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A MOSQUITO SURVEY OF CULICIDAE SPECIES AT EDİRNE CENTRAL DISTRICT FOR DISEASE VECTOR

Zafer ŞAKACI[1], Yılmaz CAMLITEPE[2*]

[1] Balıkesir University, Faculty of Science and Literature, Department of Biology, Balıkesir, TÜRKİYE
[2] Trakya University, Faculty of Science, Department of Biology, Edirne, TÜRKİYE

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Abstract: Mosquitoes are the major vectors that can transmit many diseases agents to humans and animals. This study was conducted in Edirne central district between July 2017 and July 2018 to identify important mosquito vector species, to determine their seasonality and distribution pattern in general terms. Larvae, pupae, and adults were collected from areas assessed as being particularly suitable for medically important species of the genus *Aedes* Meigen, *Culex* Linnaeus, and *Anopheles* Meigen. In addition to the foci naturally found in the areas, ovitraps placed in suitable places for ovipositing were also used. As a result, a total of 3155 females and 353 males belonging to 11 species of 5 genera were obtained. Among these species, *Anopheles sacharovi* Favre (the primary vector of malaria in Turkey) and *Culex pipiens* s.l. Linnaeus (the primary vector of West Nile Fever) has been recognized as a public health threat to the province. *Anopheles sacharovi* was present at a very low population level, while *Cx. pipiens* s.l. was determined as the most common and numerous species in the study area. Known to have a high preference for warmer climate compared to members of the *Anopheles maculipennis* s.l. Meigen, *An. sacharovi* has the risk of increasing its population in the region with possible global warming in the future. The importance of this risk increases even more since rice production is widespread especially in Edirne and this species can use the paddy fields as an effective breeding place. While *Aedes caspius* Pallas was commonly encountered, *Aedes albopictus* Skuse was not found during the field observation and ovitrap controls.

Özet: Sivrisinekler birçok hastalık etkenini insanlara ve hayvanlara bulaştırabilen en önemli vektörlerdir. Bu çalışma, önemli vektör sivrisinek türlerini belirlemek, genel anlamda mevsimselliklerini ve dağılım şekillerini belirlemek amacıyla Temmuz 2017-Temmuz 2018 tarihleri arasında Edirne merkez ilçesinde yürütülmüştür. Medikal olarak önem arz eden *Aedes* Meigen, *Culex* Linnaeus ve *Anopheles* Meigen cinslerine ait türler için özellikle uygun olduğu değerlendirilen alanlardan larva, pupa ve erginler toplanmıştır. Bunun yanı sıra uygun noktalara ovitraplar yerleştirilmiştir. Sonuç olarak elde edilen 3155 dişi ve 353 erkek sivrisineğin 5 cinse ait 11 tür olduğu saptanmıştır. Bu türlerden *Anopheles sacharovi* Favre (Türkiye'de sıtmanın birincil vektörü) ve *Culex pipiens* s.l. Linnaeus (Batı Nil Ateşinin birincil vektörü), il için bir halk sağlığı tehdidi olarak kabul edilmiştir. *Anopheles sacharovi* çok düşük bir popülasyon düzeyinde temsil edilirken, *Cx. pipiens* s.l. çalışma alanında en yaygın ve yoğun tür olarak belirlenmiştir. *Anopheles maculipennis* Meigen tür kompleksi üyelerine kıyasla daha sıcak iklimi tercih ettiği bilinen *An. sacharovi*, gelecekte olası bir küresel ısınma ile bölgedeki nüfusunu artırma riski taşımaktadır. Özellikle Edirne'de çeltik üretiminin yaygın olması ve bu türün çeltik tarlalarını etkili bir üreme yeri olarak kullanabilmesi nedeniyle bu riskin önemi daha da artmaktadır. *Aedes caspius* Pallas türüne yaygın olarak rastlanırken, arazi gözlemleri ve ovitrap kontrolleri sırasında *Ae. albopictus* Skuse türüne rastlanmamıştır.

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Kadri KIRAN

****Corresponding Author:***
Yilmaz Camlitepe
yilmazc@trakya.edu.tr

ORCID iDs of the authors:
ZŞ. orcid.org/ 0000-0002-6736-3792
YC. orcid.org/ 0000-0002-9759-2609

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Introduction

Mosquitoes are the main factor for the spread of many diseases such as dengue fever, yellow fever, chikungunya, West Nile fever, encephalitis, malaria, and filariasis. Due to this vectorial capacity and the high adaptability to new areas, systematic follow-up of these mosquito species is very important, particularly in areas that pose a risk for mosquito-mediated diseases. (Becker *et al.* 2010). Although many studies on mosquito fauna have been conducted in Turkey, most of them have been based on the identification of species and roughly determining their distribution characteristics (Parrish 1959, Ramsdale *et al.* 2001, Günay 2015).

Edirne, which is located between important rivers and therefore frequently subjected to floods, is a province where the most paddy cultivation is carried out with an area of 443,097 acres in Turkey (Taşlıgil & Şahin 2011). Besides the city has favourable climatical factors for mosquito development, it has also suitable conditions where new mosquito species entering the region can easily reproduce and establish. In addition, Edirne is a place used by irregular migrants from the Middle East, Asia, and Africa as a gateway to Europe (Deniz 2015). The reasons mentioned above provide advantages in terms of the entry and spread of mosquito-borne diseases in Edirne.

West Nile virus (WNV) cases are seen because of the coexistence of birds and vector mosquitoes in areas located on wild bird migration routes around the world. In Eastern Thrace, including Edirne, one of the most important bottle necks takes place on the wild bird migration routes between Africa and Europe (Kirwan *et al.* 2014). In molecular studies conducted in this region, *Cx. pipiens* Linnaeus was found to be the major dominant species (Günay 2015). Therefore, the current situation has made Eastern Thrace a hot spot where WNV is endemic today.

The mosquito fauna of Turkey is represented by 61 species (Parrish 1959, Ramsdale 2001, Günay 2015). In Eastern Thrace, 6 species belonging to 3 genera in Çanakkale; 28 species belonging to 6 genera in Edirne; 7 species belonging to 4 genera in İstanbul (European part); 27 species belonging to 6 genera in Kırklareli; 16 species belonging to 5 genera were identified in Tekirdağ (Öter 2007, Çağlar *et al.* 2008, Sevgili 2009, Koçak & Kemal 2014, Öter & Tüzer 2014, Günay 2015, Akbay 2016, İpek 2016, Sarıkaya 2017).

This study aimed to determine the important vector mosquito species in Edirne central district by evaluating the larvae, pupae and adult forms collected at different times from areas suitable for mosquito species in the study area and to correlate the data to be obtained with previous studies to form an assessment about possible risks that may threaten public health in the future.

Materials and Methods

Geographical and climatic characteristics of the study area

Edirne (41°40'37.09"N, 26°33'21.41"E) is located in the Thrace part of Turkey. The most important stream of the province is the Meriç River, which forms a natural border with Greece. The Arda, Tunca and Ergene rivers join the Meriç River in Edirne. Three types of vegetation can be seen in the province, namely maquis, steppe and forest. The climate is warm and temperate. The annual average temperature is 13.5°C. Annual average precipitation per square meter is 597 mm (Climate-data.org 2021). The averages of temperature and humidity levels in the province throughout the study are given below (Fig. 1).

Study area

The study was carried out in 30 localities selected in the central district of Edirne province between July 2017 and July 2018. Larvae, pupae, and adults were collected in areas that were found to be suitable for breeding of different mosquito species. In addition, ovitraps were also set up in selected localities. The localities where the field studies were carried out and the sampling methods are given below (Fig. 2).

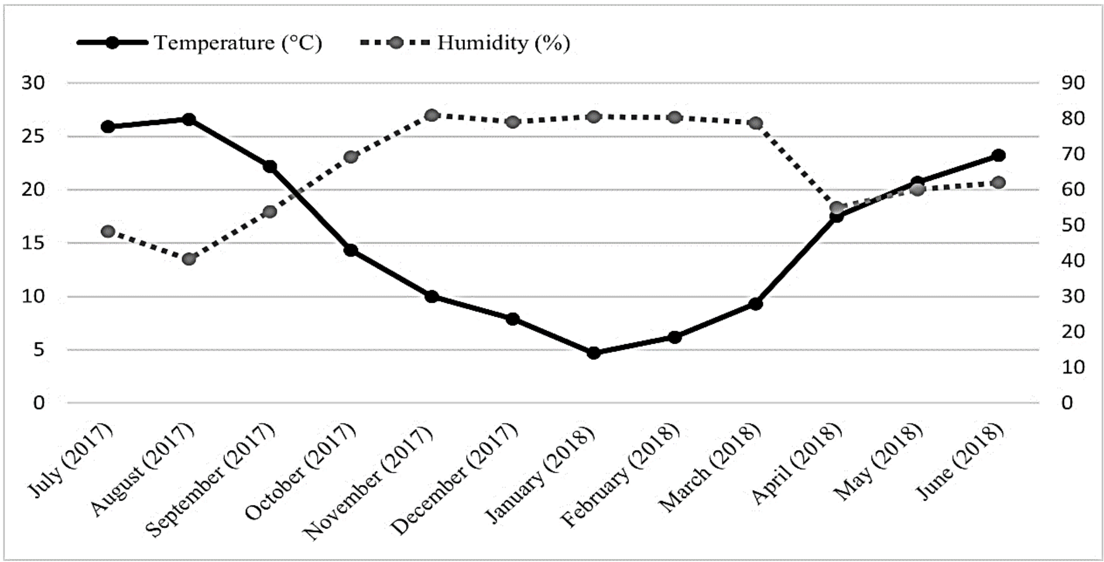

Fig. 1. Average temperature and humidity level of Edirne central district during the study period (Data from Turkish State Meteorological Service, between the years 2017-2018 (https://mgm.gov.tr/eng/forecast-cities.aspx).

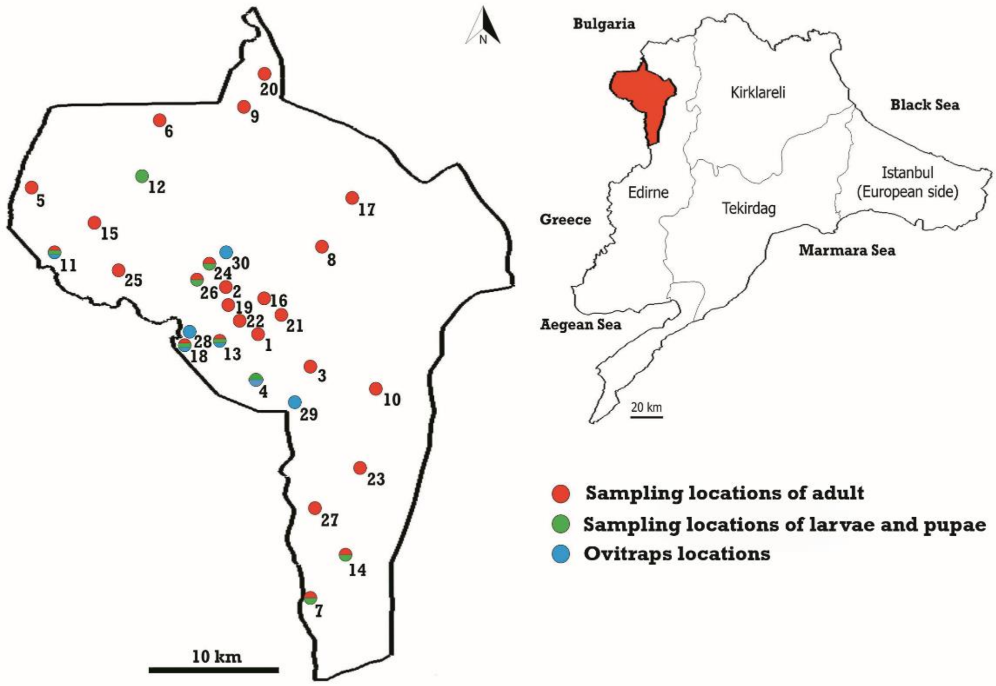

Fig. 2. The localities where the field studies were carried out and the sampling methods are presented on the map, during July 2017-July 2018 in the central district of Edirne (For locality names and distribution of mosquito species, please see Table 1)

<u>*Sampling of adults*</u>

Mosquito adults were collected from indoor and outdoor areas with a mouth aspirator during the resting and over-wintering periods. Diurnal and crepuscular species were collected from the exposed limbs and other parts of people's bodies by human-landing catches (HLC) method using a manual aspirator (Service 1993). Particular attention was paid to sampling the adults who took shelter in closed areas during the sudden rain showers observed immediately after the extremely dry periods. Adults collected with mouth aspirators were brought to the laboratory in 500 ml plastic bottles with field data records. The bottles were stored at -20°C until species identification.

<u>*Sampling and rearing of larvae and pupae*</u>

Larvae and pupae were collected from aquatic habitats with a small larval dipper and a Pasteur pipette. It was then brought to the laboratory with some habitat water together with field data records in 1200 ml volume containers. Samples were taken into plastic containers (bottom dimensions: 18x25 cm; height: 15 cm; ceiling dimensions: 20x29 cm) up to 5 cm high, placed in their habitat water, and stored at 24-25°C. The upper surfaces of the containers placed in a part of the laboratory that are not exposed to direct sunlight are covered with mesh. During the rearing process, the larvae were checked once a day. They were offered some baby fish food (Mikromin®), especially on days when there was excessive activity in the larvae, which indicates food-seeking. Feeding was completely discontinued when the pupal stages predominated. The emerging adults were collected from the containers with a mouth aspirator and transferred to 500 ml plastic bottles. The bottles were stored at -20°C until species identification.

<u>*Construction, placement and control of ovitraps*</u>

Plastic containers with a diameter of 12 cm at the top, 7.5 cm at the bottom, 11 cm in height and with a volume of 1000 ml were used as ovitrap. A water drainage hole with a diameter of 1 cm was opened 4 cm below the top of the containers that were painted black. The top is covered with a thick wire mesh. Two strips of 12 cm length and 2.5 cm width, one made of masonite and the other made of poplar wood, were used to be placed in the container. Both surfaces of the strips have been sanded. During the three-day period prior to the setup of the ovitraps, the strips were soaked in dechlorinated water for two days and left to dry for one day. Wire fasteners are made to fix the strips in the container.

For the supply of *Ae. albopictus* Skuse eggs, ovitraps were established in the areas suitable for the bio-ecology of the species (Arda-2 pieces (41°39'35.59"N, 26°29'42.96"E, 38 m), Bosna Village-1 piece (41°39'35.59"N, 26°29'42.96"E, 38 m), Karaağaç-4 pieces (41°38'3.35"N, 26°31'51.02"E, 35 m), Kapıkule-7 pieces (41°42'36.33"N, 26°22'28.67"E, 42 m), Pazarkule-2 pieces (41°39'15.56"N, 26°29'24.12"E, 43 m), Sarayiçi-2 pieces (41°41'29.68"N, 26°33'35.40"E, 40 m), Topsöğüt-2 pieces (41°36'47.94"N, 26°35'52.08"E, 32 m). Ovitraps were placed in areas that are green, wooded, shaded, easily accessible, with a space of at least 1 m high at the top (Carrieri *et al.* 2011), and less affected by wind (Suter *et al.* 2016). The containers were tied to either a tree or a wood driven into the ground, in contact with the ground. Ovitraps filled with water (600 ml) up to the discharge hole were fixed in opposing positions with one masonite and one poplar strip wire fasteners with an inclination of 45°.

Ovitraps were used during the period of 20 July-30 September, during which time the presence of eggs in the strips was checked with a magnifying glass once every ten days. In addition, the presence of larvae, pupae and their exuvia in the ovitrap was also checked. At each control, the water of the ovitrap was replaced with freshly dechlorinated water.

Identification of mosquitoes

Species identification of the adult mosquitoes obtained from the field surveys and immature stages reared under laboratory conditions were identified using a stereomicroscope (Olympus SZ51) based on the morphological keys described before (Gutsevich *et al.* 1974, Darsie & Samanidou-Voyadjoglou 1997, Becker *et al.* 2010).

Results

This study was carried out in 88 foci in 30 localities selected in the central district of Edirne province between July 2017 and July 2018. Common species in the sampling area were *Ae. caspius* Pallas (14/30), *An. maculipennis* s.l. Meigen (except *An. sacharovi*) (20/30), *Cx. pipiens s.l.* Linnaeus (20/30). The locality information of the collected species is given in Table 1.

Table 1. Distribution of mosquito species collected in Edirne central district during the period of July 2017-July 2018.

Number of location	Locality	Habitat	Number of foci	Coordinates	Altitude	Number of species	Mosquito species
1	Abdurrahman Neighborhood	Urban	2	41°39'59.29"N, 26°33'56.38"E	39 m	2	*Ae. caspius* *Cx. pipiens* s.l.
2	Babademirtaş Neighborhood	Urban	1	41°40'52.90"N, 26°33'23.62"E	60 m	1	*Cx. pipiens* s.l.
3	Balkan Campus	Urban	5	41°38'13.78"N, 26°36'38.06"E	43 m	8	*Ae. caspius* *Ae. geniculatus* *An. maculipennis* s.l.* *An. sacharovi* *Cs. annulata* *Cs. longiareolata* *Cx. pipiens* s.l. *Ur. unguiculata*
4	Bosna Village	Rural	2	41°37'31.10"N, 26°33'55.33"E	34 m	1	*Ae. caspius*
5	Budakdoğanca Village	Rural	1	41°45'39.55"N, 26°20'31.84"E	112 m	1	*An. maculipennis* s.l.*
6	Büyükismailce Village	Rural	2	41°48'55.25"N, 26°28'06.95"E	184 m	1	*An. maculipennis* s.l.*
7	Doyran Village	Rural	4	41°29'20.35"N, 26°36'27.64"E	34 m	2	*Ae. caspius* *An. maculipennis* s.l.*
8	Hasanağa Village	Rural	1	41°43'26.92"N, 26°37'32.08"E	60 m	1	*Ae. caspius*
9	Hatip Village	Rural	1	41°49'17.31"N, 26°33'24.11"E	42 m	1	*An. maculipennis* s.l.*
10	İskender Village	Rural	3	41°37'47.30"N, 26°40'22.80"E	87 m	3	*Ae. caspius* *Ae. rusticus* *Cx. pipiens* s.l.
11	Kapıkule	Urban	8	41°42'47.94"N, 26°22'05.70"E	44 m	3	*Ae. caspius* *An. maculipennis* s.l.* *Cx, pipiens* s.l.
12	Karabulut Village	Rural	1	41°46'06.09"N, 26°26'13.41"E	119 m	4	*An. maculipennis* s.l.* *Cx. pipiens* s.l. *Cx. theileri* *Ur. unguiculata*
13	Karaağaç	Urban	13	41°39'20.84"N, 26°31'36.92"E	36 m	6	*Ae. caspius* *Ae. geniculatus* *Ae. vexans* *An. maculipennis* s.l.* *Cs. annulata* *Cx. pipiens* s.l.
14	Karakasım Bucağı Village	Rural	3	41°31'0.82"N, 26°38'38.45"E	39 m	3	*Ae. caspius* *An. maculipennis* s.l.* *Cx. pipiens* s.l.

15	Kemalköy	Rural	2	41°44'7.85"N, 26°23'40.79"E	83 m	1	*An. maculipennis* s.l.*
16	Kıyık	Urban	1	41°40'45.01"N, 26°34'23.97"E	102 m	3	*An. maculipennis* s.l.* *Cs. longiareolata* *Cx. pipiens* s.l.
17	Küçükdöllük Village	Rural	2	41°45'13.39"N, 26°40'0.75"E	81 m	2	*An. maculipennis* s.l.* *Cx. pipiens* s.l.
18	Pazarkule	Urban	8	41°39'15.46"N, 26°29'23.96"E	43 m	4	*An. maculipennis* s.l.* *An. sacharovi*, *Cx. pipiens* s.l. *Ur. unguiculata*
19	Sabuni Neighborhood	Urban	1	41°40'27.76"N, 26°33'23.71"E	50 m	2	*Ae. caspius* *Cx. pipiens* s.l.
20	Suakacağı Village	Rural	2	41°50'29.69"N, 26°35'9.75"E	52 m	2	*An. maculipennis* s.l.* *Cx. pipiens* s.l.
21	Şükrüpaşa Neighborhood	Urban	1	41°40'14.46"N, 26°35'36.95"E	95 m	2	*An. maculipennis* s.l.* *Cx. pipiens* s.l.
22	Talatpaşa Neighborhood	Urban	1	41°40'8.37"N, 26°33'34.24"E	36 m	2	*An. maculipennis* s.l.* *Cx. pipiens* s.l.
23	Tayakadın Village	Rural	4	41°34'25.73"N, 26°39'35.78"E	53 m	2	*Ae. caspius* *An. maculipennis* s.l.*
24	Yeniimaret Neighborhood	Urban	2	41°41'18.14"N, 26°32'29.61"E	37 m	1	*Cx. pipiens* s.l.
25	Yenikadın Village	Rural	2	41°42'1.78"N, 26°26'19.35"E	63 m	3	*An. maculipennis* s.l.* *An. sacharovi* *Cx. pipiens* s.l.
26	Yıldırım Beyazıt Neighborhood	Urban	4	41°40'46.08"N, 26°31'37.61"E	55 m	3	*Ae. caspius* *An. maculipennis* s.l.* *Cx, pipiens* s.l.
27	Üyüklütatar Village	Rural	4	41°32'47.96"N, 26°36'31.11"E	41 m	3	*Ae. caspius* *An. maculipennis* s.l.* *Cx, pipiens* s.l.
28	Arda	Urban	2	41°39'35.59"N, 26°29'42.96"E	38 m	0	-
29	Topsöğüt	Rural	2	41°36'47.94"N, 26°35'52.08"E	32 m	0	-
30	Sarayiçi	Urban	3	41°41'29.68"N, 26°33'34.10"E	39 m	4	*Ae. caspius* *Ae. geniculatus* *Ae. vexans* *Cx. pipiens* s.l.

* except *An. sacharovi*

As a result of the research, 5 genera and 11 species belonging to 2 subfamilies were determined. In total, 3,155♀/353♂ adult individuals obtained during the study period: 217 (177♀/40♂) *Ae. caspius* Pallas, 7 (♀) *Ae. geniculatus* Olivier, 2 (♀) *Ae. rusticus* Rossi, 17 (♀) *Ae. vexans* Meigen, 646 (594♀/52♂) *An. maculipennis* s.l. Meigen, 3 (♀) *An. sacharovi* Favre, 4 (3♀/1♂) *Cs. annulata* Schrank, 3 (♂) *Cs. longiareolata* Macquart, 2594 (2338♀/256♂) *Cx. pipiens* s.l. Linnaeus, 4 (♀) *Cx. theileri* Theobald, and 11 (10♀/1♂) *Ur. unguicualta* Edwards.

While *Ae. caspius*, *An. maculipennis* s.l. and *Cx. pipiens* s.l. were the most common species in our study area *Ae. geniculatus, Ae. rusticus, Ae. vexans, An. sacharovi, Cs. annulata, Cs. longiareolata, Cx. theileri, Ur. unguiculata* were less common (Fig. 3).

The collected materials and collecting methods are given in Table 2.

Terrestrial and aquatic habitats where the species were collected are presented in Table 3.

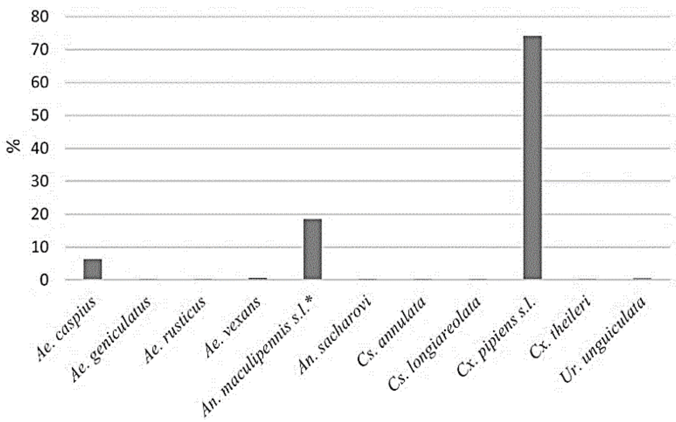

Fig. 3. The distribution rates of the species collected in Edirne central district during the period of July 2017-July 2018.

Table 2. The distribution rates for the species according to collection method.

Collection Methods (Stage)	Counts of samples collected using mouth aspirator (Adult) (%)		Counts of samples collected using larval dipper and/or pasteur pipette (Larva-pupa) (%)	Counts of samples collected using ovitrap (Egg-larva) (%)
Species	**Indoor (%)**	**Outdoor (%)**		
Ae. caspius (Pallas, 1771)	42 (19.35)	72 (33.18)	103 (47.47)	-
Ae. geniculatus (Olivier, 1791)	1 (14.29)	6 (85.71)	-	-
Ae. rusticus (Rossi, 1790)	-	2 (100)	-	-
Ae. vexans (Meigen, 1830)	-	17 (100)	-	-
An. maculipennis s.l.* Meigen, 1818	607 (93.96)	-	39 (6.04)	-
An. sacharovi Favre, 1903	3 (100)	-	-	-
Cs. annulata (Schrank, 1776)	4 (100)	-	-	-
Cs. longiareolata (Macquart, 1838)	3 (100)	-	-	-
Cx. pipiens s.l. Linnaeus, 1758	2119 (81.69)	-	361 (13.92)	114 (4.39)
Cx. theileri (Theobald, 1903)	-	-	4 (100)	-
Ur. unguiculata Edwards, 1913	3 (27.27)	-	8 (72.73)	-
Total number of species (%)	**2782 (79.30)**	**97 (2.77)**	**515 (14.68)**	**114 (3.25)**
Mouth aspirator distribution	**2782 (96.63)**	**97 (3.37)**	-	-
Methods distribution	**2879 (82.07)**		**515 (14.68)**	**114 (3.25)**

Table 3. Terrestrial and aquatic habitats where the species were collected.

Species	Adult sampling																Larval and pupa sampling									
	Indoor														Outdoor											
	Tree hollow	Concrete structure	house	Barrel (without water)	Hospital	Cow barn	Cafe	Sheep barn	Well (without water)	School	Bus	Restaurant	Hennery	public WC	Forest	Bus station	Waste water channel	Marsh	Paddy irrigation canal	Paddy field	Corn irrigation canal	Ovitrap	Cattle urine collection duct	Leaking trough water	Puddle	Water trough
An. maculipennis s.l.*	X	X		X		X		X	X	X			X	X					X					X		
An. sacharovi		X				X			X																	
Ae. caspius	X	X	X	X		X	X	X		X	X	X		X	X			X		X	X				X	
Ae. geniculatus										X					X											
Ae. rusticus																X										
Ae. vexans															X											
Cx. pipiens s.l.	X	X	X	X	X	X		X	X	X			X	X	X		X	X		X		X	X	X	X	X
Cx. theileri																								X		
Cs. annulata		X								X																
Cs. longiareolata		X								X																
Ur. unguiculata		X								X														X		X

Adults of *Anopheles sacharovi* were collected by mouth aspirator in 3 localities; Balkan Campus (loc. no. 3/n=1), Pazarkule (loc. no. 18/n=1), and Yenikadın village (loc. no. 25/n=1).

No egg, larva and pupal exuviae of *Ae. albopictus* were found as a result of the control of ovitraps placed at 20 points in 7 localities during the period of 20 July-30 September. In addition, after the ovitraps control procedure, no adult forms were found in the controls made with a mouth aspirator around the ovitraps. In the ovitraps we placed in Karaağaç and Kapıkule, *Cx. pipiens* larvae were detected instead of the targeted species *Ae. albopictus.*

According to the monthly count, the most adult sampling was carried out in December. Sampling could not be carried out as samples were not found in February and March (Table 4).

Discussion

In this research a total of 11 species was identified under two genera belonging to two subfamilies (Anophelinae and Culicinae). A total of 3155 females and 353 males were collected belonging to *Anopheles maculipennis* s.l. (except *Anopheles sacharovi*), *Anopheles sacharovi*, *Aedes caspius*, *Aedes geniculatus*, *Aedes rusticus*, *Aedes vexans*, *Culex pipiens* complex s.l., *Culex theileri*, and *Culiseta annulata*. The data revealed that *Ae. caspius*, *An. maculipennis* s.l. and *Cx. pipiens* s.l. are the most widespread species. Any biological stages of *Aedes albopictus*, whether eggs, larvae or adults, were not found in the research area.

In the last 10 years before this study, *An. sacharovi* was recorded in two localities in Enez district (Enez-İpsala road and Gala Lake side), one locality in the central district (Üyüklütatar village) (Çağlar *et al*. 2008) and İpsala district (Sevgili & Simsek 2012). During this study, *An. sacharovi* was identified in 3 localities (Balkan Campus, n=1/locality no. 3; Pazarkule, n=1/locality no. 18; and Yenikadin village, n=1/locality no. 25) in the central district of Edirne indicating that the species is distributed throughout the province, albeit at low population levels. These findings and the previous records, which have been reported mostly from Anatolian and lesser European parts (Çağlar *et al.* 2008, Simsek *et al.* 2011, Sevgili & Simsek 2012, Günay 2015, Yavaşoğlu *et al.* 2019), have shown that *An. sacharovi*, the primary malaria vector in Turkey (Alten *et al.* 2000, Özbilgin *et al.* 2011), continues to exist. Although malaria has been largely eliminated in Turkey, it is known that endemism continues in the south-eastern Anatolia Region (Akıner & Çağlar 2010). All these facts indicate that the risk of local malaria outbreaks continues due to the increasing pressure of irregular migration movements from malaria endemic countries and increasing average air temperatures (WHO 2013).

Table 4. Monthly count of adults collected during the period of July 2017-July 2018.

Months	Total mosquito count	Mosquito species	Number of mosquitoes (♀-♂)
July 2017	183	*Ae. caspius*	128 (102-26)
		Ae. geniculatus	4 (4-0)
		An. maculipennis s.l.*	9 (7-2)
		Cx. pipiens s.l.	42 (34-8)
Aug 2017	700	*Ae. caspius*	69 (58-11)
		An. sacharovi	1 (1-0)
		An. maculipennis s.l.*	447 (427-20)
		Ur. unguiculata	4 (4-0)
		Cx. pipiens s.l.	175 (118-57)
		Cx. theileri	4 (4-0)
Sept 2017	271	*Ae. caspius*	4 (3-1)
		An. sacharovi	2 (2-0)
		An. maculipennis s.l.*	75 (45-30)
		Cx. pipiens s.l.	186 (116-70)
		Ur. unguiculata	4 (3-1)
Oct 2017	247	*Ae. caspius*	7 (5-2)
		Cs. longiareolata	1 (0-1)
		Cx. pipiens s.l.	239 (134-105)
Nov 2017	242	*Ur. unguiculata*	2 (2-0)
		An. maculipennis s.l.*	64 (64-0)
		Cs. longiareolata	2 (0-2)
		Cs. annulata	2 (2-0)
		Cx. pipiens s.l.	172 (158-14)
Dec 2017	1690	*Ur. unguiculata*	1 (1-0)
		An. maculipennis s.l.*	51 (51-0)
		Cs. annulata	1 (1-0)
		Cx. pipiens s.l.	1637 (1635-2)
Jan 2018	1	*Cs. annulata*	1 (0-1)
Feb 2018	-	-	-
Mar 2018	-	-	-
Apr 2018	34	*Ae. geniculatus*	1 (1-0)
		Cx. pipiens s.l.	33 (33-0)
May 2018	61	*Ae. caspius*	3 (3-0)
		Ae. vexans	2 (2-0)
		Ae. geniculatus	2 (2-0)
		Ae. rusticus	2 (2-0)
		Cx. pipiens s.l.	52 (52-0)
June 2018	79	*Ae. caspius*	6 (6-0)
		Ae. vexans	15 (15-0)
		Cx. pipiens s.l.	58 (58-0)
Total	3508		

It is important to examine the question of whether the malaria vector *Anopheles* detected so far in Eastern Thrace is capable of carrying *P. vivax* and *P. falciparium* parasites. With this study, when historical and recent studies are evaluated, there is no doubt that *An. sacharovi* stands out compared to other *Anopheles* species. In experimental infection carried out in laboratory conditions, *An. sacharovi* has been shown to be highly susceptible to *P. vivax* strains originating from Africa, Asia and South America. In experiments investigating the development cycle of imported *P. falciparum* strains, parasites did not develop in most experiments, but oocysts and sporozoites were found in mosquitoes in some experiments (Daskova & Rasnicyn 1982). Again, it has been stated that the species is highly sensitive to European strains of *P. falciparum* (Alten & Çağlar 1998). In the comprehensive malaria surveillance screening conducted throughout Edirne between 1994 and 2002, *P. vivax* was detected in 280 of the 317,087 blood donors selected from military unit personnel and local people, and *P. ovale* was found in one student from Afghanistan. In a study, in which it was stated that domestic cases had not been seen since 1998, it was shown that external cases were caused by military personnel, especially from the south-eastern Anatolia Region (Ay *et al.* 2002). On the other hand, in 1999 in Aydın (Ertuğ *et al.* 2002) and between the period of 2013 and 2014 in Bursa (Alver & Ener 2018), *P. vivax* was registered in a person who was reported to have come to these provinces from Edirne. According to Edirne Provincial Health Directorate data, *P. vivax* originated from Afghanistan in 2015 and from Pakistan in 2016. According to Trakya University Hospital data, *P. falciparum* and *P. malariae* were detected in 5 people with a history of traveling to Burkino Faso, Angola, Ivory Coast and Equatorial Guinea in the 2013-2016 period (Figen Kuloğlu, pers. comm.). Between 2011 and 2012, in Greece, bordering Edirne province, 38 imported malaria cases among immigrants from malaria endemic countries and 46 local *P. vivax* malaria cases broke out in the local population. It has been suggested that this transmission is due to the interaction of *An. sacharovi*, which is common in the area where malaria cases occur and throughout Greece, and immigrants (Tseroni *et al.* 2015). Under this circumstance, if malaria is not followed up on irregular migrants crossing to Europe using Edirne as a gateway, and especially on military personnel coming from the south-eastern Anatolia Region, the risk of malaria cases breaking out in the province in the coming years because of their association with *An. sacharovi* should not be ignored. Although faunistic studies on *An. sacharovi* mostly focus on the southern parts of Turkey, investigation of the status of *An. sacharovi* populations in the north-western (Marmara Region) part in detail is important for public health since it is the last stop for irregular immigrants before they leave for Europe, and they stay in this region longer than other regions. Moreover, since the climate in the north-western parts have become continental recently and temperatures have increased, it is predicted that the risk will increase in the future for *P. falciparum* and *P. vivax* as the development cycle in the mosquito becomes shorter and conditions become more suitable for these two malarial parasites. The fact that *An. sacharovi*, which has an anthropophilic character, is a vector that cannot be ignored in the transmission of the malaria parasite (Alten & Çağlar 1998), and although the number of cases in the province is low, necessary surveillance programs for vector *Anopheles* species must be followed due to the risk that exogenous cases may turn into local cases.

Aedes albopictus is the major invasive mosquito species (Medlock *et al.* 2015). It was first recorded in Europe in 1979 in Albania and in 1990 in Italy. Today, it is found in more than 25 countries in the region including Bulgaria, Greece, Romania, Serbia and other Balkan countries, Italy, France, Germany, Malta, Sicily, Spain, Switzerland, Russia's Black Sea coast, Georgia, Lebanon, Israel, Syria, Saudi Arabia, and Yemen in the Middle East (Medlock *et al.* 2015, Akiner *et al.* 2016). In Turkey, established colonies were found in the Eastern Black Sea region (Akiner *et al.* 2016), almost along the entire Black Sea coastline of Thrace and Istanbul (Şakacı 2021). Previously, *Ae. albopictus* eggs were encountered in Keşan and İpsala districts of Edirne during a field surveys carried out in 2011 using ovitrap (Oter *et al.* 2013). However, in the surveys conducted in the following years targeted to the adult mosquito, no specimens were encountered in the same region (Sırrı Kar, pers. comm.). In 2018, a species was found in a locality with intense mosquito complaints on the Marmara coast of Kocaeli (Şakacı 2021). Models created for the whole of Europe demonstrated that particularly the coastal parts of the Marmara Region, the whole of Thrace, the entire Black Sea coast, and the Eastern Mediterranean Region (seacoasts of Adana, Hatay, Osmaniye) are ideal residential areas for *Ae. albopictus* (Cunze *et al.* 2016). Although its role in nature for many disease agents has not been fully elucidated, *Ae. albopictus* has the potential to transmit at least 32 viruses, including DENV (Dengue fever virus), CHIKV (Chikungunya fever virus), WNV (West Nile fever virus), ZIKV (Zika virus), and YFV (Yellow fever virus) (Gratz 2004, Paupy *et al.* 2009, Vanlandingham *et al.* 2016). In our study, *Ae. albopictus* eggs, larvae, pupae and adults were not found. Although Edirne has suitable areas (especially Karaağaç, Sarayiçi) specified in the literature (Thavara *et al.* 2001, Paupy *et al.* 2009, Higa 2011, Little *et al.* 2017), it seems that the species does not have a resident population. In the light of all these data, periodic follow-up of the species, which has entered Edirne before and has not established colonies in the province today, should be carried out in the areas where it can be reintroduced in the future.

In this study, adults of *Ae. caspius* were found in May, June, July, August and September, and larvae were found in October. Our findings were compared with a study investigating the seasonal population dynamics of the mosquito fauna in the Po delta of Italy (Verenossi *et al.* 2012), where rice cultivation is carried out intensively just like Edirne. It has been stated that *Ae. caspius* can feed on humans and animals in rural and urban areas (Gutsevich *et al.* 1974). In the study area, it is not surprising that the species shows both resting and feeding behaviour on human and even ungulates in indoor places (i.e., house, cow barn, sheep pen, school, restaurant, coffee shop, public toilet, bus) when temperature and humidity parameters affect the species negatively. As a matter of fact, intense mosquito complaint phone calls, petitions and e-mails were sent to Edirne Municipality Public Health Unit between 15 July 2017 and 10 August 2017.

When the results of our field studies are evaluated, it can be seen that the mosquito problem that continues from the second half of July to August is clearly caused by *Ae. caspius* bites. In the first half of August, *Ae. caspius* which does not exist outdoors, turns completely indoors and therefore may have been the cause of complaints to the unit throughout August, if indoor bites were not carried out by another species.

During July, large-scale mass attacks caused by *Ae. caspius* raise the question of where the species' breeding habitat is. As a matter of fact, as a result of the larvae samplings carried out in July, 59 mosquito larvae were detected in 100 ml of water sample taken from the rice field of Karakasım Subdistrict (loc. no: 14), and these larvae were almost monoculture *Ae. caspius* species (36♀/19♂ *Ae. caspius*, 3♀/1♂ *Cx. pipiens* s.l.). Even in such a small sampling of rice water, the large number of *Ae. caspius* larvae points out that the primary source of the *Ae. caspius* problem is the paddy fields, which are represented by very high decare areas in Edirne. In the province, the level of the *Ae. caspius* population is closely related to the water yield to the paddy pans and the water cut periods. The first water is given to the paddy pans in May, and this management seems to be the first spark for the population of the species. The highest water yield occurs in July first and August second. With the increase in the amount of water used in July, the increase in the population level of the species was clearly reflected in the field. As the temperature increased and humidity decreased in August, an increase in the population level was detected indoors due to the tendency of the species to sheltered areas. In September, the water yield to the pans is cut off and the harvest time of the paddy begins at the end of September and the first week of October (Tuna 2012). Among the species identified, *Ae. caspius* is the main species that bites people outdoors, especially in July. Intensive paddy cultivation was carried out and in Edirne, where the *Ae. caspius* reproduces so much in rice, plans should be developed to save people from the disturbance caused by the species. In the solution of this problem, the approach to be followed in the paddy fields will be decisive.

During the study, mosquitoes were collected from indoors using aspirator immediately after periods of extremely dry and rapid rain transitions. During these periods, it has been observed that mosquitoes gather by moving indoors. It was observed that the samples obtained using this collecting approach, which was carried out with aspirator, were morphologically suitable for species identification. It has been observed that indoor collections made in extreme weather conditions allow mosquitoes to congregate indoors, thus providing access to a dense mosquito population of the region. In addition, it was observed that unexpected species were encountered in unexpected periods, as many local mosquito species that do not normally use indoors due to challenging weather conditions took shelter indoors. Indeed, in such a period *An. sacharovi* was found inside a reinforced concrete structure. In addition, even outdoor mosquitoes such as *Ae. caspius* which has a strong exophilic character have been found indoors (Gutsevich *et al*. 1974). If the suction tube is combined with other mosquito trapping methods, it is likely that it is an instrument that can increase the success of the detection of important vectorial species and shorten the detection time.

Conclusion

With the predicted global temperature increase in the future, Edirne is expected to be an effective gateway for the entry of malaria agents such as *P. vivax* and *P. falciparum* to the Thrace Region and subsequently to Europe. An organized mosquito monitoring and control program is urgently needed in the region where many disease agents could be established in the area.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

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