J. Entomol. Res. Soc., 25(2): 417-438, 2023
https://doi.org/10.51963/jers.2023.98

Research Article
Online ISSN:2651-3579

New Faunal Data for Black Flies (Diptera: Simuliidae), with the Evidence of COI Sequences, from Mediterranean Region of Türkiye

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ABSTRACT

The present study provides faunal data for Turkish Black flies (Simuliidae, Diptera) from the Central and Western Mediterranean Region in Türkiye. Among the specimens collected from 221 different running water sites, 17 species in 3 genera and 5 subgenera were identified. Two species, *Simulium* (*Nevermannia*) *ibleum* (Rivosecchi 1966) and *Simulium* (*Nevermannia*) *brevidens* (Rubtsov 1956), were recorded for the first time from Türkiye, and 5 additional species were reported from the study area for the first time. The morphological identifications were tested by phylogenetic analyzes using mitochondrial cytochrome oxidase I (COI) barcode sequences. The COI analysis results overlapped with the morphotaxonomic identification results for eight of the 15 species. The first genetic data of 4 species (for World) and 5 species (from Türkiye) were stored in GenBank (NCBI).

Keywords: Simulium, Prosimulium, Anatolia, DNA barcoding, Cox1.

Şirin, D. Ü., Fidan, E. C., Çalışkan, H. (2023). New faunal data for black flies (Diptera: Simuliidae), with the evidence of COI sequences, from Mediterranean Region of Türkiye, *Journal of the Entomological Research Society*, 25(2), 417-438.

Received: January 02, 2023 *Accepted: March 20, 2023*

INTRODUCTION

Black flies (Diptera: Simuliidae) represent 26 genera and 2415 species (2398 living and 17 fossil) and have a wide geographic distribution occurring in all continents except Antarctica (Adler, 2022). These flies are key organisms in both aquatic and terrestrial ecosystems, but are perhaps best known for the blood-sucking habits of adult females. The females need blood to mature the eggs and this requirement makes this family important as biting pests and vectors of parasites and pathogens to humans and other warm-blooded animals (Crosskey, 1990). Due to the extensive cryptic speciation and high morphological homogeneity observed in Simuliidae, taxonomists emphasize that the diversity within the family has not been adequately revealed (Adler, Currie, & Wood, 2004; Andrade-Souza, Silva, & Hamada, 2017).

Türkiye, with its rich biodiversity, is still one of the countries in the Palearctic region with limited knowledge of its blackfly fauna. The first information about the Simuliidae in Türkiye was the description of *Simulium pulchripes* Austen, 1925 from Canakkale. However, until the 1990s, research on the family in Türkiye was almost nonexistent. Kazancı & Clergue-Gazeau (1990), in the first comprehensive study of black flies in the country, listed 21 species. 15 years later, 8 more species of the family were firstly reported from Anatolia by Şirin & Şahin (2005). Crosskey & Zwick (2007) published a checklist with 9 new records and distribution information, as well as all records known for the country up to that time. Çağlar & İpekdal (2009) listed 45 species and evaluated the Simuliidae fauna of Türkiye by comparing it with neighboring countries. Şirin, Çalışkan, & Şahin (2015) reported 17 species from the Turkish Thrace, with one species newly recorded for the country. In 2015, a new species belonging to the genus *Prosimulium* was described by Adler & Şirin (2015), and another new one of the genus *Metacnephia* was described by Şirin & Adler (2015). Başören & Kazancı (2016) published a checklist for the species of Simuliidae of Türkiye. An understanding of the Turkish simuliid fauna also benefited from cytotaxonomical studies (Adler & Şirin, 2014; Adler et al, 2015). In recent years, there has been an increase in studies on the fauna of Simuliidae in Türkiye. Furthermore, information about the presence and distribution of these flies in many areas of the country is still limited. The latest edition of the world Simuliidae checklist reports 57 species in Türkiye (Adler, 2022). Additionally, Fidan & Şirin (2022) added a new record for the fauna. This number corresponds to about 2.4% of all species in the family and about 9% of the Palearctic fauna. It is possible that more species of this family live in the country.

Cryptic species are common in the Simuliidae family and morphotaxonomic methods may be insufficient in the identification of some species (Adler et al, 2004). *Simulium*, the largest genus of the family, comprises many species complexes (Adler, 2022). However, cryptic taxa can display key differences that are important for ecological and epidemiological reasons for taxa whose bioindicator and vector species are widespread, such as Simuliidae. Therefore, accurate identification at the species level is very important for biomonitoring and vector control programs. In recent years DNA barcode approach has been widely used in studies focusing on phylogeny, population genetics and phylogeography in simuliids and revealing cryptic diversity (Ruiz-Arrondo et al, 2018). In DNA barcode studies, the gene cytochrome c oxidase subunit I (COI) is mostly preferred as a marker for species identifications of black flies, as in many other animals (Andrade-Souza et al, 2017).

In this study, it was aimed to determine the black fly species living in the Central and Western Mediterranean region of Türkiye by both morphotaxonomic and molecular taxonomic methods.

MATERIALS AND METHODS

Specimen collection

The study material consisted of 10605 larvae, 8792 pupae and 172 reared adults (99 males and 73 females) from 221 different running waters in the Central and Western Mediterranean region and is deposited in the Eskişehir Osmangazi University Entomology Collection. Larvae and pupae were collected into 80% ethanol for morphotaxonomic examination and absolute ethanol for molecular analyses. Reared flies with their pupal exuviae were fixed in 80% ethanol.

Sampling localities information and dates are listed in Table 1 and the positions of the sites are shown on the map in Fig. 1. Numbers on the map and in the Table 1 provide correlation with the site records listed for each species in result section.

Figure 1. Map of collecting sites for black flies in Central and Western Mediterranean Region in Türkiye.

Table 1. Collecting sites for black flies in the Central and Western Mediterranean Region of Türkiye.

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
1	Burdur	37°46’53.12”	30°22’38.91”	930	25.03.2015
2	Burdur	37°38’59.36”	30°17’4.00”	1126	25.03.2015
3	Burdur	37°38’53.35”	30°17’46.47”	1170	25.03.2015

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
4	Burdur	37°35'27.47"	30°16'46.93"	1251	25.03.2015
5	Burdur	37°33'54.21"	30°16'48.08"	1380	25.03.2015
6	Burdur	37°30'44.74"	30°18'59.11"	1379	25.03.2015 / 26.06.2015
7	Burdur	37°45'55.42"	30°23'58.20"	1121	26.03.2015 / 24.05.2015
8	Isparta	37°44'36.16"	30°28'58.42"	1269	26.03.2015 / 30.03.2016
9	Isparta	37°47'43.28"	30°46'16.06"	1581	26.03.2015
10	Isparta	37°51'34.85"	30°48'6.89"	1060	26.03.2015 / 25.05.2015
11	Isparta	37°43'13.32"	30°56'5.65"	1170	26.03.2015 / 25.05.2015
12	Isparta	37°41'18.34"	30°57'1.94"	12313	26.03.2015
13	Isparta	37°39'19.59"	30°58'37.95"	1186	26.03.2015 / 25.05.2015
14	Isparta	37°36'41.64"	30°59'21.27"	1070	26.03.2015
15	Isparta	37°35'47.65"	30°59'12.52"	1050	26.03.2015 / 25.05.2015 / 05.05.2016
16	Isparta	37°32'36.43"	30°58'44.18"	924	26.03.2015 / 25.05.2015 / 05.05.2016
17	Isparta	37°33'2.89"	30°51'2.06"	583	26.03.2015
18	Burdur	37° 7'29.30"	29°41'17.04"	1021	27.03.2015
19	Burdur	36°57'52.55"	29°46'55.08"	1371	27.03.2015 / 27.04.2015
20	Burdur	36°49'35.90"	29°42'3.43"	1490	27.03.2015
21	Antalya	36°50'2.08"	29°47'0.64"	1207	27.03.2015
22	Antalya	36°26'0.79"	29°36'2.17"	1310	28.03.2015 / 25.06.2015
23	Antalya	36°25'8.22"	29°35'54.93"	1219	28.03.2015
24	Antalya	36°19'19.87"	29°28'11.42"	973	28.03.2015
25	Antalya	36°14'51.70"	29°28'5.33"	720	28.03.2015
26	Antalya	36°28'36.94"	30° 6'13.05"	289	28.03.2015 / 23.05.2015
27	Antalya	36°28'35.07"	30° 5'49.04"	550	28.03.2015
28	Antalya	36°27'54.94"	30°20'23.74"	533	28.03.2015 / 28.03.2016
29	Antalya	36°28'5.17"	30°20'21.45"	552	29.03.2015
30	Antalya	36°30'0.46"	30°19'43.94"	750	29.03.2015 / 28.03.2016
31	Antalya	36°34'50.80"	30°22'2.07"	1130	29.03.2015
32	Antalya	36°39'24.75"	30°24'27.45"	1119	29.03.2015
33	Antalya	36°45'44.12"	30°27'5.76"	946	29.03.2015 / 25.04.2015 / 23.05.2015
34	Antalya	36°55'1.64"	31° 1'33.15"	53	30.03.2015
35	Antalya	36°58'37.57"	31°12'3.75"	34	30.03.2015
36	Antalya	36°46'58.14"	31°43'16.30"	158	30.03.2015
37	Antalya	36°48'55.70"	31°53'14.23"	773	30.03.2015 / 22.05.2015 / 24.06.2015
38	Antalya	36°52'39.45"	31°45'57.53"	487	30.03.2015 / 21.05.2015
39	Antalya	36°56'7.51"	31°45'11.29"	642	30.03.2015
40	Antalya	37° 2'16.98"	31°43'59.62"	934	30.03.2015
41	Antalya	37° 4'48.90"	31°39'24.55"	456	30.03.2015 / 23.06.2015
42	Antalya	37° 7'41.15"	31°47'53.43"	1224	30.03.2015
43	Antalya	37°10'59.07"	31°46'38.91"	1195	30.03.2015
44	Antalya	37°14'9.33"	31°46'11.78"	1233	30.03.2015
45	Konya	37°22'20.05"	31°43'0.50"	1430	30.03.2015
46	Konya	37°32'59.61"	31°34'0.50"	1192	30.03.2015
47	Burdur	37°14'57.71"	29°32'44.53"	978	1.05.2014
48	Burdur	37° 1'45.04"	29°23'14.99"	1176	1.05.2014
49	Burdur	36°59'14.31"	29°24'26.59"	1105	1.05.2014
50	Burdur	36°59'18.42"	29°32'59.44"	1248	1.05.2014
51	Burdur	36°58'51.34"	29°33'20.32"	1347	1.05.2014
52	Burdur	36°52'21.29"	29°26'18.55"	1470	1.05.2014
53	Muğla	36°49'38.58"	29°33'45.71"	1124	1.05.2014

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
54	Muğla	36°49'7.76"	29°39'1.84"	1231	1.05.2014 / 27.04.2015
55	Antalya	36°49'16.24"	29°47'37.27"	1416	1.05.2014
56	Denizli	37°29'5.26"	29°24'37.21"	873	2.05.2014
57	Denizli	37°30'53.18"	29°30'54.94"	1092	2.05.2014
58	Burdur	37°25'7.18"	28°47'47.12"	1232	2.05.2014
59	Burdur	37°20'21.13"	29°56'53.55"	1073	2.05.2014
60	Burdur	37°34'25.12"	30°25'2.63"	1166	2.05.2014
61	Burdur	37°38'47.94"	30°25'19.35"	1264	2.05.2014
62	Burdur	37°38'21.82"	30°33'37.43"	1113	2.05.2014
63	Konya	38°17'20.41"	31°26'20.34"	1481	22.04.2015
64	Konya	38°14'58.69"	31°21'48.41"	1611	22.04.2015
65	Isparta	37°56'1.51"	31°18'12.95"	1128	22.04.2015
66	Isparta	37°42'18.94"	31°25'51.01"	1169	22.04.2015
67	Antalya	37° 6'19.14"	30°56'23.68"	40	23.04.2015
68	Antalya	37° 8'53.89"	30°54'45.56"	56	23.04.2015
69	Antalya	37° 8'49.51"	30°55'58.39"	56	23.04.2015
70	Antalya	37°10'32.74"	30°56'35.69"	150	23.04.2015
71	Antalya	37°12'59.32"	30°57'36.87"	271	23.04.2015 / 25.06.2015
72	Antalya	37°13'47.95"	30°57'52.72"	301	23.04.2015 / 25.06.2015
73	Antalya	36°30'14.94"	32° 9'18.09"	380	24.04.2015
74	Antalya	36°30'9.31"	32°10'45.70"	618	24.04.2015
75	Antalya	36°26'35.09"	32°11'32.87"	26	24.04.2015
76	Antalya	36°25'56.04"	32°16'19.68"	40	24.04.2015
77	Antalya	36°29'29.71"	32° 9'0.95"	140	24.04.2015 / 24.06.2015
78	Antalya	36°28'33.18"	32°16'23.34"	182	24.04.2015
79	Antalya	36°15'25.71"	32°19'57.01"	35	24.04.2015 / 02.05.2016
80	Antalya	36°15'43.20"	32°22'49.86"	88	24.04.2015
81	Antalya	36°15'1.46"	32°24'6.90"	87	24.04.2015
82	Antalya	36°47'45.15"	30°30'9.78"	644	25.04.2015 / 23.05.2015
83	Antalya	36°46'33.08"	30°25'38.44"	972	25.04.2015 / 23.05.2015
84	Antalya	36°42'26.38"	30°26'43.55"	1233	25.04.2015 / 23.05.2015
85	Antalya	36°40'30.05"	30°25'46.20"	1127	25.04.2015
86	Antalya	36°32'53.74"	30°20'47.84"	979	25.04.2015
87	Antalya	36°28'52.78"	30°19'59.78"	524	25.04.2015 / 23.05.2015 / 28.03.2016
88	Muğla	36°28'38.43"	29°24'31.48"	122	25.04.2015
89	Muğla	36°29'2.56"	29°18'49.24"	81	25.04.2015
90	Muğla	36°37'31.33"	29°20'49.02"	119	25.04.2015 / 23.05.2015
91	Muğla	36°42'42.72"	29° 2'56.65"	13	26.04.2015
92	Muğla	36°43'51.83"	29° 1'28.97"	11	26.04.2015
93	Muğla	36°54'40.28"	28°46'22.40"	48	26.04.2015
94	Muğla	36°56'15.37"	28°48'35.53"	171	26.04.2015
95	Muğla	36°56'29.93"	28°47'59.28"	178	26.04.2015
96	Muğla	36°59'20.20"	28°38'19.88"	6	26.04.2015
97	Muğla	37° 0'54.09"	28°20'37.88"	15	26.04.2015
98	Muğla	36°43'6.74"	29°11'7.84"	439	27.04.2015 / 24.05.2015 / 26.06.2015
99	Muğla	36°45'33.96"	29°14'5.19"	526	27.04.2015
100	Muğla	36°43'38.02"	29°21'25.63"	180	27.04.2015
101	Muğla	36°46'45.61"	29°28'34.06"	1084	27.04.2015
102	Muğla	36°54'40.17"	29°39'44.46"	1230	27.04.2015
103	Burdur	37° 0'58.50"	29°43'25.34"	1074	27.04.2015

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
104	Antalya	37° 1'55.45"	29°59'48.62"	1520	27.04.2015
105	Antalya	37° 0'13.60"	30°15'35.75"	922	27.04.2015
106	Antalya	37°14'58.30"	30°14'0.49"	955	28.04.2015
107	Burdur	37°35'45.60"	30°30'39.96"	1050	28.04.2015
108	Burdur	37°38'51.34"	30°36'27.35"	1035	28.04.2015
109	Burdur	37°38'6.93"	30°36'56.61"	1021	28.04.2015 / 26.06.2015
110	Isparta	37°39'48.60"	30°40'6.40"	785	28.04.2015
111	Isparta	37°42'45.79"	30°39'12.33"	880	28.04.2015
112	Burdur	37°31'37.84"	30°45'23.96"	350	28.04.2015
113	Burdur	37°26'37.10"	30°47'1.53"	280	28.04.2015
114	Isparta	37°48'24.09"	30°55'3.83"	1170	29.04.2015
115	Isparta	37°48'51.10"	30°55'57.49"	1172	29.04.2015
116	Isparta	37°47'10.10"	30°58'48.74"	1190	29.04.2015
117	Isparta	37°47'50.05"	31° 0'36.60"	1205	29.04.2015
118	Isparta	37°43'59.06"	31° 1'29.46"	1165	29.04.2015
119	Isparta	37°40'54.11"	31° 1'38.22"	1103	29.04.2015 / 27.06.2015 / 05.05.2016
120	Isparta	37°55'40.44"	30°55'47.08"	951	29.04.2015
121	Isparta	38° 0'43.03"	30°57'50.03"	954	29.04.2015
122	Isparta	38°12'36.74"	31° 6'40.64"	1020	29.04.2015
123	Konya	38°16'36.80"	31°25'22.45"	1484	21.05.2015
124	Isparta	38°12'20.72"	31°15'46.96"	1104	21.05.2015
125	Isparta	38° 3'12.73"	31°24'10.12"	1191	21.05.2015 / 23.06.2015
126	Konya	37°31'6.95"	31°48'34.15"	1126	21.05.2015
127	Antalya	36°58'55.29"	31°43'36.99"	763	21.05.2015
128	Antalya	36°43'47.22"	31°35'41.85"	12	22.05.2015
129	Antalya	36°47'22.72"	31°51'41.73"	485	22.05.2015
130	Antalya	36°49'21.53"	31°59'34.61"	890	22.05.2015 / 24.06.2015
131	Antalya	36°45'38.41"	32° 1'28.47"	250	22.05.2015 / 24.06.2015
132	Antalya	36°37'5.80"	31°52'49.34"	89	22.05.2015
133	Antalya	36° 7'23.68"	32°34'24.47"	24	22.05.2015 / 08.05.2016
134	Antalya	36°29'33.22"	30° 3'58.43"	394	23.05.2015
135	Antalya	36°25'38.81"	29°55'25.66"	405	23.05.2015
136	Antalya	36°20'42.48"	29°48'1.23"	220	23.05.2015
137	Antalya	36°16'40.57"	29°43'30.55"	213	23.05.2015
138	Muğla	36°49'59.03"	29°10'29.57"	918	24.05.2015 / 26.06.2015
139	Muğla	36°51'27.23"	29°10'42.99"	1305	24.05.2015 / 26.06.2015
140	Denizli	36°56'6.71"	29° 8'24.98"	996	24.05.2015 / 26.06.2015
141	Denizli	36°59'2.34"	29°12'3.71"	827	24.05.2015 / 26.06.2015
142	Denizli	37° 6'6.65"	29°24'0.61"	1319	24.05.2015 / 26.06.2015
143	Burdur	37° 7'27.31"	29°29'48.24"	1097	24.05.2015 / 26.06.2015
144	Burdur	37° 9'22.58"	29°36'47.06"	959	24.05.2015 / 26.06.2015
145	Burdur	37°30'49.79"	29°43'35.11"	1159	24.05.2015
146	Burdur	37°39'10.97"	30°10'36.58"	914	24.05.2015
147	Isparta	37°27'44.06"	30°54'33.13"	330	25.05.2015
148	Isparta	37°33'52.62"	30°58'45.03"	940	25.05.2015
149	Isparta	37°33'16.01"	31° 8'8.88"	1389	25.05.2015
150	Isparta	37°33'41.08"	31° 8'0.82"	1380	25.05.2015
151	Isparta	38°11'30.50"	31°14'42.31"	1094	23.06.2015
152	Konya	37°36'50.42"	31°35'30.42"	1144	23.06.2015
153	Konya	37°23'35.65"	31°41'19.53"	1408	23.06.2015

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
154	Antalya	36°39'25.77"	31°52'30.26"	148	24.06.2015 /03.05.2016
155	Antalya	36°31'55.43"	32°18'57.36"	364	24.06.2015
156	Antalya	36°26'41.22"	32°12'52.27"	74	24.06.2015
157	Antalya	36°57'12.07"	30°57'55.22"	22	25.06.2015
158	Antalya	37° 7'44.00"	30°55'3.41"	62	25.06.2015
159	Antalya	37°11'17.86"	30°57'33.30"	177	25.06.2015
160	Antalya	36°54'55.92"	30° 3'24.00"	1257	25.06.2015
161	Antalya	36°52'20.69"	30° 1'3.58"	1158	25.06.2015
162	Antalya	36°37'9.51"	29°46'45.95"	1068	25.06.2015 / 06.05.2016
163	Antalya	36°33'33.86"	29°37'48.16"	1680	25.06.2015
164	Antalya	36°33'29.95"	29°37'57.18"	1500	25.06.2015
165	Antalya	36°23'20.88"	29°31'6.98"	877	25.06.2015
166	Burdur	37°30'54.97"	30° 4'37.87"	990	26.06.2015
167	Burdur	37°29'40.15"	30° 9'3.70"	1103	26.06.2015
168	Burdur	37°26'11.71"	30°15'9.27"	1292	26.06.2015
169	Isparta	37°48'43.08"	31° 0'45.64"	1202	27.06.2015
170	Isparta	37°48'40.02"	31° 5'3.93"	1331	27.06.2015
171	Isparta	37°47'57.81"	31° 6'52.83"	1257	27.06.2015
172	Isparta	37°43'26.17"	31° 8'23.99"	1252	27.06.2015
173	Isparta	37°42'58.60"	31°14'41.71"	1373	27.06.2015
174	Isparta	37°42'2.57"	31° 1'58.27"	1124	27.06.2015
175	Muğla	37°17'46.31"	28°10'9.74"	399	27.03.2016
176	Muğla	37°17'42.40"	28° 8'59.87"	363	27.03.2016
177	Muğla	37°11'23.15"	28°34'37.27"	790	27.03.2016
178	Muğla	37° 9'22.26"	28°34'10.19"	78	27.03.2016
179	Muğla	37° 2'17.25"	28°30'23.28"	100	27.03.2016
180	Muğla	37° 0'15.55"	28°33'1.41"	88	27.03.2016
181	Muğla	36°55'35.41"	28°49'42.45"	136	27.03.2016
182	Muğla	36°44'46.03"	28°59'27.59"	180	27.03.2016
183	Muğla	36°42'23.62"	29° 2'54.05"	22	27.03.2016
184	Muğla	36°31'27.30"	29°24'4.81"	228	28.03.2016
185	Muğla	36°30'5.62"	29°19'43.57"	85	28.03.2016
186	Antalya	36°39'1.55"	30°25'58.25"	1120	28.03.2016
187	Antalya	37°0'49.49"	30°49'47.99"	61	29.03.2016
188	Antalya	37°11'27.64"	30°47'50.91"	49	29.03.2016
189	Antalya	37° 3'9.65"	31° 6'15.93"	148	29.03.2016
190	Antalya	36°58'39.93"	31° 7'33.71"	47	29.03.2016
191	Antalya	37° 2'41.55"	31° 6'12.33"	155	29.03.2016
192	Antalya	37° 0'59.03"	31° 7'22.42"	64	29.03.2016
193	Antalya	37° 0'47.42"	31°11'49.91"	20	29.03.2016
194	Antalya	37° 3'19.78"	31°14'14.05"	95	29.03.2016
195	Antalya	37° 7'54.33"	31°12'36.87"	112	29.03.2016
196	Burdur	37°20'33.46"	30°48'31.64"	193	29.03.2016
197	Burdur	37°36'58.12"	30° 4'11.34"	862	30.03.2016
198	Burdur	37°40'53.64"	30° 1'3.61"	928	30.03.2016
199	Burdur	37°42'48.95"	30° 0'56.30"	1050	30.03.2016
200	Burdur	37°39'19.66"	29°49'24.53"	1085	30.03.2016
201	Burdur	37°37'19.88"	30°21'0.11"	1197	30.03.2016
202	Antalya	36°51'28.60"	31°25'53.02"	25	3.05.2016
203	Antalya	36°51'41.56"	31°33'59.46"	59	3.05.2016

table continued

Locality No	City	Latitude (N)	Longitude (E)	Altitude (m)	Sampling Date
204	Antalya	36°37’52.47”	31°54’37.97”	142	3.05.2016
205	Antalya	36°59’25.76”	30°33’53.47”	314	3.05.2016
206	Antalya	37° 1’38.06”	30°16’31.99”	920	4.05.2016
207	Antalya	37°26’35.35”	30°47’53.25”	306	4.05.2016
208	Isparta	37°34’52.34”	30°49’27.02”	404	5.05.2016
209	Burdur	37°12’43.82”	29°43’28.76”	1202	6.05.2016
210	Antalya	37°0’11.36”	29°56’36.81”	1405	6.05.2016
211	Antalya	36°52’34.42”	30° 1’17.48”	1161	6.05.2016
212	Antalya	36°34’39.01”	29°43’32.58”	1156	6.05.2016
213	Antalya	36°25’42.23”	29°36’21.08”	1250	6.05.2016
214	Antalya	36°18’11.03”	29°27’17.89”	1018	6.05.2016
215	Antalya	36°53’26.28”	29°39’43.39”	1080	7.05.2016
216	Denizli	37°14’42.01”	29°31’20.96”	920	7.05.2016
217	Denizli	38° 9’32.12”	29°38’47.76”	812	7.05.2016
218	Isparta	37°58’55.42”	30°58’10.96”	953	5.05.2016
219	Antalya	37°38’42.17”	30°59’31.66”	1189	5.05.2016
220	Antalya	36°49’49.58”	29°33’42.72”	1120	7.05.2016
221	Antalya	36°56’33.73”	29°38’13.10”	1400	7.05.2016

Identifications

Material was studied under a stereomicroscope (Leica MZ 16), according to methods described by Bass (1998) and identified by using the keys by Rubtsov (1956), Knoz (1965), Crosskey (2002), Bass (1998), Crosskey & Malicky (2001), Yankovsky (2003), Crosskey & Zwick (2007) and Jedlicka, Kudela, & Stloukalova (2004). The nomenclature follows that of Adler (2022).

DNA Extraction, polymerase chain reaction and sequencing

The cytochrome c oxidase subunit I (COI) gene region of mitochondrial DNA (mtDNA) was analyzed. We used GenBank accessions and new sequences acquired in this study. Total DNA was extracted via Macherey-Nagel Animal Genomic DNA Extraction Kit. The universal primers LCOI (5′-GGTCAACAAATCATAAAGAT ATTGG-3) and HCOI (5′TAAACTTCAGGGTGACCAAAAAATCA-3′) were used for amplification (Simon et al, 1994).

Polymerase chain reaction (PCR) was carried out in 50 µl volume; 0.2 µl from each primer (100 pm), 1 µl Deoxynucleotide solution mix (10 mM), 4 µl 50 mM MgCl2 (25 mM), 5 ml 10X Standart Taq reaction Buffer [containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl], 0.25 Taq DNA polymerase (New England Biolabs), and 3 µl of 50–70 ng sample DNA. PCR cycling parameters were as follows: denaturation at 95℃ for 30 sec, 35 cycles of 95℃ for 20 sec., annealing at 41℃ for 30 sec, elongation at 72℃ for 1 min 40 sec. and final extension at 72℃ for 5 min. Results were visualized with agarose gel electrophoresis including ethidium bromide stain. Sanger sequence analysis and purifications were carried out by Macrogen Europe (Amsterdam, the Netherlands).

Molecular Data Analysis

The analyses were conducted with 109 COI sequences from 19 species of Simuliidae. We used NCBI GenBank accessions (52 sequences+4 sequences as outgroups) and 53 new sequences acquired in this study. All sequences were checked manually with SEQUENCER v. 4.1 (Gene Codes Corporation). The alignment was made with Mafft version 7 (https://mafft.cbrc.jp/alignment/server/), following the auto strategy. The number of conservatives, variable and parsimony-informative sites were calculated using MEGA7 (Kumar, Stecher, & Tamura, 2016). Haplotypes were determined by DnaSP v.5 (Librado & Rozas, 2009) and haplotype frequencies were calculated. Sequences are deposited in the Genbank database. The best-fit evolutionary model for our data matrix was estimated by jModelTest v.0.1.1 (Posada, 2008).

Aligned sequences were analyzed with Maximum parsimony (MP), with 100 random additions, nearest neighbor interchange (NNI) algorithm and heuristic search approach by PAUP Version 4.0b10 (Swofford, 2002), and Maximum Likelihood (ML) with raxmlGUIversion 1.5 (Silvestro & Michalak, 2012) and ML-rapid 1000 bootstrap option. MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) program was used for Bayesian phylogenetic inference (BI), with four simulations of Markov chains, 10 M generation and sampling every 100th generations and with 1000 trees discarded as burn-in. As outgroups we used two species of Blephariceridae: *Elporia barnardi* (GenBank acc. num: AF427037.1 and AF427038.1) and *Liponeura cinerascens cinerascens* (GenBank acc. num: MW181342.1 and MW181343.1). While the relationship between haplotypes was carried out through median-joining, MJ network approach Network 4.5.1.6 (http://www.fluxux-engineering.com, access date: 19.09.2022.) program, mutational distances between haplotypes were analyzed with SplitsTree v.4.11.3 (Huson & Bryant, 2006) program.

DNA sequence-based analysis TCS (Clement et al, 2000) and Automatic Barcode Gap Discovery (ABGD) programs were applied to the dataset for the species delimitation test. The distance-based test SpeciesID (speID) was conducted to estimate large range supported cluster numbers in the range of 0.5–7% threshold values (Shiyang, Vaidya, & Ng, 2006).

NCBI accession codes and registration data for each type of COI sequences used in the study are listed in Table 2.

Table 2. Species and the NCBI GenBank accession number of the COI sequences included in this study.

Species	Accession No	Country	Author
Prosimulium rachiliense Djafarov, 1954	OK073998	Türkiye	This study
	OK073996		
	OK073997		
	OK073991		
	OK073993		
	OK076952		
	OK076955		
	OK076957		
	OK076954		
	OK076713		
	OK066328		

table continued

Species	Accession No	Country	Author
Prosimulium hirtipes (Fries, 1824)	MF197685.1	Sweden	Kudela et al, 2018
	MF197686.1		
	KP861150.1	United Kingdom	Unpublished
	KP861151.1		
	KP861149.1		
	KP861148.1		
	KP861147.1		
Metacnephia subalpina (Rubtsov, 1956)	OK076953	Türkiye	This study
	OK076956		
Metacnephia lyra (Lundstrom, 1911)	KT278290.1	Sweden	Unpublished
	JQ220531.1	Finland	Unpublished
Simulium petricolum (Rivosecchi, 1963)	OK066351	Türkiye	This study
	OK066352		
	OK066353		
	OK066354		
	OK076714		
	OK066575		
	OK066576		
	OK066577		
	OK067201		
	GQ465967.1	United Kingdom	Unpublished
	GQ465951.1		
	GQ465952.1		
	GQ465950.1		
Simulium ibleum (Rivosecchi, 1966)	OK073994	Türkiye	This study
Simulium angustitarse (Lundstrom, 1911)	KP861164.1	United Kingdom	Unpublished
	KP861165.1		
Simulium brevidens (Rubtsov, 1956)	OK046460	Türkiye	This study
	OS046728		
	OK046735		
	OK047087		
	MG894322.1	Spain	Ruiz-Arrondo et al, 2018
	MG894229.1		
	MG894186.1		
	MG894181.1		
Simulium costatum Friederichs, 1920	OK047370	Türkiye	This study
	OK047418		
	OK047419		
	OK073995		
	OK047420		
	GU072929.1	Sweden	Unpublished
	GU072928.1		
	GU072927.1		
	GU072926.1		
Simulium cryophilum (Rubtsov, 1959)	OK047421	Türkiye	This study
	OK073992		
	OK073999		
	MG894307.1	Spain	Ruiz-Arrondo et al, 2018
	MG894188.1		
	MG599017.1	United Kingdom	Unpublished
	MG599016.1		

table continued

Species	Accession No	Country	Author
Simulium vernum Macquart, 1826	GU072982.1	Sweden	Unpublished
	GU072980.1		
Simulium bezzii (Corti, 1914)	OK047354	Türkiye	This study
	OK047368		
	OK047369		
	MK545146.1	Iran	Khanzadeh et al, 2019
	MK545145.1		
	MK545144.1		
	MK545143.1		
Simulium auricoma Meigen, 1818	OK148435	Türkiye	This study
Simulium kiritshenkoi Rubtsov, 1940	OK047478	Türkiye	This study
	OK047480		
	OK047479		
	MK545139.1	Iran	Khanzadeh et al, 2019
	MK545138.1		
	MK545136.1		
	MK545135.1		
Simulium ornatum sp-comp.	OK047481	Türkiye	This study
	OK047483		
	OK047482		
Simulium ornatum Meigen 1818	KP861038.1	United Kingdom	Unpublished
	KP861037.1		
	KP861036.1		
Simulium variegatum Meigen, 1818	OK066329	Türkiye	This study
	MG894323.1	Spain	Ruiz-Arrondo et al, 2018
	MG894321.1		
	MG894319.1		
	MG894301.1		
Simulium balcanicum (Enderlein, 1924)	OK047477	Türkiye	This study
	MH587353.1	Serbia	Đuknić et al, 2019
	MH587353.1		
	MH587354.1		
	MH549570.1		
	MH549569.1		
Simulium pseudequinum Séguy, 1921	MH587349.1	Greece	Đuknić et al, 2019
	MH587348.1		
	MH587350.1	Bulgaria	Đuknić et al, 2019
	MH587347.1	Croatia	Đuknić et al, 2019
	OK047270	Türkiye	This study
	OK041122		
	OK047269		
	OK046148		
	OK046149		
Simulium paraequinum Puri, 1933	OK039228	Türkiye	This study
	MT309529.1	Iran	Unpublished
	MT309530.1		
	MT309535.1		
	MT309537.1		

RESULTS

Morphotaxonomic analyses

A total of 19569 individuals (10605 larvae, 8792 pupae and 172 reared adults) collected from 221 rivers in the study area were evaluated and 17 species were identified. Information about the species is given below.

Prosimulium rachiliense Djafarov, 1954

Material examined: A total of 843 pupae, 2170 larvae, 5 males and 3 females collected from 67 sites (6, 7, 9, 10, 12, 13, 14, 15, 16,19, 20, 21, 22, 23, 24, 25, 28, 29, 30, 31, 32, 33, 40, 42,43, 44, 45, 46, 50, 52, 54, 55, 57, 63, 64, 65, 73, 74, 77, 82, 83, 84, 85, 86, 87, 101, 102, 103, 104, 108, 111, 113, 115, 117, 119, 121, 123, 142, 144, 149, 150, 163, 186, 190, 213, 215, 221) were examined.

Metacnephia subalpina (Rubtsov, 1956)

Material examined: A total of 259 pupae, 1007 larvae and 2 males collected from 24 sites (11, 13, 16, 23, 36, 45, 52, 54, 55, 64, 66, 72, 73, 101, 102, 111, 119, 121, 123, 124, 130, 149, 153, 221) were examined.

Simulium (*Eusimulium*) *angustipes* Edwards, 1915

Material examined: A total of 9 pupae and 10 larvae collected from 3 sites (8, 10, 79) were examined.

Simulium (*Eusimulium*) *petricolum* (Rivosecchi, 1963)

Material examined: A total of 1340 pupae, 978 larvae, 21 males and 15 females collected from 74 sites (1, 2, 3, 4, 7, 13, 19, 25, 33, 34, 35, 37, 38, 56, 57, 59, 62, 65, 69, 76, 77, 80, 81, 82, 83, 87, 90, 91, 92, 93, 98, 99, 100, 103, 105, 106, 112, 113, 119, 126, 127, 130, 131, 132, 134, 135, 136, 138, 140, 142, 143, 145, 146, 148, 154, 159, 165, 167, 168, 174, 176, 178, 179, 180, 182, 183, 193, 203, 204, 206, 215, 217, 218, 219) were examined.

Simulium (*Nevermannia*) *ibleum* (Rivosecchi, 1966)

Material examined: A total of 16 pupae and 17 larvae collected from 4 (6, 7, 22, 23) sites were examined.

Remarks: *Simulium ibleum,* new record for Türkiye, was previously described by Rivosecchi (1971) as a subspecies of *Simulium angustitarse* (Lundström, 1911). *Simulium angustitarse,* also reported from a few regions in Anatolia, is mostly distributed in Central and Northern European countries. Whereas, *S. ibleum* is found in Mediterranean countries (Adler, 2022). The gill filament branching angles and lengths of the pupae in our material, the shape of the postgenal cleft, the structure of the hypostomal and mandibular teeth, and the morphology of the ventral plate extracted from mature male pupae are confirmed with the desription of *S. ibleum* in Rivosecchi (1971).

Simulium (*Nevermannia*) *brevidens* (Rubtsov, 1956)

Material examined: A total of 60 pupae, 93 larvae and 1 male collected from 13 sites (37, 40, 71, 77, 82, 83, 86, 95, 98, 117, 119, 129, 139) were examined.

Remarks: *Simulium brevidens* is a member of the *S. vernum* species group, the largest species group in the genus *Simulium* and new for Turkish fauna. The distinctive features of this species in the pupal stage are the pattern and length of the anterior projection of the cocon, the shape of the common stalk of the gill filaments and the branching directions, and the double branched structure of the pupal thoracic trichomes. All these characters are observed in our pupae. Similarly, the shape of the postgenal cleft of the larvae confirms to the description of the species given by Knoz (1965).

Simulium (*Nevermannia*) *costatum* Friederichs, 1920

Material examined: A total of 34 pupae and 35 larvae collected from 8 (11, 16, 27, 28, 33, 63, 164, 210) sites were examined.

Simulium (*Nevermannia*) *cryophilum* (Rubtsov, 1959)

Material examined: A total of 23 pupae, 20 larvae and 2 males collected from 5 sites (15, 26, 33, 81, 82) were examined.

Simulium (*Nevermannia*) *vernum* Macquart, 1826

Material examined: A total of 29 pupae, 3 larvae, 1 male and 1 female collected from 2 sites (11, 44) were examined.

Simulium (*Simulium*) *bezzii* (Corti, 1914)

Material examined: A total of 142 pupae, 115 larvae, 7 males and 7 females collected from 18 sites (9, 10, 52, 56, 57, 61, 83, 85, 141, 142, 194, 196, 199, 211, 212, 218, 219, 220) were examined.

Simulium (*Simulium*) *kiritshenkoi* Rubtsov, 1940

Material examined: A total of 2885 pupae, 2967 larvae, 30 males and 27 females collected from 108 sites (3, 6, 7, 8, 10, 11, 13, 18, 22, 23, 25, 26, 27, 34, 35, 36, 38, 50, 51, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 69, 71, 75, 77, 79, 82, 83, 87, 91, 92, 93, 95, 96, 98, 100, 103, 106, 107, 108, 109, 112, 117, 119, 123, 124, 125, 126, 129, 130, 131, 133, 134, 135, 136, 138, 139, 140, 141, 142, 143, 144, 145, 148, 150, 151, 152, 153, 154, 161, 162, 166, 168, 169, 170, 171, 172, 175, 176, 181, 183, 185, 188, 191, 197, 198, 199, 200, 201, 203, 204, 205, 209, 210, 212, 213, 214, 215, 220, 221) (5, 16, 19, 31, 70) were examined.

Simulium (*Simulium*) *ornatum* species complex

Material examined: A total of 70 pupae, 90 larvae and 1 female collected from 5 sites were examined.

Remarks: This species belongs to the *S. ornatum* species group, and was found at 5 different localities. It is very similar morphologically to *S. kiritshenkoi* but differs in the common stems and height of the pupal gill filaments. Since we did not have suitable material to examine the male genitalia, it could not be determined which of the species in the species group.

Simulium (*Simulium*) *variegatum* Meigen, 1818

Material examined: A total of 1323 pupae, 1385 larvae, 11 males and 7 females collected from 56 sites (7, 8, 11, 13, 16, 17, 19, 23, 26, 27, 28, 29, 30, 31, 32, 33, 36, 38, 39, 41, 71, 73, 74, 77, 81, 86, 87, 88, 89, 91, 95, 101, 110, 113, 118, 119, 120, 122, 124, 125, 130, 131, 139, 144, 145, 147, 148, 150, 153, 155, 170, 173, 177, 186, 215, 221) were examined.

Simulium (Obuchovia) auricoma Meigen, 1818

Material examined: A total of 15 pupae, 22 larvae, 2 males and 2 females collected from 10 sites (9, 15, 38, 73, 101, 119, 131, 169, 174, 201) were examined.

Simulium (Wilhelmia) balcanicum (Enderlein, 1924)

Material examined: A total of 231 pupae, 256 larvae and 1 male collected from 19 sites (47, 67, 75, 79, 93, 126, 128, 144, 145, 146, 158, 162, 166, 176, 183, 185, 193, 217, 220) were examined.

Simulium (*Wilhelmia*) *paraequinum* Puri, 1933

Material examined: A total of 533 pupae, 351 larvae, 9 males and 5 females collected from 19 sites (25, 35, 36, 47, 48, 49, 67, 68, 119, 133, 144, 157, 158, 183, 190, 197, 199, 209, 216) were examined.

Simulium (*Wilhelmia*) *pseudequinum* Séguy, 1921

Material examined: A total of 980 pupae, 1086 larvae, 7 males and 5 females collected from 75 (6, 7, 8, 13, 19, 56, 57, 59, 69, 75, 76, 77, 78, 79, 81, 83, 90, 91, 92, 93, 96, 97, 98, 100, 109, 112, 117, 126, 128, 131, 132, 133, 134, 136, 137, 141, 142, 145, 146, 154, 156, 160, 162, 165, 166, 167, 169, 172, 175, 176, 180, 182, 184, 185, 187, 188, 189, 192, 193, 194, 195, 200, 202, 203, 204, 205, 206, 207, 208, 212, 215, 217, 219, 220, 221) sites were examined.

Phylogenetic analyses

We created our dataset with 19 Simuliidae species (15 species from the western Mediterranean and 4 species from NCBI) and a total of 109 COI sequences from them were used for phylogenetic analyses. All 109 files were checked with the BLAST program for correcting the gene region after alignment. The sequences changed between 580 bp and 650 bp before they were organized into data blocks with MEGA7. As a result, data sets of 105 sequences (with 4 outgroup sequences) and 560 base pairs were obtained. Of these, 285 sites were conservative, 275 were variable, and 209 were parsimony-informative. The file was converted into different formats for use in phylogenetic analysis with MEGA, DnaSP, Mesquite and DAMBE programs. Of 87 haplotypes gathered from the data block, four belonged to outgroup sequences and 76 were unique (each individual created a haplotype), whereas 11 were shared within species (two or three specimens of same species created haplotypes). Haplotype diversity was calculated as 0.9951 by DnaSP v.5.The jModelTest v.0.1.1 suggested the evolutionary model as GTR+I+G (general time reversible+ invariable sites + gamma), according to AIC (Akaike Information Criterion), with p-inv = 0.4110 gamma shape = 0.5050.

All phylogenetic analyses (ML, MP, BI) resulted in similar tree topology, which can be viewed in the BI tree (Fig. 2). Eight of the species identified in the study (*Simulium balcanicum, S. paraequinum, S. pseudequinum, S. petricolum, S. ibleum, S. auricoma, S. bezzii* and *Metacnephia subalpina*) were branched as separate monophyletic species in the tree, agree with the morphotaxonomic identifications. Species delimitation tests also confirmed the results for these species. On the other hand, for the other 7 species (*Simulium brevidens, S. cryophilum, S. ornatum, S. ornatum species complex, S. costatum, S. variegatum* and *P. rachiliense*), both phylogenetic analyzes and species delimitation tests yielded interesting results.

First of all, it is surprising that all haplotypes of *Simulium brevidens* and *S. cryophilum* (both our own and NCBI records) were grouped together and were not separated as two different species in all species delimitation tests. A similar result was observed for *S. kiritshenkoi* and *S. ornatum species* complex. The haplotypes of *S. kiritshenkoi* and *S. ornatum species* complex in our study material, and *S. kiritshenkoi* registered to NCBI from Iran and *S. ornatum* haplotypes registered to the UK were all grouped as a single species in the phylogenetic tree. All species delimitation tests also indicated that these haplotypes belong to a single species.

Another interesting result that had appeared in both phylogenetic analyzes and species tests was for *S. costatum*. The haplotypes of our *S. costatum* material branched separately from the haplotypes registered with the NCBI from Sweden. Furthermore, all species tests also confirmed that these are different species. Additionally, *P. rachiliense*, which is common in the study area, was separated as a monophyletic species in phylogenetic analyzes as expected from *P. hirtipes*, another species in the same species group obtained from NCBI. TCS and SpeID tests showed some *Prosimulium rachiliense* haplotypes as different species, but phylogenetic analyzes did not support this conclusion except for the “OK073991” coded haplotype. On the other hand, the “OK073991” haplotype, surprisingly emerged as a different species in both phylogenetic analyzes and species tests. There could be three possible explanations. The haplotype could be (i) ancestral haplotype, (ii) a cryptic species or two cytoforms suggested by Adler & Şirin (2014) for *P. rachiliense* Anatolian populations, and (iii) a pseudogene.

The last species that gave different results than expected in phylogenetic analyzes and species tests is *S. variegatum*. For it, a single haplotype of a single individual was included in the data set from the West mediterranean region. However, this haplotype was distinguished from the Spanish haplotypes which downloaded from NCBI with high branch support value in all three phylogenetic analyses. This result was also supported by the species delimitation tests.

According to the results of network analysis; the haplotypes shown in red in the middle, indicates the (Hypothetic) haplotypes that are not included in the study data set but should be found among the existing haplotype connections. As a result of the analysis, it is seen that the species do not share haplotypes with others and they are grouped similarly in the phylogenetic tree (Fig. 3). Similar results were obtained in the SplitsTree analysis, which was applied to support the Network analysis (Fig . 4).

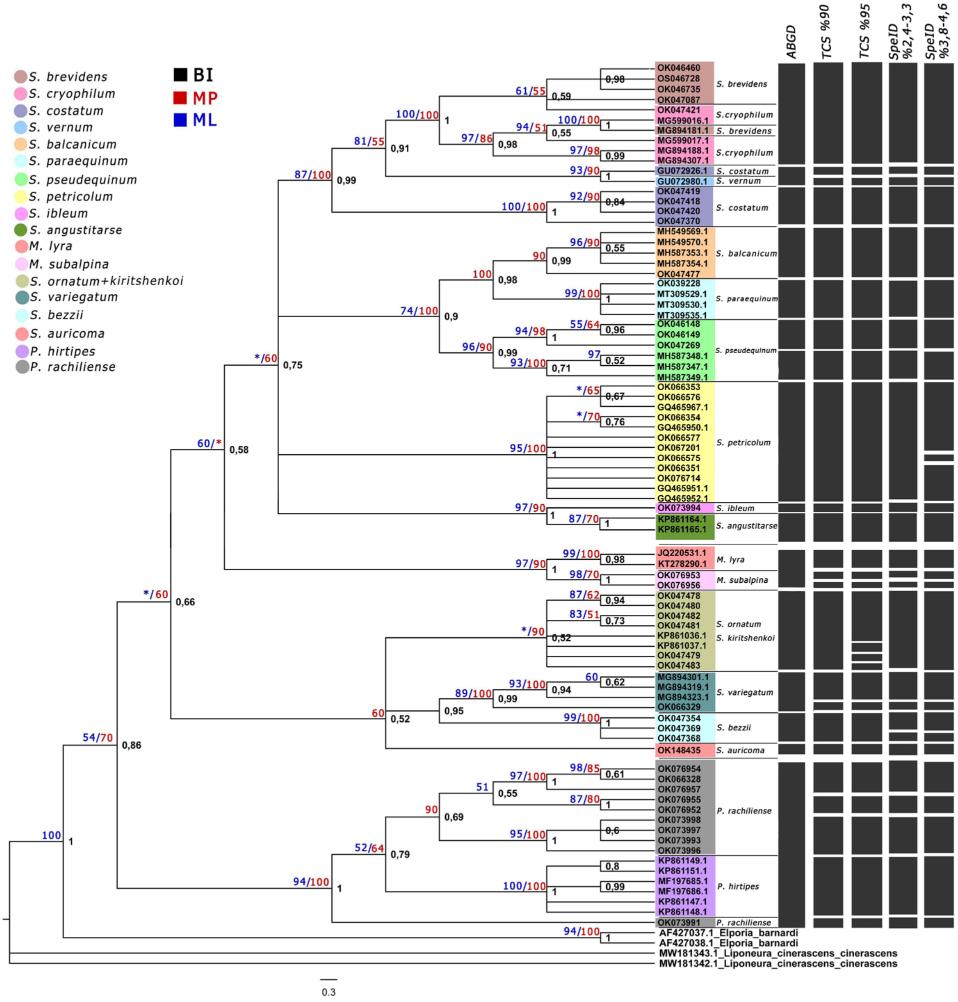

Figure 2. Phylogenetic tree for all analyses of Simuliidae from Central and Western Mediterranean Region of Türkiye. The numbers on the nodes indicate bootstrap values (MP-ML) or posterior probability values (BI). Black is for BI, red is for MP and blue is for ML respectively. (*) indicates 50% and below values or not supported by the respective analyses. Species delimitation tests are mapped on the tree (ABGD-TCS-SpeID).

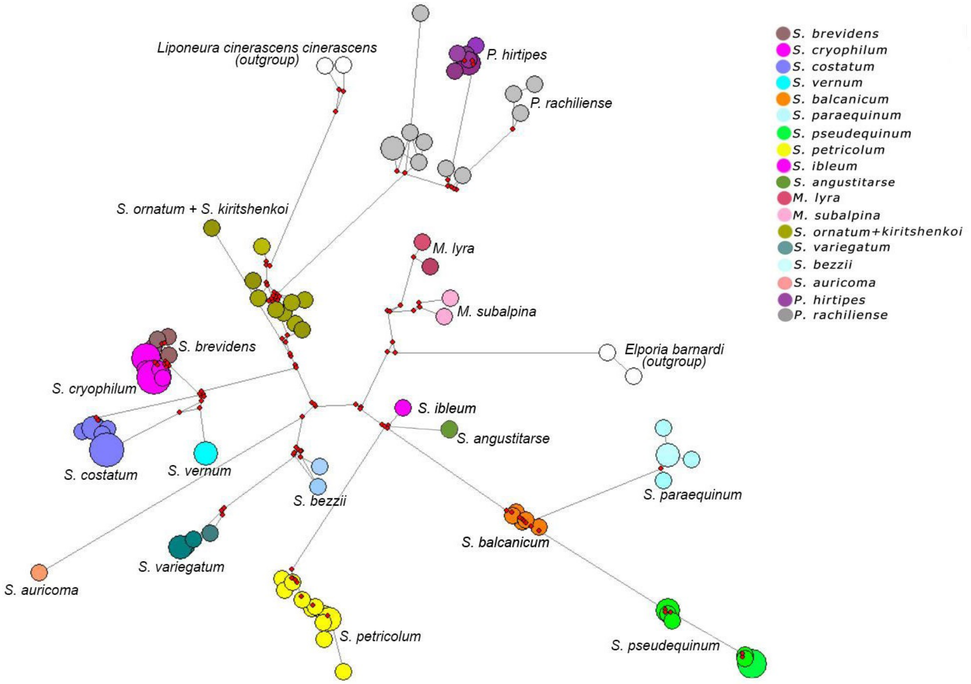

Figure 3. Haplotype network analysis created with COI 87 haplotype data set (Network programme).

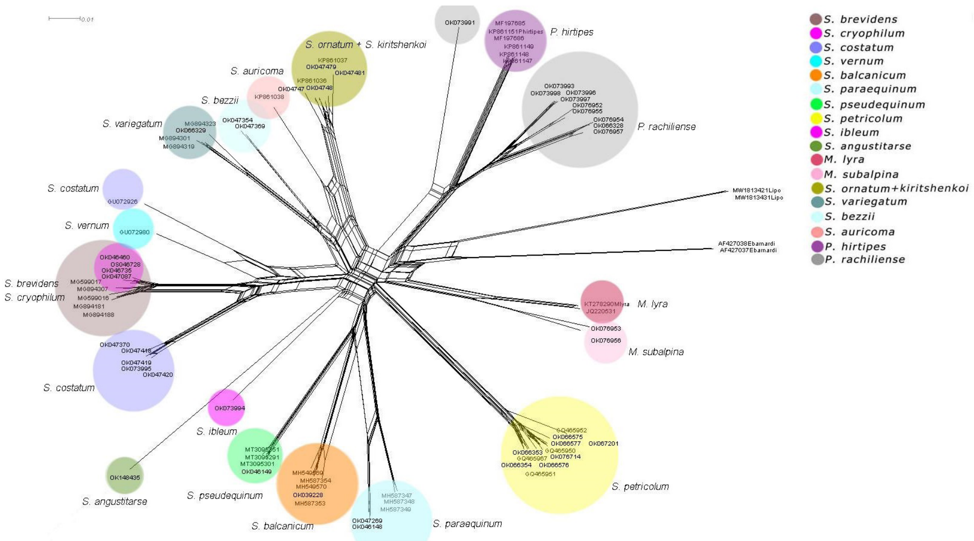

Figure 4. Haplotype network analysis created with COI 87 haplotype data set (Splitstree programme).

CONCLUSION

The results of the study have presented new contributions to the information about the Anatolian Simuliidae fauna. *Simulium ibleum* (Rivosecchi, 1966) and *S. brevidens* (Rubtsov, 1956), common species of other Mediterranean countries, were recorded in Anatolia for the first time in this study and the number of known species of the family in Türkiye has increased to 60. In addition, five species; *Metacnephia subalpina* (Rubtsov, 1956); *Simulium (Eusimulium) petricolum* (Rivosecchi, 1963); *Simulium (Nevermannia) costatum* Friederichs, 1920; *Simulium (Simulium) kiritshenkoi* Rubtsov, 1940 and *Simulium (Obuchovia) auricoma* Meigen, 1818 were reported for the first time in the Central and Western Mediterranean region and new locality records have been provided for other species as well. The species identified in the region are common in the central and southwestern Palearctic (Adler, 2022), as expected (Adler, 2022). *S. kiritshenkoi* was the dominant species found in 109 localities in the region. *S. pseudequinum* (83 localities) and *S. petricolum* (74 localities) also were extensively collected in the study area. The abundance of these three species may be due to the fact that they have multiple generations per year and wide habitat preferences.

Phylogenetic analyses performed with COI sequences confirm morphotaxonomic identifications of 11 species, while revealing some problematic situations for others. For example, the COI data revealed that *S. ibleum* is a distinctly separate species from *S. angustitarse*, of which it is a subspecies before. However, the analyses failed to separate *S. cryophilum* and *S. brevidens* reported for the first time from Türkiye and not registered in the NCBI. Both species are in the *S. vernum* species group, but *S. brevidens* is distinguished from *S. cryophilum* and other members of the group in the pupa by the following characters: upper and lower gill filaments at an acute angle; thoracic tubercles smooth and round; dorsal projection of cocoon short and irregular; and thoracic trichomes dichotomously branched (Jedlicka et al, 2004). We also observed these characters in our material, although the analysis of the COI data did not reveal a clear difference in the species status of *S. brevidens* and *S. cryophilum*. We suggest that additional genes, total mitochondrial genome or microsatellite studies could be used to investigate this taxonomic problem.

We also observed problematic results for *S. kiritshenkoi* and the species we identified as *S. ornatum* species complex. Many authors have stated that members of the *S. ornatum* species group are difficult to separate morphologically and genetically (Adler, Werner, & Kampen, 2021). Fidan (2020) investigated the phylogeny of Anatolian populations of the *S. ornatum* species group, using three different genes (COI, NADH2 and ITS1-2), and compared them with European populations. She concluded that *S. kiritshenkoi* and the *S. ornatum* have incomplete linage sorting and are not two separate species. It is seen that we have experienced a similar pattern in the study, when the phylogenetic tree and haplotype network analyzes are examined.

The COI data for the species identified as *S. costatum* in our study also differ from the data in the NCBI and indicate a different species according to phylogenetic and haplotype network analyses. In both network and phylogenetic analysis, *S. costatum* haplotypes studied within this study were grouped separately. The morphological characters of the larvae used in the diagnosis of this species conformed to those given by Bass (1998). More extensive genetic analyses and morphotaxonomic studies are needed to determine if our material is a different species from *S. costatum*.

The most surprising result obtained in the study is the difference between the COI data from *S. variegatum* in our study area and the data in NCBI from Spain. The data point to two separate species, according to all species delimitation tests in our study. However, it is known that this species is easily distinguished from all other species of the family by two large thoracic bulges (patagia) anterodorsally in the pupal thorax, as in our material. This result suggests that the individuals from whom COI sequences were obtained may have been misidentified or the material using in DNA extraction may have been confused with another species. The result should be tested with more comprehensive material and genetic data.

The genetic information of 4 species *(Prosimulium rachiliense, Metacnephia subalpina, Simulium ibleum, S. auricoma)* was entered into GeneBank (NCBI) for the first time and the first NCBI record of 5 species (*S. petricolum, S. brevidens, S. costatum, S. cryophilum, S. variegatum*) from Türkiye were recorded through this publication.

Finally, our results emphasize that morphological and molecular data should be used together in order to accurately identify black fly species. This integrated approach will facilitate the identification of black fly species and the implementation of properly targeted vector control and pest management strategies. Our study area is a tourism region with considerable livestock activities. Thus, determining the presence of anthropophilic and mammophilic species such as *S. kiritshenkoi* and *S. pseudequinum* could contribute to the planning of biomonitoring programs to ensure human and animal health.

ACKNOWLEDGEMENTS

We are thankful to Dr. Peter H. Adler (retired from Clemson University, USA) for his useful advice concerning the manuscript and checking the English. This work has been supported by Eskisehir Osmangazi University Scientific Research Projects Coordination Unit under grant number ESOGUBAP-201419051.

AUTHOR CONTRIBUTIONS

ÜDŞ, ECF and HÇ collected the study material from running waters. ÜDŞ identified all species morphotaxonomically. ECF and ÜDŞ performed DNA extraction and phylogenetic analysis. ÜDŞ was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

The authors declare that they have no conflict of interest.

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