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Culicoides (Diptera: Ceratopogonidae) Fauna in Central Tunisia

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Abstract

For a better understanding of the *Culicoides* spp biodiversity of the Center of Tunisia, an entomological survey was carried out between 2009 and 2012 in four districts. A total of 9275 biting midges were collected from different sites in the Center of Tunisia using CDC and OVI light traps as well as emergence in the laboratory from mud sampling.

Twenty two species were identified of which two were newly recorded for the Tunisian fauna. The most abundant *Culicoides* spp occurring on the Center of Tunisia were *Culicoides imicola* (6.9%) captured by light traps and *Culicoides circumscriptus* (70.93%) from mud. Other less abundant species were also identified including *Culicoides paolae* (15.04%); *Culicoides sahariensis* (12.45%); *Culicoides jumineri* (9.72%); *Culicoides cataneii* (66.07%); *Culicoides puncticollis* (5.34%); *Culicoides newsteadi* (3%); *Culicoides kingi* (1.36%); *C. circumscriptus* (0.71%); *Culicoides* spp near kibunensis (0.58%); *Culicoides. pseudojumineri* (0.31%); *Culicoides heteroclitus* (0.10%); *Culicoides pseudopallidus* (0.03%); *Culicoides saevus* (0.15%); *Culicoides submaritimus* (0.05%); *Culicoides langeroni* (0.05%); *Culicoides punctatus* (0.05%); *Culicoides pseudolangeroni* and *C. jumineri var* (0.03%). *Culicoides kurensis* and *C. puncticollis* (0.01%).

During this study two species of Culicoides were reported for the first time: *Culicoides sergenti* (3.1%) (Region of Kairouan) and *Culicoides semimaculatus* (0.04%) (Region of Sidi Bouzid). An updated checklist is provided for the 35 species of *Culicoides* now known to occur on Tunisia.

This study has the potential to significantly improve our understanding of the epidemiology of Bluetongue (BT) in Tunisia in recent years.

Keywords: *Culicoides*; Light traps; Emergence; Center of Tunisia; Species distribution

Abbreviations:

CDC: Centre of Disease Control; OVI: Onderstepoort Veterinary Institute; BT: Bluetongue; EHDV: Epizootic Haemorrhagic Disease; SBV: Schmallenberg Virus

Introduction

Culicoides biting midges (Diptera:Ceratopogonidae) are small, blood-sucking insects that feed on a wide range of hosts, and act as vectors for pathogens responsible for animal and human diseases worldwide. Of more than 1316 biting midges species which have been described to date, approximatively 50 arboviruses have been isolated from species of *Culicoides* [1,2]. Most recently, *Culicoides* have been acknowledged as a vector of the newly identified Schmallenberg bunyavirus (genus *Orthobunyavirus*; Bunyaviridae) (SBV) in Europe [3,4].

An outbreak of BT (serotype 2) occurred in Tunisia between 1999 and 2002 [5]. The first such outbreak appeared during autumn 1999 in the eastern part of the country along the coast. The overall morbidity and mortality rates were 8.35% and 5.5% respectively [5]. In 2000, 72 outbreaks were reported between June and October affecting 6.120 sheep in the eastern and central parts of the country.

Entomological investigations have reported an increasing number of species during the last decades. Indeed, in the early eighties' 19 species were identified [6] including 10 new ones in Tunisia. In 2005, the number of the recorded species was 22 with three new ones: *C. paolae*, *C. imicola* and *C. newsteadi* [5]. In 2008, Hammami et al. identified 14 species with one new for the fauna: *C. punctatus*. Finally, Sghaier et al. [7] identified 25 species of which 7 were identified for the first time: *Culicoides obsoletus*, *Culicoides fascipennis*, *Culicoides subfasciipennis*, *Culicoides santonicus*, *C. submaritimus*, *Culicoides univittatus* and *Culicoides indistinctus*.

Despite the reports of severe outbreaks of arboviral diseases in domestic animals in Center of Tunisia, the studies interested in vector potential of *Culicoides* are disparate and sparse. Thus updated information on species composition and distribution is required to assess the economic losses due to this serious hematophagous pest.

The present study aimed to improve the knowledge of the *Culicoides* fauna in Tunisia by studying the biodiversity using light trapping and breeding site sampling.

Materials and Methods

Study area

An entomological investigation was carried out in both Eastern and Western Centre of Tunisia including Monastir, Mahdia, Kairouan and Sidi Bouzid region (Table 1 and Figure 1).

Regions	Surface (Km2)	Estimated population	Climate		Animal fauna
			Rain (mm)	Temperature °C	
Monastir	1024	548 828	280-400	7.5-32	Pet Animal (Dog and cats)
Mahdia	2966	410 812	200-300	23	Livestock (Cattle, sheep, horses, goats and chickens)
Kairouan	6712	570 559	250-400	5-42	
Sidi Bouzid	6994	429 912	234	13.1-27.5	

Table 1: Geographical information of the four regions study.

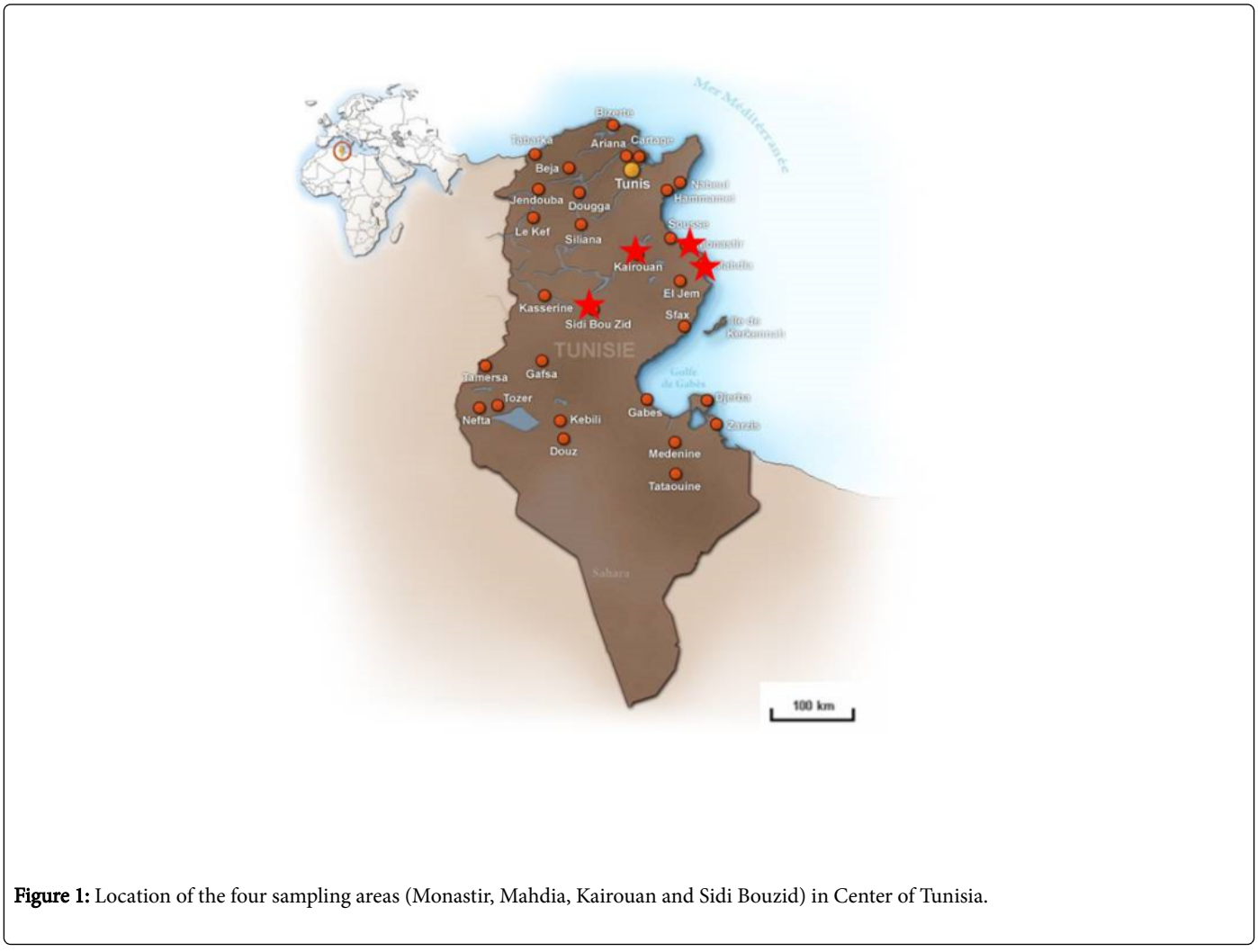

Figure 1: Location of the four sampling areas (Monastir, Mahdia, Kairouan and Sidi Bouzid) in Center of Tunisia.

Field capture of biting midges

Light traps: Biting midges were collected between 2009 and 2012 using two light traps models: home-made miniature CDC (Centre of Disease Control, Atlanta, USA) and OVI (Onderstepoort Veterinary Institute). All collections were done in human-inhabited biotopes where domestic animals (i.e., cattle, horses, dogs, goats, and chicken) are present (Table 2). The traps were installed no more than 1 m from the ground near to animals, either outside or inside shelters (Table 2). Traps were set before sunset and collected the next morning.

Period of Collections	Locality		Type of trap	Trap localisations	GPS location
16-06-2009		Khniss (S1)	OVI	Outside	N: 35°43° 34″/E : 10° 49°.3″
17-06-2009		Khniss (S2)	OVI	Outside	N: 35°44°22″/E : 10°48° 49″
05-10-2009		Bir zira Khniss (S3)	OVI	Outside	N: 35°44'41″/E : 10°49'77″
12-10-2009		Khniss (S4)	OVI	Inside	N: 35°46'15"/E : 10°47'34"
14-10-2009		Skanes (S5)	OVI	Outside	N: 35°46'15"/E : 10°47'34"
19-10-2009		Châaba khniss (S6)	OVI	Outside	N: 35°45'51"/E : 10°47'32"
14-07-2010		Touza-jemmel (S7)	CDC	Outside	N: 35°37'61"/E : 10°49'65"
14-07-2010		Touza-jemmel (S8)	CDC	Outside	N: 35°37'61"/E : 10°49'65"
14-07-2010		Beni hassen (S9)	CDC	Inside	N: 35°34'11"/E : 10°48'87"
14-07-2010		Sayada (S10)	CDC	Inside	N: 35°40'12"/E : 10°53'99"
15-07-2010		Zaouiet kontech (S11)	CDC	Inside	N: 35°38'65"/E : 10°45'36"
15-07-2010		Zaouiet kontech (S12)	CDC	Inside	N: 35°38'65"/E : 10°45'36"
15-07-2010		Sahline (S13)	CDC	Outside	N: 35°34'0"/E : 10°43'12"
21-07-2010		Zéramdine (S14)	CDC	Outside	N : 35°34'28"/E: 10°43'12"
21-07-2010		Jemmel (S15)	CDC	Outside	N: 35°37'96"/E :10°45°78"
03-10-2012	Monastir	Bembla (S20)	CDC	Outside	N: 35°40'96"/E :10°45°86"
20-09-2011					
07-10-2011					
25-10-2011					
28-10-2011					
28-01-2012	Mahdia	Elyana(19)	CDC	Outside / Inside	N: 35°3'06"/E: 11°2'95'
10-04-2011	Kairouan	Elmrazig (16)	CDC	Outside	N: 35°34'28"/E: 10°43'12"
15-04-2011		Elbraga (17)			
15-04-2011	Sidi Bouzid	Bir Elhfay (18)	CDC	Outside	N: 35.04°/E : 9.49°

Table 2: Geographical and ecological characteristics of regions where *Culicoides* spp were collected in Center of Tunisia.

Breeding sites: Mud samples were collected from different breeding sites (Figure 2). The dominant vegetation is Juncus and Salicornia. Mud samples of 750 cm^3 and were scraped from the soil surface using a flat trowel in a line parallel to the water's edge. Samples containing the *Culicoides* larvae at different instars were transported to the laboratory and placed in crystallizers closed by a glass plate to prevent the escape of adults.

Figure 2: Biodiversity descriptors cover breeding sites of *Culicoides*.

Morphological identification: The head, wings and genitalia of individual biting midges were cut off within a drop of ethanol and slide-mounted in Canada balsam. *Culicoides* spp were morphologically identified and separated using their wing patterns according to the key of [8,6,9,10].

Statistical analysis: The analysis of the faunistic data was conducted according to the methods of standard community analysis described by [11] using "PRIMER 6" (Plymouth Routines in Multivariate Ecological Research) package. The multivariate analysis of the faunistic sites affinity was carried out by non-parametric multidimensional scaling (NMDS) ordination on the basis of Bray-Curtis similarity. For estimation of similarity and differences in the Ceratopogonidae community composition, cluster analysis was used. Similarity among sites was determined using the Bray-Curtis similarity index.

Sites	RS	J'	H'
Monastir	23	0,4301	1,349
Kairouan	5	0,803	1,292
Sidi Bouzid	7	0,7332	1,427
Mahdia	10	0,71	1,635

Table 3: Degree of similarity between regions.

Several indexes were calculated for each site in order to assess the species assemblages: species richness (number of species: S), diversity (Shannon-Wiener index H' = –å (Pi × Log2 Pi)) and evenness (Pielou index J'=H'/ Log2S), where H' is the diversity index and RS is the species richness (Table 3).

Results

Culicoides spp presence and abundance in Central Tunisia. Between 2009 and 2012 a total of 5325 specimens of biting midges (3589 females and 1736 males) were collected using light traps. Thus, 19 species of *Culicoides* were identified. Diversity of *Culicoides* varied depending on sites, reflecting the environmental differences in Center of Tunisia. A summary of the frequency of each of these species and their abundance in the study area is provided Table 4.

		Jun-09	Oct-09	Jul-10	Apr-11		Sep-11	Oct-11	Jul-12	Oct-12	Total en %
Subgenera	**Culicoides spp**	**Monastir**			**Kairouan**	**Sidi Bouzid**	**Mahdia**			**Monastir**	
Avaritia	*C.imicola*	10	1277	2383	0	0	0	0	0	0	68.92
Beltranmyia	*C. circumscriptus*	4	20	3	0	2	0	8	1	0	0.71
Culicoides	*C.punctatus*	0	0	3	0	0	0	0	0	0	0.05
	C.newsteadi	27	42	82	1	0	0	7	0	1	3
Monoculicoides	*C. puncticollis*	0	0	1	0	0	0	0	0	0	0.01
Oecacta	*C.jumineri*	54	283	104	0	0	23	54	0	0	9.72
	C.jumineri var	2	0	0	0	0	0	0	0	0	0.03
	C. pseudopallidus	0	0	9	0	0	0	0	0	0	0.16

	C. pseudojumineri	0	17	0	0	0	0	0	0	0	0.31
	C. cataneii	5	0	1	0	0	0	1	0	0	0.13
	C.submaritimus= maritimus	0	3	0	0	0	0	0	0	0	0.05
	Culicoides spp near kibunensis	0	0	31	0	0	0	0	0	0	0.58
Pontoculicoides	*C. saevus*	1	0	0	0	7	0	0	0	0	0.15
Remmia	*C. kingi*	0	22	0	0	0	0	0	0	28	0.93
Synhelea	*C.sahariensis*	0	1	0	0	0	0	0	0	0	0.01
Miscellanoues	*C. paolae*	1	16	768	0	1	2	2	0	11	15.04
	C. kurensis	0	0	1	0	0	0	0	0	0	0.01
	C. langeroni	0	3	0	0	0	0	0	0	0	0.05
	C.pseudolangeroni	0	2	0	0	0	0	0	0	0	0.03
Total		104	1686	3386	1	10	25	72	1	40	100%

Table 4: Total number of the *Culicoides* spp trapped in Tunisia.

Among these species, the principal vectors of BTV in Bassin Mediterranean, *Culicoides imicola* represented 68.92% of the total identified species which is consistent with the distribution of BTV in this region. In our collection, nine subgenera are represented: Avaritia, Beltranmyia, *Culicoides*, Monoculicoides, Oecacta, Pontoculicoides, Remmia, Synhelea and Miscellaneous.

A total of 62 mud samples were collected from different breeding sites. Of these samples, 3 950 specimens of *Culicoides* biting midges (2088 males and 1862 females) were collected. They belonged to13 different species (Table 5).

Culicoides **spp**	**Total**	**Number of females**	**Number of males**	**% Females**	**% Males**
C. circumscriptus	2802	1196	1606	30.27	40.65
C. puncticollis	211	146	65	3.69	1.64
C. sahariensis	492	272	220	6.88	5.57
C. jumineri	54	46	8	1.16	0.2
C. kingi	17	6	11	0.15	0.27
C. cataneii	235	123	112	3.11	2.83
C. langeroni	3	3	0	0.07	0
C. pseudojumineri	5	3	2	0.07	0.05
C. sergenti	123	60	63	1.51	1.59
C.heteroclitus	4	4	0	0.1	0
C. paolae	1	1	0	0.02	0
C.pseudolangeroni	1	1	0	0.02	0
C. semimaculatus	2	1	1	0.02	0.02

Total	3950	1862	2088	47.14	52.86

Table 5: Total number of *Culicoides* spp obtained by emergence.

The most abundant species were *Culicoides circumscriptus*, *Culicoides sahariensis*, *Culicoides cataneii*, *Culicoides puncticollis* and *Culicoides sergenti* with proportion of 70.92% (2802), 12.45% (492), 5.94% (235), 5.33% (211) and 3.10% (123) respectively. Two *Culicoides* spp [*C. sergenti* (Kieffer), *Culicoides semimaculatus* (Clastrier)] were reported for the first time in the Center of Tunisia. *C. sergenti* emerged from mud with presence of vegetation (*Typha*) and was found in the district of Kairouan. For *C. semimaculatus* was emerged from watercourse in the district of Sidi Bouzid.

Species	**Sampling Date**	**Environment near the sampling place**			
		Vegetation	**Water**	**Human housing**	**Farms**
C. circumscriptus	2009: June, October and November	*Juncus*, *Salicornia* and *Typha*	Present (Oued)	Present	Present
	2010: February, July				
	2011: January, February, April and May				
	2012: April and May				
C. puncticollis	2009: June, October and November	Oliviers, *Juncus*	Present (Well)	Present	Present
	2010: February				
	2011: February				
C. sahariensis	2009: June, October	*Juncus*, *Salicornia* and *Thypha*	Present	Present	Present
	2011: February, April and August				
C. jumineri	2009: June	*Juncus*, *Salicornia* and *Thypha*	Present	Present	Present
	2011: February				
C. kingi	2009: June	*Juncus*, *Salicornia* and *Thypha*	Present	Present	Present
	2011: February				
C. cataneii	2009: June	*Juncus*, *Salicornia* and *Thypha*	Present	Present	Present
	2011: February				
C. langeroni	2009: June	*Salicornia*	Present	Present	Present
C. pseudolangeroni	2009: June, October and November	*Juncus*, *Salicornia* and *Thypha*	Present (Wadi)	Present	Present
C. pseudojumineri	2009: June	*Juncus*, *Salicornia* and *Thypha*	Present	Present	Present
C. sergenti	2011: February	*Thypha*	Present	Present	Present
C.hetroclitus,	2009: June	*Juncus*, *Salicornia* and *Thypha*	Present	Absent	Absent
C.semimaculatus,	2011: February	*Juncus*, *Salicornia* and *Thypha*	Present	Absent	Absent
C. paolae	2012: April	*Juncus*, *Salicornia*	Present	Present	Present

Table 6: Ecological data and characterization of the sampling place.

Table 5 shows the results of the environmental surveys for the investigated sites. The analysis of the faunistic data using according to table 6, *Culicoides* spp frequent biotope represented by vegetation, water, human housing and farms. An updated checklist of all 34 species of the genus *Culicoides* recorded from Tunisia is provided in Table 7 and including the references of the first report of each species.

Subgenus	Species	Reference of the record in Tunisia
	Culicoides imicola kieffer, 1913	Chaker et al. [12]
Avaritia	*Culicoides obsoletus* Meigen, 1818	Sghaier et al. [7]
Beltranmyia	*Culicoides circumscriptus* Kieffer, 1918	Chaker and Kremer [6]
	Culicoides newsteadi Austen, 1921	Chaker et al. [12]
Culicoides	*Culicoides punctatus* Meigen, 1804	Hammami et al. [13]
	Culicoides parroti Kieffer, 1922	Chaker and Kremer [6]
	Culicoides puncticollis Becker, 1903	Chaker and Kremer [6]
Monoculicoides	*Culicoides riethi* Kieffer, 1914	Chaker and Kremer [6]
	Culicoides cataneii Clastrier, 1957	Chaker and Kremer [6]
	Culicoides corsicus Kremer, 1971	Chaker and Kremer [6]
	Culicoides gejgelensis Dzhafarov, 1964	Chaker and Kremer [6]
	Culicoides griseidorsum Kieffer, 1918	Chaker and Kremer [6]
	Culicoides heteroclitus Kremer and Callot,1965	Chaker and Kremer [6]
	Culicoides jumineri Callot and Kremer, 1969	Chaker and Kremer [6]
	Culicoides longipennis Khalaf, 1957b	Sghaier et al. [7]
	Culicoides maritimus Kieffer, 1924	This study
	Culicoides pseudopallidus Khalaf, 1961	This study
	Culicoides santonicus Callot, Kremer, Rault and Bach, 1966	Hammami et al. [13] Sghaier et al. [7]
	Culicoides semimaculatus[*] Clastrier 1958a	Sghaier et al. [7]
	Culicoides sergenti[*] Kieffer, 1921h	Chaker and Kremer [6]
	Culicoides submaritimus=C. maritimus Borkent 2008	Chaker and Kremer [6]
Oecacta	*Culicoides univittatus* Vimmer, 1932	Sghaier et al. [7]
Pontoculicoides	*Culicoides saevus* Kieffer, 1922g	Sghaier et al. [7]
Remmia	*Culicoides kingi* Austen, 1912	Chaker and Kremer [6]
	Culicoides fascipennis Staeger, 1839	Hammami et al. [13]
Silvaticulicoides	*Culicoides subfasciipennis* Kieffer, 1919a	Chaker and Kremer [6]
Synhelea	*Culicoides sahariensis* Kieffer, 1923a	Chaker and Kremer [6]
Miscellaneous	*Culicoides kurensis* Dzhafarov, 1960	Chaker and Kremer [6]
unplaced	*Culicoides langeroni* Kieffer, 1921	Chaker and Kremer [6]
	Culicoides lailae A	Sghaier et al. [7]
	Culicoides lailae B=C. odiatus Borkent 2008	Chaker et al. [12]
	Culicoides marcleti Callot, Kremer and Basset, 1968	Chaker and Kremer [6]
	Culicoides odiatus Austen 1921	
	Culicoides indistinctus=C. odiatus Borkent, 2008	
	Culicoides paolae Boorman, 1996	

	Culicoides pseudolangeroni Kremer, Chaker and Delécolle, 1981	
*New to Tunisia		

Table 7: Updated checklist of the 35 species of the genus *Culicoides*.

Moreover, the distribution of *Culicoides* spp based on geographic characteristics of the studied region is shown in Figure 3.

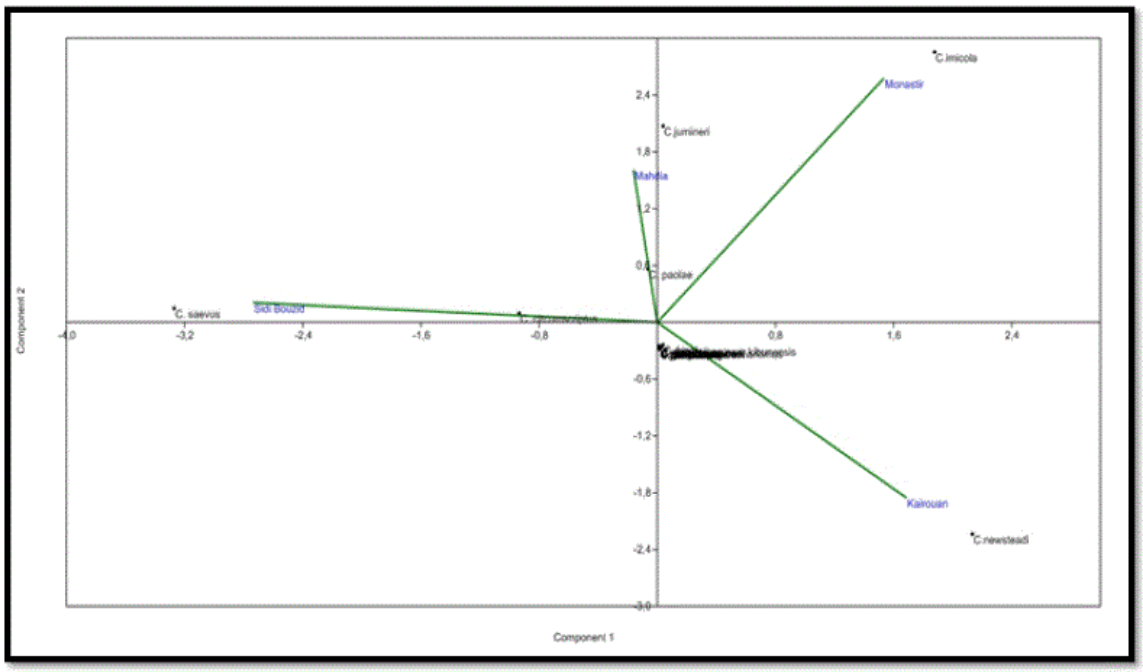

Figure 3: Abundance of *Culicoides* spp according to geographical characteristics in the studied region.

Based on this Figure 3, we observed that *Culicoides imicola* were the principal species present in the Central zones (region of Monastir). This observation was confirmed by trapping results. Moroever, in the Kairouan region, *Culicoides newsteadi* were the most prevalent species. About Sidi Bouzid the Figure 3 showed the presence of *Culicoides paolae*. Figure 4 present the degree of similarity between regions' and give slightly difference. Three different regions (Mahdia, Kairouan and Sidi Bouzid) were found in both clusters. This difference was also observed by the NMDS.

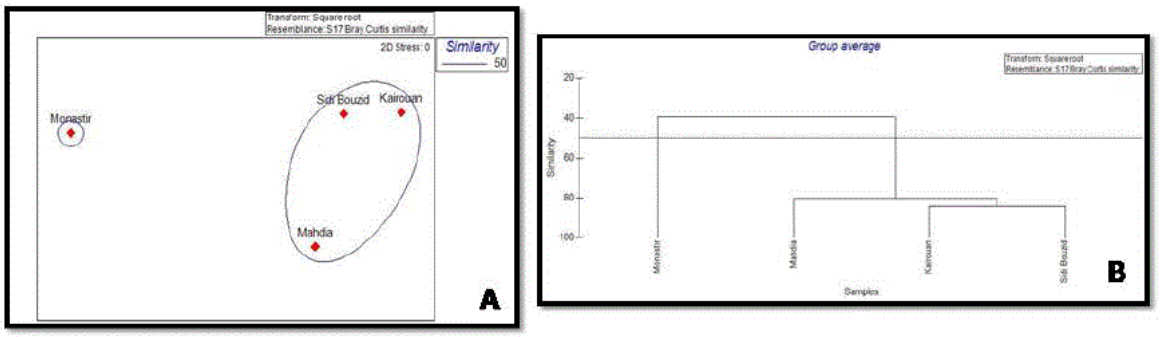

Figure 4: Degree of similarity between regions.

Finally, Figures 5 and 6 shows the different part of the *Culicoides* spp reported for the first time in this study.

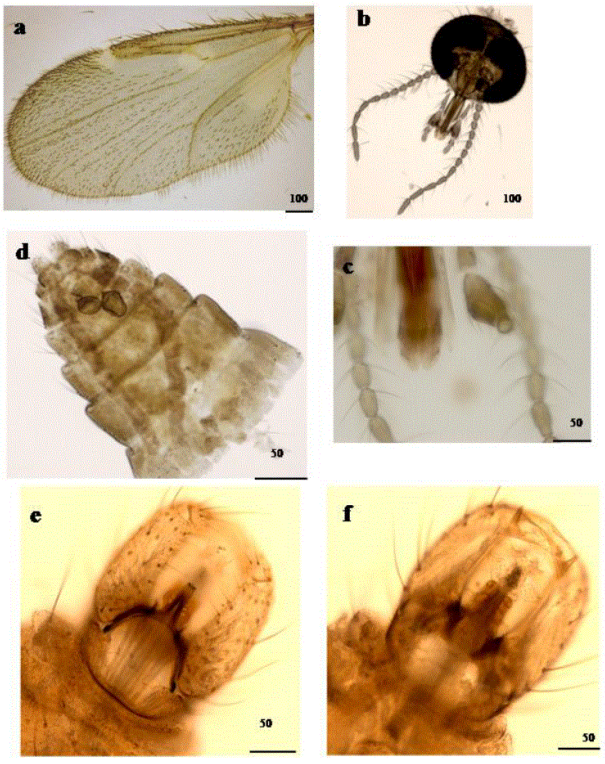

Figure 5: *Culicoides semimaculatus* female and male.

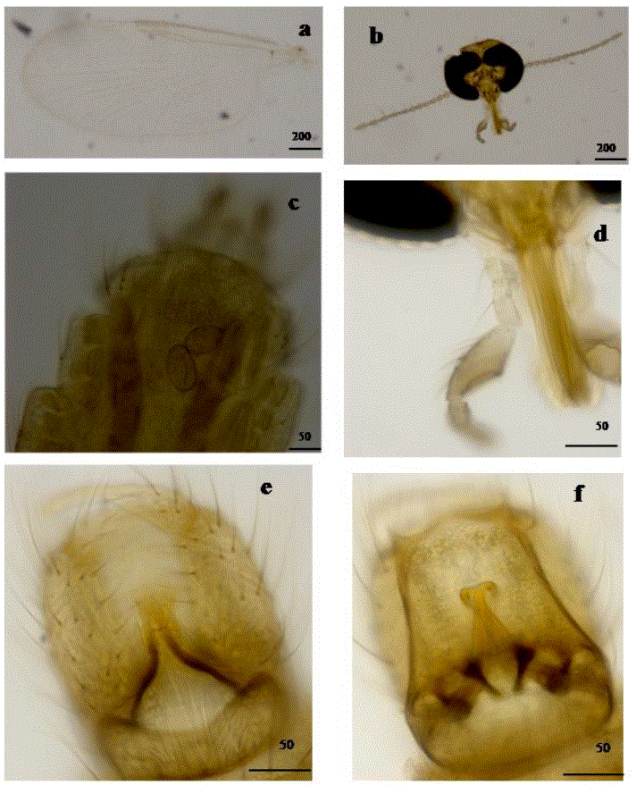

Figure 6: *Culicoides sergenti* (Kieffer) female and male.

Discussion

The objective of this study was to update knowledge on the *Culicoides* fauna present in Center of Tunisia. In fact, few data were found describing the *Culicoides* population composition in Tunisia [6,12,13,7]. Ceratopogonidae in Tunisia have received modest attention from collectors and their biology is poorly understood. Only restricted geographical areas have been intensively surveyed and

because of this the Ceratopogonidae fauna of many regions remains largely unknown.

In the present study, 22 *Culicoides* spp were identified in Central Tunisia including two newly described species: *C. sergenti* (district of Kairouan) and *C. semimaculatus* (region of Sidi Bouzid), which is less than the previously reported study. In fact, [6] identified 10 species newly introduced for the fauna. Evenly, Chaker and co-workers identified three new ones [12]. Moreover, [7] identified 7 for the first time. This may be due to the trap type, position and the period of study. Long-term trapping is recommended for a better assessment of the number of *Culicoides* spp present in the country.

C. sergenti was reported from Algeria and Maroc [5]. Therefore, it is important to assess the potential role and the host preference of *C. sergenti*.

C. semimaculatus has previously been reported to be geographically limited to southern Europe and detected in proximity to cattle, goats and sheep [14]. So it's important to capture this species and to analyze its potential role to transmit disease.

The most abundant species collected were *C. imicola*, *C. paolae* and *C. jumineri*. This finding agrees with those of previous surveys in Tunisia [12,15] in which these species, were found to represent >90% of all biting midges captured. Even more interesting, *C. imicola* was trapped in high numbers in the governorate of Monastir. The high abundance of this species is can be ascribed to: (1) the use of the Onderstepoort blacklight (UV) suction traps [16]. In fact, [17] have demonstrated that Blacklight was more attractive to vector insects than white light and increases monitoring sensitivity in areas where vector abundances are low; (2) the biotic and abiotic factors linked to the availability of suitable breeding habitats which did not occur locally and equally for all species. Indeed, in Sardinia, *C. imicola* and *C. newsteadi* occur more frequently in coastal areas at lowers altitudes, whereas *C. obsoletus* and *Culicoides pulicaris* are restricted to more mountainous central areas [14]. Previous studies have suggested that the high abundance of *C. imicola* is most likely linked to its preference for breeding in areas where soil is moist and nutrient-rich and with full exposure to sunlight, characteristics which are more commonly found in the centre [18]. Statistical analysis of our data suggested that *C. imicola* was significantly more abundant in the region of Monastir than in other regions. We therefore assume that *C. imicola* populations is likely to be settled in Tunisia and seem to have a major role in BT outbreaks. It's necessary to verify the presence of other potential vector than *C. imicola* in Center of Tunisia.

The second abundant species was *C. paolae*. This species was reported from Malta, Sardinia, Corsia, Tunisia, France, Algeria, Greece and Spain in chronological order [19]. In Tunisia, it was reported for the first time in the governate of Monastir from Central Tunisia [12]. Previous studies, have proposed that *C. paolae* from southern Italy feed on horses [20]. Although its antennal and palpal morphology have led other authors to speculate that it feeds preferentially on birds [2]. Even so, the establish of the host preference and larval habitat of *C. paolae* is crucial, since this species is very widespread and abundant in the Center of Tunisia.

C. jumineri was caught in light traps. However, only low numbers were reared from mud near an irrigation channel. This finding corroborates earlier observations made by [21] who reported that *C. jumineri* emerged from mud near irrigation channel. Also, [22] suppose that *C. jumineri* was reared in the reach environment with vegetation like the gross-covered pool.

C. circumscriptus were the most abundant species obtained by emergence. As previously reported, this species prefers puddles of water contaminated with animal excreta [23] and in the absence of surface water occurs in wet soil rich in organic matter [24]. In our study, *C. circumscriptus* were reared in almost all collected mud samples and seems to be plastic in its tolerance for the range of environmental conditions that existed. Even more interesting is that *C. circumscriptus* sharing the same biotope with *C. puncticollis*, *C. cataneii* and *C. sahariensis*. The current result corroborates the findings of [22] who demonstrated the association of *C. circumscriptus* with *C. cataneii* and *C. sahariensis*.

Information on the species composition and distribution of *Culicoides* vectors is a necessary prerequisite to understand the epidemiology of *Culicoides*-borne pathogens.

The results of the survey described here therefore have the potential to significantly improve our understanding of the epidemiology of BT in Tunisia in recent years. *Culicoides* are important vectors for a range of pathogens causing diseases with veterinary and public health importance including BTV, AHSV, epizootic haemorrhagic disease (EHDV), filarial diseases and the recently discovered SBV. It is important to note that the high abundance of *C. imicola* in the Center Tunisia underlines the real risk of spreading new disease. Thus, it is important to improve our understanding of climatic factors in *C. imicola* activity influencing their distribution and seasonal pattern. Further study, are needed to continue the monitoring of other potential vectors in an attempt to limit the potential incursion and spread of the disease in other region of Tunisia like southern Tunisia.

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