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New record of *Aedes vittatus* and *Culiseta subochrea* (Diptera: Culicidae) and their distribution from Shadegan Wetland, South Western Iran

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Abstract
This study was conducted in Shadegan International wetland area, south western Iran. Mosquito larvae were collected from an urban site in the east of the wetland, using dipping method in December 2011. Mosquito larvae were identified using the morphology-based keys. The water quality parameters were measured during mosquito larvae collection using HQ40d Portable Multi-Parameter Meter, titration method and turbidity meter from the late October to late December 2011. A total of 1071 larvae were collected. Four species belonging to four genera including *Aedes vittatus* (Bigot), *Culex pipiens* Linnaeus, *Culiseta subochrea* (Edwards) and *Ochlerotatus* (=*Aedes*) *caspius* (Pallas) *sensu lato* were identified and their distribution and some other aspects were reviewed and discussed. Recorded parameters in the present study indicated that the water quality of the studied area was in a poor condition according to Environmental Protection Agency and World Health Organization water quality standards ($p < 0.05$). As per the authors' knowledge, this was a new record for the studied area and Khuzistan province regarding the occurrence of *Ae. vittatus and Cs. subochrea*.

Keywords: *Aedes vittatus*, *Culiseta subochrea*, Culicidae, distribution, new record

1. Introduction

Mosquito-borne diseases such as malaria, Japanese encephalitis [1], and Rift Valley fever, are a major public health threat in some Asian countries, including Islamic Republic of Iran. Also, mosquitoes transmit other diseases such as *Dirofilaria immitis* (Leidy) (dog heart worm) and *D. repens* Railliet and Henry (dirofilariasis) [2], as well as the mosquito-borne nematode *Setaria* (setariasis) [3] in Iran. Some mosquito-borne arboviral outbreaks like Japanese encephalitis and Rift Valley fever in the WHO Eastern Mediterranean Region, including Islamic Republic of Iran, are possible to occur [4]. *Culex tritaeniorhynchus* Giles was mentioned by Travis and Labadan (1967) as a vector of Japanese encephalitis in Islamic Republic of Iran and Iraq. Although there is no formal recent record of dengue fever in Islamic Republic of Iran [5], there are some doubtful old records of this virus in the country [6]. These reports show the status of mosquitoes as a threat to human health.

Wetlands take on characteristics that distinguish them as distinct ecosystem. They are the most productive ecosystems in the world [7, 8]. Shadegan International wetland in Khuzistan province, south western Iran, is considered to be one of the most wonderful natural attractions of the world because of its unique biodiversity and link to Jarahi river and Persian Gulf waters. In fact, the presence of fresh water and saline water in the wetland which is regarded a rare phenomenon has led to the diversity in the wetland's marine ecosystem. It creates a suitable habitat for a large number of migratory birds that fly and arrive to this area from north Europe, Canada and Siberia every year. The high diversity of plant and animal species in the Shadegan wetland has caused the International Supreme Council for the Environment to register it as an International protected zone.

Although Shadegan International wetland has the potential to become a tourism destination and bears many socioeconomic advantages for local residents and ecoenvironmental conditions to prevent the dust phenomena in recent decade, high density of mosquitoes threaten human health and prevent tourist activities.

Also as mentioned some mosquito-borne arboviral outbreaks like Japanese encephalitis and Rift Valley fever in the WHO Eastern Mediterranean Region, including Islamic Republic of Iran, is possible to occur [4] and correct identification is essential for effective control of problematic mosquitoes [9]. In Khuzistan province malaria vectors are studied well as compared to other mosquitoes. In a recent study conducted by Navidpour *et al*. (2012) on adult mosquito collection, five mosquito species including *Culex pipiens* L., *Cx. tritaeniorhynchus* Giles, *Cx. sinaiticus* Kirkpatrick, *Cx. modestus* Ficalbi, *Ochlerotatus caspius* (Pallas) were detected and reported [10]. Thus a study was designed to collect and identify mosquito larvae from Shadegan wetland. The aim of this study was to obtain new data, which would be valuable to develop programmes for future planning of mosquito control in this area of south western Iran.

2. Materials and Methods

2.1 Geographical information: This study was conducted in Shadegan International wetland area in Khuzistan province, south western Iran which is one of the 18 International wetlands registered on UNESCO's Natural Heritage List. It is the largest wetland in Iran which covers an area of 537,700 hectares, located 52 km far from Abadan and 40 km far from Ahvaz (the capital city of Khuzistan province) and surrounded from north to Shadegan city and Khor Doraq, from south to Bahmanshir river, from west to Darkhovien and Abadan road and from east to Khure-Musa. The surface is covered by vegetation and water supply is mainly through Karoun river. The area has a hot and humid climate with coordinates: 48° 17'- 48° 50'E 30° 17'- 30° 58'N [11].

2.2 Mosquito larvae collection and identification: Shadegan wetland mosquito fauna was investigated from October 2011 to September 2012. Because we encountered a high population mosquito larvae occurred after sever rainfall in the late December 2011 [11]. So a site of Shadegan International wetland located at the east of the wetland between Shadegan city and wetland where urban waste released into the wetland was selected. Immature mosquito stages were collected in aquatic habitats using the standard larval dipping technique in the December 2011. The specimens were identified using the morphology-based keys [12, 13, 14, 15, 16, 17].

2.3 Measure of water quality parameters: The water quality parameters measured during mosquito larvae collections were water temperature, electrical conductivity (EC), dissolved oxygen (DO), pH, hardness, total dissolved solids (TDS), total suspended solids (TSS), turbidity, and salinity using the HQ40d Portable Multi-Parameter Meter, titration method and turbidity meter from October 2011 to September 2012 and compared with Environmental Protection Agency (EPA) and World Health Organization (WHO) water quality standards [18, 19] using One-Sample T Test (Data not shown) [11]. In this study only the data of the water quality parameters of the late October to late December 2011 were shown.

3. Results and Discussion

In the present investigation, a total of 1071 mosquito larvae were collected from the site of the Shadegan wetland in December 2011 (Table 1). Four species belonging to four genera were identified: *Aedes vittatus* (Bigot), *Culex pipiens* Linnaeus, *Culiseta subochrea* (Edwards) and *Ochlerotatus* (=*Aedes*) *caspius* (Pallas) *sensu lato* (*s.l.*) [20] (Table 1). Table 2 shows the water quality parameters measured from the mosquito larvae collection site of the Shadegan International wetland, during sampling from the late October to late December 2011. Based on the parameters accessed, the water was in a poor condition in Shadegan wetland, according to Environmental Protection Agency (EPA) and WHO water quality standards. The electrical conductivity (EC), hardness, total dissolved solids (TDS), total suspended solids (TSS) and turbidity were higher than the EPA and WHO water quality standards (Data not shown) [18, 19, 11]. One-Sample T Test indicated that there was a significant difference between these water parameters and the EPA and WHO water quality standards (all p-values < 0.05) (Data not shown) [11].

Table 1: Frequency of Culicinae mosquito larvae collected in Shadegan wetland, south western Iran, December 2011

Species	Number	Percentage
Aedes vittatus	1	0.09
Culex pipiens	2	0.19
Culiseta subochrea	109	10.18
Ochlerotatus caspius s.l.	959	89.54

Table 2: Water parameters measured in the Shadegan wetland during sampling from late October to late December 2011

Month	Temperature (°C)	pH	DO	Salinity	Hardness	TDS	TSS	Turbidity (NTU)	EC ($\mu s.cm^{-1}$)
			($mg.L^{-1}$)						
October	23.6	7.4	10	386.7	5225	6290	21	47	25260
December	21.5	7.8	14	181	1343.5	3860	14.7	21	7740

In a study conducted by Navidpour *et al*. (2012) [10] five species including *Culex pipiens* L., *Cx. tritaeniorhynchus* Giles, *Cx. sinaiticus* Kirkpatrik, *Cx. modestus* Ficalbi, *Ochlerotatus caspius* were detected [10], but in this study four species belonging to four genera of mosquito including *Aedes vittatus*, *Culex pipiens*, *Culiseta subochrea* and *Ochlerotatus caspius s.l.* were identified. *Oc. caspius* was the most frequent species in both studies. *Cs. subochrea* was the second but the others had a very low prevalence (Table 1). *Aedes vittatus* and *Culiseta subochrea* are the first record of these mosquito species on the Shadegan International wetland area in Khuzistan province, south western Iran. *Aedes vittatus* has worldwide distribution [21] including Afrotropic (Ethiopian), Oriental (Indo-Malayan) and Palearctic zoogeographic regions. Although, *Aedes vittatus* was reported from Iran previously [15] and has been mentioned recently, in the checklist of Iranian Culicidae by Azari-Hamidian [5], but has not been collected in the current decade studies which were conducted in Iran. Because presence of *Ae. vittatus* has been recorded after the onset of the rainy season [22] and this study was conducted after rainfall, naturally it is found. *Aedes vittatus* lays its eggs out of water on damp surfaces in natural rock hollows. When rains occur, the rock hollows fill with water and the non submerged eggs are stimulated to hatch. Thus, in countries with a distinct dry season, the eggs must be resistant to prolonged dehydration, while awaiting the rains. The most common larval habitats of *Ae. vittatus* were puddles followed by rock holes. A number of studies have investigated *Aedes* egg survival under natural conditions. Thus in Africa (mainly Nigeria), field studies have been carried out on *Ae. vittatus* [23], the larvae of this species are generally known to occupy rock holes [24], however in this study the larvae of this species are generally known to occupy the marginal reedy areas

of the entrance Shadegan International wetland which covers the surface of the Shadegan urban wastewater where released into the wetland. Although larvae of *Ae. vittatus* has been already found in puddles in Midwestern Nigeria [25].

Aedes vittatus identified as a rural and/or sylvan yellow fever virus (YFV) vector in the Central African Republic. YFV occurs naturally in an enzootic cycle involving sylvatic mosquito species such as *Ae. vittatus*, which breed in natural sites (e.g. bamboo stumps, bromeliads and tree holes). In human settlements, YFV is transmitted during epidemics by *Ae. vittatus* in rural areas [26]. In a study conducted by Angel and Joshi (2008) in arid and semi-arid areas of Rajasthan, India, *Ae. vittatus* showed highest infection (20%) of vertically transmitted dengue virus followed by *Ae. albopictus* (18.7%) [27], however area of the Shadegan International wetland in Khuzistan province, south western Iran has a tourism destination and has the same arid and semi-arid condition as area of Rajasthan, India, which was *Ae. vittatus* reported from it may be potentially dengue transmission in this area of Iran. Also, *Ae. vittatus* has potential vectors of arbovirus pathogens such as CHIK virus which was tested positive for CHIK infection and the possibility of CHIK transmission in the southern France area where this species are found [28].

Culex pipiens is a species of the *Culex* genus that is the most widely distributed mosquito in the world and found on every continent except Antarctica [15]. It is a domesticated species developing in close association with man which is a troublesome species that commonly infests houses and bites at night and is said to display a preference for avian blood [29].

Previous studies showed that *Cx. pipiens* is widely distributed in world and Iran and recent studies in Iran including: Guilan [30], Sistan and Baluchistan [31], Ardebil [32], Kurdistan [33], Hamadan [34], North Khorasan [35] and Qom [36] Provinces, and also in this study in areas of the Shadegan International wetland in Khuzistan province, south western Iran are confirmed it.

Culex pipiens larvae have been found in many different natural and artificial habitats including underground ones such as flooded cellars, drains, wells, septic tanks, underground train systems, abandoned mine tunnels, and coal mines [15]. The wide variety of the breeding places can explain why this species is one of the most abundant and common mosquito species in Iran [30] as well as in the Shadegan International wetland in Khuzistan province, southwestern of Iran. In Iran, this species was found mostly in natural habitats, as well as in this study was found in the marginal reedy areas of the Shadegan wetland entries, but Moosa-Kazemi *et al*. (2000) collected it only in rice fields (artificial habitat) [37] and Azari-Hamidian *et al*. (2002) found it mainly in man-made habitats [38].

In the Old World, some mosquitoes in the *Culex pipiens* complex are excellent enzootic vectors of West Nile virus, circulating the virus among birds, whereas others bite mainly humans and other mammals [30, 39]. Recent, indirect evidence based on blood-meal analysis and theory suggests that *Cx. pipiens* serves as both an enzootic and an epidemic (i.e., "bridge") vector [40]. Overally, *Culex pipiens* serve as vector of important medically arboviruses (arthropod-borne virus) diseases, such as West Nile virus, Japanese encephalitis, St. Louis encephalitis, Western Equine encephalitis, Eastern Equine encephalitis, Sindbis, La Crosse, Tahyna and Rift Valley fever. Also, it carries a number of other diseases, especially filariasis such as *Wuchereria bancrofti* and *Dirofilaria immitis*, avian malaria and Pox (Fowl) [32].

The genus *Culiseta* includes five species in Iran [5]. Most *Culiseta* are cold-adapted species which only occur in warmer climates during the colder parts of the year or at higher elevations where temperatures are low. The larvae of most species are found in ground waters such as bogs, marshes, ponds, streams, ditches and rock-pools, but an African species occurs in tree-holes, a common eastern Palaearctic species occurs in wells and rock pools, and several Australian species occur underground. Little is known about the blood-feeding habits of females. Most species feed on birds and mammals, but a few feed on reptiles. Several species attack domestic animals and occasionally humans [41].

Culiseta subochrea is distributed in Egypt, United Kingdom, Israel, Netherlands, Lebanon, Syria, Palestinian Territory, Belgium, Germany and Ireland which is included Palearctic zoogeographic region [42], however in Iran, reported (as subspecies of *Cs. annulata*) in Iran. Zaim and Cranston (1986) mentioned *Cs. anuulata* in their checklist and *Cs. subochrea* in their keys [14]. AzariHamidian (2005) found *Cs. annulata* in northern Iran [43]. The taxonomy and distribution of these two species need more investigation in Iran [43]. Recent studies showed that *Cs. subochrea* was found in Ardebil [32], Kurdistan [33], Hamadan [34] and Qom [36] Provinces, and also in this study in areas of the Shadegan International wetland in Khuzistan province, southwestern of Iran.

Five species of the genus *Culiseta* including *Cs. alaskaensis* Ludlow, *Cs. annulata* (Schrank), *Cs. longiareolata*, *Cs. morsitans* (Theobald) and *Cs. subochrea* are recorded in Iran [5] and the Middle Asia countries [35]. However, only *Cs. subochrea* was found during the present study.

Oc. caspius s.l. is widely distributed in Europe and occurs in a variety of habitats. Recent studies showed that *Oc. caspius* was found in Ardebil [32], Sistan and Baluchistan [31], Iranian Persian Gulf Islands [9, 44], Kurdistan [33], North Khorasan [35], Hamadan [34] and Qom [36] Provinces, and have also been recorded in southern Iran [9] and also *Oc. caspius* was detected in this study in areas of the Shadegan International wetland in Khuzistan province, southwestern of Iran. Although previous studies indicated that *Oc. caspius* is widely distributed in Iran, however in this study was detected with the highest prevalence (Table 2). Seasonally high abundances of this species, as observed in this study (% 89.54), cause it to be an economically significant pest in areas of the Shadegan International wetland in Khuzistan province, south western Iran as mentioned by Becker *et al*. (2003) [45], mainly along the Mediterranean coast [46]. Moreover, *Ae. caspius* successfully was infected from Pakistan with West Nile virus (WNV) in the laboratory, and has been isolated from this mosquito species in the Ukraine. During the last 10 years, equine West Nile epizootics were recorded around the Mediterranean basin and *Ae. caspius* may be involved in the transmission of this virus along the Mediterranean coast [47]. This data proved that *Ochlerotatus caspius* can be a threat to areas of the Shadegan International wetland in Khuzistan province, south western Iran as a vector of diseases which will be investigated as a new project in the future.

Medicaly, *Oc. caspius s.l.* is known as the potential or proven vector of different human and domesticated animal pathogens that cause diseases in different areas of the world [15, 35, 36].

More than 23 mosquito species have been implicated as potential bridge vectors or epidemic vectors, i.e., those responsible for transmission to humans [48]. Certain species of the mosquitoes are involved in the transmission of the various arboviral and filarial diseases to humans and domesticated animals and/or are important for their biting nuisance in different parts of the world.

The mosquito fauna of Iran includes 64 species and three subspecies representing seven genera [5, 32]. Present study revealed that *Aedes vittatus* and *Culiseta subochrea* caught had not been previously recorded in areas of the Shadegan International wetland in Khuzistan province, south west of Iran.

The occurrence of some mosquito-borne infections such as malaria, West Nile, Sindbis [49, 50, 51, 52] and an imported case of Dengue fever in Iran [51], though there is no known vector of it in the country, shows the importance of mosquito and mosquito-borne disease surveillance. Although a rare research of mosquito medical importance such as a focus of dirofilariasis in northwestern Iran has been conducted [32], it seems will be initiated programme of researches to monitor the mosquito-borne arboviral diseases of mosquito potential along the different regions of Iran. Because of growing concerns that mosquito-borne arboviral disease transmissions in Iran neighbors and mosquito passenger arrival diseases from other countries increase in recent years might threaten for Iran and countries of this regions in the near future, the results of this study will provide valuable information to help estimate the vector potential of mosquito species.

4. Conclusion

In the present investigation, new data, which would be valuable to develop programmes for future planning of mosquito control in Shadegan wetland, south western Iran was obtained. As per the authors' knowledge, this was a new record for the studied area and Khuzistan province regarding the occurrence of *Ae. vittatus and Cs. subochrea*.

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