Chapter

Morphological Keys for the Identification of Tunisian *Culicoides* Biting Midges (Diptera: Ceratopogonidae)

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Abstract

Culicoides biting midges are tiny blood-feeding insects of several diseases with veterinary and public health significance, including Bluetongue in ruminants, African horse sickness in equids and filarial diseases like Onchocercosis and Mansonellosis affecting various species such as humans. Their identification depends basically on the microscope examination of key morphological characters. Consequently, identification keys are important to any non experiment working with these biting midges. The Tunisian fauna of *Culicoides* biting midges consists of 35 species, whose morphological delineation may be troublesome for non-taxonomists. In response to this situation, and for the first time a key to the adult *Culicoides* species in Tunisia was prepared.

Keywords: *Culicoides*, morphological identification, taxonomy, Tunisia, vectors

1. Introduction

Culicoides Latreille, 1809 (Diptera: Ceratopogonidae) is a genus of biting midges, containing 1368 species divided into numerous subgenera [1]. Indeed, they are vectors of a variety of pathogens, including protozoans [2], filarial parasites [3] such as avian haemosporidians [4] and Tetrapetalonema spp. [5–7]. More than 50 viruses have been isolated from *Culicoides* spp. worldwide [8], such as African horse sicknes virus (AHSV), Bluetongue virus (BTV), Epizootic hemorrhagic virus (EHDV), Schmallenberg virus (SBV), or Oropouche virus (OROV) [9]. These viruses are responsible for outbreaks of non-contagious disease in ruminants, causing severe economic loses [10]. In the Mediterranean basin as well as sub-saharan Africa, the main vector of BTV and AHSV is *Culicoides imicola* [11]. Moroever, other Paleartic *Culicoides* species, within the subgenera Avaritia, such as *C. obsoletus*, *C. scoticus*, *C. dewulfi* and *C. pulicaris* are also known or potential BTV vectors [12, 13]. The recent BTV outbreaks in Tunisia demonstrate how a relatively neglected arthropod vector group can rapidly augment in interest. Thus identification of the vector is an important step in the epidemiology of vector born diseases. For instance, Tunisian fauna of *Culicoides* includes 35 distinct species [14]. Indeed, at the regional level, there are no proper morphological keys to Tunisia *Culicoides* Species. This can cause a major confront in the control effort as the accurate

identification of vectors is crucial for vector incrimination. In this context, a morphological identification key was prepared for the adult of the recorded *Culicoides* species in Tunisia as simply as possible, using the most important characters. Hopefully, this will aid public health workers, students and entomologists for rapid and accurate identification of *Culicoides* to the genus and species levels.

2. Methods

2.1 Trapping methods

Culicoides specimens used herein were collected at different locations, in studies conducted in Tunisia. Collection sites were selected based on their characteristics, including presence of animals, type of vegetation, and degree of urbanization. Two models of light traps: home-made miniature using CDC (Centre of Disease Control, Atlanta, USA) and OVI (Onderstepoort Veterinary Institute) were used.

2.2 Samples processing

All insects were collected in a beaker filled with 70% ethanol. Sampled *Culicoides* were separated from other insects and identified according to wing characters using a stereomicroscope. *Culicoides* midges were separated according to sex. Specimens were dissected on a glass slide separating the terminal part of the abdomen, wings and the entire head with a fine needle and mounted in a mix of Balsam Alcohol-Phenol for later identification. Species identification was made according to different morphological keys [15–17].

2.3 Morphological characterization

The morphological characteristics used here were based on original observations and previous usage in the litterature [15, 17, 18] (**Figure 1**). Several morphological characters were examined during the preparation of this morphological identification keys. The following main features were considered: *Head* (Eyes; Antenna (short segments: shape; sensilla coeloconica (Presence); antennal ratio XI/X ratio,

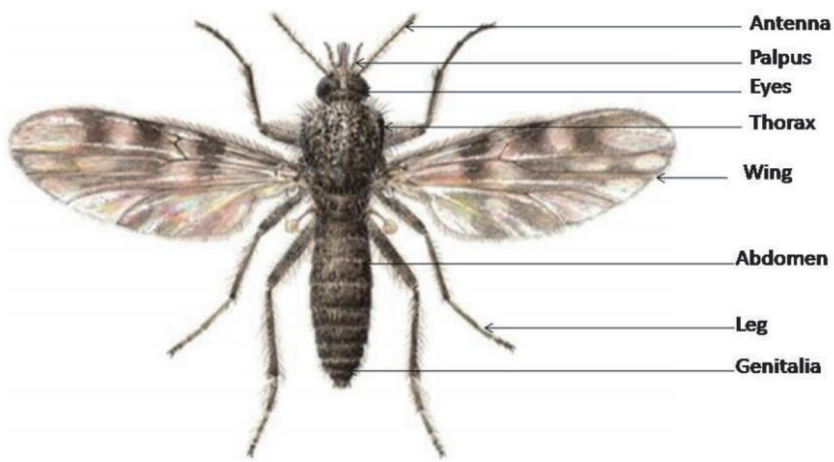
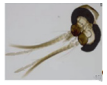
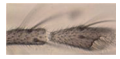
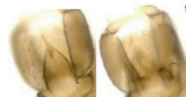
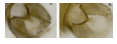
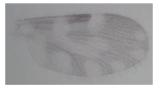
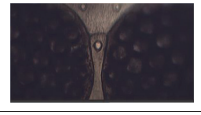
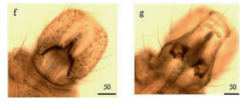
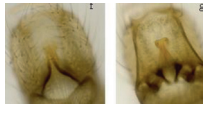
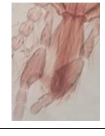
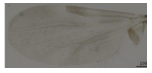
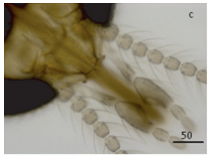
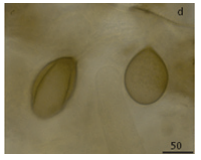
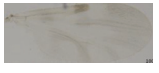
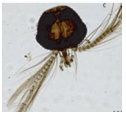
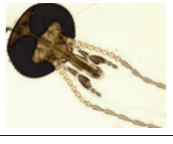
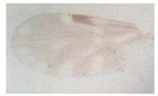
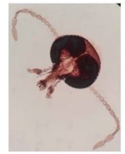
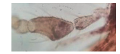
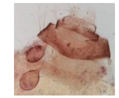
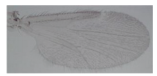
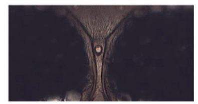
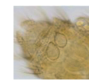
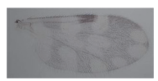
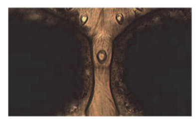
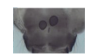
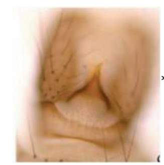
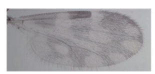
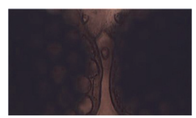
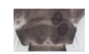
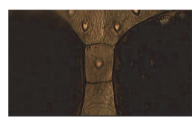
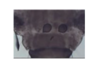
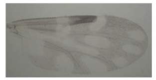
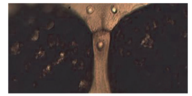
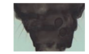
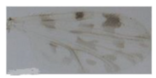
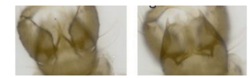
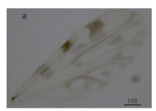
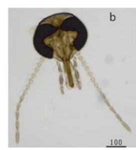
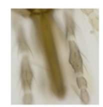
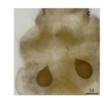
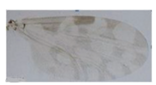
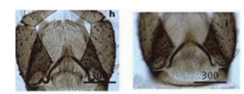


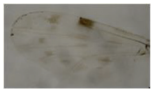
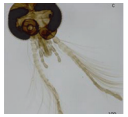
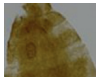
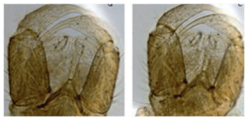
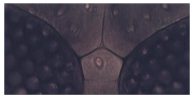
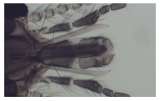
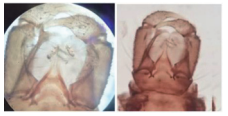
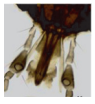
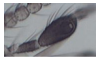
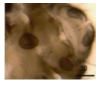
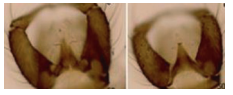
Figure 1.
Adult of Culicoides (Diptera: Ceratopogonidae).

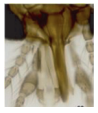
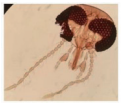
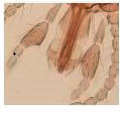
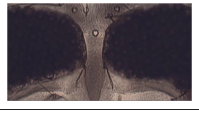
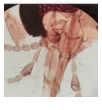
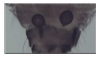
Sub-genera	Species	Wing	Head		Palpus	Spermathecae	Genitalia of male
			Female	Male			
Avaritia	*C. imicola*						
Oecacta	*C. jumineri*						
	*C. gejgelensis or C. cataneii variation**						NA
	C. semimaculatus						
	C. sergenti						
	C.jumineri var			NA			NA

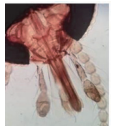
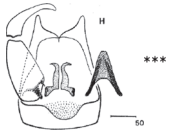
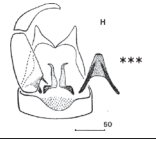
Sub-genera	Species	Wing	Head		Palpus	Spermathecae	Genitalia of male
			Female	Male			
	C.pseudopallidus			NA			NA
	C. cataneii						
	C.pseudojumineri						
	C.sp near kibunensis						NA
	C.submaritimus : C.maritimus						NA

Sub-genera	Species	Wing	Head		Palpus	Spermathecae	Genitalia of male
			Female	Male			
	C.longipennis						NA
	*C. corsicus**						NA
	C. heteroclitus						NA
	*C.griseidorsum**						 **
	*C.maritimus**						NA
	*C. santonicus**						NA

Sub-genera	Species	Wing	Head		Palpus	Spermathecae	Genitalia of male
			Female	Male			
	*C. univittatus**						NA
Synhelea	*C.sahariensis*						
Culicoides	*C.newsteadi*						
	C.punctatus			NA			NA
Beltranmyia	*C.circumscriptus*						

Sub-genera	Species	Wing	Head		Palpus	Spermathecae	Genitalia of male
			Female	Male			
Monoculicoides	*C.puncticollis*						
	*C.parroti**						
	*C.riethi**						NA
Remmia	*C.kingi*						
Pontoculicoides	*C.saevus*			NA			NA
Miscellanoues	*C.paolae*						

Sub-genera	Species	Wing	Head		Palpus	Spermathecae	Genitalia of male
			Female	Male			
	C.kurensis						NA
	C.langeroni						
	C.pseudolangeroni						NA
	*C. marcleti**						NA
	C. odiatus		NA				NA
	*C.indistinctus**						NA

Sub-genera	Species	Wing	Head		Palpus	Spermathecae	Genitalia of male
			Female	Male			
Silvaticulicoides	*C. fascipennis*						
	*C. subfascipennis**		NA				

**: photo of different part (wing, head, 3rd palpal and spermatheca) of* Culicoides *species were taken from Mathieu et al 2012*

***: photo of genitalia taken from Turgut F, 2018*

****: photo of drawn part of* Culicoides *were taken from Delecolle JC, 1985*
Other image illustarting the different parts of Culicoides *body in this chapter were taken from the Laboratory of Medical and Molecular Parasitology-Mycology LP3M (code LR12ES08), Department of Clinical Biology B, University of Monastir, Tunisia*

Table 1.
Key to Culicoides *species in Tunisia based on female and male morphology.*

length of segment XI divided by length of segment X; sensilla coeloconica, segments III to VI (Presence); sensilla coeloconica, segments VII to X (Presence); sensilla coeloconica, segments XI to XV (Presence)); *Palpus* (3rd palpal segment (shape, number of sensory pits, single sensory pits (opening versus depth)); *Abdomen*: Spermathecae (spermathecal duct at the end of the sclerotized ring, number, size, shape, abdominal sclerites (presence or absence)); *Wings* pale or dark spots (presence/absence), distribution, size.

Representative *Culicoides* specimens for each species available were selected and relevant characters were image captured with a digital camera through Leica microscopy. For some illustrations of Tunisian *Culicoides* species we used a drawn image used by (18).

3. Results and discussion

The present works illustrated the identification keys to adults of 35 *Culicoides* species (Diptera: Ceratopogonidae) which have been recorded in Tunisia (**Table 1**). Indeed, comprehensive identification keys of genera and species of *Culicoides* were elaborated. The simplified keys allow distinguishing each genus, subgenus and species based on the most important morphological features. Nevertheless, identification keys are fundamental for anyone dealing with insects of medical and veterinary significance, such as *Culicoides* biting midges. They are aimed to provide a guide for those interested to identify field-collected specimens obtained for many purposes and different type of studies. Indeed, identification keys especially those accompagnied by digital photographs and line drawing illustarting taxonomically relevant characters, are useful for species identification of *Culicoides* midges [15]. In Tunisia, even though the first record of *Culicoides* was documented in 1981 [15], field identification of this medically important group of insects has been carried out referring to the basic features and published literature. Indeed, in the case of the Tunisian biting midge fauna, [15] (in French) contain only restricted geographical areas, do not contain the new species records [14, 16, 19]. In the morphological identification keys presented here are accompanied by digital photographs taken from original specimens, which make the keys more user-friendly and facilitating the accurate identification of species.

One of the main limitations of the keys is that the males are incomplete. Therefore, species characterizations were not illustrated in the identification key based on male morphology. Furthermore, some species were not of good quality to take a clear photograph. For this, keys were supplemented with illustrations adapted from published literature [17, 18].

To the author's knowledge, these are the first keys prepared for Tunisian Culicoides fauna which are meant as an aid to the rapid identification.

Key for *Culicoides* (Diptera: Ceratopogonidae) females from Tunisia

1	1 functional spermathecae.....	**2**
	2 functional spermathecae.....	**5**
	3 functional spermathecae.....	***C. saevus***
2	Sensilla coeloconica: presence on segments III to XI, presence on segments XII to XIIII.....	
	Spermatheca narrow opening.....	**3**
	Sensilla coeloconica: absence on segments XII to XV, presence on segments III, VIII and X.....	

	3rd palpal segment slender with slightly swollen, multiple irregular pits.....	
	Spermatheca wide opening, oval, elongated, heavily curved in the middle.....	**4**
3	Pharynx posterieur armature: presence.....	
	Sensilla coeloconica: absence on segment XV.....	
	Wing: r-m cross vein, presence on dark spot in the corner with M1 vein.....	***C. circumscriptus***
4	Pale wing with only 1 dark spot cover the second radial cell.....	
	Antennal XI/X ratio: inferior or equal to 2.....	***C. parroti***
	Wing with 1 ore more pale spots.....	
	Antennal XI/X ratio: inferior or equal to 2.....	***C. puncticollis***
5	Eyes: inter-ocular space: joined.....	
	3rd palpal segment: Multiple irregular pits.....	
	Wing: 1 ore more pale spots well defined.....	
	Pharynx posterieur armature: Presence.....	***6***
	Eyes: Separated.....	
	3rd palpal segment: One single sensory pit, triangular, wide opening and shallow pit.....	
	Wing: 1 or more pale spot.....	
	Pharynx posterieur armature: absence.....	7
6	Wing: 2 pale spots fused in the middle basal of M1.....	
	Presence of dark spot in r3.....	
	Leg: Middle leg: Absence of spines on 4th tarsomere	***C. newsteadi***
	Wing: pale spot in the base of M1	
	Leg: Middle leg: Presence of spines on 4th tarsomere.....	***C. punctatus***
	Wing: pale spot over r-m crossvein fused with the m2.....	
	Presence of dark spot in r3.....	
	Leg: Middle leg: Absence of spines on 1st to 3rd tarsomere.....	***C. imicola***
7	Wing: Absence of pale spot (base wing).....	
	Antenna: antennal XI/X ratio: inferior or equal to 2.....	**8**
	Wing: Presence of pale spot at thebase of the wing.....	
	Antenna: I.A: total lengthe of 5 apical segments [11–15]/total length of 8 basal segments [3–10]: superior to 1,12.....	***C. jumineri***
8	Presence of sensilla coeloconica on the 6th segment.....	**9**
	Absence of sensilla coeloconica on the 6th segment.....	***C. pseudojumineri***
9	Size of 2 spermatheca equal to 50 µ.....	***C. jumineri var***
	Size of 2 spermatheca superior to 30 µ.....	***C. heteroclitus***
	Size of 2 spermatheca inferior to 58 µ.....	**10**
10	Presence of sclerotized ring at the end of the spermatheca duct...	***C. gejgelensis or C. cataneii var***
	Absence of sclerotized ring at the end of the spermatheca duct...	**11**
11	Length of palp superior to 185 µ.....	***C. pseudopallidus***
	Length of palp superior to 164 µ.....	**C. cataneii**

12	Absence of sensilla coeloconica on the segment XV.....	**13**
	Absence of sensilla coeloconica on all segments.....	**16**
13	Length of total antennal segment superior to 517 µ.....	***C.odiatus***
	Length of total antennal segment inferior to 488 µ.....	**14**
14	Palp (3/1 + 2) ratio, length of 3rd palpal segment divided by length of 1st and 2nd palpal segment: inferior to 1, 07.....	**15**
15	2 spermathecae: pyriform, size: superior to 58 µ.....	***C. langeroni***
	2 spermathecae ovoid, size: inferior to 50 µ.....	***C.pseudolangeroni***
	Wing: absence of pale spots in the distal part of r3, m1, m2.....	
	3rd palpal: swollen with narrow opening and deep pit.....	***C. semimaculatus***
	Presence of more than one pale spot in the distal part of r3, m1, m2.....	
	3rd palpal segment strongly swollen, with narrow opening single sensory pits and deep pit.....	***C. paolae***
	Length of total antenna: inferior to 300 µ.....	***C. marcleti***
	Length of total antenna: superior to 363 µ.....	***16***
16	I.A: inferior to 1.03.....	***C. kingi***
	I.A superior to 1.20.....	***17***
17	Palp (3/1 + 2) ratio: inferior to 1.13.....	***C. corsicus***
	Palp (3/1 + 2) ratio: superior to 1.26.....	***18***
18	Presence of chitinized thorn at the beginning of the pharynx posterior.....	***C. sahariensis***
	Presence of 1 to 3 thin thorns at the beginning of the pharynx posterior.....	***C. longipennis***
	Presence of chitinized thorn at the beginning of the pharynx posterior.....	***19***
19	Presence of pale spot r3, m1, m2, anal cell, pale spot in distal part: Presence of 2 pale fused, 2 pale spot separated.....	***C. maritimus***
	Absence of pale spot r3, m1, m2 and anal cell.....	***20***
20	Absence of pale spots.....	
	Presence of sensilla coeloconica on all short segments and absent on all long segments.....	***C. sergenti***
	Presence of pale spots.....	
	Sensilla coeloconica not present on all short segments.....	***21***
21	Presence of pale spots	
	Sensilla coeloconica on all short segments but often absent on segment X.....	
	I.A: superior to 1 µ.....	
	3rd palpal segment, triangular, swollen, sensory pit wide opening	***C. sp near kibunensis***
	Presence of pale spot.....	
	Presence of sensilla coeloconica on segments VII, VIII, IX and X	
	Presence of sensilla coeloconica on segments XI to XIIII.....	
		I.A inferior to 1 µ
	3rd palpal segment, triangular and moderate swollen, single sensory pit, wide opening and swollen pit.....	***C. kurensis***

Key for *Culicoides* (Diptera: Ceratopogonidae) males from Tunisia.

1	Aedeagus: bifid.....	***2***
	Aedeagus: not bifid.....	***3***
2	Presence one dark spot on the 2nd rad cell.....	***C. parroti***
3	Welded paramers with distal part consisting of spoon shaped.....	***C. heteroclitus***
	Paramers separated, pointed with 7 to 8 sawteeth on its posterior edge.....	***C. sergenti***
	Paramers terminated by a succesion of thorns and presenting a lobe.....	***4***
	Paramers with tapered pointed end.....	***7***
4	Arm of the aedeagus with lateral process.....	***5***
	Arm of aedeagus without lateral process.....	***6***
5	Body of aedeagus: rectangular, elongated.....	***C. corsicus***
	Body of aedeagus: short.....	***C. marcleti***
6	Body of aedeagus: triangular with truncated end terminating in tiny teeth.....	***C. longipennis***
	Body of aedeagus: Triangular with pointed distal end.....	***C. sahariensis***
7	Ventral membrane spiculated.....	***8***
	Ventral membrane not spiculated.....	***12***
8	Body of aedeagus: short and large.....	***9***
	Body of aedeagus: Triangular.....	**10**
	Body of aedeagus with rounded apex.....	
	Absence of ventral apodeme.....	***C. circumscriptus***
	Body of aedeagus without rounded apex.....	
	Well developped ventral apodeme.....	***C. kingi***
10	Body of aedeagus large in the distal part, rounded end	***C. imicola***
	Center of the aedeagus body occuped by a chitinized point directed forward.....	***11***
11	Basal quarter of the wing covered by a pale spot.....	***C. jumineri***
	Slight lightening at the base of the wing.....	***C. pseudopallidus***
	Wing with pale spots.....	***16***
	Absence of pale spots at the base.....	***17***
12	Body of aedeagus: triangular, wide basal arch andrectangular distal part.....	***C. paolae***
	Body of aedeagus: triangular.....	***13***
	Body of aedeagus: rectangular.....	***15***
13	Body of aedeagus: very little developped.....	
	Arm of aedeagus: very long.....	***C. saevus***
	Arm of aedeagus: well developped.....	
	Arm of aedeagus: short.....	***14***
14	Process: very short, broad-based.....	***C. langeroni***
	Process: long, with a narrow base.....	***C. pseudolangeroni***

15	Body of aedeagus: rectangular, large.....	***C. cataneii***
	Body of aedeagus: elongated, narrow.....	***C. gejgelensis or C. cataneii var***
16	Single and big pale spot in the basal half of m1.....	***C. punctatus***
	2 or bilobed spot in the basal half of m1.....	***C. newsteadi***
17	Arms of appendix: horse ear shaped well developped.....	
	Paramers with additional lobe well developped, wide terminal part, with 5 to 6 thiks tips.....	***C. semimaculatus***
	Aedeagus without additional appendices on the arms.....	
	Paramers without additional lobe.....	***18***
18	Presence of spot (proximal part of m1).....	***C. pseudojumineri***
	Presence of pale spot m4 cut by the edge of the wing	
	Presence of spot (proximal part of m1).....	
	Presence of pale spot m4 not cut by the edge of the wing	***C. jumineri var***

4. Conclusion

The simplified keys, would contribute towards improving research capacity among researchers in Tunisia as the identification is a fundamental requirement for anyone dealing with medically important insects.

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Conflict of interest

The authors declare no conflict of interest.

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