REVIEW OF MOSQUITO (DIPTERA: *CULICIDAE*) SPECIES RECORDS IN THE REPUBLIC OF MOLDOVA

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Rezumat

În lucrare sunt analizate rezultatele cercetărilor recente, şi celor efectuate în anii precedenţi, privind ţînţarii hematofagi răspîndiţi pe teritoriul Republicii Moldova. Lista adnotată a speciilor de ţînţari este completată cu şase specii noi pentru fauna republicii: *Anopheles pseudopictus, Aedes geminus, Culex torrentium, Culiseta longiareolata, Coquillettidia buxtoni şi Uranotaenia unguiculata*. În perioada aa. 1947-2011 în Republica Moldova au fost semnalate 40 specii de ţînţari, 8 dintre care aparţin subfamiliei Anophelinae şi 32 de specii – subfamiliei Culicinae, din 8 genuri: *Aedes, Aedimorphus, Dahliana, Ochlerotatus, Culex, Culiseta, Coquillettidia* şi *Uranotaenia*.

Cuvinte-cheie: Culicidae – ţînţari - specii noi - forme adulte – larve - pupe - populaţii - abundenţa speciilor - vectorul malariei.

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Introduction

Mosquitoes are the most important group of arthropods of medical and veterinary significance, belonging to a single family of Diptera, the *Culicidae*. There are over 3500 species and subspecies of currently known mosquitoes. Mosquitoes are found everywhere in the world where standing water occurs, which is needed for the development of their aquatic immature stages [1]. There are three subfamilies recognized within the family *Culicidae*. The species of importance from standpoint of public health are contained in the subfamilies of *Anophelinae* and *Culicinae*. About 40 species of *Anopheles* are considered by medical entomologists as important malaria vectors in some parts of the world. Many species of *Ochlerotatus* and *Culex* are vectors of arboviruses that infect various vertebrates, including humans. Thus *Culex pipiens* is a vector of Tahyna virus and Sindbis virus in Europe, St. Louis encephalitis virus in North America, and West Nile virus on several continents [13, 14]. Females of species in a third subfamily *Toxorhynchitinae*, lack mouthparts adapted for sucking blood from vertebrates [6]. There are no representatives of *Toxorhynchitinae* in the Republic of Moldova. Eight species of *Anophelinae*, all belonging to the genus *Anopheles* and thirty-two species of *Culicinae* in eight genera: *Aedes*, *Aedimorphus*, *Dahliana*, *Ochlerotatus*, *Culex*, *Culiseta*, *Coquillettidia* and *Uranotaenia* were identified in Moldova [19, 20, 21, 22, 23, 27, 28, 29, 30]. The Moldavian Culicidae together with adjacent areas of Ukraine were first reviewed by Prendel (1947, 1950, 1956, 1962, 1965). Letter there have been appeared several publications which have affected the mosquito faunal list: Sinelshikov (1973), Tihon (1981, 1984), Gutsevich & Dubitskiy (1987), Uspensky et al

(1990, unpublished reports of IZ ASM). Few papers have been dedicated to *Anopheles maculipennis* s.l. and its control due to the malaria outbreaks in the 40-50's in the Republic of Moldova: Satov (1925), Markovich et al. (1949), Sergeeva (1953a, 1953b), Stegnii & Kabanova (1978) [17, 24, 25, 26, 11].

This paper presents the results of recent survey of mosquitoes from the Republic of Moldova with a review of past records from 1947 to 2011. An updated checklist with 6 new species records has been provided. The data for the period from 1986 to 1990 have been published with the permission of Uspenski A.

Material and methods

Since 2008 until 2011, the species composition, and density of mosquitoes have been monitored between early April and middle November at 33 localities along 245 km between natural reserves "Prutul de Jos" and "Padurea Domneasca" in Moldova. Immature and mature mosquito habitats such as floodplains of the Prut and Dniester Rivers, temporary and permanent pools, tree-holes, artificial containers, open pastures, forests, cattle sheds and basements have been surveyed. Larvae and pupae were collected using large or small dippers or pipettes, according to the size and the type of larval habitat. Larval sampling was conducted using standard dipping techniques. Ten to thirty dipper samples, depending on the size of aquatic habitat, were taken at intervals along the edge of each larval habitat [10]. The immature stages were transported to the laboratory in plastic containers and reared to the adult stage. Adult mosquito surveys were conducted using aspirators, test tubes and entomological nets for mosquito catches from human volunteers, vegetation and cattle sheds. Females of the Culex pipiens Complex (*Culex pipiens* biotype *pipiens* (Linnaeus), *Cx. pipiens* biotype *molestus* (Forskal) and *Cx. torrentium* (Martini) collected in the floodplains, forests and open area were identified as *Cx. pipiens s.l./Cx. torrentium*, and females or males collected from the village basements and cattle sheds were identified as *Cx. pipiens s.l.* The eggs of some females *An. maculipennis s.l.* collected in the field were checked on color pattern [1, 2]. Fourth instar larvae and adults were identified to species according to taxonomic keys of Gutsevich et al. (1970), Cranston (1987), Becker (2010) [1, 2, 3]. The siphonal index was used to distinguish the fourth instar larvae *Cx. pipiens* biotype *molestus* from *Cx. pipiens* biotype *pipiens* [15]. The mails of *Aedes vexans* (Meigen) (=*Aedimorphus vexans* sensu Reinert et al., 2009), *Ae. geminus* Peus, *Ochlerotatus geniculatus* Olivier (=*Dahliana geniculata* sensu Reinert et al., 2006), *Oc. annulipes* Meigen, *Oc. caspius* Pallas, *Oc. sticticus* (Meigen), *Cx. modestus* Ficalbi, *Cx. pipiens*, *Cx. theileri* Theobald, *Cx. torrentium*, *Cx. territans* Walker, *Culiseta longiareolata* (Macquart), *Cs. annulata* (Schrank), *Coquillettidia buxtoni* (Edwards), *Cq. richiardii* (Ficalbi) and *Uranotaenia unguiculata* Edwards, were identified to species on diagnostic hypopygial structures at microscope Leica (Germany).

The systematic arrangement of species takes into account the systematic classification used in the reference works of Knight & Stone (1977, 1978), Ward (1992) and Reinert et al. (2006, 2009) [5, 8, 9, 12].

Results and Discussions

During the four-year study a total of 4459 adult mosquitoes, belonging to 34 species or species complexes have been collected in the forests, open area, floodplains, cattle

sheds and basements. *Aedes vexans* was the most abundant species (34.2%), followed by *Cx. pipiens s.l.* (16.7%), *Cx. modestus* (6.6%), *Da. geniculata* (5.8%), *An. maculipennis s.l.* (5.8%), *Cx. pipiens s.l./Cx. torrentium* (4.2%), *Oc. sticticus* (4.1%), *Cq. richiardii* (2.6%), males of *Cx. torrentium* (2.4%), *Oc. annulipes* (2.3%), *Oc. cantans* (2.0%), *Oc. cataphylla* (1.8%), *Oc. excrucians* (1.7%), *An. plumbeus* (0.7%) and number of all other species amounted to (>2%) (Table 1).

Table 1. Recorded mosquito species from 1947 to 2011 in the Republic of Moldova.

Species	Authors			
	Prendel 1947-1965	**Tihon 1973-1981**	**Uspensky 1986-1990**	**Sulesco et al 2008-2011**
Anopheles claviger (Meigen, 1804)	++	++	+	+
An. maculipennis sensu lato Meigen, 1818	++++	+++	+++	[+++]
An. atroparvus Van Thiel, 1927	++	+	-	-
An. maculipennis s.s. Meigen, 1818	++++	?	+++	+++
An. messeae Falleroni, 1926	++++	+	+	-
An. sacharovi Favre, 1903	+	-	+	+
An. plumbeus Stephens, 1826	++	+	+	+
An. hyrcanus (Pallas, 1771)	+++	++	++	+
An. pseudopictus Grassi, 1899*	-	-	-	+
Aedes cinereus Meigen, 1818	+++	+++	+++	+
Ae. geminus Peus, 1970*	-	-	-	+
Aedimorphus vexans (Meigen, 1830)	++++	+++	++++	++++
Dahliana geniculata (Olivier, 1791)	+++	+++	+++	+++
Ochlerotatus refiki (Medschid, 1928)	-	-	(+)	-
Oc. rusticus (Rossi, 1790)	-	-	(+)	-
Oc. diantaeus (H.D.K, 1913)	+	-	-	(+)
Oc. annulipes (Meigen, 1830)	-	-	+	++
Oc. behningi (Martini, 1926)	(+)	-	-	+
Oc. cantans (Meigen, 1818)	+	+	+	++
Oc. caspius (Pallas, 1771)	+++	+++	+++	++
Oc. cataphylla (Dyar, 1916)	++	-	-	++
Oc. communis (De Geer, 1776)	++	++	++	+
Oc. dorsalis (Meigen, 1830)	+	+	+	+
Oc. excrucians (Walker, 1856)	+	-	+	++
Oc. flavescens (Muller, 1764)	+	-	+	+
Oc. mariae (Sergent & Sergent, 1903)	+	-	(+)	-
Oc. pulcritarsis (Rondani, 1872)	+	-	-	+
Oc. punctor (Kirby, 1837)	+	+	+	(+)

Table 1. (continued)

Oc. riparius (Dyar and Knab, 1907)	-	-	+	+
Oc. sticticus (Meigen, 1838)	+	+++	++	+++
Culex modestus Ficalbi, 1889	+++	+++	++	+++
Cx. pipiens s.l. Linnaeus, 1758	++++	++++	++++	++++
Cx. p. biotype *pipiens* Linnaeus, 1758	?	?	?	[+++]
Cx. p. biotype *molestus* Forskal, 1775	[+]	?	?	[++]
Cx. theileri Theobald, 1903	+	+	-	(+)
Cx. torrentium Martini, 1925*	-	-	-	++
Cx. territans Walker, 1856	+	-	+++	++
Culiseta longiareolata (Macquart, 1838)*	-	-	-	+
Cs. alaskaensis (Ludlow, 1906)	+	-	+	-
Cs. alaskaensis indica (Edwards, 1820)	+	-	-	-
Cs. annulata (Schrank, 1776)	++	++	+	+
Coquillettidia buxtoni (Edwards, 1923)*	-	-	-	(+)
Cq. richiardii (Ficalbi, 1889)	+++	++	+	++
Uranotaenia unguiculata Edwards, 1913*	-	-	-	+
No. of species	**30**	**19**	**28**	**34**

Occurrence: ++++ = massive; +++ = abundant; ++ = frequent; + = regularly; (+) = rare; [ ] = not counted in the species lists; ? = uncertain, * = new species records.

Species abundance as well was assessed within the larval populations from 57 breeding sites. A total of ~4000 larval specimens were collected and their identification revealed the presence of 17 species. *Cx. pipiens s.l.* was the most abundant with ~ 52% of all collected larvae, followed by *An. maculipennis s.l.* (12%), *Da. geniculata* (11%), *Cx. torrentium* (8%) and *Cx. modestus* (8%). Larvae of *Ae. vexans*, *Oc. caspius*, *Cx. territans* and *Ur. unguiculata* were relatively abundant in the temporary and permanent pools. *An. sacharovi*, *An. plumbeus*, *An. hyrcanus*, *Ae. geminus*, *Cs. longiareolata*, *Cs. annulata* and *Oc. dorsalis* were less abundant. Only one immature specimen of *Cx. theileri* was found.

The 6 new species records in 6 genera (*Anopheles*, *Aedes*, *Culex*, *Culiseta*, *Coquillettidia* and *Uranotaenia*) were obtained for the Republic of Moldova during our investigations.

Anopheles pseudopictus was first collected in Palanca, VIII-17-2008, near the border with Ukraine as 15 ♀♀ on human baits. This species is very simmilar to *An. hyrcanus* and can be separated by its entirely white tarsomere IV of the hind legs. It has been recorded in Turkey, Iran, Afghanistan, Italy, Danube delta in Romania. Because of its exophilic behaviour *An. pseudopictus* has never been regarded as a dengerous vector of malaria in the Mediterranean region [4, 1, 13].

Aedes geminus was first collected as five 4th instar larvae in the "Codrii Reserve", VII-29-2010 from the temporary ground pools along with 17 larvae *Cx. pipiens*. Larvae were grown up in laboratory till adults. *Ae. geminus* closely resembles *Ae. cinereus* and

can be identified with certainty solely through hypopygial characteristics. The species is recorded with certainty from England, northwestern France, Germany, Poland, Czech Republic and southern Sweden. It has also been identified from the southern and eastern shores of the Baltic Sea [1, 4].

For the first time immature and mature stages of ***Culex torrentium*** were recorded in seven localities: Ghidighici VI-29-2008, Chisinau VII-10-2008, "Codrii Reserve" VII-21-2009, "Padurea Domneasca" Reserve IX-18-2010, Leuseni X-13-2010, Cotul Morii IX-23-2010, Congaz IX-23-2010, using male hypopygial characters. This ornithophilic species is morphologically similar to *Cx. pipiens* and there was a high degree of association between these taxa in the breeding sites. In northern Europe Ockelbo virus (Alphavirus) has been isolated from *Cx. pipiens/Cx. torrentium.* In the laboratory the vector competence of *Cx. torrentium* was much higher than that of *Cx. pipiens* [1, 4, 14].

Culiseta longiareolata was first collected as two 4th instar larvae in Anenii Noi, IX-08-2008 and Ceadir-Lunga, X-03-2010 as 25 larvae and 5 pupae in the artificial containers together with larvae *Cx. pipiens*. The females of *Cs. longiareolata* rarely bite humans and appear to be ornithophilic, and are regarded as vectors of blood parasites in birds [1]. *Cs. longiareolata* is a vector for brucellosis, avian influenza and West Nile encephalitis [18].

Today *Cs. longiareolata* is distributed in Albania, Azores, Botswana, Bulgaria, Canary islands, Croatia, Cyprus, Djibouti, Egypt, southern England, Ethiopia, France as far north as Paris, Greece, Hungary, India, Iran, Iraq, Israel, Italy, Jordan, Lebanon, Lesotho, Madeira, Mauritania, Morocco, Namibia, Pakistan, Portugal, Romania, Russia, Slovakia, Somalia, South Africa, Spain, Sudan, Switzerland, Syria, Tajikistan, Tunisia, Turkey, Ukraine and the lower Volga area as far as the northern slopes of Caucasus as well as in Yemen [4, 1, 7].

Coquillettidia buxtoni was first captured as 1♂ from vegetation in Hincesti, VI-21-2011. This species has limited distributional range and rarity; the data on the biology of the species are scanty. *Cq. buxtoni* is distributed in Mediterranean subregion of the Palaearctic. In Europe the species is present in Spain, France, and Italy, and also reported from Romania and Ukraine [1, 4].

Uranotaenia unguiculata was first sampled as 1♀, 1♂ in Hincesti, IX-23-2010, from the coastal vegetation of the lake. Subsequently 4♀ and 40 larvae *Ur. unguiculata* were collected from Leuseni X-13-2010, Iabloana IX-18-2010, Chisinau IX-29-2010, Cotul Morii IX-23-2010, Congaz IX-23-2010 and Danceni VII-07-2011. *Ur. unguiculata* is a frequent species throughout the Mediterranean region. In Europe, its distribution range stretches as far north as Germany [7]. In Eastern Europe the species can be found in the southern Ukraine and the Volga delta with further occurrence in middle and southwest Asia to Iran and Pakistan [1, 4].

Conclusions

1. Altogether 40 mosquito species records: eight species of *Anophelinae* and thirty-two species of *Culicinae* in eight genera: *Aedes, Aedimorphus, Dahliana, Ochlerotatus, Culex, Culiseta, Coquillettidia* and *Uranotaenia* have been obtained between 1947 and 2011 in the Republic of Moldova.

2. During our investigations 34 mosquito species have been identified in Moldova from 2008 to 2011. *Ae. vexans* and *Cx. pipiens s.l.* are the massive species; *Da. geniculata, Oc. sticticus* and *Cx. modestus* are the abundant species; *An. maculipennis s. l., Oc. annulipes, Oc. cantans, Oc. caspius, Oc. cataphylla, Oc. excrucians, Cx. territans* and *Cq. richiardii* - frequent species; *Anopheles claviger, An. hyrcanus, An. sacharovi, An. plumbeus, An. pseudopictus, Aedes cinereus, Ae. geminus, Oc. behningi, Oc. communis, Oc. dorsalis, Oc. flavescens, Oc. pulcritarsis, Oc. riparius, Cx. torrentium, Cs. longiareolata, Cs. annulata* and *U. unguiculata* - regularly species; *Oc. diantaeus, Oc. punctor, Cx. theileri* and *Cq. buxtoni* - rare species.

3. Species *An. messeae, An. hyrcanus* and *Ae. cinereus* were massive or abundant in the past and *An. claviger, Oc. communis* and *Cs. annulata* were frequent for our region. Recent faunal and ecological surveys revealed the reduction in their distribution and abundance due to mosquito control in the past and anthropogenic pressure.

4. For the first time 6 new species records in 6 genera (*Anopheles, Aedes, Culex, Culiseta, Coquillettidia* and *Uranotaenia*) were obtained for the Republic of Moldova during our investigations: *Anopheles pseudopictus, Aedes geminus, Culex torrentium, Culiseta longiareolata, Coquillettidia buxtoni* and *Uranotaenia unguiculata*.

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