

Culicoides Latreille, 1809 (Diptera: Ceratopogonidae) species in the Western Black Sea Region of Turkey, new records for the Turkish fauna

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Abstract: This study was carried out to detect biting midges of the *Culicoides* (Diptera: Nematocera: Ceratopogonidae) fauna in the Western Black Sea Region of Turkey between June and August 2014. In this study, 34,047 *Culicoides* samples were captured and 35 *Culicoides* species were identified. *C. obsoletus* (Meigen), 1818 was found to be the most dominant species. It was followed by *C. picturatus* Kremer & Deduit, 1961; *C. punctatus* (Meigen), 1804; and *C. pulicaris* (Linnaeus). All of the *Culicoides* species detected in the study were reported for the first time in the Western Black Sea Region. *C. alazanicus* Dzshafarov, 1961 and *C. deltus* Edwards, 1939 (*C. lupicaris*) were recorded as new records for the first time in Turkey.

Key words: *Culicoides*, biting midges, Western Black Sea, Fauna, Anatolia, Turkey

1. Introduction

Culicoides biting midges are bloodsucking insects and they disturb human beings and animals by causing allergic reactions in the host. They also decrease animal products and kill animals as they transmit diseases (1–5).

The number of the studies conducted to detect *Culicoides* species in Turkey increased in the last 25 years. Serious efforts to identify and document *Culicoides* species in Turkey began with Navai's PhD thesis (6), in which she documented the presence of 19 *Culicoides* species from Turkey. Jennings et al. (7) recorded 18 *Culicoides* species from the southwestern part of Turkey.

Dik and Dinçer (3) collected 14,098 *Culicoides* specimens from Konya Province in the Central Anatolian Region. Yılmaz (8) detected 42 *Culicoides* species in Elazığ Province in the Eastern Anatolian Region of Turkey. In a study (9) carried out on *Culicoides* species in the Mediterranean Region, 24 *Culicoides* species were detected. The *C. imicola* Kieffer, 1913 and *C. schultzei* (Enderlein), 1908 complexes were reported to be in lower numbers, while the *C. obsoletus* complex was captured in high numbers in the Aegean Region of Turkey (10). Other studies carried out in relatively restricted areas followed these studies (11,12). The results of the studies performed in Turkey demonstrated that the *C. imicola* and *C. schultzei* complexes existed in the Mediterranean and Aegean

regions while the *C. pulicaris* and *C. obsoletus* complexes were found in Central Anatolia and other parts of Turkey (13). There are two studies on the *Culicoides* fauna in the Black Sea Region of Turkey (11,14). However, they were carried out in the Central Black Sea Region, and there is no study found on *Culicoides* species in the Western Black Sea Region of Turkey. In this respect, the present study aimed to detect the *Culicoides* species in the Western Black Sea Region of Turkey.

2. Materials and methods

2.1 Localities

This study was carried out in the Western Black Sea Region of Turkey: Bolu (31.78790°E 40.76908°N, 31.78449°E 40.76591°N, 31.65090°E 40.71857°N, 32.03119°E 40.77508°N, 32.12446°E 40.75224°N), Düzce (31.23387°E 40.83242°N, 31.10784°E 41.07611°N, 31.30424°E 40.77368°N, 31.32021°E 40.78865°N), Zonguldak (31.44630°E 41.14327°N, 32.07146°E 41.44087°N, 32.08950°E 41.37797°N, 31.87624°E 41.09844°N), Bartın (32.336634°E 41.559359°N, 32.317185°E 41.587467°N, 32.280395°E 41.613904°N, 32.293438°E 41.615372°N, 32.620191°E 41.616457°N, 32.614114°E 41.566770°N), Karabük (32.615°E 41.153°N, 32.618329°E 41.220235°N, 32.269116°E 41.214819°N), and Kastamonu (33.676893°E 41.456923°N, 33.547726°E 41.210323°N, 33.629702°E

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41.242928°N, 34.133861°E 41.491733°N, 34.092790°E 41.482169°N) between June and August 2014 (Figure 1).

2.2. Methods

Onderstepoort-type light traps with white or black tubes were used for the collection of *Culicoides* samples. The light traps were placed either in or near sheep and cattle pens or poultry and turkey houses at sunset and were maintained for 1–3 days. A total of 80 light traps; 36 were used in June, 22 in July, and 22 in August, and 18 of them were placed in Bolu, 14 in Düzce, 12 in Bartın, 18 in Zonguldak, 4 in Karabük, and 14 in Kastamonu. *Culicoides* specimens were identified using a light microscope in accordance with the description and illustrations in relevant papers (8,15–22). The chi-square test was used for statistical analysis in this study.

3. Results

In this study, 34,047 *Culicoides* samples were captured and 35 species were identified. Almost all of them were females, and male specimens of *C. nubeculosus* (Meigen), 1830 and *C. obsoletus* were caught rarely. More specifically, 12,288 *Culicoides* samples were collected in Bartın, 11,743 in Bolu, 6833 in Zonguldak, 2417 in Kastamonu, 765 in Düzce, and 1 in Karabük (Table 1). As seen in Table 1, the greatest number of *Culicoides* specimens (27 species) were caught in Bolu, and Bartın (24 species), Zonguldak (23 species), Kastamonu (19 species), Düzce (18 species), and Karabük (1 species) followed, respectively. Distribution

of *Culicoides* species according to the provinces and the months is shown in Table 2. The most dominant species, *C. obsoletus*, was mostly captured in Bartın and Kastamonu in June, in Bolu in July, and in Düzce and Zonguldak in August. The second most dominant species, *C. picturatus*, was mostly caught in Bartın in June and in Bolu in July, and it was detected in lower numbers in the other provinces. The third most dominant species, *C. punctatus*, was detected in the highest number in Bartın in June, in Kastamonu in July, and in Düzce and Zonguldak in August.

The highest number of *Culicoides* specimens was collected in June, July and August followed it. Considering *Culicoides* samples captured, they were more abundant in Bartın and Kastamonu in the month of June, in Bolu in July, and in Düzce and Zonguldak in August (Figure 2).

Statistically, differences in the distribution of the *Culicoides* samples by month were found to be significant ($P < 0.05$). *C. obsoletus* was detected as the most dominant species in the study; *C. picturatus*, *C. punctatus*, *C. pulicaris*, and *C. subfasciipennis* Kieffer, 1919 followed it, respectively (Figure 3).

The greatest number of *C. obsoletus* specimens was collected in Bartın (7981), and 4302 specimens in Zonguldak, 3873 specimens in Bolu, and 1377 specimens in Kastamonu followed it, respectively. The second most dominant species, *C. picturatus*, was mostly caught in Bolu

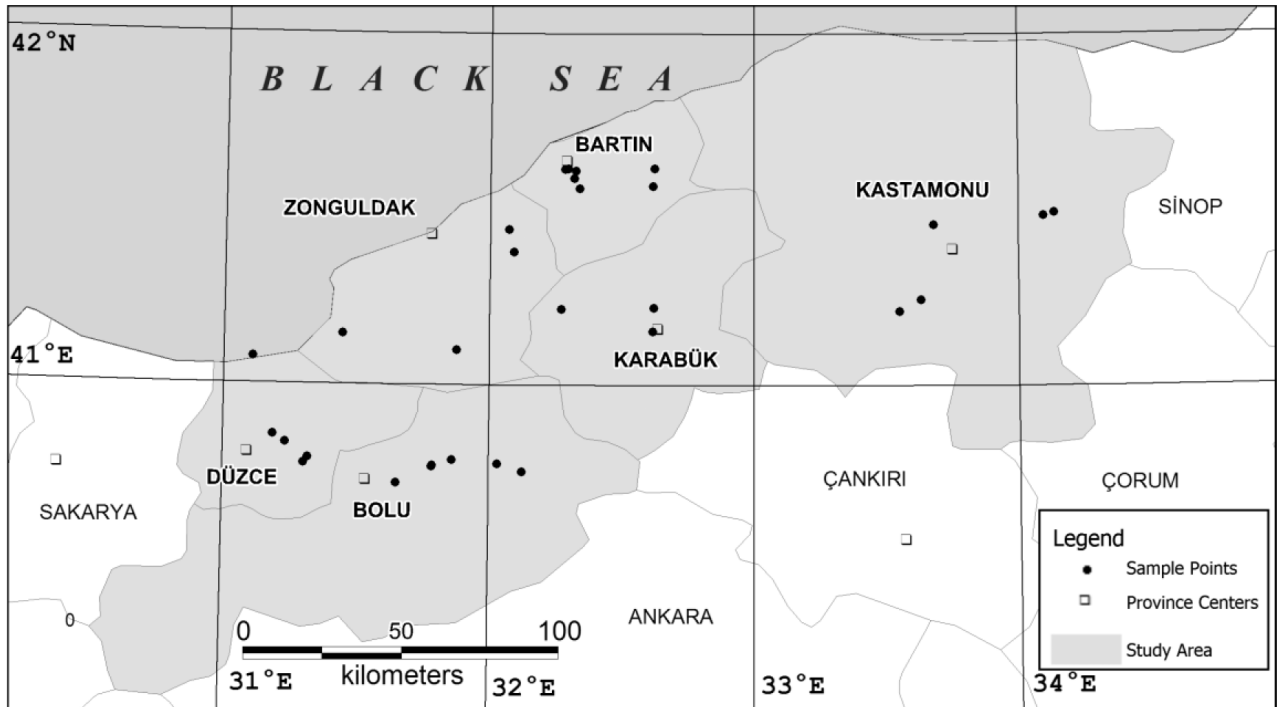


Figure 1. Outline map of the Western Black Sea Region of Turkey detailing the collection sites of *Culicoides* specimens.

Table 1. General distribution of the *Culicoides* species according to the collection centers.

<i>Culicoides</i> species	Collection centers						
	Bartın	Bolu	Düzce	Karabük	Kastamonu	Zonguldak	Total
<i>C. achrayi</i>	237	63	2	-	-	94	396
<i>C. alazanicus</i>	5	-	-	-	-	5	10
<i>C. brunnicans</i>	20	17	1	-	2	-	40
<i>C. circumscriptus</i>	81	34	-	-	9	10	134
<i>C. deltus</i>	-	-	-	-	-	124	124
<i>C. dzhafarovi</i>	1	1	-	-	-	-	2
<i>C. fagineus</i>	47	11	115	-	-	189	362
<i>C. fascipennis</i>	34	-	1	-	22	3	60
<i>C. festivipennis</i>	16	38	19	-	24	17	114
<i>C. flavipulicaris</i>	4	15	4	-	27	36	86
<i>C. furcillatus</i>	139	25	1	-	1	1	167
<i>C. gejelensis</i>	27	38	2	-	3	157	227
<i>C. imicola</i>	-	-	1	-	-	-	1
<i>C. kibunensis</i>	-	1	-	-	2	1	4
<i>C. longipennis</i>	11	5	-	-	2	2	20
<i>C. maritimus</i>	2	3	-	-	-	8	13
<i>C. newsteadi</i>	117	73	-	-	-	1	191
<i>C. nubeculosus</i>	11	39	2	-	7	4	63
<i>C. obsoletus</i>	7981	3873	508	1	1377	4302	18,042
<i>C. pallidicornis</i>	7	23	2	-	1	18	51
<i>C. parroti</i>	53	1	-	-	1	3	58
<i>C. pictipennis</i>	-	2	-	-	-	-	2
<i>C. picturatus</i>	1981	5645	2	-	75	38	7741
<i>C. pulicaris</i>	884	443	18	-	151	80	1576
<i>C. pumilus</i>	-	-	1	-	-	-	1
<i>C. punctatus</i>	592	799	82	-	691	1737	3901
<i>C. puncticollis</i>	-	2	-	-	-	-	2
<i>C. schultzei</i>	2	1	3	-	-	2	8
<i>C. scoticus</i>	-	53	-	-	-	-	53
<i>C. simulator</i>	3	-	-	-	-	-	3
<i>C. slovacus</i>	-	7	-	-	-	-	7
<i>C. subfasciipennis</i>	17	506	-	-	16	1	540
<i>C. tauricus</i>	-	-	-	-	1	-	1
<i>C. truncorum</i>	-	-	1	-	-	-	1
<i>C. vexans</i>	-	15	-	-	4	-	19
Unidentified	16	10	-	-	1	-	27
Total	12288	11743	765	1	2417	6833	34,047

Table 2. Distribution of the *Culicoides* species according to the collection centers and months.

Culicoides species	Collection centers			Bartın			Bolu			Düzce			Karabük			Kastamonu			Zonguldak				
	Months	June	July	August	June	July	August	June	July	August	June	July	August	June	July	August	June	July	August	June	July	August	
<i>C. achroyi</i>		236	1	-	4	59	-	-	-	-	-	2	-	-	-	-	-	-	-	87	7	-	
<i>C. alazanicus</i>		1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	
<i>C. brunnicans</i>		20	-	-	-	16	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. circumscriptus</i>		34	46	1	8	1	25	-	-	-	-	-	-	-	-	-	3	-	6	1	1	8	
<i>C. deltus</i>		-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	14	-	-	-	-	-	108
<i>C. dzhagarovi</i>		-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. fagineus</i>		36	9	2	-	9	2	-	-	-	-	115	-	-	-	-	-	-	-	-	1	118	
<i>C. fascipennis</i>		26	8	-	-	-	-	-	-	-	-	-	-	-	-	3	19	-	-	-	3	-	
<i>C. festvipennis</i>		3	7	6	2	-	36	-	-	-	19	-	-	-	-	3	1	20	-	-	5	12	
<i>C. flavipulicaris</i>		2	-	2	3	9	3	-	-	-	4	-	-	-	-	3	9	15	-	-	2	34	
<i>C. furcillatus</i>		137	2	-	-	25	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	
<i>C. geigelensis</i>		18	5	4	-	-	38	-	-	-	2	-	-	-	-	1	-	2	-	4	11	142	
<i>C. imicola</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	
<i>C. kibunensis</i>		-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	
<i>C. longipennis</i>		2	6	3	-	-	5	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2	
<i>C. maritimus</i>		-	2	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	
<i>C. newsteadi</i>		82	35	-	65	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	
<i>C. nubeculosus</i>		1	5	5	-	-	39	2	-	-	-	-	-	-	-	7	-	-	-	1	2	1	
<i>C. obsoletus</i>		6544	1420	17	1600	2078	195	87	199	222	1	-	-	-	-	1343	19	15	-	38	1974	2290	
<i>C. pallidicornis</i>		5	2	-	1	21	1	-	-	2	-	-	-	-	-	1	-	-	-	-	2	16	
<i>C. parroti</i>		3	46	4	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	3	
<i>C. pictipennis</i>		-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. picturatus</i>		1839	121	21	-	5641	4	-	2	-	-	-	-	-	-	27	48	-	-	8	26	4	
<i>C. pulicaris</i>		869	13	2	160	243	40	1	4	11	-	-	-	-	-	120	8	9	-	8	6	66	
<i>C. pumilus</i>		-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. punctatus</i>		431	152	9	29	381	384	4	33	45	-	-	-	-	-	40	612	39	-	-	58	1679	
<i>C. puncticollis</i>		-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. schultzei</i>		2	-	-	1	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	2	
<i>C. scoticus</i>		-	-	-	53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. simulator</i>		3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. slovacus</i>		-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. subfascipennis</i>		16	1	-	2	502	2	-	-	-	-	-	-	-	-	3	13	-	-	-	1	-	
<i>C. tauricus</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	
<i>C. truncorum</i>		-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. vexans</i>		-	-	-	-	15	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	
Unidentified		16	-	-	-	10	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	
Total		12,288			11,743		765				1					2417						6833	

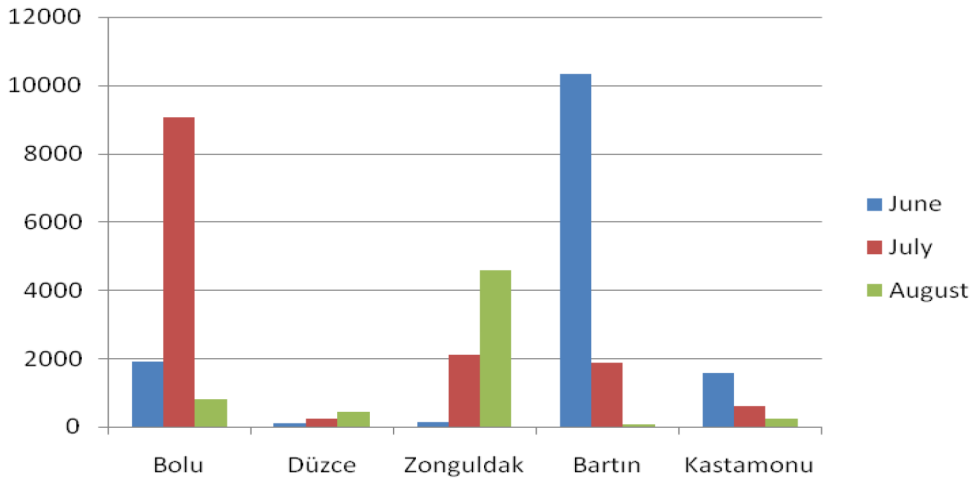


Figure 2. Distribution of the *Culicoides* species according to the collection centers and months.

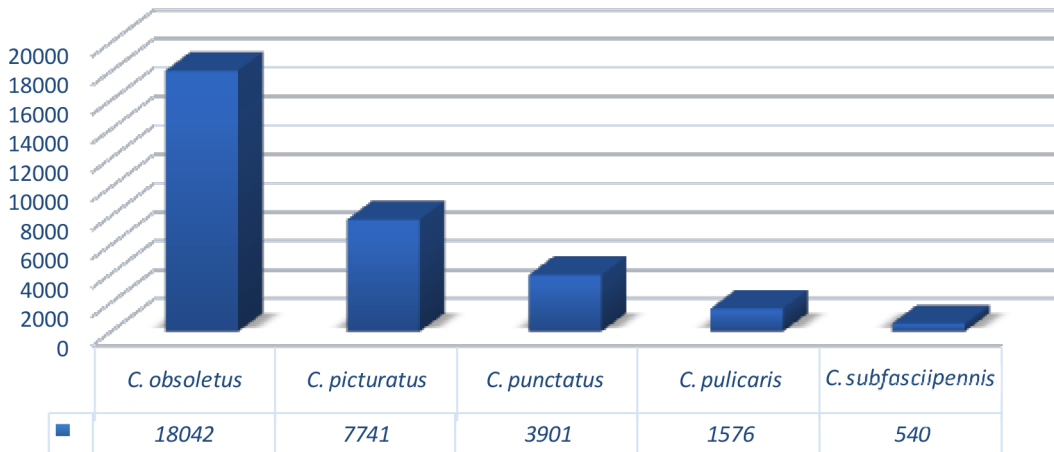


Figure 3. Domination status of the *Culicoides* species.

(5645) and Bartın (1981), and this species was detected in lower numbers in the other collecting centers. The third dominant species, *C. punctatus*, was mostly captured in Zonguldak (1737), and Bolu (799), Kastamonu (691), and Bartın (592) were the subsequent provinces.

4. Discussion

Although the *Culicoides* fauna in some regions or districts of Turkey is either not studied or studied only a little, 59 *Culicoides* species were recorded to date (14,23). There are two former studies performed on *Culicoides* species in the Black Sea Region of Turkey (11,14). In those studies, 14 (11) and 30 (14) *Culicoides* species were reported in the Central Black Sea Region, but there was no study on *Culicoides* species in the Western Black Sea Region. Therefore, this study was the first study on *Culicoides* biting midges in the region. A total of 34,047 *Culicoides* samples were caught in the Western Black Sea Region and 35 species were detected

in this study. Thus, all of the species are reported here for the first time in the Western Black Sea Region of Turkey. Thirty-three of the species had already been recorded in different regions of Turkey. *C. alazanicus* (Figure 4) and *C. deltus* [(=*C. lupicaris* Downes & Kettle, 1952) (24)] (Figure 5) were reported for the first time in Turkey in this study. However, only female specimens of these species were detected in the study. The *C. schultzei* complex and *C. imicola* complex were recorded as dominant species in the southern (9,25), southeastern (23), and Aegean (10) regions of Turkey. However, they were caught rarely in this study. *C. slovacus* Országh, 1969 was previously recorded only in Elazığ, eastern Turkey (8), and was also captured in Bolu in this study.

The females formed the largest part of the *Culicoides* samples caught in this study. However, male specimens of *C. nubeculosus*, *C. obsoletus*, *C. circumscriptus* Kieffer, 1918, and *C. gejjelensis* Dzhafarov, 1964 were detected

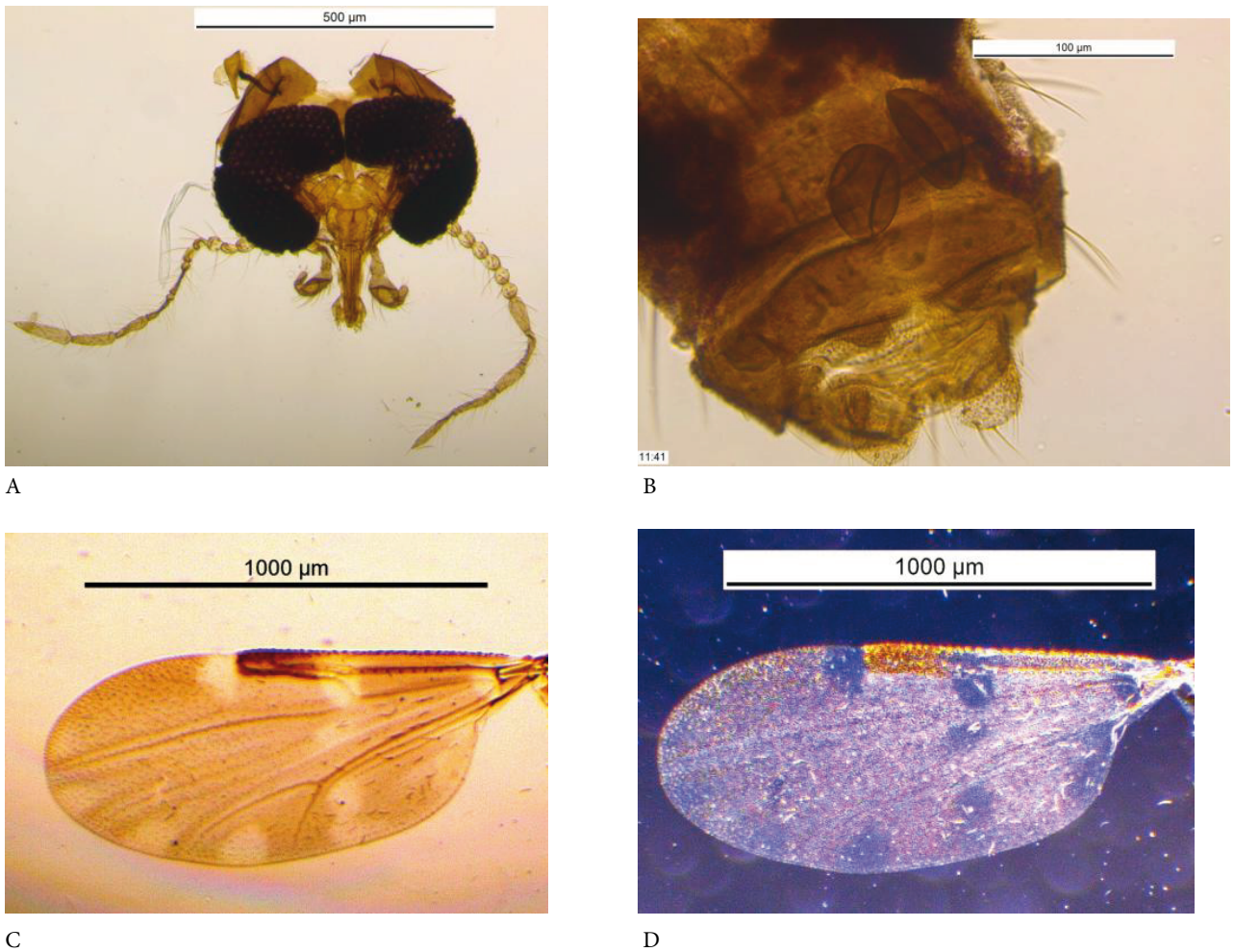


Figure 4. *Culicoides alazanicus*, female: A- head, B- posterior end of abdomen, C- wing (light microscope), D- wing (phase-contrast microscope), original.

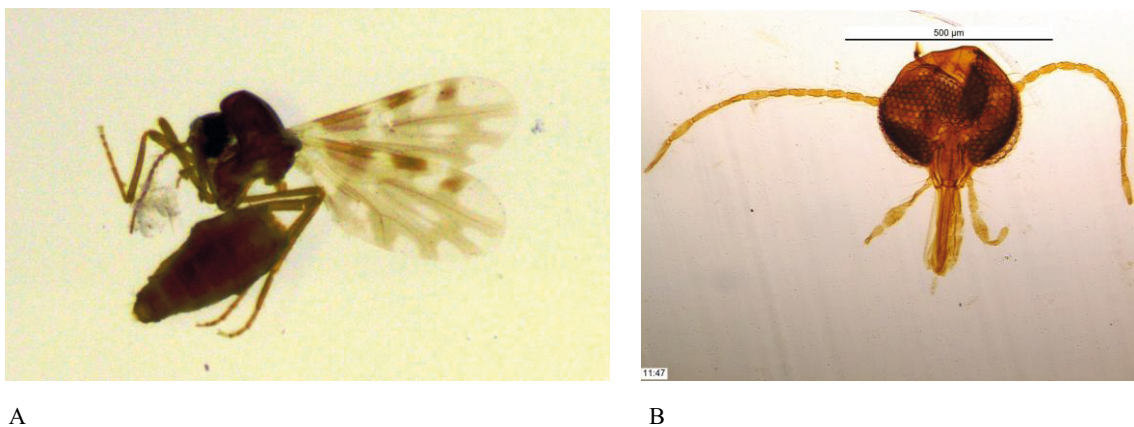


Figure 5. *Culicoides deltus* (= *C. lupicaris*): A- female, B- female, head, original.

occasionally. Females of *Culicoides* species feed on blood, while males do not take blood and feed on the nectar of flowers. The light traps were placed in or near sheep and

cattle pens and/or poultry houses. Therefore, females who came into the pens or houses for feeding were caught in greater numbers compared to males.

Most of the *Culicoides* samples were captured in Bartın, and Bolu, Zonguldak, and Kastamonu followed it. Generally, the number of the *Culicoides* species was parallel to the number of the *Culicoides* samples captured in the collecting centers, and 27 species in Bolu, 24 species in Bartın, 23 species in Zonguldak, 19 species in Kastamonu, 18 species in Düzce, and 1 *Culicoides* species in Karabük were detected. The *Culicoides* specimen in Karabük was caught in June. Light traps failed to be placed in July, and the tubes consisting of August samples collected in Yenice, Karabük, were broken during the study. For these reasons, only one *Culicoides* sample could be collected in Karabük. The sheep pen in which two light traps were placed in June was actually very suitable for breeding of *Culicoides* as thousands of *Psychoda* Latreille, 1796 (Diptera: Psychodidae) samples were captured there, so it is interesting that only one *Culicoides* specimen could be caught there in this study.

It was stated that the number of *Culicoides* increased in summer and reached the highest numbers in July in Turkey (3,26). In this study, the highest number of *Culicoides* specimens was captured in June (14,039), while 13,892 *Culicoides* specimens could be caught in July and 6116 in August. At first view, these results seem different from the findings of the previous researchers (3,26). In fact, of 80 light traps used in the study, 36 were placed in June and 22 in both July and August. Therefore, the average number of the *Culicoides* specimens was 390 in June, 632 in July, and 278 in August. These results show that *Culicoides* species have the highest flight activity in July in Turkey, as reported in the previous studies. *Culicoides* sampling in June was especially done in rainy conditions in Bolu and Düzce. However, the highest number of *Culicoides* specimens was captured in Bolu, while they were caught in lower numbers in Düzce, Zonguldak (Çaycuma), and Karabük (Safranbolu) in June. During the Bartın and Kastamonu sampling in June, the rain had stopped a few days ago and the weather was sunny, and probably those weather conditions caused the increase in the number of the *Culicoides* specimens caught in Bartın and Kastamonu. It was stated that slight breeze did not have any effect on flight activities of *Culicoides*, while they were unable to fly in strong wind and rain (17). The data given above support Dzhafarov's findings. The number of light traps used in July was lower than that of June. Moreover, sampling failed in Karabük and a light trap placed near a cattle pen was removed by the farmer in July. In spite of this, more *Culicoides* samples were caught in July due to the increase in temperature and breeding sites of *Culicoides* after the rain.

Most of the traps were placed in Bolu and Zonguldak (18 traps in each); Düzce and Kastamonu (14 traps in each), Bartın (12 traps), and Karabük (4 traps) followed them. Considering the number of light traps, the highest

number of *Culicoides* samples was captured in Bartın (877.7/trap), and Bolu (652.4) and Zonguldak (379.6) followed it. The average number of *Culicoides* samples for each trap in Bartın in June was very high, because they were mostly caught in the Balıcak and Aşağı Çerçi villages in Ulus, a district of Bartın. However, the number of *Culicoides* samples captured in Bartın in July and August decreased.

The most dominant species, *C. obsoletus* was detected in all collecting centers; *C. fascipennis* (Staeger), 1839; *C. festivipennis* Kieffer, 1914; *C. flavipulicaris* Dzhafarov, 1964; *C. furcillatus* Callot, Kremer & Paradis, 1962; *C. gejelensis*; *C. nubeculosus*; *C. pallidicornis* Kieffer, 1919; *C. picturatus*; *C. pulicaris*; and *C. punctatus* were also caught in all collecting centers except Karabük in the study. On the other hand, *C. tauricus* Gutsevich, 1959 in Kastamonu; *C. pumilus* (Winnertz), 1852 and *C. truncorum* Edwards, 1939 in Düzce; *C. pictipennis* (Staeger), 1839, *C. puncticollis* (Becker), 1903, *C. scoticus* Downes & Kettle, 1952, and *C. slovacus* in Bolu; and *C. vexans* (Staeger), 1839 in Bolu and Kastamonu were detected occasionally.

More than 50 viruses have been isolated from *Culicoides* species to date (4). The most important ones are African horse sickness virus (AHSV), bluetongue virus (BTV), epizootic hemorrhagic disease virus (EHDV), Akabane virus (AKAV), bovine ephemeral fever virus (BEFV), and Schmallenberg virus (SV) (5,23). The primary vectors of these viruses in North Africa, the Middle East, and South Europe is *C. imicola*. *C. obsoletus*; *C. scoticus*; *C. dewulfi* Goetghebuer, 1936; *C. chiopterus* (Meigen), 1830; *C. pulicaris*; and *C. punctatus* are also important vectors in Europe (27–37). During the BTV outbreak in Europe and the Thrace Region of Turkey in 1999, *C. obsoletus* was found as a common species, while the main vector of BTV, *C. imicola*, could not be found in the region or other countries (4,5). *Culicoides*-borne diseases such as BTV, AKAV, EHDV, BEFV, and SA have occurred in different regions of Turkey from time to time (38–40). *C. imicola*, *C. pulicaris*, and *C. obsoletus* complexes as important vectors of BTV were detected in different parts of Turkey (2,3,8–10,13,14,23).

There are three studies conducted on detecting vector species of *Culicoides* in Turkey (23,41). Dik et al. (41) also carried out a study to detect possible vectors of BTV and EHDV in the Mediterranean and Aegean regions of Turkey. In the last study performed to detect *Culicoides* vectors of BEFV in the southern and southeastern parts of Turkey in 2012, during a BEFV outbreak on the Syrian border, Dik et al. (23) could not detect any viral genome in any of the *Culicoides* species. However, *C. schultzei* and *C. imicola* complexes were reported to have been dominant species in these regions. Seroprevalence of BTV and AKAV in the cattle in the Central Black Sea Region of Turkey was found

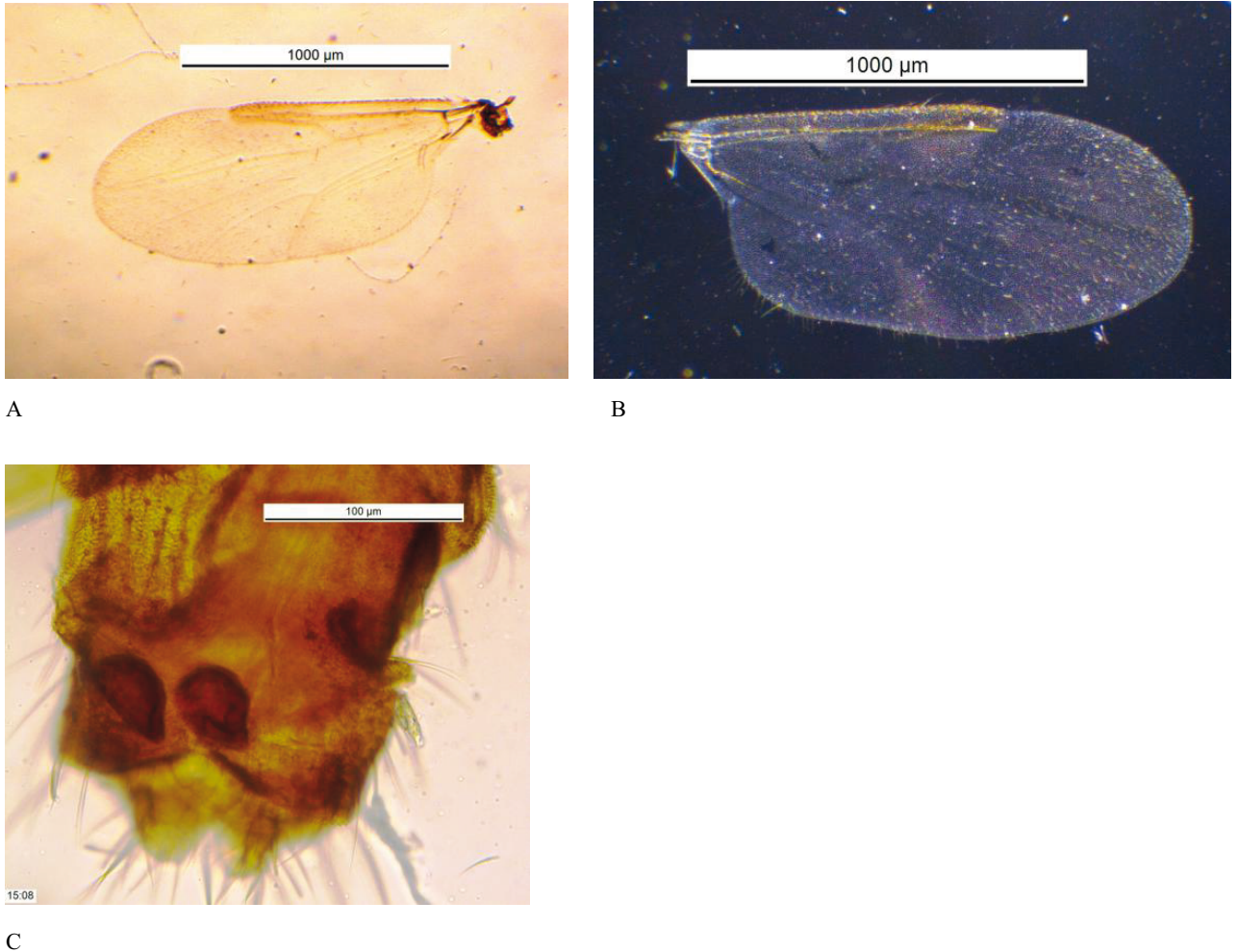


Figure 6. *Culicoides brunnicans*: A- female, wing (in light microscope), B- wing (phase-contrast microscope), C- abdominal end of female, original.

as 11% and 22%, respectively (38), but there was no study carried out on either *Culicoides* species or *Culicoides*-borne diseases in the Western Black Sea Region of Turkey. *C. imicola*, the primary vector of BTV and AKAV, and *C. schultzei*, a possible vector of these diseases, were caught in low numbers in the study. *C. dewulfi* and *C. chiopterus*, other vectors of BTV in Europe, could not be detected in this region. However, *C. obsoletus* sensu stricto (s.s.), *C. punctatus*, and *C. pulicaris* s.s, main vectors of BTV in Europe, were detected as dominant species in the Western Black Sea Region. Therefore, these species could be serving as vectors of BTV in the region.

In conclusion, among the species detected in the region by this study, *C. alazanicus* and *C. deltus* were recorded for the first time in Turkey. It was very difficult to see dark spots on the wing of *C. brunnicans* (Figure 6). For this reason, a phase-contrast microscope should be carefully used to see dark spots on the wing. In consideration of mean intensity per trap, *Culicoides* specimens were mostly captured in

July. *C. obsoletus*, *C. picturatus*, *C. punctatus*, *C. pulicaris*, and *C. subfasciipennis* were detected as dominant species in the region. These findings indicate that the common species, *C. obsoletus* s.s. and *C. pulicaris* s.s., are important risk factors for the region since they can serve as vectors in transmitting and spreading BTV and AKAV. In spite of the findings, further studies are needed not only to complete the faunistic list of the *Culicoides* fauna in Turkey but also to provide information about phylogenetic relationships among species and host-parasite associations.

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