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Preliminary Results for the Species Composition and Dynamics of Mosquito Family (Culicidae) in the City of Plovdiv

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Abstract. The mosquitoes from the Culicidae family (Insecta: Diptera) up to this moment comprise more than 3600 species in the world and are found almost in all geographical regions. They are some of the most important insects in terms of epidemiology. They are carriers of more than 50 viral and bacterial infections and parasitoses (malaria, yellow fever, viral encephalitis, dengue fever, tularemia, philariasis etc.). The latest studies of the species composition of the mosquito fauna in the region of Plovdiv date back to 1997. On the basis of research data and own preliminary studies, the entire available information has been compiled and summarized about the species composition, the ecology and the importance of mosquitoes as vectors on the territory of the city of Plovdiv, as well as in the region. The preliminary study was conducted in consecutive years (2011 and 2012) from May to October. In the period from May – December 2011 and 2012, for each of the months, samples were taken, using the method of "hand collection". For the territory of the city of Plovdiv 8 biotopes were identified. The purpose of the study is to investigate the species composition, the morphology and the biology of the mosquitoess from the Culicidae family in the urbanized biocenoses (in the city of Plovdiv). The article provides information about the species composition and the dynamics of the mosquitoes in the city of Plovdiv, about 13 species from 6 genera which have been identified so far, and about the dynamics broken down by months of the period under study.

Key words: mosquitoes, Diptera, Culicidae, Plovdiv, Bulgaria.

Introduction

The mosquitoes (Diptera: Culicidae) are widespread bloodsucking insects. They inhabit almost all regions of the world, except the areas covered with permanent ice and comprise more than 3600 species. In our country there 46 known species from 9 genera (MIKOV, 2011).

The mosquitoes are specific carriers of over 50 transmissible infections and parasitoses, such as malaria, dengue fever, yellow fever, philariasis, tularemia, viral encephalitis, etc. (PETROV, 1984).

In their development the mosquitoes pass through a water environment, in which they develop eggs, larvae and pupae and an air environment – as adult specimen. In its lifespan (on average up to 3 months, rarely 6 months) one female mosquito lays from 80 to 300 eggs. The places which the mosquitoes prefer are permanent or temporary water basins – swamps, river spills, puddles and tree holes etc., places with stagnant water. They are not found only in the fast-flowing waters of big rivers, lakes and the seas.

The male specimen feed on plant juices, while the female are bloodsucking. The blood is necessary for the maturation of the eggs. The female mosquito actively seeks hosts (animals or people) and after filling up with blood finds a peaceful and quiet place where it stays until the maturation of the eggs is complete. They are most active during the evening and early in the morning and the optimal temperature for them is 25–26°C. The distances which the mosquitoes cover while flying in their natural conditions differ. Usually they cover a distance of 3 to 5 km, and some of them, carried by the wind, may fly over a distances up to 80 km.

More than half of the world's human population lives with the risk of infection from mosquitoes, which carry agents of diseases, such as malaria, dengue fever, chikungunya, West Nile fever, Japanese encephalitis or lymph philariasis.

The estimates made by the World Health Organization (WHO) show that 247 million people have become sick in 2006 and around one million people have died (WHO, 1992) from diseases carried by mosquitoes. Although around three quarters of all mosquito species are found in the tropical and subtropical regions, the mosquitoes are not a problem only for those regions. The mosquitoes have a significant impact and can transmit pathogens to people in the temperate geographical latitudes, for example, the West Nile Fever - a viral epidemic in the USA, or high temperature fever, caused by chikungunya in Italy in 2007 (DEPOORTERE *et al*., 2008).

The mosquitoes also cause significant economic damage to agriculture. There are known case in which animals, as a result of large amount of bites, reduce their milk yields with 15–30% or die from intoxications. It has been determined that labor productivity of forest workers during the summer season, when the mosquitoes are most active, may decrease with 30—40% (TURELL *et al*., 2001, MCABEE *et al*., 2008, BECKER, 2003; BECKER *et al.,* 2003).

In the last 100 years the climate in Europe has become warmer on average with 0.8 ^{o}C as a result of human activity and the greenhouse effect (GITHEKO *et al.,* 2000).

The increase of the average temperature and the warming of the climate would lead to a widening of the ranges, upsurge of the population wave of the carrier to the north. The period which is favorable for the development of mosquitoes would become longer. The epidemic potential of non-endemic or slightly endemic area would increase and in regions where malaria has been eradicated, there would be an increase of the risk of new appearance of the disease (MARTENS *et al.,* 1995; 1997; JETTEN *et al*., 1996; MCCARTHY *et al.,* 2001).

Health problems of importance mosquitoes and measures to prevent and combat it is designed in an article on the problem of PETROV & BILEVA (2010). The interest in mosquitoes, their ecology and the use of appropriate methods for taking samples dates back from the beginning of the nineteenth century with the discovery that mosquitoes can act as carriers of diseases which affect humans and domestic animals.

According to SOUTHWOOD & HENDERSON (2000) perhaps the most important factor in choosing the appropriate methodology for taking samples is careful formulation of the specific ecological problems, which the investigator wishes to take into account. This will determine the time and length of the period for taking samples, the specific program and methodology, whether the study would be wide-reaching, attempting to describe a large scale distribution and abundance of the species composition or whether it would focus on a particular species or population or on the intensive study of a particular population, usually with objective of determining absolute or relative size of the population and of the factors, which regulate its numbers.

The malaria representatives of the mosquito fauna which are found in the region of Plovdiv are: *Anopheles superpictus* Grassi; *Anopheles maculipennis* Mg.; *Anopheles claviger* (= *A. bifurcatits*) Lav.; *Anopheles (myzirrhynchus) pseadopictus* (= *A. hyrcanus*)

Grassi (MARKOV, 1929). HRISTOVA *et al*. (1969) conducted a study of the species composition of the mosquito fauna in Plovdiv in 1967-1968. The study confirms the studies done so far, summarizing that the most widespread mosquito species in the city of Plovdiv are from the genus *Anopheles* and genus *Culex*.

The last study of the species composition of bloodsucking mosquitoes in the city of Plovdiv was conducted in 1996-1997 (HRISTOVA *et al*., 2000) and the results encompass the following species:

Subfamily Anophelinae

Genus *Anopheles* Meigen

- *Anopheles (Anopheles) maculipennis* Meigen, 1818

Subfamily Culicinae

Genus *Culex* Linnaeus

- *Culex (Culex) pipiens* Linnaeus, 1758
- *Culex pipiens pipiens* Linnaeus, 1758
- *Culex pipiens molestus* Forsk, 1775

Genus *Culiseta* Felt

- *Culiseta (Culiseta) annulata* Schrank, 1776
- *Culiseta (Culiseta) alaskaensis* Ludlow
- *Culiseta (Allotheobaldia) longiareolata* Maqcuart, 1838

This article has been prepared on the basis of the preliminary results from a two-year (2011-2013) study, which aims to investigate the species composition, the morphology, the biology, the ecology of the mosquito population from Culicidae family in urbanized areas (in the city of Plovdiv).

Materials and Methods

Method for collection of imaginal stage specimens.

In the beginning of the study 4 Malaise traps were made and mounted on the work sites in the city of Plovdiv. To collect and store the trapped insects and specifically the mosquitoes, we used 75% ethyl alcohol (C_2H_5OH) for laboratory samples.

The Malaise traps are widely used for collecting flying insects. They operate and provide representative information for the flying insects throughout the season. They are convenient for ecological, faunistic and monitoring studies. Their operation is simple and consists of replacing the container with preserving liquid and providing security for the trap (MALAISE, 1934).

In the beginning of the study specific biotopes were identified to be studied, both in the city of Plovdiv and in the protected area "The River Mouth of the Veleka River" and the Ropotamo river.

In April-May 2011 over 25 biotopes and work sites were surveyed for the upcoming terrain and field practice and from these 4 biotopes and 4 work sites were selected. For the territory of the city of Plovdiv 8 biotopes were identified, of which 4 for positioning of Malaise traps for the entire season from May to October, on one of those we used a mosquito traps BG-Sentinel in two versions - with the addition of CO_2 and with placing an insecticide lamp, and 4 biotopes for mosquito larvae, of various types.

The Malaise traps were processed every two weeks for 5 months, two collections of 4 work sites provide a collection of 72 samples with imaginal adult stage specimen, for two years.

On one of the work sites we used a mosquito trap BG-Sentinel. BG-Sentinel ™ in two versions - with the addition of CO_2 and with the addition of an insecticide lamp. The catch is in a double bag and is located above a fan, the specimen are not destroyed and are preserved, and can easily be identified and used to identify the disease agents.

Hand collection of adult mosquitoes using the test-tube method for collection of mosquitoes (WHO, 1992).

In the period from May – November 2011 and 2012, for each of the months, samples were taken, using the method of "hand collection". This method was applied at 7 sites, differentiated in three groups:

1. Residential buildings
2. Public buildings
3. Agricultural buildings

Methods of collection of larvae and pupae.

Method of submerging of the "white tray" (20 x 15 x 3 cm) for determining of the density at the larval stage of development and method of submerging of hydrobiological net mounted on a circular frame (15 cm diameter) and attached to a wooden or aluminium handle (1.50 m).

Collection of larvae and pupae by means of a trap of automobile tires (SCOTT & CRANS, 2003) and various containers as traps for larvae.

The larval stage specimen were collected from 4 biotopes - biotope 1 is natural and temporary, biotope 2 – artificial and temporary, biotope 3 – artificial and permanent, biotope 3 artificial and temporary.

The characteristic features of each biotope (topography, approximate dimensions, water movement, water purity, water temperature, pH (acidity), hardness, temporary or permanent, natural or artificial) are given in Table 1.

Table 1. Characteristic features of the biotopes for collection of larvae.

Biotopes	Year	Area in sq. m	Water movement	Water purity	Number of checks	pH	Water temperature	Hardness (in German degrees)	Plant life
Biotope No. 1 spills of the Maritsa river near the Food Technologies University	2011 2012	1800	Slow current	Low contamination	10/3* 6/2*	7,5	17 – 30 °C 18-32 °C	14,00	*Typha* sp. * grassy
Biotope No. 2 Aquaduct, Bunardzhika hill	2011 2012	70	Stagnant	Not contaminated	10/3 6/1	6,8	15 - 34 °C 15 - 35 °C	13,72	*Typha* sp.
Biotope No. 3 fountain in the Tsar Simeon garden	2011 2012	650	Stagnant	Not contaminated	10/0 6/0	7,4	19 – 31 °C 19 – 33 °C	14,00	Algae
Biotope 4 – automobile tires, residential area "Trakiya"	2011 2012	5	Stagnant	Contaminated	10/5 6/3	7,9	18 – 35 °C 18 – 37 °C	14,56	Decaying leaves

*The first digit shows the total number of the checks, and the second – the number of the checks where mosquito larvae were found.

The species determination is made by morphological characteristics of imago and larvae. The classification of the species is according to SNOW & RAMSDALE (2003).

Works by DRENSKI (1938), BOZHKOV (1991) and HARBAH (2001) are used for the species identification. Species are confirmed by Dr Ognyan Mikov (National Centre of Infectious and Parasitic Diseases - Sofia).

Results and Discussion

In the period from May 2011 to November 2012, 20 assemblies of imaginary forms were made from 4 workstations with malaise traps (Fig. 1). 75% of the samples were positive, 15% of the total had no reported samples due to technical or weather reasons.

Using the "hand assembly" method, 228 adult mosquitoes were collected in 14 samples from 7 sites (Fig. 2). Relevant to the research, an increased number of individuals indoors under the constitutional evolutionary factors and adverse abiotic environmental factors of the environment is established in October and November (MARTENS *et al.*, 1995; 1997; JETTEN *et al.*, 1996; MCCARTHY *et al.*, 2001). The preparation for diapause provided a large number of mosquitoes indoors.

Data from the assemblies in one of the workstations, in residential district Trakia with mosquito trap BG-Sentinel ™, is given in table 2. When using two methods, with the addition of CO_2 and insecticidal lamp, a greater amount of imaginary forms is established when using light as "bait".

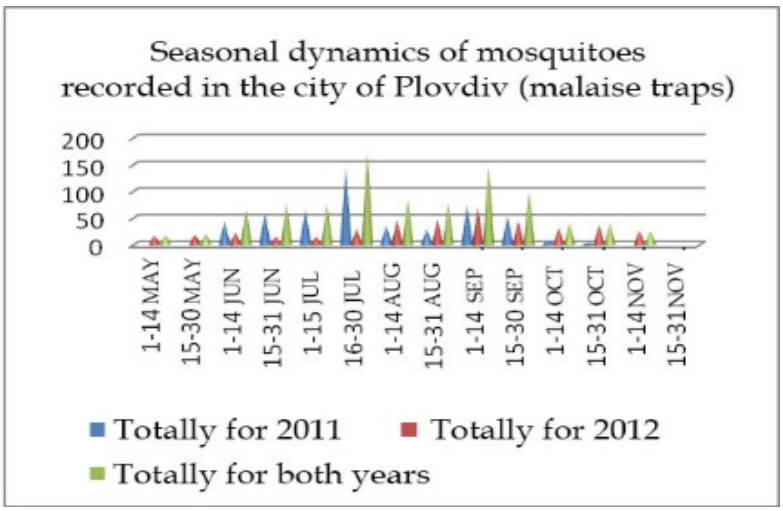

Fig.1. Seasonal dynamics of mosquitoes recorded in the city of Plovdiv (malaise traps) - sample distribution by numbers, workstations and weeks.

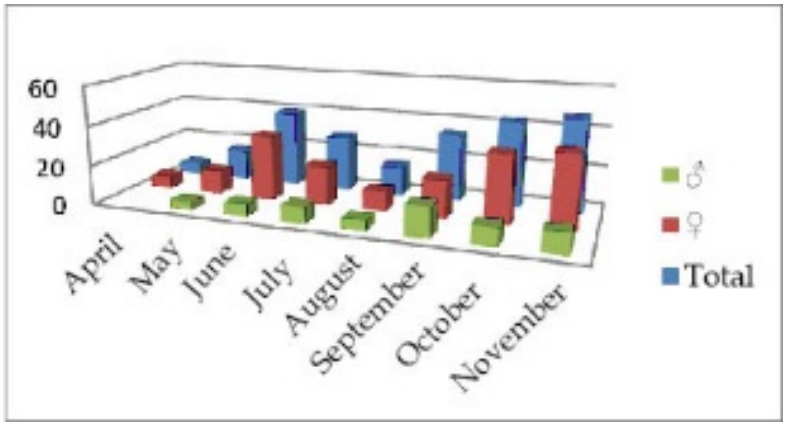

Fig. 2. Data on assemblies of imaginary mosquitoes using the "hand assembly" method in the period from 2001 to 2012 by number, sex and date.

Table 2. Data on assemblies, total and percentage of collected imaginary forms by BG-Sentinel with CO_2 and by BG-Sentinel with insecticidal lamp

Assembly by months	**Total with BG-Sentinel with CO_2**	♀	♂	**%**	**Total with BG-Sentinel with insecticidal lamp**	♀	♂
May	9	*7*	*2*	11,25 %	12	*10*	*2*
June	11	*6*	*5*	13,75 %	19	*14*	*5*
July	11	*8*	*3*	13,75 %	25	*17*	*8*
August	12	*9*	*3*	15 %	19	*15*	*4*
September	16	*12*	*4*	20 %	32	*26*	*6*
October	10	*8*	*2*	12,5 %	18	*15*	*3*
November	11	*7*	*4*	13,75%	11	*7*	*4*
Total:	**80**	**57**	**23**	**100%**	**136**	**104**	**32**

Seasonal dynamics of mosquitoes recorded in the city of Plovdiv, caught with BG-Sentinel trap, with CO_2 and insecticidal lamp indicates that the most favourable months for the development of mosquitoes are June and July, with peaks in numbers in September and the first half of October. (Fig. 3)

1,401 mosquitoes, 853 ♀♀ and 548 ♂♂, were collected during the whole period of study from all biotopes. The collected larvae during this period are 197 and the positive samples are only 27.5%.

Seasonal dynamics of mosquitoes recorded in the city of Plovdiv indicates that the most favourable months for the development of mosquitoes are May, June, July and August, with peaks in numbers in the second half of July, September and early October (Fig. 4).

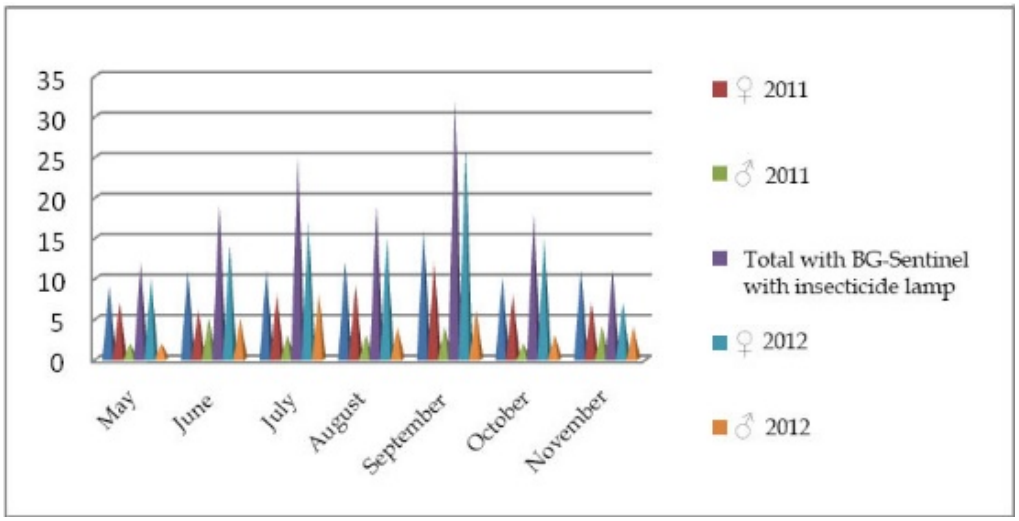

Fig. 3. Data on the collected adult mosquitoes with BG-Sentinel by date and sex.

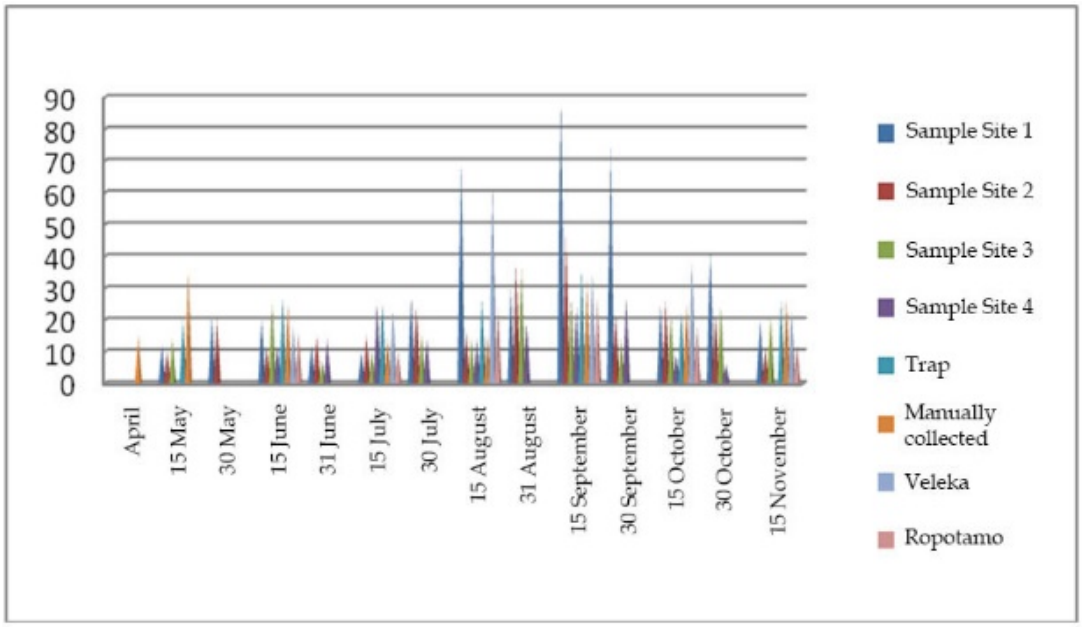

Fig. 4. Seasonal dynamics of mosquitoes recorded in the city of Plovdiv and in the control stations.

The amount of mosquitoes is directly dependent on the temperature and atmospheric moisture. The research gives data shift from the previous studies that the greatest occurrence of mosquitoes is recorded in the months that coincide with the second and third generation, namely July and August, while at the end of September and October their number is reduced. These results show that the favorable period for the development of mosquitoes is prolonged, leading to an expansion of the areas and population boom in the north.

Most male individuals prove that the assembly is carried out very close to the place where they proliferate (Fig. 5).

Anthropogenic influences significantly affect populations of mosquitoes in urban areas. Among the bloodsucking mosquitoes in urban areas, the study of the mosquito *Culex pipiens* (VINOGRADOVA, 1997) is of great scientific and practical importance.

During the two years of the study, some part of the collected material was defined. Until now, in the fauna of Plovdiv representatives of 6 genera, including 13 species presented in a systematic list that includes genus, species, author and year of description of the species have been identified (Table 3).

Conclusions

As a result of the studies, conducted bet-

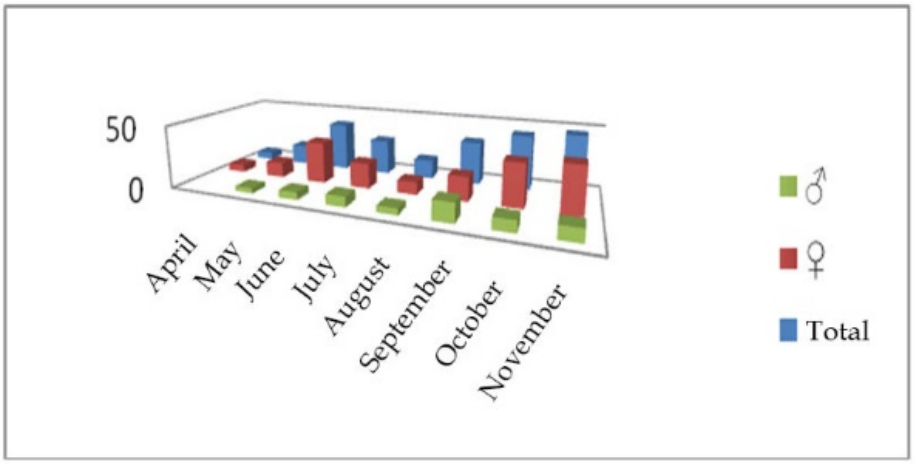

Fig. 5. Data on assemblies of imaginary mosquitoes using the "hand assembly" method in the period from 2001 to 2012 by number, sex and date

Table 3. Systematic list of the mosquito fauna (Culicidae) in the city of Plovdiv.

№	Genus	Species	Author	Year
1.	*Anopheles*	*claviger*	Meigen	1804
2.	*Anopheles*	*hyrcanus*	Pallas	1771
3.	*Anopheles*	*maculipennis*	Meigen	1818
4.	*Anopheles*	*messeae*	Falleroni	1926
5.	*Anopheles*	*superpictus*	Grassi	1899
6.	*Aedes*	*cinereus*	Meigen	1818
7.	*Culex*	*pipiens*	Linnaeus	1758
8.	*Culex*	*territans*	Walker	1856
9.	*Culiseta*	*glaphyroptera*	Schiner	1864
10.	*Culiseta*	*longiareolata*	Macquart	1938
11.	*Culiseta*	*morsitans*	Theobald	1901
12.	*Ochlerotatus*	*cataphylla*	Dyar	1916
13.	*Uranotaenia*	*unguiculata*	Edwards	1913

ween 2011 and 2012, on the populations of bloodsucking mosquitoes (Culicidae) in the city of Plovdiv, data on the dynamics, species composition, biological and environmental conditions is obtained.

In the seasonal dynamics of mosquitoes recorded in the city of Plovdiv, there is an extension of the favourable development period (May - October). Increase in population wave is observed in June, July and August, with peaks in numbers in the second half of July, September and early October.

Preliminary results of the study indicate the presence of vertical migration with establishing *Uranotaenia unguiculata*.

According to the preliminary results in the city of Plovdiv, 6 genera (*Anopheles, Aedes, Culex, Culiseta, Ochlerotatus, Uranotaenia*), including 13 species are established. The species composition of the mosquito fauna has been increased by three new genera (*Culiseta, Ochlerotatus* and *Uranotaenia*), in comparison with the recent studies by HRISTOVA *et al*. (2000) and others.

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