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MORPHOLOGICAL CHARACTERISTICS OF TWO *Culicoides* Latreille BITING MIDGES (DIPTERA, CERATOPOGONIDAE) WITH A NEW RECORD FOR TURKISH FAUNA

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Abstract: This study was carried out in May-October 2013 and May-November 2017 in Sinop Province (Western Black Sea Region-Turkey). The *Culicoides* Latreille biting midges were collected with light traps and mounted on microscope slides in phenol-Canada balsam. The basic morphological features of *Culicoides clastrieri* Callot, Kremer & Deduit, 1962 and *Culicoides tbilisicus* Dzhafarov, 1964 in Sinop (Turkey) province were studied. *Culicoides clastrieri* is recorded for the first time from Turkey. Thus, the number of *Culicoides* species in Turkey reached to 72. In addition, the first taxonomic measurements and data of males and females of the poorly-known *C. tbilisicus*, whose distribution in Turkey was reported recently, were evaluated.

Özet: Bu çalışma, 2013 yılının Mayıs-Ekim ve 2017 yılının Mayıs-Kasım ayları arasında Sinop ilinde (Batı Karadeniz Bölgesi-Türkiye) gerçekleştirilmiştir. *Culicoides* Latreille cinsine ait sinekler ışık tuzakları ile toplanmış ve fenol-Kanada balzamı içinde mikroskop lamlarına yerleştirilerek prepatları hazırlanmıştır. Sinop (Türkiye) ilinde tespit edilen *Culicoides clastrieri* Callot, Kremer & Deduit, 1962 ve *Culicoides tbilisicus* Dzhafarov, 1964 türlerinin temel morfolojik özellikleri çalışılmıştır. *Culicoides clastrieri* türü Türkiye için yeni kayıttır. Böylece Türkiye *Culicoides* faunasından bildirilen tür sayısı 72'ye ulaşmaktadır. Türkiye'deki yayılışı tespit edilen ve az bilinen *C. tbilisicus* türünün erkek ve dişilerine ait ilk taksonomik ölçümler ve veriler paylaşılmıştır.

Introduction

Flies belonging to the genus *Culicoides* Latreille of the Ceratopogonidae family, known as biting midges, are distributed in all terrestrial habitats of the world reaching to the mountain tops at an altitude of approximately 4.000 m above sea level except the extreme polar regions and New Zealand. They are particularly widespread in temperate and tropical regions (Mellor *et al.* 2000, Borkent 2017).

Biting midges are found along rivers and lakes, in areas with rich vegetation, and near animal shelters. During the day, they hide in trees, shrubs, barns, and rocky places (Dik 2017). Most species are active in the twilight of the morning and evening and at night. During their activity periods, female biting midges take nourishment by sucking blood from their hosts including livestock such as cattle, sheep, goats, horses, donkeys, poultry; domestic animals such as cats, dogs; and humans (Mellor *et al.* 2000, Mullen 2002, Santiago-Alarcon *et al.* 2012, Hadj-Henni *et al.* 2015, Slama *et al.* 2015). Birds, rodents (*Mus musculus* L., *Mustela nivalis* L.), reptiles (*Carlia fusca* Duméril & Bibron), and even *Aedes* spp. and *Drosophila melanogaster* Meigen have also been established to be among the hosts of *Culicoides* (Slama *et al.* 2015). Their bites, despite their small body, are painful and can cause burning and itchiness in humans (Borkent & Spinelli 2007, Borkent 2017). They also act as vectors for many viruses, filarial nematodes and protozoa. Thus, they cause livestock, particularly cattle, sheep, goats, horses to develop sickness and therefore lead to economic losses (Dik *et al.* 2006, Dik 2017).

The distribution of 117 *Culicoides* species in Europe is known currently (Borkent *et al.* 2022). The genus is widespread in Turkey and represented by 71 species (Korkmaz *et al.* 2021), of which 50 species have been reported from the Black Sea Region of the country (Dik *et al.* 2008, Turgut & Kılıç 2015, Dik *et al.* 2017, Turgut 2018a, 2018b, Korkmaz *et al.* 2021, Turgut & Küçük

2022). Recent studies show that, apart from the reported species, the distribution of many biting midges species in Turkey is highly probable. Therefore, with this study, it is aimed to determine these species and to reveal their morphological characteristics.

Materials and Methods

The specimens were collected from Sarıkum Nature Reserve Area in Sinop Province (Western Black Sea Region-Turkey) during the periods from May to October 2013 and May to November 2017 using a Centers for Disease Control (CDC) miniature light trap with 6 V halogen bulb and an 18 W, 12 V black fluorescent light trap. Collected insects were preserved in 70% ethanol. Sampling studies were carried out every 10-15 days. A total of 101 specimens of *Culicoides clastrieri* Callot, Kremer & Deduit, 1962 and *C. tbilisicus* Dzhafarov, 1964 species were mounted on microscope slides in phenol-Canada balsam as described by Wirth & Marston (1968). Specimens were randomly selected to represent each field study. The slides were examined and photographed through a LEICA, DFC 450C & DM 2500 microscope. Species identification was achieved using the interactive species identification key developed by Mathieu *et al.* (2012). Taxonomic measurements were calculated according to Navai (1977) and Szadziewski (1988): Palpal Ratio (PR): ratio of the length of the third segment of the maxillary palp to its widest; Female Antennal Ratio (AR): the ratio of the total length of the distal five flagellomeres to the total length of eight proximal flagellomeres; Male Antennal Ratio (AR): the ratio of the total length of the distal four flagellomeres to the total length of the nine proximal flagellomeres; Costal Ratio (CR): the ratio of costal vein length to wing length (the costal vein length and wing length were measured starting from the basal arculus); Tarsal Ratio (TR): it is calculated by dividing the length of the first tarsomere (basitarsus) by the length of the second tarsomere; TR(I) for forelegs, TR(II) for middle legs and TR(III) hind legs. Materials were deposited in the Sinop University, Vocational School of Health Services, Department of Medical Services and Techniques, Sinop, Turkey.

Results

Systematics

Family CERATOPOGONIDAE Newman

Subfamily Ceratopogoninae Newman

Tribe Culicoidini Kieffer

Genus *Culicoides* Latreille

Subgenus *Sensiculicoides* Shevchenko

Culicoides clastrieri Callot, Kremer & Deduit, 1962

Material examined: Sinop, Centrum, Sarıkum Nature Reserve Area (42.012776 N, 34.929193 E), sea level, 28.06.2017, 1♀.

Distribution

Bosnia and Herzegovina, Britain I., Corsica, French mainland, Germany, Italian mainland, Poland, Slovakia, Slovenia, Spanish mainland, Ukraine (Szadziewski 1985, Sarvašová *et al.* 2014, Borkent & Dominiak 2020, Borkent *et al.* 2022). Gutsevich (1973) and Glukhova (1989) reported this species from Transcarpathian region (Mukachevo) and Remm (1988) reported from France, Yugoslavia, Carpathians. New record for Turkey.

Description

Female (n=1): Eyes bare and separated by a distance less than 1 facet (Fig. 1a). Palpus 5-segmented and 208 µm in length. Third palpal segment moderately swollen, with a broad and shallow sensory pit (Fig. 1b, c). PR 2.57. Pharynx posterior armature present (Fig. 1d). Flagellum length 675 µm. AR 1.38. Sensilla coeloconica present on flagellomeres 1, 7-13 and 1, 8-13.

Thorax and legs yellowish brown, halters pale (Fig. 1e). Tibial comb with 4 spines, the first and the second from the spur the longest. TR (I) 2.5, TR (II) 3, TR (III) 2.1.

Wings with prominent pale spots (Figs. 1f, g). Wing length 1201 µm, width 567 µm. CR 0.6. A large pale spot over the r-m crossvein extending from costal margin to m cell. Second radial cell wholly dark. Poststigmatic pale spot that distal to the second radial cell extends to M1 vein and touch the pale spot on M1 vein. Pale spots merge with wing margin at the apex of the r3, m1, m2 and cua1 cells. It is the largest in cell r3 and extends along the wing margin. Also, a rounded small pale spot in the base of m1 cell. A pale spot in m cell elongates to m2 cell but does not touch with pale spot over the r-m vein; m cell with indistinctly rounded a small pale spot at the base. Anal cell with fused two pale spots. Wing has pale spots over the basal arculus and at the base of anal cell.

Two functional spermathecae, spherical and with short sclerotized necks. Rudimentary spermatheca present. Functional spermathecae 39 × 36 µm and 38 × 32 µm (The posture of the first is not smooth). Sclerotized ring at the end of the spermathecal duct present (Fig. 1h).

Subgenus *Oecacta* Poey

Culicoides tbilisicus Dzhafarov, 1964

Material examined: Sinop, Centrum, Sarıkum Nature Reserve Area (42.012776 N, 34.929193 E), sea level, 16.05.2013, 121♀♀; 24.05.2013, 117♀♀, 2♂♂; 04.06.2013, 12♀♀; 19.06.2013, 39♀♀; 26.06.2013, 3♀♀; 31.05.2017, 1♀; 14.06.2017, 7♀♀; 28.06.2017, 21♀♀; 12.07.2017, 4♀♀; 21.07.2017, 3♀♀.

Distribution in Turkey: Kastamonu, Sinop, Van (Korkmaz *et al.* 2021).

General Distribution: Azerbaijan, Britain I., Bulgaria, China (Tibet), Corsica, French mainland, Georgia, Italian mainland, Near East (Remm 1988, Borkent & Dominiak 2020, Borkent *et al.* 2022). Glukhova (1989) reported this species from the Transcaucasia (Aras River Valley, Tbilisi surroundings, Abkhazia) in the former USSR.

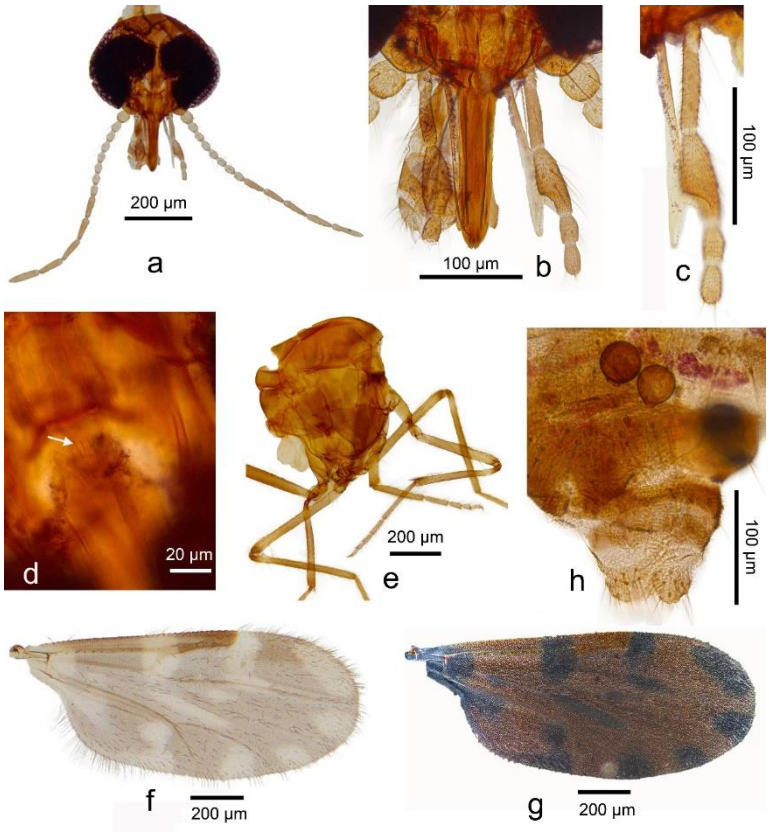

Fig. 1. *Culicoides clastrieri* female. **a**. Head, **b**. mouthparts, **c**. maxillary palp, **d**. pharynx posterior armature, **e**. thorax, **f**. wing (bright field image), **g**. wing (dark field image), **h**. abdomen.

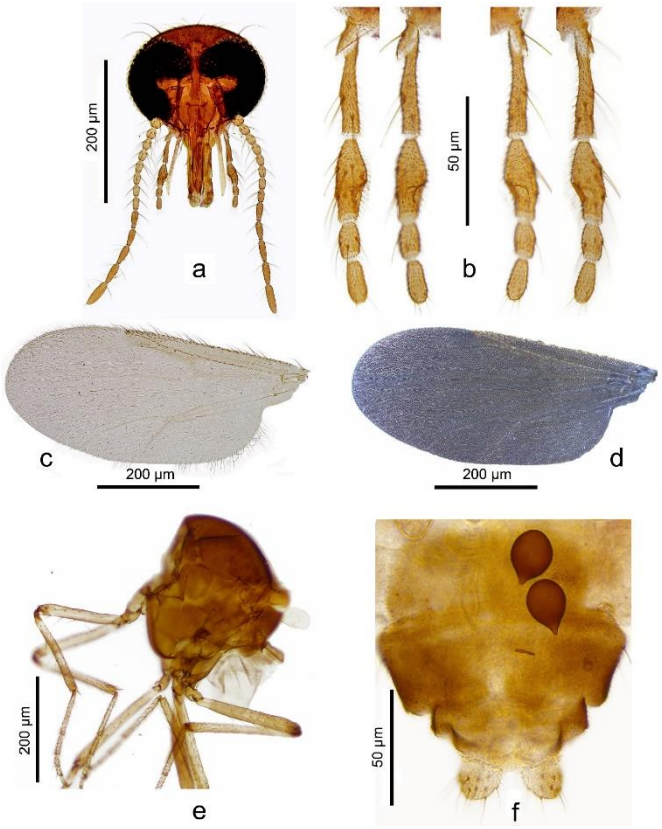

Fig. 2. *Culicoides tbilisicus* female. **a**. Head, **b**. maxillary palps (viewed from different angles), **c**. wing (bright field image), **d**. wing (dark field image), **e**. thorax, **f**. abdomen.

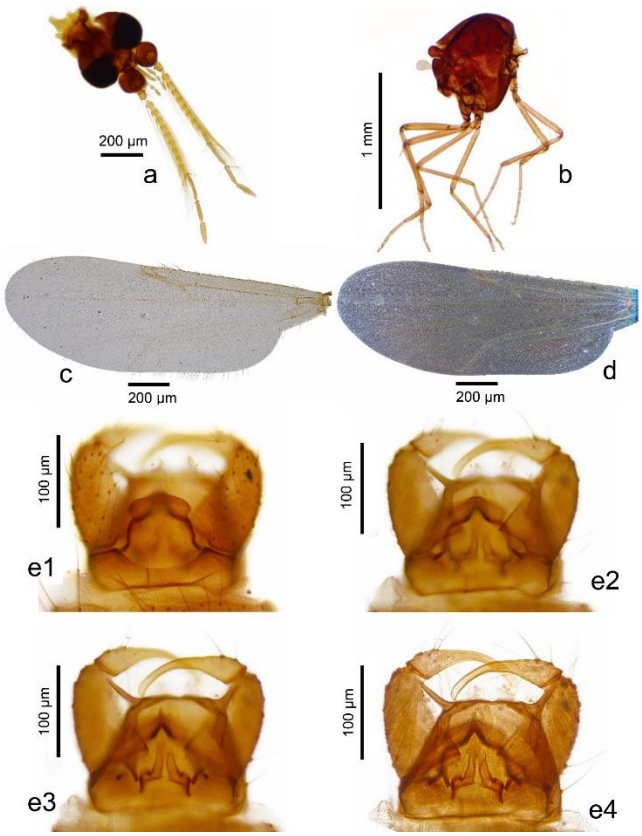

Fig. 3. *Culicoides tbilisicus* male. **a**. Head, **b**. thorax, **c**. wing (bright field image), **d**. wing (dark field image), **e**. genitalia (viewed from different angles).

Description

Female (n=10): Eyes bare and separated by a distance less than a facet (Fig. 2a). Palpus 5-segmented and 117 µm in length (104-124 µm). Third palpal segment moderately swollen, with multiple irregular pits (Fig. 2b). PR 2.61 (2.36-3.06). Flagellum length 314 µm (259-333 µm). AR 1.10 (1.06-1.14). Sensilla coeloconica present on flagellomeres 1, 9-13. In some specimens, sensilla coeloconica were observed on the 8th flagellomere. Sensilla coeloconica are small, light colored and difficult to see.

Wings without markings (Figs. 2c, d), length and width 612 × 288 µm (524-660 × 255-312 µm). CR 0.59 (0.57-0.61). Thorax brown, halters pale and legs light brown (Fig. 2e). Tibial comb with 4 spines, the second from the spur the longest. TR (I) 2.17 (2.07-2.28), TR (II) 2.68 (2.52-3.06), TR (III) 1.98 (1.91-2.06).

Two functional spermathecae, equal, ovoid and with short, sclerotized necks. Rudimentary spermatheca present. Functional spermathecae length and width 27 × 19 µm (25-30 × 17-21 µm), 26 × 19 µm (24-29 × 17-21 µm). Sclerotized ring at the end of the spermathecal duct absent (Fig. 2f).

Male (n=1): Head as in Fig. 3a. Palpus 5-segmented and 215 µm in length (n=2). Third palpal segment slightly swollen and with small sensory pit. PR 3.85 (3.69-4.02; n=2). Flagellum with 13 flagellomeres, length 859 µm (n=1). AR 0.94 (n=1). Flagellomeres 1-10 with long setae and flagellomeres 2-10 fused. Sensilla coeloconica present on flagellomeres 1, 11-13.

Thorax brown and legs yellowish brown (Fig. 3b). Tibial comb with 4 spines, the second from spur longest. TR (I) 2.02 (1.98-2.05; n=2), TR (II) 2.51 (2.43-2.58; n=2), TR (III) 1.87 (1.82-1.92; n=2). Wing without markings (Figs. 3c, d). In one example, wing width and length 1402 × 516 µm, while in the other example 1351 × 511 µm. CR 0.55 (n=2).

Male genitalia as in Fig. 3e. Sternite 9 with a deep and wide caudomedian excavation, membrane without spicules. Apicolateral processes of Tergite 9 long, slender and divergent. Dorsal apodeme of gonocoxite long but ventral apodeme small. Gonostylus curving with slender. Gonostylus broad basally, tapering and curving in the middle and a pointed tip. Parameres separate. Base of parameres bent laterally. Basal knobs large and sclerotized their extensions elongate parallel anteriorly. Also, protrusions in the form of thorns facing each other on the base of the parameres. Stem of parameres moderatly stout, apically swollen. Parameres with 4-5 pointed extensions laterally at the apex. Aedeagus with a very deep, broad, sclerotized basal arch with a pointed tip posteriorly. Lateral arms slender and curved, directed laterally at base. Shoulders of aedeagus remarkably broad and swollen. Distal median process long and slender, tapering to a rounded apex.

Discussion

Culicoides clastrieri is recorded for the first time from Turkey. Addition of this species in the fauna increased the reported number of *Culicoides* species in Turkey to 72. In addition, the number of *Culicoides* biting midges detected in Sinop is 30 (Dik *et al.* 2008, Turgut & Kılıç 2015, Dik *et al.* 2017, Turgut 2018a, 2018b, Korkmaz *et al.* 2021, Turgut & Küçük 2022). The distribution of *C. alazanicus* Dzhafarov, 1961, *C. delta* Edwards, 1939 (as *C. deltus*) (Dik *et al.* 2017), *C. brunnicans* Edwards, 1939, *C. griseidorsum* Kieffer, 1918 (Turgut & Kılıç 2015), *C. chiopterus* (Meigen, 1830), *C. comosioculatus* Tokunaga, 1956, *C. haranti* Rioux, Descous & Pech, 1959 has been reported only from the Black Sea Region in Turkey. Thus, *C. clastrieri* was included in these seven species.

Culicoides clastrieri is similar to *C. festivipennis* Kieffer, 1914 in terms of morphology. As a result of recent molecular studies, it has been revealed that these two species are also genetically close to each other (Sarvašová *et al.* 2014). However, due to the differences in their morphology, ecology, and trophic preferences, the opinion that these two species should be considered as different species has gained acceptance (Sarvašová *et al.* 2014). One of the main morphological differences between the two species is the dispersion of sensilla coeloconica in their antennae. In *C. clastrieri*, sensilla coeloconica are found in flagellomeres 1, 9-13, while in *C. festivipennis*, it is found in flagellomeres 1-13 (Mathieu *et al.* 2012; Sarvašová *et al.* 2014). In the specimen examined in this study, sensilla coeloconica were detected in flagellomeres 1, 7-13 on one antenna, and in flagellomeres 1, 8-13 on the other antenna, demonstrating the variation of the species in terms of the dispersion of sensilla coeloconica. A similar example of variation was noted by Szadziewski (1985). He reported that there were sensilla coeloconica on the flagellomere 3 in one antenna and on the flagellomere 4 in the other antenna in a specimen he examined. Another important morphological difference between the two species is seen in wing patterning. In *C. clastrieri*, the pale spot in the cell r5 is large and extends towards the second radial cell. However, the pale spot in the r5 cell in *C. festivipennis* is small. In addition, *C. festivipennis* contains 2-3 separate pale spots in m cells. But in *C. clastrieri*, the m cell has a large pale spot that does not merge with the pale spot on the r-m vein and extends towards the m2 cell (Figs. 1f, g) (Mathieu *et al.* 2012).

Culicoides tbilisicus is morphologically similar to *C. furcillatus* Callot, Kremer & Paradis, 1962. However, they differ in wing patterning and sensory pit structure in their third palpal segment. There are two pale spots on *C. furcillatus* wing and there is a small shallow sensory pit on its third palpal segment. However, the wing of *C. tbilisicus* is unpatterned (Figs. 2c, d) and carries multiple irregular sensory pits in its third palpal segment (Fig. 2b) (Mathieu *et al.* 2012). *Culicoides tbilisicus* bears sensilla coeloconica on flagellomeres 1, 9-13. It has been detected that the majority of the examined *C. tbilisicus* specimens have this sensilla coeloconica dispersion. Yet, in some specimens, it was observed that there was sensilla coeloconica on the flagellomeres 2, 3, 4, 7 and 8. Similarly, Glukhova (1989) reported that *C. tbilisicus* carries sensilla coeloconica on flagellomeres 1, 5-13 or 6-13.

As a result of the recent faunistic studies on biting midges in Turkey, important contributions have been made to the fauna of *Culicoides*. The number of species, which reached to 72 with the addition of *C. clastrieri* to the Turkish biting midges fauna is relatively high when compared to the 117 species in Europe. This situation reveals once again the importance of Turkey in terms of biodiversity.

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Conflict of Interest: The author has no conflicts of interest to declare.

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