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Taxonomic study of Black-flies (Diptera: Simuliidae) larvae in Al Arab River (Lattakia, Syria)

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Abstract— Black-flies larvae samples were collected monthly from one fixed site in AL Arab river (Lattakia, Syria) for a year during the period from December 2017 till October 2018. Slides were prepared and morphological characteristics of last-instar larvae (matured) and pupae were used to determine the classification of species. As a result, eleven species (Metacnephia persica; Prosimulium tomosvaryi; Simulium. (Eusimulium) angustipes; S. (welhelmia) pseudoequinum; S. (simulium) kiritshenkoi; S. (Obuchovia) auricuma; S. (Simulium) trifasciatum; S.(Nevermannia) ruficorne; S. (Eusimulium) . sp and S. (welhelmia) paraequinum) belonging to three genera (Metacnephia, Prosimulium and Simulium) were reported, ten species of them were reported for the first time in Syria. This is the first study of black-flies larvae taxonomy in Syria.

Keywords—Simuliidae, Fauna, Runningwater, Syria.

I. INTRODUCTION

Black-flies, Simuliidae are the most abundant insects in the running water ecosystems. At present, over 2310 species of black flies were recorded around the world [1] This group is an important medical and veterinary small blood-sucking insects. The adult female's bites cause a wide range of problems for humans and other vertebrates. For example, certain species of Simulium are the vectors of Onchocerca volvulus Leuckart nematode. The parasite causing a human onchocerciasis disease, which has a great social impact in tropical regions of Africa and America. Thus, in addition to explaining patterns in streams, knowledge of simuliid diversity can provide insight into the dynamics of onchocerciasis transmission and the transmission of other serious human diseases (e.g. human river blindness)[2]. Immature simuliids are dominant members of the aquatic insect communities and play an important trophic role as detritivores in lotic ecosystems, they occupy habitats ranging from temporary stream trickles to large rivers. Black fly larvae can also play a major role as principal processors of plant materials in running streams. In fact, larvae are passive filter feeders, they filter suspended particulate organic matter from water, making it available for a considerable number of other invertebrates, such as Thrichoptera, which massively feed on them.

Rest of the paper is organized as follows, Section I contains the introduction of black-flies, Section II contain the related work of many studies of black-flies larvae, Section III explain the methodology with flow chart, Section IV describes results and discussion and Section V concludes conclusion and future scope.

II. RELATED WORK

Scientists have studied insects for a long time. they separated the species on the basis of morphological properties. We remark the study which recorded eight species belonging to two genera *Prosimulium* and *Simulium* in Turkey [3], in another study the species listed out which was reported from Turkey and the neighboring countries [4]. In New Zealand sub region 19 species of Simuliidae were reported [5]. In Brazil the larva, pupa, male, and female of new species *Simulium bifenestratum* were described and illustrated [6]. While 20 species were collected from three areas in Brazil distinct in relation to environmental variables [7], mainly owing to differences in altitude and water pH.

In Thailand *Simulium lomkaoense* and *Simulium piroonae* were described in two different studies in the same year based on the females, males, pupae and mature larvae characters and provided a keys of 11 species of the varicorne species-group [8, 9]. Several studies on black fly communities associated with environmental factors have been reported from different parts of the world, such as the first study on seasonal biodiversity of black flies and evaluation of ecological factors affecting their distribution in northern Thailand, larvae were collected from six fixed-stream sites in relation to altitude gradients yielded 5475 last-instar larvae, belonging to 29 black flies species [10], and the black flies of urban area of Rome, Italy were studied and concluded that black flies can be considered a good environmental indicators for assessing the ecological

health of both watercourses and surrounding landscapes [11]. In Germany, the behavior of different black-fly species (*Simulium noelleri*, *Simulium ornatum* and *Simulium variegatum*) was studied using a video equipment in two flowing velocities [12]. In America the taxonomy and ecology of Simuliidae were studied [13, 14]. In Nigeria the larvae of six species and pupae of other three to be colonizing of different substrates were recorded [15].

In Iraq, nine species of Simuliidae (S. (B) buxtoni, S. (E) paucicuspis, S. (N) Ruficorne, S. (S) bezzi, S. (S) Kiritshenkoi, S (W) dahestanicum, S. (W) lineatum, S. (W) paraequinum, S (W) pseudequinum) were reported [16].

So far, only four species were reported in Syria namely; Levitinia freidbergi Beaucournu-Saguez & Braverman, Greniera dobyi Beaucournu-Saguez & Braverman , Simulium (Nevermannia) ruficorne Macquart and Simulium (Wilhelmia) golani Beaucournu-Saguez & Braverman [17].

Simullidae is rarely studied insect group in Syria despite of presence of large freshwater bodies, therefore, we opined that this study should be carried out.

III. METHODOLOGY

Study area

One fixed-site on AlArab river located in Mediterranian basin, Northern Lattakia (35° 38' 51" N, 35° 50' 14" E and 54 m sea level) was selected and it's characteristics are as follows: Riverbed has a mud and rocks, width is about 3 m and altitude is about 1 m. Larval and pupal substrates are rocks, plants and fallen tree leaves trailing in the river and there are trees surrounding the area.

Collecting Samples

Animal samples were collected monthly between December 2017 and October 2018. Larvae and pupae were collected by special net consists of a semicircle hank with a winch (1 m), the fabric with 0.3 inch holes, or by hand using fine forceps from available substrates in streams, such as fallen leaves, mud or rock surfaces, and trailing grasses. Collected larvae samples were preserved in a 70% ethanol solution till slides were prepared.

Slide preparation procedure

Larvae were kept in 10% sodium hydroxide solution over night at room temperature, which were previously preserved in 70% alcohol solution (NAOH digests the inner muscle tissue and leaves the sclerotized portion of the larval exoskeleton, including body). Larvae were cleared next morning by keeping them in distilled water for 5-10 minutes. Followed by water bath specimens were placed in (30-50-70-90) % alcohol solution respectively for 5 minutes to each concentration. Specimens were placed in alkzailol solution for 5 minutes. 1-2 drops Of Canada Balsam mountant were placed on clean slide. Specimens were placed in the mountant, lying larvae ventral side up and head pointed up. Coverslip was lowered over the mountant at an angle using forceps. Then slides were placed in drying oven (didn't exceed 55 ⁰C) for 2 to 4 days. Slides were labeled for site and date of collection.

Species identification

Morphological characteristics of last-instar larvae (matured) and pupae were compiled and compared with the taxonomic keys of [2, 3, 5, 8, 16].

IV. RESULTS AND DISCUSSION

Eleven species of black fly larvae were reported in Al Arab river site. Ten species were reported for first time in Syria.

Checklist of recorded species during this study

Genus Metacnephia Crossky 1- M. persica Robtsov Genus Prosimulium Roubaud 2- P. tomosvaryi Enderlein Genus Simulium Latreille Subgenus Eusimulium Roubaud

- 3- S. (E) angustipes Edwards
- 4- S. (E) aureum Fries
- 5- S.(E) sp.

Subgenus Nevermannia Enderlein

- 6- S. (N) ruficorne Macquart
- Subgenus *Obuchovia* Rubtsov 7- S. (O) auricoma Meigen
- Subgenus *Simulium* Latreille s. str.
 - 8- S. (S) kiritshenkoi Rubtsov
 - 9- S. (S) trifasciatum Curtis

Subgenus Wilhelmia Enderlein

- 10- S. (W) paraequinum Puri
- 11- S. (W) pseudequinum Seguy.

1- Simulium (Eusimulium) angustipes:

Head capsule brown yellowish; cephalic apotome with distinct marks and widest near the posterior edge; posteromedian spot slender; cervical sclerites visible as two brown spots (Fig. 1A); postgenal cleft present, well developed and not extending forwards so far as to reach base of hypostomium; postgenal bridge therefore complete behind hypostomium (Fig. 1B);; median tooth of hypostoma narrow and not trifid (Fig. 1C); gill histoblast angulate anteroventrally (Fig. 1D); shape of mandible as (Fig. 1E); ventral papillae present on last abdominal segment; three anal gills without branches (simple) (Figure1F); shape of anal sclerite as (Figure1G).

Remarks: This species is widely distributed especially in the neighboring countries. It was reported from Britain, Algeria, Armenia, Austria, Belarus, Bosnia and Herzegovina, China (Hebei) Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland,

Italy, Kazakhstan, Latvia, Lebanon, Luxembourg, Macedonia, Mongolia, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Spain, Sweden, Switzerland, Tunisia, Turkey and Ukraine Adler (2019). This species was reported for the first time in Syria.

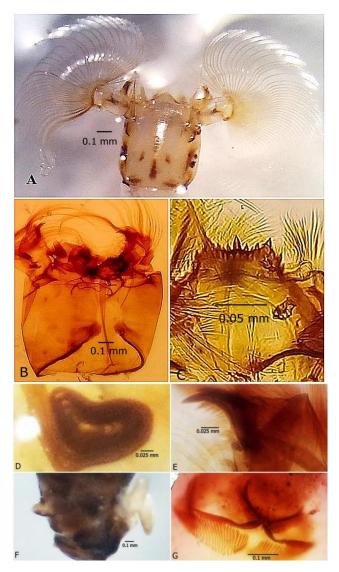


Figure 1. *Simulium (E.) angostipes* larva A. Cephalic apotome; B. Postgenal cleft; C. Hypostomium; D.Gill histoblast; E. Mandibula; F. Posterior proleg; G. anal sclerite.

2-Simulium (Welhelmia) pseudequinum:

Head capsule brown yellowish; cephalic apotome widest near the posterior edge. Cervical sclerites visible as two brown spots; median tooth of hypostoma narrow and not trifid; shape of hypostomal teeth various and Length of median tooth not distinctly longer than the other teeth (Fig. 2B); postgenal cleft present, well developed and not extending forwards so far as to reach base of hypostomium; postgenal bridge therefore complete behind hypostomium (Fig. 2A);; filaments of gill histoblast short, inflated and slightly curved, showing outwardly; very large wrinkled branches directed downwards (Fig. 2C distinct quality) and all of it separating from each other; preapical teeth and preapical spines of mandible short and slender (Fig. 2D); ventral papillae absent or inconspicuous; three simple anal gills (Fig. 2E); posterior circlet wider in ventral than dorsal; shape of anal sclerite as (Fig. 2F).

Remarks This species was reported from the most of the neighboring countries. Distribution of this species is in, Canary Islands, Algeria, Armenia, Austria, Azerbaijan, Bosnia and Herzegovina, Britain, China, Croatia, Cyprus, France, Greece, Georgia, India, Iran, Iraq, Italy (incl. Sardinia, Jordan, Kazakhstan, Kyrgyzstan, Lebanon, Libya, Macedonia, Montenegro, Morocco, Pakistan, Portugal, Romania, Russia (Caucasus), Serbia, Slovakia, Slovenia. Tajikistan, Spain, Tunisia, Turkey. Turkmenistan, Ukraine, Uzbekistan and Czech Republic Adler (2019). By this study, this species is reported for the time first in Syria.

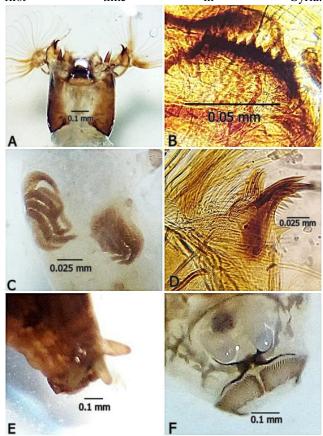


Figure 2. *Simulium (welhelmia) pseudoequinum* larva, A. Postgenal cleft ; B. Hyposomium C. Gill histoblast ; D. Mandibula ; E. Posterior proleg ; F. anal sclerite.

3-Simulium (Simulium) kiritshenkoi:

Head capsule yellowish; cephalic apotome widest near the posterior edge; cervical sclerites visible as two brown spots (Fig. 3A); median tooth of hypostoma narrow and not trifid; ventral papillae absent or represented by slight swelling in front of posterior circlet; hypostomal teeth very small and subequal in length Median tooth and sublateral teeth well developed and longer than the lateral teeth (Fig. 3C); preapical teeth and preapical spines of mandible short and slender (Fig. 3D); shape of mandible as (Fig. 3D);

posterior circlet nearly same width in dorsal and ventral; postgenal cleft smaller, subquadrate or slightly pentagonal, extending less than half way towards base of hypostomium; shape of anal sclerite as (Fig. 3E); anal gills without branches; gill histoplast with eight filaments composed of circularly coiled fine filaments.

Remarks

This species is distributed over the Mediterranean basin to Western and Central Asia. Iran, Armenia, Azerbaijan, Bulgaria, Cyprus, Georgia, Iraq, Pakistan, Romania, Russia (Caucasus), Tajikistan, Turkey, Ukraine Adler (2019).

This species was reported for the first time in Syria.

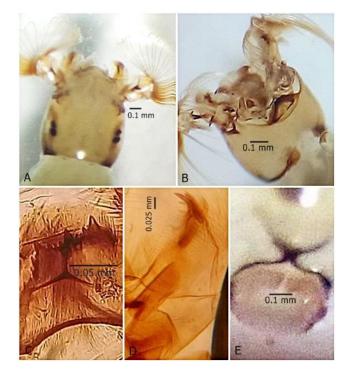


Figure 3. *Simulium (simulium) kiritshenkoi* larva, A. Cephalic apotome; B. Postgenal cleft; C. Hypostomium; D. Mandibula; E. anal sclerite.

4- Simulium (Trichodagmia) auricoma:

Head capsule yellowish; cephalic apotome with distinct dark marks and widest near the posterior edge; posteromedian spot slender (Fig. 4A). Cervical sclerites visible as two brown spots (Fig. 4B). Postgenal cleft less deep than wide. Median tooth of hypostoma narrow, not trifid, corner and not strongly produced. Median tooth and lateral teeth well developed and longer than the sublateral teeth (Fig. 4C). Gill histoplast angulate anteroventrally. Ventral papillae absent or inconspicuous. Preapical teeth and preapical spines of mandible long and strong, Shape of mandible as (Fig. 4D). Shape of anal sclerite as (Fig. 4E).

Remarks Reporting of this species is restricted to the Palearctic region (Western Asia and some parts of Europe) Austria, Andorra, Bulgaria, Croatia, Cyprus, Czech Republic, France, Germany, Greece, Italy, Kazakhstan,

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Lebanon, Morocco, Poland, Portugal, Romania, Serbia, Slovakia, Spain, Turkey, Ukraine Adler (2019).

This species was reported for the first time in Syria.

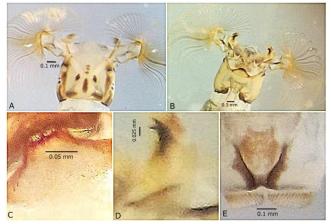


Figure 4. *Simulium (E.) auricoma* larva, A. Cephalic apotome; B. Postgenal cleft; C. Hypostomium; D. Mandibula; E. anal sclerite.

5- Simulium (Simulium) trifasciatum:

Head capsule yellowish. Cephalic apotome widest near the posterior edge (Fig. 5A). Cervical sclerites visible as two brown spots . Postgenal cleft rounded or flat. Anterior margin of postgenal cleft well visible and postgenal cleft rectangle. Median tooth of hypostoma narrow and not trifid. Shape of hypostomal teeth various. Median tooth and lateral teeth well developed and longer than the sublateral teeth (Fig. 4B). Ventral papillae absent or inconspicuous. Preapical teeth and preapical spines of mandible short and slender. Filaments of gill histoblast long, slender and curved. Posterior circlet nearly same width in dorsal and ventral (Fig. 4C).

Remarks: This species is distributed in Palearctic region, Britain, Algeria, Andorra, Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Morocco, Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, Switzerland, Turkey and Ukraine Adler (2019).

This species was reported for the first time in Syria.



Figure 5. *Simulium (E.) trifasciatum* larva, A. Lateral view of mature larva; B. Postgenal cleft; C. Posterior proleg.

6- Simulium (Nevermannia) ruficorne:

Head capsule brownish. Cephalic apotome widest near the posterior edge. Posteromedial spot on cephalic apotome triangular. Cervical sclerites visible as two brown spots. Hypostomium with median and corner teeth very long, prominent, narrow and not trifid (Fig. 5B). Postgenal cleft smaller and slightly deeper than its width. Postgenal cleft not extending forwards so far as to reach base of hypostomium, postgenal bridge therefore complete behind hypostomium, Postgenal cleft extended more than 1/6 distance to hypostomal groove (Fig. 5C). Ventral papillae present on last abdominal segment, conical and obvious. Dorsal filament of gill histoblast rounded anteroventrally. Rectal papillae branched.

Remarks This species is widely distributed in Afrotropic and Palearctic realm, Algeria, Angola, Benin, Burkina Faso, Cameroon, Canary Islands, Cape Verde Islands, Central African Republic, Chad, Comoro Islands, Congo, Democratic Republic of Congo, Egypt, Eritrea, Ethiopia, Ghana, Guinea, Guinea, Bissau, Iraq, Ivory Coast, Jordan, Kenya, Lebanon, Liberia, Libya, Madagascar, Madeira, Malawi, Mali, Malta, Mauritius, Morocco, Mozambique, Namibia, Niger, Nigeria, Oman, Portugal, Saudi Arabia, Senegal, Sierra Leone, Somalia, South Africa, South Sudan, Spain, Sudan, Syria, Tanzania, Togo, Tunisia, Uganda, Yemen, Zambia, Zimbabwe (Adler, 2019).

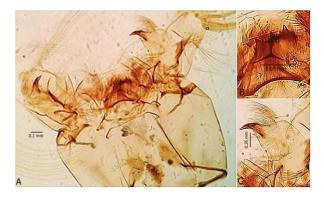


Figure 6. *Simulium (N) ruficorne* larva, A. Postgenal cleft, B. Hypostomium. C. Mandibula

7- Metacnephia persica:

Head capsule brown yellowish. Cephalic apotome widest near the posterior edge. Cervical sclerites visible as two brown spots; Postgenal cleft extending far forwards and reaching base of hypostomium, postgenal bridge therefore incomplete. Postgenal cleft narrow and regularly tapering towards base of hypostomium (Fig. 7A). Hypostomial teeth small but visibly protruding beyond line of apical flange. Median tooth of hypostoma narrow and not trifid. Hypostomal teeth very small and subequal in length (Fig. 7B). Ventral papillae absent or inconspicuous. Gill histoplast with 20–22 filaments.

Remarks

this species is reported from Iran; Armenia, Azerbaijan, Kazakhstan, Lebanon, Romania, Turkmenistan and Uzbekistan Adler (2019). This is a new record of this species in Syria.

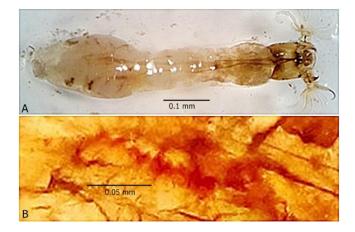


Figure 7. *Metacnephia* (*M*) *persica* larva, A. ventral view of last instar larva; B. Hypostomium.

8- Prosimulium tomosvaryi:

Head capsule brownish. In dorsal view of head, cephalic apotome narrowed posteriorly, with a change in direction of the lateral suture bounding the apotome at a definite point about one quarter head-length from the posterior end of the head. Outermost hypostomial tooth on each side enlarged, as high as the median tooth. Median tooth of hypostoma large and trifid (Fig. 8A). Anal gills simple.

Remarks

this species is distributed in Germany; Andorra, Austria, Armenia, Belgium, Bosnia and Herzegovina, Britain (En, Sc), Bulgaria, Croatia, Czech Republic, France, Georgia, Greece, Hungary, Ireland, Italy (incl. Sicily), Luxembourg, Morocco, Poland, Portugal, Romania, Russia (Caucasus), Serbia, Slovakia, Slovenia, Spain, Switzerland, Turkey, Ukraine Adler (2019). This species was reported for first time in Syria.

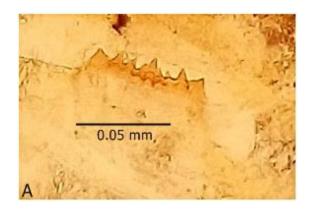


Figure 8. Prosimulium tomosvaryi larva, A. Hypostomium.

9- Simulium aureum:

Posteromedial spot on cephalic apotome triangular. Cervical sclerites visible as two brown spots. Posteromedian mark not triangular, rather a narrow rectangle, both postero-median and antero-median marks are thus rather linear and elongate, with the gap between them smaller than the length of either mark, anal gills simple. Postgenal clift. Head capsule brown yellowish, Cephalic apotome with distinct marks and widest near the posterior edge, Posteromedian spot slender. Cervical sclerites visible as two brown spots. Postgenal cleft present, well developed and not extending forwards so far as to reach base of hypostomium, postgenal bridge therefore complete behind hypostomium (Fig. 9A). Median tooth of hypostoma narrow and not trifid (Fig. 9B). gill histoblast angulate anteroventrally. Ventral papillae present on last abdominal segment. Anal gills without branches (simple). Shape of mandible as (Fig. 9C).

Remarks

S. aureum reported from several countries like, Sweden; Austria, Belarus, Belgium, Britain (En, Sc, Wa), China (He, Sh), Czech Republic, Denmark, Estonia, Finland, France, Germany, Iceland, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Russia (CR, NR, SR), Siberia (WS), Slovakia, Spain, Sweden, Switzerland, Ukraine, Uzbekistan, Bulgaria, India, Japan , Kazakhstan , Pakistan , Romania , Russia (Caucasus) Adler (2019). This is a new report in Syria.

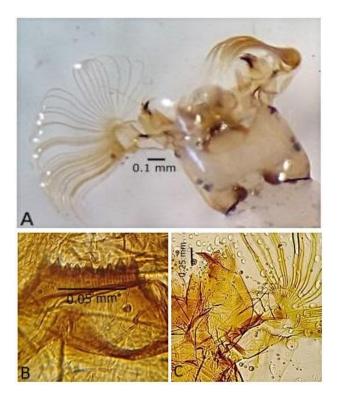


Figure 9. *Simulium (E.) aureum* larva, A. Postgenal cleft ; B. Hypostomium; C. Mandibula.

10- Simulium (Eusimulium). sp:

Head capsule brown yellowish, Cephalic apotome with distinct marks and widest near the posterior edge, Posteromedian spot slender. Cervical sclerites visible as two brown spots (Fig. 10B). Postgenal cleft present, well developed and not extending forwards so far as to reach base of hypostomium, postgenal bridge therefore complete behind hypostomium (Fig. 10C). Median tooth of hypostoma narrow and not trifid. gill histoblast angulate anteroventrally . Ventral papillae present on last abdominal segment. Larva without anal gills without branches. Shape of hypostomium as (Fig. 10D). Shape of anal sclerite as (Fig. 10E). Shape of pupa as (Fig. 10F).

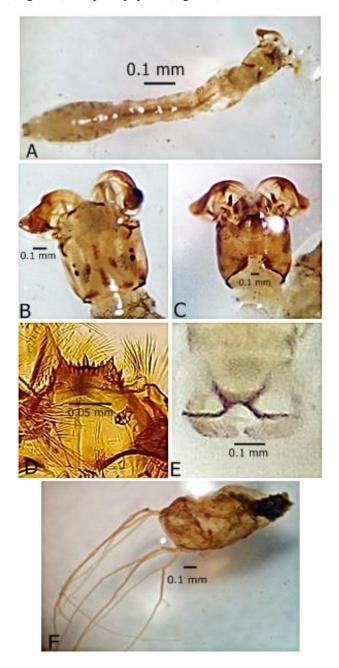


Figure 10. *Simulium (E). sp*, A. Ventral view of mature larva; B. Cephalic apotome; C. Postgenal cleft ; D. Hypostomium; E.anal sclerite; F. pupa.

11- Simulium paraequinum:

Head capsule brownish. Cephalic apotome widest near the posterior edge (Fig. 11A). Hypostomium with median and corner teeth very long, prominent, narrow and not trifid. Ventral papillae present on last abdominal segment, conical and obvious. Dorsal filament of gill histoblast rounded anteroventrally. Postgenal cleft not extending forwards so far as to reach base of hypostomium, postgenal bridge therefore complete behind hypostomium, Postgenal cleft extended more than 1/6 distance to hypostomal groove (Fig. 11B). Anal gills simple. Gill spot large wrinkled branches directed downwards.

Remarks

Pakistan; Armenia, Azerbaijan, Bulgaria, Croatia, Greece, Hungary, Iran, Iraq, Italy, Jordan, Lebanon, Romania, Russia (SR), Serbia, Turkey, Ukraine (Crimea)Adler (2019). This is a new report in Syria.

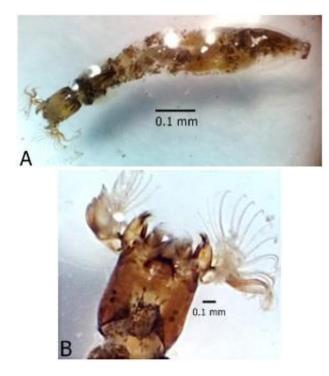


Figure 11. Simulium paraequinum larva, A. view of mature larva; B. postgenal cleft.

V. CONCLUSION AND FUTURE SCOPE

The most important taxonomic keys were used to identify species after slide preparation of Blackflies last instar larvae. Eleven species belonging to three genera *Metacnephia*, *Prosimulium* and *Simulium* were reported from a station on Al Arab river during this study namely, *Metacnephia persica; Prosimulium tomosvaryi; Simulium.* (*Eusimulium*) angustipes; S. (welhelmia) pseudoequinum; S. (simulium) kiritshenkoi; S. (Obuchovia) auricuma; S. (Simulium) trifasciatum; S.(Nevermannia) ruficorne; S. (Eusimulium) aureum; S. (Eusimulium). sp and S. (welhelmia) paraequinum. Ten species of them were reported for the time in Syria.

Because of the presence and multitude of large freshwater bodies in Syria, we suggest continuing taxonomic studies of black-flies larvae should be continued, and ecological studies should be under taken as well.

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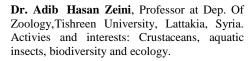
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