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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

The Distribution and Relative Abundance of the Mosquıto Specıes in Eastern and Middle Black Sea Area

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Abstract: Mosquitoes (Diptera: Culicidae) are among the most important vectors for the transmission of medically significant pathogens and parasites to humans and animals. This study aimed to determine the species composition, distribution, and relative abundance of mosquito fauna in the Eastern and Middle Black Sea regions of Türkiye between 2014 and 2022. A total of 46081 adult specimens were collected from 1700 sampling points in 513 localities across 14 provinces using larval dipping, light traps, BG-Sentinel traps, and human landing catches. Morphological identification revealed 31 mosquito species belonging to six genera: *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Orthopodomyia*, and *Uranotaenia*. *Aedes albopictus* was the most abundant species (40.5%), followed by *Culex pipiens* s.l. (37.3%) and *Anopheles maculipennis* s.l. (5.75%). The highest species richness was observed in Rize, Artvin, and Samsun, while Ordu and Amasya showed the lowest diversity indices. The widespread occurrence of the invasive *Aedes albopictus* and its rapid expansion across the region highlights the increasing risk of arboviral transmission, particularly for West Nile virus, dengue, and chikungunya. Climatic factors, vegetation types, land use, and vertebrate host availability appeared to influence spatial distribution and diversity patterns. The findings underline the necessity for continuous entomological surveillance and vector control strategies to mitigate potential mosquito-borne disease risks in this ecologically sensitive region.

Keywords: *Aedes albopictus*, black sea region, mosquito fauna, species richness, Türkiye, vector ecology.

Doğu ve Orta Karadeniz Bölgesindeki Sivrisinek Türlerinin Dağılımı ve Göreceli Bolluğu

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Öz: Sivrisinekler (Diptera: Culicidae), tıbbi açıdan önemli patojen ve parazitleri insanlara ve hayvanlara bulaştıran en önemli vektörler arasında yer almaktadır. Bu çalışma, 2014 ile 2022 yılları arasında Türkiye'nin Doğu ve Orta Karadeniz bölgelerindeki sivrisinek faunasının tür bileşimini, dağılımını ve göreceli bolluğunu belirlemeyi amaçlamıştır. Toplam 14 ilde, 513 lokalitede 1700 örnekleme noktasından larva kepçeleme, ışık tuzakları, BG-Sentinel tuzakları ve insan üzerine konma yöntemleri kullanılarak toplam 46.081 ergin birey toplanmıştır. Morfolojik tanımlama sonucunda *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Orthopodomyia* ve *Uranotaenia* olmak üzere altı cinse ait 31 sivrisinek türü belirlenmiştir. En bol tür *Aedes albopictus* (%40,5) olup, bunu *Culex pipiens* s.l. (%37,3) ve *Anopheles maculipennis* s.l. (%5,75) takip etmiştir. En yüksek tür zenginliği Rize, Artvin ve Samsun illerinde gözlenirken, Ordu ve Amasya illeri en düşük çeşitlilik indekslerini göstermiştir. İstilacı *Ae. albopictus*'un yaygın olarak görülmesi ve bölge genelinde hızla yayılması, özellikle Batı Nil virüsü, dang humması ve chikungunya açısından artan arboviral bulaşma riskine işaret etmektedir. İklimsel faktörler, bitki örtüsü tipleri, arazi kullanımı ve omurgalı konakların bulunabilirliği, mekânsal dağılım ve çeşitlilik desenlerini etkilemiş görünmektedir. Bulgular, bu ekolojik olarak hassas bölgede potansiyel sivrisinek kaynaklı hastalık risklerini azaltmak için sürekli entomolojik sürveyans ve vektör kontrol stratejilerinin gerekliliğini vurgulamaktadır.

Anahtar Kelimeler: *Aedes albopictus*, karadeniz bölgesi, sivrisinek faunası, Türkiye, tür zenginliği, vektör ekolojisi.

INTRODUCTION

Mosquitoes are among the most significant arthropods because they can transmit medically important pathogens and parasites to humans and animals (Azari-Hamidian et al., 2019). In the last decade, the incidence of mosquito-borne diseases has increased in parallel with expanded trade and transportation, climate changes, and invasive mosquitoes settling in new areas (Petrić et al., 2014;

Weaver & Reisen, 2010). Due to their vectorial competence and ability to adapt to new areas, mosquito survey studies are the most important step to preventing disease and vector control studies (Şakaci & Çamlitepe, 2022). 63 mosquitoes were reported in Türkiye up to 2016 (Akiner et al., 2016; Demirci, 2021; Günay, 2015). In 2017, two new species were reported in Adana from Incirlik Air Base and so the fauna of Türkiye was started to represent by 65 mosquito species (Reeves et al., 2017). The number of mosquito species in Türkiye is expected to increase gradually. Although many studies on mosquito fauna have been conducted in Turkey, including local and national (Günay, 2015; Parrish, 1959; Ramsdale et al., 2001; Şakaci & Çamlitepe, 2022), the mosquito fauna of the Black Sea region has not been studied extensively studied (Failloux et al., 2017). On the other hand, in the eastern Black Sea region, local *Cx. pipiens* s.s. and invasive *Aedes* species have been studied in detail (Akıner et al., 2022; Demirci et al,, 2021; Öztürk & Akiner, 2023a, 2023b).

The Eastern Black Sea region provides favorable climatic parameters for mosquito development and allows establishment and introduction of invasive species (Akiner et al., 2016; Merdivenci, 1984). On the other hand, the Black Sea region has one main bird migration route (Artvin-Borçka) and one secondary bird migration route (Özkan, 2019). It has become a favorite many tourists related to the highland areas and favorite climate condition in the summertime (Akıner et al., 2022).

The presence of mosquito-borne diseases has been previously reported in Türkiye and Black Sea Region. Malaria has affected almost the entire Anatolian geography since ancient times. Despite being eliminated many times, it has re-emerged when the fight against malaria has been interrupted (Piyal et al., 2013). The last malaria cases were reported again in 2010 in southeastern Anatolia (Şanlıurfa Diyarbakır Mardin) (Özbilgin et al., 2011). Many transport malaria cases have been reported since then. West Nile Virus infection has been shown in Türkiye since then 1970s, and some human cases reported resulting in death in 2010 (Kalaycioglu et al., 2012). Furthermore, WN virus detected in the Black Sea region recently in *Ae. albopictus* and *Cx. pipiens* (Akıner et al., 2019). Dengue occurred in 6 provinces (İzmir, Manisa, Antalya, Çanakkale, Istanbul, and Trabzon) between 1889 and 1945 years (Schaffner & Mathis, 2014). On the other hand, transport positive cases of dengue, zika and chikungunya have been reported in Türkiye (Ergunay et al., 2010; Sezen et al., 2018; Yağcı et al., 2012).

This study aims to determine species composition and distribution areas of mosquito species in the region by collecting larvae, pupae and adult samples of mosquito species found in the eastern Black Sea region of Türkiye. Thus, this data may help to understand future possible risks of mosquito-borne diseases in the area.

MATERIAL AND METHOD

Materials and Methods

Study Area: The study area is in the northeastern part of Turkey and and shares a border with Georgia border is located on the easternmost coast. The area generally includes the coast and tiny inland regions. The Eastern and Middle Black Sea areas differ in climate and agricultural structure. The Eastern Black Sea region generally has a more humid climate, and its vegetation structure includes tea, kiwi, and hazelnut plantations, and dense and lush forest areas. The Middle Black Sea is relatively warmer and less humid, and vegetation generally includes agricultural areas. Two large delta areas (Kizilirmak and Yesilirmak Rivers) are in the region. Furthermore, many small river deltas are in throughout their coastal zone to the Georgian border.

Mosquito Collections, Identification: Mosquito sampling was undertaken at 1700 sampling points in 513 localities from 14 provinces of Middle and Eastern Black Sea area between active season (May to October) of 2014 and 2022 (Figure 1). Samples were collected both indoors and outdoors as larvae, pupae, and adults that are suitable for breeding of different mosquito species. Geographical information of sampling was detected in decimal degrees using a GPS device (eTrex Vista HCx, Garmin, Olathe, KS, USA). Larvae and pupae were collected using larval dipping, and adults were caught using light traps, BG-Sentinel traps, and human-landing catches (HLC) methods by using Hepa Filter Mouth Aspirators and Prokopack Aspirator (John W. Hock Company, Gainesville, FL, USA) as described by European Center of Disease Control previously (Medlock et al., 2018). Immature specimens were reared until adults emerged under laboratory conditions (at 27±1 °C, 75±5% RH, and a 14:10 (L:D) h photoperiod). All samples were identified under a computer-aided Leica Microsystem EZ4® (Leica Microsystems, Wetzlar, Germany) stereo microscope using morphological keys (Becker et al., 2010; Schaffner et al., 2001). Subsequently, all specimens were pooled according to the collection site and year, species, and stored at -80°C.

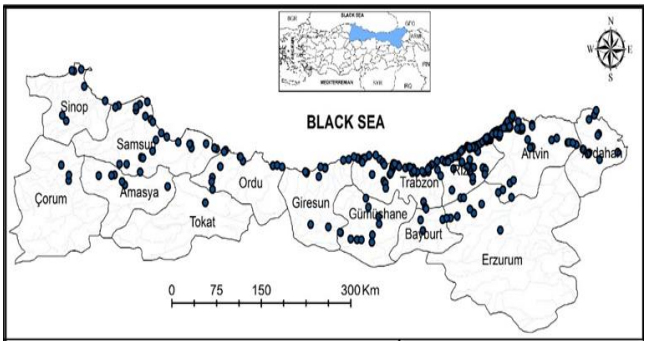

Figure 1. Study area map showing the sampling localities across 14 provinces in the Eastern and Middle Black Sea regions of Türkiye (2014–2022)

Abundance: The density (relative abundance) of mosquito species during the whole study period in the middle and eastern Black Sea area was calculated separately for total and genus based according to the following formula (Rydzanicz & Lonc, 2003):

D=l/L x 100%

Where D is the density, l is the number of samples of each mosquito species, and L is the number of all samples.

RESULTS

A total of 46081 adult mosquito specimens were collected from 14 provinces in the Black Sea Region of Türkiye. The specimens represented six genera: *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Orthopodomyia*, and *Uranotaenia*. Cumulative and city-based distribution of the species are presented in Table 1 and Figure 2. According to the data, *Ae. albopictus* was the most abundant species with 18663 individuals (40.5%) in the whole region. *Cx. pipiens* s.l. was the second most abundant species, accounting for 17184 individuals (37.29%) of all collected specimens. *Aedes rusticus* and *Culiseta fumipennis* were the least abundant species in the areas around %0.001 (Figure 2, Figure 3).

In *Aedes* genus, 9 mosquito species were identified in the area. While *Ae. albopictus* (40.50%) has the highest density, *Ae. rusticus* (0.01%) has the lowest density and was detected in low numbers only in three cities. The third least abundant species was *Aedes refiki* (0.05%), it was found only in one city (Figure 2, Figure 3).

In *Anopheles* genus, Although *An. maculipennis* s.l. (5.75%) was found most abundant species, *Anopheles plumbeus* (0.23%) was least abundant. *An. maculipennis* s.l. was distributed in all cities, *An. plumbeus* found only 6 cities in low number except Sinop (Figure 2, Figure 3).

In *Culex* genus, 12 mosquito species were identified. Although *Cx. pipiens* s.l. was most abundant species, *Culex territans* was least aboundant species in the region. It was found in four of the 14 cities. Interestingly *Culex modestus* (0.06%) was found in just only two cities. Although *Cx. territans* appear to have the lowest density, it was sampled in 4 different cities, albeit in low numbers (Figure 2, Figure 3).

The genus *Culiseta* was represented by three species in the study area. *Culiseta longiereolata* (3.51%) was found in 9 cities, *Cu. fumipennis* (0.01%) was found in one city (Figure 2, Figure 3).

Other genera, including *Coquilettidia*, *Orthopodomyia* and *Uranotaenia* represent each one species and found low density. Although *Uranotenia unguiculata* was found in four cities, *Orthopodomyia pulcripalpis* was found one and *Coquilettidia richiardii* was found two cities (Figure 2, Figure 3).

Species/City	Amasya	Ardahan	Artvin	Bayburt	Çorum	Erzurum	Giresun	Gümüşhane	Ordu	Rize	Samsun	Sinop	Tokat	Trabzon	Total collected specimens	Relative abundance in whole aera	Relative abundance in genus
AeAe	-	-	312	-	-	-		-	-	419		-	-	15	**746**	**1,62**	**3,60**
AeAl	-	-	6091	-	-	-	211	-	33	8744	118	-	-	3466	**18663**	**40,50**	**90,06**
AeCa	-	46	28	56	135	26	-	95	-	17	121	180	9	-	**713**	**1,55**	**3,44**
AeCi	-	-	-	5	-	-	6	-	-	11	-	-	-	-	**22**	**0,05**	**0,11**
AeCr	-	3	-	-	-	-	-	-	-	36	21	-	-	-	**60**	**0,13**	**0,29**
AeGe	-	29	15	-	-	-	-	-	-	-	7	-	-	-	**51**	**0,11**	**0,25**
AeRe	-	26	-	-	-	-	-	-	-	-	-	-	-	-	**26**	**0,06**	**0,13**
AeRu	-	-	-	-	3	-		-	-	1	4	-	-	-	**8**	**0,02**	**0,04**
AeVe	-	-	12	-	138	84	115	24	-	1	-	47	12	-	**433**	**0,94**	**2,09**
AnCl	-	28	56	-	-	-	3	-	16	17	4	37	-	8	**169**	**0,37**	**5,21**
AnHy	111	18	-	-	111	-	-	-	-	-	50	17	8	-	**315**	**0,68**	**9,70**
AnMa	234	283	275	145	112	141	77	164	235	67	598	259	25	39	**2654**	**5,76**	**81,76**
AnPl	-	-	1	-	-	-	8	-	-	1	4	91	-	3	**108**	**0,23**	**3,33**
CoRi	5	-	-	83	-	-	-	-	-	-	-	-	-	-	**88**	**0,19**	**100,00**
CxDe	-	-	4	7	-	13	51	-	-	6	15	-	-	-	**96**	**0,21**	**0,48**
CxHo	59	77	198	423	-	59	108	40	9	302	-	-	3	17	**1295**	**2,81**	**6,54**
CxIm	-	2	4	-	-	-	-	-	-	-	-	27	-	2	**35**	**0,08**	**0,18**
CxLa	-	-	12	-	-	-	-	13	-	79	26	-	-	-	**130**	**0,28**	**0,66**
CxMa	-	-	4	-	-	-	21	-	-	6	-	-	-	-	**31**	**0,07**	**0,16**
CxMi	41	-	102	-	-	-	-	-	-	-	6	31	12	9	**201**	**0,44**	**1,01**
CxMo	-	-	-	-	-	-	-	8	-	-	25	-	-	-	**33**	**0,07**	**0,17**
CxPe	-	-	-	-	-	-	27	-	23	3	-	-	-	-	**53**	**0,12**	**0,27**
CxPi	726	266	2772	1156	58	752	1740	1330	1326	2837	1777	1042	124	1278	**17184**	**37,29**	**86,77**
CxTe	8	-	-	-	-	-	5	8	-	2	-	-	-	-	**23**	**0,05**	**0,12**
CxTh	-	83	4	150	107	80	-	-	-	34	18	156	9	-	**641**	**1,39**	**3,24**
CxTo	-	-	9	18	-	55	-	-	-	-	-	-	-	-	**82**	**0,18**	**0,41**
CuAn	-	-	-	-	-	80	-	7	-	13	-	196	26	-	**322**	**0,70**	**16,50**
CuFu	-	-	9	-	-	-	-	-	-	-	-	-	-	-	**9**	**0,02**	**0,46**
CuLo	59	-	-	182	137	299	54	614	-	203	15	58	-	-	**1621**	**3,52**	**83,04**
OrPu	-	23	-	-	-	-	-	-	-	-	-	-	-	-	**23**	**0,05**	**100,00**
UrUn	-	-	-	-	109	111	-	18	-	-	-	-	8	-	**246**	**0,53**	**100,00**
Total	**1243**	**884**	**9908**	**2225**	**910**	**1700**	**2426**	**2321**	**1642**	**12799**	**2809**	**2141**	**236**	**4837**	**46081**	**100**	

Figure 2. Spatial distribution and relative abundance of mosquito species collected across the study area

Abbreviations in the table refer to AeAe = *Aedes aegypti*, AeAl = *Aedes albopictus*, AeCa = *Aedes caspius*, AeCi = *Aedes cinereus*, AeCr = *Aedes cretinus*, AeGe = *Aedes geniculatus*, AeRe = *Aedes refiki*, AeRu = *Aedes rusticus*, AeVe = *Aedes vexans*, AnCl = *Anopheles claviger*, AnHy = *Anopheles hyrcanus*, AnMa = *Anopheles maculipennis* s.l. AnPl = *Anopheles plumbeus*, CoRi = *Coquillettidia richiardii*, CxDe = *Culex deserticola*, CxHo = *Culex hortensis*, CxÝm = *Culex impudicus*, CxLa = *Culex laticinctus*, CxMa = *Culex martinii*, CxMi = *Culex mimeticus*, CxMo = *Culex modestus*, CxPe = *Culex perexiguus*, CxPi = *Culex pipiens* s.l., CxTe = *Culex territans*, CxTh = *Culex theileri*, CxTo = *Culex torrentium*, CuAn = *Culiseta annulata*, CuFu = *Culiseta fumipennis*, CuLo = *Culiseta longiareolata*, OrPu = *Orthopodomyia pulcripalpis*, UrUn = *Uranotaenia unguiculata*

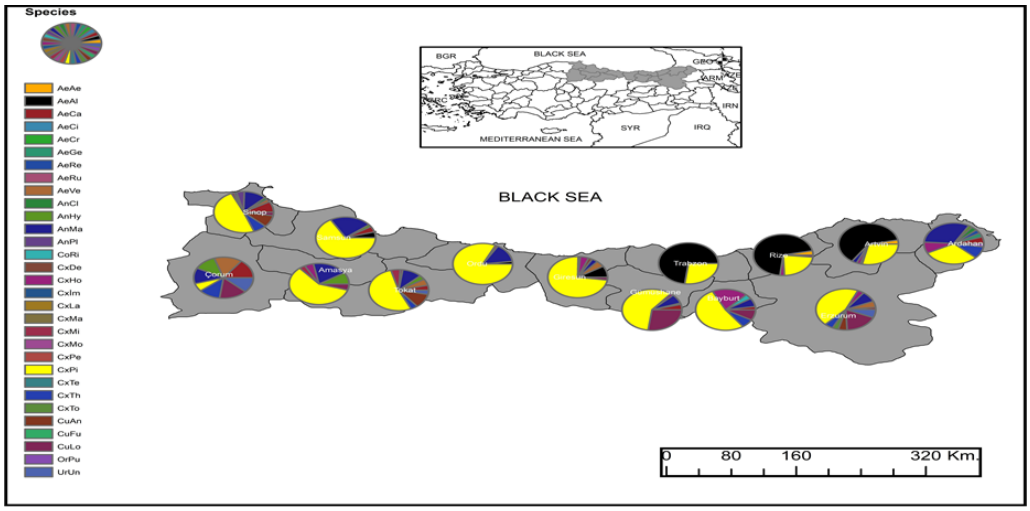

Figure 3. Proportional representation of the six mosquito genera identified in the Eastern and Middle Black Sea regions.

City Patterns of Mosquito Species Richness and Diversity Across Provinces: A total of 31 mosquito species were identified across 14 provinces, from 1700 collection points of 513 localities. Species richness and diversity indices exhibited substantial spatial heterogeneity, reflecting varying ecological dynamics across the region. The highest species richness was observed in Rize (n=20), Artvin (n=18), and Samsun (n=16). In contrast, Ordu (n=6) and Amasya (n=8) recorded the lowest richness values.

Shannon diversity index (H′), showed its highest absolute values in Çorum (H = 2.07), Ardahan (H = 1.85), and Erzurum (H = 1.82). Conversely, Ordu (H = 0.66) and Trabzon (H = 0.69) had the lowest values.

Simpson's index corroborated the Shannon-based patterns. The highest diversity (values approaching 1, indicating greater evenness) was noted in Çorum (D = 0.87), Ardahan (0.78), and Erzurum (0.75). In contrast, Ordu (D = 0.33) and Trabzon (D = 0.42) again reflected reduced species evenness, reinforcing the conclusion of localized species dominance (Table 1).

Table 1. Species richness and Shannon and Simpson diversity index across provinces.

Provinces	Total Species Richness	Number of collection points	Shanon (H)	Simpson' (D)
Amasya	8	5	1.3007	0.610287
Ardahan	12	18	1.847	0.784694
Artvin	18	103	1.08736	0.541542
Bayburt	10	8	1.50652	0.676641
Çorum	9	3	2.06884	0.871014
Erzurum	11	10	1.81626	0.753276
Giresun	13	11	1.15513	0.471816
Gümüşhane	11	10	1.22947	0.594716
Ordu	6	11	0.66282	0.326851
Rize	20	113	0.99488	0.482206
Samsun	16	25	1.22563	0.550408
Sinop	12	7	1.68087	0.724328
Tokat	10	18	1.65598	0.692968
Trabzon	9	167	0.69722	0.416726
Total	31	1700	1.35204	0.69061

DISCUSSION AND CONCLUSION

Although surveys of mosquito species of public health importance related to mosquito-borne diseases and mosquito control dated back to first decade of the Turkish republic, exact lists and first comprehensive publication released 1950s (Parrish, 1959). According to this report, Türkiye divided four topographic areas and Black Sea region is comprised three parts of it (Coastal plain, Anatolia, and Eastern Anatolia). Rainfall regime and driving climate forces of the Black Sea area are different from those in other regions and convenient of the many native and invasive species for arthropods. Thirty-one species were identified from middle and eastern Black Sea area including 14 provinces. Although nine species belong to *Aedes*, four species *Anopheles*, twelve species *Culex*, three species Culiseta genera, three genera represent one species each (*Coquilettidia*, *Orthopodomyia*, and *Uranotenia*).

Most important finding is invasive species distribution and relative abundance in the area (most abundant species in whole region). *Ae. albopictus* was first recorded in the eastern part of the area in 2015 and just only found three cities (Akiner et al., 2016). It spread all eastern and middle parts of the Black Sea coastal area except Sinop for the next seven years. Although *Aedes aegypti* was also found in this area and recorded together with *Ae. albopictus* for the first time in 2015, it hadn't been shown spreading pattern coastal or inland area (Demirci et al., 2021; Öztürk & Akiner, 2023a). Historically, malaria is an important parasitic disease and West Nile virus at least as important as malaria in these region (Evered and Evered, 2011; Kalaycioglu et al., 2012). Our findings indicate that the second and third most prevalent species were *Cx. pipiens* s.l. and *An. maculipennis* s.l. in this area. Furthermore, Akıner et al. (2019) reported that the *Ae. albopictus* potential for WNV circulation and their findings about the specimens showed has a great potential of the WNV circulation of the area after two-year introduction. Last situation of the three species indicated that the dynamics and distribution patterns of these species in the area should be carefully monitored.

Many entomological surveys have been conducted in the Türkiye but mosquito surveys very restricted and generally one species/group related to the perceived medical, epidemiological importance (Akiner et al., 2016; Akıner et al., 2022; Demirci et al., 2021; Öztürk and Akiner, 2023a, 2023b). Species distribution is influenced by various parameters such as temperature, humidity, and geographic barriers of the area. Furthermore, one of the most important parameters of the mosquito distribution is vertebrate host distribution and abundance (Muja-Bajraktari et al., 2019). The diversity indexes showed difference between the cities and inland areas results found higher than coastal areas. This result may be related to the differences inland and coastal habitat, although the number of species found is higher in coastal cities. Land use and area dynamics in the coastal area are very different from the inland areas. Fragmented habitat structures in the inland areas may be supporting this richness together with vertebrate host richness and distribution. Devi and Jauhari (2005) and Muja-Bajraktari et al. (2019) also indicated that this situation and climatic parameters effects mosquito distribution. The mosquito fauna comprises 65 mosquito species in Türkiye and additionally two biotypes of *Cx. pipiens* s.l., plus unconfirmed *Aedes koreicus* situation increased 68 species according to the last records (Akiner et al., 2016; Günay, 2015; Ramsdale et al., 2001; Reeves et al., 2017). Study area includes nearly half of the species and includes four most important vector species although *Ae. aegypti* distribution and abundance very restricted. Kuçlu and Dik (2018) described 13 species belonging to 4 genera in the Western Black Sea region. Results of this study showed species similarity except two *Aedes* species (*Aedes flavescens*, *Aedes pullatus*). They also find most abundant species is *Ae. caspius*. This difference may be related to the collection procedures, collection areas profile and limited time of their study. Interestingly they found *Cx. pipiens* forth most abundant species in this study in contrast to our study. In another study conducted in the eastern part of the country (neighboring cities of our study) showed that the two different profiles of the mosquito composition (Demirci, 2021). Demirci, (2021) described 12 species belonging to 5 genera were sampled and different species compositions were determined in two different areas (11 species in Aras valley, 7 species in Kars-Ardahan plateau). Furthermore, this study showed that *Aedes caspius* most abundant species for Aras valley, and *Culex theileri* is the most abundant for Kars-Ardahan plateau. *Ae. caspius* is the seventh most abundant species in our area in contrast to the above-mentioned two studies. *Cx. theileri* ranked (eighth) after *Ae. caspius* for abundance in our study area. Area's condition and elevation may be shown in this pattern. Although these study areas ranged from 812m to 2238m, our study areas ranged from 0m to 2000m height. All described species were found in our area except *Culiseta subochera*. Although, we found almost three times more species in the middle and eastern Black Sea area, fewer species were detected in the east and west neighboring areas.

Emerging new breeding sites related to the rainfall regime, deforestation of the areas for tea and nut farming, the emergence of new settlements and the expansion of cities can be considered the most important driving forces of the mosquito distribution and abundance in the area. Furthermore, area's location of main routes of the east west road or vice versa and tourist profiles from the mosquito borne diseases endemic countries (mostly Arabian Peninsula) make the situation more complicated for mosquito borne diseases. Therefore, surveillance and control activities are the most important topic for the public health specialist in this area. Prediction of the climate scenarios, invasive species distribution of the many areas showed that the urgent action of the areas for future perspectives.

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