Journal of Wildlife and Biodiversity 3(1): 31-39 (2019)

by Arak University, Iran (http://jwb.araku.ac.ir/)

DOI: 10.22120/jwb.2018.93291.1031

Research Article

Chironomidae (Insecta: Diptera) biodiversity at generic level in Lar River, Tehran Province with introducing two new genera for Iranian fauna

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Received: 8 September 2018 / Revised: 29 September 2018 / Accepted: 22 November 2017 / Published online: 30 November 2018. Ministry of Sciences, Research and Technology, Arak University, Iran.

Abstract

Chironomidae flies (Insecta: Diptera) are known for their ecological importance; they are bio-indicator of aquatic ecosystems and play important roles in different levels of food chain. Although they serve as food supply for fish and others aquatic organisms, they are good predators of many Arthropoda species. The present study was conducted to investigate the chironomid variation in Lar River (Lar and Polour), eastern part of Tehran province, Iran through spring and summer 2014 in several replications. Shannon-Wiener and Simpson diversity indices were calculated for the sampling station separately. Sorenson`s coefficient was calculated to assess the similarity between the sampling areas. The results showed that there were a great variety of Chironomidae in Lar River composed of two sub-families and 6 different genus; Chironominae (*Chironomus, Stictochironomus, Polypedilum, Endochironomus, Hyporhygma*) and Tanypodinae (*Brundiniella*) which 2 of them are recorded for the first time for Iranian fauna. Among the collected genera, *Polypedilum* was the most abundant genus especially in Lar station rather than Polour. According to the diversity indices, genera diversity in Polour station was higher than Lar.

Keywords: *Chironomidae, Lar River, Polypedilum, Shannon-Wiener Index*.

Introduction

Chironomidae is one of the most important unique organisms in aquatic ecosystems in all around the world (Callisto *et al.* 2002). These insects compromise the most larval abundance populations in rivers and streams with density of aquatic plant (Milakovic *et al.* 2001). The family belongs to the order of Diptera and the premature stages (eggs, larvae and pupa) develop in water and compose one of the main food regime for fish and other aquatic macro-organisms (Armitage *et al.* 1995). They are so valuable as bio-filtering agents (Henriques-Oliveira *et al.* 2003) and bio-indicators of water pollutions (IAEA 2014). The family is composed of 11 subfamilies, several hundreds genera and more than 4000 species (Armitage *et al.* 1995).

Typically, Chironomidae has been shunned by many benthologists because of perceived difficulties in specimen preparation, identification, taxonomy, morphology and literature (Epler 2001); this led to the point that most of the recent studies have ended to subfamilies and genus. On the other hand, as Chironomidae resemble other insects, so the documentation of this fauna throughout the world is not fullfied (Armitage *et al.* 1995). In Iran, classification of Chironomidae have been reported from Anzali Wetland (Valipour 1997), southern coastline of Caspian Sea

(Ahmadi and Mousavi Nanekaran 2002), *al.* 2014), Ghazal Ozan river in Zanjan province (Navan Maghsoodi 2013), Zayandehrood river in Isfahan province (Ebrahimnezhad and Fakhri 2005, Shayeghi *et al.* 2014), Golpaygan river (Ebrahimnezhad and Allahbakhshi 2013), Haji Abad river in Hormozgan province (Khosravani *et al.* 2014) and different rivers in Tehran province (Arkia 2016, 2017, Alvari 1997). Karami *et al.* (2014) provided a checklist and key identification of Chironomidae Larvae in Marbor River (Isfahan, Iran) and reported 39 genera from four subfamilies: Chironominae (15 genera), Diamesinae (2 genera), Orthocladiinae (17 genera) and Tanypodinae (5 genera), which from these, 13 genera were reported for the first time in Iran. Sharifinia (2015) reviewed the macroinvertebrates of the Iranian running waters through 15 years of recent studies until 2015 and showed that Arthropoda comprised the most taxa with 34 described genera of Chironomidae as the dominant family. Following the previous studies, because of the lack of information about Chironomidae diversity in aquatic systems of Iran, especially around Tehran province and the need to improve the ecological substrate for their survive, this paper has focused of the Chironomidae diversity in Lar river, in east of Tehran province.

Material and methods

Sampling area

The river originates from Kaloon Bastak Mountains where rain and snowfalls are in plentiful. These mountains are to the east and north east of the province and at an attitude of 3000 m and flows to the southern range of Alborz mountains which leads to the eastern edge of Tehran province (Fig. 1). Two stations were selected for sampling at two different height above the sea: Lar at the height of 2259 meters above sea.(35°50′22″N, 52°2′38″E) and Polour at the height of 2307 meters above sea (35°49′16″N, 52°2′13″E).

Marbor river in Isfahan province (Karami *et*

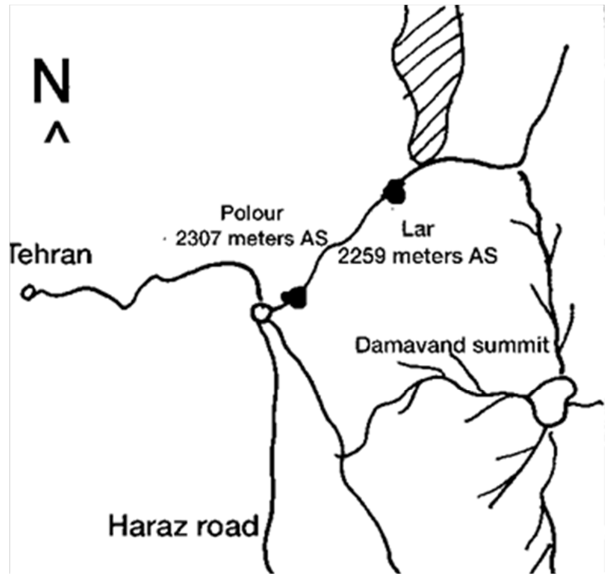

Figure 1. Lar river district in east of Tehran province; sampling areas were marked by the height above sea level.

Sampling and preservation method

Samples of larvae were taken monthly through spring and summer, resulting in four replicas for each station. Samples were taken by dredge and kept in Ethanol 70% until the laboratorial identifications. For better systematic identification, permanent mountains were prepared. For this purposes, samples were soaked in a 10% solution of potassium hydroxide (KOH) on a hot plate at the temperature of 70° c. Ten minutes in acetic acid was followed by transfer to absolute alcohol. The dehydration process was performed to prepare the samples transferring to slide. Samples were glutted by a drop of Canada Balzam and a cover slide and the sampling features such as date and place of sampling were written on it.

Systematic identification

To identify the samples, valuable and available identification keys such as, Karami et al. (2014) Ebrahimnezhad and Allahbakhshi

(2013) and Epler (2001) were used to the generic level. In order to identify the genera, head capsules and body characteristics of the larvae were studied under the microscope.

Statistic analysis

In order to determine any significant differences in larval abundance among different stations, mean and standard Error of larval abundance were calculated for each sample in each site. The null hypothesis (that there was no difference between mean abundance of different genera among the stations was tested using a one way ANOVA and Duncan method. Simple T-student method was applied to show any difference between two stations.

For comparison the biodiversity of Chironomidae genera between two sampling area, Shannon index and Simpson Index were calculated for Lar and Polour separatedly. The equations for the two indices are:

Shannon index (H)= -Σ pi ln pi

Simpson index (D)= 1/(Σ pi 〖^2〗)

The Shannon index is an informative statistic index which assumes all species are presented randomly in a sample but the Simpson index is a dominance index which gives more weight to common or dominant species. Pi in both of them is a fraction of the species abundance to the total collected samples. The bigger the index, the more diverse the area. Sorenson coefficient was calculated to show the similarity level between two sampling stations by the following equation:

Sorenson`s Coefiecient (CC)= 2C/(S1+S2)

Where C is the number of species the two sampling areas have in common, S1 is the total number of species or genera in this case in sampling area 1 and S2 is the similar count for area 2).

Results

Totally 48 samples were collected from Lar river which were consisted of 6 genera belonged to two subfamilies Chironominae (5 genera) and Tanypodinae (1 genus), two genera were collected for the first time for the Iranian fauna which are indicated by a star. Subfamilies, genera and tribes are shown in Table 1; among the 48 samples, 22 samples were belonged to Polour and 26 samples collected from Lar area.

Table 1. Genera collected in Lar river; the genera marked by * are reported for the first time for Iranian fauna

Subfamily	Tribe	Genera	Lar station	Polour station
Tanypodinae	Macropelopiini	*Brundiniella**	+	+
Chironominae		*Chironomus*	+	+
		Stichtochironomus	-	+
		Polypedilum	+	+
		Endochironomus	+	-
		*Hyporhygma**	+	-

Subfamily TANYPODINAE

***Brundiniella** Roback 1978**

Larvae of *Brundiniella* live in rivers and distinguished by the weak lateral setal fringe on the body; maxillary palp with ring organ near middle; rounded head capsule; yellow-brown ligula with inner teeth directed more or less straight forward; well developed dorsomental plates each with 5 large teeth and two smaller teeth, and with a thin, pointed, medial extension that reaches or almost reaches the pseudoradula; and the expanded base of the small claw of the posterior parapod (Epler 2001). Totally 10 samples were collected, 5 samples from each station equally (Fig. 2).

Subfamily Chironominae:
***Endochironomus* Kieffer 1918**

Just 2 larvae were collected from Lar station (35°50′22″N, 52°2′38″E). The genus is reported for the first time for the Iranian fauna (Fig. 2). The genus was distinguished by the 3-4 median teeth on mentum separated from the lateral portion by a distinctive line which runs posteriorly from the median teeth to the anteromedial corner of the ventromental plates; ventromental plates with anterior and posterior margins parallel for most of their length, and lateral apex rounded; and the tuberculate anterior margin of the cardo.

***Chironomus* Meigen, 1803**

Twelve larvae of the genus were sampled totally which one of them were from Lar station and the rest were collected from Polour .Larvae are distinguished by the presence of a frontoclypeal apotome and one medial labral sclerite; pecten epipharyngis a single multi-toothed comb; mandible with basal radially arranged grooves and simple seta subdentalis; 0-1 pairs of caudolateral tubules and 0, 1 or 2 pairs of ventral tubules (figure 2).

***Hyporhygma* Reiss 1982**

Two larvae were collected from Polour station (Fig. 2). The genus was distinguished by the frontal apotome, with 2 median labral sclerites anterior to it; pectin epipharyngis of three scales, each with 46 smaller teeth on surface; mentum with 2 median teeth lower and smaller than first lateral teeth; ventromental plates with scalloped anterior margin and strong striae and sculpturing; and leaf-mining habit.

***Polypedilum* Kieffer 1912**

19 *Polypedilum* larvae were totally collected which the majority of 15 samples were collected from Lar station and the rest belonged to Polour. The distinctive mentum, with median and second lateral teeth longer than first lateral teeth, will distinguish most members of the genus. Other larvae may be identified by the frontoclypeal apotome with straight anterior margin expanded laterally into lobes in which the S 3 setae are located (except in *P. ontario*); 5 segmented antennae (4 segmented in 2 species); and the 4 median teeth of the mentum not separated from the rest of mentum by a distinct line. (figure 2).

***Stichtochironomus* Kieffer & Theinemann, 1919**

Just three larvae of the genus were collected from Lar station. Larvae were reddish minute vermiform with distinct dark pattern on the back of their head capsule. Mentum was provided with four middle teeth with two long outer. The genus is mostrly found in dense popuplation in several aquatic habitats (figure 2).

Statistical analysis

Frequency of different genera collected in this paper are presented in table 2. As it can be seen most of the samples were belonged to *Polypedilum* and the least samples were *Endochironomus* totally. Comparing the mean number of samples collected in 4 replications in different genera showed no significant difference (df= 5, F= 1.200 and Sig.= 0.409). The most abundant genus through sampling procedure was *Polypedilum* (4.75 ± 2.28) and the least genus was Endochironomus (0.25 ± 0.07) which is in accordance with the result of table 2. Comparing frequency of the found genera in two stations by use of T-student test showed no significant difference (df=1, F= 0.537, Sig.=0.482); the mean number of the collected samples was calculated for four genera in Polour station as 3.44 ± 0.33 and the same parameter for five genera in Lar station was 5.3 ± 0.406.

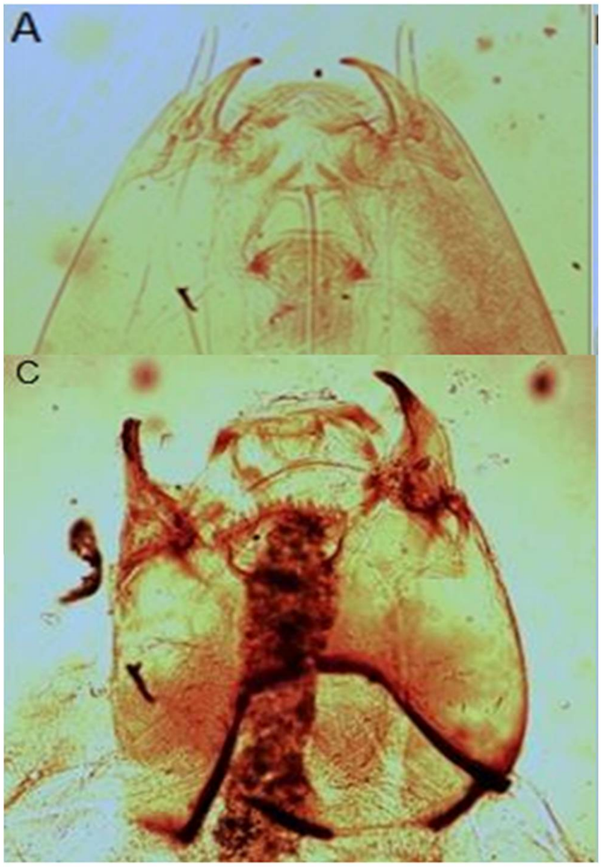

Figure 2. Head capsule of (A) *Brundiniella*, (B) *Radotanytarsus,* (C) *Endochironomus* and (D) *Hyporhygma*

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Table 2. Mean percentage of the genera found in Lar River

Genus	Frequency	percentage
Chironomus	12	25
Endochironomus	2	4.16
Hyporhygma	2	4.16
Polypedilum	19	39.58
Stichtochironomus	3	6.25
Brundiniella	10	20.83

Shannon and Simpson indices were calculated for whole the sampling area as H=1.354 and D= 3.720, respectively. Calculating the Shannon index for Lar and Polour areas separately showed that the index were H=1.2012 and H=1.2109, respectively. Simpson index was calculated as D= 2.5621 for Lar and D= 2.917 for Polour area.

Sorenson`s coefficient for Lar and Polour Stations was calculated as CC=0.6667; which showed relevance to high similarity between two sampling areas. These sampling areas had quite a bit of overlap or similarity.

Discussion

According to Ash *et al.* (1987), Tanypodinae genera are belonged to the Palearctic and Nearctic regions which 26 genera are common to both regions; other genera like *Brundiniella* have been reported from Nearctic region including north America; some researches admitted this suggestion and reported *Brundiniella* from United States of America (Alaska Energy Authority 2013). Recent study on Tanypodinae of eastern China (Niitsuma, 2003) and Thailand (Epler 2001) showed that *Brundiniella* could be found in Palearctic region, too. Phylogeny of different species of *Brundiniella* was studies by the same researchers.

Among Chironominae genera, *Chironomus* has been reported at generic level from different parts of Iran, such as Hablehrood river (Arkia *et al.* 2017), Jajrood river (Arkia *et al.* 2016), Marbor river (Karami *et al.* 2014), Zayandehrood river (Shayeghi *et al.* 2014; Ebrahimnezhad and Fakhri 2005), Golpayegan river (Ebrahimnezhad and Allahbakhshi 2013) in Isfahan province, the ponds around Tehran province (Alvary 1997), Anzali Lagoon in Gilan province (Valipour 1997) and southern shores of Caspian sea (Mousavi 1995). Rajabipour *et al.* (2011) reported *Chironomus aprilinus* Meigen in sediments of the earthponds of Bafq area in center of Iran. Sahragard and Rafatifard (2010) studies the biology and effect of temperature on larval development time of *Chironomus riparius* Meigen as a brilliant index of water pollution and in another study tried to mass rearing of the same species under laboratorial condition (Sahragard and Rafatifard 2006). *Chironomus albidus* was reported from southern coast of the Caspian Sea (Ahmadi and Mousavi 2002). This genus belonged to Palearctic region has been reported at species level from lake of Uluabat in Turkey (Arslan *et al.* 2010), the same study suggested that the presence of *Chironomus* in fresh waters could be considered as an index for chemical and organic contamination; more population, less contamination. Rawal (2014) studies *Chironomus* larvae in Udaipur region in India to show the role of habitat features like pH, temperature, chloride content, dissolved oxygen, COD and BOD on the ecological bio-indicators such as *Chironomus* and concluded that these insects can be used as water pollution index. In this study, *Chironomus* was found in two stations with considerable density that composed 22.6% of the collected samples. As there was no significant difference between two stations in *Chironomus* density, it would be concluded that water features are similar through Lar River.

Hyporhygma firstly reported from Nearctic region (Francis 2004) and it is the first time which this genus is reported from Palearctic region. There is a limited studies on the genus which highlights the need for more studies on the genus and its distribution in Iran and other parts of the world.

Endochiromnomus has been previously reported at generic level from Iranian fauna (Alvari 1997) and at species level from water ponds near to apples orchards of Slovakia (Hemerlik *et al.* 2016), water bodies of the Volga region in Russia (Durnova *et al.* 2015), Phong river in Thailand (Sriariyanuwath *et al.* 2015), some European countries like the Netherlands, United Kingdom, Norway, Belgium, Belarus, Sweden and Germany which are listed in Global Biodiversity Information Facility and heated lakes of Poland (Cieminski and Zdanowski 2009). Camur-Elipek *et al.* (2010) reported several species of *Endochironomus* from Gala Lake in Turkey. *Endochironomus tendens* reported as large population of mining larvae in fresh water of river Tisza and vegetable moist lands from Hungary (Toth *et al.* 2008). As it seems the genus has global distribution and more research studies are needed to maintain its species in Iranian aquatic resources.

Polypedilum and *Stichtochironomus* have been reported from different parts of Iran like Hablehrood river (Arkia *et al.* 2017), Marbor river (Karami *et al.* 2014), Zayandehrood river (Ebrahimnezhad and Fakhri 2005) and Golpayegan river (Ebrahimnezhad and Allahbakhshi 2013) in Isfahan province. *Polypedilum* showed the highest density among the collected genera in this study and as mentioned previously in similar studies in Ergen river basin of Turkey (Ozkan *et al.* 2010), Phong river in Thailand (Sriariyanuwath *et al.* 2015) and small rivers in Lithuania (Virbickas and Pliuraites 2002), it would be considered as a bio-indicator for oil pollution. According to IAEA (2014), presence of *Stichtochironomus* has negative correlation with vanadium, copper and chrome pollution. The genus has been reported from Lithuania (Virbickas and Pliuraites 2002). Harper *et al.* (1998) represented *Stichtochironomus* as an indicator of sited riffles and runs of eastern England which would be applied in limnological studies. According to low density of the genus in this study, it would be concluded that the sampled water in Lar river is polluted in some aspects which is needed to be measured.

In a study on the Shannon-Wiener index of Chironomidae in Brazil, the index was calculated for four sites through Vacacai-Mirim river microbasin. Shannon-Wiener index for Chironomidae was higher in Rocky sampling sites (Konig and Santos 2013). Rawal (2014) calculated the Shannon-Wiener index of *Chironomus* species in Ayad river of Udaipur city in India and showed that Shannon diversity index was measured is H`= 1.21 which is so similar to the results of this paper. Unfortunately any Iranian study focusing on determining the diversity indices for Chirnomidae was not found. According to Nemati Varnosfaderany *et al.* (2010) the benthic macroinvertebrate communities of the Zayandeh Rud River were dominated by Chironomid larvae (30.51%) but they did not focus on the family diversity among different sampling station in Zayandehroud river. Moghdani *et al.* (2013) introduced Chironomidae with two genera as the dominant group of Mond protected area in Bushehr province. Amri *et al.* (2014) studied the diversity of macrobenthos communities in Jajroud River, Iran and found out that although Ephemeroptera was the most abundant taxon in Jajroud river, Chironomidae and two other dipteran families were the most dominant families. They discussed that the diversity of Chironomidae was mainly correlated with water temperature.

Conclusion

It can be concluded from this paper that Lar river had a great resource of Chironomidae species with at least 6 genera which two of them, *Brundiniella* and *Hyporhygma* were reported as the first record for Iranian fauna. Shannon-Wiener and Simpson indices indicated that Polour sampling area with higher aquatic habitats was more diverse.

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