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Research Article

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 catanei (66.07%); Culicoides puncticollis (5.34%); Culicoides newsteadi (3%); Culicoides kingsi (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 lanceroni (0.05%); Culicoides punctatus (0.05%); Culicoides pseudojumineri 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 sergentii (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 subfuscipennis, 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                         |
| Mahdia     | 2966          | 410 812              | 200-300  | 23                             |
| 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**

| Date       | 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°44’42″/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 | 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)   | Outside / Inside    | N: 35°3’06″/E: 11°2’95″ |
| 10-04-2011 | Kairouan                | Elmrazig (16)| Outside            | N: 35°34’28″/E: 10°43’12″ |
| 15-04-2011 |                         | Elbraga (17) | 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 cm³ 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.
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 | 0.430| 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 $H' = -\sum (P_i \times \log_2 P_i)$) and evenness (Pielou index $J' = H' / \log_2 S$), 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.

| Subgenera       | Culicoides spp | Jun-09 | Oct-09 | Jul-10 | Apr-11 | Sep-11 | Oct-11 | Jul-12 | Oct-12 | Total en % |
|-----------------|----------------|--------|--------|--------|--------|--------|--------|--------|--------|------------|
| Avaritia        | *C. imicola*   | 10     | 1277   | 2383   | 0      | 0      | 0      | 0      | 0      | 68.92      |
| Beltranmyia     | *C. circumscriptus* | 4     | 20     | 3      | 0      | 2      | 0      | 8      | 1      | 0.71       |
| Culicoides      | *C. punctatus* | 0      | 0      | 3      | 0      | 0      | 0      | 0      | 0      | 0.05       |
|                 | *C. newsteadi* | 27     | 42     | 82     | 1      | 0      | 7      | 0      | 1      | 3          |
| Monoculicoides  | *C. puncticollis* | 0     | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0.01       |
| Oecacta         | *C. jumineri*  | 54     | 283    | 104    | 0      | 23     | 54     | 0      | 0      | 9.72       |
|                 | *C. jumineri var* | 2     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0.03       |
|                 | *C. pseudopallidus* | 0     | 0      | 9      | 0      | 0      | 0      | 0      | 0      | 0.16       |
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 to 13 different species (Table 5).
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.

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 |
|---------------|---------------------------|------------------------------------|
| Avaritia      | Culicoides imicola kieffer, 1913 | Chaker et al. [12]                  |
|               | Culicoides obsoletus Meigen, 1818 | Sghaier et al. [7]                  |
| Beltranmyia   | Culicoides circumscriptus Kieffer, 1918 | Chaker and Kremer [6]               |
| Culicoides    | Culicoides newsteadi Austen, 1921 | Chaker et al. [12]                  |
|               | Culicoides punctatus Meigen, 1804 | Hammami et al. [13]                |
| Monoculicoides| Culicoides parroti Kieffer, 1922 | Chaker and Kremer [6]               |
|               | Culicoides puncticollis Becker, 1903 | Chaker and Kremer [6]               |
|               | Culicoides riethi Kieffer, 1914 | Chaker and Kremer [6]               |
| Oecacta       | Culicoides catanei Clastrier, 1957 | Chaker and Kremer [6]               |
|               | Culicoides corsicus Kremer, 1971 | Chaker and Kremer [6]               |
|               | Culicoides gejgelensis Dzhafarov, 1964 | Chaker and Kremer [6]               |
|               | Culicoides griseldorum 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 martimus 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 sergentii Kieffer, 1921h | Chaker and Kremer [6]               |
|               | Culicoides submaritimus=C. martimus Borkent 2008 | Chaker and Kremer [6]               |
|               | 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]               |
| Silvaticulicoides | Culicoides fascipennis Staeger, 1839 | Hammami et al. [13]                |
|               | Culicoides subfuscipennis Kieffer, 1919a | Chaker and Kremer [6]               |
| Synhelea      | Culicoides sahariensis Kieffer, 1923a | Chaker and Kremer [6]               |
| Miscellaneous | Culicoides kurensis Dzhaferov, 1960 | Chaker and Kremer [6]               |
|               | Culicoides tangeroni Kieffer, 1921 | Chaker and Kremer [6]               |
|               | Culicoides falae A | Sghaier et al. [7]                  |
|               | Culicoides falae B=C. odiatus Borkent 2008 | Chaker et al. [12]                  |
|               | Culicoides marcelli Callot, Kremer and Basset, 1968 | Chaker and Kremer [6]               |
|               | Culicoides odiatus Austen 1921 | Chaker and Kremer [6]               |
|               | Culicoides indistinctus=C. odiatus Borkent, 2008 | Chaker and Kremer [6]               |
|               | Culicoides paolae Boorman, 1996 | Chaker and Kremer [6]               |
**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. Moreover, 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.
Discussion

The objective of this study was to update knowledge on the Culicoides fauna present in Central 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 Morocco [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 Ondersteoport 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 some authors to speculate that it feeds preferentially on birds [2]. Even so, the establishment 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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