Simulium (*Boophthora*) *erythrocephalum* (De Geer, 1776) – a subgenus and species new to Armenia

(Diptera: Simuliidae)

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Abstract. Distributional records and ecological data for the black fly *Simulium erythrocephalum* (De Geer, 1776) of the subgenus *Boophthora* Enderlein, 1925 (Diptera: Simuliidae) are reported for the first time from Armenia. Developmental stages and adults were found at six sites in 2010. The Armenian populations were characterized morphologically, cytogenetically, and by DNA sequence, revealing close similarities to European populations.

Key words. Simuliidae, *Simulium*, *Boophthora*, *erythrocephalum*, Palaearctic, Armenia, distribution, Caucasus.

Introduction

In contrast to other Eurasian countries, the Armenian simuliid fauna has been studied relatively well. In total, 54 species in five genera are now known from Armenia (ADLER & KACHVORYAN 2001, ADLER & CROSSKEY 2012). These do not include *Simulium erythrocephalum*, a species first described from Sweden by DE GEER in 1776, which has been shown to be widely distributed in at least 34 countries of the Palaearctic (ADLER & CROSSKEY 2012). In Central Europe, *S. erythrocephalum* occurs in various kinds of flowing water systems such as lowland streams, outflows from ponds, irrigation constructions and even large rivers. Under optimal conditions, it can develop mass populations and then be a serious pest and nuisance to humans and farm animals (e.g. IGNJATOVIĆ-ĆUPINA et al. 2006).

Specimens of *S. erythrocephalum* were found in our collections from Armenia in the context of studies of black flies tolerating polluted water and organic contamination, and of species developing mass populations and causing problems for human and animal health. This finding represents the first report of this species in Armenia and the most southeastern collection site in the western Palaearctic region.

Material and methods

Black flies were collected in April 2010 and were primarily larvae and pupae but included a few adults, either field-collected by netting or reared from pupae. Pinned and ethanol-preserved specimens are deposited in the Clemson University Arthropod Collection (SC, USA), the Diptera collection of the Museum für Naturkunde at Humboldt University of Berlin (Germany), and in the private collection of the first author (DW).

Collection sites and dates. Specimens were collected in the Yerevan region (Fig. 1) – Armarvir Region (20 April 2010): 1) Vardanashen, 40°03'N, 44°11'E, 846 m; 2) Yeraskhahun, 40°04'N, 44°12'E, 847 m (Yeraskhahun River); 3) Yeraskhahun, 40°04'N, 44°13'E, 840 m (Metsamor River); 4) Lusagyugh, 40°05'N, 44°16'E, 843 m; 5) Hovtashat, 40°05'N, 44°16'E, 845 m; 6)

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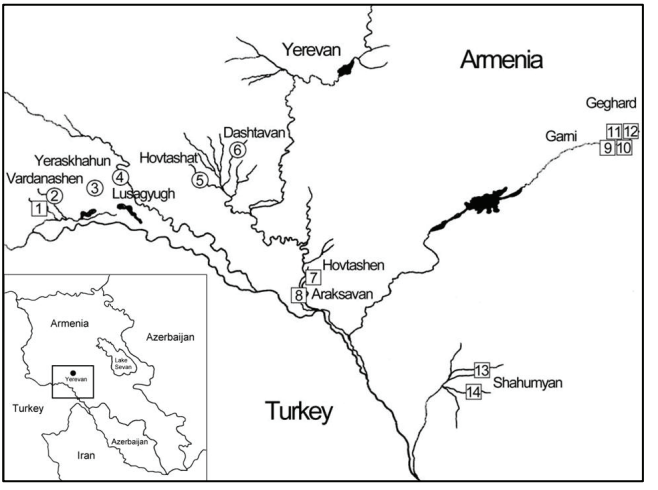


Fig. 1. Sampling sites in Armenia, with records (encircled numbers) and without records (numbers in squares) of *Simulium erythrocephalum*.

Dashtavan 40°06'N, 44°22'E, 840 m. – Ararat Region (16 April 2010): 7) Hovtashen, 40°01'N, 44°27'E, 832 m; 8) Araksavan, 40°00'N, 44°26'E, 831 m; 9) Garni, 40°06'N, 44°43'E, 1167 m (Azat River); 10) Garni, 40°06'N, 44°43'E, 1167 m (Goght River); 11) Garni, 40°06'N, 44°43'E, 1157 m (small stream, inlet to Azat River); 12) Garni, 40°06'N, 44°43'E, 1280 m (small stream, inlet to Azat River); 13) Shahumyan, 39°55'N, 44°34'E, 835 m; 14) Shahumyan, 39°55'N, 44°33'E, 823 m a.s.l.

Species identification and characterization. Morphological identifications were made according to the keys of RUBTSOV (1956), KNOZ (1965), and BASS (1998). The posterior half of the larval body was severed, opened ventrally with fine dissecting needles, and Feulgen-stained (ROTHFELS & DUNBAR 1953). Gender was determined by gonadal examination (elongate in females, round in males). The giant polytene chromosomes were dissected from the silk glands, placed in a drop of 50% acetic acid, and squashed under a coverslip. Banding patterns of the polytene chromosomes were compared with standard maps of WERNER & ADLER (unpubl.). Three genetic loci commonly used for insect identification, characterization, and phylogenetics were analysed: the nuclear ribosomal internal transcribed spacers 1 and 2 (ITS1, ITS2; PERRIN et al. 2006, GOMULSKI et al. 2005), and the mitochondrial cytochrome c oxidase subunit I gene (COI; DALLAS et al. 2003). Parts of these DNA regions (ITS1: ca. 180 bp, ITS2: ca. 520 bp, COI: ca. 450 bp) were sequenced and compared among specimens from Armenia (Metsamor River; n=3), Germany (n=2), and Poland (n=1).

Results

Larvae and pupae of *S. erythrocephalum* were found in Yeraskhahun River (site 2; Fig. 2) and Metsamor River (site 3), as well as in other inlets to River Hrazdan in Lusagyugh (site 4), Hovtashat (site 5), and Dashtavan (site 6). Swarming males and females searching for blood hosts and oviposition sites were netted in Hovtashat (site 5) near an inlet to River Hrazdan. Although black flies were collected in Aragazoth Region, Kotajk Region, Wajoz Dsor Region, and around Lake Sevan in Gegharkunik Region, *S. erythrocephalum* was collected only in the region enlarged in Fig. 2.

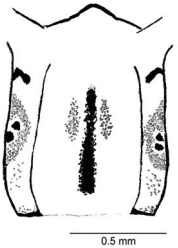
Fig. 2. Armenian sampling site near Yeraskhahun, Metsamor River, 40°04'N, 44°13'E (photo: WERNER, 20 April 2010).

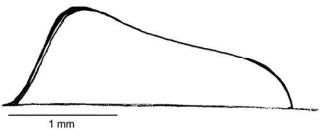
Our specimens were identified as *S.* (*Boophthora*) *erythrocephalum* on the basis of morphological characteristics. The species can be identified morphologically by the pattern of the larval head capsule, the branching pattern of the six pupal gill filaments, the shape of the pupal cocoon, and the structure of the female and male genitalia.

Larva: Identification is ideally made with the last larval instar. The body is greyish with reddish-brown bands, and has a length of 5.0–7.5 mm which is reduced to about 3.5 mm in the summer. The conspicuous bands are a good character for field identification. A pair of inconspicuous dorsolateral papillae is located on each body segment. The head capsule is greyish-yellow and entirely pale yellow laterally. Apotome markings are variable. The posteromedian and anteromedian head spots can be separate or fused (Fig. 3). They are dark brown in spring and pale brown in summer. The head spots are not surrounded by infuscation. The posterolateral head spots are rather faint. The postgenal cleft is large, wide, and rounded, with an ill-defined border. Ventral tubercles are conspicuous. Rectal papillae have three lobes, but secondary lobules are possible.

Pupa: The cocoon is densely woven of fine silk and is of simple construction. It typically has a thin frontal rim but lacks a horn or elongation. The length of the pupal body is 2.3–3.5 mm (Fig. 4). Each pupal gill consists of six filaments arranged in three pairs (Fig. 5). The second or middle pair (filaments 3 and 4) arises directly from the base of the common trunk, without a separate common stalk. In anterior view, the middle pair is in a horizontal plane while the upper and lower pairs are in a longitudinal plane.

Male/female: The body length of adult specimens is 2.2–3.8 mm. The scutum and scutellum are blackish with a shiny silver pattern. The male genitalia have a species-specific shape, with the gonostyles shorter than the gonocoxites (see DAVIES 1968: 82; RUBTSOV 1956: 600).





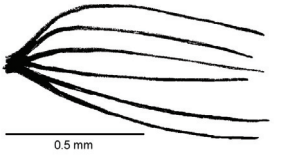


Fig. 3 (left). Head capsule of Armenian larva of *Simulium erythrocephalum* with typical apotome marks (dorsal view). – Fig. 4 (middle). Habitus of pupal cocoon (lateral view). – Fig. 5 (right). Pupal gill (lateral view).

Cytological identification was based on two female and three male larvae collected in Yeraskhahun (Metsamor River). Sex chromosomes were microscopically undifferentiated and all larvae had the standard banding sequence of WERNER & ADLER (unpubl.; CHUBAREVA & PETROVA 2008). The larvae had six different polymorphic inversions – IS-2, IS-3, IL-9, IIS-1, IIL-4, and IIIL-3 – all of which also have been found in European populations. The average number of heterozygous inversions in the 5 larvae was 2.00. *S. erythrocephalum* is chromosomally a highly polymorphic species, and the Armenian larvae also show a high level of polymorphism, even though they represent a geographically peripheral population.

Among the six larval specimens tested, DNA sequences were nearly identical, with one nucleotide exchange in one Armenian specimen in the ITS1 region, one nucleotide exchange in the Polish specimen in the ITS2 region, and two identical insertions of two nucleotides in two Armenian specimens in the COI region, thus suggesting conspecificity and close genetic relatedness. The sequences obtained have been deposited in GenBank under accession numbers JQ285853-58 (COI), JQ285859-64 (ITS1) and JQ285865-70 (ITS2).

Discussion

Since DE GEER (1776) described *S. erythrocephalum* from his property in Leuvsta, Sweden, as *Tipula erythrocephala*, this taxon has been recorded many times in the Palaearctic region. Its distribution extends from Scandinavia in the north (JENSEN 1997) to the Iberian peninsula in the southwest (VALLE, pers. comm.), and from the UK in the west (BASS 1989) to Turkey (ŞIRIN & ŞAHIN 2005) and Russia including Siberia, Ukraine, and Kazakhstan in the east (RUBTSOV 1956). Material collected in 1970 from various parts of Turkey, the geographical bridge between Europe and the Caucasus, and examined by CROSSKEY & ZWICK (2007), however, did not include *S. erythrocephalum*. ŞIRIN & ŞAHIN (2005) reported the species from the upper parts of the Sakarya River basin near Seyitgazi in Turkey. This area previously had been the southeasternmost location known for *S. erythrocephalum*. The collection localities in Armenia are approximately 2000 km further to the east, representing a new southeasternmost distribution border, separate from the finding of *S. erythrocephalum* in the more northerly Kazakhstan. The detection of the species at several sites, with considerable population densities (up to 20 % of all larvae collected), suggests successful establishment in Armenia.

Breeding sites of *S. erythrocephalum* are generally weedy lowland streams and rivers, such as the Danube (Germany, Serbia), the Spree (Germany), the Warta (Poland), and the Morava (Czech Republic) (BASS 1998, RÜHM 1998, WERNER unpubl.). In such water systems, developmental stages of *S. erythrocephalum* have been found during the total vegetation period, with a tendency to produce mass populations in spring and early summer. The hematophagous females can cause severe medical and veterinary problems under such conditions (WERNER 2007). In Armenia, the aquatic stages did not occur in river situations where the current velocity was less than 20 cm/s, but could be found in large numbers where the water was turbulent. All tributaries with *S. erythrocephalum* were organically enriched, indicating a certain tolerance to pollution. This situation, however, is not comparable to that of *S. ornatum*, which breeds in extremely polluted streams (WICHARD 1976). At all collection sites, plant material was available as substrate for larvae and pupae, which were attached to *Sparganium* sp. and *Ranunculus* sp. Few larvae of *S. erythrocephalum* showed small secondary lobes of the rectal organ. This phenomenon is sometimes found in rivers with high pollution (ZWICK, pers. comm.). Larvae of *S. erythrocephalum* with this variation were described first by PURI (1925), but were henceforth found throughout Europe.

Adults of both genders were caught in Hovtashat during sunny periods around midday and in the afternoon. Typical for *S. erythrocephalum*, males swarmed near bushes and grassy structures hanging from a bridge. Females presumably searching for oviposition sites flew above the water surface of streams where aquatic plants were floating. Host-seeking females approached the excursion group in close proximity to the parked car.

Wherever *S. erythrocephalum* was found in Armenia, the aquatic stages of the subgenus *Wilhelmia* were always present, while those of the *S. ornatum* group were sometimes present. Species of these taxa frequently occur together and build up characteristic biocenoses in simuliid breeding habitats of the Palaearctic (WERNER et al. 2012). Larvae of *Wilhelmia* and the *S. ornatum* group can be distinguished by their greenish brown and grey colour, respectively, and well-defined apotome marks, postgenal clefts, and pupal gill histoblasts. In all streams, *S. erythrocephalum* was outnumbered by larvae and pupae of *Wilhelmia*, suggesting that the developmental stages of the species of these two subgenera occur sympatrically.

The distribution of *S. erythrocephalum* is thus far greater, and extends much more toward southeastern Asia, than previously thought. Chromosomal and molecular evidence suggests that the Armenian population is closely related to European populations.

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