Original Article

A Pictorial Key for *Culex pipiens* Complex (Diptera: Culicidae) In Iran

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Abstract

Background: The aim of this study was to design pictorial key and taxonomic literature of *Culex pipiens* complex in Iran.

Methods: Larvae were collected using standard dipping methods in 13 randomly selected areas of Bushehr, Hamedan, Kerman, Khorasan-e-Razavi, Khuzistan, Mazandaran, Tehran, Sistan and Baluchistan and Yazd Provinces from April 2009 to October 2010. The data were analyzed using SPSS Ver. 11.5.

Results: *Culex pipiens* larvae were identified based on the Seta 1 of the abdominal segments III–IV in north and central parts of Iran. This diagnostic character had some variation among the *Cx. quinquefasciatus* collected from south of the country. The identification value of intersection of costa, subcosta and bifurcation of R2+3 of female veins, was calculated as 90–100 % for *Cx. pipiens*. This diagnostic character was varied among the *Cx. quinquefasciatus* specimens. The male genitalia found as the main characters to distinguish of *Cx. quinquefasciatus* from *Cx. pipiens*.

Conclusion: It is necessary more studies on the behavior and genetic variations of *Cx. pipiens* complex in Iran.

Keywords: Pictorial key, Taxonomy, *Culex pipiens* complex, Iran

Introduction

Culex pipiens complex species have been known as important vectors of medical and veterinary arthropod-borne diseases (Kasai et al. 2008). Some vector borne diseases such as filariasis, West Nile fever, Western and Eastern Equine encephalitis, Japanese encephalitis and St Louis encephalitis are transmitted by these species complex (Smith 1973, Vinogradova 2000, Kasai et al. 2008). *Cx. pipiens* transmits West Nile virus among wild birds and plays an important role in enzootic cycles (Hayes et al. 2005).

Some pathogens transmitted by culicine mosquitoes such as West Nile and Sindbis viruses, *Dirofilaria immitis* (dog heartworm) and *D. repens* (dirofilariasis), and have been reported in Iran (Naficy and Saidi 1970, Saidi et al. 1976, Azari-Hamidian et al. 2007). Moreover, potential outbreaks of some mosquito-borne arboviral diseases such as Japanese encephalitis (JE) and Rift Valley fever reported in the Eastern Mediterranean Region (WHO 2004).

The morphological and ecophysiological variations of *Cx. pipiens* complex have been an important topic in extensive researches (Harbach 1985, 1988, Vinogradova 2000, 2003, Azari-Hamidian and Harbach 2009, Dehghan et al. 2010, 2011, 2014). Because of the complicities, more than 75 synonyms have been

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proposed for this complex species (Knight and Stone 1977). Barr (1982) reported a wide geographical distribution, and morphological variations among the *Cx. pipiens* and *Cx. quinquefasciatus* populations in the world. There are variations among the diagnostic characters of these species complex that attributed to some degree of expression such as species, subspecies, variety or forms (Ishii 1991).

Culex quinquifasciatus is distributed in the tropical areas with various hosts, whereas, *Cx. pipiens* found in the moderate areas with host preferences of nest maker birds (Vinogradova 2000).

Shahgudian (1960) and Lotfi (1976) provided identification keys for Iranian *Anopheles* and *Culex* species, respectively. The study on physiology and behavior of *Cx. pipiens*, the nominotypical and *molestus* form, was described in Iran (Lotfi 1970, 1973, 1976, Amirkhanian 1974, Zaim and Cranston 1986). Zaim (1984) reported six genera and 55 species of Iranian mosquitoes. Some years later a checklist and systematic key was provided for Iranian Culicinae by Zaim and Cranston (1986). Although, in the previous study had not been mentioned about diagnostic characters of *Cx. pipiens* and *Cx. quinquefasciatus* in the systematic keys (Zaim and Cranston 1986), but Azari-Hamidian and Harbach (2009) addressed these characters.

The present article, reviews some morphological characters of *Cx. pipiens* complex species, collected in some parts of Iran to facilitate conducting comprehensive research about systematics, ecology, medical and veterinary importance of the complex. Until now, seven genera, 64 species, and three subspecies of Iranian mosquitoes was reported (Azari-Hamidian 2007a).

Culex pipiens complex belongs to the Pipiens group, and was divided to several subgroups and subtypes (Harbach 2011, 2013).

Culex pipiens including form *molestus* and *Cx. quinquefasciatus* have been reported in Iran (Azari-Hamidian 2007a, Azari-Hamidian and Harbach 2009). Furthermore, the larval habitat of *Cx. pipiens* form *molestus* was reported by Golestani (1967) in Tehran City. The polygene chromosomal pattern of the autogenous Tehran strain of *Culex pipiens molestus* was described by Amirkhanian in 1974. Further studies about distribution of *Cx. pipiens* have been reported in Iran (Zaim 1987, Azari-Hamidian et al. 2005, 2011, Azeri-Hamidian 2007b, Moosa-Kazemi et al. 2009, 2010, Dehghan et al. 2013). Distribution of *Cx. quinquefasciatus* has been reported in Yazd and Kermanshah Provinces (Ghaffary 1955). More studies reported distribution of this species in southern parts of Iran (Zaim 1987, Mousa-kazemi 2000, Azari-Hamidian et al. 2005, Moosa-Kazemi et al. 2009, Azari-Hamidian et al. 2010, Moosa- Kazemi et al. 2010, Khoobdel et al. 2012). The distribution of this species has been reported in tropical areas in south of Iran based on molecular identification (Azari-Hamidian et al. 2010, Dehghan et al 2013).

Morphological studies on *Cx. pipiens* complex, revealed that, male genitalia and DV/D ratio are the main morphological characters for distinguishing adults of *Cx. quinquefasciatus* and *Cx. pipiens* (Sundararaman 1949). The main reliable character for identification in larval stage has been known as the number of branches of seta 1 on the abdominal III–IV segments (Harbach 1988, Dehghan et al. 2013).

Since there are scatter studies about the morphological variations and distribution of *Cx. pipiens* complex in Iran, (Zaim 1987, Azari-Hamidian and Harbach 2009, Azari-Hamidian et al. 2010) it is necessary to obtain more accurate data on the variability of the species. On the other hand, the final decision about taxonomic status needs more complete data that will be obtained from further studies in different geographical areas. The results of this study can be useful to identify *Cx. pipiens* complex species, as well as designing plans for vector control programs in the future.

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Materials and Methods

Study area

Chabahar (25°17′N, 60°37′E) and Nikshahr (26°04′N, 60°37′E) counties were selected from Sistan and Baluchistan Province with tropical warm and humid climate. In southern Iran, Jiroft (28.5°N, 57.8°E) from Kerman, Borazjan (29°15′N, 51°12′E) from Bushehr, Ahvaz (31°19′N 48°41′E) and Bostan (31°27′N 48°04′E) counties from Khuzistan Province with subtropical warm and humid climate were selected. Yazd, Zarch (54°04'N 31°59'E) from Yazd and Kerman (30°17′N 57°04′E) from Kerman Province with hot and dry desert climate were selected. For cool and moist Mediterranean climate Neka (36°42′N 53°33′E) county from Mazandaran and Mashhad (36°18′N 59°36′E) from Khorasan-e-Razavi Province were chosen. Hamedan (34°48′N 48°31′E) from Hamedan, and Tehran (35°45′N 51°35′E) from Tehran Province were selected from cold and dry climate (Fig. 1).

Mosquito sampling and morphological studies

This cross sectional study was conducted in 13 randomly selected areas in Iran. Larval stages of the mosquitoes were collected, using standard dipping technique from April 2009 to October 2010.

The larvae were collected from different regions of the country using WHO standard dipping method (WHO 1992). The samples were transferred to the Entomology Laboratory, Department of Entomology and Parasitology, School of Medical Sciences, Tarbiat Modares University. The mosquitoes' larvae maintained in specific cage for rearing in insectarium condition (22–25 ºC, 70–75% RH). Microscopic slides were used to mounting of some parts of the adult body such as wings and maxillary palps using Canada balsam diluted with xylen. The caudal abdominal segment of males were removed, and placed in KOH 10% for 20 to 30 minutes, then washed with distilled water and placed in ethanol 96% for dehydration (Barr 1957, Jakob et al. 1979). Microscope slides of the samples were prepared using Puri's medium. The taxonomic figures were drawn using light Zeiss microscope with a Nikon drawing tube accessory long arm (9.1 inches) (22.5 cm). The morphological features were used to identify the *Cx. pipiens* complex includes:

In larval stage

a. Seta 1 of abdominal segments III–IV (Fig. 2) (Azari-Hamidian and Harbach 2009).
b. Seta 1a–S and 1b–S in the siphon (Fig. 3) (Harbach 1988).
c. Shape of the siphon (Fig. 3) (Harbach 1988, Azari-Hamidian and Harbach 2009).

In adult stage (Male genitalia)

a. DV/D: The ratio used for adult identification, also used as a confirmation for molecular studies. DV was described as the distance between two tips of dorsal and ventral arms and D was defined the distance between two tips of dorsal arms (Fig. 4) (Mattingly et al. 1951, Barr 1957, Kamura 1959, Vinogradova 2003, Smith and Fonseca 2004).

Female Characters (Wing venation)

a. Costa and subcosta intersection with R2+3 furcation (Fig. 5) (Harbach 1985, Azari-Hamidian and Harbach 2009).

Data were analyzed using SPSS Ver. 11.5.

Results

Overall, 304 larvae and 419 adults (177 males and 242 females) were randomly selected. The branch number of Seta 1 on abdominal segments III–IV, 1a–S tuft, 1b–S tuft and siphon shape are showed in Table 1. *Culex pipiens* larvae were identified in the samples of Mashhad, Tehran, Neka, Yazd and Zarch1 areas whereas, *Cx. quinquefasci-*

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atus was found in Zarch 2 and Kerman based on the Seta 1 abdominal segments III–IV. The findings showed variations at the mentioned character among the samples collected from Borazjan, Chabahar, Jiroft, and Nikshahr.

The mean average of 1a–S tuft branches were calculated 2.7 to 4.7 and range of 2 to7 and 6.2 to 7.9, range 2 to 10 for *Cx. pipiens* and *Cx. quinquefasciatus* respectively. The mean average of 1b–S tuft branches were counted 2.8 to 4.4, rage 2 to7 and 6 to**7**.8, range 3 to 13 for *Cx. pipiens* and *Cx. quinquefasciatus* respectively.

The siphon shape of *Cx. pipiens* larvae were found gradually narrowing toward the end of siphon in all samples that were collected from Mashhad, Tehran, Neka, and Zarch1 whereas, this character included 84.2 % of *Cx. pipiens* samples collected from Yazd. The variation was found among the siphon shape of *Cx. quinquifasciatus* larvae in Borazjan, Chabahar, Jiroft, Kerman, and Nikshahr samples (Table 1).

The species of *Cx. pipiens* were identified based on the morphological characters of female wings vegetation in Hamedan, Yazd and Bostan, while it was found varied among the samples collected from Neka, Zarch, Ahvaz, Chabahar, Nikshahr and Jiroft. *Culex quinquefasciatus* was identified in Chabahar (97%), Ahvaz (88.9%), and Nikshahr (73.7 %) (Table 2).

Table 3 shows the DV/D and D/V ratios. The DV/D ratio calculated -1.23–0.12 in mean average and range -0.21–0.2 in *Cx. pipiens*. This ratio was calculated in mean average of 0.5–1.09 and range 0.33–2.37 for *Cx. quinquefasciatus*. The D/V ratio of *Cx. pipiens* samples were calculated in mean average of 0.95–1.35, and range of 0.7–1.75. In addition, mean average of this ratio was calculated 0.35–0.64 and the range was 0.17–0.78 in *Cx. quinquefasciatus*.

Table1. The variations of some morphological characters of *Culex pipiens* and *Cx. quinquefasiatus* larvae, Islamic Republic of Iran, 2009–2010

Areas	No	Seta 1 abdominal segment III – IV		Seta 1a-S			Seta 1b-S			Siphon shape	
		Single	Double	Mean	Max	Min	Mean	Max	Min	Gradually narrowing	Widen in middle
Mashhad▲	21	0 (0%)	21(100%)	3.2	7	2	3.2	5	2	21 (100%)	0 (0%)
Neka▲	29	0 (0)	29 (100)	2.7	5	2	2.8	4	2	29 (100)	0 (0)
Tehran▲	50	0 (0)	50 (100)	4.3	7	2	4.4	7	2	45 (100)	5 (0)
Yazd▲	19	0 (0)	19 (100)	4.7	7	3	4.4	6	3	16 (84.2)	3 (15.8)
Zarch1▲	46	0 (0)	46 (100)	4.5	6	3	4.2	7	2	46 (100)	0 (0)
Borazjan△	21	4 (19)	17 (81)	6.3	8	4	6.2	10	3	1 (5)	20 (95)
Chabahar△	40	20 (50)	20 (50)	6.9	10	4	6.9	10	4	4 (10)	36 (90)
Jiroft△	23	4 (17.4)	19 (82.6)	7.9	9	6	7.8	13	6	17 (74)	6 (26)
Kerman△	16	0 (0)	15 (100)	6.7	10	4	6.3	8	4	15 (93.7)	1 (6.3)
Nikshahr△	24	8 (33.3)	16 (76.7)	6.2	9	4	6	10	4	4 (16.7)	20 (83.3)
Zarch 2△	15	0 (0)	15 (100)	6.3	8	2	6.5	8	5	0 (0)	15 (100)
Total	304	–	–	–	–	–	–	–	–	–	–

▲All of the species identified as *Culex pipiens*
△All of the species identified as *Culex quinquefasciatus*

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Table 2. The variations of some morphological characters of wings in the females of *Culex pipiens* and *Cx. quinquefasciatus*, Islamic Republic of Iran, 2009–2010

Areas	No	Costa and Subcosta Intersection			
		At or beyond level of Furcation of R2+3 (*Cx. pipiens*)		Before level of Furcation of R2+3 (*Cx. quinquefasciatus*)	
		Prevalence	%	Prevalence	%
Hamedan▲	16	16	100	0	0
Neka▲	34	33	97.06	1	2.94
Yazd▲	5	5	100	0	0
Zarch▲	84	75	90	9	10
Ahvaz△	27	3	11.1	24	88.9
Bostan▲	9	9	100	0	0
Chabahar△	33	1	3	32	97
Jiroft△	15	14	93.33	1	6.67
Nikshahr△	19	5	26.3	14	73.7
Total	242	–	–	–	–

▲All of the species identified as *Culex pipiens*
△All of the species identified as *Culex quinquefasciatus*

Table 3. The ratio variations of male genitalia in *Culex pipiens* and *Cx. quinquefasciatus*, Islamic Republic of Iran, 2009–2010

Areas	No	DV/D			D/V		
		Mean	Max	Min	Mean	Max	Min
Hamedan▲	20	0.09	0.05	-0.17	1.27	1.51	0.92
Neka▲	25	0.07	0.14	- 0.21	1.21	1.75	0.78
Tehran▲	9	-1.23	0.00	- 0.19	1.35	1.61	1.00
Yazd▲	30	0.12	0.20	- 0.19	0.95	1.63	0.70
Ahvaz△	24	0.90	1.54	0.47	0.35	0.55	0.25
Chabahar△	22	0.50	1.55	0.41	0.64	0.78	0.24
Jiroft△	19	1.09	2.29	0.33	0.36	0. 60	0.18
Kerman△	7	0.90	1.68	0.40	0.38	0.56	0.23
Nikshahr△	21	0.79	2.37	0.38	0.46	0.62	0.17
Total	177	–	–	–	–	–	–

▲All of the species identified as *Culex pipiens*
△All of the species identified as *Culex quinquefasciatus*

Fig. 1. The study areas and distribution of *Culex pipiens* and *Culex quinquefasciatus* in different stratum of Iran, 2010

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Published Online: January 06, 2016

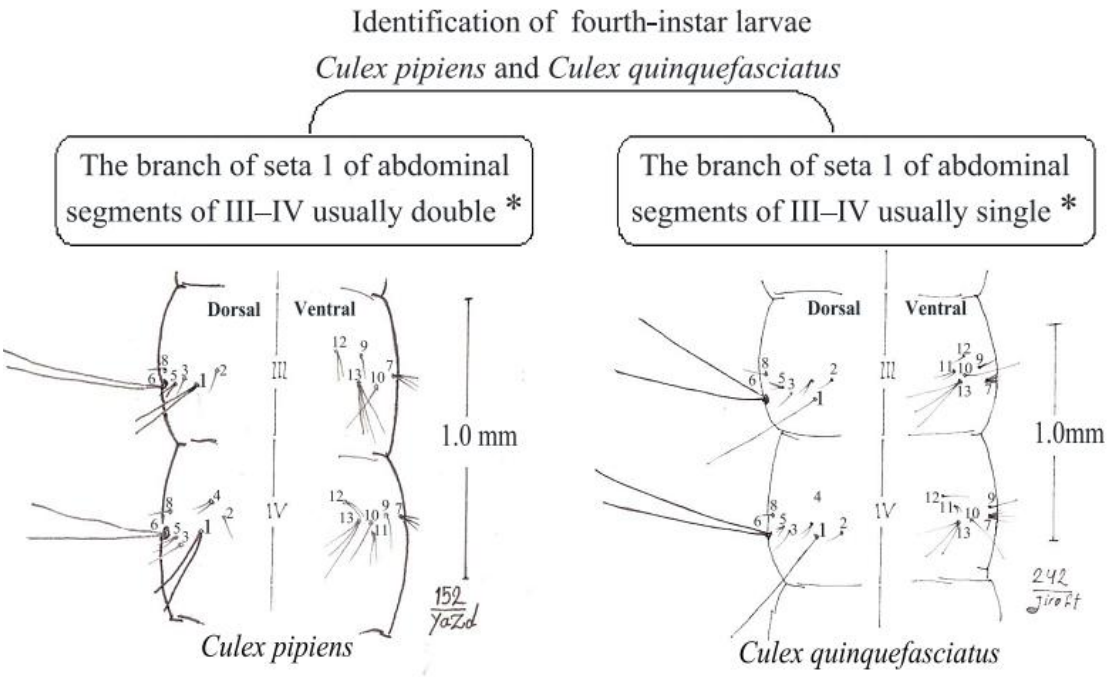

Fig. 2. Identification of *Culex pipiens* and *Culex quinquefasciatus* larvae based on abdominal segments of III-IV

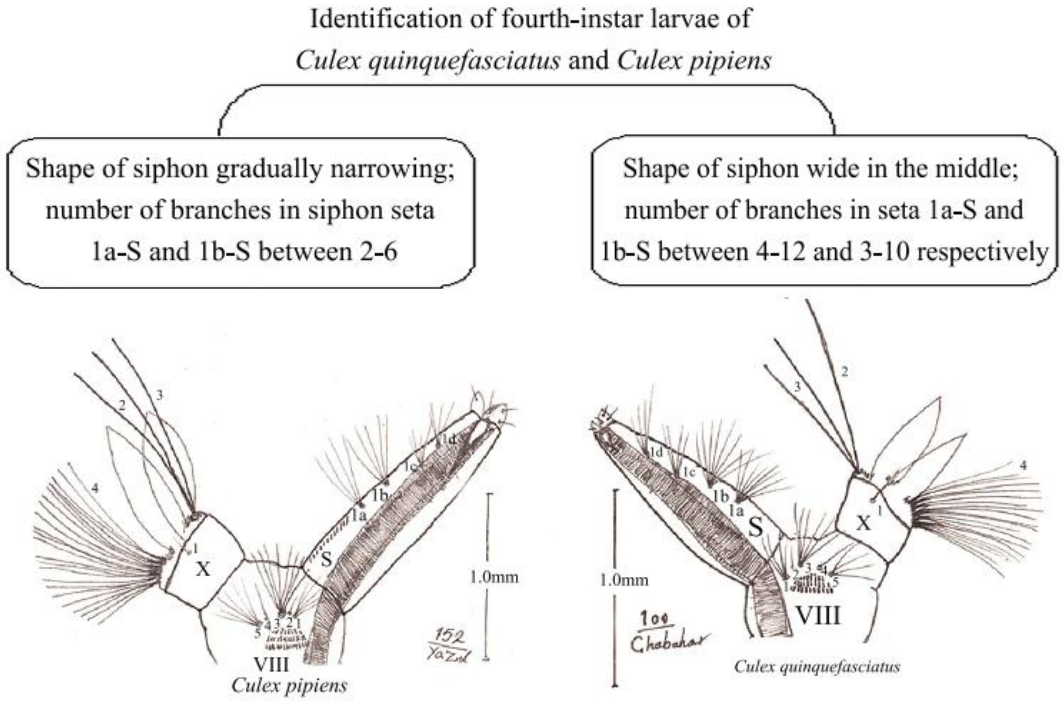

Fig. 3. Identification *Culex pipiens* and *Culex quinquefasciatus* larvae based on siphon characters

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Published Online: January 06, 2016

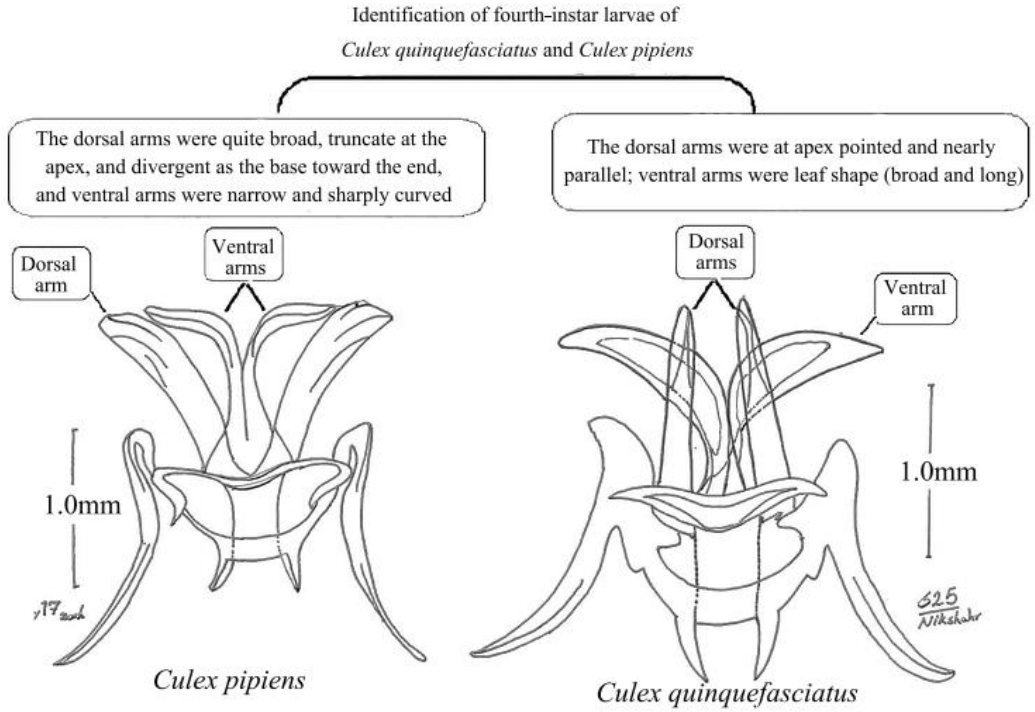

Fig. 4. Identification of adult male in *Culex pipiens* and *Culex quinquefasciatus* by male genitalia and different of dorsal and ventral arms

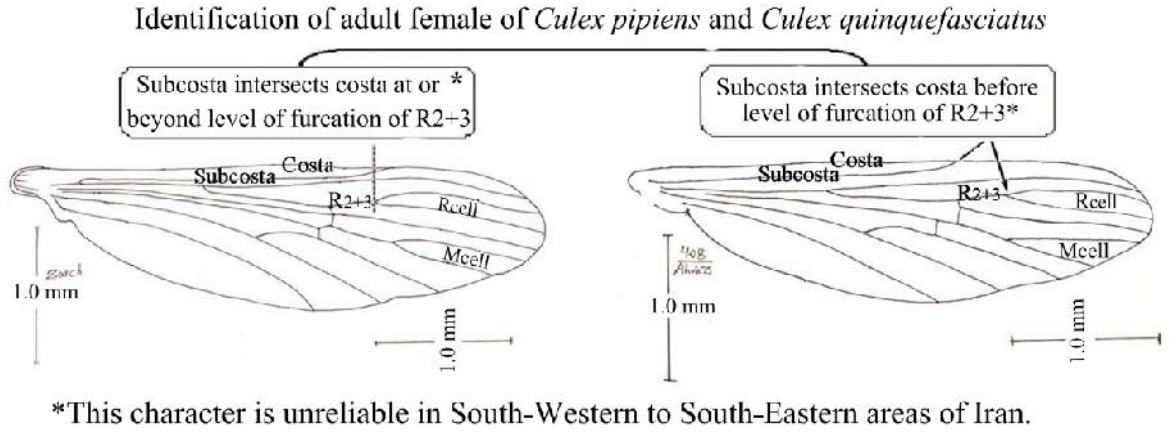

Fig. 5. Identification of adult female in *Culex pipiens* and *Culex quinquefasciatus* based on vegetation of wings

Discussion

In present research, the seta 1 on the abdominal segments III–IV was found the main reliable characters for identification of *Cx. pipiens* and *Cx. quinquefasciatus* in north and central of the Country. This character was not found a reliable character for

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Cx. quinquefasciatus in southern parts of the country. Moosa-Kazemi et al. (2010) was reported the occurrence of *Cx. pipiens* in Kurdistan Province, northeast of Iran.This study showed that seta 1 of abdominal segments III–IV of *Cx. pipiens* had two branches. Harbach (1988) and Azari-Hamidian and Harbach (2009) described this character in *Cx. pipiens* as well.

Our finding showed, siphonal seta 1a-S and 1b-S of *Cx. pipiens* had range of 2–7 branches. These results are supported by previous study, Knight and Malek (1951) reported an average 4 and a range 2–9 branches for *Cx. pipiens*.

Results of this study showed the branch number of seta 1a-S and 1b-S had a mean average of 6–7.9 and range of 2–13 for *Cx. quinquefasciatus*. Similarly, Harbach (1988) reported more branches in *Cx. quinquefasciatus* than *Cx. pipiens*.

In this study, shape of the siphon was studied among 165 larvae samples and confirmed vast most of the *Cx. pipiens* species. As mentioned above, there are character variations among the samples which were collected from south of Iran. Generally, the length of siphon in *Cx. pipiens* was reported longer than in *Cx. quinquefasciatus* (Harbach 1988, Azari-Hamidian and Harbach 2009).

According to our observations, the seta 1 branches of abdominal segments III–IV and shape of the siphon in larval samples were found as valuable characters, which can easily used to distinguish of *Cx. pipiens* from *Cx. quinquefasciatus*. In our study, some characters were overlapped between *Cx. pipiens* and *Cx. quinquefasciatus* species, therefore, it is recommended the whole characters should be evaluated to accurate identification.

Culex pipiens complex is considered cosmopolitan species. Although, the distribution patterns of the complex species have been reported in Iran (Zaim 1987, Harbach 1988, Azari-Hamidian et al. 2010) results of this study indicated the distribution of *Cx. pipiens* in Bostan, Yazd, Neka, Mashhad, Hamedan and Tehran. Nevertheless, the distribution of *Cx. quinquefasciatus* was limited in Chabahar, Nikshahr, Jiroft, Kerman, Borazjan, Ahvaz and Zarch.

The occurrence of *Cx. quinquefasciatus* in the Iranian Persian Gulf islands had been reported previously (Azari-Hamidian et al. 2010, Khoobdel et al. 2012). In addition this species was reported in Kermanshah, western Iran (Ghaffary 1954), and in Bandar-e Anzali Northern Iran (Harbach 1988). There are scattered data about the species composition of *Cx pipiens* and *Cx. quinquefasciatus* in southern Iran. As mentioned, the male genitalia reported as the main morphological characters to identification of the *Cx. pipiens* complex species. In our research DV/D ratio of *Cx. quinquefasciatus* was more than 0.4 (0.56–1.89, mean 1.03), But in *Cx. pipiens* this ratio found ranges -0.14 to zero (Harbach 1988). The DV/D ratio of *Cx. pipiens*, calculated at mean average of -1.23–0.12 and range of 0.21–0.2 while, calculated at mean average of 0.5–1.09 and ranges 0.33–2.37 for *Cx. quinquefasciatus*.

The D/V ratio was calculated at mean average of 0.95–1.35, and range of 0.7–1.75 for *Cx. pipiens* samples collected in south of the country, whereas it was calculated at mean average of 0.35–0.64 and range of 0.17–0.78 for *Cx. quinquefasciatus*. Further supports for these results also came from some previous studies, Sasa (1967) express the D/V ratio in range of 0.4–0.9 in *Cx. pipiens pallens* and less than 0.3 in *Cx. quinquefasciatus* and average of 1.2 in *Cx. pipiens* form *molestus*. Moreover, Choochote (1987) reported the D/V ratio at average of 0.35 for *Cx. quinquefasciatus*. In parallel to the present research, Mohsen et al. (1995) reported D/V ratio as 1.43 for *Cx. pipiens* form *molestus* and 0.337 for *Cx. quinquefasciatus*.

In recent research, dorsal arms of male genitalia of *Cx. quinquefasciatus* described

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as narrow, sharp apex and parallel as the base toward the end. In addition, the ventral arms were flat and leaf shape. Whereas, in *Cx. pipiens* dorsal arms were described quite broad, truncate at the apex, and diverges in the base toward the end of arms, which, indicated the occurrence of *Cx. pipiens* in north to central parts of Iran. Further support to these results also came from a previous study, Harbach (1988) described the dorsal arms of phalosoma as divergent, broad and nearly truncate at the apex for *Cx. pipiens*.

Conclusion

Identification of the *Cx. pipiens* complex species using morphological key have some difficulties because of occurring variations among closely related complex species. In present pictorial key, the colors of the siphon and male's genitalia are not referring to the color of original samples. However, the described taxonomical characters in this article should be included in other characters, which were reported in the previous literatures. The most complex species as well as *Cx. pipiens* complex should be identified by morphological characters in the first step, although some researchers prefer molecular studies such as Ace. 2 gene, microsatellite loci and COI gene for solving the morphological taxonomic problems.

Comprehensive studies such as phylogenic and molecular are necessary to obtain new information for identification of *Cx. pipiens* complex species in the future.

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