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Prevalance of Simulium Species in the Kızılırmak River Basin in Ankara and Kırıkkale Territories

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Abstract
This study aimed to determine the fauna of Simuliidae within the boundaries of Kizilirmak River Basin in Kirikkale and Ankara. The materials of the study were composed of a total of 8877 samples including 7509 larva, 372 end-stage larva and 996 pupae collected from nine different locations (Kalecik, Bala, Yahşihan, Merkez (Kirikkale), Bahşılı, Keskin, Karakeçili, Çelebi, Sulakyurt), through which Kızılırmak river running between March 2009 and October 2010. The samples were assessed by a stereomicroscope. In examination, *S. petricolum*, *S. equinum*, *S. pseudequinum*, *S. lineatum*, *S. balcanicum*, *S. erythrocephalum* and *S. alajense* were the species identified. In addition, *S. angustipes* and *S. ornatum* were classified into groups. *Simulium petricolum* (25.73%) was the most prevalent specie identified while *S. alajense* (0.02%) was the least. In conclusion, while the presence of *S. lineatum*, *S. pseudequinum*, *S. balcanicum* and *S. alajense* were reported previously, *S. petricolum, S. equinum* and *S. erythrocephalum* were recorded the first time in Kızılırmak.

Keywords: Ankara, identification, Kirikkale, Kızılırmak River Basin, prevalence, *Simulium* spp.

INTRODUCTION

Simulium are flies belonging to Arthropoda root, Insecta class, Diptera order, Nematocera subset and Simuliidae family.The most common lineage in the Simuliidae family is Simulium.In this lineage, there are 38 sub-lineages and around 1000 species (Wal and Shearer 2001; Şirin 2007).

Apart from the Antarctic continent, some deserts and islands where there are no river sources, 2101 living and 12 extinct species have been identified that are widespread all over the world (Adler and Crosskey 2010).

Adults of the Simuliidae species found in the nematocera suborder are flies with short chunky bodies, large wings, strong legs, short antennae and short proboscis.Body length varies between 1.2 - 6.0 mm depending on the species.The larvae hatching in the form of worms.The body structure of the larvae consists of 3 parts as caput, thorax and abdomen.Caput is well developed.The largest part of the body is the thorax.Abdomen consists of 8 segments (Crosskey 1990).Simuliidae pupae are usually found in a cocoon.The wings and legs are fixed to the nested abdomen, and the abdomen is relatively immobile.Body length varies between 2-7 mm (Jedlicka and Stloukalova 1997; Jensen 1997).

Flies in this family are the food source of various living organizms such as trout (Ozvarol et al. 2011). The flies in the Simuliidae family feed on plant juice. The females also suck blood from human and warm-blooded animals to complete their life-cycle (Crosskey 1990). Because of this biological feature, they cause numerous pathological conditions. Simulium sp. with toxic, traumatic and, startling propertiesactivate some pathological mechanisms. They are also mechanical or biological vectors for some viral, bacterial and parasitic diseases. They cause anemia in animals due to blood sucking; thus,the incidence of death occursin affected animals time to time (Tüzel et al. 1997; Wal and Shearer 2001; Van Emden 2004; Charles and Hendrix 2006; Yaman 2006). Simuliumsp. are the vector of onchocerciasis (Albany 1997; Charalambous et al. 2005; İnci and Düzlü 2009), commonly known as the river blindness in humans in the tropic lands. Moreover, they are responsible for the transport of Onchocerca species, which are observed in cattle, various deer species and wild boar (Güralp 1997; Toparlak and Tüzer 2005). They are also vector for Mansonella ozzardi, which is found in the peritoneal cavity of humans in tropical countries and its microphiesare found blood and skin. In poultry, Simulium sp. are vector for Splendido flaria falliensis, Leucocytozoon and some Trypasoma species (Tüzer and Toparlak 1999; Service 2001). Simuliidae flocks collectively fly children's nostrils and may cause occlusion that may result in death (Demirsoy 2003). Periodic outbreaks threaten human and animal health by disturbing their psychology. As a consequence,they may cause serious economic losses especially in agriculture and tourism (Taylor et al. 2003; Şirin 2007, Sarıözkan et al 2014).

There are regional faunistic studies of Simulium species in our country (Jedlicka 1975; Kazancı and Ciergue-Gazeau 1990; Şirin 2001; Şirin and Şahin 2005; Kazancı 2006; Crosskey and Zwick 2007). However, these studies are not sufficient to reveal the complete fauna of the country as previously concluded by the previous studies (Ertunç et al. 2008; Kazancı and Ertuç a 2009; Kazancı and Ertuç b 2009, Kazancı and Ertuç 2010).

In this study, we aimed to reveal the presence of Simuliidaesp. in the river Kizilirmak River Basinand classify them into species or groups.

MATERIALS AND METHODS

In this study, larvae and pupae belonging to Simulium species were collected from 9 different stations (Kalecik, Bala, Yahşihan, Merkez (Kırıkkale), Bahşılı, Keskin, Karakeçili, Çelebi, Sulakyurt) through which the River passes between March 2009 and October 2010.Samples were collected at the intervals of 15 days: once in March, twice in April, May, and June and once a month in July, August and September, and the stations were visited 10 times a year for two years following the same sequence.

Samples were collected at 15-day intervals, once in March, twice in April, May, June and 30-day intervals in July, August and September. Following the same sequence, the stations were visited 20 times for a period two years.

Plants, stones and litter, which are located in shallow parts of the river with a slow flow rate, were quickly removed from the water. The pupae and larvae which are adhered to the smooth and clean surfaces of these substances were collected from the surfaces carefully with pincers or watercolor brush. Samples were collected into 3.5 mm plastic caps and fixed in 80% ethanol.

The samples were first examined under a stereo dissection microscope (Olympus SZ61 brand).Each sample was separated into young larvae, old larvae and pupae separately for the collected site. Larvae and pupae were counted separately and stocked. For diagnosis, pupae and aged larvae were examined and photographed according to diagnostic keys.

Samples were identified and categorized according to the descriptions made by Adler and Crosskey 2010;Bass 1998;Belqat and Dakki 2004;Crosskey1986, Crosskey (a) 1991;Crosskey (b) 1991;Crosskey 1998;Crosskey 2002;Crosskey and Adler 1996;Crosskey and Crosskey 2007, Jedlicka 1975;Jensen 1984, 1997;Crosskey and Malicky 2001, Knoz 1998 and Rubtsov 1990. For species distribution in Turkey and the world, the checklists cited by Kazancı and Ertunc (2009) and Adler and Crosskey(2010) were used.

RESULTS

A total of 8877 specimens, 7897of which were in larvae stage and 996 were in pupae stage, were evaluated. Among 7897 larvae, 372 were in the last period of larva stage. In 2009 and 2010, 4897 and 3980specimens were collected, respectively. Microscopic examination revealed presence of 7 different species. However, 2 different Simulium spp. could not be identified morphologically in specie level. The identified Simulium spp. were given below:

***Simulium (Wilhelmia) equinum*(Linnaeus, 1758)**

Simulium (Wilhelmia) equinum samples were collected from the leaves and stems of plants found the shallow sites of the river. A total of 47 end-stage larvae and pupae, constituting 3.43% of the samples, belonged to this specie. Samples were found in districts of Kalecik, Yahşihan, Kırıkkale-Center and Bahşılı in July 2009, Yahşihan in June 2010 and Bahşılı and Keskin in August 2010. The morphological characteristics of this species were depicted in Figure 1, Figure 2.

Figure 1. By*Simulium equinum*(A) last-instarlarvaes ventral viewing of head capsule and length of the postgenal slot (black arrow)(4.5X), (B) last-instarlarvaes dorsal viewing of head capsule and anterolateral spots (black arrow) (4.5X).

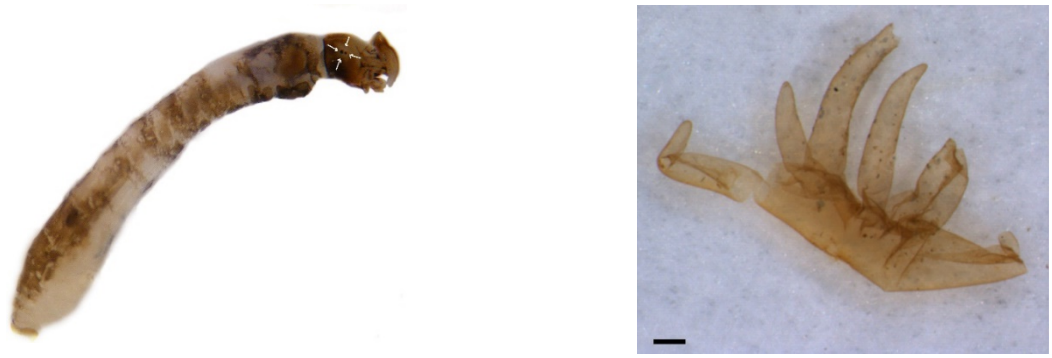

Figure 2. By*Simulium equinum*last-instarlarvaes lateral viewing. The eye spots on the sides of the head and around the irregularly shaped white areas (Left)(white arrow) (0,67X), pupal structure of the gill filaments (right) (Bar: 200µm).

***Simulium (Wilhelmia) pseudequinum (*Seguy, 1921)**
342 end-stage larvae and pupae belonging to this species were found in the plant stem and leaves in the shallow areas of river. These samples constituted 25% of the samples diagnosed. The larvae and pupae of this specie were found in the district of Yahşihan in July 2009, Kalecik, Yahşihan, Kırıkkale-Center and Bahşılı in August 2009, Yahşihan and Kırıkkale-Center in September 2009, and Kalecik and Yahşihan in October 2009.

***Simulium (Wilhelmia) lineatum*(Meigen, 1804)**
Samples of this species, 257 end-stage larvae and pupae, were found in the plant stem, leaves as well as stones found in shallow areas of the river. The number of samples belonging to this specie constituted 18.78% of the samples identified. Samples fo this specie were found in the districts of Kalecik, Yahşihan and Bahşılı in July 2009, Kalecik, Yahşihan and Bahşılı in August 2009, Bahşılı in September 2009, Kalecik and Bahşılı in October 2009, Karakeçili in May 2010, Kalecik in July 2010, Kalecik and Keskin in July and August 2010.

***Simulium (Wilhelmia) balcanicum*(Enderlein, 1924)**
This species has been identified mostly as in co-presence with *S. equinum* and *S. pseudequinum*. Among the species identified, 235 end-stage larvae and pupae, constituting 17.17% of the diagnosed samples, belonged to this species. The larvae and pupae of this species were collected in the districts of Kalecik, Yahşihan and Bahşılı in July 2009, Bahşılı in August 2009, Kalecik and Bahşılı in September 2009, Kalecik in June 2010, Yahşihan and Keskin in July 2010, Kalecik, Yahşihan, Kırıkkale-Center, Bahşılı and Keskin in August 2010 and Kalecik and Kırıkkale-Center in September 2010.

***Simulium (Eusimulium) angustipes (*Edwards, 1915)**
This species, which generally lives in small and warm streams, has been found on the plant stem and leaves in vegetation-rich waters.78 end-stage larvae and pupae of this species were collected and constitute 5.7% of the identified samples.Examples were found in Kalecik, Bahşılı and Keskin districts in August 2009, Kırıkkale-Merkez district in September 2009, Yahşihan district in October 2009, and Keskin districts in July 2010.

***Simulium (Eusimulium) petricolum* (Rivosecchi, 1963)**
Samples of *Simulium (Eusimulium) petricolum* were found in small branches of the river that formed and seen time to time depending on the seasons and frequently seen in the summer. Constituting 25.73% of the samples collected, 352 end-stage larvae and pupae were belonged to this specie. Samples of the species were found in the districts of Yahşihan and Kırıkkale-Center in July 2009 and August 2009, Kırıkkale-center in September 2009, Kalecik and Yahşihan in October 2009, and Yahşihan in May and June 2010. The morphological characteristics of this species were depicted in Figure 3, Figure 4.

Figure 3. By*Simulium petricolum*; The general viewing of last-instar larvae, shape ofpupal gill histoblast (arrow). (left) (0.67X), last-instar larvaes ventral viewing of head capsule and length and width and of the postgenal slot (right)(white arrow)(4.5X).

Figure 4. *Simulium (Eusimulium) petricolum*general viewing of pupa, gill filaments branching patterns (0.67X).

***Simulium (Boophthora) erythrocephalum* (DeGeer, 1776)**
This species, which usually lives in warm, flowing but slow flowing waters, has been found attached to plants in this particular water.Among the samples collected for the study, only 3 pupae (0.21%) were collected from Kalecik and Yahşihan districts in July 2010 and Bahşılı district in August 2010. The morphological characteristics of this species were depicted in Figure 5.

Figure 5. By *Simulium erythrocephalum* the general viewing of pupae and coccon (left)(0.67X), pupal structure of the gill filaments (right)(Bar: 200µm).

***Simulium*(*Simulium*) *alajense* (Rubtsov, 1938)**
Simulium(*Simulium*) *alajense* samples were collected from the stem and leaves of plants found in small streams leaving off the main stream with a high discharge rate and vegetation. This species as in pupae stage (0.07%) was encountered in July 2010 in the disrict of Kalecik.

***Simulium ornatum* (Meigen, 1818)**
The *Simulium ornatum* larvae and pupae of this species were found in the plant stem and leaves found in small streams leaving off the main stream with a high discharge rate. Since the morphological characteristics of the species in the *Simulium ornatum* group show similarities, the samples were sub classified into species-level by means nomenclature. Constituting 3.87% samples collected, 53 end-larvae and pupae were belonged to this group and collected from the districts of Yahşihan and Kırıkkale-center in July 2009, and Yahşihan in October 2009 and April as well as May 2010.

DISCUSSION

To date, 2101 species have been identified belonging to 26 genera in the Simuliidae family. These species are further classified into subgroups. In Turkey, there are a limited number studies on Simulium fauna. According to the publication by Adler and Crosskey (2010), 49 Simulium species were found in Turkey. In this study, we identified 7 species of the Simuliidae family in the Kızılırmak river basin in Kırıkkale and Ankara. In addition, two groups belonging to the Simuliidae family were also found. As the samples could not be subclassified morphologically into subspecies. For this group, further molecular studies would make a difference by means of sub classification to species. Although the identified species and groups have been previously reported from Turkey (Adler and Crosskey, 2010), this report is the first to report the presence of *Simulium (Wilhelmia) equinum, S. (Boophthora) erythrocephalum, and S.(Eusimulium) petricolum* in the province of Kırıkkale and Ankara.

The most common Simuliidae species identified in the present study was S. petricolum, constituting 25.73% of the larvae and pupae samples of the total number of each species. The other species were as follows: S. pseudequinum (25%), S. lineatum (18.78%), S. balcanicum (17.17%), S. equinum (3%).43), and S. erythrocephalum (0.22%). The least common species was S. alajense (0.02%). In addition, two groups of Simuliidae were also found: S. ornatum (3.87%) and S. angustipes (5.7%). Taking the publication into consideration by Adler and Crosskey (2010) citing presence of 49 Simuliidae species, it can be said that Kızılırmak river basin does not foster these 49 species at least the districts and the time points of sample collection in the present study.

In the study, Simulium (Eusimulium) petricolum (Rivosecchi, 1963) species of the genus Eusimulium (Roubaud, 1906) of the subgenus of Simulium were determined. This species has been identified in Italy, Austria, Bosnia, Britain, Cyprus, Czech Republic, France, Greece, Ireland, Libya, Morocco, Portugal, Serbia, Spain and Russia(Crosskey 1998, Adler and Crosskey2010). In this study, the specified species were found in the districts of Kalecik, Yahşihan, and Kırıkkale-Central. Presence of *Simulium petricolum* in Turkey has been reported first from the province of Afyon. Although it was the most prevalent species found by the present study, this publication is the first report for the presence of this species in the province of Kırıkkale.

The present study found 4 specie in the subgenus of Simulium (Latreille, 1802), genus of Wilhelmia (Enderlein, 1921). *Simulium pseudequinum, S. equinum, S. balcanicum and S. lineatum.* Of these, *Simulium (Wilhelmia) pseudequinum* (Seguy, 1921) has been reported from Canary Islands, Algeria, Armenia, Austria, Azerbaijan, Bosnia, Britain, Cyprus, France, Greece, Georgia, India, Iran, Iraq, Israel, Italy, Jordan, Kazakhstan, Kyrgyzstan, Lebanon, Libya, Macedonia, Morocco, Pakistan, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Tajikistan, Tunisia, Turkmenistan, Ukraine, Uzbekistan and Czech Republic. In Turkey, ıth has been reported from the Altindere River in Trabzon, Büyük Menderes River Basin, Coruh, the Euphrates River, Kızılırmak River Basin, Namnam River, Sakarya, Seyhan, Yesilirmak River Basin, Muğla Koycegiz Protected Area, Zamantı river (Ertuncet al 2008; Kazancı and Ertunç b 2008). S. pseudequinum was the second most prevalent species found in samples collected from the district of Kalecik, Yahşihan, Merkez, Bahşılı,

Karakeçili and Keskin.

Simulium (Wilhelmia) lineatum (Meigen, 1804) has been reported from Germany, Afghanistan, Armenia, Austria, Azerbaijan, Belarus, Belgium, Bosnia, Britain, Bulgaria, China, Czech Republic, Denmark, France, Hungary, Iran, Iraq, Ireland, Italy, Kazakhstan, Latvia, Lithuania, Lebanon, Switzerland, Pakistan, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, Turkmenistan, Kyrgyzstan, Ukraine and Uzbekistan (Kazancı and Ertunç b 2008). In Turkey, it has been reported from the Kızılırmak River Basin (Yılmaz et al. 2007), Sakarya River Basin (Şirin 2001), Yeşilırmak River Basin (Kazancı and Ertunç 2010), Muğla Province Köyceğiz Protected Area (Kazancı and Ciergue 1990; Kazancı and Ertunç 2010), Büyük Menderes RiverBasin was determined in Çoruh River Basin (Kazancı and Ertunç b 2008). Simulium (Wilhelmia) lineatum, found in the districts of Kalecik, Yahşihan, Merkez, Bahşılı, Karakeçili and Keskin, was the third most common species in the present study. This species caused an epidemic in the Middle Kızılırmak Basin between 2006 and 2007. It was reported that the intensive fly population caused loss of yield in animals as well as tourism in Kayseri and Nevşehir as the quality of life style were negatively affected (Yılmaz et al. 2007). Thus, the Kırıkkale province is under the risk of such an epidemic. The authorities should mind this and take necessary measures to prevent such epidemics.

Simulium (Wilhelmia) balcanicum (Enderlein, 1924) has been reported from Bulgaria, Austria, Belarus, Bosnia, Germany, Greece, Hungary, Italy, Macedonia, Poland, Romania, Russia, Serbia, Slovakia, Ukraine and Lithuania (Kazancı and Ertunç b 2008). In Turkey, it has been reported from the Sakarya river Basin (Şirin 2001), Yesil irmak river Basin (Kazancı and Ertunc 2010), Mugla Province Koycegiz protected Area (Kazancı and Ciergu 1990; Ertunc et al. 2008), Büyük Menderes river Basin, and the Kızılırmak river Basin (Kazancı and Ertunç 2010). In the present study, Simulium (Wilhelmia) balcanicum was frequently found as in co-presence with S. equinum and S. pseudequinum in the districts of Kalecik, Yahşihan, Merkez, Bahşılı and Keskin.

Simulium (Simulium) ornatum is a group under the Simuliidae family. A population of larvae and pupae in the present study was identified as in a group of Simulium ornatum since the morphological characterization did not allow further sub-classification of these larvae and pupae into species. The group of Simulium ornatum includes 25 species and 90 subspecies with worldwide distribution (Adler and Crosskey, 2010). The present study found its presence the districts of Yahşihan and Kırıkkale-centrum.

Simulium (Wilhelmia) equinum (Linnaeus, 1758) was found Armenia, Austria, Belarus, Belgium, Bosnia, Bulgaria, Czech Republic, China, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Lithuania, Macedonia, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Siberia, Slovakia, Slovenia, Spain, Sweden, Switzerland and Ukraine (Kazancı and Ertunç b 2008). In Turkey, it has been reported from the Sakarya River Basin (Şirin, 2001). The present study found larvae and pupae of *Simulium (Wilhelmia) equinum* in the districts of Kalecik, Yahşihan, Kırıkkale-centrum, Bahşılı, and Keskin. In addition, the present study also reports that *Simulium (Wilhelmia) equinum* mostly co-existed with S. pseudequinum and S.balcanicum.

Simulium angustipes (Roubaud 1906), is a group under the Simuliidae family Similar to Simulium (Simulium) ornatum, a population of larvae and pupae in the present study was identified as in a group of Simulium angustipes since the morphological characterization did not allow further sub-classification of these larvae and pupae into species. This group has 36 species and 46 subspecies reported worldwide as well as in Turkey (Adler and Crosskey, 2010). The present study found the larvae and pupae of this group in the districts of Kalecik, Yahşihan, Kırıkkale-centrum and Bahşılı.

Simulium (Boophthora) erythrocephalum (De Geer, 1776) is the only species belonging to the Boophthora subgenus of the genus Simulium. As cited by Kazancı and Ertunc (2010), it has been reported from Sweden, Austria, Belarus, Belgium, Britain, China, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Kazakhstan, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Siberia, Slovakia, Slovenia, Spain, Switzerland, Ukraine and Moldova. In Turkey, it has been reported from the Büyük Menderes river Basin and the Sakarya river Basin (Sirin, 2001). In the present study, Simulium (Boophthora) erythrocephalum were only found in pupae stage. The pupae of this species were found in the districts of Kalecik, Yahşihan and Bahşılı. Simulium (Boophthora) erythrocephalum can suck blood and may lead to death of the attacked humans and animals, especially cattle (Crosskey 1990; İnci and Düzlü 2009; Güralp 1997).

Simulium (Simulium) alajense (Rubtsov, 1938) is another species identified. It has been reported from Kyrgyzstan, Afghanistan, Armenia, Bulgaria, China, India, Kazakhstan, Mongolia, Pakistan, Tajikistan, Turkmenistan, Uzbekistan and Romania. In Turkey, it has been reported from the Kızılırmak River Basin (Kazancı and Ciergu 1990) and the Sakarya River Basin (Sirin, 2001). The present study reports its presence in the district of Kalecik. Only the pupae stage could be collected.

In addition to being a faunistic study, the data presented in this report are also important to alert for the transmitted diseases by the Simuliidae family. Species in the Simuliidae family transmit a number of disease agents. S. jeningsi, S. ornatum and S. erytrocephalum are the vectors for Onchocercalinearis (O. gutturosa) that causes disease in cattle, zebu and buffaloes (Opener 19944). The presence of S. ornatum and S. erytrocephalum strains in the studies areas is a warning as these species transmit onchocerciasis. Thus, authorities should take measures against potential diseases that bear risks for animal population in this region of the country.

CONCLUSION

As a result, this study revealed the presence of larvae and pupae of seven species and two groups of the Simulium family in the Kızılırmak river basin in Kırıkkale and Ankara. The study provided comprehensive information about the Simuliidae fauna, which is useful for further faunistic studies. The data generated in the present study are important not only for illumination of Simulium fauna but also alerting for possible risks posed by the identified species and groups of Simulium fauna for the animals and humans.

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