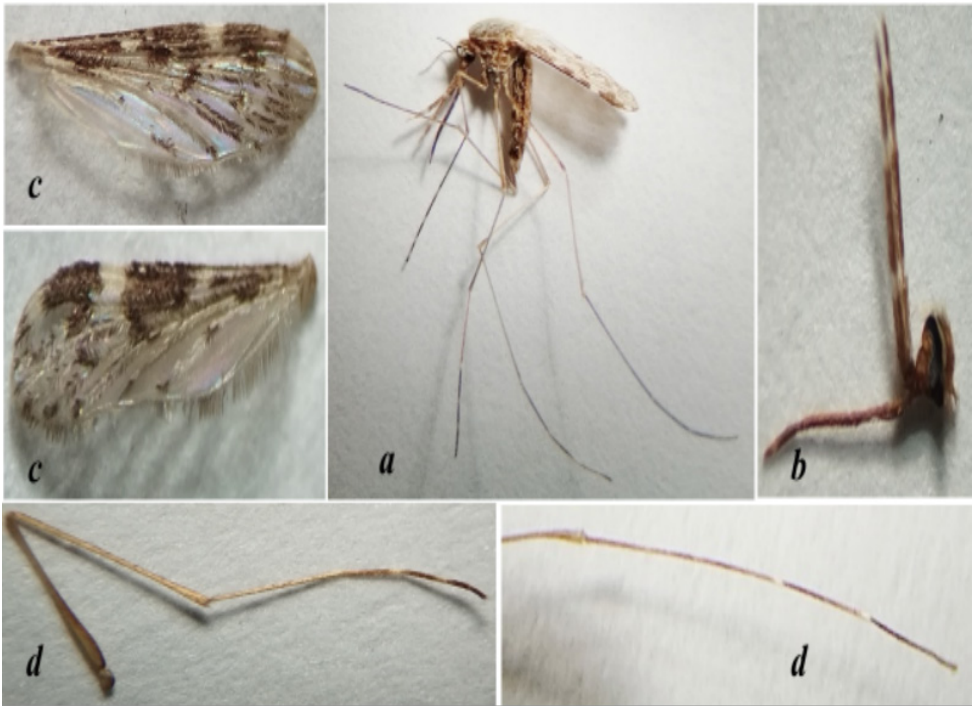

Figure 4. *An. hyrcanus*: a – general view; b – head (proboscis, palps, antennae); c – wings; d – hind limb.

2. A. claviger

"Spring malarial mosquito", formerly *An. bifurcatus* (Linn). No spots on the wings.

Gonotrophic development. Egg (embryo) from 40 hours to 8 days. Larva 5-30 days. Pupa 2-2.5 days. Imago 1-2 months. A full cycle takes 10-40 days. Fertility 100-500 eggs.

Size. Eggs 0.6-0.8 mm, larvae 1.0 mm, adults 6-7 mm, wings 2.0-2.2 mm, proboscis 2.1-2.2 mm, antennae 1.1-1.3 mm.

Morphology. The general coloration is yellowish (in the south) or light chocolate (in the north) (Fig. 5a). On the forehead there is a bunch of white scales and hairs directed forward (Fig. 5b). Mesoscutum brown with whitish-gray longitudinal stripe. Wings without spots (5c). Tarsi uniformly brown (5d). Hypopygium of males: at the base of the first valvular segment, there are three large setae, of which two lateral setae are contiguous at their bases and branched. The inner one, located closer to the aedeagus, sits on the tubercle and is not branched (Fig. 5).

Mosquito *An. claviger* large is distinguished by a whitish-gray longitudinal stripe on the meconium, without spots on the wings.

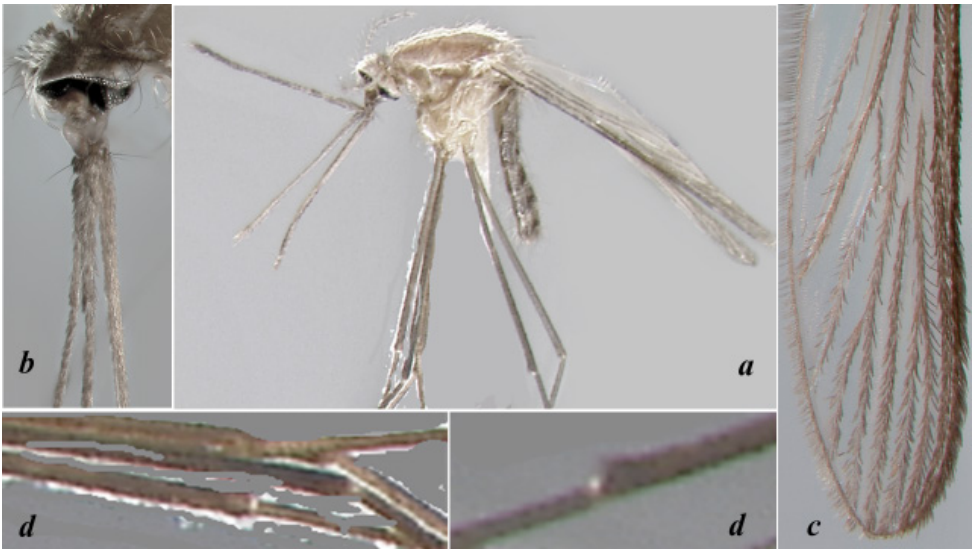

Figure 5. *An. claviger*: a – general view; b – head (proboscis, palps, antennae); c – wings; d – hind limb.

3. An. pulcherrimus

"White malarial mosquito" – the body as a whole is whitish-gray-yellowish. The head is covered with white scales. Between a pair of eyes there is a tuft covered with white hairs.

Gonotrophic development. Egg (embryo) 3-6 days. Larva 5-7 days. Pupa 2-2.5 days. Imago 1-2 months. A full cycle takes 10-20 days. Fertility 100-500 eggs.

Size. Eggs 0.6-0.8 mm, larvae 1.0 mm, adults 5-6 mm, wings 2.0-2.1 mm, proboscis 2.1-2.2 mm, antennae 1.1-1.2 mm, the length of the antennae in females is 1.2-1.3 mm and 1.1-1.2 mm in males.

Morphology. *An. pulcherrimus* is the most beautiful species of mosquito, covered with white and golden scales (Fig. 6a). The head is covered on the sides with

light brown, and above with pure white protruding scales; on the forehead between a pair of eyes there is a bunch of white hairs directed forward (Fig. 6b). The head and scutellum are covered with white fluffy long hairs. Shupik on head with four pale stripes. Antennae of females are dark brown, covered with short white and fluffy scales, each segment bearing a rosette of long white hairs. The ends are brown. Antenna segments I, VII, and VIII are additionally covered with pure white scales. Antennae of males are brown with long golden hairs. The chest is covered with scales. The middle part of the chest is covered with gray scales. The proboscis is covered with light brownish adjoining scales. Abdomen covered with broad light scales with protruding tufts of dark scales on the posterior lateral surface. Wings on anterior margin with four dark areas and six black spots (6c). There are two dotted spots at the base of the wing, and black spots are also scattered over the entire surface of the wing. Tarsi with white rings, front ones covered with white scales, hind legs purple with wide rings (6d).

In males, five hairs are located at the base of the first pair of hypopygium. The location of five hairs at the base of the first pair of hypopygium valves confirms that this species is *Anopheles pulcherrimus* (Fig. 6).

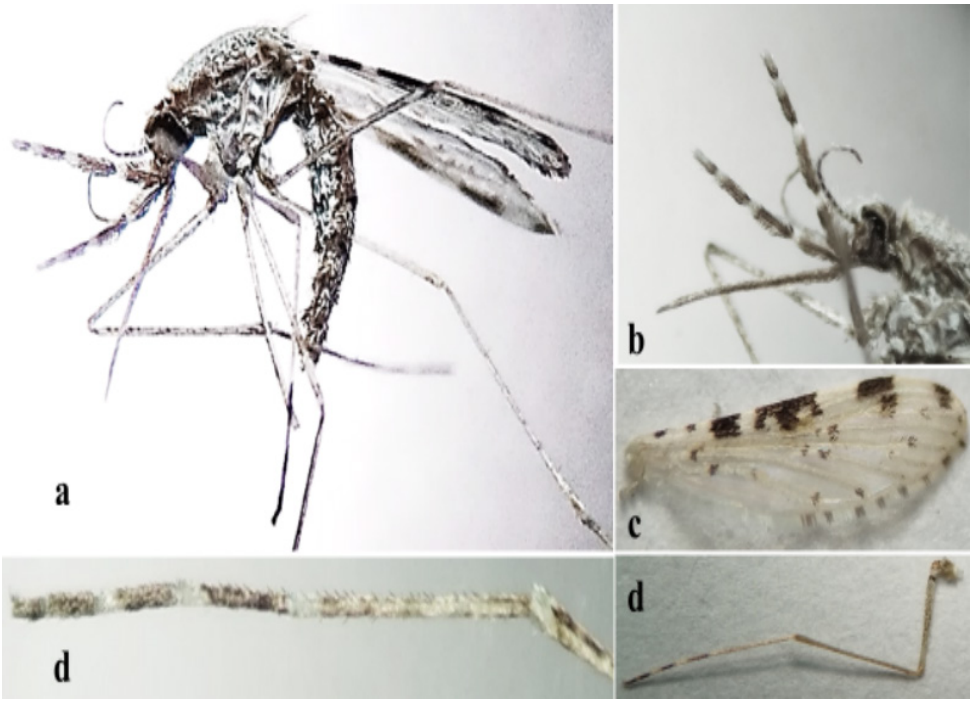

Figure 6. *An. pulcherrimus*: a – general view; b – head (proboscis, palps, antennae); c – wings; d – hind limb.

4. An. superpictus

"The Painted Malaria Mosquito". Body color is grey-yellow. On the sides of the head is covered with brown scales, and in the upper part white.

Gonotrophic development. Egg (embryo) 3-6 days. Larva 5-7 days. Pupa 2-2.5 days. Imago 1-2 months. A full cycle takes 12-13 days. Fertility 100-500 eggs.

Morphology. Egg body size 0.6-0.8 mm, larva 1.0 mm, adults 5-6 mm, wings 3.5-4.5 mm, proboscis 2.1-2.2 mm, antennae 1.2-1 .3 mm.

An. superpictus is gray or gray-green (Fig. 7a). The head is golden yellow with well-defined dark brown spots, with brown scales on the sides and with white protruding scales on top (Fig. 7b). Antennae dark brown, apex whitish with three white spots and with rosettes of brown hairs, shorter by half the length of the head and almost straight. The proboscis is long, thin, with a light apex. The palps are black, with sharp white rings and a white apex. Thorax pinnate, mesonotum light brown, slightly darker laterally, covered with sparse light brown hairs, with narrow whitish scales densely covering meconium, barrels with a grayish tinge light brown.

The abdomen is brown, covered with long delicate light yellowish hairs, not covered with scales. The anterior margin of the wings has four white spots covered with white scales in the basal part of the costal vein; the intermediate sections of the anterior margin of the wing are covered with black-brown scales on the common stem r1+r3, in the basal part (Fig. 7c). The legs are dark brown, at the junction of the thigh and lower leg. (Fig.7d). Legs have narrow white rings. The hind tarsi are uniform brown with light rings (Fig. 7).

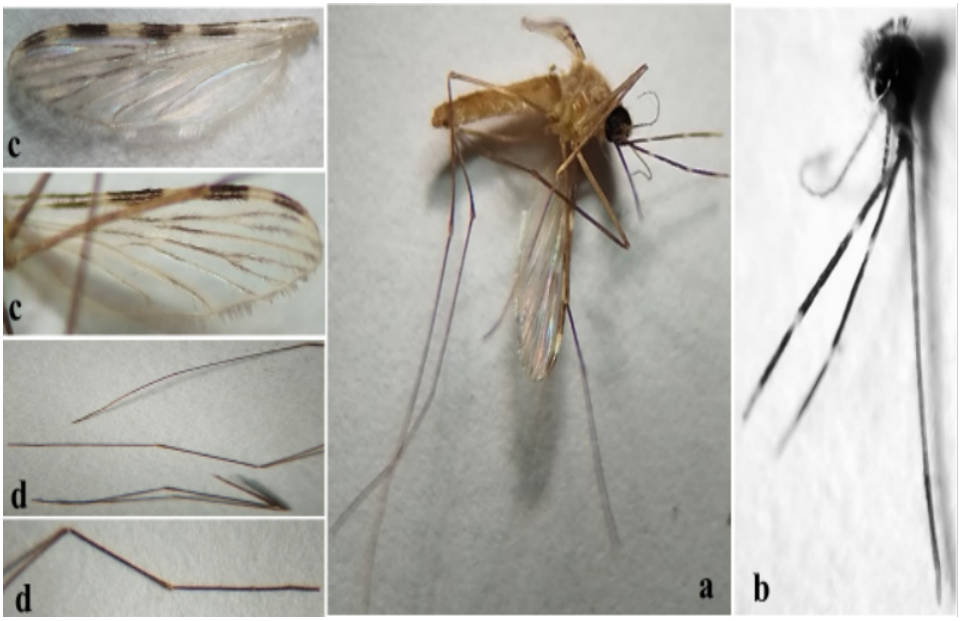

Figure 7. *An. superpictus*: a – general view; b – head (proboscis, palps, antennae); c – wings; d – hind limb.

The arrangement of five strong setae at the base of the hypopygium confirms that this species is *An. superpictus*.

According to the results of the morphological studies of mosquitoes collected in Surkhandarya region, it was found that 4 species of malarial mosquitoes of the genus *Anopheles* of the Palearctic dominion live in the studied region: *An. hyrcanus*, *An. claviger*, *An. pulcherrimus* and *An. superpictus*. Additional molecular genetic studies were carried out to confirm their species.

Molecular studies have shown that in the mtDNA COI of species *An. hyrcanus*, *An. pulcherrimus* and *An. superpictus*, 653 base pairs of nucleotide sequences were isolated. The resulting nucleotide sequences were closest to *An. hyrcanus* (KU743225), *An. pulcherrimus* (JX255710) and the sequence was compared with *An. superpictus* (JX255708) (Fig. 8).

An. hyrcanus sequence was obtained from a database with a type (Genbank, NCBI). Similarities between *An. hyrcanus* (KU743225) was 99.85%. Between them there was a replacement of a single nucleotide, that is, 575th nucleotide position T-thymine changed to C-cytosine.

The next round was with *An. pulcherrimus*. Similarities between *An. pulcherrimus* and *An. pulcherrimus* (JX255710) based on Genbank was 99.85%. It was also found that 1 nucleotide was swapped between these species, which was explained by the fact that it was swapped between T-thymine and C-cytosine at 341th nucleotide position.

Similarity between *An. superpictus* and *An. superpictus* (JX255708) was 99.85%. The difference between them was also a single nucleotide. This difference was eliminated by the exchange of T-thymine nucleotides for C-cytosine in the 397th nucleotide position.

So, all three studied species were confirmed by data from the *Anopheles* mosquito Genbank database, and their similarity was 99.85%.

The difference among nucleotides between *An. hyrcanus* and *An. pulcherrimus* was 10.8%, *An. hyrcanus* and *An. superpictus* was 11.2%, *An. pulcherrimus* and *An. superpictus* was 9.9%.

Phylogenetic analysis. The phylogenetic tree of *Anopheles* mosquitoes studied by us and obtained from the GenBank, is shown in Figure 9.

Based on the mtDNA COI sequence in the phylogenetic tree, *Anopheles* species have been identified as monophyletic clusters. Regardless of where they were collected, all individuals of the same species are cohesive in a group close to each other. Studies have identified *An. superpictus* and *An. pulcherrimus* with 100%, *An. hyrcanus* with 97% index occupy their place in the phylogenetic tree. However, differences in the nucleotide sequence were found in the samples of *An. superpictus* and *An. pulcherrimus*. In studies, a certain species of *An. hyrcanus* and pseudopictus species (MT993487; FJ210896) obtained from the GenBank of Great Britain and Greece, nucleotide sequences between clusters were identified (Fig. 9).

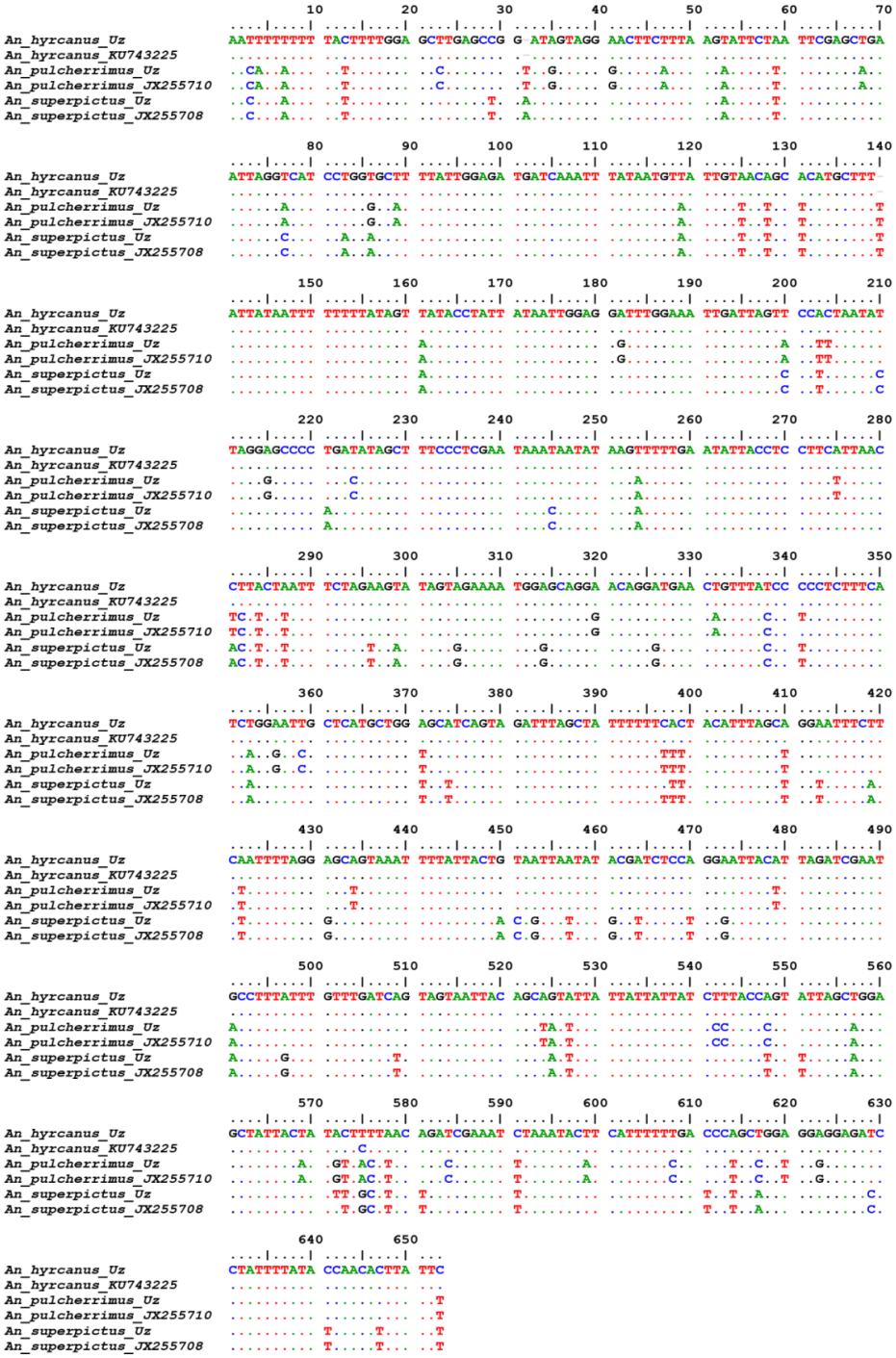

Figure 8. The mtDNA COI domain of the genus *Anopheles* is a partial comparison of nucleotide sequences (domain defined in the 5' to 3' direction, with the same nucleotide bases as dots).

Eventually, studies in the regions of Surkhandarya region of Uzbekistan gave a morphological and molecular genetic description of mosquitoes of the genus *Anopheles*. Based on the morphological description, 4 species were identified, of which 3 species were confirmed in accordance with mtDNA COI regions.

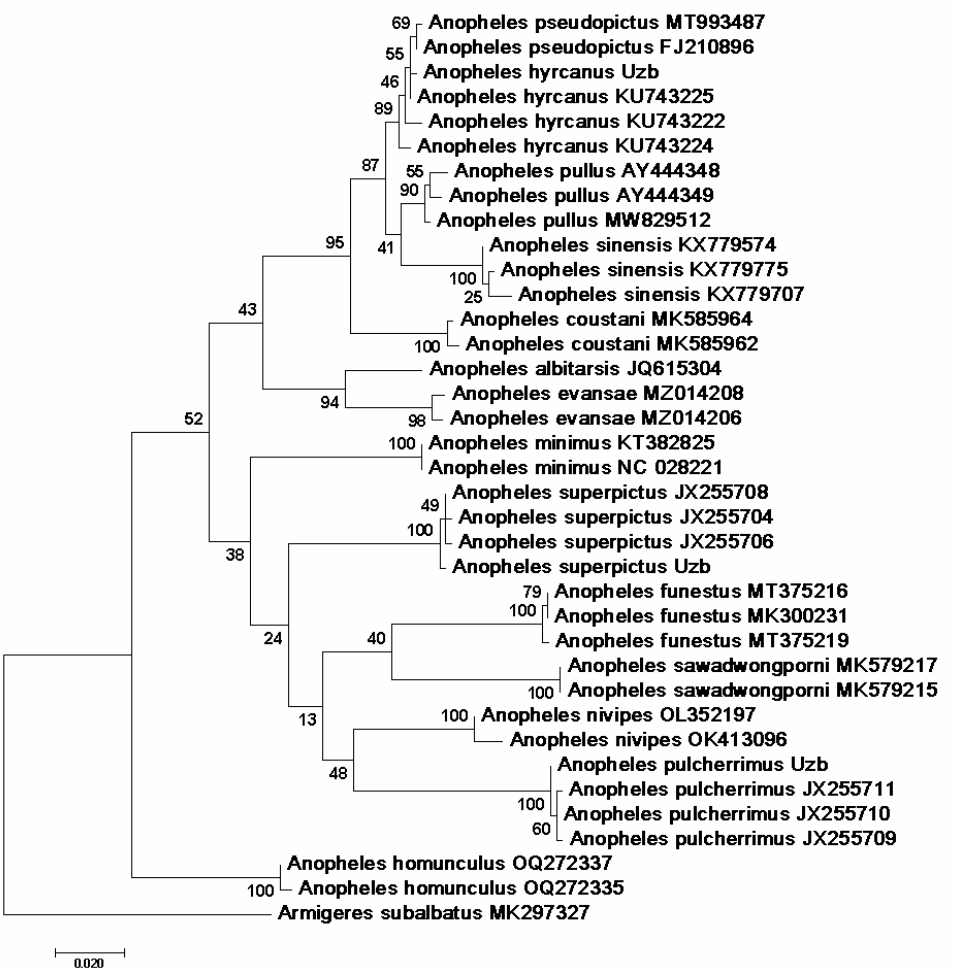

Figure 9. Phylogenetic tree created using the method of ML the genus *Anopheles* based on the mtDNA COI gene. Bootstrap numbers are given in corresponding branches (500 bootstrap repetitions).

Conclusion

In various natural landscapes of Surkhandarya region of Uzbekistan, 4 mosquito's species belonging to the genus *Anopheles* are common: *An. hyrcanus*, *An. claviger*, *An. pulcherrimus* and *An. superpictus*, to the family Culicidae of the order Diptera and confirmed by the results of morphological studies. The ratio of male and female

mosquitoes of the genus *Anopheles* is 1:1. Molecular genetic analysis of 3 species was carried out: *An. hyrcanus*, *An. pulcherrimus* and *An. superpictus*. The nucleotide sequence similarities of the mtDNA COI domain of the selected 3 mosquito species, when compared the obtained samples from Genbank, were 99.8-100%. The results of morphological and molecular genetic analysis show the wide distribution of *An. hyrcanus*, *An. pulcherrimus* and *An. superpictus* in Surkhandarya region. It is believed that these species of mosquitoes are of epidemiological significance as distributors of malarial plasmodium in the south of the republic. The obtained COI regions of 3 mosquito species in this study were placed in NCBI as follows: *An. hyrcanus* KU743225; *An. pulcherrimus* JX255710; *An. superpictus* JX255708.

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