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## Mosquito (Diptera: Culicidae) fauna of the Velyka Dobron' Game Reserve (West Ukraine) with new distribution data and medical risk assessment

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**Abstract:** The distribution of mosquito species in the Transcarpathian part of Ukraine is poorly studied. Here we present the results of a four-year survey carried out in the Velyka Dobron' Game Reserve, and provide the actualized checklist of the Transcarpathian Culicidae fauna containing 32 species. Four of the sampled species have not been formerly known in Transcarpathia: *Anopheles hyrcanus*, *Ochlerotatus cataphylla*, *Ochlerotatus nigrinus*, *Uranotaenia unguiculata*. Based on quantitative composition of studied assemblage, vector potential of species and distribution of transmitted pathogens we evaluate the medical and veterinary risk caused by mosquitoes in Transcarpathian region.

**Keywords:** Ukraine, vector, infection risk, *Anopheles*, *Ochlerotatus*

### 1. Introduction

Mosquitoes are known as an important group of aquatic macroinvertebrates. They belong to the most essential elements in both water and terrestrial food webs (Leopoldo, 2008). Their larvae are present in almost every aquatic habitat from clear to heavily polluted waters, while the habitat preference of different species strongly differs. Due to these characteristics, they are one of the most important and sensible indicators of aquatic environment and are generally used in the ecological water quality classification (Paine and Graufin, 1956).

The survey of mosquito fauna has great emphasis in the planning, supervision, and estimation of possible effects of mosquito control and in the assertion of claims of tourism (Szabó, 2007a, 2007b). An important aspect of mosquito control is the maximization of effectiveness with the minimization of environmental damages. The key precondition is the mapping of breeding sites and the revealing of the quantitative and qualitative composition of local mosquito assemblages (Tóth, 2003).

Beyond human annoyance and painful bites, mosquitoes are important vectors of many human and animal diseases; thus their faunistic examination is important in public health. Results of monitoring can be used in the early detection and prevention against serious

epidemics. Mosquitoes can transmit different pathogens like bacteria (e.g. *Rickettsia* sp.), viruses (e.g. West Nile virus), protozoans (e.g. *Plasmodium* sp.), and nematodes (e.g. *Dirofilaria* sp.) (Gazzavi-Rogozina et al., 2017; Pavlichenko et al., 2017). One of the most commonly known diseases transmitted by mosquitoes is malaria, which was common in Ukraine even in the first half of the 20th century (Leonard, 1959). Presently, the occurrence of malaria is showing an increasing trend in Ukraine and throughout Europe due to the climate change and migration processes. In Ukraine, 10% of malaria patients die. To solve this problem, in addition to the development of medical systems, extensive entomological studies are also necessary. To reduce the risk of spreading malaria and other mosquito-borne diseases, regional monitoring of the species composition of the vector organisms and their habitats are required (Pavlichenko et al., 2017).

Our knowledge of mosquito fauna in different parts of Ukraine shows spatial differences. The mosquito fauna of some main cities (Kiev, Kharkiv, Odessa, Zhytomyr) and protected natural areas (e.g., Kaniv Nature Reserve and Pyryatyn National Nature Park) are well known due to recent investigations (Kilochytska and Stetsenko, 2015; Kilochytska and Kilochytskiy, 2017; Kilochytska, 2012, 2014; Yasynska and Korzh, 2013; Levyskyi, 2016; Gazzavi-

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Rogozina et al., 2017). Nevertheless, the distribution of mosquito species in West Ukraine is almost unknown, especially considering the area of Transcarpathia. The last known checklist of mosquitoes in this region was published more than 20 years ago (Sheremet, 1998).

Although mosquito assemblages in the Hungarian part of the Bereg Plain is well examined (Szabó, 2007b; Szabó et al., 2011), but our knowledge of Ukrainian Transcarpathia, however, is rather poor. The Velyka Dobron' Game Reserve is located on the Transcarpathian lowlands in the Ukrainian part of the Bereg Plain. Our goals were to collect actual data from this region, provide the first record of mosquito fauna in the Velyka Dobron' Game Reserve, and create a new actualized checklist of Transcarpathia.

## 2. Materials and methods

### 2.1. Methods of data collection

Our investigations were carried out between 2014 and 2017. During this period 79 samplings were made in the summer and autumn swarming periods of those years except 2016, when samplings were made also in spring. In 2014 sampling was made with a mercury vapor lamp (125 WHgLi) combined with a large white sheet (4×3 m, 1.5 m height). The specimens were collected from a sheet with an insect aspirator. Ethylacetate was used to kill caught specimens. Since 2015, samples have been collected using a Jermy type automatized light trap containing the same mercury vapor lamp (125 W HgLi) (Nowinszky, 2003). The samplings were repeated 2-3 times a week (depending on the weather conditions) during the swarming periods. In this case, chloroform (CHCl<sub>3</sub>) was used as killing agent. The mosquitoes were identified based on the keys of Mihályi and Gulyás (1963), Mohrig (1969) and Kenyeres and Tóth (2008). In the case of nomenclature, Kenyeres and Tóth (2008) were followed.

### 2.2. Sampling sites

The samplings were carried out in the Velyka Dobron' Game Reserve, located in the Ukrainian part of the Bereg Plain. The Bereg Plain belongs to the Upper Tisza Region in the northeastern part of the Carpathian Lowlands (Great Hungarian Plain). This area has a cold, wet continental climate with strong Carpathian mountainous effects (Szanyiet al., 2015). The manual light trapping was performed in different sampling sites in the reserve, while the Jermy type light trap located at 48°26'39.27" N; 22°24'17.98" E.

The Velyka Dobron' Game Reserve is a zoological reserve with state importance. It is located in a lowlands area with an average altitude of 105 m. This area is a marginal part of the former Szernye Marsh; thus, it is still aqueous and wet despite the drainage. The territory is intersected by the Szernye Canal (which constructed during the drainage of Szernye-bog) and the Latorca River.

On the floodplain these watercourses have a continuous water supply with a maximum level in spring. Beyond that the reserve is surrounded by many artificial canals, which are periodically drying out, and greater or lesser ephemeral ponds and puddles, which are ideal breeding habitats for mosquitoes. The area is mostly covered by mixed hardwood forest, but on the floodplains of the Latorca River and the canals willow poplar softwood woodlands are also extended.

## 3. Results and discussion

During our four-year study, 889 specimens of 19 mosquito species were collected and identified. The checklist of Sheremet (1998) mentioned 28 mosquito species from all the Transcarpathia (Table 1). Since we found 68% of this fauna in a relatively small area, the Velyka Dobron' Game Reserve has relatively species rich Culicidae fauna. Four of the 19 caught species are new for the Transcarpathian fauna (*Anopheles hyrcanus*, *Ochlerotatus cataphylla*, *Ochlerotatus nigrinus*, *Uranotaenia unguiculata*); thus, the actualized check list of the region contains 32 Culicidae species.

All of the 4 recently found species are already known from other parts of Ukraine since they are characteristics for the steppe and forest steppe region. Beyond that *Anopheles hyrcanus* and *Uranotaenia unguiculata* has been published from the marshy Polyssia region (NW Ukraine) and the Carpathians (Sheremet, 1998; Levitskyi, 2016) while *An. hyrcanus* also has been found in cities of Kharkiv (Gazzavi-Rogozina et al., 2017) and Donetsk (E Ukraine) (Ryazantseva, 1971). *Ochlerotatus nigrinus* is also distributed in the Carpathians (Sheremet, 1998), while in the Pyryatyns'kyi region (N Ukraine) only one specimen was sampled by Kilochytska and Stetsenko (2017). *Ochlerotatus cataphylla* has an old data from Dnipropetrovsk (Voronova et al., 2009) and recently it has not been validated, but it can be found in the Charpathians (Sheremet, 1998), cities of Kahrkiv (Gazzavi-Rogozina et al., 2017), Kiev (Kilochytska, 2012, 2013; Kilochytska and Stetsenko, 2017) and Zhytomir (Yasynska and Korzh, 2013) and in many points of the Polyssia region (Levitskyi, 2016). Although all of them occur in Hungary, *Oc. nigrinus* and *Ur. unguiculata* have never been found in the neighboring fauna in the Hungarian part of the Bereg Plain. *An. hyrcanus* is rare in the Pannonian Lowland (Szabó et al., 2011), while *Oc. cataphylla* is common in the hilly regions of Hungary (Tóth, 2004; Kenyeres and Tóth, 2008) and less common in the Bereg Plain as seen in Table 1 (Szabó et al., 2011).

The species composition of the studied area is similar to the fauna in the Hungarian part of the Bereg Plain. To date, 21 species have been published from the Hungarian part. Among them five are differential species for the Hungarian part, while there are three species (*Ochlerotatus nigrinus*,

**Table 1.** Checklist of the Transcarpathian mosquito (Culicidae) fauna by sources (VD: our samplings in the Velyka Dobron' Game Reserve, TR: Sheremet (1998) Transcarpathian checklist) with relative frequencies (RF%) of species in samples taken between 2014-2017 and the checklist in the Hungarian part of the Bereg Plain (HUN, Szabó 2007b; Szabó et al., 2011). Bold: species with new data for Transcarpathia. \*Species with significant medical and/or veterinary importance.

Species	VD (RF%)	TR	HUN
Anopheles algeriensis (Theobald, 1903)	-	-	+
Anopheles atroparvus (van Thiel, 1927)*	0.44	+	+
Anopheles claviger (Meigen, 1804)*	0.11	+	+
<b>Anopheles hyrcanus (Pallas, 1771)</b>	<b>0.44</b>	-	+
Anopheles maculipennis (Meigen, 1818)*	16.35	+	+
Anopheles messeae (Falleroni, 1926)	0.11	+	+
Anopheles plumbeus (Stephens, 1828)	-	+	-
Orthopodomyia pulcripalpis (Rondani, 1872)	-	+	-
Aedes cinereus (Meigen, 1818)*	14.68	+	+
Aedes rossicus (Dolbeskin, G. & M., 1930)	0.22	+	+
Aedes vexans (Meigen, 1830)*	41.27	+	+
Ochlerotatus annulipes (Meigen, 1830)	2.89	+	+
Ochlerotatus cantans (Meigen, 1818)	0.44	+	+
Ochlerotatus caspius (Pallas, 1771)	0.33	+	+
<b>Ochlerotatus cataphylla (Dyar, 1916)</b>	<b>0.11</b>	-	+
Ochlerotatus communis (De Geer, 1776)	-	+	-
Ochlerotatus diantaeus (Howard, D. & K., 1913)	-	+	-
Ochlerotatus excrucians (Walker, 1856)	0.11	+	+
Ochlerotatus flavescens (Müller, 1764)	-	-	+
Ochlerotatus geniculatus (Olivier, 1791)	-	+	-
Ochlerotatus intrudens (Dyar, 1919)	-	+	-
<b>Ochlerotatus nigrinus (Eckstein, 1918)</b>	<b>0.44</b>	-	-
Ochlerotatus pullatus (Coquillett, 1904)	-	+	-
Ochlerotatus pulcristarsis (Rondani, 1872)	-	+	-
Ochlerotatus punctor (K. in R., 1837)	-	+	-
Ochlerotatus rusticus (Rossi, 1790)	-	+	-
Ochlerotatus sticticus (Meigen, 1838)	7.56	+	+
Culiseta annulata (Schränk, 1776)	-	-	+
Culiseta glaphyoptera (Schiner, 1864)	-	+	-
Culiseta longiareolata (Macquart, 1838)	-	+	+
Culex hortensis (Ficalbi, 1890)	-	-	+
Culex modestus (Ficalbi, 1890)*	1.45	+	+
Culex pipiens (Linnaeus, 1758)*	11.35	+	+
Culex territans (Walker, 1856)	-	+	-
Coquillettidia richiardii (Ficalbi, 1889)	1.45	+	-
<b>Uranotaenia unguiculata (Edwards, 1913)</b>	<b>0.22</b>	-	-
Number of species	19	28	21

Coquillettidia richardii and Uranotaenia unguiculata) that have been found only in the Ukrainian part of the Bereg Plain. Thus, the known Culicidae fauna of the Bereg Plain contains 24 species.

The only dominant species was *Aedes vexans* (RF = 41.3%). In the rank structure, it was followed by the subdominant *Anopheles maculipennis* (16.4%), *Aedes cinereus* (14.7%), *Culex pipiens* (11.3%), and *Ochlerotatus sticticus* (7.6%) (Table 1). All these frequent species are common and abundant in Europe and some even in the Holarctic region. They can develop in wide range of stable and ephemeral water bodies. Sum of their relative frequency was more than 90% and all of them have medical or veterinary importance due to transmitting different diseases especially viruses. Beyond behavior and environmental requirements that increase their danger, *Ae. vexans* and *Oc. sticticus* can migrate far from the water bodies while *An. maculipennis* can be developed even in polluted habitats.

The subdominant *An. maculipennis* has an outstanding role in the spread of malaria as seen in Table 2 (Mihályi and Gulyás, 1963; Tóth, 2004; Hubálek, 2008; Kenyeres and Tóth, 2008; Piperaki and Daikos, 2016; Helge et al., 2016) which is an endemic disease in the Carpathian Basin (Trájerand Hammer, 2018). The global warming and the intensified human migration increase the risk of larger epidemics through Europe. In Ukraine, one in ten

infection caused to death of the patient that shows the magnitude of the danger that might be caused by this disease (Pavlichenko et al., 2017).

*Ae. vexans* and *Cx. pipiens* are both potential vectors of West Nile virus that recently appear in more European countries (Table 2). In Ukraine this pathogen caused an epidemic in 1985 with 38 patients (Hubálek and Halouzka, 1999; Ziegler et al., 2013). Between 2007 and 2016 the total number of cases was 86. The most infected regions were East Ukrainian Zaporizhzhya, Poltava, and Donetsk (Demchyshyna et al., 2018). In the central and eastern part of the country 13.5% of samples taken from horses (n=310) contained the virus (Ziegler et al., 2013). More than 300 cases were reported in Serbia in 2012, when a large epidemic was occurred, while in Bucharest (Romania) more than 400 cases were registered in 1996 (Szentpáli-Gavallér et al., 2014). Only 31 patients could be found in neighboring Hungary between 2010 and 2012. These two species also can transmit the *Dirofilaria* sp. pathogen of dirofilariasis. *D. repens* is one of the most rapidly spreading parasites in Europe, having both medical and veterinary importance. It was first recorded in Kharkiv in 1927, but between 1997 and 2012 nearly 1500 cases were recorded in the central and east part of the country (Salamatin et al., 2013).

Dominant and subdominant mosquito species of studied assemblages can transmit further pathogens e.g. *Francisella tularensis* (Tularemia), USUTU virus and

**Table 2.** Characteristics and medical and/or veterinary importance of dominant and subdominant Culicidae species lives in Velyka Dobron' Wildlife Reserve based on (Mihályi and Gulyás, 1963; Tóth, 2004; Hubálek, 2008; Kenyeres and Tóth, 2008; Piperaki and Daikos, 2016; Helge et al., 2016).

Species	Character	Transmitted diseases
<i>Ae. vexans</i>	widespread, migrate far from hatching sites, in forest has all day activity	- West Nile Fever (West Nile virus) - Tularaemia ( <i>Francisella tularensis</i> ) - Dirofilariasis ( <i>Dirofilaria</i> sp.) - Tahyna virus
<i>An. maculipennis</i>	widespread in Europe and West Asia, can live in disturbed and polluted water bodies	-Malaria ( <i>Plasmodium</i> spp.)
<i>Ae. cinereus</i>	Holarctic, common and even abundant in lowlands and lower mountains	-Tularaemia ( <i>Francisella tularensis</i> ) - Ockelbo virus - Tahyna virus
<i>Cx. pipiens</i>	Holarctic, not migratory, generally abundant, can develop in small ephemeral water bodies	- West Nile Fever (West Nile virus) - Avian malaria ( <i>Plasmodium</i> spp.) - Dirofilariasis ( <i>Dirofilaria</i> sp.) -USUTU virus - Tahyna virus - Ockelbo virus - Batai virus
<i>Oc. sticticus</i>	Holarctic, common and abundant, migrate far from hatching sites, active all day long	- Tahyna virus

Tahyna virus which are present in Transcarpathia or in the surroundings.

Considering the relatively high ratio of vector species and especially their dominance in the mosquito assemblages of the Velika Dobron' Game Reserve and probably in the whole region the risk of a potential epidemic is significant and may increase in the future. Thus, the further study of the neighboring regions and monitoring of the local mosquito and pathogen populations are an important topic of local government and of the non-governmental

organizations. During further investigations studies on dominant and subdominant species should be prioritized.

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