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# Ecological Requirements of Larval Simuliidae (Insecta, Diptera) Species of some Streams in Camili Valley (Artvin, Turkey)

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**Research Article** 

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#### Abstract

This study was carried out in July 2016 in Camili (Macahel) which is Turkey's first and only biosphere reserve area. This study aimed to investigate the Simuliidae species of some streams in Camili Valley, to identify the "reference site" which are important for the Water Framework Directive (WFD), to determine ecological characteristics of the studied sites according to System A and System B Classification of Water Framework Directive (WFD), to explore the relationship between the recorded species and some environmental variables of streams by using CCA technique. For this purpose, 257 individuals of Simuliidae were sampled from nine studied sites. Physicochemical variables (dissolved oxygen, pH, water temperature, electrical conductivity, velocity) were measured at each studied site. In Camili Valley the following species were collected: *Simulium (Nevermannia) angustitarse, Simulium (Nevermannia) cryophilum, Simulium (Nevermannia) vernum, Simulium (Simulium) tuberosum, Simulium (Simulium) variegatum*. Because anthropogenic stresses were insignificant in the research area, studied sites and Simuliidae fauna of the area were not affected negatively. Also, six of the studied sites have reference habitat conditions.

Keywords: Canonical Correspondence Analysis, Camili (Macahel), physicochemical variables, reference site, Simuliidae.

Camili Vadisi'ndeki (Artvin, Türkiye) bazı akarsuların larval Simuliidae (Insecta, Diptera) türlerinin ekolojik gereksinimleri

#### Özet

Bu çalışma, Temmuz 2016 tarihinde Türkiye'nin ilk ve tek biyosfer rezerv alanı olan Camili (Macahel) bölgesinde gerçekleştirilmiştir. Çalışma ile Camili Vadisi'nde bulunan bazı akarsulardaki Simuliidae türlerinin ve Su Çerçeve Direktifi (SÇD) için önem taşıyan "referans istasyon" ların belirlenmesi, Su Çerçeve Direktifi (SÇD)'nin Sistem A ve B sınıflandırmasına göre çalışılan istasyonların ekolojik karakterlerinin tespit edilmesi, CCA analizi kullanılarak bazı çevresel değişkenler ile kaydedilen türler arasındaki ilişkinin ortaya çıkarılması amaçlanmıştır. Bu amaçla, dokuz örnekleme noktasından Simuliidae familyasına ait 257 birey toplanmıştır. Her örnekleme alanında fizikokimyasal değişkenler (su sıcaklığı, pH, elektriksel iletkenlik, çözünmüş oksijen, akıntı hızı) ölçülmüştür. Teşhisler sonucunda *Simulium (Nevermannia) angustitarse, Simulium (Nevermannia) cryophilum, Simulium (Nevermannia) vernum, Simulium (Simulium) bezzii, Simulium (Simulium) tuberosum, Simulium (Simulium) variegatum türleri tespit edilmiştir. Çalışma alanında antropojenik baskı önemsiz düzeyde olduğu için çalışılan istasyonlar ve Simuliidae faunası olumsuz etkilenmemiştir. Ayrıca, istasyonlardan altı tanesi referans istasyon özelliği göstermektedir.* 

Anahtar Kelimeler: Camili (Macahel), fizikokimyasal değişkenler, Kanonik Uyum Analizi, referans istasyon, Simuliidae.

#### INTRODUCTION

The family Simuliidae has spread on all continents except Antarctica. According to the most recent edition of the inventory of world Simuliidae (Adler, 2020), Simuliidae fauna comprises 2348 species (2331 living and 17 fossils). Larvae of Simuliidae, an important member of aquatic invertebrate communities, play a significant role in aquatic food webs (Crosskey, 1990). Filter-feeding larvae feed on dissolved organic matter (DOM) and suspended particles (Wotton, 2009; Ciborowski et al., 2017).

Simuliidae species can be used as a bioindicator for indicating habitat degradation of streams (Feld et al., 2002). Many environmental factors such as dissolved oxygen, pH, electrical conductivity, water temperature, substrate structure influence the distribution of blackflies (Ross and Merritt, 1978;

Lautenschlager and Kiel, 2005). Some Simuliidae species are very sensitive to environmental changes, while some species are tolerant to changes in habitats (Seitz, 1992; Feld et al., 2002; Kazancı, 2006).

The European Water Framework Directive (WFD), which is the most comprehensive water legislation of the European Union (EU), aims to protect the ecological status of aquatic ecosystems, to prevent their degradation, to restore all water bodies, and to conserve water resources in Europe (Council of European Communities, 2000). Biomonitoring of the ecological quality of aquatic ecosystems is very important and necessary for WFD studies. Benthic macroinvertebrates are widely used as bioindicators for monitoring habitat quality (Rosenberg and Resh, 1983; De Pauw et al., 2006). Another purpose of WFD is to determine which water bodies could be classified as "reference conditions". The WFD defines the reference conditions as the conditions that prevail in the absence or near absence of human disturbance or alteration (Council of European Communities, 2000).

The number of WFD studies using macroinvertebrates has increased in last 10 years in Turkey (Kazancı and Ertunç, 2010; Kazancı et al., 2010a; Kazancı et al., 2010b; Duran and Akyıldız, 2011; Ekingen and Kazancı, 2012; Kazancı et al., 2013a; Kazancı et al., 2013b; Zeybek et al., 2014; Kazancı et al., 2015; Türkmen and Kazancı, 2015; Arslan et al., 2016a; Arslan et al., 2016b; Başören and Kazancı, 2016; Bolat et al., 2016; Türkmen and Kazancı, 2019; Gültekin et al., 2017; Kazancı et al., 2017; Zeybek, 2017; Akay and Dalkıran, 2019; Gültekin, 2019).

Eastern Black Sea Region, known as a sub-ecoregion of the Caucasus Biodiversity Hotspot, which is one of the 25 World Biodiversity Hotspot regions (Myers et al., 2000; Kazancı et al., 2011). The Caucasus is one of the Worldwide Fund for Nature's (WWF) Global 200 Ecoregions, identified as globally outstanding for biodiversity. Camili (Macahel) is surrounded by Karçal Mountains on three sides. This area was declared by United Nations Educational, Scientific, and Cultural Organization to be the first and only "Biosphere Reserve Area" in Turkey (UNESCO, 2005).

This study aimed to investigate the Simuliidae species of some streams in Camili Valley, to identify the "reference site" which are important for the Water Framework Directive (WFD), to determine ecological characteristics of the studied sites according to System A and System B Classification of WFD, to explore the relationship between the recorded species and some environmental variables of streams by using CCA technique.

## **MATERIAL AND METHODS**

Camili (Macahel), which is in Borçka district of Artvin province in northeastern Turkey, is located in a valley on the slopes of Karçal Mountains (Figure 1). Since this area has not been much impacted by anthropogenic activities, the streams, especially at higher altitudes, are slightly polluted or unpolluted.

Nine (9) sites were sampled from running waters which are located in Camili in July 2016. In each site, water temperature, pH, electrical conductivity, dissolved oxygen concentration, and velocity were measured in the field by using a YSI 556 multi-probe system and Hydro Bios current meter RHCM. The water quality classes of the studied sites were evaluated by using the Surface Water Quality Regulation Annex-5 (Anonymous, 2015).

Larvae and pupae of Simuliidae were collected by a standard D-shaped pond net and by hand. Samples were preserved in 80% ethyl alcohol. Leica MZ75 stereomicroscope and Olympus CX21FS1 binocular microscope were used for identifications. Simuliidae species were identified according to Rubtsov, 1990; Crosskey, 2002; Jedlicka et al., 2004; Lechthaler and Car, 2005; Crosskey and Zwick 2007.

To investigate the relationships between Simuliidae species and the environmental variables, canonical correspondence analysis (CCA) was performed using the ECOM 2.1.3.137 version (Henderson and Seaby, 2007) software programs.

Some geological characteristics required for System A and System B of WFD and physical variables of the studied sites were recorded for the definition of the stream types.

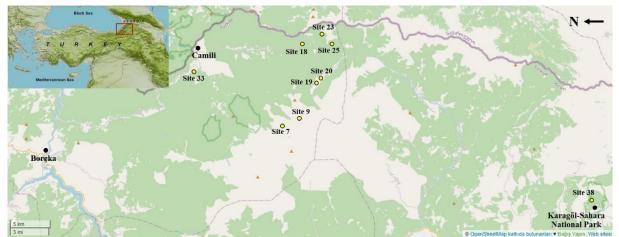


Figure 1. Studied area and collecting sites of Camili Valley.

## RESULTS

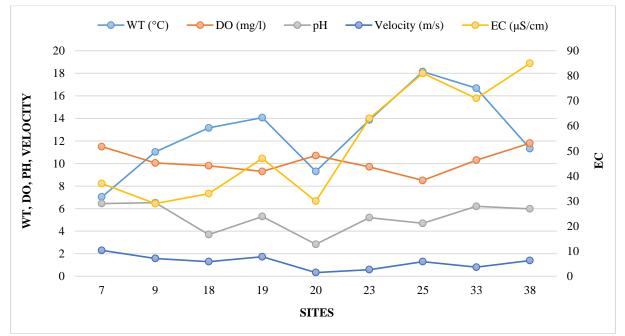
A total of 257 blackfly larvae and pupae were collected, and six species were identified from nine studied sites. Identified species were *Simulium (Nevermannia) angustitarse, Simulium (Nevermannia) cryophilum, Simulium (Nevermannia) vernum, Simulium (Simulium) bezzii, Simulium (Simulium) tuberosum, Simulium (Simulium) variegatum (Table 1).* According to this table, the highest species number (4) was found in Site 9 and 20 while the lowest species number (1) was found in Site 25 and 33.

Table 1. Simuliidae species list of collecting sites.

Species/Sites	7	9	18	19	20	23	25	33	38
S. (N.) angustitarse					*	*			*
S. (N.) cryophilum		*			*	*			
S. (N.) vernum			*		*				
S. (S.) bezzii		*		*					
S. (S.) tuberosum	*	*	*	*	*				*
S. (S.) variegatum	*	*	*	*			*	*	*

The results of the physicochemical variables (water temperature, pH, electrical conductivity, dissolved oxygen concentration, velocity) were given in Figure 2.

The water temperature values recorded were between 7.03 and 18.15 °C. The dissolved oxygen values recorded were between 8.5 and 11.8 mg/l. The pH values recorded were 2.84 and 6.53. The electrical conductivity values recorded were between 29 and 85  $\mu$ S/cm. The velocity values recorded were between 0.33 and 2.33 m/s.



**Figure 2.** Values of water temperature (°C), dissolved oxygen (mg/L), pH, electrical conductivity (µS/cm), and velocity (m/s)

The nine sites and six species detected in these sites were shown in the CCA diagram (Figure 3). The order of significance of environmental variables was found to be, from most significant to least significant: velocity, electrical conductivity, water temperature, dissolved oxygen concentration, and pH.

Some physical and geological characteristics of studied sites required for System A and System B of WFD were given in Table 3.

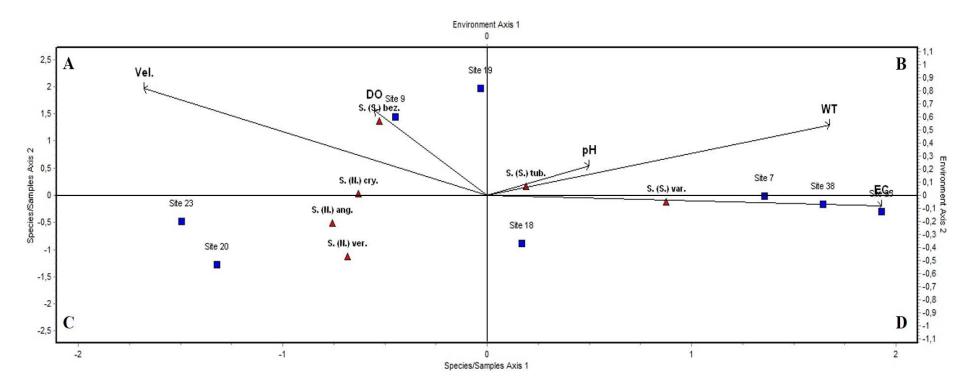
Sites	Site name	WT (°C)	DO (mg/l)	pН	EC (µS/cm)	Final Water Quality Class
7	Lekoban Plateau-1	Ι	Ι	Ι	Ι	III
9	Lekoban Plateau-2	Ι	Ι	Ι	Ι	Ι
18	Merata Plateau-1	Ι	Ι	IV	Ι	IV
19	Merata Plateau-2	Ι	Ι	IV	Ι	IV
20	Merata Plateau-3	Ι	Ι	IV	Ι	IV
23	Çurupira Plateau-1	Ι	Ι	IV	Ι	IV
25	Çurupira Plateau-3	Ι	Ι	IV	Ι	IV
33	Gohinav Stream	Ι	Ι	III	Ι	III
38	Karagöl-1	Ι	Ι	IV	Ι	IV

**Table 2.** Water quality classes of the studied sites according to the physicochemical variables (Anonymous, 2015)

(WT: temperature, DO: dissolved oxygen, EC: electrical conductivity)

	Site 7	Site 9	Site 18	Site 19	Site 20	Site 23	Site 25	Site 33	Site 38
Ecoregion (System A)	Y	Y	Y	Y	Y	Y	Y	Y	Y
Altitude (System A)	High (>800m)	High (>800m)	High (>800m)	Medium (200m-800m)	High (>800m)				
Altitude (System B)	2386m	2388	2092	2171	2190	2190m	1963m	563m	1642m
Catchment Area (System A)	Medium (100-1000km <sup>2</sup> )	Medium (100-1000km <sup>2</sup> )	Medium (100-1000km <sup>2</sup> )	Medium (100-1000km <sup>2</sup> )	Medium (100-1000km <sup>2</sup> )				
Geology (System A and System B)	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous
Latitude (System B)	41° 24' 0.84" N	41° 24' 0.61" N	41° 29' 0.90" N	41° 26' 5.41" N	41° 26' 4.94" N	41° 30' 0.28" N	41° 29' 6.59" N	41° 27' 5.4" N	41° 18' 34" N
Longitude (System B)	42° 02' 2.87" E	42° 03' 3.85" E	42° 03' 9.81" E	42° 04' 5.53" E	42° 04' 6.90" E	42° 05' 2.79" E	42° 06' 1.28" E	41° 53' 54.32" E	41° 28' 56" E
Substratum	30% rock, 50% stone, 15% gravel, 5% sand	40% rock, 40% stone, 15% gravel, 5% sand	40% rock, 40% stone, 15% gravel, 5% sand	20% rock, 50% stone, 25% gravel, 5% sand	20% rock, 50% stone, 20% gravel, 10% sand	20% rock, 50% stone, 20% gravel, 10% sand	40% rock, 40% stone, 15% gravel, 5% sand	30% rock, 40% stone, 20% gravel, 10% sand	20% rock, 35% stone, 30% gravel, 15% sand
Stream Zone	Hypocrenon	Hypocrenon	Hypocrenon	Epirhithron	Epirhithron	Epirhithron	Epirhithron	Metarhithron	Epirhithron
<b>Riparian vegetation</b>	0%	10%	10%	10%	0%	40%	10%	100%	50%
Stream width in dry period	1.5m	1.5m	2m	4m	1m	2m	2m	9m	4m
Stream width in wet period	2.5m	3.5m	1m	15m	1m	7m	8m	15m	8m

Table 3. Geological and physical characteristics of sampling sites according to System A and System B Classification of WFD



Abbreviations: S. angustitarse – S. ang; S. cryophilum – S. cry; S. vernum – S. ver; S. bezzi – S. bez; S. tuberosum – S. tub; S. variegatum – S. var; velocity – Vel; dissolved oxygen – DO; water temperature – WT; electrical conductivity – EC.

Figure 3. CCA diagram with environmental variables and Simuliidae species (▲: Species, ■: Sites)

## **DISCUSSION AND CONCLUSION**

Investigation of the Simuliidae fauna of Turkey is necessary and important for biological monitoring studies and WFD research. In this study, the Simuliidae species composition of nine sites in Camili Valley were determined, information on their habitat preferences was given, and some physicochemical variables of sites were measured. The pH values were low in almost all studied sites because of snowmelt and rainfall runoff in the research area. These events during spring and early summer, cause a short-term decline in alkalinity. This process is called episodic acidification and was reported by Kazancı (2009) for the first time in the Eastern Black Sea Region and the Yeşilırmak River Basin.

In almost all studied sites, pH value was associated with Class III and Class IV water quality while other physicochemical variables were associated with Class I water quality. Blackflies may be more tolerant to decreases in pH value than another group of freshwater macroinvertebrates (Bernard et al., 1990; Chmielewski and Hall, 1992). In other words, many blackfly species will be able to cope with the short-term decline in alkalinity. Therefore, Simuliidae species were sampled in our studied sites, even under low pH conditions caused by episodic acidification.

Six of the studied sites (Site 7, 9, 18, 19, 20, and 23) have reference habitat conditions. There are no anthropogenic impacts or habitat degradation in the study area and all sites have high water quality according to physicochemical variables (except pH values due to episodic acidification). Site 25 and 33 were located near the villages. Site 38 was located in Karagöl-Sahara National Park and samples were collected from inlet stream. Also, there was a regulator near Site 38. Therefore, these sites have not to reference habitat conditions.

The relationship between six Simuliidae species and some physicochemical variables (water temperature, dissolved oxygen, pH, electrical conductivity, and velocity) of streams were shown in the CCA diagram (Figure 3). The determinant environmental variables in quadrant A were velocity and dissolved oxygen. According to the CCA diagram, *S. (S.) bezzii* was positively correlated with these two variables. *S. (S.) bezzii* usually lives in fast-flowing unimpaired running waters, but it also can live in degraded eutrophic waters (Seitz, 1994; Lechthaler and Car, 2005). This species prefers oligosaprobic and betamesosaprobic habitats (CSN 75 7716, 1998). There is no information concerning stream zonation preferences in the literature. Başören and Kazancı (2017) recorded this species from crenon, epirhithron, and metarhithron zone of the streams in the Eastern Black Sea Region. Site 9 and 19 were placed in quadrant A. *S. (S.) bezzii* was recorded only from these sites. Site 9 was located in hypocrenon zone of the mountain stream in Lekoban Plateau and has Class I water quality. Site 19 was located in epirhithron zone of the mountain stream in Merata Plateau and has Class IV water quality because of pH value.

The determinant environmental variables in quadrant B were pH and water temperature. S. (S.) tuberosum was placed in this quadrant but it was close to the center of the CCA diagram because of a weak correlation with the environmental variables. This species was the second common species after S. (S.) variegatum in this study. It was recorded from six sites (Site 7, 9, 18, 19, 20, and 38). S. (S.) tuberosum was previously recorded by Kazancı and Ertunç (2008) in Artvin province. It inhabits mostly medium sized upland streams with fast-flowing, but it can be found in wide streams with rich vegetation and slow current (Zahar, 1951; Bass, 1998). This species primarily prefers oligosaprobic and betamesosaprobic habitats, but it also occurs in xenosaprobic habitats. It has wide habitat preferences range from epirhithron to epipotamon, but S. (S.) tuberosum prefers mainly metarhithron and hyporhithron of streams (Lechthaler et al., 2017). Site 7 was placed in between quadrant B and D. In this site, only S. (S.) tuberosum and S. (S.) variegatum were recorded. Therefore, they were closely located in the CCA diagram. Site 7 was located in hyporenon zone of the mountain stream in Lekoban Plateau and has Class I water quality. S. (S.) tuberosum was collected from the other two sites (Site 9 and 18) in hypocrenon zone of streams. That is, it can be said that this species can also prefer this region of streams.

There was no determinant environmental variable in quadrant C. S. (S.) angustitarse and S. (S.) vernum were placed in this quadrant. They were negatively correlated with water temperature and pH. S. (S.) angustitarse has a widespread distribution through entire Europe. It is a stenothermal species and predominates in clean and cold waters near the source of rivers (Lechthaler and Car, 2005). Therefore, they were placed opposite the representing water temperature variable. However, this species can survive in organically polluted water (Rubtsov, 1990). It mainly prefers betamesosaprobic

habitats, but it also occurs in oligosaprobic and alphamesosaprobic habitats. The stream zonation preferences of this species are epirhithron, metarhithron and hyporhithron mainly, but it occurs also hypocrenon zone of streams (Lechthaler et al., 2017).

S. (S.) vernum can live in a wide range of running waters from small mountain streams to large rivers (Scheder and Waringer, 2002). It is generally found in oligosaprobic and betamesosaprobic habitats, but it can be inhabited in xenosaprobic and alphamesosaprobic habitats. The stream zonation preferences of this species are epirhithron and metarhithron mainly, but it occurs also hypocrenon and hyporhithron zone of streams. (Lechthaler et al., 2017). S. (S.) vernum was recorded from Site 18 and 20 with the lowest pH values (3.7 and 2.84 respectively) in this study. Similarly, this species was found by Basören (2015) at three different sites with low pH value (4 and 5.5) in Aksu Stream (Giresun, Turkey). For this reason, it is expected that it was placed opposite the arrow representing pH variable. Site 20 and 23 were placed in quadrant C. S. (S.) angustitarse, S. (S.) vernum and S. (N.) cryophilum were recorded mostly from these sites. S. (N.) cryophilum was placed in between quadrant A and C. This species is one of the most common blackfly species in undisturbed mountain brooks (Scheder and Waringer, 2002; Crosskey and Howard, 2004). It prefers also cold and fast-flowing running waters (Kazancı, 2006). Therefore, S. (N.) cryophilum was placed opposite the representing water temperature variable and close the representing velocity variable. This species is generally found in oligosaprobic and betamesosaprobic habitats, but it can be inhabited in xenosaprobic and alphamesosaprobic habitats. The stream zonation preference of this species is epirhithron mainly, but it occurs also hypocrenon and metarhithron zone of streams (Lechthaler et al., 2017). Site 20 and 23 that were placed in quadrant C, were located in epirhithron zone of the mountain streams in Merata and Çurupira Plateau respectively. These studied sites have Class IV water quality because of pH value.

The determinant environmental variable in quadrant D was electrical conductivity. S. (S.) variegatum was placed in this quadrant. According to the CCA diagram, S. (S.) variegatum was positively correlated with this variable. This species was the most abundant (52%) in this study. It is widespread from Europe to the Caucasus (Crosskey and Howard, 2004). It mostly lives in mountain streams and small rivers with high dissolved oxygen concentrations (Kiel, 2001; Scheder and Waringer, 2002). S. (S.) variegatum is generally found in oligosaprobic and betamesosaprobic habitats, but it can be inhabited in alphamesosaprobic habitats. Epirhithron, metarhithron, and hiporhithron are stream zonation preferences of this species (Lechthaler et al., 2017). In Basören and Kazancı (2017), this species was recorded mainly from epirhithron zone of the streams in the Eastern Black Sea Region. Additionally, this species was also found in crenon zone. Site 18, 25, 33, and 38 were placed in quadrant D. Sites 25 and 33 do not appear clearly in the CCA diagram because they overlap. The reason for this situation is that only S. (S.) variegatum was recorded from both sites. Since Site 25, 33, and 38 have the highest EC values (81, 71, 85 µS/cm respectively), they are expected to be positioned next to the arrow representing EC. S. (S.) variegatum has the highest number of individuals (111 individuals) in Site 38. Site 18 was in hypocrenon zone of the mountain stream in Merata Plateau and has Class IV water quality. Site 25 was in epirhithron zone of the mountain stream in Curupira Plateau and has Class IV water quality. Site 33 was in metarhithron zone of the Gohinav streams and has Class III water quality. Site 38 was situated in Karagöl-Sahara National Park and there was a regulator near the sampling site. This site also has Class IV water quality. Site 25 and 33, which were located near the villages, and Site 38 have not to reference habitat conditions. The reason why these four studied sites have Class IV water quality is the pH value.

Camili (Macahel) declared as the first and only biosphere reserve area of Turkey is one of the biologically richest regions. Anthropogenic impacts on this area are insignificant and six of the studied sites also showed reference habitat conditions. However, biological degradation, tourism activities, hydroelectric power plant construction and road construction have recently threatened aquatic habitats in this region. Because our studied sites were generally in isolated areas, Simuliidae fauna was not affected by these negative impacts. All sites are suitable for the survival of Simuliidae species. In this study, *S.* (*N.*) angustitarse, *S.* (*N.*) cryophilum, *S.* (*N.*) vernum, *S.* (*S.*) bezzii, *S.* (*S.*) tuberosum, and *S.* (*S.*) variegatum were recorded from Camili Valley. However, it can be expected that much more Simuliidae species will be found because this region is rich in biodiversity.

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