Original Article

Some New Records of *Culicoides* Species (Diptera: Ceratopogonidae) from Iran

***Mohammad Abdigoudarzi**

Department of Parasitology, Razi Vaccine and Serum Research Institute, Alborz, Iran

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Abstract

Background: Biting midges of the genus *Culicoides* act as vectors for important diseases affecting humans and both wild and domestic animals. Collection of adult *Culicoides* specimens in the near vicinity of vertebrate hosts is the major part of any bluetongue surveillance plan. There are old records of *Culicoides* species dated from 1963, 1968 and 1975. Therefore, it was decided to collect different ceratopogonids members using a light trap.

Methods: One night catching using light traps with a suction fan was performed at representative sites (25 places) located in North Western Provinces (Ardebil, Eastern Azerbaijan, Western Azerbaijan and Zanjan) of Iran (suspected farms for clinical records of Bluetongue virus or serodiagnosis of the Bluetongue virus). Samples were detected and identified primarily and were sent to a reference center for final verification.

Results: Seven *Culicoides* species including (*Culicoides circumscriptus*, *C. flavidus*, *C. longipennis*, *C. pulicaris*, *C. puncatatus*, *C. nubeculosus*, and three species from *Culicoides* (*Oecacta*) are under study in reference laboratory in Poland and *C. puncticollis* were confirmed from Iran.

Conclusion: Morphological and explanation of each species was regarded in this study. In comparison to old record, there are four new records of *Culicoides* species from Iran and one species is regarded suspected for viral transmission.

Keywords: Ceratopogonidae, *Culicoides*, Iran

Introduction

Biting midges of the genus *Culicoides* Latreille, 1809 act as vectors for important diseases affecting humans and both wild and domestic animals. There is an important problem regarding these insects in European countries then it forcing international offices to get instant records of related disease from public sectors.

Medical and veterinary importance of *Culicoides* species is regarded by different scientists. Bluetongue virus was initially isolated from *C. obsoletus* midges in Cyprus (Mellor and Pitzolis 1979), and African horse sickness virus from a mixed pool of this species and Pulicaris group midges in Spain (Mellor 1990). *Culicoides obsoletus* has been confirmed as bluetongue vector in northern and southern Europe. *Culicoides obsoletus* can also cause an allergic response to its bite in sheep and goats (Connan and Lloyd 1988).

Ceratopogonid midges are small nematocerous diptera, the female members have biting mouthparts and mandibles, which work rather as a pair of scissors. They are mostly one to two millimeters long, the largest British species spans four millimeters or less. The body is stout and the wings are held flat and folded over the abdomen when at rest. They can easily be mistaken for members of the Chironomidae, indeed, they were formerly included in this family. They may be distinguished, however, by the short front legs (in the Chironomidae the front legs are usually much longer than the others), the wing venation and by the biting

***Corresponding author:** Dr Mohammad Abdigoudarzi, E-mail: m.abdi@rvsri.ac.ir

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mouthparts (non-biting mouthparts in the Chironomidae). The wing venation is simple with (usually) two radial cells. In most species the wings are clear, without markings, but many species of *Culicoides* (and of some species of other genera) have easily recognizable patterned wings (some Chironomidae have patterned wings, but then the venation is different). A good formal diagnosis of the family is given in (Downes and Wirth 1981), and a key to the British genera of Ceratopogonidae is provided in Boorman and Rowland (1988).

Mesghali (1963) recorded 22 species from the genus *Culicoides* from Iran. His collection methods was using aspirator collecting flies resting on the body of livestock, collecting flies attracted to the light from the window, collecting midges when there was a screening plan for collection of *Anopheles* members by total catch (insecticide spray) method in malaria campaign, stick trap and light trap. He also noted that except *C. puncticollis* and *C. similis* the rest of midges were recorded as new records on that time. The reason of that study by Mesghali was the induction of African horse sickness in 1959 in Southern parts of Iran, which the vector is *Culicoides* members where 60% up to 80% of horses died after the disease.

Twenty-six species have been recorded by Navai and Mesghali (1968). From 26 species of *Culicoides* reported on that time, 14 species were recorded for the first time in Iran: *C. bulbostylus*, *C. flavidus*, *C. flavisimilis*, *C. ibericus*, *C. koreensis*, *C. longipennis*, *C. nagahanai*, *C. odibilis*, *C. pallidicornis*, *C. pallidipennis*, *C. pictimargo*, *C. pulicaris*, *C. punctatus*, and *C. similisbaghdadensis*.

Navai's collection methods were Light trap, tent trap and adults emerged after pupa collection from aquatic habitats. Later, two new species, *C. mesghalii* and *C. shahgudiani* were recorded from the Persian Gulf area of Iran (Navai 1973). *Culicoides* members from south-west Asia have been studied by Navai (1977). There are records of *Culicoides* vector members from the nearby country like Turkey (Dik et al. 2006).

The distribution pattern of *Culicoides* spp. is according to Iranian geographical coordination (Northern latitude 40°, Southern latitude 25°) (Western longitude 44° and Eastern longitude 63.5°). Due to warming up of the north hemisphere, the spread of *Culicoides* species has been moved from 40° to 43° (Northern latitude) (Djuricic et al. 2003).

In any bluetongue surveillance system, the principal aim is to capture adult *Culicoides* in the near vicinity of vertebrate hosts, and to employ a powerful trap (to enhance surveillance sensitivity at low *Culicoides* population levels and, furthermore, to increase the number of captured midges for virus isolation studies) (Goffredo and Meiswinkel 2004). The results of a new serological study in Iran, showed that the Bluetongue virus seroprevalence of sheep in West-Azerbaijan (64.86%) was the highest and lowest prevalence was seen at Qom (12.1%) (Khezri and Azimi 2013).

According to above old records and the importance of the study of ceratopogonids in Iran, screening *Culicoides* spp. for Blue tongue control in sheep, increased warming up of the north hemisphere and its effect on distributional pattern of *Culicoides* spp, it was decided to collect different ceratopogonids members using light trap. A collection method as one night catching on site using light traps with a suction fan was performed at representative sites in northwestern provinces of Iran (suspected farms for clinical records of Bluetongue virus or serodiagnosis of the Bluetongue virus).

Materials and Methods

New collection field trips were designed according to Bluetongue seropositive records in livestock in North Western Provinces of Iran.

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Collection method (one night catching on site using light traps with a suction fan) (Sanyo–IK-150) was performed at representative sites in (Ardebil, Eastern Azerbaijan, Western Azerbaijan and Zanjan) (Table 1) (suspected farms for clinical records of BT virus or serodiagnosis of the BT virus).

From Sep 2004 up to early Oct 2005, (it was done mostly on late August and early September (late summer time in Iran) adult members of *Culicoides* were collected and transferred to the laboratory near ice pack in an isolator box (at arrival time they were fresh and intact for better inspection).

Culicoides members were separated and have been mounted on slides using a clearing agent then Hoyer'medium was applied and different characters including wing patterns and venation was regarded using identification key (Rawlings 1996).

Accidentally collected ceratopogonids during an agricultural research on insect pests were sent to Razi Institute and were studied too.

Parallel samples were sent to Dr Ryszard Szadziewski (Poland) and Dr Shahin Navai (Germany).

Results

From September 2004 up to October 2005, *Culicoides* specimens from 25 different regions were collected by light trap. Different members of Chironomidae, Psychodidae, Sciaridae and Cecidomyiidae families from diptera have been confirmed, *Forcipomyia* (Ceratopogonidae) also noted and some lepidoptera and hymenoptera were recorded. Ceratopogonidae members were selected. *Culicoides* spp. were separated and were studied under an anatomical microscope. Nine *Culicoides* species including (*Culicoides circumscriptus*, *C. flavidus*, *C. longipennis*, *C. pulicaris*, *C. puncatatus*, *C. nubeculosus*, and three species from *Culicoides* (*Oecacta*) were confirmed from Iran. Data for geographical location, time range and type species and number of samples are included (Table 1 and 3).

Fig. 1. *Culicoides pulicaris* (wing pattern is distinctive), (Original photo)

Table 1. Data for Different species of *Culicoides* collected according to Bluetongue seropositive records from livestock in Iran

Province	Place	Time Range	Collected Insect type
Ardbil	Khalkhal road, Heleh abad	14th Sep. 2005	*Culicoides* (*Culicoidea) punctatus* (Meigen, 1804), 7 f *Culicoides* (*Oecacta*) sp (under verification) B, 3 f *Culicoides* (*Culicoidea*) *punctatus* (Meigen, 1804), 8 f *Culicoides* (*Oecacta*) sp (under verification.)C, 3 f *Culicoides* (*Beltranmyia*) *circumscriptus* Kieffer, 1918, 1 m, Chironomidae 6 f,

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Table 1. Continued…

			Cecidomyiidae 4 specimens
Eastern Az.		21st -22nd Aug. 2005 6th -13th Sep. 2005	
Eastern Az.	Marand, Yamchi, Amir abad	6thSep. 2005	*Culicoides* (*Monoculicoides*) *nubeculosus* (Meigen, 1830) 3 f, 3 m, *Culicoides* (*Oecacta*) sp (under verification.)C, 2 f
	Jolfa, Galin ghiye	6thSep. 2005	*Culicoides* (*Monoculicoides*) *nubeculosus* (Meigen, 1830) 4 f, Chironomidae 8 specimens
Western Az.	Poldasht, Vet. Office	11th Aug. 2005	Chironomidae, 10 f *Culicoides* (*Oecacta*) sp (under verification) C, 4 f
		12th Aug. 2005	Chironomidae, 1 f *Culicoides* (*Oecacta)* sp I(under verification) A, 1 f *Culicoides* (*Oecacta*) sp Indet. C, 4 f
Western Az.		14th -18th Sep. 2004 12th -14th June 2005 10th -12th Aug. 2005	Not included
Zanjan		9th -11th June 2005 16th -17th Aug. 2005 4th Oct. 2005	Not included
Zanjan	Tarom, Gilvan	4th Oct. 2005	*Culicoides* (*Beltranmyia*) *circumscriptus* Kieffer, 1918, 3 f *Culicoides* (*Oecacta*) sp (under verification.)A, 1 f Chironomidae (2), Psychodidae (1), Sciaridae (1), Cecidomiidae (1), Ceratopogonidae (Forcipomyia) (1 f) *Culicoides* (*Oecacta)* sp (under verification.)C, 1 m

Table 2. Different recorded *Culicoides* species from Iran

Mesghali (1963)	**Navai and Mesghali (1968)**	**Abdigoudarzi (2008)**
***Culicoides caspius* Gutzevich, 1959**	*Culicoides circumscriptus*	*Culicoides circumscriptus*
***Culicoides circumscriptus* Kieffer, 1918**	*Culicoides flavidus*	*Culicoides flavidus*
***Culicoides dendrophilus* Amosova, 1957**	*Culicoides flavisimilis*	*Culicoides longipennis*
***Culicoides firuzae* Dzhafarov, 1958**	*Culicoides grisescens*	*Culicoides pulicaris*
***Culicoides grisescens* Edwards, 1939**	*Culicoides neliophilus*	*Culicoides puncatatus*
***Culicoides halophilus* Kieffer, 1924**	*Culicoides ibericus*	*Culicoides nubeculosis*
***Culicoides heliophilus* Edwards, 1921**	*Culicoides kurensis*	**Culicoides* (*Oecacta*) species A
***Culicoides Kurensis* Dzhafarov, 1960**	*Culicoides longipennis*	**Culicoides* (*Oecacta*) species B
***Culicoides maritimus* Kieffer, 1924**	*Culicoides nagahani*	**Culicoides* (*Oecacta*) spcies C
***Culicoides parroti* Kieffer, 1922**	*Culicoides odililis*	
***Culicoides omogensis* Arnaud, 1956**	*Culicoides pauidicornis*	
***Culicoides pictipennis* staeger, 1839**	*Culicoides pallidicornis*	
***Culicoides puncticollis* Becker, 1902**	*Culicoides pictimargo*	
***Culicoides riethi* Kieffer, 1914**	*Culicoides pictipennis*	
***Culicoides saeous* Kieffer, 1922**	*Culicoides pulicaris*	
***Culicoides sejfadinei* Dzhafarov, 1922**	*Culicoides puncatatus*	
***Culicoides schultzei* Enderlin , 1908**	*Culicoides puncticillus*	

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Table 2. Continued…

***Culicoides similis* Carter, Ingram and Macfi, 1920**	*Culicoides riethi*
	Culicoides saevus
***Culicoides simulator* Edwards, 1939**	*Culicoides schuttzei*
***Culicoides sinanoensis* Tokunga, 1937**	*Culicoides bulbostylus*
***Culicoides subfascipennis* Kieffer, 1919**	*Culicoides seifadinei*
***Culicoides turkmenicus* Guttzevich, 1959**	*Culicoides similisbaghda*
	Culicoides dansis
	Culicoides subfacipennis
	Culicoides turkmensicus

*Note: Three new species are under verification

Table 3. Different species and number of samples (Final Verification from Dr Szadziewski)

No.	Location	No. of specimens	Final Verification (Dr. Szadziewski)
1-	Iran-Az. Gharbi -PoldashtVet.Service20-584(11thAug.2005)	**10**	**Sample 1. Chironomidae, 10 females.**
2-	Iran-Az. Gharbi -Poldasht Vet. Service 20-5-84(11th Aug. 2005)	**6**	**Sample 2. *Culicoides* (*Oecacta*) new species are under study, 4 females.**
3-	Iran-Az. Gharbi -Poldasht Vet. Service 20-5-84(11th Aug. 2005)	**6**	**Sample 3. Chironomidae, 1 female. *Culicoides* (*Oecacta*) sp new species are under study A, 1 female *Culicoides* (*Oecacta*) sp new species are under study, 4 females.**
4-	Iran-Zanjan- Tarom- Gilvan-Abbar 12-7-84(4th Oct.2005)	**4**	**Sample 4. *Culicoides* (*Beltranmyia*) *circumscriptus* Kieffer, 1918, 3 females *Culicoides* (*Oecacta*) sp new species are under study, 1 female.**
5-	Iran-Zanjan- Tarom- Gilvan-Abbar 12-7-84(4thOct.2005)	**6**	**Sample 5. Chironomidae 2 Psychodidae 1 Sciaridae 1 Cecidomyiidae 1 *Forcipomyia* (Ceratopogonidae) 1 female *Culicoides* (*Oecacta*) new species are under study C, 1 male.**
6-	Iran- Ardbil- khalkhal-road Heleh Abad 23-6-84-1(14thSep.2005)	**10**	**Sample 6. *Culicoides* (*Culicoidea*) *punctatus* (Meigen, 1804), 7 females *Culicoides* (Oecacta) new species are under study, 3 females.**
7-	Iran- Ardbil- khalkhal-road Heleh Abad23-6-84-2(14th Sep.2005)	**11**	**Sample 7. *Culicoides* (*Culicoidea*) *punctatus* (Meigen, 1804), 8 females *Culicoides* (Oecacta) new species are under study, 3females.**
8-	Iran- Ardbil- khalkhal-road Heleh Abad23-6-84(14thSep.2005)	**12**	**Sample 8. *Culicoides* (*Beltranmyia*) *circumscriptus* Kieffer, 1918, 1 male Chironomidae 6 females Cecidomyiidae 4 specimens.**
9-	Iran- Az. Sharghi- Marand- Amir abad 15-6-84(6th Sep.2005)	**8**	**Sample 9. *Culicoides* (*Monoculicoides*) *nubeculosus* (Meigen, 1830, 3 females, 3 males *Culicoides* (*Oecacta*) new species are under study, 2 females.**
10	Iran- Az. Sharghi- Marand- Jolfa-Galin Ghieh 15-6-84(6th Sep.2005)	**12**	**Sample 10. *Culicoides* (*Monoculicoides*) *nubeculosus* (Meigen, 1830, 4 females Chironomidae 8 specimens.**
Total		**85**	

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Discussion

Mesghali, 1963, recorded 22 species from the genus *Culicoides* Latreille, 1809 from Iran (Mesghali 1963). Navai and Mesghali, 1968 recorded 26 species from Iran (Navai and Mesghali 1968). Navai's collection methods were Light trap, tent trap and adults emerged after pupa collection from aquatic habitats (Table 2).

Regarding above data and precise attention to these records and after taking the advancement of synonymy of *C. circumscriptus* with *C. puncticollis* twenty-two recorded species from Mesghali (1963) should be reduced to twenty-one and there are eleven shared recorded species between data from Navai and Mesghali (1968) and Mesghali (1963). Totally, regarding past and present studies there are forty recorded *Culicoides* species from Iran.

In a study on *Culicoides* species in Portugal 66% of collected specimens in summer were *C. imicola*, but *C. obsoletus* and *C. pulicaris* were highly collected during winter. The authors of this study in Portugal believed that *C. pulicaris* could preserve the virus of African horse sickness by overwintering and act as a reservoir of this virus (Capela et al. 2003).

Entomological investigation of the presence of *Culicoides* species in Bosnia and Herzegovina was conducted in 2007. During the investigation, 2,256 *Culicoides* midges were collected and only one species (*Culicoides obsoletus* Meigen, 1818) was identified (Omeragic et al. 2009). In a study by Talavera et al. (2011), newly recorded species are: (1) *C. yemenensis*, new record for Europe, (2) *C. coluzzii* and *C. sejfadinei*, being new records for the Iberian Peninsula, and (3) *C. pseudopallidus*, which is new record for Spain. *Culicoides sejfadinei* was recorded from Iran by Mesghali (1963) and Navai and Mesghali (1968) too. This species was not included in new collection data from Iran, and then additional collection studies were done.

Accidentally collected ceratopogonids during an agricultural campaign in (Arak, Iran) was regarded and finally six females from *Culicoides puncticollis* were confirmed in this study (Alikhani, unpublished data).

Nine *Culicoides* species including (*C. circumscriptus*, *C. flavidus*, *C. longipennis*, *C. pulicaris*, *C. puncatatus*, *C. nubeculosus*, and three species from *Culicoides* (*Oecacta*) were confirmed from Iran. Data for geographical location, time range and type species and number of samples are included (Table 1 and 3).

There are morphological and explanation of each species as follows:

1- *Culicoides* (*Beltranmyia*) *circumscriptus* Kieffer, 1918

Culicoides circumscriptus and *C. festivipennis* were seen as dominant species especially in organic matter rich mud near the water reservoirs. *C. circumscriptus* was found in a wide variety of habitats (Uslu and Dik 2007). This species has been reported by Mesghali (1963) and Navai and Mesghali (1968) (Table 2). It has not been confirmed as a vector for bluetongue virus. This species could be distinguished regarding the color pattern of the wing and wing venation.

2- *Culicoides flavidus*

As a new species of the genus *Culicoides* found in the valley of the Araks River (Armenias lowest elevation is found in the Araks River valley) is described (Dzhafarov 1959)**.** *Culicoides flavidus* Dzhafarov, belongs to the group of species with non-spotted wings. It is similar to the recently described *C. firuzae* from the same locality, the characteristic differences being the light yellow color of the entire body, including the mesonotum and scutellum, and the different structure of the male hypopygium.

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3- *Culicoides longipennis* (Khalaf 1957)
= *Culicoides flavisimilis* Dzhafarov

The wings are greyish with prominent pale spots. It closely resembles *C. sahariensis*, but in that species the pale spot at the base of cell M2 overlaps vein m1. In male, the posterior part of the aedeagus of *C. longipennis* terminates in a few bristles, not in a point as in either *C. sahariensis* or *C. similis*.

4- *Culicoides pulicaris* (Linnaeus 1758)

Seventeen collected samples during present study were sent to Dr Navai and this species was confirmed by her. The collection place was Ardbil, Khalkhal, Heleh Abad and date of collection was (14th Sep 2005).

There was also one record of *C. pulicaris* from Azerbaijan Sharghi, Marand, Jolfa and date of collection was (6th Sep 2005) (Fig. 1).

5- *Culicoides punctatus* (Meigen 1804)
= *Culicoides pulicaris* Kieffer
= *Culicoides kasachstanicus* Shakirzjanova

The tips of wing veins M1, M2 and CU1 typically end at the wing tip in small pale spots, distinguishing this species from *C. pulicaris*, but the distinction is not always clear-cut. Both of these species are variable in the extent and intensity of the wing markings.

6- *Culicoides nubeculosus* (Meigen 1830)
= *Culicoides puncticollis* Goetghebuer 1912

This is one of the largest of the British *Culicoides*. The female may be distinguished from others of the subgenera by the dark markings on a pale ground, the dark second radial cell, the single ovoid spermatheca, and the presence of a yellowish spot in the centre of the scutellum. It is very similar to *C. puncticollis*, but in that species, the spermatheca is sausage-shaped and the male aedeagus is different.

***7- Culicoides obsoletus* (Meigen 1818)**

In the Western Palaearctic region, *C. obsoletus* is by far the most commonly encountered species on farms and stables and can be present in high abundance (light trap catches can exceed 30 000 individuals/ night). The above species feed on a wide range of mammals including humans, cattle, horses, sheep and deer. It also feeds on avian hosts. They are commonly known as the 'Garden midge' due to occasional presence in semi-urban habitats.

The entomological survey of vectors should be limited to high-risk areas in the Western Provinces of Iran. Trapping site should be selected by several criteria, such as farm type (sheep or cattle), average temperature, average rainfall and humidity or vicinity to (stand water). The choice of trapping sites should be made as either cattle, sheep, goats or horses must be present (large livestock holdings are preferred) (>10 animals, livestock to be located in the near vicinity of the light trap all night; stabling can be of any type (but must be open) priority should be given to farms where conditions such as pools of water or mud are found, created either naturally (rain) or by irrigation or overflows.

Conclusion

Ten *Culicoides* species including (*C. circumscriptus*, *C. flavidus*, *C. longipennis*, *C. pulicaris*, *C. puncatatus*, *C. nubeculosis*, *Culicoides* (*Oecacta*) species A, *Culicoides* (*Oecacta*) species B, *Culicoides* (*Oecacta*) species C and *C. puncticollis* were confirmed from Iran. There are four new records of *Culicoides* species from Iran and *C. pulicaris* is regarded suspected for viral transmission.

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