

Original Articles**The Morphological Variations of *Culex pipiens* Larvae (Diptera: Culicidae) in Yazd Province, Central Iran**H Dehghan¹, J Sadraei¹, *SH Moosa-Kazemi²¹Department of Medical Entomology and Parasitology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran²Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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Abstract**Background:** *Culex pipiens* complex shows variations in morphological and biological characters including different biological forms and has medical and veterinary importance. Because of having morphological variations, sometimes it is not easy to separate this species from *Cx. quinquefasciatus* and *Cx. torrentium*. The aim of this study was to identify the *Culex pipiens* complex species in order to use in control programs in the future.**Methods:** This study was carried out in two randomly selected rural villages in Yazd County, eastern Iran using dipping technique from April to October 2009. The data were analyzed using SPSS software version 16.**Results:** Average of siphon index in fourth-instar larvae was 3.86 ± 0.03 , the minimum and maximum were calculated 2.43 and 5.14, respectively. Siphon/Saddle index was measured as average, minimum and maximum 3.2 ± 0.2 , 2.78, and 4.42 respectively. In our study, only 4 specimens had single seta 1 on segments III and VI (2.5%) and the remaining beard double seta (97.5%). The maximum 3-6 branches seta 1a-S and 1b-S (95%) were observed on siphon.**Conclusion:** More populations of *Culex pipiens* from different areas of Iran need to be studied to gain complete information about the taxonomy and ecology of the species in the country.**Keywords:** *Culex pipiens* complex, larvae, taxonomy, Iran**Introduction**

Culex pipiens Linnaeus shows a wide morphological and biological variations and well known as a broad research topic by many researchers (Knight and Malek (1951). This complex includes several members of the same morphological form and has different variations in physiology and behavior. The species group includes more than 75 binomial names due to the complexity of synonyms (Knight 1978). Barr (1982) believes that the concept of classical complex for the geographical populations. He mentioned *Cx. pipiens* and *Cx. quinquefasciatus* as a simple geographical population, which has a great distribution. Ishii (1991) expressed that there are variation among the characteristics of

this complex and should not considered being independent species. There are some degree of intra species such as subspecies, variety and form among the members of this complex (Vinogradova 2000). *Culex pipiens*, *Cx. quinquefasciatus* and *Cx. pipiens* form *molestus* are the most common members of this complex and are widespread in the world (Cornel et al. 2003).

Culex pipiens has wide distribution from Europe, next to tropical regions of Asia and Africa till central part of North America, and one third of South Australia and South America. *Culex quinquefasciatus* has been distributed in tropical areas and has overlap extensively with *Cx. pipiens* (Vinogradova 2000).

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Hybrid forms of this species have been distributed in Australia, Africa, the Middle, and Far East of Asia, North and South America. The habitat of *Cx. pipiens* form *molestus* is temperate climates while *Cx. quinquefasciatus* considered cosmopolitan species (Bourguet et al. 1998). *Culex pipiens* has more adaptation to larval habitats such as the pit containing high degree of waste water up to cavity include dishes of fresh water and presented as the most common species in the subgenus *Culex* in southwestern Asia (Harbach 1988). In Iran, *Cx. pipiens* and its form *molestus* and *Cx. quinquefasciatus* are recorded (Azari-Hamidian 2007). *Culex pipiens* (including form *molestus*) has a wide distribution in most areas of Iran and *Cx. quinquefasciatus* is recorded in seven southern provinces of the country (Zaim 1987).

Culex pipiens has the major role in human nuisance due to biting. *Wuchereria bancrofti* and *Brugia malayi* transmitted by *Cx. pipiens* and distributed in tropical and subtropical areas of Africa, Asia, Australia, and Pacific Islands. Some arbovirus infections have been transmitted by *Cx. pipiens* such as West Nile fever, Sindbis, Japanese Encephalitis, St Louis Encephalitis, Western Equine Encephalitis, Rift Valley fever, Tahyna, and Oropouche. *Plasmodium gallinaceum* and *P. relictum*, causes of avian malaria, are transmitted by this species (Vinogradova 2000).

Culex pipiens form *molestus* is separated from *Cx. pipiens* mainly by ecological and physiological characters. Larvae of this species have been found in underground habitat including the storage of water, septic tanks and sanitary space. *Culex pipiens* larval habitat is open space accumulated with artificial water.

The main and reliable characters for diagnosis in larval stage are siphon index, siphon/saddle index, the branch number of seta 1a-S and 1b-S and seta 1 of the abdominal segments of III and IV, and the shape of siphon (Harbach 1988, Azari-Hamidian and Harbach 2009).

The Siphon Index has been used widely for separating the members of this complex. The index is defined as the ratio of siphon length to its basal width. Many researchers have been reported considerable variations in *Cu. pipiens* and *Cx. pipiens* form *molestus*, also Harbach (1988) has rejected this character as diagnostic feature for these forms. The siphon index of *Cx. pipiens* larvae changes based on geographical and ecological variations (Vinogradova 2000). In addition, considerable variations have been cited, for example siphon index of species are grown in contaminated water are shorter than the species reared in the fresh water. Nevertheless, there are considerable ranges due to length of siphon inside the complex species (Harbach 1988). Changes in habitats, locations, sex composition is reflected in larval siphon and can be used for diagnosis analysis among the complex species (Vinogradova 2003). The average of siphon index is correlated to the certain types of larval habitats in ground or underground places. The average index siphon not only correlated with larval habitats, but also has relation to geographical distribution. This index for species in above ground habitats is stronger than other species based on comparing larval habitats. *Culex torrentium* shows close relationship to the members of *Cx. pipiens* complex based on morphological characters, even some researchers proposed it as a member of *Cx. pipiens* complex (Vinogradova 2000, Smith and Fonseca 2004). Harbach (1988) classified *Cx. torrentium* with *Cx. vagans* in the Trifilatus Subgroup of the Pipiens Group. This species with *Cx. pipiens* complex is sympatric in Europe and some areas of Asia including Iran (Azari-Hamidian et al. 2009).

According to Harbach (1988), the most important diagnostic characters of *Cx. torrentium*, *Cx. quinquefasciatus* and *Cx. pipiens* larvae are the branch of seta 1 of abdominal segment of III–V. *Cx. quinquefasciatus* and *Cx. pipiens* are sympatric in some

parts of the world that their distributions have overlap situation (Jupp 1978).

Since the morphological variations and biological diversity of *Culex pipiens* can be found in different populations, each study possibly obtains more accurate data of the variability of *Cx. pipiens* complex to provide a more reasonable discussion about the applied taxonomic issue. It is evident that the final decision about the taxonomic status needs more complete information which should be obtained from different ways among the complex populations.

The aim of this study was to identify the *Culex pipiens* complex species in order to use in control programs in the future.

Materials and Methods

Study area

A study was carried out from April to October 2009 in Yazd Province, Iran. This study was performed in Zarch and Shahediyeh, two randomly selected rural villages in Yazd County (54°04'N–31°59'E). The province is bounded by Isfahan Province in the west, South Khorasan Province in the east, Kerman and Fars Provinces in the south, Razavi Khorasan and Seman Provinces in the north (Fig. 1). In 2009 the maximum and minimum mean monthly temperatures were 41° C in July and -4.4 °C in January, respectively. The total annual rainfall was 62 mm, the minimum 0.3 mm in May and maximum 18 mm in March. The mean annual relative humidity was 37%. The main occupations are agriculture and husbandry of cow and goat. Based on the available epidemiological data from Yazd Health Center, there are no villages under the entomological survey. Based on this information, two rural areas with 889 houses and 24358 populations were selected.

Mosquito sampling and morphological studies

In the present study, two cities of Shahediyeh and Zarch were selected and sampling

was carried out in selected larval habitats. The larvae of each habitat collected by dipping technique, were separately transported to the laboratory of Medical Entomology, School of Public Health, Tehran University of Medical Science, Iran and identified using the keys of Zaim and Cranston (1986) and Azari-Hamidian and Harbach (2009).

In order to morphometric studies, the samples of *Culex pipiens* larvae were separated and sent to the Entomology Laboratory, Department of Entomology and Parasitology, School of Medical Sciences, Tarbiat Modarres University. The fourth-instar larvae were preserved in lactophenol and the microscope slides were prepared using d'Faure's medium. The diagnostic characters of larvae were measured carefully using a microscope equipped with a glass lens dial.

The data were recorded in specific forms according to sampling method, location, and date of collection and were analyzed using SPSS software ver. 16.

Results

The siphon index was measured in 176 fourth-instar larvae. The average siphon index was 3.86 ± 0.03 , the minimum were calculated 2.43 and the maximum 5.14. This index had the most frequency in 159 specimens (90%) in the range of 3.3–4.8. Siphon/Saddle index of 176 larvae was measured as average, minimum and maximum 3.2 ± 0.2 , 2.78, and 4.42 respectively. This index showed the most prevalence in 174 larvae (99%) with the range of 2.7–3.95.

In addition, the larval abdominal seta 1 on segments III and IV and its branches were studied. This character was analyzed in 159 larvae, only 4 had single seta (2.5%) and the rest bifid seta (97.5%) indicating the presence of *Culex pipiens* in the area. The number of the branches of seta 1a-S and 1b-S was 3–8 and the most prevalence branches were 3–6 (95%) on siphon. The most num-

ber of seta on the siphon was 8–9. In our research, the siphon was gradually narrows in 96% of the samples from base toward the

apex. These features of *Cx. pipiens* larvae are presented in Table 1.

Table 1. The variations of some morphological characters of siphon in *Culex pipiens* larvae, Yazd Province, Central Iran, 2009–2010

Character	n	Distinctiveness	Prevalence	%
Seta 1 abdominal segment III and IV	159	Single	4	2.5
		Double	155	97.5
Siphon Seta 1a-S and 1b-S	158	3	22	14
		4	52	33
		5	52	33
		6	24	15
		7	6	4
		8	2	1
Siphon seta number	127	6	12	9.5
		8	85	67
		9	18	14
Siphon shape	181	10	12	9.5
		Gradually narrowing	173	96
		Wide in the middle	8	4

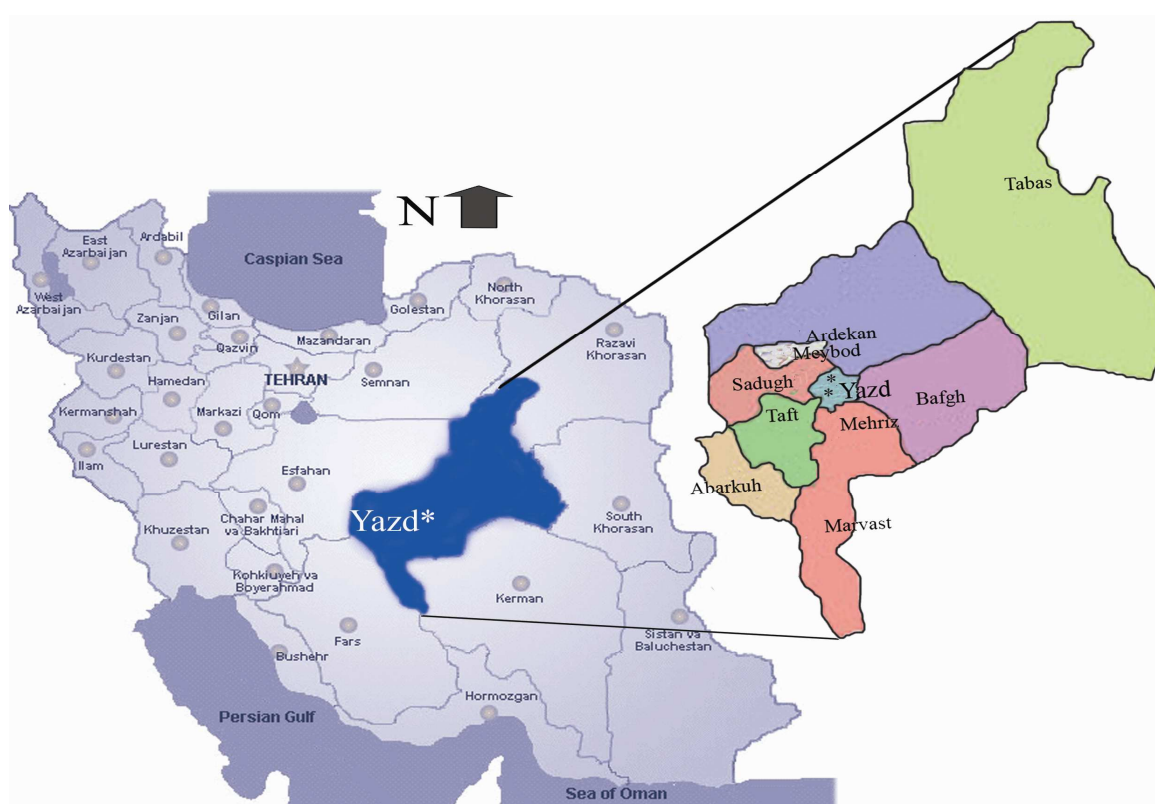


Fig. 1. Map of Iran indicating the location of Yazd County situated in the center of Yazd Province

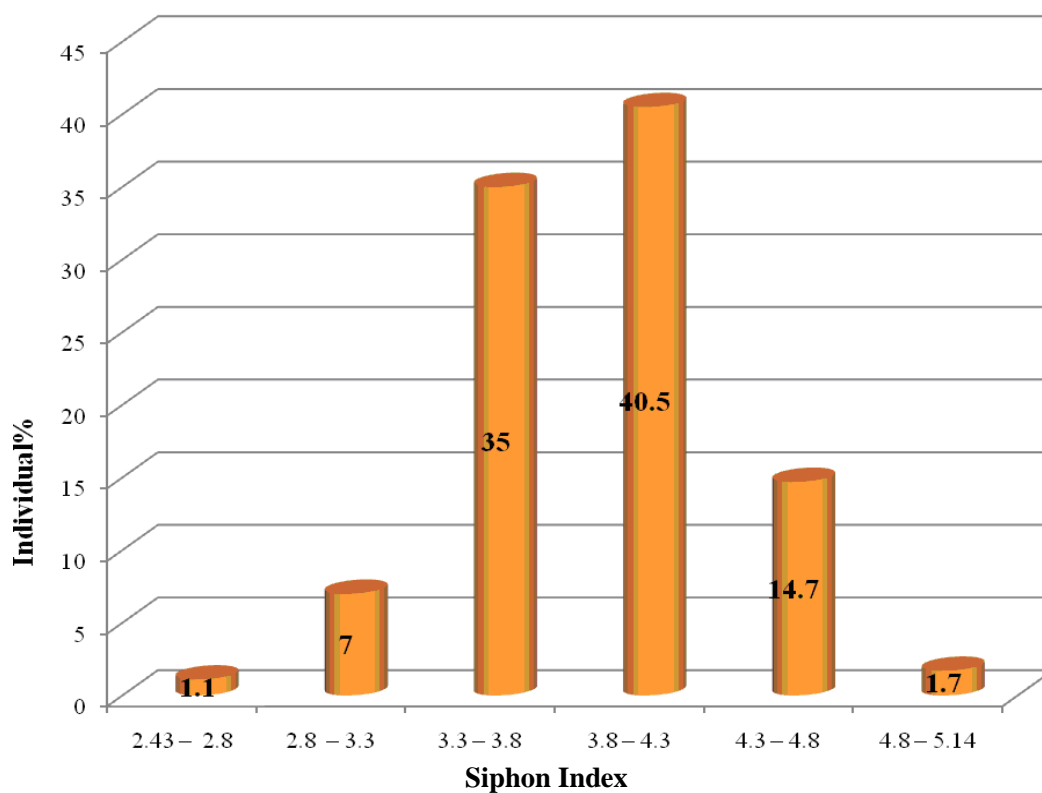


Fig. 2. The range of siphon index in *Culex pipiens*, Yazd Province, Iran, 2009-2010

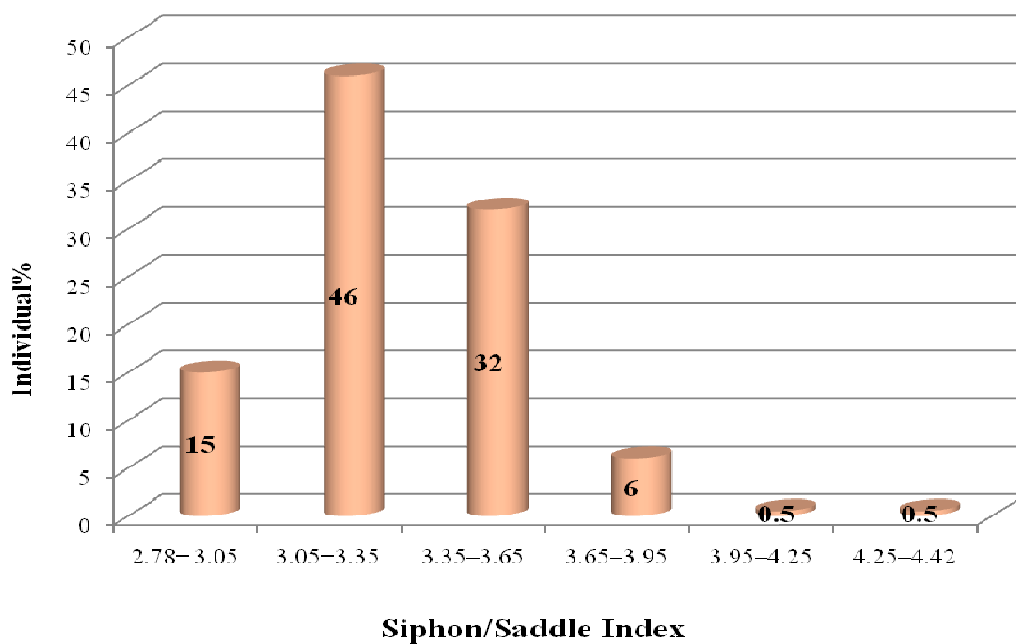


Fig. 3. The range of siphon/saddle index in *Culex pipiens*, Yazd Province, Iran, 2009-2010

Discussion

Culex pipiens complex is cosmopolitan species. The distribution of the member of this complex in Iran is not completely known using reliable characters for identification and a few studies have been performed in this field (Zaim 1987, Azari-Hamidian and Harbach 2009, Azari-Hamidian et al. 2009, Azari-Hamidian et al. 2010).

The larval chaetotaxy and siphon characters are very important in the taxonomy of the genus *Culex*. In our research three related characters including: siphon index, siphon/saddle ratio, and chaetotaxy were studied.

Siphon index was studied in 176 samples and was obtained in average 3.86 and the range of 2.43–5.14. Siphon index was calculated as 2.8–4.8 in 97% of the samples and shown with a scattering in Fig. 2. In similar study in Egypt, this index was reported as 3.7 in average and the range 2.7–4.7 for *Cx. pipiens* (Knight and Malek 1951). Harbach (1988) reported the average 4.58 and the range 3–5.8 for *Cx. pipiens*. Ishii (1991) stated the overall ratios of less than 4 as indicator for mosquito larvae developed in pollutant water, whereas high ratio greater than 4.4 for the specimens caught in larval habitats with fresh water. On the other hand, there are some reports about relation between the average of siphon index and underground or above ground larval habitats. *Culex pipiens* found in above ground habitats, whereas *Cx. pipiens* form *molestus* chooses underground habitats. The range of siphon index was reported 4.8–6.2 for 80% of *Cx. pipiens* populations and was found 3.3–4.4 for 90% of the *Cx. pipiens* form *molestus* populations (Vinogradova 2000).

Considering the samples collected in Yazd Province from above ground in all larval habitats, siphon index was compared based on the average amount of water pollution with organic matter. The results of the finding showed that the average of the index was

3.65 in 63 samples found in sewage water near the households. The average index was 4 in 113 specimens collected from the cistern water pool near homes and livestock shelters. The diversity of this index shows relation to larval habitats with fresh water to contaminated water with high levels of organic matter.

The siphon/saddle index and seta 1 of abdominal segment of III–IV are the main characters for the reliable differentiation of *Cu. pipiens* from *Cx. quinquefasciatus* (Harbach 1988, Azari-Hamidian and Harbach 2009). Moosa-Kazemi et al. (2010) identified *Cx. pipiens* using these characters in Kurdistan Province. In our study, the average rate of the siphon/saddle index was 3.2 with the maximum 4.42, and the minimum 2.78. The diversity of this index was low and in 99% of the samples was calculated as a range between 2.7–3.95 (Fig. 3). The average of this index was reported 4.08 and the range 3.48–4.63 for *Cx. pipiens* and was mentioned the range 2.77–3.41 for *Cx. quinquefasciatus* by Harbach (1988). The ratio of this index was reported more than 3.45 for *Cx. pipiens* and less than 3.45 for *Cx. quinquefasciatus* by Azari-Hamidian and Harbach (2009).

In our study, we found the present of double seta 1 of abdominal segment of III–IV in 96.9% of the samples. In parallel to Harbach (1988) and Azari-Hamidian and Harbach (2009), this character attributed to *Culex pipiens*.

Siphonal seta 1-S of *Culex pipiens* complex, especially 1a-S and 1b-S, was used for identification, particularly *Cx. pipiens* and *Cx. quinquefasciatus*. In our study, siphonal seta 1a-S and 1b-S were observed in 158 samples, and was found range 3–8 branches, whereas 95% of the samples have 3–6 branches. Harbach (1988) cited the variation of the number of branches and mentioned the more branches of seta in *Cx. quinquefasciatus* than *Cx. pipiens*. In agree with the present research,

Knight and Malek (1951) reported an average 4 and a range 2–9 branches in *Cx. pipiens*. In our study, seta 1-S was counted in 127 larval samples and 8–9 setae were observed in 103 samples (81%). The most number of tuft setae (10 setae) were counted in 12 samples (Table 1). Harbach (1988) mentioned four pairs of seta 1-S in *Cx. pipiens*, sometimes fifth tuft was observed on one or both sides of siphon.

Shape of siphon was studied in relation to siphon width in the middle and its base in 181 larvae. This character in the middle was less than in the base in 173 larvae samples, and was wide in the middle of others (Table 1). In general, siphon of *Cx. pipiens* is longer than in *Cx. quinquefasciatus* (Harbach 1988, Azari-Hamidian and Harbach 2009). This feature can be used for the differentiation of *Cx. pipiens* and *Cx. quinquefasciatus*.

In conclusion, our study indicated that siphon/saddle index is specific diagnostic character, in contrast, siphon index has relative diagnostic value due to more scattering range (Fig. 2 and 3). According to our observations, branch of seta 1 of abdominal segment of III–IV and shape of siphon in larval samples are valuable characters which can easily separate *Culex pipiens* and *Cx. quinquefasciatus*. According to the results reported previously, some characters were overlap in *Cx. pipiens* and *Cx. quinquefasciatus*, therefore to determine the species it is recommended studying all characters together and then making a decision about such a complex species. Based on our study, *Cx. pipiens* larvae were detected in Yazd area and in order to determination of the distribution of the complex species more study should be carried out in other areas of Iran.

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