Chapter 10

Culicoides spp. (Diptera: Ceratopogonidae) in Tunisia

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Additional information is available at the end of the chapter

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Abstract

Culicoides is a genus of biting midges in the family Ceratopogonidae. The female midges require blood meals for egg production. There are over 1000 species in the genus, which is divided into many subgenera. Several species are known to be vector of many diseases and parasites, which can affect animals. As vectors of viruses, *Culicoides* species are of the higher veterinary importance. More than 75 arboviruses, belonging to Bunyaviridae, Reoviridae and Rabdoviridae families, were isolated from different *Culicoides* species. In Mediterranean region, the principal vector of Bluetongue virus is represented by *Culicoides imicola*, and also other European *Culicoides* biting midges are implicated in virus transmission. Despite the virulence of these species and his colonisation in Tunisia, they are still considered as neglected area due to the rarity or the absence of programmes to control these biting midges. Thus, the available data on species composition, dominant species, breeding sites and host preferences are urgently needed to better understand these biting midges and to develop reliable tools to prevent the spread of other diseases that threaten human and animal life.

Keywords: *Culicoides*, biting midges, Tunisia, species, geographical distribution

1. Introduction

Culicoides biting midges are important vectors of a number of arboviruses causing disease in domesticated livestock such as bluetongue (BT) and African horse sickness. These midges are smaller (1–3 mm) than mosquitoes and can be much more abundant [1] (**Figure 1**).

The development cycle of *Culicoides* consists of egg, four larval instars, pupa and adult. Almost, 1400 extant and extinct species of *Culicoides* have been described from a highly diverse range of ecosystems, and the genus is present in all major land masses with the exception of Antarctica and New Zealand and at altitudes of up to nearly 4000 masl. The first description

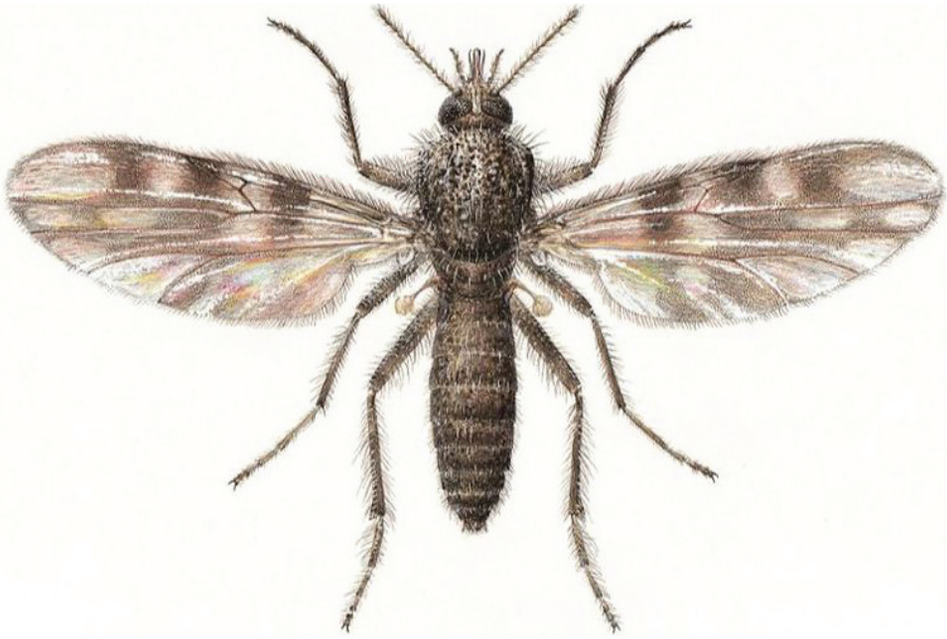

Figure 1. Female of *Culicoides* sp.

to these biting midges is by reverend W. Derham who described their life history and biting biotops in 1731. The primary studies on sub-Saharan *Culicoides* date to 1908 when two species were described from Namibia [2].

The bites of females species of *Culicoides* cause skin lesion and comprise dermatitis in livestock [3, 4], affecting the general health status of domestic animals and wildlife [4, 5]. Regardless of transmitted disease, *Culicoides* midges play an important role in human health. In fact, they can transmit Oropouche virus, leading in severe cases to febrile illness Oropouche fever, between humans beings [1–7]. Oropouche virus is currently restricted to the Neotropics and infects humans, causing major outbreaks of febrile illness. After Bluetongue (BT) appeared, entomological studies were implemented to establish which species of *Culicoides* had acted as vectors.

The aim of this chapter is to review epidemiological features of *Culicoides* species in Tunisia.

2. Life cycle of *Culicoides*

Almost all *Culicoides* exige moisture-rich habitats for development of egg, larval and pupal forms and the availability of these environments is a key determinant for their distribution, abundance and seasonal occurrence [1]. All *Culicoides* species present a complete metamorphosis life cycle. The female midges require blood meals for the completion of the gonotrophic cycle, but those of a few species are autogenous and therefore may produce an initial batch of eggs without feeding using reserves stored from the larval period (**Figure 2**).

2.1. Eggs

The eggs are usually about 400–500 μm in length. They are laid in wet soil in boggy flushes, mires and in the transition zone at the edge of bogs. The eggs have an elongate, curved and

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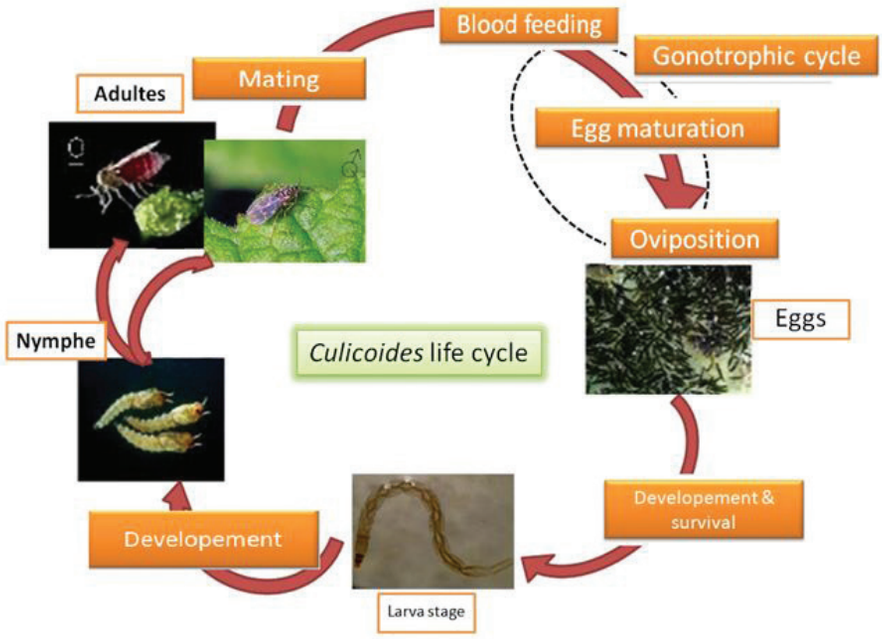

Figure 2. Life cycle of *Culicoides* vectors.

pointed form at each end. Concerning the number of eggs produced, this later varies among species and also size of blood meal. It seems agreed that a blood meal is important in the egg laying in *Culicoides*.

Species	Breeding sites	References
Culicoides imicola	Cow dung rich in organic material, grassed margins of streams, muddy habitats	[11–15]
Culicoides sonorensis	Edges of waste water, irrigation run off in pasture, puddles, trough spillover	[16–18]
Culicoides brevitarsis	Cattle dung	[19, 20]
Culicoides oxystoma	Paddy fields, stream edges, pond margins	[20, 21]
Culicoides obsoletus	Cattle dung, cowshed, dried dung on the walls of the building, leaf compost, tree holes	[22–26]
Culicoides scoticus		
Culicoides dewulfi	high soil moisture, cattle dung breeders	[27]
Culicoides chiopterus		
Culicoides cataneii	Ponds and river margins, rich organic matter, soils poor in organic matter, unpolluted sites, grass covered pool	[12, 15, 28, 29, 30, 31]
Culicoides sahariensis	Mud in drainage channels rich in organic matter, mud fringing a salt lake, unvegetated pond, shorelines of the unvegetated pond and the grass covered pool moist	[12, 15, 29, 30]
Culicoides circumscriptus	Puddles of water contaminated withy animal excreta, inundated soils	[15, 28]
Culicoides newsteadi	Breeding in shallow, brackish pools, lined with decaying vegetable materialx	[31]

Species	Breeding sites	References
Culicoides jumineri	Mud near irrigation channel	[32]
Culicoides nubeculosus	Mud rich in dying near the water reservoirs and in mud from swap, organic matter	[33]
Culicoides puncticollis	Sites rich in organic matter, mud rich of dung near water reservoirs and mud from swamps and less in mud from reed sites areas	[11, 33]
Culicoides gejgelensis	Mud with poor organic matter alongside streams, mud from around dams, mud from reed sites	[33]
Culicoides riethi	Rich organic matter, mud swamps contaminated by feces of poultry animals, mud rich in dung near water reservoirs	[33, 34]

Table 1. Some example of breeding sites for certain *Culicoides* species.

Figure 3. Breeding sites of some *Culicoides* species. Photograph: LP3M: Laboratory of Medical and Molecular Parasitology-Mycology, University of Monastir Tunisia.

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2.2. Larva

The larvae are vermiform, usually pale. They have a distinct head capsule with toothed mandibles and eyespots. There are three thoracic and nine abdominal segments. The larvae are narrow and worm-like, and they live in the soil. Neverthless, the larvae of some species are omnivorous, and their diet includes small animals such as Nematodes, other insect larvae, fungi and parts of plants. They grow slowly when compared to some other species in the genus, due to the nutrient-poor soil [8]. According to *Culicoides* species, the breeding sites of *Culicoides* larva were very variable, usually defined as humid rich and enriched in animal or vegetal organic matter. Many larval biotopes are defined worldwide including damp or wet decomposing vegetation, wet leaf packs, manure, many different types of tree holes, swamps, ponds, lakes, streams and river margins, bogs and salt marshes [9] (**Table 1**), (**Figure 3**). Such great variety of habitats of many species of biting midges makes it difficult to find out the immature stages [10]. Nevertheless, it was state that the last stage larvae overwinter and pupate the following spring to early summer.

2.3. Pupa

The pupal stage is formed in the same site as the last larval stage. Pupal colour can be pale yellow to light brown. They are 2–5 mm in length with an unsegmented cephalothorax that has a pair of respiratory horns that may bear spines or wrinkles. The pupae of most *Culicoides* species are aquatic and have the ability to float.

3. Disease transmission and distribution of *Culicoides* sp.

3.1. Disease transmission

Biting midges of the genus, *Culicoides* play a big threat role, and this when several species serve as biological vectors of pathogens of medical and veterinary importance. Almost, over 50 arboviruses have been isolated from species of *Culicoides* [1, 35, 36]. In addition, only few *Culicoides* species have a significant deleterious impact on human existence. Opportunistic feeding of *Culicoides* species on humans can have impact on tourism, forestry and agricultural industries [1]. Actually, the major economic impact of *Culicoides* resides in their ability to transmit bluetongue virus (BTV), epizootic haemorrhagic disease virus (EHDV) and African horse sickness virus (AHSV). These arboviruses are of greatest importance in ruminants and equines. The biting midges have recently been identified as the vector of the Orthobunyavirus, Schmallenberg virus [37].

In the context of pathogen transmission to or between humans, *Culicoides* include a range of filarial nematodes transmitted between humans, especially *Mansonella ozzardi*, *M. perstans* and *M. streptocerca* [38].

It is noteworthy that biologically transmitted *Culicoides* species have the ability to transmit Oropouche virus (OROV), the aetiological agent of the febrile illness Oropouche fever, between human beings [1, 38]. Indeed, the symptoms of Oropouche fever include headache and also lead to generalised arthralgia, anorexia and in rare cases meningitis [1]. **Table 2** summarises major disease transmitted by *Culicoides* species in worldwide.

		Viruses							Filarial Nematodes							Parasites					
		BTV	AHSV	EHDV	EEV	OROV	Vesicular stomatitis Indiana	West Nile	Mansonella ozzardi	M. perstans	M. streptocerca	Onchocerca cervicalis	Onchocerca gibsoni	Onchocerca reticulata	Haemoprote-us	Plasmodium	Leucocytozoon	Hepatocystis	Leishmania	References
Subgenera	**Vectors**																			
Amossovia	*C. arboricola*							x												[39]
Avaritia	*C. bolitinos*		x																	[1]
	C. brevitarsis	x																		[40]
	C. dewulfi	x																		[42]
	C. fulvus	x																		[42]
	C. grahami									x	x									[43]
	C. imicola[*]	x	x		x														x	[1, 40, 42, 43]
	C. obsoletus	x																		[40]
	C. orientalis												x							[41]
	C. pungens												x							[41]
	C. wadai	x																		[40]
Beltranmyia	*C. circumscriptus*[*]														x	x			x	[42, 44]
Haematomyidium	*C. debilipalpis*								x											[41]
	C. paraensis								x											[41, 45]
	C. lahillei								x											[41]
Hoffmania	*C. guttatus*								x											[41]
	C. insignis	x							x											[41]

		Viruses							Filarial Nematodes								Parasites				References
		BTV	AHSV	EHDV	EEV	OROV	Vesicular stomatitis Indiana	West Nile	Mansonella ozzardi	M. perstans	M. streptocerca	Onchocerca cervicalis	Onchocerca gibsoni	Onchocerca reticulata	Haemoprote-us	Plasmodium	Leucocytozoon	Hepatocystis	Leishmania	
Macfiella	*C. phlebotomus*								x											[41]
Monoculicoides	*C. varriipennis var sonorensis*	x			x															[40]
	C. nubeculosus											x		x					x	[41, 43]
	C. sonorensis	x					x													[40, 46]
Meijerehelea	*C. arakawei*																x			[47]
	C. distinctipennis									x										[41]
Miscellaneous	*C. adersi*																	x		[47]
Oecacta	*C. furens*								x			x								[41]
	C. stellifer							x												[39]
Remmia	*C. oxystoma*												x							[41]
Silvaticulicoides	*C. biguttatus*							x												[39]
Trithecoides	*C. fulvithorax*									x										[41]
Unplaced	*C. austeni*										x									[41]
	C. bwambanus									x										[41]
	C. inornatipennis									x										[41]

(*detected in Culicoides spp. In Tunisia)

Table 2. Pathogen associated with the genus *Culicoides* worldwide.

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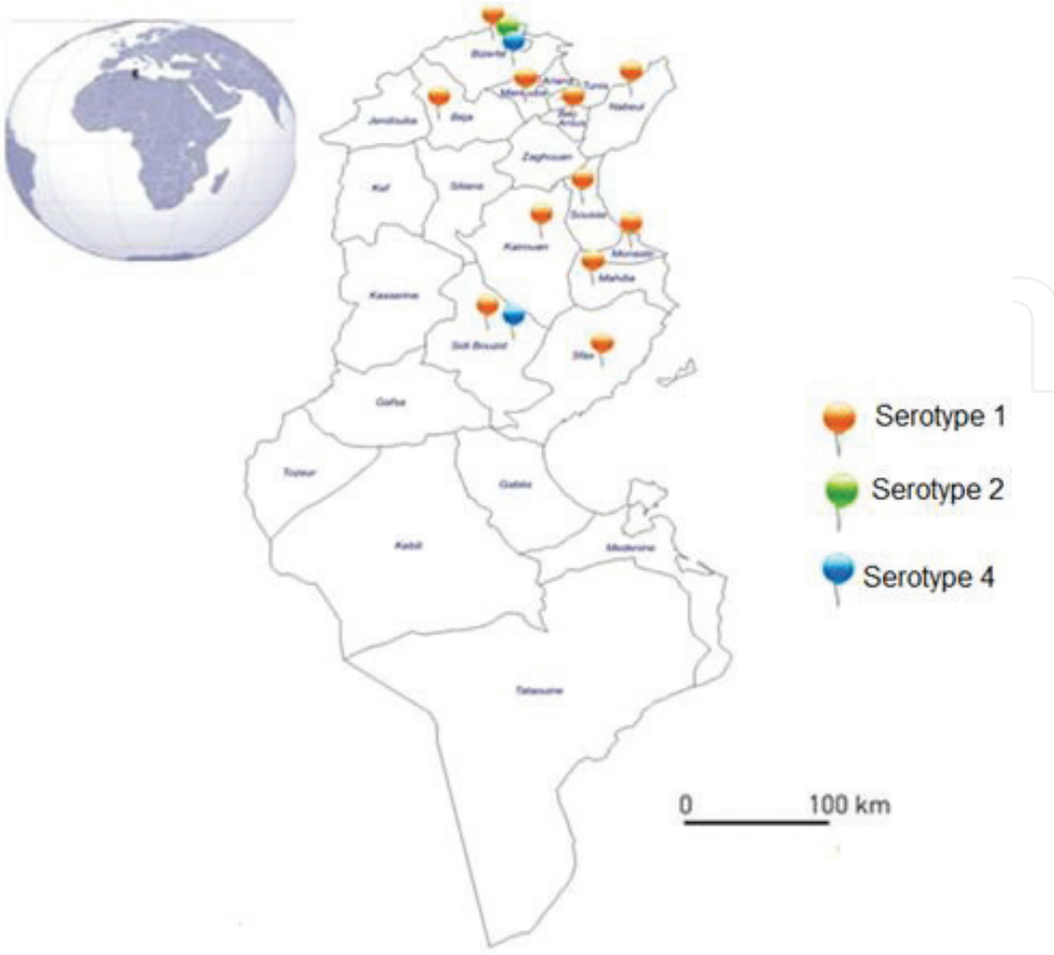

Figure 4. BTV serotype distribution in Tunisia.

4. Distribution of *Culicoides* spp.

Culicoides midges are found on all large landmasses ranging from the tropics to the tundra, with the exception of Antarctica and New Zealand.

In Tunisia, first incursion of BTV dates from 1999 (serotype 2), where the autumn was characterised by high temperatures and heavy rain. This weather created favourable conditions for BTV vector activity. It is noteworthy that the optimum conditions for activity of these biting midges are temperatures of 18–29°C and high humidity [48]. During this first incursion, severe clinical signs were observed in affected sheep: high temperature (41–42°C), nasal discharge, salivation, oedema and congestion of the head and the mucous membranes. Affected sheep flocks were located in the eastern part of Tunisia along the cost. The overall morbidity and mortality rates were 8, 35% and 5, 5%, respectively. In 2000, 72 outbreaks of BT were reported during the period extending from June to October. Indeed, 6120 clinical cases were diagnosed in sheep, of which 1318 died. Moreover, outbreaks were reported in 10 districts with most cases appeared in the eastern and central parts of the country [49].

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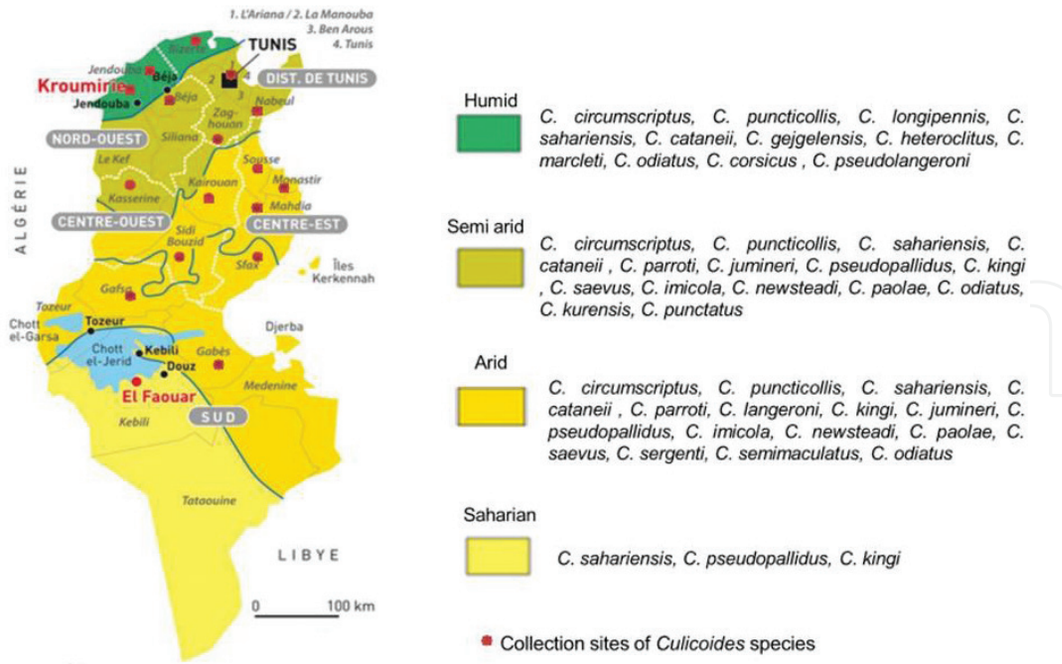

Figure 5. Distribution of *Culicoides* species in Tunisia. Source of the map of Tunisia from [54].

In total, three serotypes of BTV were reported in Tunisia: serotype 1, 2 and 4. **Figure 4** shows the distribution of BTV serotype in Tunisia.

Since the epizootic of vector-borne disease (AHS in 1966 and BT in 1999) in Tunisia, the veterinary authorities of the region have implemented surveillance programmes to detect and identify the presence and the distribution of the known vectors of disease, notwithstanding that fewer studies have been made in comparison with other Mediterranean countries. Indeed, in 1981, a study of [29] reported that the presence of 22 *Culicoides* species, with the most abundant species, was *C. circumscriptus, C. sahariensis, C. longipennis* and *C. puncticollis*. However, no *C. imicola* was detected. It is only to be expected that the presence of *C. imicola* in Tunisia was reported only in 2005 in the Monastir governate [50]. Evenly, Hammami et al. [51] reported 14 species with one new for the fauna *C. punctatus*. Since 2009, the national veterinary authorities of Tunisia have implemented an epidemiovigilance programme. Entomological studies have been conducted aimed to the detections of any new competent vectors. Thereby, Sghaier et al. [52] have identified 25 species of which seven were identified for the first time: *C. obsoletus, C. submaritimus, C. santonicus, C. univittatus, C. fascipennis, C. subfasciipenis* and *C. indistinctus*. However, this study was conducted in different regions: eastern and northern part of the country. Since this date, no studies were made to update knowledge on the *Culicoides* fauna present in Tunisia. But in 2016, Slama et al. [53] identified 22 species in Central Tunisia (Monastir, Kairouan and Sidi Bouzid). This study reported the presence of two new species: *C. semimaculatus* and *C. sergenti*. Indeed, the numbers of the *Culicoides* species recorded from Tunisia were increased to 35 species. **Figure 5** represents the distribution of *Culicoides* species in different regions of Tunisia.

5. Tools for *Culicoides* species identification

The most common method of *Culicoides* identification relies in the use of taxonomic keys. The observation of wing patterns allows the classification of the insects into vector relevant groups of *Culicoides* spp. Moreover, certain species can be identified based on wing pattern, while others need microscopic analysis of slide-mounted parts of bodies [55, 56]. Morphological identification can be a time-consuming procedure and laborious process that requires intensive training and most importantly that the biting midges be undamaged. If for any reason the *Culicoides* specimen of interest is damaged, morphological identification may not be possible. Withal, some species can only be identified by differences in the male genitalia, making it difficult or impossible to identify adult females, or may even be morphologically indistinguishable [57]. Many PCR-based tests have been used for identification of *Culicoides* spp., targeting the mitochondrial cytochrome oxidase I gene (mt COI) and the ribosomal RNA genes internal transcribed spacer 1 or 2 (ITS1, ITS2). Moreover, the fused carbamoylphosphate synthetase, aspartate transcarbamylase and dihydroorotase (CAD) nuclear marker have also developed for its utility in differentiating species [58]. **Table 3** summarises the molecular markers used for molecular analysis within *Culicoides*.

Another molecular technique (matrix-assissted laser desorption/ionisation time of flight mass spectrometry, MALDI-TOF MS) has proven its benefits for rapid, simple and cost-effective characterisation and identification of biting midges [59].

Genomic region	Molecular marker	References
Mitochondrial	COI	[57, 60–67, 78]
	COII	[68–70]
	28S	[71]
	18S rRNA	[72]
	16S rRNA	[73, 74]
	Cytb	[66]
Ribosomal	ITS1	[75–78]
	ITS2	[79]
Nuclear	CAD	[58]

Table 3. Molecular markers used for *Culicoides* species identification.

6. Conclusion

Despite the fact that the epidemiological studies realised till now, *Culicoides* species in Tunisia are yet neglected vectors. Their geographical propagation is increasing because of the environmental changes. For this reason, more epidemiological studies and many surveillance and control systems are required to be created.

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