الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي و البحث العلمي

Université Ferhat Abbas Sétif 1
Faculté des Sciences de la
Nature et de la Vie

جامعة فرحات عباس، سطيف 1
كلية علوم الطبيعة و الحياة

DEPARTMENT OF BIOLOGY AND ANIMAL PHYSIOLOGY

N°...../SNV/**2020**

THESIS

Presented by

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For the fulfillment of the requirements for the degree of

DOCTORAT 3 CYCLE

Field: **BIOLOGICAL SCIENCES**

Specialty: **ANIMAL ECOLOGY**

TOPIC

Inventory, Biology and Ecology of Culicidae (Diptera) of Setif region and control tests using plant extracts

Presented publically 14/10/2020

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DEDICATION

I DEDICATE THIS WORK TO MY FATHER, MY HUSBAND, MY SISTERS, MY CHILDREN, MY GRANDMOTHER AND MY UNCLE. ALL OF YOU WERE BESIDE ME AND SUPPORTED ME, SO THE WORDS WILL NEVER BE ENOUGH TO THANK YOU. BUT THANK YOU FROM ALL MY HEART.

IN MEMORY OF MY MOTHER AND MY GRANDFATHER, MAYBE YOU LEFT US SINCE A LONG TIME, BUT YOU LIVE IN MY SOUL AND YOU WERE WITH ME ALWAYS, I HOPE YOU ARE PROUD OF ME.

ACKNOWLEDGEMENT

Thanks, to may supervisor Pr. Mustapha Bounechada that supported me and acts when he should act to help me.

I sincerely thank the members of the jury for the honor they have given me for agreeing to evaluate my thesis. Thus, I warmly thank the Presendent of this jury Pr. Farida Benia. I would also like to thank Dr. Fouad Meradsi and Dr. Abdelkrim Laatamna, as well as Dr. Linda Aissaoui for agreeing to be the examiners of my thesis.

Thanks, to Dr, Mathieu Bruno (Medicine faculty, Institute of Parasitology and Tropical Pathology (IPPTS)), I have learn more from you, thank you for giving me the opportunity to work in your laboratory and to share with me your experience.

Thanks, to the institute of parasitology and tropical pathology, Strasbourg (France), for having me and for funding my molecular analyses.

Thanks, to Pr. Saliha Dahamna, for here support, I will never forget what you did for me, and the wonderful way you act with me.

Thanks, Mrs. Safia Zeghbib, you were always beside me, thank you for helping me to publish my mosquito sequences in Genbank. May Allah help you and guide you.

ملخص

أفراد أسرة البعوضيات (Culicidae) هي عبارة عن حشرات ثنائية الأجنحة يتم دراستها على نطاق واسع. اعتبر الباحثون في مختلف المجالات أن البعوض هو مادة بيولوجية محورية لإنجاز مختلف الدراسات بسبب المشكلات التي تسببها سواء على صعيد صحة الإنسان أو الحيوان. البعوض الناقل هي أنواع من عائلة البعوضيات التي غالبًا ما تشارك في نقل العديد من الأمراض القاتلة والخطيرة مثل الملاريا وحمى الضنك والشيكونغونيا والحمى الصفراء وفيروس النيل الغربي وزيكا ... إلخ. في العقود الماضية ، شهدت الجزائر تفشي الأمراض المتعلقة بالبعوض ؛ بالإضافة إلى ذلك ، تتعرض في الحاضر لاستقرار الأنواع الغازية الزاعجة البيضاء (*Aedes albopictus* (Skuse 1894)). ومع ذلك ، فإن دراسة التنوع البيولوجي للبعوض في الجزائر ما زال غير كاف إذ هناك نقص في المعلومات المتعلقة بالكثافة السكانية وأنماط التوزيع لمجموعات البعوض كما أن جرد الأنواع يعتمد فقط على تحديد الهوية المورفولوجية. في هذا السياق ، أجرينا جردًا للبعوض في منطقة سطيف (السهول الجزائرية العالية) من 2016 إلى 2019 ، من أجل توفير قائمة بأنواع البعوض في منطقة الدراسة وتحليل تنوعها البيولوجي وكثافتها وتوزيعها حسب منطقتين مناخيتين مختلفتين (مناخ البحر المتوسط في الشمال CSA و مناخ شبه قاري في مناطق السهوب BSk) باستخدام اختبارات إحصائية مختلفة .تم تحديد الأنواع باستخدام كل من التعريف المورفولوجي (مفاتيح التشخيص) والتعريف الجزيئي (تحليل PCR-RFLP للمورث Cytochrome c Oxidase subunit 1 gene COI). تمكنا من تحديد تسعة أنواع من البعوض بما في ذلك ناقلات الملاريا *An c hispaniola* (Theobald 1901) و *Anopheles labranchiae* (Falleroni 1926) (4.4٪) (0.5٪) .كما تم توفير تسلسل COI لستة أنواع من البعوض الموفرة على منصة Genbank حسب الارقام التسلسلية MK047302-MK047315. من مجموع البعوض الذي تم أخذ عينات منه، كان النوع *Culex pipiens* (Linnaeus 1758) هو الغالب (46.9٪) والأكثر شيوعًا (التردد = 61٪) بينما أظهرت *Culiseta longiareolata* (Macquart 1838) أعلى كثافة (51.2 ± 63.7). علاوة على ذلك، لقد كشفنا عن وجود علاقة عالية وإيجابية بين *Cx. theileri* (Theobald 1903)و *An labranchiae* (r_s= 0.89, p> 0.001) ، مما يطرح إمكانية استخدام *Cx. theileri* كمؤشر وجودي للنوع *An labranchiae* .كذلك ، أكدت المقارنة الزوجية والتحليلات المقابلة وجود ارتباط كبير بين توزيع الأنواع / الكثافة والمناطق المناخية في منطقة الدراسة (KW U = 51, p> 0.01) ، وأكدت تأثير تغير المناخ على مجموعات البعوض. من ناحية أخرى ، أظهر أعضاء مجموعة *Cx. pipiens* تباينًا في التكوين المورفولجي الخاص بهم ، لذلك قمنا بتحديد التكوين المورفولوجي لمركب *Cx. pipiens* من أجل تسهيل عمليات الجرد المستقبلية وتمييز الأنواع المحلية . أخيرًا، فإن السيطرة على مجموعات البعوض أمر أساسي أين تعتبر الزيوت الأساسية بدائل محتملة للمبيدات الحشرية الاصطناعية. لذلك فقد سعينا إلى تقييم قدرة الزيوت الأساسية المستخرجة من نباتات تم جمعها من شمال شرق الجزائر على السيطرة على كثافة يرقات بعوضة *Cs longiareolata* والتي تعتبر أحد أهم الأنواع الناقلة لطفيليات البلازموديوم (*plasmodium*) عند الطيور كما تعتبر من أكثر الأنواع انتشارا وكثافة في منطقة الدراسة. تم اختبار الزيوت الأساسية المستخرجة من الزعتر البري الشائع *Thymus vulgaris* و الشيح *Artemisia herba-alba* والعرعار *Juniperus phoenicea* و أكليل الجبل *Rosmarinus officinalis* و الكاليتوس *Eucalyptus globulus*، ضد الطور الثالث والرابع ليرقات *Cs longiareolata* ؛ تعرضت اليرقات لسلسلة من تركيزات الزيوت الأساسية المختبرة لمدة 24 ساعة. تم تكرار التركيزات التي تسببت في وفاة ما بين 10 ٪ و 90 ٪ أربع مرات كما تم تكرار

التجربة كاملة ثلاث مرات. تم استخدام البيانات التي تم جمعها لتحديد قيم LC_{50} وLC_{90} . كشفت نتائج الاختبار عن وجود نشاط مبيدي فعال للزيوت الأساسية المختبرة ، حيث أظهر الزعتر البري الشائع معدل وفيات 100 ٪ عند التركيز النهائي 80 جزء في المليون ، في حين أن معدل الوفيات بنسبة 100 ٪ عند باقي الزيوت كان عند التركيز النهائي 200 جزء في المليون. علاوة على ذلك ، كانت التركيزات القاتلة التي تسببت في وفيات بنسبة 50 ٪ و 90 ٪ (LC_{50},LC_{90}) متباينة حيث كان زيت الزعتر البري الشائع أكثر الزيوت الأساسية كفاءة (LC_{50} = 25,64 ppm, LC_{90} = 50,53 ppm) يليه زيت العرعار (LC_{50} = 59,83 ppm, LC_{90} = 137,68 ppm) ثم زيت إكليل الجبل (LC50 = 64,18 ppm, LC_{90} = 96,55 ppm) ثم زيت الشيح (LC_{50}= 86,67 ppm, LC_{90} = 139,55 ppm) وفي المرتبة الأخيرة زيت الكاليتوس (LC_{50} = 95,83 ppm, LC_{90} = 168,25 ppm). وبالتالي ، فإن استخدام الزيوت الأساسية أو مكوناتها النشطة الرئيسية مثل α-pinene و 1،8 cineole- و Camphor قد يكون وسيلة صديقة للبيئة للتحكم في يرقات البعوض. لذلك نستطيع أن نقول في الأخير أن الدراسة التي قمنا بها في مجملها توفر برنامجا متكاملا للمراقبة و التحكم في مجموعات البعوض في منطقة سطيف.

الكلمات المفتاحية: البعوض, منطقة سطيف, التنوع البيولوجي, دراسة البيئة, نشاط مبيد اليرقات.

ABSTRACT

The members of the family Culicidae, commonly known as mosquitoes, are Diptera insects widely studied. Researchers in various fields have considered mosquitoes as a focal biological material to study because they carry and spread disease to both humans and animals. Mosquito vectors are species of the family Culicidae often involved in the transmission of many deadly and dangerous diseases like malaria, dengue, chikungunya, yellow fever, West Nile virus, Zika…etc. In the last decades, Algeria has experienced outbreaks related to mosquitoes; additionally, it is exposed at the present to the installation of the invasive species *Aedes albopictus* (Skuse 1894). However, the mosquito biodiversity in Algeria is poorly studied, likewise, information about density and distribution patterns of mosquito populations is missed and the inventories were depended only on morphological identification. In this context, we performed a mosquito inventory in the Setif region (Algerian high plains) from 2016 to 2019, in order to provide the list of mosquito species in the study area and analyze their biodiversity, density and species distribution across two climate zones (Mediterranean Csa and steppe BSk Zones) using different statistical tests. The identification of species was done using a combination of morphological (diagnostic keys) and molecular (PCR-RFLP analysis of Cytochrome c Oxidase subunit 1 gene) approaches. The sampling yielded the identification of nine mosquito species including the malaria vectors *Anopheles labranchiae* (Falleroni 1926) (4.4%) and *An cinereus hispaniola* (Theobald 1901) (0.5%). The COI sequences of six species are provided (Accession numbers MK047302-MK047315). From the total sampled mosquitoes, *Culex pipiens s.l* (Linnaeus 1758) was the predominant (46.9 %) and the most frequent species (f=61%) while *Culiseta longiareolata* (Macquart 1838) showed the highest density (51.2±63.7). Further, we have revealed a high and positive correlation between *Cx. theileri* (Theobald 1903) and *An labranchiae* (r_s=0.89, $p<0.001$), which poses the possibility of using *Cx. theileri* as species indicator of *An labranchiae*. Moreover, the pairwise comparison and Ordination Corresponding Analyses ascertained the presence of a significant association between species distribution/density and climate zones in the study area (K-W U=51, $p<0.01$), and confirm the effect of the climate changes on the mosquito population. Furthermore, the members of *Cx. pipiens s.l* population showed a variation in their morphology, we demonstrated the unusual keys to facilitate the morphological identification in future inventories and to discriminate local species of *Cx. pipiens* complex. Finally, mosquito control is indispensable and the use of essential oils in

mosquito control is considered as a potential alternative of synthetic insecticides; therefore, we aimed to assess the larvicidal activity of the essential oils extracted from five medicinal plants collected from northeastern Algeria against *Cs longiareolata* larvae, a vector of *Plasmodium* species in birds and one of the most abundant mosquito species at the studied region. The essential oils extracted from: *Thymus vulgaris*, *Artemisia herba-alba*, *Juniperus phoenicea*, *Rosmarinus officinalis* and *Eucalyptus globulus*, were tested against the 3rd and 4th instar *Culiseta longiareolata* larvae; the larvae were exposed to a series of concentrations of the tested essential oils for 24h. The concentrations that caused between 10% and 90% mortality was replicated four times, the entire test was repeated three times. The collected data was used to determine the LC_{50} and LC_{90} values. The tested oils revealed an efficient larvicidal activity, *T. vulgaris* showed 100% mortality at 80ppm final concentration, while the other tested oils showed 100% mortality at 200ppm. Furthermore, the lethal concentrations that caused 50% and 90% mortality (LC_{50} and LC_{90}) were varying, *T. vulgaris* was the most efficient essential oil (*LC_{50}=25.64ppm, LC_{90}=50.53ppm), followed by *J. Phoenicea* (LC_{50}=59.83ppm, LC_{90}=137.68ppm), *R. officinalis* (LC_{50}= 64.18ppm, LC_{90}= 96.55ppm), *A. herba-alba* (86.67ppm LC_{50} and LC_{90}=139.55ppm), then *E. globulus* (LC_{50}=95.83ppm, LC_{90}= 168.25ppm). Thus, The use of essential oils or their principal active components as α-pinene, 1,8-cineole and Camphor may serve as an eco-friendly method to control mosquito larvae. Consequently, the study provides a comprehensive program to control the mosquito population in the Setif region.

Keyword: Culicidae, Setif region, Biodiversity, Ecology, Larvicidal activity.

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Résumé

Les membres de la famille des Culicidae, communément appelés moustiques, sont des insectes Diptères largement étudiés. Des chercheurs de divers domaines ont considéré les moustiques comme un matériel biologique essentiel à étudier en raison des problèmes qu'ils posent toujours, qui menacent à la fois la santé humaine et animale. Les espèces vecteurs de la famille des Culicidae sont souvent impliquées dans la transmission de nombreuses maladies mortelles et dangereuses telles que le paludisme, la dengue, le chikungunya, la fièvre jaune, le virus du Nil Occidental, Zika… etc. L'Algérie a connu des épidémies liées aux moustiques au cours des dernières décennies. De plus, il est actuellement exposé à l'installation de l'espèce invasive *Aedes albopictus* (Skuse 1894). Cependant, la biodiversité des moustiques en Algérie est peu étudiée. De même, les informations sur la densité et les schémas de répartition des populations de moustiques sont manquantes et les inventaires ne reposent que sur l'identification morphologique. Dans ce contexte, nous avons réalisé un inventaire des moustiques dans la région de Sétif (hautes plaines Algériennes) de 2016 à 2019, afin de fournir la liste des espèces de moustiques dans la zone d'étude et d'analyser leur biodiversité, leur densité et leur répartition entre espèces dans deux zones climatiques (Csa dans le nord et BSk dans les région semi-aride) en utilisant différents tests statistiques. L'identification des espèces a été réalisée à l'aide d'une combinaison d'approches morphologiques (clés de diagnostic) et moléculaires (analyse PCR-RFLP du gène de la sous-unité 1 du cytochrome c oxydase COI). L'échantillonnage a permis d'identifier neuf espèces de moustiques, notamment les vecteurs du paludisme *Anopheles labranchiae* (Falleroni 1926) (4,4%) et *An c hispaniola* (Theobald 1901) (0,5%). les séquences de COI de six espèces sont fournies (numéros d'accès MK047302-MK047315). Parmi tous les moustiques échantillonnés, *Cx. pipiens s.l* (Linnaeus 1758) était l'espèce prédominante (46,9%) et la plus fréquente (f = 61%), tandis que *Culiseta longiareolata* (Macquart 1838) présentait la densité la plus élevée (51,2 ± 63,7). De plus, nous avons révélé une forte corrélation positive entre *Cx. theileri* (Theobald 1903) et *An labranchiae* (rs = 0,89, p> 0,001), ce qui laisse entrevoir la possibilité d'utiliser *Cx. theileri* comme indicateur d'*An labranchiae*. Après, la comparaison par paires et les analyses d'ordination correspondantes ont permis d'établir la présence d'une association significative entre la répartition / densité des espèces et les zones climatiques dans la zone d'étude (KW U = 51, p> 0,01), et de confirmer l'effet des changements climatiques sur les populations de moustiques. En outre, la morphologie des membres de la population de *Cx. pipiens s.l* variait,

nous avons démontré les clés inhabituelles permettant de faciliter l'identification morphologique dans les futurs inventaires et de discriminer les espèces locales du *Cx. pipiens* complexe. Enfin, le control de moustique est une chose indispensable et l'utilisation des huiles essentielles dans la lutte contre les moustiques est considérée comme une alternative potentielle aux insecticides de synthèse. Nous avons donc cherché à évaluer l'activité larvicide des huiles essentielles extraites de cinq plantes médicinales recueillies dans le nord-est de l'Algérie contre les larves de *Culiseta longiareolata*, l'espèce vecteur du *Plasmodium* chez les oiseaux et l'une des espèces de moustiques les plus abondantes dans la région étudiée. Les huiles essentielles extraites de: *Thymus vulgaris*, *Artemisia herba-alba*, *Juniperus phoenicea*, *Rosmarinus officinalis* et *Eucalyptus globulus* ont été testées contre les larves de 3ème et 4ème stades de *Culiseta longiareolata*; les larves ont été exposées à une série de concentrations des huiles essentielles testées pendant 24h. Les concentrations qui ont causé une mortalité comprise entre 10% et 90% ont été répliquées quatre fois, l'essai entier a été répété trois fois. Les données collectées ont été utilisées pour déterminer les valeurs de la LC_{50} et de la LC_{90}. Les huiles testées ont révélé une activité larvicide efficace, *T. vulgaris* a présenté une mortalité de 100% à une concentration finale de 80 ppm, tandis que les autres huiles testées ont présenté une mortalité de 100% à 200 ppm. En outre, les concentrations létales ont causées des valeur de mortalité à 50% et 90% (LC_{50} et LC_{90}) variée, *T. vulgaris* étant l'huile essentielle la plus efficace (LC_{50} = 25,64 ppm, LC_{90} = 50,53 ppm), suivie de *J. Phoenicea* (LC_{50} = 59,83 ppm, LC_{90} = 137,68 ppm), *R. officinalis* (LC50 = 64,18 ppm, LC_{90} = 96,55 ppm), *A. herba-alba* (LC_{50}= 86,67 ppm, LC_{90} = 139,55 ppm), puis *E. globulus* (LC_{50} = 95,83 ppm, LC_{90} = 168,25 ppm). Ainsi, l'utilisation d'huiles essentielles ou de leurs principaux composants actifs tels que l'α-pinène, le 1,8-cinéole et le camphre peut servir de méthode écologique pour lutter contre les larves de moustiques. Par conséquent, la présente étude à fournit un programme complet pour contrôler la population de moustiques dans la région de Sétif.

Mot clés: Culicidae, région de Sétif, Biodiversité, Ecologie, Activité larvicide.

ABRIVIATIONS

A.

Ae: *Aedes*

An: *Anopheles*

Arithmetic mean AM

C.

CCA: canonical corresponding analysis

Cs: *Culiseta*

Csa : warm temperate climate with warm and dry summer

Cx: *Culex*

B.

BSk : semi-arid; cold and dry climate

D.

DENV: Dengue fever

DDT: Dichloro-diphenyl-trichloroethane

E.

EO: essential oils

H.

HCH: γ-hexachlorocyclohexane

L.

LC_{50}: concentrations that cause 50% of death

LC_{90}: concentrations that cause 90% of death

O.

Oc: *Ochlerotatus*

OHF: Omsk haemorrhagic fever virus

S.

SE: Standard Error

W.

WNV: West Nile Virus

Y.

YFV: Yellow fever virus

Z.

ZIKV: Zika virus

PUBLICATION AND COMMUNICATION

PUBLICATION

Nabti I. & Bounechada M., 2019. Larvicidal Activities of Essential Oils Extracted from Five Algerian Medicinal Plants against *Culiseta longiareolata* Macquart. Larvae (Diptera: Culicidae). *European Journal of Biology*. 78(2).

Nabti I. & Bounechada M., 2019. Mosquito biodiversity in Setif region (Algerian High Plains), density and species distribution across two climates zones. *Entomologie faunistique – Faunistic Entomology* 2019 72, 161-173

COMMUNICATION

Mosquito control using *Juniperus phonecea* L. essential oil as an alternative of chemical insecticides. Nabti I. & Bounechada M. International symposium entitled "environment & Sustainable Development", Relizane, (Algeria) 10-11 February 2020

Habitat preferences of mosquito species in the Algerian high plains (Setif region). Nabti I. & Bounechada M. National symposium entitled "L'Apport des Biotechnologies sur la protection de l'environnement", M'sila, (Algeria) 15-16 December 2019.

Variation écologique et morpho-métriques observées sur les larves de *Culex pipiens* s.l dans les hautes plaines Algeriennes. Nabti I. & Bounechada M. National symposium entitled "l'environnement et le développement durable", Mascara, (Algeria) 22-23 January 2020.

Cooccurrence des larves de moustique et la possibilité d'utiliser le niveau de corrélation pour l'utilisation d'espèce indicatrices dans la détection des espèces vectrices. Nabti I. & Bounechada M. National symposium entitled "1^{ere} journée nationale d'entomologie et de parasitologie", Mascara, (Algeria) 11 March 2020.

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GENERAL INTRODUCTION

Insects are beneficial and important living organisms that participate in the different levels of food chains whether as prey, predators, scavengers or decomposers. However, many insect families include vector species capable to transmit viruses, bacteria and parasites to both humans and animals, threatening the public health. The family Culicidae (mosquitoes) is one of the most important and largest insect families, and it also includes a group of the most competent vector species.

Mosquitoes exist everywhere in the globe except Antarctica; they are characterized by high adaptability to different types of climate. Its life cycle pass through two important phases: aquatic and aerial. The female mosquitoes lay their eggs on the water surface, and then the eggs hatch and live in the water as larvae and pupae until they reach the adult stage; after, the adult mosquitoes leave the surface of the water and start their lives very close to the human environment. Mosquitoes can breed very quickly and in high numbers, and constitute a major inconvenience to humans. Unfortunately, mosquitoes inconveniences do not depend exclusively on their annoying bites, but they transmit diseases that caused millions of deaths (Water and Organization, 2004).

The vector ability of some mosquito species made them an important biological material for conducting various types of research due to the urgent need to control them and the public health problems that they cause. The information provided by these studies can be linked and used to control mosquito populations, and the information provided on the environmental characteristics of mosquitoes may be the most important. The biological and ecological characteristics of mosquito populations provide information about mosquito breeding sites, their density and their distribution by species and region. It also analyzes the environmental and climatic factors that can affect the density and distribution of mosquitoes by geographical dimensions and climatic zones. Mosquito biodiversity is undergoing continuing changes explained by the modification in population richness noted by inventorying studies (Benedict *et al.*, 2007; Berti *et al.*, 2015; Linares *et al.*, 2016). Further, mosquito species live in a particular area act as a population that interacts beyond a defined dynamic affected by competition and climatic conditions (Kingsolver, 1989; Alto *et al.*, 2005; de Oliveira *et al.*,

2017). Therefore, the study of mosquito biodiversity must consider co-occurrence, density and distribution patterns.

However, good ecological study has to start with a good inventory. The Identification is of huge importance in the medical entomology, Jourdain *et al.* (2018) gave a set of blind samples consisting of adult mosquitoes and larvae to participant laboratories for genus and species identification. This evaluation showed that all identifications were exclusively morphological. 81% of identifications were correct at the genus level, 64% at the species level. Thus, the morphological identification of mosquitoes, when used alone, may lead to false lists of species. Recently, DNA-based identification was adopted to support mosquito inventories using the sequence divergence at cytochrome c oxidase subunit 1(COI) to discriminate closely related mosquito species (Laboudi *et al.*, 2011; Engdahl *et al.*, 2014; Afizah *et al.*, 2019). Consequently, the integrative taxonomy approach represented in the combination of morphological and molecular identification results more assured mosquito species lists.

The accurate inventory lists and data collected from the inspection filed will definitely provide sufficient information to implement a control program to monitor mosquito populations; nevertheless, we will finally need to use insecticides to reduce mosquito density and control their numbers. However, chemical pesticides routinely used can pose an environmental risk vis-a-vis other organisms. So much research has been done to find environmentally friendly alternatives that enable us to monitor mosquitoes without causing collateral damage. For instance, the enhancement of behavior-based control tools and the development of repellent and toxic products based on botanic components can target different mosquito life stages (Benelli, 2015; Benelli *et al.*, 2016). Essential oils (EOs) extracted from different parts of plants were frequently tested for their mosquitocidal activity (Pavela, 2015); these primary botanic materials present various biological activities, they can act as insecticides where they can affect either the oviposition, survival, larval duration, pupation and insect emergence (Bakkali *et al.*, 2008; Bessah and Benyoussef, 2015). However, the larvae stage appears to be more appropriate to control mosquito populations because of the high reproduction rates and larvae food mechanisms that allow a high number of mosquito individuals to be targeted simultaneously. Therefore, the assessment of the larvicidal efficacy of various plant derivatives was the main objective of many research papers (Markouk *et al.*, 2000; Park *et al.*, 2002; Elimam *et al.*, 2009; Ghosh *et al.*, 2012).

In this study, we fixed several objectives:

- The first part will demonstrate a general review of the biology and the ecology of mosquitoes plus to the most important eco-friendly methods evaluated to control mosquito populations.
- In the second part, we will investigate mosquito populations in the Setif region using the most accurate method, the integrative method, that confound both the morphological identification that based on pictorial keys for basic information, and the molecular method represented in the DNA-based identification of the COI barcode to obtain an ascertained species list.
- Simultaneously, we will analyze the collected ecological data using the most accurate bio-statistical software in order to extract the maximum information about mosquito density, fluctuation, distribution, and breeding site preferences. The results will lead to designate cartography of the distribution of mosquito populations in the Setif region and highlight its distribution patterns.
- Finally, we will test in the last part, the larvicidal activity of the essential oils extracted from five aromatic medicinal plants on *Culiseta longiareolata* (Macquart 1838) larvae as an alternative natural insecticide to control mosquitoes.

Indeed, the entire work constitutes a program model to control mosquito population.

CHAPTER 1 BIBLIOGRAPHIC REVIEW

1 Systematic

After discovering the involvement of mosquitoes in transmitting microfilaria and protozoa, Chamberlain J the secretary of State for the Colonies of Britain appointed a committee to collect mosquitoes from British colonies and send them to British museum that subsequently appointed Theobald FV to prepare the description of mosquitoes of the world; the published book "a monograph of the Culicidae of the world" (Theobald, 1901) illustrated in its five editions first descriptions of many mosquito species. In the midst of continuous new records, the classification of Culicidae has undergone several changes. According to Becker *et al.* (2003), Edwards (1932) established three subfamilies in the family Culicidae: Anophelinae, Culicinae, and Toxorhynchitinae. A more recent study conducted by Harbach and Kitching (1998) confirmed the position of the Anophelinae and Culicinae as a subfamily within the family Culicidae; whereas, they considered the Toxorhynchitinae as a tribal rank within the Culicinae sub-family. Classifying mosquitoes in lower levels has never been easier; several researchers as Edwards FW, Belkin JN, Knight KL, Stone A, Reinert JF and Harbach RE have constantly revised the mosquito taxonomy. The constant review of mosquito lists is in fact due to several factors, the most important being the presence of highly morphologically close species, as well as the presence of species complexes characterized by complete morphological similarity while differing genetically, behaviorally, and/or physiologically. A list of the last valid species is provide by Harbach (2013a).

1.1 Culicidae sub-families

1.1.1 Culicinae

Culisinae sub-family is subdivided into eleven tribes: Aedeomyiini, Aedeni, Culicini, Culisetini, Ficalbiini, Hodgesiini, Mansoniini, Orthopodomiini, Sabethini, Toxorhynchitini, Uronotaeniini. The tribes that include the most familiar species are Aedeni, Culicini, and Culisetini, the largest tribe in the Culicinae subfamily the Aedini tribe regroups 64 genera of which the oldest species discovered belong. The polyphyletic genera *Aedes* (Meigen 1818) and *Ochlerotatus* (Reinert 2000) are the most common in the Aedini tribe, they were first included in the same genus *Aedes*, then they were divided into two genera by Reinert (2000).

The Culicini tribe regroups the cosmopolitan genus *Culex* (Linnaeus 1758) that includes the worldwide complex species *Culex pipiens* s.l commonly known as house mosquito.

1.1.2 Anophelinae

Anophelinae sub-family includes three genera: the cosmopolitan *Anopheles* (Meigen 1818), the Australasian *Bironella* (Theobald 1905), *Chagasia* (Cruz 1906). However, the genus *Anopheles* considers the most important because it lists the most important Malaria vector specie in humans (Calderaro *et al.*, 2013). According to Harbach (2013b), the genus *Anopheles* is subdivided into seven subgenera classified based on the number and positions of setae on the male genitalia gonocoxites: *Anopheles* (Meigen 1818), *Baimaia* (Harbach, Rattanarithikul and Harrison 2005), *Cellia* (Theobald 1905), *Kerteszia* (Theobald, 1905), *Lophopodomyia* (Antunes 1937), *Nyssorhynchus* (Blanchard 1902) and *Stethomyia* (Theobald 1902). However, the new molecular approaches and phylogenic studies still propose new classifications. A study conducted in 2017 by Foster *et al.* (2017) analyzed the amino acid sequences of 150 newly sequenced mitochondrial genomes of Anophelinae and suggested modifications to the Anophelinae classification.

1.2 Complex species

"The knowledge of species complexes containing species that are morphologically very similar but differ greatly in their vector competence, has generated interest in the control of malaria by genetic manipulation" (Becker *et al.*, 2003). The closely related species was of crucial importance in taxonomic studies. Entities morphologically similar such as species complexes and subspecies or more deeply related species which are genetically, behaviorally, or physiologically different constitute a difficulty of discrimination. The most common related species are known among *Anopheles* and *Culex.*

1.2.1 Anophelinae complex

in Thailand, six malaria vectors: *Anopheles dirus* (Peyton and Harrison 1979), *An. minimus* (Theobald 1901), *An. maculates* (Theobald 1901), *An. sundaicus* (Rodenwaldt 1925), *An. barbirostris* (van der Wulp 1884) and *An. leucosphyrus* (Dönitz 1901) were proven to be species complexes (Saeung, 2012). *An. claviger s.l* (Meigen 1804) is another *Anopheles* complex usually found in Europe and the Mediterranean region (Schaffner *et al.*, 2000). Additionally, *Anopheles cinereus* s.l is a complex species regroups two subspecies *Anopheles cinereus cinereus* (Theobald 1901) distributed in Arabian Peninsula, Ethiopia and Sudan, and

Anopheles cinereus hispaniola (Theobald 1903) issued from the Mediterranean region and the Equatorial Africa (Ramsdale, 1998). All the previous complexes are considered malaria vectors; further, *Anopheles maculipennis* s.l Meigen complex is likewise an important Palaearctic *Anopheles* complex, it comprises over 12 closely related species that are able to transmit *Plasmodium* species in humans (Laboudi *et al.*, 2011).

1.2.1.1 *Anopheles maculipennis* complex

In the 20th century, endemic malaria in Europe was related to the presence of *Anopheles maculipennis* complex; however, after malaria eradication and the reduction in natural breeding sites the Malaria disappeared but *Anopheles maculipennis* s.l still existed. The concept of Anopheles without malaria was confusing; thus, following researches illustrated the fact that *Anopheles maculipennis* s.l is a complex of dozen separate species (Becker *et al.*, 2003). In 1976, the genetically independent member of the complex the species *Anopheles beklemishevi* (Stegnii and Kabanova 1976) was newly described. After, White (1978) discussed the *Anopheles* complex species list, he recognized 13 members species in which nine are Palaearctic: *An. atroparvus* (Meigen 1927), *An. bekilemishevi*, *An. labranchiae* (Falleroni 1926), *An. maculipennis s.s* (Meigen 1818), *An. martinius* (Shingarev 1926), *An. melanoon* (Hackett 1934), *An. messeae* (Falleroni 1926), *An. sacharovi* (Favre 1903) and *An. sicaulti* (Roubaud 1935), and four are Nearctic: *An. aztecus* (Hoffmann 1935), *An. earlei* (Vargas 1943), *An. freeborni* (Aitken 1939) and *An. occidentalis* (Skuse 1889). On the basis of egg morphology, we can differentiate certain *Anopheles maculipennis* s.l. species (Figure 1.1). Another character could be used to differentiate maculipennis species, a key was given to use the variation in the wing ornamentation to separate some species (Ungureanu and

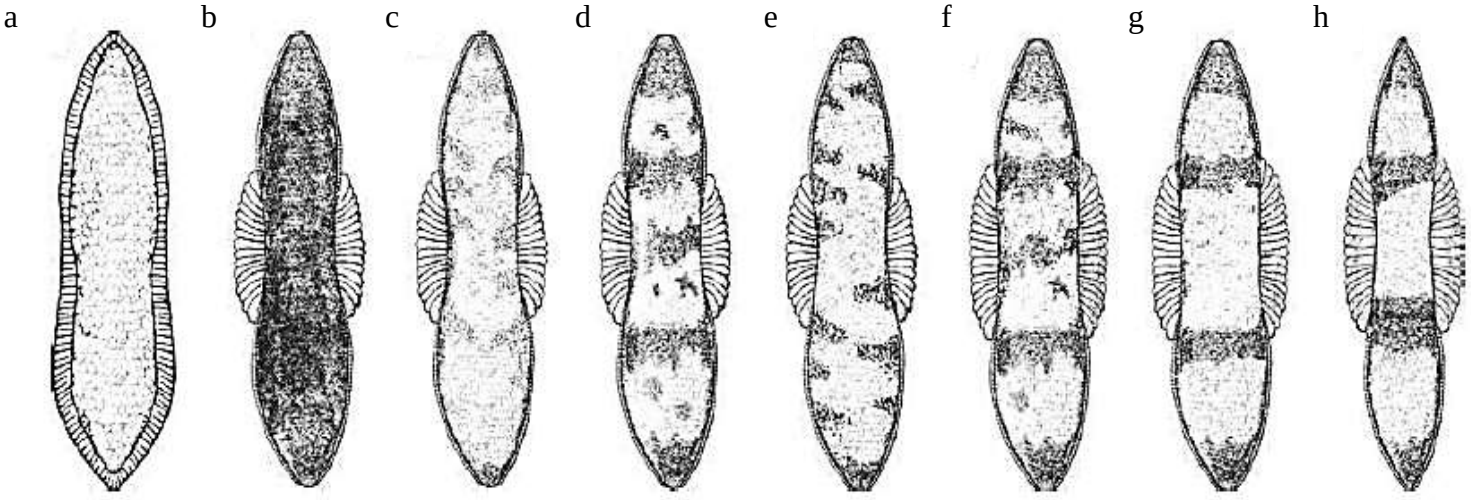


Figure 1.1. Eggs of *Anopheles maculipennis* complex: a. *An. sacharovi*, b. *An. melanoon*, c. *An. atroparvus*, d. *An. subalpinus*, e. *An. labranchiae*, f. *An. messea*, g. *An. maculipennis s.s*, h. *An. beklemishevi* (Becker *et al.*, 2003)

Shute, 1947). However, the disponibility of eggs is not always possible; in addition, many species are conspecific and the wing ornamentation cannot be useful to separate closely related species. The novel molecular methods that use DNA barcoding are currently developed to distinguish *Anopheles maculipennis* s.l subspecies. The COI-based DNA barcoding was found useful to complement morphological identification of mosquito species (Chan *et al.*, 2014; Werblow *et al.*, 2016). Laboudi *et al.* (2011) have used COI analyses to determine if the Moroccan *Anopheles maculipennis s.l* (*An. labranchiae* and *An. sicaulti*) populations are genetically isolated from those of Algeria, and investigate the presence of more than one member of the Maculipennis Group in the Arab Maghreb. Further, the second internal transcribed spacer (ITS2) of ribosomal DNA (rDNA) was marked as an intraspecific sequence variation in *Anopheles maculipennis* s.l subspecies and was exploited as specific molecular method for maculipennis species differentiation (Proft *et al.*, 1999; Sevgili and Simsek, 2012; Gholami *et al.*, 2019; Tagliapietra *et al.*, 2019).

1.2.1.2 *Anopheles cinereus* complex

Anopheles cinereus cinereus was mentioned as *An. cinereus* in previous inventory studies in Ethiopia (Animut *et al.*, 2012), Southwestern Asia and Egypt (Glick, 1992). While in the past, *Anopheles cinereus hispaniola* was mentioned as *An. hispaniola* in inventory studies in Spain (Galliard, 1928), Morocco (Callot *et al.*, 1946; Ristorcelli *et al.*, 1946; Guy, 1962), Nord-Tchad (Chabaud *et al.*, 1959). Nevertheless, in the recent studies in Morocco the same species is mentioned as *An. cinereus* (Trari *et al.*, 2004; Faraj *et al.*, 2009), further in an update checklist of mosquitoes of Morocco published by Trari *et al.* (2017) they mentioned *An. c hispaniola* as a previous usage or a synonym of *An. cinereus*. *An. c hispaniola* was confirmed by numerous researches to be a subspecies of *An. cinereus;* the separate distribution of *An. c cinereus* (Arabian Peninsula, Ethiopia and Sudan to Cape Province) and *An. c hispaniola* (Mediteranean region, Equatorial Africa) appeared to be the only criterion to distinguish the two subspecies (Ramsdale, 1998). No works on genetic evidences were found.

1.2.2 Culicinae complexes

1.2.2.1 *Ochlerotatus caspius* complex

Certain authors still conjoin the *caspius* species to the genus *Aedes*; whereas, in the last valid list by Harbach (2013a) *caspius* is belong to the *Ochlerotatus* genus. *Ochlerotatus caspius* seems to be a complex species, the first detection of the two forms of *Oc. caspius*

form A and form B was in Italy (Becker *et al.*, 2003). A more recent study conducted by Wassim *et al.* (2013) confirmed the existence of a genetic distance between the two morphologically identical forms of *Oc. caspius*.

1.2.2.2 *Culex pipiens* complex

The identification of the members of the complex *Culex pipiens* s.l was handled by numerous authors (Di Luca *et al.*, 2016; Shaikevich *et al.*, 2016; Zittra *et al.*, 2016; Beji *et al.*, 2017) based on polymorphisms in COI and the flanking region of CQ11 microsatellite locus (Bahnck and Fonseca, 2006). While the identification of subspecies, biotypes and physiological variants remained inconstant, Harbach (2012) reviewed the taxonomic history of the complex *Culex pipiens* s.l; he concluded that: *Cx. p pipiens* and *Cx. p quinquefasciatus* are separate species, *Cx. molestus* is a phenotypic and physiotype of *Cx. p pipiens*; and that there is no evidence that suggest other species included in the Pipiens Assemblage.

1.3 Mosquitoes in North Africa: review

Since the detection of the involvement of mosquitoes in the disease transmission, inventories in different regions of the world were conducted in order to define a list of local mosquito species. These lists are useful to highlight the list of vector species in the investigated area and serve as a tool of good control; they help likewise in recognizing new invasive species. We mention here the latest lists provided in new reviews in North Africa; in Tunisia (Tabbabi *et al.*, 2017), 43 mosquito species were reported. They have recorded *Anopheles labranchiae* as the malaria vector in Tunisia. Other inventories reviews were published in Morocco (Trari *et al.*, 2017) and Libya (Gawhari *et al.*, 2018). So far, there is no published article that reviews the mosquitoes of Algeria. However, investigations of mosquito fauna were carried out in Algeria in different regions. No reference publications were supported with molecular identification. Lafri *et al.* (2014) have conducted a mosquito survey in 15 departments; 17 species were identified: *Ae. Albopictus*, *Aedimorphus vexans* (Meigen 1830), *An. labranchiae*, *An. multicolor* (Cambouliu 1902), *Cx. deserticola* (Kirkpalrick 1924), *Cx. hortensis* (Ficalbi 1889), *Cx. pipiens*, *Cx. territans* (Walker 1856), *Cx. theileri*, *Cs. litorea* (Shute 1928), *Cs. longiareolata*, *Oc. coluzzii* (Rioux, Guilvard & Pasteur 1998), *Oc. detritus* (Haliday 1833), *Oc. dorsalis* (Meigen 1830), *Oc. flavescens* (Muller 1764), *Oc. geniculatus* (Olivier 1791) and *Uranotaenia unguiculata* (Edward 1913). Bouabida *et al.* (2012) has

recorded additionally *Cx. laticinctus* (Edwards 1912), *Cx. perexiguus* (Theobald 1901), *Cs. annulata* (Schrank 1776), and *Cs. subochrea* (Edwards 1921).

2 Mosquito biology and ecology

2.1 Mosquito biology

The mosquito life passes through two important stages: aquatic and aerial. In the aquatic stage, the mosquito develops from an egg into a pupa through four larval instars. The mosquito female lays the eggs on the water surface and sometimes on wet soil or other areas prone to flooding from rain, either individually or in rafts depending on the female's species.

2.1.1 Oviposition

After the blood meal, mosquito females head for the water surface to lay their eggs. The oviposition behavior depends on mosquito species; the majority of mosquito species laid their eggs on the water surface of their preferred breeding sites singly like in *Anopheles* or in rafts like in *Culex* and *Culiseta*. Other species of *Aedes* and *Ochlerotatus* lay their eggs singly on moist soil (Figure 1.2.)

The life of mosquitoes begin when the egg find the appropriate conditions to hatch, that is why mosquito females select their breeding sites carefully. *Trichoprosopon digitatum* (Rondani 1848) mosquitoes lay their eggs in small pots and guards their eggs until the hatch, even when choosing their breeding sites, they prefer sites that contain a female guarding their eggs (Sherratt and Church, 1994). In another study, a laboratory experiment showed that *Toxorhynchites amboinensis* (Doleschall 1857) females avoided laying in pots containing starved larvae (Linley, 1988). *W. smithii* (Coquillett 1901) showed in an experiment conducted by Heard (1994), more probability to lay eggs in experimental water-holding

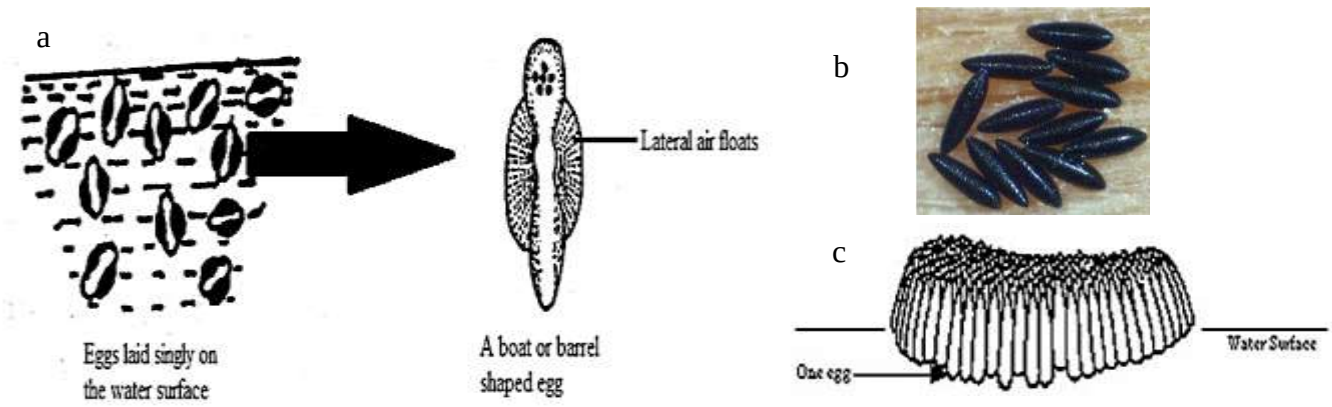


Figure 1.2. Mosquito eggs: a) *Anopheles*, b) *Aedes*, c) *Culex* and *Culiseta* (Mehlhorn, 2001) modified.

pitcher plant leaves containing mosquito or midge larvae . *Cs. longiareolata* females prefer to lay their eggs in sites characterized by food availability; they are simultaneously prudent to lay in sites containing developed stages of tadpoles according to an experience conducted by Blaustein and Kotler (1993). The existence of late stages of conspecific larvae produced negative effect in the oviposition selection of *An. coluzzii* (Coetzee and Wilkerson 2013) (Mwingira *et al.*, 2019). *Cx. annulirostris* (Skuse 1889) avoided oviposition in water derived from habitats containing fish even with the absence of fish in the experiment containers (Hanford *et al.*, 2019). *Cx. quinquefasciatus* is a mosquito species may relies on olfactory clues to choose oviposition sites, while visual clues may play more important role in oviposition sites for other *Culex* species (Shin *et al.*, 2019). *Cx. restuans* (Theobald 1901) showed an oviposition preference to sites rich of sod and grass, while *Cx. pipiens* prefer sites rich of rabbit chow according to an experiment conducted by Lampman and Novak (1996). *Orthopodomyia* species in Illinois are usually found in artificial breeding sites according to Hanson *et al.* (1995). *Coquillettidia* species need the availability of host plants in the water to breath, therefore, the existence for *Coquillettidia* species is always associated with the presence of larval host plants; further, Sérandour *et al.* (2010) confirmed that also the water quality affect the habitat selection in *Coquillettidia* species where they prefer water with low salt concentration and neutral pH. Another research on *Cq. richiardii* (Ficalbi 1889) showed that the environmental light and oxygen concentrations are influencing factors for larval attachment, and that a dark anoxic environment is more favorable for *Cq. richiardii* larvae; the same experiment leads that the Carbon dioxide produced by plant roots attract *Cq. richiardii* larvae (Sérandour *et al.*, 2006). The majority of the species previously reported laid their eggs in surface water, stocked or fluent. In contrary, *Ae. vexans* (Meigen 1830) and *Oc. sticticus* (Meigen 1836) from the floodwater mosquitoes, lay their eggs on suppose flood areas (Gjullin *et al.*, 1950). Other mosquito species that behave similarly to floodwater mosquitoes as *Oc. communis* (de Geer 1776), *Oc. punctor* (Kirby 1837) and *Oc. cantans* (Meigen 1818) are snow-pool mosquitoes; these species lay one time per year, and their eggs need to be frozen before hatching (Dargahi, 2019).

2.1.2 Larvae

After mosquito eggs hatch, larvae live in water from 1 to 7 days, sometimes more if the conditions are not optimal, and likewise, some species may hibernate during the larval stage. Mosquito larvae lose their skin four times and pass through four instars L1, L2, L3, and L4. During their aquatic life, mosquito larvae come to the surface to breathe through a siphon in

Culicines and through the spiracles in *Anopheles* (Figure 1.3). Because of the difference in the food mechanism; the Culicinae rest at an angle to the water surface, while *Anopheles* species rest in parallel to water surface (Figure 1.4). The spiracle lobs within the respiratory segment opened when breathing and closed when larvae leave the water surface. The *Coquillettidia* and *Mansonia* breath in another way, they submerge to roots and aquatic plants (Figure 1.5), where they pierce plant tissues with hooks and teeth contained in the distal part of spiracles to get oxygen (Becker *et al.*, 2003). Just as mosquito species differ slightly in breathing mechanisms, they may differ mainly in their feeding behavior. In a recent laboratory experiment, Yee *et al.* (2004) observed a difference in foraging behavior between *Aedes albopictus* and *Ae. aegypti*, where *Ae. albopictus* showed superior resource-harvesting ability and therefore greater survivorship regarding *Ae. aegypti*. Further, the first instar larvae (L1 and L2) of *Ae. aegypti* showed higher digestion of food comparing to *Ae. albopictus*, while the digestive enzyme were more active in further instars (L3 and L4) of *Ae. albopictus* (Ho *et al.*, 1992). On the other hand, *Anopheles* species reach the surface of the water to breath as previously mentioned; however, a study conducted by Merritt *et al.* (1992) on *An. quadrimaculatus* (Say 1824) discussed the interfacial feeding of *Anopheles* larvae. The experience showed that *An. quadrimaculatus*, while positioned at the water surface rotates its head against the air and moves its lateral palatal brushes to create a water current that directed the nutrient particles towards the head.

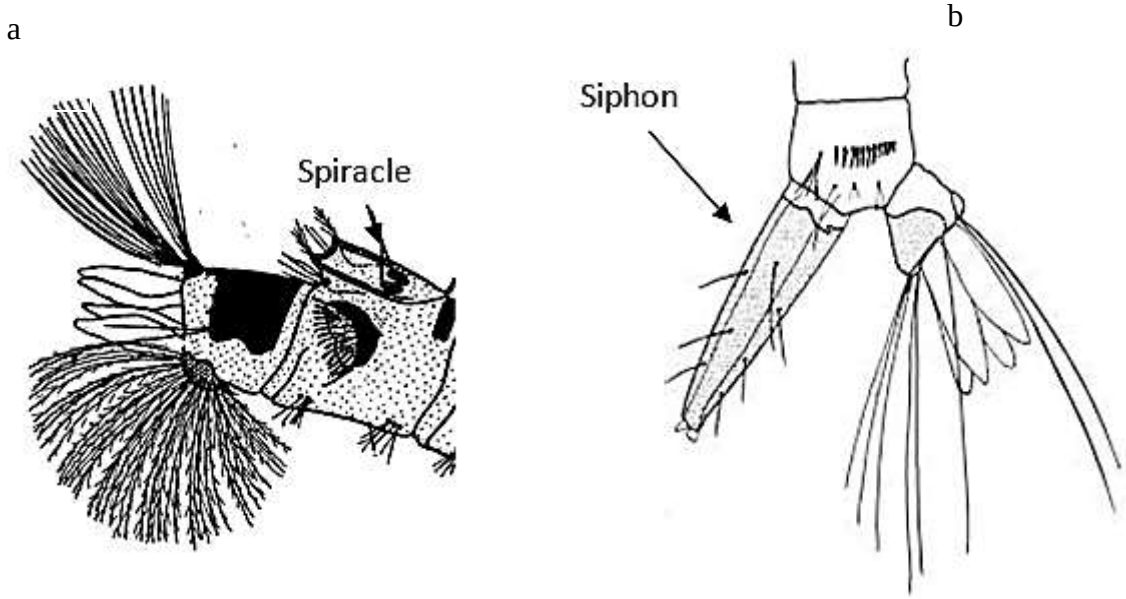


Figure 1.3. Respiratory segment a) Anopheles: spiracle, b) Culicinae: siphon (UCR, 2019) modified.

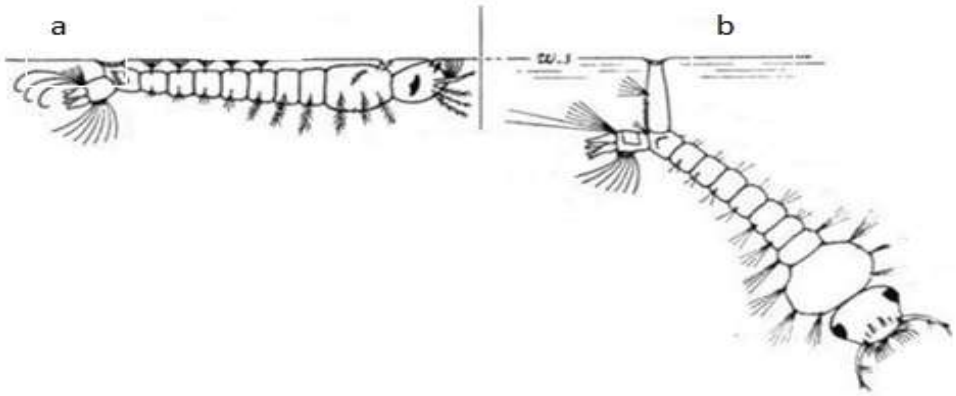


Figure 1.4. Mosquito larvae resting, a) Anopheles, b) Culicinae (Mehlhorn, 2001) modified.

Figure 1.5. *Coquillettidia* and *Mansonia* submerge to aquatic plants (ICPMR, 2019).

2.1.3 Pupae

When mosquito larvae achieve the fourth instar L4, they molt and become nymphs or pupae. The pupae stage is the interval between larvae and adult stages in mosquitoes; this stage extends from 1 to 4 days. Pupae do not eat; however, they reach the water surface to breath exactly as larvae. Pupae breathe using respiratory trumpets (Figure 1.6), which are modified in *Coquillettidia* and *Mansonia* to be able to penetrate plant tissue and take the oxygen from the plant aerenchyma.

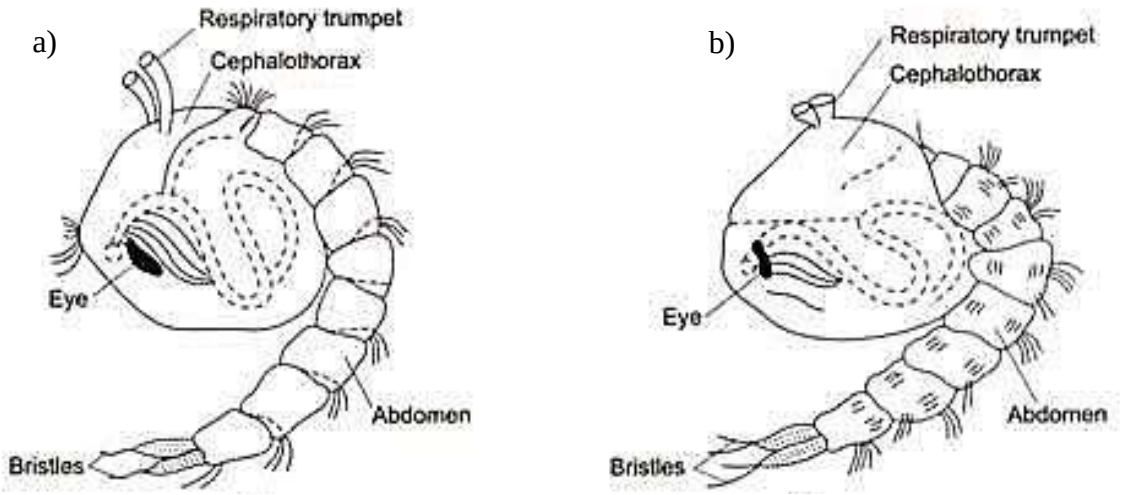


Figure 1.6. Mosquito pupae: a) *Culex*, b) *Anopheles* (Becker *et al.*, 2003) modified.

2.1.4 Adult

When *Anopheles* adults rest on solid surfaces, their body forms an angle of 40° to 90°; whereas, the Culicinae rests in almost a parallel position with the resting surface (Figure 1.7). The adult mosquito leaves the water surface to start aerial stage, they are next capable to fly for long distances. In general, both mals and females feed on nectars; nevertheless, the food composition may afect differently the longivety of the tow sexes. According to an experiment conducted by (Vrzal *et al.*, 2010), *Culex quinquefasciatus* adults live longer when exposed to diet high in amino acid and sugar, whereas larval diet quantity affect differently males and females.

Male and females mate after 3 to 5 days after emergence. The male life is relatively short as its role finish by mating. On the other hand, the life duration of mosquito females exstend more, and it can vary by species the fact that females are responsible for laying and ensuring generations' survival; females need therefore a blood meal to lay eggs (anautogeny). However, the *molestus* form of *Culex pipiens* can autogenously reproduce; it means that they can reproduce without the need for a blood meal (Gao *et al.*, 2019). The tow form of *Cx. pipiens* differ not only in autogeny (pipiens form) and anautogeny (molestus form), but also in mating mechanisms, stenogamy (mating in a restricted space) in molestus form and eurygamy (mate in open space) in pipiens form; and possibility of diapauses overwinter for pipiens form contrary to molestus form which does not hibernate (Becker *et al.*, 2012; Korba *et al.*, 2016; Shaikevich *et al.*, 2016).

Mosquito females can bite indoor (inside habitation) or outdoor (ouside habitation) as *Anopheles gambiae* s.s. (Faye *et al.*, 1997), an *Aedes* species (Mukwaya, 1974; Kamgang *et*

al., 2012). After the blood meal, where mosquito females rest is of important information since it defined whether outdoor spraying is more efficient or indoor spraying. Mosquito females can rest indoor as *An. parensis and An. funestus* (Gillies and De Meillon, 1968; Mouatcho *et al.*, 2007), *An. arabiensis* (Sharp and le Sueur, 1991) *Ae. aegypti* (Pant and Yasuno, 1970; Perich *et al.*, 2000; Chadee, 2013; Dzul-Manzanilla *et al.*, 2016) and molestus form of Culex pipiens assemblage (Gomes *et al.*, 2013) or outdoor. A study aimed by Das *et al.* (2004) mentioned the outdoor collection of *Cx. tritaeniorhynchus, Cx. bitaeniorhynchus* and *Cx. gelidus* and in indoor collection of *Cx. quinquefasciatus, Cx. tritaeniorhynchus, An. vagus* and *An. subpictus*. While some species may resting and biting both indoor and outdoor as in *An. gambiae* s.s. (Omer and Cloudsley-Thompson, 1970; Lines *et al.*, 1986; Faye *et al.*, 1997; Okech *et al.*, 2003; Odiere *et al.*, 2007), and some *Culex* species (de Meillon *et al.*, 1967; Gad *et al.*, 1995; Das *et al.*, 2004; Gomes *et al.*, 2013)

Generally, mosquito adults behavior differ by species just as in the case of larvae; in an ancient study aimed by Laurence (1960), the biology of *Mn. africana* (Theobald 1901) and *Mn. uniformis* (Theobald 1901) has been compared. The authors noted that the *Mn. uniformis* is more active after the blood-meal, the two species do not interbreed, and that they show the same adult behavior at mating, feeding and when ovipositing; they are both stenogamous. Likewise, Taye *et al.* (2016) noted variance within *Anopheles* species behavior. The differences in mosquito specie' behavior may appear equally in their feeding preferences, anthropophily (prefer human blood) or zoophily (prefer animal blood), and their association to human habitat, exophilic (independent of humans) or endophilic (associated with humans). In a more recent study the behavior among *Anopheles* species was studies by Trung *et al.* (2005),

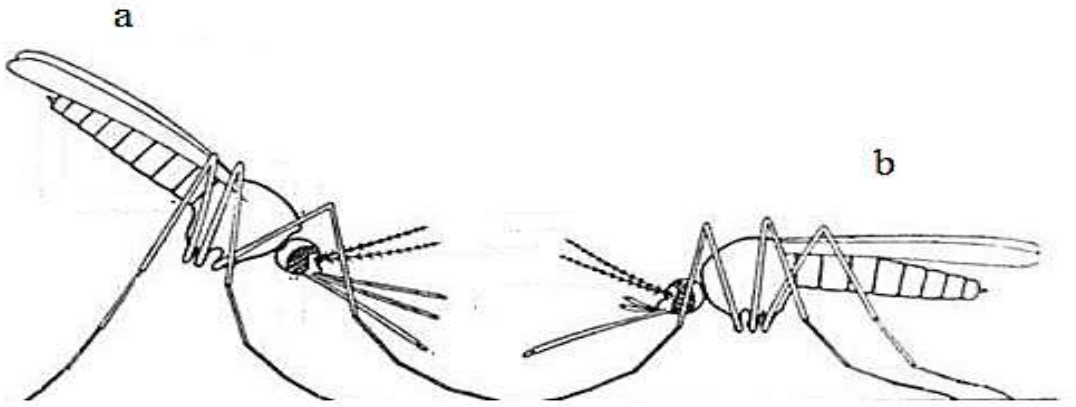


Figure 1.7. Mosquito adult resting: a) *Anopheles*, b) Culicinae. (Mehlhorn, 2001) modified.

they noted that *An. minimus, An. campestris, An. nimpe, An. sinensis, An. maculatus, An. aconitus, An. dirus,* and *An. sundaicus* differs on term of food preferences, degree of anthropophily, and biting activity. *An. gambiae* s.s. and *An. funestus* are characterized by high anthropophily (Seyoum *et al.*, 2012; Dadzie *et al.*, 2013), while *An. arabiensis* note more flexibility in its feeding preferences (Takken and Verhulst, 2013) however it was considered before as more zoophilic (Mnzava *et al.*, 1995) it is also highly endophilic (Mahande *et al.*, 2007). *Aedes* species showed more plasticity toward host preferences (Lyimo and Ferguson, 2009); likewise, *Cx. pipiens* from *Culex species* showed tendency for four mammal species primarily humans, in addition to avian hosts (Hamer *et al.*, 2008; Gomes *et al.*, 2013). Other mosquito host preferences in Cameron (Rickenbach *et al.*, 1974)

2.2 Characterization of mosquito species

The identification of mosquito species is of critical importance in the determination of vector capacity. The basic identification articulates on the morphological characteristics, however, other features can distinguish some genera in terms of biological and behavioral properties.

2.2.1 Identification based on pictorial keys

The morphological identification of mosquitoes is based on pictorial keys; therefore, it is substantial to recognize the mosquito anatomy before starting any identification.

2.2.1.1 Larvae morphology

The larva is composed of a head, thorax, abdominal segments, saddle and siphon (except for *Anopheles*, *Bironella* and *Chagasia*). The larvae body is covered with setae where the localization and number form pictorial keys that can be used to differentiate species. Regardless the difference in the respiratory segment in *Anopheles* and *Culicines*, *Anopheles* species are characterized with palmate setae which are absent in *Culicines* species (Figure 1.8).

2.2.1.2 Adult morphology

Mosquito adults differ from other insects in the form: Head, abdomen and thorax covered with setae and scales, and the scaled proboscis is longer than the thorax.

The shape and color of the different parts of mosquito females are important elements in the morphological identification; the head; the thorax, the legs and the wings (Figure 1.9). The head is constituted of a proboscis, maxillary palpus and antenna (Figure 1.10). The thorax contains separate areas that include discriminatory setae and the scutellum (figure 1.11). The abdomen is constituted of eleven segments, the dorsal plate or tergite is covered with setae, scales and lateral tuffs (Figure 1.12). The wings possess a venation that is characteristic in some species; consists of longitudinal veins, connected by six cross veins (Figure 1.13). Contrariwise, the mosquito males' identification is based only on genitalia characterization.

2.2.1.3 Pictorial keys

To obtain a good morphological discrimination, it will be better to go through ordered steps. The better method to do this is to use Xper2 software with the database provided by Gunay *et al.* (2018) which is regularly updated; in addition to other literature support as A monograph of the Culicidae of the world (Theobald, 1901), Mosquitoes and their control (Becker *et al.*, 2003) and neotype designations of mosquito species (Huang, 1968; Sirivanakarn and White, 1978; Harbach *et al.*, 1984; Harbach, 1992; Flores-Mendoza *et al.*, 2004; Rueda *et al.*, 2004; Rueda *et al.*, 2005; Harbach and Chen, 2006; Gonzalez and Sallum, 2009).

Mosquito species can be recognized in both larval and adult stages. However, some species are very close morphologically in the larvae stage which recommends rearing the mosquito into the adult stage to identify it. Nevertheless, they exist general keys that allow a facilitate genera identification.

2.2.1.3.1 Larvae genera identification

Looking to Figure 1.14, the principle genera could be recognized from the form of the respiratory segment of mosquito larvae; after, we can verify the absence or the presence of abdominal plates. The position and insertion of siphonal tufts is likewise a very important character to differentiate the principal genera *Culex, Aedes, Culiseta,* and *Coquillettidia* (Figure 1.14). The identification of mosquito larvae can be less efficient in some cases because of the similarity in some species' morphology; therefore, the resort to adult identification becomes necessary.

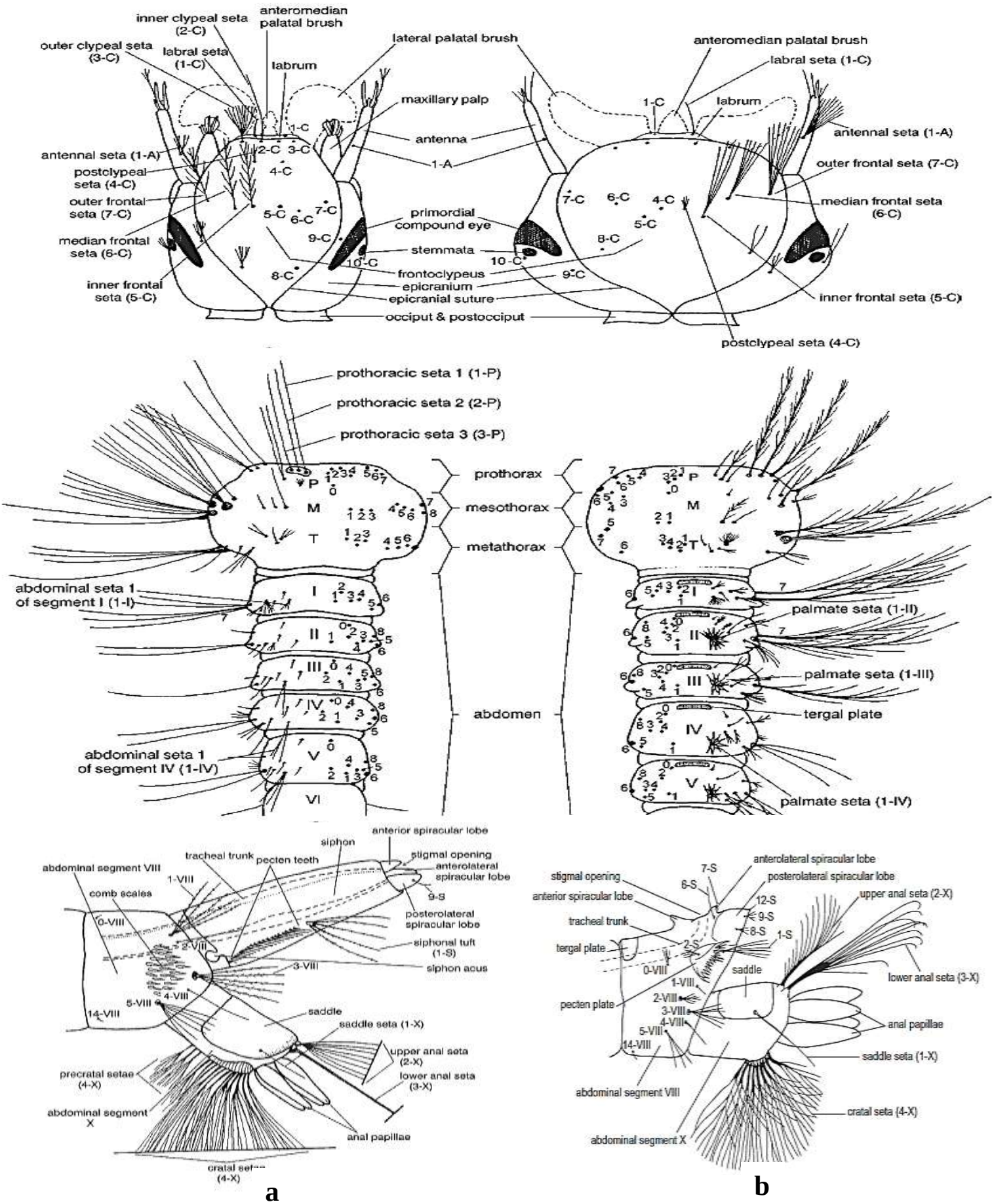


Figure 1.8. Mosquito larvae morphology: a) Culicinae, b) Anophelinae (Becker *et al.*, 2003).

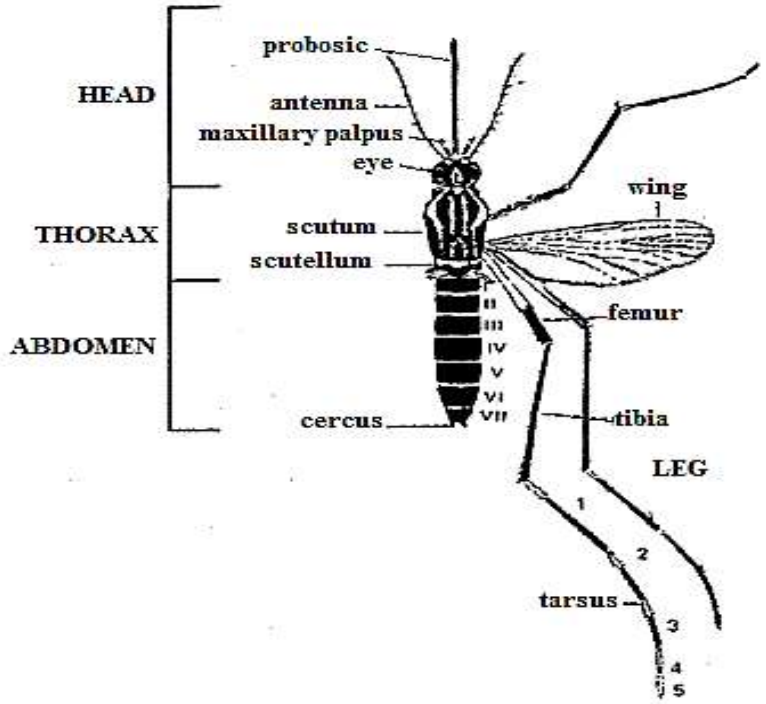


Figure 1.9. General morphology of adult mosquitoes (Mehlhorn, 2001).

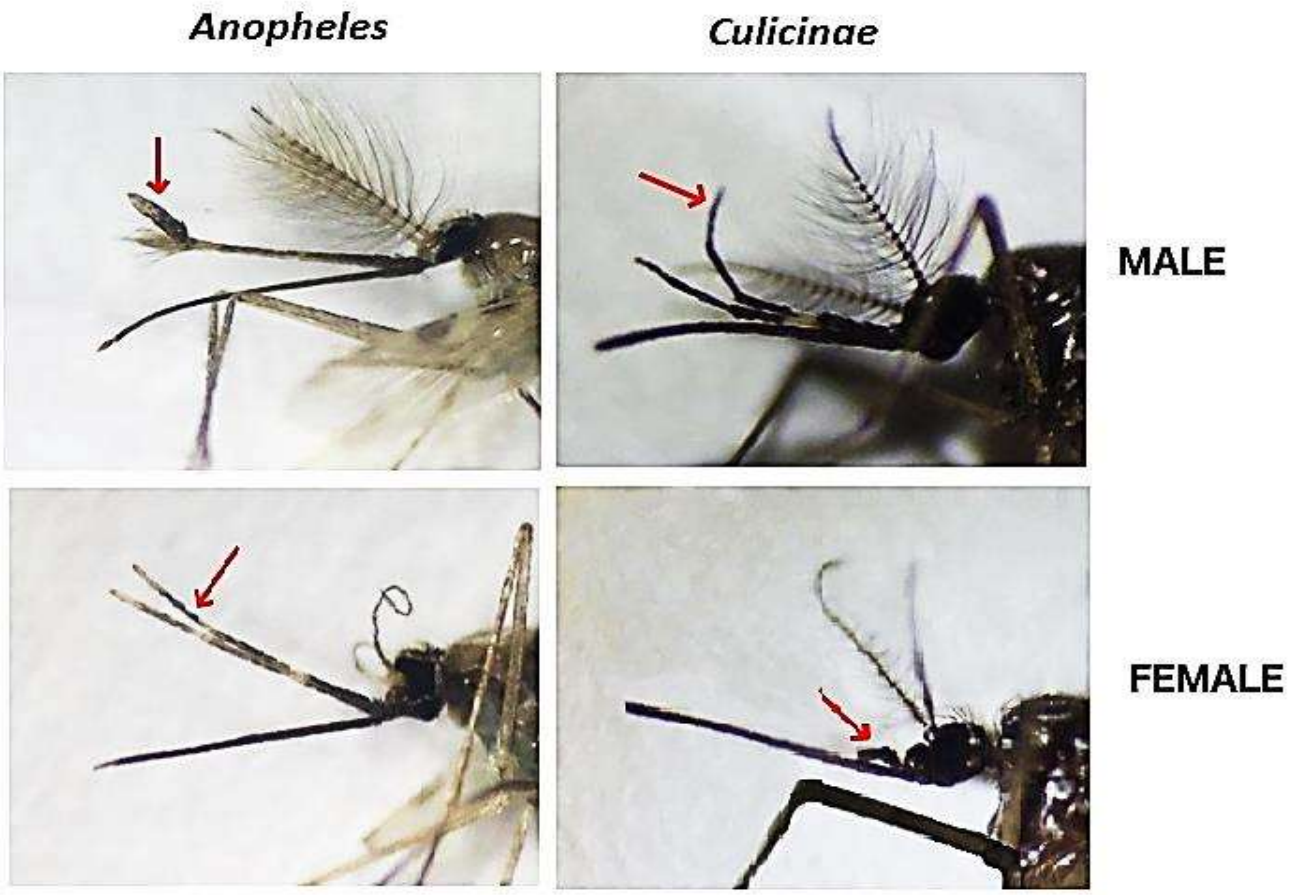


Figure 1.10. Head of male and female *Anopheles* and Culicinae adults (Gunay *et al.*, 2018)

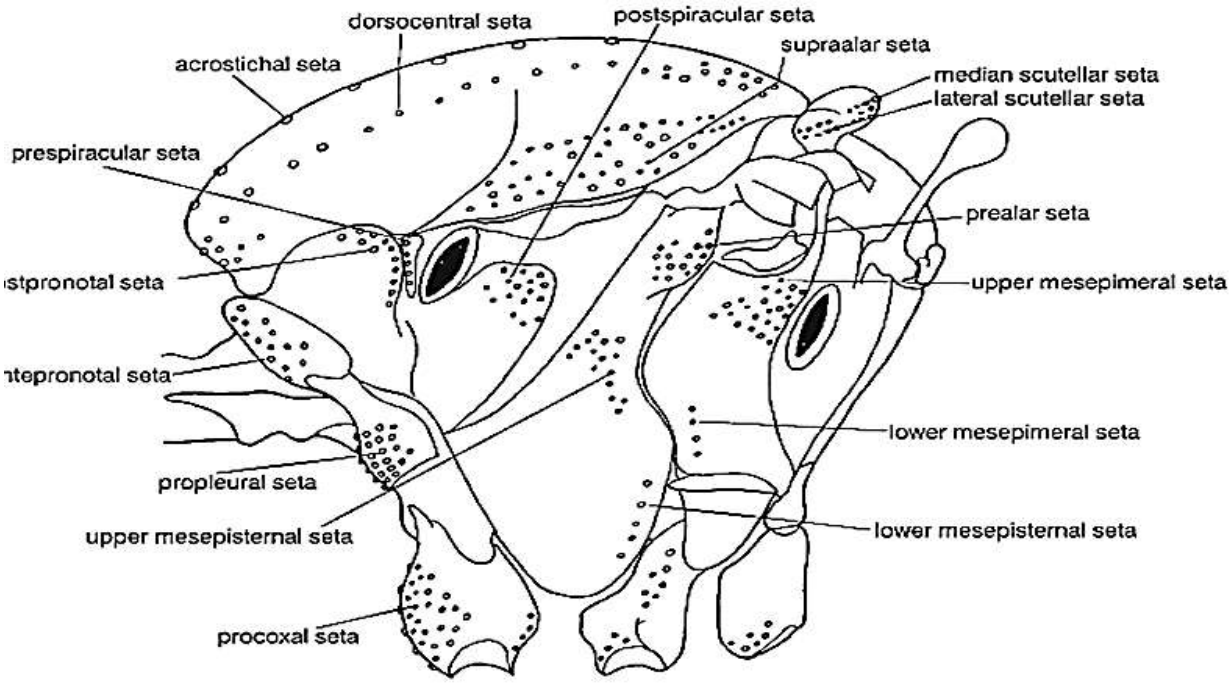


Figure 1.11. Thorax setation in lateral view (Becker *et al.*, 2003)

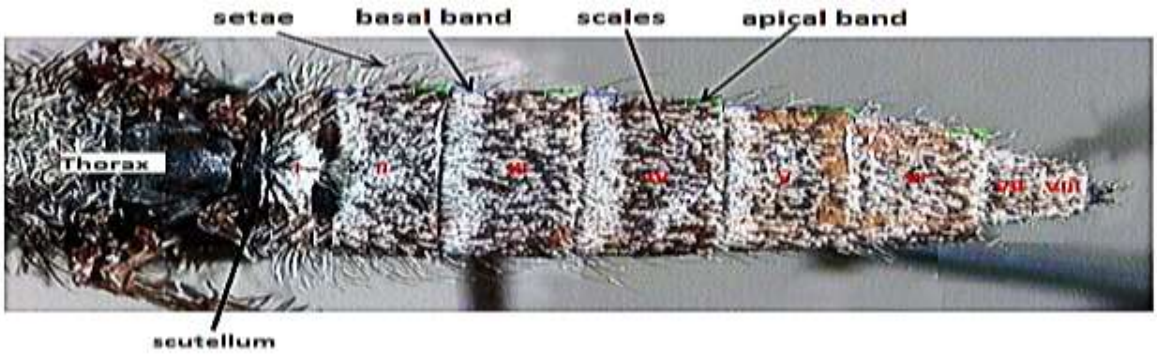
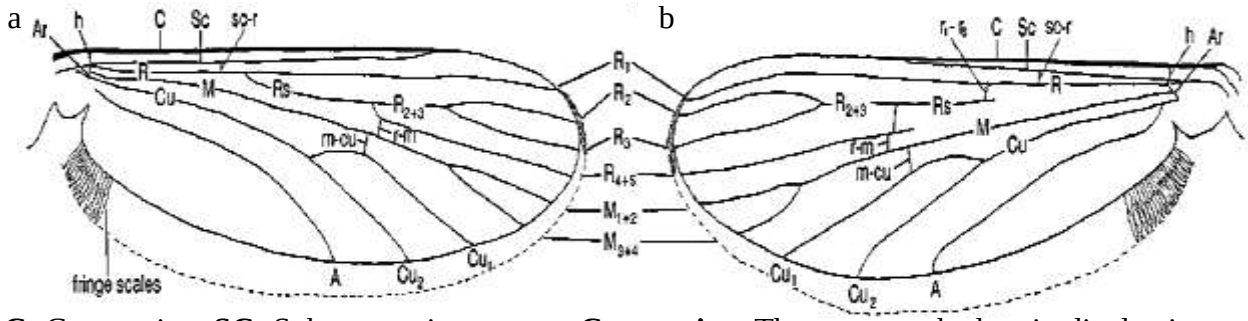


Figure 1.12. General morphology of mosquito adult abdomen (Gunay *et al.*, 2018) modified.



C: Costa vein **SC:** Subcosta vein
R: Radius vein forks into R1
Rs: Radial vein sector forks into R_{2+3} and R_{4+5}
R_{2+3} forks into R2 and R3
M: Media vein forks into M_{1+2} and M_{3+4}
Cu: Cubitus vein forks into Cu_1 and Cu_2
A: Anal vein

Cross veins: That connect the longitudinal veins
h: Humeral vein
Ar: Arcilus
sc-r: Subcostal-radial vein
r-r_s: Sectorial vein
r-m: Rafio-medial vein
m-cu: Radio-medial vein

Figure 1.13. Wing venation : a) Culicinae, b) Anophelinae (Becker *et al.*, 2003) modified.

2.2.1.3.2 Adult genera identification

The identification of mosquito adults is based on the external morphology in females and the genitalia in males. The Anophelinae females are easily recognized by their long maxillary palpus and their simple Scutellum. If the mosquito's maxillary palps are short we can look to the wings; the *Uranotaenia* genus has wings without a fringe scale. For the rest of the genera, the legs can serve as an organ with specific characters that can differentiate *Orthopodomyia* from *Culex, Aedes, and Culiseta* (Figure 1.15). It exist pictorial keys for the identification of mosquito female species; however, it is impossible in many cases to separate some close species. In this case, we can refer to the males' identification. The general form of males' hypopygium is different in *Aedes, Culex* and *Anopheles* genera as illustrated in Figure 1.16.

2.2.2 Molecular Identification

The morphological identification of mosquito species needs long experience; and even for experts, species morphologically close are sometimes undistinguishable. The development of molecular approaches facilitated the mosquito identification and provided more ascertained results. The CX1 gene newly annotated cytochrome oxidase subunit 1 (COI) is the marker used for mosquito systematic (Hebert *et al.*, 2003); the amplification of the fragment 5' of COI gene used for DNA barcoding proved its ability to discriminate mosquito species. The COI locus was used to identify 28 indigenous and non-indigenous mosquito species by Werblow *et al.* (2016), it was used to confirm the presence of a single member of *Anopheles maculipennis* s.l group in Morocco and Algeria (Laboudi *et al.*, 2011), and it was used in multiple complementing morphological identification of mosquito species (Talbalaghi and Shaikevich, 2011; Chan *et al.*, 2014; Engdahl *et al.*, 2014; Versteirt *et al.*, 2015). However, some complex species needs more specific microsatellite markers to be separated. Although COI barcoding can, in some cases, differentiate the members of *Anopheles maculipennis* s.l complex (Laboudi *et al.*, 2011; Laboudi *et al.*, 2014), the marker ITS-2 ribosomal DNA is used us supplement analyze that provide more precise results in distinguishing *Anopheles maculipennis* s.l members (Dousti *et al.*, 2006; Danabalan *et al.*, 2014; Gholami *et al.*, 2019). While COI barcoding alone was found not able to separate the members of Culex pipiens assemblage, this is why, a supplement microsatellite CQ11 is currently used (Bahnck and Fonseca, 2006; Amraoui *et al.*, 2012; Di Luca *et al.*, 2016).

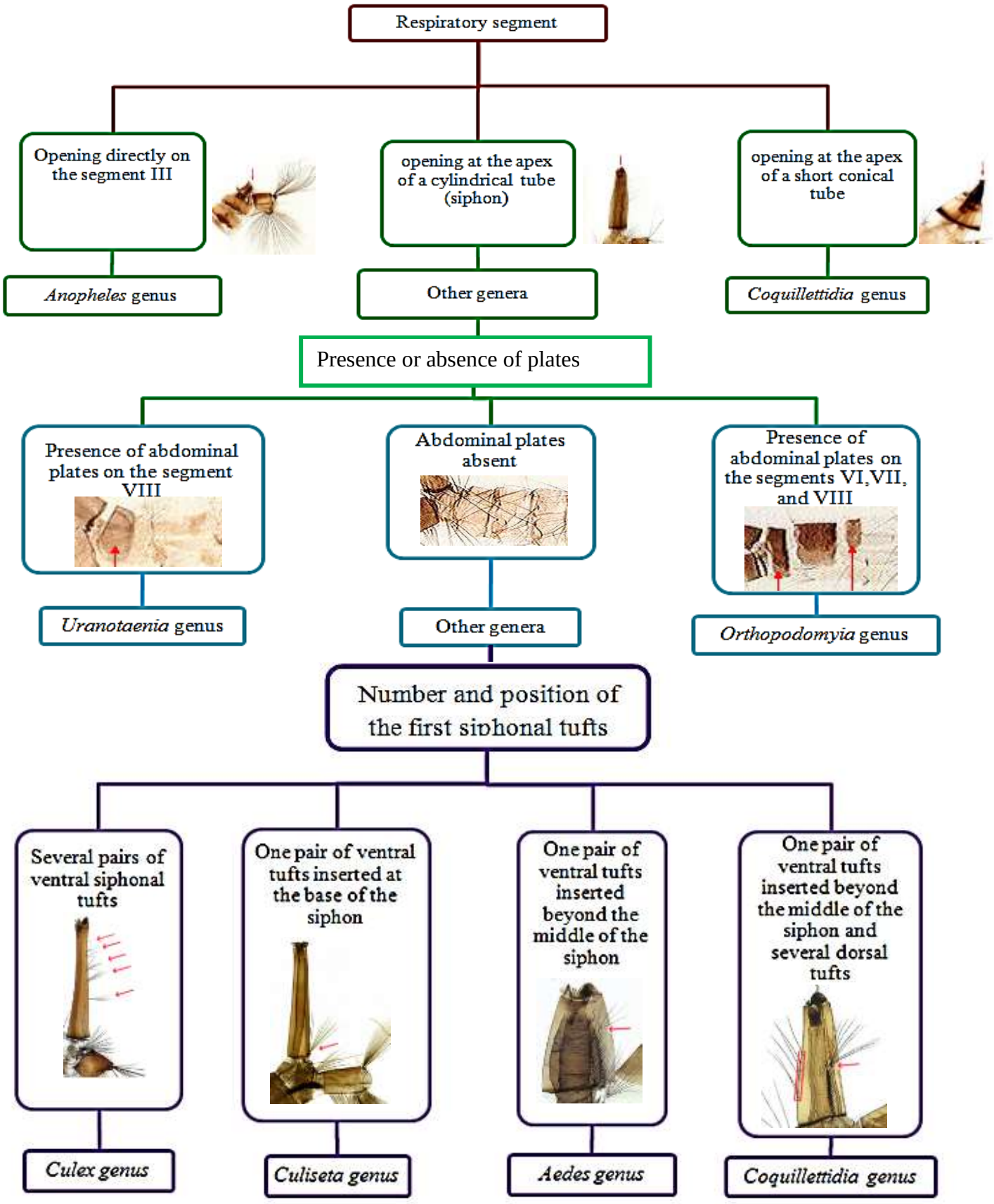


Figure 1.14. Pictorial keys for the identification of larvae mosquitoes (Culicidae) genera (photos obtained from Gunay *et al.* (2018)).

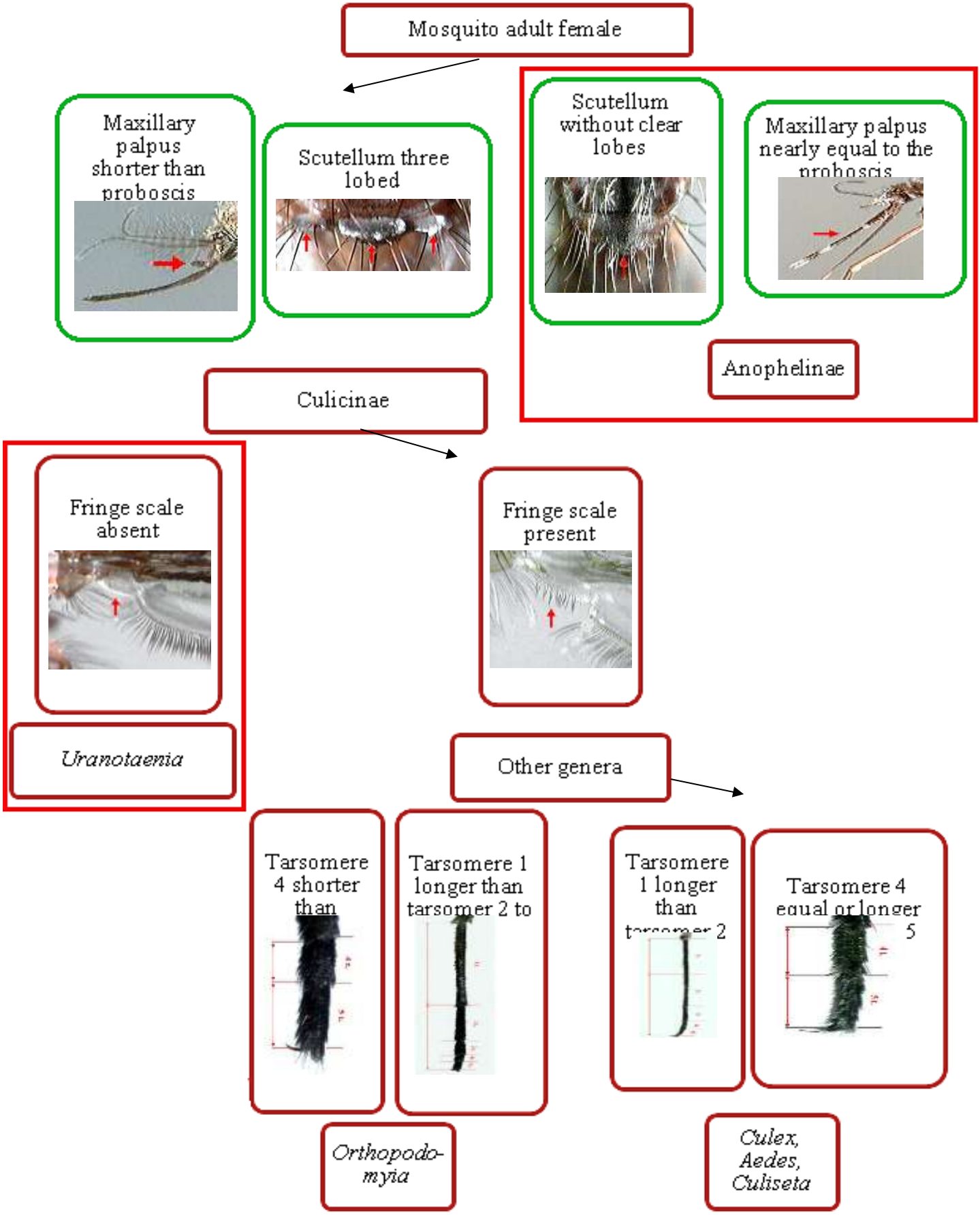


Figure 1.15. Pictorial keys for the identification of adult mosquitoes (Culicidae) genera (photos obtained from Gunay *et al.* (2018)).

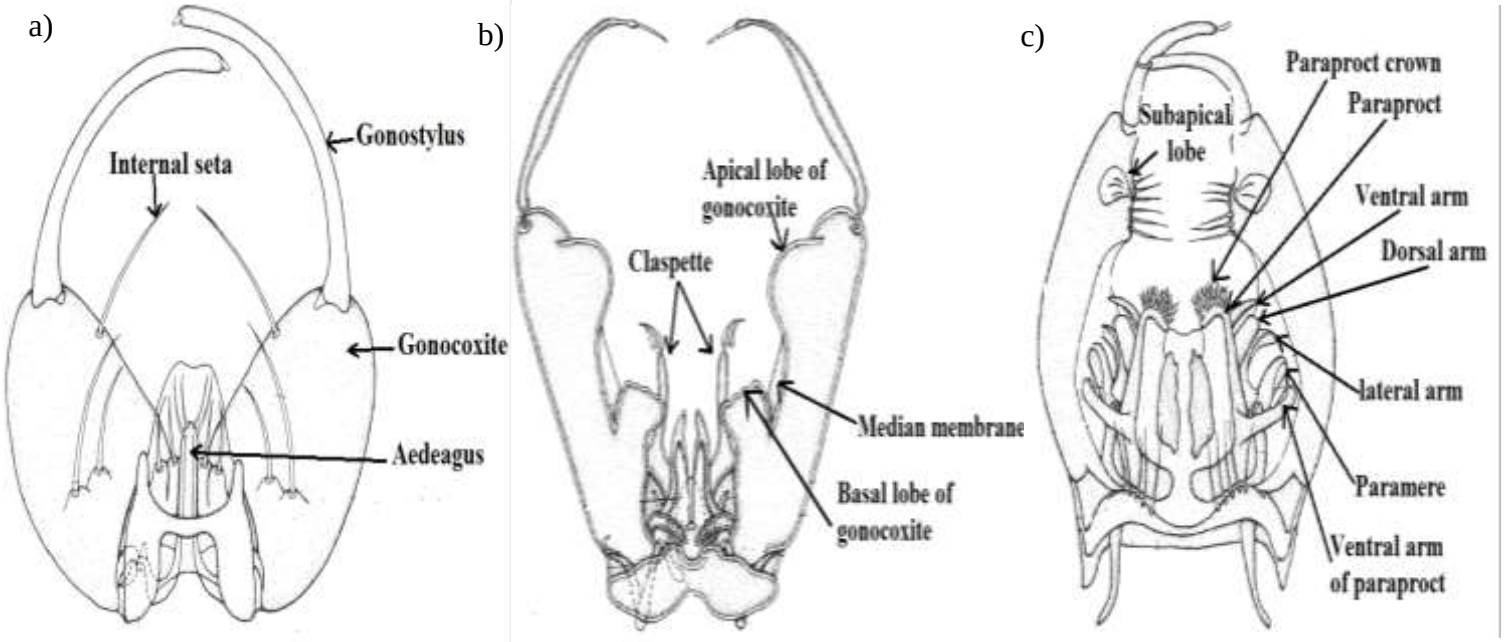


Figure 1.16. Mosquito male hypopygium: a) *Anopheles* genus, b) *Aedes/Ochlerotatus* genus, c) *Culex* genus ((Becker *et al.*, 2003) modified).

2.3 Mosquito ecology

Mosquito populations' dynamics are impacted with their environment, and various biotic and abiotic factors could lead to changes in their biodiversity. Therefore, the knowledge of the parameters of mosquitoes' biology as the breeding sites selection, development, survival and feeding of larvae, co-occurrence and predation, adult longevity...etc., in addition to the delimitation of important environment factors that affect mosquito populations, are of crucial importance for the development of novel methods for the biological control of mosquito vectors (Lees *et al.*, 2014).

2.3.1 Biotic factors

2.3.1.1 Breeding sites selection

Through the ecological and inventory studies conducted on mosquitoes, we noted habitat preferences of mosquito species. *Anopheles* larvae species are known to prefer presence in clear water (Tabbabi and Daaboub, 2017; Dom, 2019). More specifically, *Anopheles labranchiae* and *An. arabiensis* breed more in natural breeding sites and their larvae found more in natural and rural areas (Animut *et al.*, 2012; Boccolini *et al.*, 2012). However, *An. arabiensis* larvae can also be found in artificial containers and manmade ditches during the dry season (Hamza and Rayah, 2016). Likewise, *An. gambiae* and *An. darling* larvae were used to occur more in residual puddles rain puddles (Minakawa *et al.*, 2004; Etang *et al.*,

2016). While *An. darling* were encountered more frequently along river margins according to a larval habitat characterization study conducted in central America by Manguin *et al.* (1996). In the other hand, *Aedes* species have another affinity to breed in urban sites. Speaking in particular about tiger mosquito, *Aedes albopictus* was previously considered as a rural species (Higa, 2011); whereas it has adapted subsequently well with the urban and suburban environment (Wu *et al.*, 2010; Caputo *et al.*, 2012). However it breeds in natural or urban areas, *Ae. albopictus* females lay their eggs frequently in tires, brick holes, abandoned plastic containers, rock pools and tree holes (Simard *et al.*, 2005). In contrast, another study confirmed that the number of *Ae. albopictus* breeding sites was higher in urban than in rural areas, which affirm the ability of mosquito species and particularly invasive species to change their habitat preferences according to the environmental changes (Li *et al.*, 2014). For *Cx. pipiens* species we cannot the difference in habitat preferences of the two forms of *Cx. pipiens* assemblage, where molestus form prefers underground site with high organic values, in contrast, pipiens form colonizes a various aboveground breeding sites (Becker *et al.*, 2012).

2.3.1.2 Development and survival of larvae

The duration of larval development could be affected by several factors, Tun-Lin *et al.* (2000) found that the development rate and survival of *Aedes aegypti* (Linnaeus 1762) adults can be affected by the larval diet and the temperature of breeding sites. More in-depth researches were conducted to delimit the level of influence of temperature on immature stages. A study published by Rueda *et al.* (1990) found that the temperature between 20-34°C is the best range for better development of *Ae. aegypti* and *Cx. quinquefasciatus*. Kirby and Lindsay (2009) confirmed that low as well as high-temperature degrees, decrease the larvae development through a study conducted on *An. gambiae* and *An. arabiensis*. likewise, a recent study carried out by Johnson and Russell (2019) on *Cq. linealis* (Skuse 1889) and *Cq. xanthogaster* (Edwards 1924) confirmed that the development of mosquito larvae increases when temperature decrease. Further, the study conducted in laboratory conditions on *An. darlingi* (Root 1926) indicated that feeding larvae with higher food quantities, enhanced the longevity, bites frequency, blood meal duration and wing length (Araújo and Gil, 2012). Consequently, both temperature and larval diet have a major influence on the development and emergence of mosquitoes, and more on their vector capacity.

2.3.1.3 Interactions with co-occurring organisms

In the breeding sites, mosquito species may co-occur in free sites (absence of other living organisms); likewise, we can find mosquito species in the same site with aquatic insects, aquatic larvae, tadpoles or even fishes. The co-occurring organisms may act as predators, or in other cases, they can act as competitors or cannibals. The food competition exist especially between mosquito species larvae; a study carried out by Kirby and Lindsay (2009) has yielded that the survival of *An. gambiae* unaffected by the co-occurrence with *An. arabiensis*. Contrary, when reared with *An. gambiae*, the *An. arabiensis* survival reduced (20%) than when reared alone (57%). Tadpools are considered as well as important competitors of mosquito species, where they could reduce their density and survival (Mokany and Shine, 2002; Mokany and Shine, 2003). However they are not found to be predators of mosquito larvae (Weterings, 2015). Further, other research studies confirmed the existence of intra-instar cannibalism and predation between mosquito species; where an intra-instar cannibalism has been detected between *An. gambiae* and *An. stephensi* (Liston 1901), and intra-instar cannibalism and predation between the members of *An. gambiae* complex in laboratory experiences conducted by (Koenraadt and Takken, 2003); Porretta *et al.* (2016). Consequently, the competition, cannibalism and predation are important factors that control the density of mosquito larvae in the breeding.

2.3.2 Abiotic factors

2.3.2.1 Urbanization

Mosquito density is relatively related to the breeding sites' position. Frequently, the rural areas provide better conditions for mosquito species to breed and feed; therefore, the species richness in rural sites is higher than that of the urban sites. However, a study conducted in Britain confirmed that urbanization could influence the community composition, abundance and phenology of mosquitoes breeding, where the species richness in urban sites was found lower comparing to in rural sites but the density of mosquitoes was contrariwise higher (Townroe and Callaghan, 2014). In another study, Rubio *et al.* (2011) measured the incidence rate of mosquito breeding sites in urban areas, relying on used tires as habitats selected by the females of *Culex pipiens* and *Ae. aegypti*, where they found that 65.2 % of the water-filled tires were infested and that the abundance of *Ae. aegypti* was higher in largest cities. Cardo *et al.* (2018) found likewise that *Cx. pipiens* and *Cx. quinquefasciatus* adapted well to the urban sites.

2.3.2.2 Climate change

The abundance and repartition patterns of mosquito populations are influenced by climate. Reisen *et al.* (2008) found that temperature variations have affected mosquito abundance during a study carried out in California in order to determine the temporal variation of *Cx. tardis* (Coquillet 1896). Leisnham and Juliano (2012) and (Reinhold *et al.*, 2018) have confirmed in reviews the impact of climate change on *Aedes* mosquitoes by collecting evidence from previous studies.

3 Medical importance

The diseases that can be transmitted by mosquito vectors are various, dangerous and deadly in some cases. Next, we demonstrate the most common and distributed diseases with their emergence in Algeria and North Africa

3.1 Malaria

Malaria is the first to be discussed because of the high number of death signaled each year. Malaria is a human illness caused by the protozoa genus *Plasmodium* mainly *Plasmodium falciparum*: infective stage Sporozoite disease Falciparum malaria, *P oval*: infective stage Sporozoite disease oval malaria and *P vivax*: infective stage Sporozoite disease vivax malaria (Mehlhorn, 2008; Odolini *et al.*, 2012); the blood stage is the infectious stage, however, the disease may be asymptomatic where the patient notice no symptoms. The uncomplicated form is a more developed stage where the patient shows general and nonspecific symptoms. The sever form appears as a severe anemia which influence strongly on vital organs as brain, lungs, and kidneys (Phillips *et al.*, 2017). Malaria is transmitted exclusively by *Anopheles* females, according to the official WHO site in 2019, 228 million malaria cases, 405 000 malaria deaths, and 2.7 millions of available malaria resources recorded in 2018. The numerous mosquito vectors are responsible for malaria transmission, the mean malaria vectors in Africa are *An. labranchiae, An. gambiae, An. sergentii, An. multicolor* and *An. hispaniola, An. melas, An. merus, An. darlingi, An. arabiensis, An. moucheti, An. nili, An. funestus* (Faraj *et al.*, 2009; Boubidi *et al.*, 2010; Sinka *et al.*, 2010; Adlaoui *et al.*, 2011; Sinka *et al.*, 2012).

Back in time, the first discover of the blood stage of malaria was in Algeria in 1880 by Alphonse laveran (Bruce-Chwatt, 1981), Algeria has experienced malaria. An average of 5300 of malaria cases was reported in Algeria between 1948 and 1953 (WHO-Algeria, 1956). A malaria control department was established by the Institute Pasteur in 1904, however, the malaria parasites were sill reported in Tinzaouatine until 2007. According to WHO (2016), Algeria achieved more than 75% decrease in malaria cases, it reported less than 10 cases in 2013, where the malaria cases were exclusively due to *P. falciparum*; the radical treatment policy was using with primaquine for *P vivax* and gametocytocidal treatment for *P falciparum* (Figure 1.17). Algeria reported malaria-free in 2014 (WHO-Algeria, 2014) (Figure 1.18). The primary vectors responsible for malaria transmission in Algeria were *Anopheles labranchiae* and *An. sergentii*, the secondary vectors were *An. multicolor* and *An. hispaniola*, and *An. gambiae* in the south (Boubidi *et al.*, 2010; Sinka *et al.*, 2010).

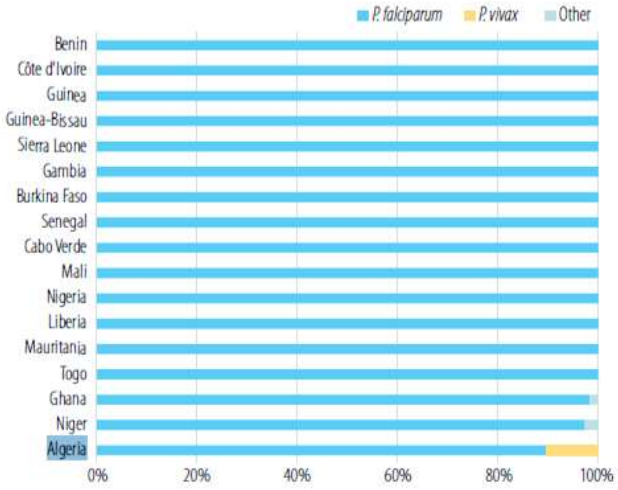


Figure 1.17. Percentage of cases due to *Plasmodium falciparum* and *P. vivax*, 2009-2013 (WHO-Algeria, 2014).

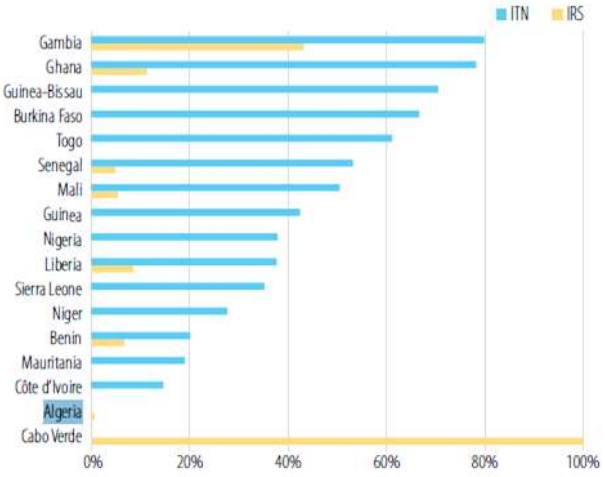


Figure 1.18. Percentage of population at risk of malaria with access to an insecticide-treated mosquito net (ITN) and percentage protected with indoor residual spraying (IRS), 2013(WHO-Algeria, 2014).

3.2 West Nile virus

West Nile Virus (WNV) is a flavivirus that affects human, equine, and avian. The virus is indigenous to all the continents accept America where it is newly introduced; birds are the natural reservoir and the principle vectors are *Culex* sp mosquitoes (Campbell *et al.*, 2002). In Morocco, WNV caused 94 equines cases and 42 died in 1996 (Murgue *et al.*, 2001), the reemerged of WNV was reported in September 2003 (Schuffenecker *et al.*, 2005); after, in 2008, a local virus circulation was detected among resident birds in a nonepidemic period

(Figuerola *et al.*, 2009). The WNV infection among humans was detected in the southern provinces of Morocco in using serological evidence 2012 (El Rhaffouli *et al.*, 2013). In Tunisia, 87% of 173 patients hospitalized for encephalitis and meningoencephalitis were diagnosed as WNV positive in 1997 (Marrakchi, 1998), further, a WNV outbreak occurred in Monastir, Tunisia in 2003 (Riabi *et al.*, 2010; Riabi *et al.*, 2014). In 2014, after the successive WNV outbreaks, for the first time in Tunisia, Wasfi *et al.* (2016) isolated WNV strain from *Culex pipiens* mosquitoes. likewise, Algeria experienced WNV outbreaks, in 1994, 50 cases were detected in Timimoun, the patients suffered of high fever and neurological symptoms, 8 patients died (Le Guenno *et al.*, 1996). Lafri *et al.* (2019) reviewed the WNV outbreaks in Algeria until 2014. Hachid *et al.* (2019) provided the first serotype evidence that confirm the circulation of WNV in Algiers. *Cx. pipiens* is the mean mosquito vector of WNV (Andreadis *et al.*, 2001; Nasci *et al.*, 2001; Wasfi *et al.*, 2016; Bennouna *et al.*, 2019); however, *Aedes* species were found susceptible to WNV after laboratory infection (Philip and Smadel, 1943; Baqar *et al.*, 1993; Turell *et al.*, 2001; Balenghien *et al.*, 2008), and isolation of WNV from captured *Aedes* mosquitoes were reported (Labuda *et al.*, 1974; Kulasekera *et al.*, 2001).

3.3 Dengue fever (DENV)

Dengue is a mosquito-borne viral disease; its patients suffer from nonspecific symptoms like fever, headache, nausea, vomiting, muscle pain and pain behind the eyes. The dengue incidence and geographical distribution have largely increased in the last years (Rigau-Pérez *et al.*, 1998; Messina *et al.*, 2019). Amarasinghe *et al.* (2011) reviewed the incidence of dengue in Africa; Botswana, Central African Republic, the Chad Republic of the Congo, Gambia, Guinea-Bissau, Guinea, Liberia, Malawi, Mauritania, Niger, Sierra Leone, and Zimbabwe reported the dengue cases between 1960 and 2010, the authors reported no information about the countries of North Africa because of nonavailability of data. After we did our researches, we found some data about dengue in Morocco; positive DENV patients were reported in Morocco in 2017 (Bajjou *et al.*, 2018; Hilali *et al.*, 2019), the infected patients were staying in Côte d'Ivoire and Burkina Faso during the outbreaks in 2016 and 2017. The mean vector of DENV is *Aedes Ae aegypti* and *albopictus* (Sharma *et al.*, 2005; Humphrey *et al.*, 2016; Amraoui *et al.*, 2019; Ong *et al.*, 2019; Shamsuzzaman, 2019).

3.4 Yellow Fever (YFV)

YFV is a mosquito-borne flavivirus disease original of tropical areas in Africa and South America (Monath, 2001; Monath and Vasconcelos, 2015). In Africa, YFV is still a major health problem since 90% of YFV cases reported in the world occurred in Africa (Mutebi and Barrett, 2002). In 1931, Specimens of blood serum were collected from Algeria, Tunisia and Morocco for an investigation of the distribution of YFV; the results purposed the lack of the disease in these counties (Sawyer and Whitman, 1936). No other documentary found about YFV status in North Africa including Algeria. The vector of YFV is meanly *Aedes aegypti* however other *Aedes* species can be involved in YFV transmission (Christophers, 1960; Mutebi and Barrett, 2002; Kamgang *et al.*, 2019; Yen *et al.*, 2019).

3.5 Zika (ZIKV)

ZIKV is as well a mosquito-borne flavivirus disease related to YFV, WNV and DENV; it was identified in the first time in monkeys during YFV surveillance in the Zika Forest Uganda in 1947, it was after detected from *Aedes africanus* mosquitoes and reported latter in humans in 1952 (Dick *et al.*, 1952; Macnamara, 1954; Campos *et al.*, 2015). ZIKV was isolated from humans in Central African Republic, Gabon, Egypt, Nigeria, Uganda, Tanzania, and Sierra Leone (Moore *et al.*, 1975; Robin and Mouchet, 1975; Fagbami, 1977; Fagbami, 1979; Wikan and Smith, 2016; Otu *et al.*, 2019; Runge-Ranzinger *et al.*, 2019). *Aedes* species are the mosquito vectors of ZIKV (Gutiérrez-Bugallo *et al.*, 2019; Gutiérrez-López *et al.*, 2019; Hugo *et al.*, 2019; McKenzie *et al.*, 2019).

4 Mosquito control

Since mosquitoes are a key threat for human and animal populations worldwide, mosquito control is, therefore, a crucial preventive tool. Trying to control mosquitoes, the researchers have tested different strategies whose chemical control was the most widely used. However, due to the secondary effects of chemical insecticides, as toxicity and resistance (Hemingway and Ranson, 2000; Mossa *et al.*, 2018), other control methods considered to be eco-friendly methods, like biological control using other co-occurred organisms, microbial control agents, behavior-based control tools, plant born mosquitocidal, insect growth regulators…etc, are more frequently aimed.

4.1 Chemical control

By mean of the development of chemical insecticides, people were able to stop genocide caused by mosquito-borne-diseases (Shretta *et al.*, 2017). The Chlorinated hydrocarbons group was known by its chemical stability, high persistence and insecticidal efficacy against mosquitoes (Mathis and Quarterman, 1953); Dichloro-diphenyl-trichloroethane (DDT) was and still on the head of the list of the chlorinated hydrocarbons group used in mosquito control (Bruce-Chwatt, 1971; van den Berg, 2009; Kasinathan *et al.*, 2019). DDT and γ-hexachlorocyclohexane (γHCH) are the chemical insecticides the most used for mosquito control and more especially for malaria control (Curtis, 2002; Sadasivaiah *et al.*, 2007); simultaneously, DDT and HCH resistance was proved (Flight activity of insecticide resistant and susceptible *Anopheles stephensi* mosquitoes in actograph chambers lined with malathion, γ HCH or dieldrin/Pyrethroid and DDT Resistance and Organophosphate Susceptibility among *Anopheles* spp. Mosquitoes, Western Kenya), further, the indoor use of contamination of stored food and feed commodities from indoor use of HCH and DDT provoked contamination of stored food, bovine milk malaria control programs and consumed vegetables (Battu *et al.*, 1989a; Battu *et al.*, 1989b; Adeleye *et al.*, 2019), also, the contamination of agriculture lands (Mitra *et al.*, 2019; Kafaei *et al.*, 2020). Organophosphates (OPs) is another group of chemical insecticide used in mosquito control, however, it is less stable than the first group but less persistent (Ageda *et al.*, 2006); for this reason, the OPs were developed us alternatives of DDT. Nevertheless, OPs are toxic for many living organisms, and they brought high resistance towards *Aedes* and *Culex* mosquitoes (Fuseini *et al.*, 2019; Prado *et al.*, 2019; Smith *et al.*, 2019; Tabbabi *et al.*, 2019). N-methyl is likewise an active Carbamates used strongly as insecticide

The chitin synthesis inhibitors and the juvenile hormone analogs belong to the insect growth regulators (IGRs) group; they constitute another type of synthetic insecticides that target the normal growth of mosquitoes (Park *et al.*, 2019; Stevens *et al.*, 2019). However, these synthetic insecticides have a side effect on non-targeted organisms as it proved in recent researches (Santorum *et al.*, 2019; Yokoyama, 2019).

4.2 Biological control

4.2.1 Plant-born insecticides

Plant derivate are used to control mosquitoes in different levels; they can affect the oviposition, survival, larval duration, pupation and insect emergence (Benelli, 2015; Benelli *et al.*, 2016). Aqueous extracts and their principle compounds extracted from different plant species and parts were used likewise to control mosquitoes; Fernandes *et al.* (2019) supported the use of *Helicteres velutina* K. as mosquitocide according to the results that they obtained by testing the principle compounds isolated from the aqueous extracts of *H. velutina* K. tested against *Ae aegypti*. We can site simultaneously other researches that confirmed the mosquitocidal activity of the aqueous extracts of other plant species: *Citrus grandis* L. (Ishtiaq *et al.*, 2019), *Annona reticulata* L. (Govindarajan and Benelli, 2016), *Bougainvillea spectabilis*, *Saraca asoca*, and *Chenopodium album* (Sharma *et al.*, 2019)…etc. Moreover, the use of sliver nanoparticles obtained from the green syntheses of aqueous extracts of different plant parts to control mosquito vectors was evaluated in various researches; the lethal concentrations that cause 50% of death (LC_{50}) of silver nanoparticles tested against *Anopheles culicifacies* (Giles 1901) were found efficient (Amerasan *et al.*, 2015). Likewise, the activity of silver nanoparticles synthesized from various aqueous plant extracts were tested against *An. stephensi*, *Cx. quinquefasciatus* and *Ae aegypti*; in the total results, the LC_{50} values were substantial (Perumal *et al.*, 2018; Alshehri *et al.*, 2019; Pilaquinga *et al.*, 2019; Saini *et al.*, 2019). Essential oils and their principal compounds were equally tested for their mosqutocidal activity as plant-born materials; various conducted researches have insured their potential use eco-friendly alternative of chemical insecticides (Azeem *et al.*, 2019; Muturi *et al.*, 2019; O'Neal *et al.*, 2019)

4.2.2 Co-occurring predators

From the past to the present, the use of co-occurring living organisms in mosquito control was always evaluated; according to an ancient study conducted by Mogi *et al.* (1984), predators killed 48.7–87.0% of *Culex* and *Anopheles* larvae before adult emergence in containers containing predators, whereas the survival of larvae populations was higher in predator-free containers. Therefore, mosquito larvae are potential prey for several aquatic organisms. Recent studies confirmed the existence of various mosquito larvae predators that could be used in biological control, since they reduce mosquito larvae density. Copepod species and odonates larvae were found to be predators of mosquito larvae (Saha *et al.*, 2012;

Früh *et al.*, 2019; Ilahi *et al.*, 2019). Further, various fish species were likewise found to be predators of mosquito larvae as *Aplocheilichthys spilauchen*, *Poecilia reticulate*, *Cnesterodon decemmaculatus* and *Jenynsia multidentata* (Bonifacio *et al.*, 2019; Deacon *et al.*, 2019; Okyere *et al.*, 2019). A mosquito predator could be also another mosquito larva. The larvae of the non-biting mosquitoes *Toxorhynchites amboinensis*, or elephant mosquito as commonly known, are also predators of other mosquito species larvae (Digma *et al.*, 2019).

4.2.3 Microbial-control

Microbial agents were involved in pest control including mosquito control. The idea of microbial-control is emphasized since mosquitoes may be naturally infected by viruses, parasites, and bacteria; if the pathogen is not infectious for animals and humans, then it can be used to control mosquitoes.

The *Nosema algerae* protozoa infect naturally mosquitoes (Vavra and Undeen, 1970), Undeen and Alger (1975) examined its infectivity against the malaria vector *Anopheles stephensis*, the authors considered that *N algerae* did not affect mainly the larvae survivor, whereas, it affected sufficiently the adult longevity. In a further work, Undeen and Alger (1976) confirmed in a laboratory experiment that *N algerae* injected by an infected mosquito will unlikely provoke any infection. However, using bacteria as microbial control agent is more frequent because of its ease of handling and cost effectiveness (Becker *et al.*, 2003). *Bacillus thuringiensis israelensis* (Bti) and *B sphaericus* (Bs) were considered as an eco-friendly mosquito control tool and their efficacy against mosquito species were proven (Becker, 1997; Wirth *et al.*, 2000; Ben-Dov, 2014; Dawson *et al.*, 2019; N'do *et al.*, 2019). The Bti control is non-toxic to the other aquatic organisms other than mosquitoes (Lagadic *et al.*, 2016; Lawler, 2017); however, Allgeier *et al.* (2019) found that Bti has a negative effect on chironomids which can perturb the food chains in multiple ecosystems. Further, *Cx. pipiens* mosquitoes collected from field after a failure Bs control developed a resistance after less than eight generations (Nielsen-Leroux *et al.*, 1997; Zahiri *et al.*, 2002).

4.2.4 Sterile Insect Technique (SIT)

The SIT is an eco-friendly, highly specific, reversible control method practiced in multiple fields and laboratories (Thomé *et al.*, 2010; Nolan *et al.*, 2011; Bouyer and Lefrançois, 2014). This method consists to produce sterile mosquito males and then release them in the nature in large numbers, the sterile males will compete with the indigenous males to fertilize the females, the fertilized females will fatherly lay sterile eggs i.e. not able to

hatch; and depending on the fact that mosquito females mate once in their life cycle, the use of sterile mosquito males will contribute to a decrease in mosquito population. According to Benedict and Robinson (2003) and Lees *et al.* (2015), SIT is an effective and safe control program. However, this method requires experience and developed laboratory material; moreover, it is not a fast strategy in the case of sudden epidemics.

CHAPTER 2

MOSQUITO BIODIVERSITY IN SETIF REGION

1 Material and methods

1.1 Study area

1.1.1 Overview

Setif region of high plains Northeastern Algeria (36°03'N 5°31'E) stretches over a surface of 6504km^2, the human population density is approximately 230 inhabitants/km^2; the population

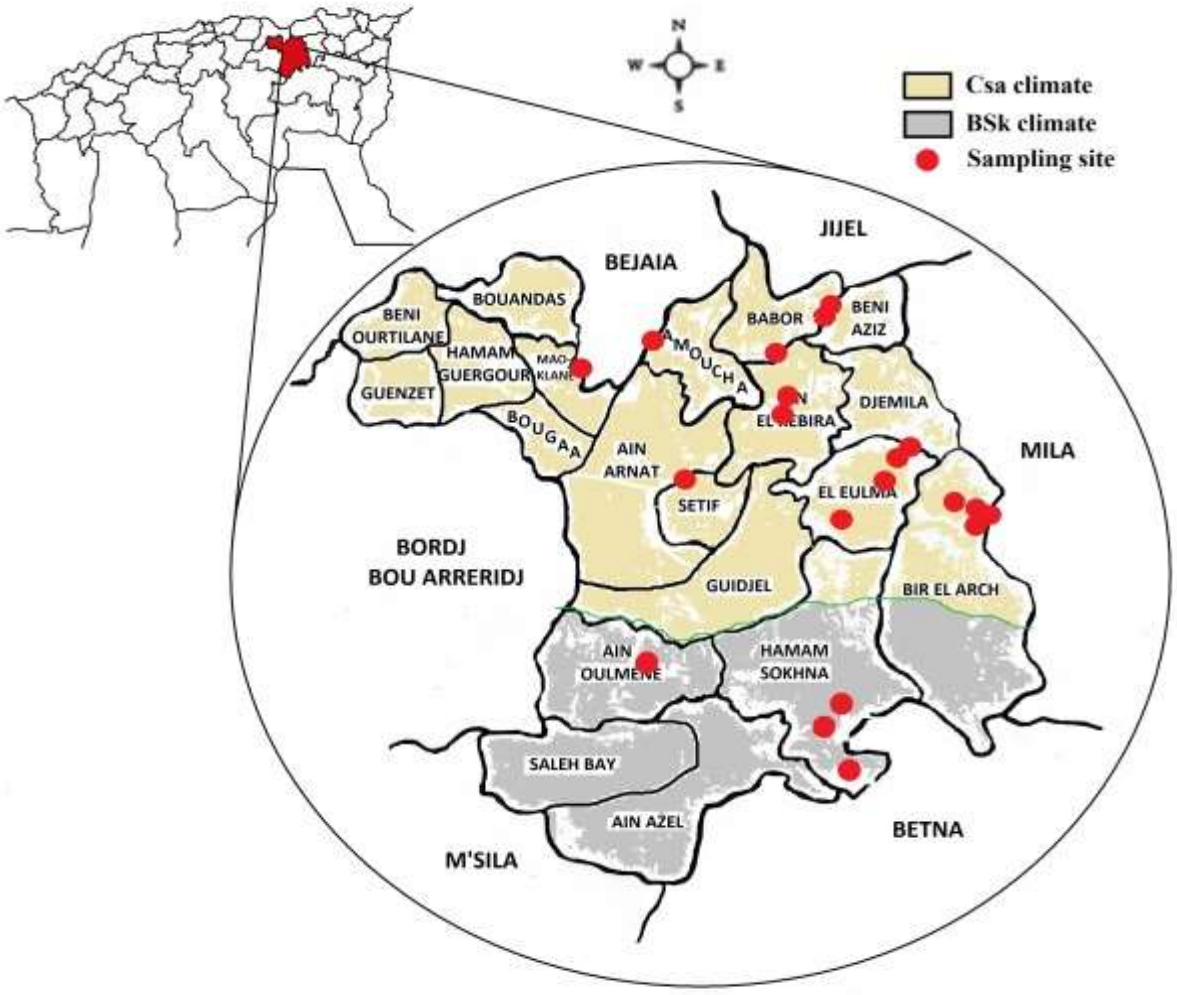


Figure 2.1. The geographical localization of Setif region and the distribution of the sampling sites (n=20) in two types of climate zones: Csa (Mediterranean climate) and BSk (steppe climate).

is distributed in the different landscape structures according to the nature of their life activities. Whereas, agriculture constitutes relatively an important sector in the study area due to the availability of farmlands and water surfaces (dams and rivers) (Rouabhi *et al.*, 2012; Rouabhi *et al.*, 2016). Setif is characterized by heterogeneity of climate according to Köppen climate classification (Köppen *et al.*, 2011). The dominant climate is Csa (warm temperate climate with warm and dry summer); however, we can differentiate two sectors: a north part of Csa climate, and a south part of BSk climate (semi-arid; cold and dry).

1.1.2 **Water surfaces**

Setif region is characterized by the disponibility of water surfaces; we can found dams: El-Maouane, Draa Addis, Bouchitat; marches: Sebkhet el Hamiet, Sebkhet Bazer,Sebkhet Sokhna, Sebkhet Melloul, Sebkhet Ain Lahdjar, Sebkhet Sed el Maleh; small reservoirs: Reggada reservoir, Ain Abassa reservoir, El Ouricia reservoir, Ouled Adouane, Oued Doulani reservoir; lakes: Kertila lake; Rivers: Oued Bou Sellam, Oued Barhoum, Oued Farmetou, Oued Khalfoune, Oued ech Chair, Oued Safsaf, Oued Dhemcha, Oued Deheb…etc. Dams, reservoirs, rivers situated in the majority in the north part of Setif region, Sebkhet (wet areas) are all situated in the south part.

1.1.3 **Temperature and precipitation during sampling period**

According to the data we recorded, the mean annual precipitation during the sampling period was 289.7mm which is considered as low comparing to the previous years (400mm before 2014 according to (Bouregaa and Fenni, 2014); the peaks were noted in January, April, and November with mean values comprised between 29 mm and 49.5mm. The coldest month was January, February and December with temperature comprised between 8°C and 9.5°C; the temperature increase slowly from Jun (31.3°C) until reaching their maximum mean in July, and then decreased in almost the same frequency (Figure 2.2).

1.1.4 **Sampling sites**

The sampling sites were randomly selected; we tried to cover all the study area as possible as we could. During the field inspection more than 6O sites were checked, 22 sites were found positive. During the field inventorying, we faced the problem of the drought of the sites every time we visited them again, which obliged us to search for new sites. 52 mosquitoes collected from sites in Mawan and Kaawen were not identified. The mosquitoes collected from the other sites (21 sites, Figure 2.1) were identified. Information about altitude and geographic

coordinates of the sampling sites are provided in Table 1.1. We provide in Figures 2.3, Figure 2.4, Figure 2.5, Figure 2.6, Figure 2.7, Figure 2.8, Figure 2.9, Figure 2.10, Figure 2.11, Figure 2.12, Figure 2.13.

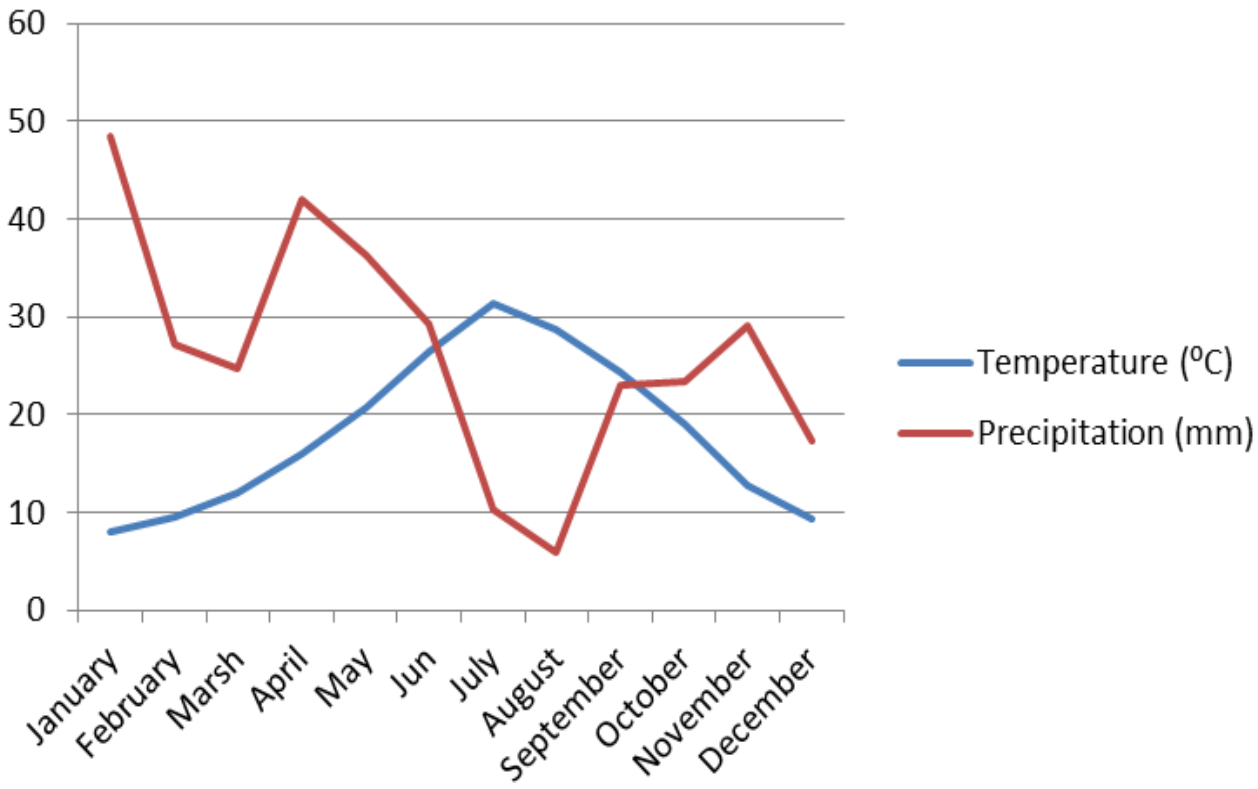


Figure 2.2. Mean temperature in ^{0}C and precipitation per month in mm in Setif region during the field inspection period 2016-2019 (ONM, Office national de la météorologique. 2019).

Table 1.1. Sites of sampling, with altitude and geographic coordinates.

Department	Sites	Number of sites	Geographic coordinates	Altitude
Belaa	Belaa	site 1	36°11'20.7"N 5°52'30.1"E	1009 m
		site 2	36°11'48.9"N 5°51'48.8"E	1024 m
		site 3	36°11'48.9"N 5°51'48.8"E	1009 m
		site 4	36°11'30.8"N 5°52'25.2"E	1009 m
		site 5	36°12'06.1"N 5°46'45.9"E	1059 m
El eulma	Guelta zargua 1	site 6	36°12'37.7"N 5°42'33.8"E	1009 m
	Guelta zargua 2	site 7	36°12'41.7"N 5°42'48.3"E	1112 m
	Tachouda	site 8	36°15'42.8"N 5°42'17.0"E	849 m
		site 9	36°15'42.8"N 5°42'17.0"E	849 m
	El eulma	site 10	36°09'31.5"N 5°41'35.4"E	964 m
	El eulma	site 11	36°09'35.6"N 5°40'41.2"E	967 m
Beni fouda	Beni fouda 1	site 12	36°15'43.4"N 5°38'04.3"E	712 m
	Beni fouda 2	site 13	36°15'08.4"N 5°37'42.2"E	870 m
	Oued dehab	site 14	36°15'27.9"N 5°36'22.5"E	835m
Beni aziz	Oued dehamcha	site 15	36°22'16.7"N 5°39'24.1"E	577.0 m
	Beni aziz	site 16	36°27'52.0"N 5°39'02.5"E	716.0 m
Bougaa	Mawklen	Site 17	36°33'44.6"N 5°18'03.1"E	99 m
Setif	Ain welmen	site 18	35°54'44.1"N 5°16'42.5"E	973.0 m
Bayda bordj	Bayda bordj	site 19	35°53'27.4"N 5°39'43.9"E	895.0 m
	Bayda bordj2	site 20	35°53'36.3"N 5°40'30.1"E	884.0 m
	Bayd bordj3	site 21	35°53'47.7"N 5°41'00.3"E	884.0 m

Figure 2.3. Site 3 (personal photo).

Figure 2.4. Site 5 (personal photo).

Figure 2.5. Site 4 (personal photo).

Figure 2.6. Site 1 (personal photo).

Figure 2.7. Site 7 (personal photo).

Figure 2.8. Site 8 (personal photo).

Figure 2.9. Site 6 (personal photo).

Figure 2.10. Site 11 (personal photo).

Figure 2.11. a) Site 12, b) Site 13, c) Site 14 (personal photo).

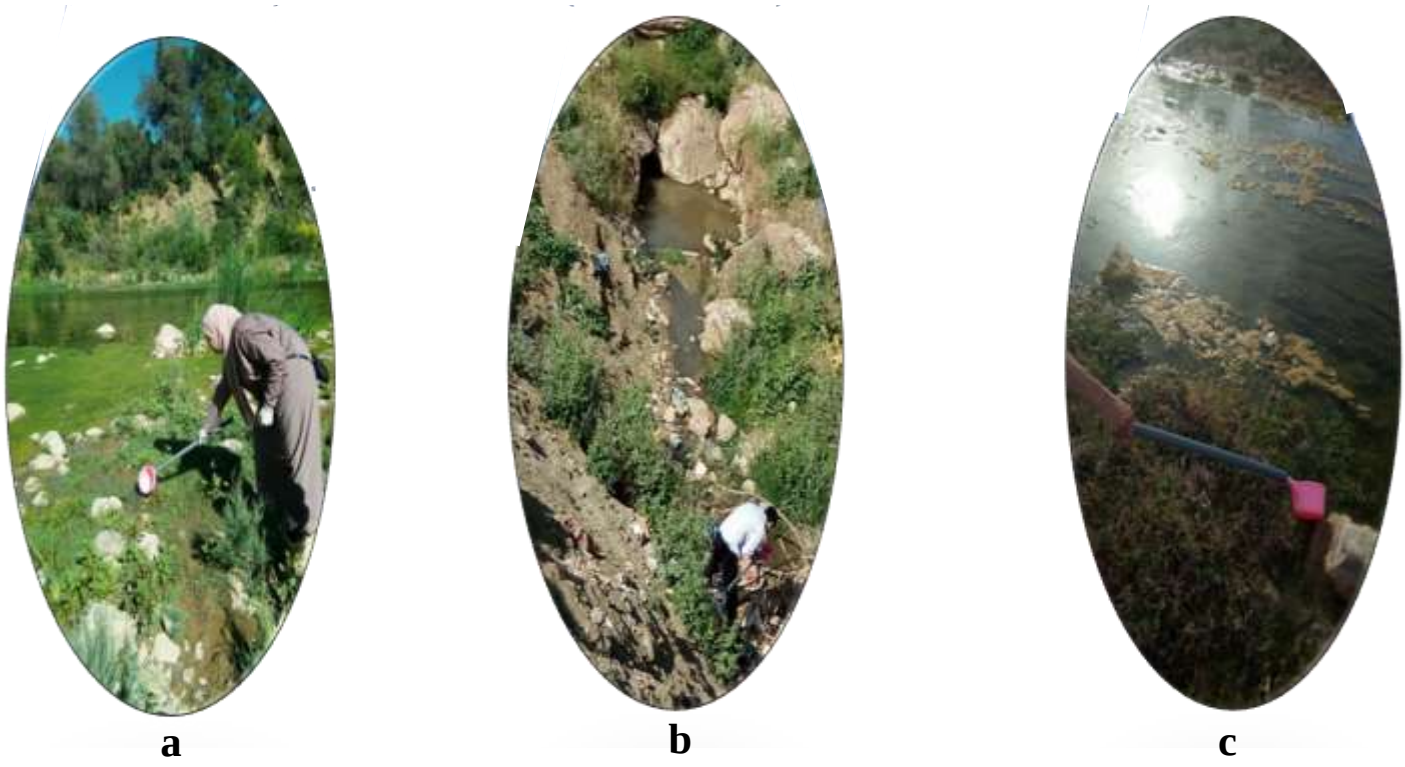


Figure 2.12. a) Site 17, b) site 16, c) site 15 (personal photo).

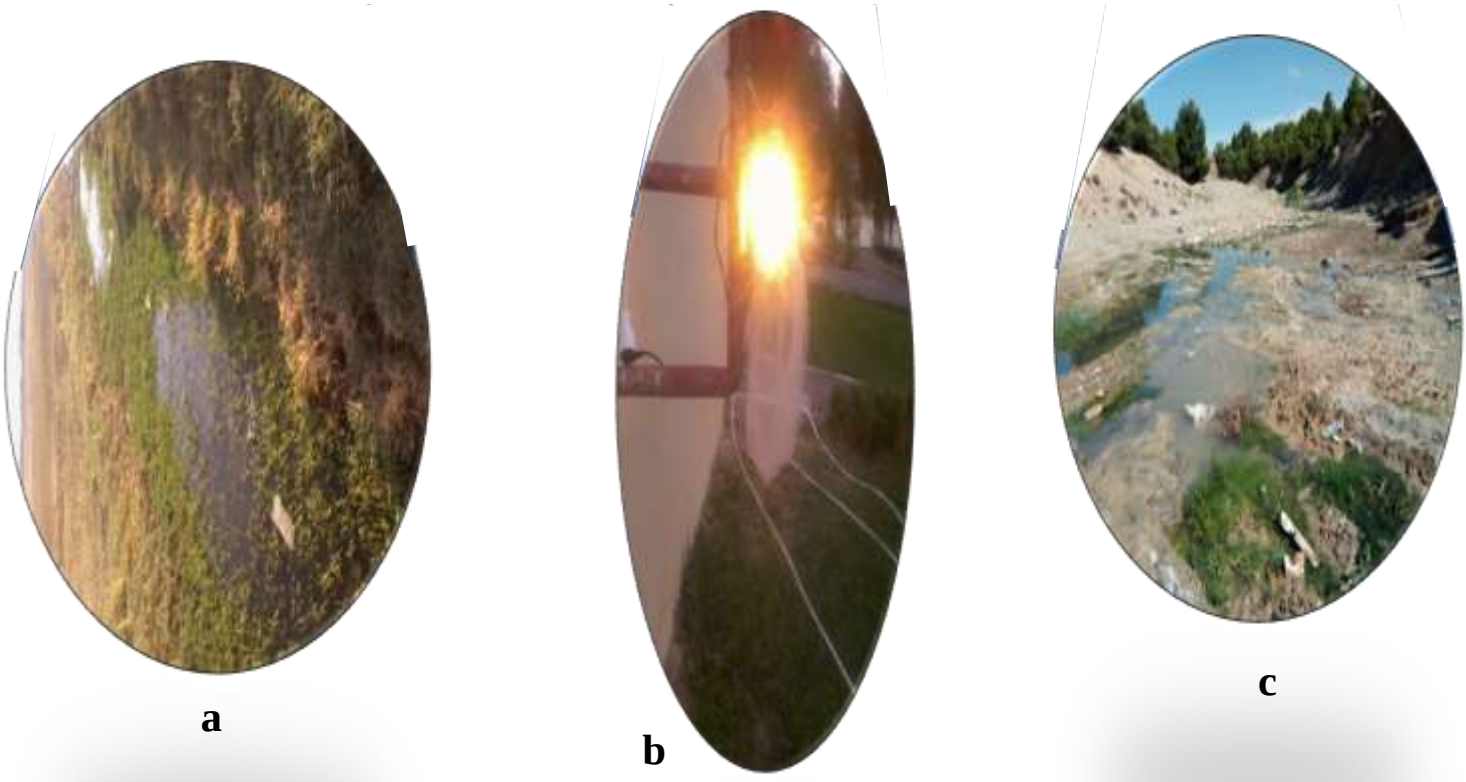


Figure 1.13. a) Site 20, b) site 19, c) site 18.

1.2 **Sampling**

The sampling was conducted during 2016-2019 and targeted larval and adult forms.

1.2.1 **Larvae sampling and preparation for observation**

The larvae sampling occurred using a standard dipper of 1L capacity, the larvae were then transferred in small containers and counted (Photo 1.1). The third and fourth instar larvae were:

- Identified alive: this operation preserves the setae that can be lost easily with the intense manipulation;
- Identified after preservation in Alcohol 70%;
- Identified after being mounted for permanent preparations (Becker *et al.*, 2003): the larvae were killed in 60° hot water, dehydrated then in increasing degrees of alcohol 70%, 90%, 100% for 15mn for each concentration. After, the larva was transferred on the glass slide and a drop of the medium Eukitt is added on, we put then on a cover glass (Photo 1.2).

First and second instar larvae were reared in breeding site water until they reach the fourth instar.

Photo 1.1. Mosquito sampling and separation for easy counting (personal photos).

Photo 1.2. Larvae preparation for permanent preservation (personal photo).

1.2.2 **Adult sampling and preparation for observation**

Adults sampling was done using simple CDC miniature light traps, 7 CDC traps were handmade using a yellow light lamp and fan "12VDC" (Photo 1.3). The adults who emerged from the rearing larvae were aspired using mouth aspirator, as well were the adults found in houses (Photo 1.4). The female adults were pinned for morphological observation as in Photo 1.5, the genitalia of male adults was prepared for microscopic observation as follows: we removed the abdomen of male adults from their thorax, the abdomen was next placed in potassium hydroxide solution and heated for 15mn; after, the abdomen was removed into an acetic acid solution for all the night; the next day, the genitalia was removed carefully and placed on a glass slide, we added a big drop of Eukitt medium and then we covered it by a cover slide.

Photo 1.3. Handmade CDC traps (personal photo).

Photo 1.4. Aspiration of emerged adults using mouth aspirator (personal photo).

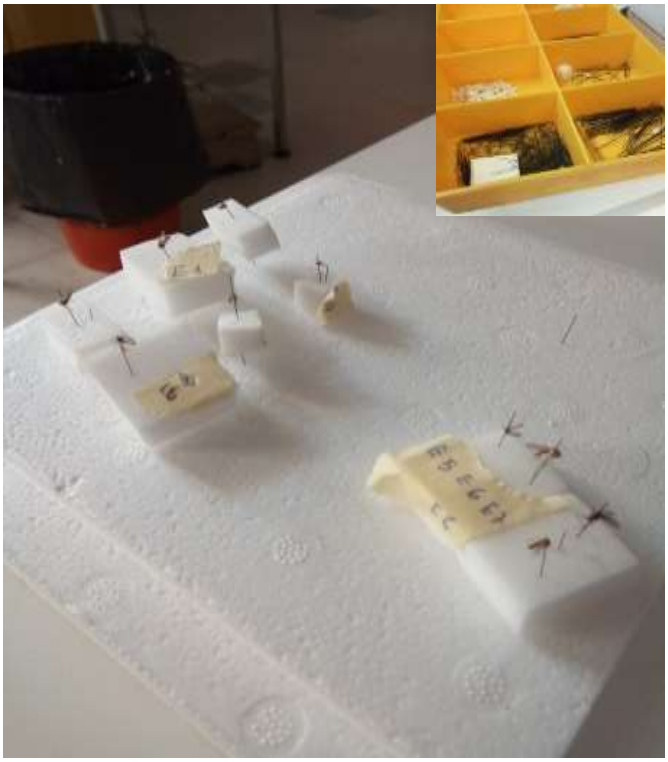


Photo 1.5. Females pinned for morphological observation (personal photo).

1.3 Morphological observation and characterization

The larvae and male genitalia observation was conducted using a Brosser LCD MICRO 5MP microscope with a camera built 5MP CNOS 1/2.5", 2560 x 1920 pixel arrays (Photo 1.7). The female adults' observation was conducted using binocular microscope loupe (Photo 1.6). The characterization of larvae and mosquito females was done using pictorial keys with help of XPER software and the last version of Moskeytool_V1.2 provided by French National Research Institute for Sustainable Development IRD (Gunay *et al.*, 2018). Male adults were identified using hypopygium pictorial keys provided by Becker *et al.* (2003).

Photo 1.7. Larvae observation using microscope LCD (personal photo).

Photo 1.6. Adult female observation using binocular microscope loupe (personal photo).

1.4 Molecular analyses

1.4.1 PCR test

The DNA of the harvested and reared adults was extracted from the legs using DNeasy blood & tissue kit (Qiagen, Hilden, Germany) by following the handbook instructions. The PCR amplification of the COI barcode was performed in a total volume of 35 µl consisting of 10x reaction buffer; 2.5 mM MgCl 2 ; 200 µM of dNTPs; 28 pmol each primer LCO1490 and HCO2191 (Vrijenhoek, 1994); 2.5 U of TaqDNA polymerase. A volume of 3µl of genomic DNA was added to each PCR reaction and samples without DNA were included to exclude carryover contamination. The PCR procedure was as follows: initial denaturation stage and activation of the enzyme at 95°C for 2 minutes; 40 cycles at 94°C for 40 seconds, 50°C for 40

seconds and 72°C for 1 minute, followed by a final extension phase at 72 °C for 7 minutes. PCR products were examined on 1% Agarose gel and the band's intensity was noted using a gel imaging system (ChemiDoc™ XRS+ System with Image Lab™ Software #1708265); both strands of the successful amplifications were sequenced at GATC Biotech (Konstanz, Germany). Sequencing results were analyzed using Geneious 10.2.3 software (https://www.geneious.com/) (Kearse *et al.*, 2012). The data of positive sequences were edited using BioEdit (Hall, 1999) and compared with sequences deposited in GenBank and Bold using the blast algorithm. COI sequences were deposited in GenBank with the accession numbers from MK047302 to MK047315.

1.4.2 **Phylogenetic analyses**

In order to evaluate the evolutionary relationships between the positive sequences and those provided in Genbank, the obtained sequences with an average of 658 bases were aligned using the Muscle algorithm. We exported sequences from Genbank to construct phylogenetic trees using the Neighbor Joining algorithm, the bootstrap support was obtained through 1000 replications. We calculated the genetic distance between the positive sequences and their congener we have obtained in the blast results (score 100) in order to analyze the phylogenetic divergence between the closely species. Analyses were conducted in MEGA7 using the Kimura 2-parameter model.

1.5 **Ecological data analyses**

Only identified specimens are included in the ecological data analyses; the density, abundance, frequency, habitat characterization, co-occurrence, ecological indices, and distribution patterns are analyzed using analysis software as follows:

- We analyzed the total and species descriptives of mosquito population using SPSS version 25 (2017) by calculating:
  - Frequency in percentage (f): Used function: (Analyze→ Descriptive statistics→ Frequencies)
  - Mean density (Arithmetic mean AM ± Standard Error ER): Used function: Analyze→ Descriptive statistics→ Descriptive.

- The effect of urbanization on the choice of breeding site can be evaluated by the analyze of the nature of breeding sites (rural or urban); likewise, the tendency of

mosquito species to lay in permanent or temporary sites provide information about the degree of adaptation of mosquitoes to their ecosystem and characterize the species' preferences. Simultaneously, we would like to determine the value of presence or absence of algae on the choice of mosquito species to their breeding sites and if this choice affects their density. During the sampling we collected the data related to the sampling sites; the observed variations in mosquito breeding site characteristics, in terms of type, nature, presence or absence were analyzed in order to characterize the habitat preferences of mosquitoes in the study area. The analysis was conducted using SPSS by calculating:

- Frequencies: Used function: Analyze→ Descriptive statistics→ Frequencies
- Crosstabs, Used function: Analyze→ Descriptive statistics→ Crosstabs
- Comparing density: we use non parametric tests in case of non-normality and heterogeneity of data ($p<0.05$): Mann-Whitney U test for two independent variables (analyze→ nonparametric tests→ legacy dialogs→ 2 independent samples) and the parametric test: independent samples t-test for two independent variables in case of normality and homogeneity of data (Analyze→ compare means→ Independent-Samples T test)

- The co-occurrence of mosquito species was used to calculate the frequency of the species association and non-association, and the level of correlation between the co-occurred species using the *cor.test* (method=Spearman's because of non-normality of data) and the *corrgram* package (Wright and Wright, 2018) in R studio (Team, 2018). The Spearman's correlation (r_s) was considered as weak if $0<r_s\leq0.4$, moderate if $0.4<r_s\leq0.7$ and strong if $0.7<r_s<1$. Only species found more than one time was included in the analyses. Species found only one time were excluded from the correlation test.

- We used PAST3 (Hammer *et al.*, 2001) software to calculate the ecological indices: species richness (S), (Simpson, 1949), Shannon index (H') (Shannon and Weaver, 1949), and Evenness (E") indices (Hill, 1973).

- To analyze the distribution patterns of mosquito species in the study area we conducted the canonical corresponding analysis (CCA) to compare the mosquito

clusters between the two climate regions Csa and BSk using PAST3. And we compared the difference in mosquito density across the climate regions Csa and BSk using SPSS software and the Mann-Whitney U test for two independent variables: analyze→ nonparametric tests→ legacy dialogs→ 2 independent samples.

2 Results

2.1 Species identification

The sampling yielded the identification of nine mosquito species; the list of the sampled species are classified according to the last revision of the online Mosquito Taxonomy Inventory (Harbach, 2013a).

Subfamily : Culicinae

Genus: *Coquillettidia*

Subgenus : *Coquillettidia*

Species : ***Coquillettidia richiardii*** (Ficalbi 1889)

Genus : *Culiseta*

Subgenus : *Allotheobaldia*

Species : ***Culiseta longiareolata*** (Macquart 1838)

Genus : *Ochlerotatus*

Subgenus : (subgenus uncertain)

Species : ***Ochlerotatus caspius*** (Pallas 1771)

Genus : *Culex*

Subgenus : *Culex*

Species : ***Culex theileri*** (Theobald 1903)

Species: ***Culex simpsoni*** (Theobald 1905)

Species : ***Culex pipiens*** (Linnaeus 1758)

Subgenus : *Maillotia*

Species : ***Culex hortensis*** (Ficalbi 1889)

Subfamily : Anophilinae

Genus : *Anopheles*

Subgenus : *Anopheles*

Species : ***Anopheles labranchiae*** (Falleroni 1926)

Subgenus : *Cellia*

Species : ***Anopheles cinereus hispaniola*** (Theobald 1901)

2.1.1 **Morphological identification**

2.1.1.1 ***Coquillettidia richiardii* larvae**

- Siphon opening at the apex of a short conical tube (Photo 2.1);
- Integument of the saddle with spicules grouped by two (Photo 2.2).

Photo 2.1. *Coquillettidia richiardii* larvae (personal photo).

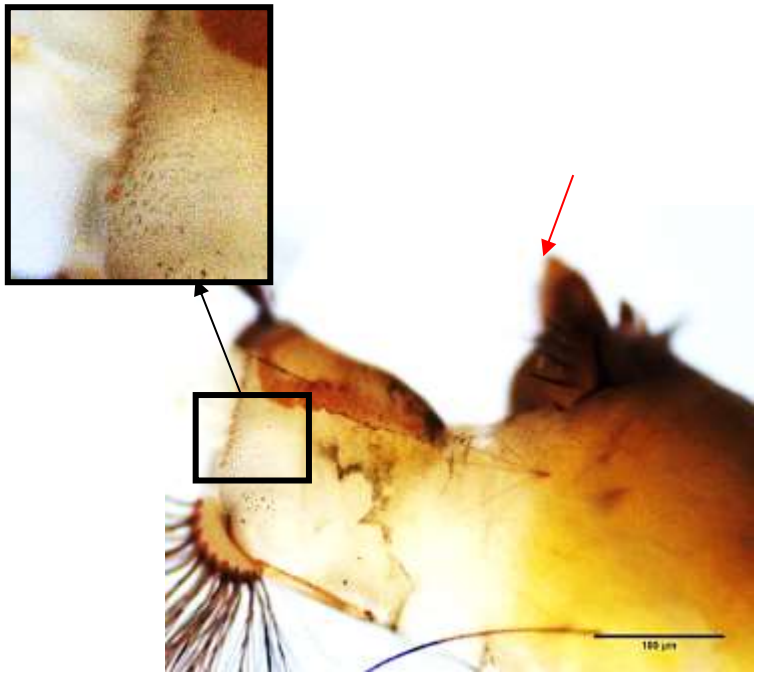


Photo 2.2. *Coquillettidia richiardii*: short conical respiratory tube and integument of the saddle with spicules grouped by 2 (personal photo magnification 10x).

2.1.1.2 *Culiseta longiareolata*

2.1.1.2.1 *Culiseta longiareolata* Larvae

- Siphon constitute a cylindrical tube
- Abdominal plates absent;
- Siphon: one pair of siphonal tufts inserted at the base of the siphon; pecten's ornamentation with spines only; the pecten extend near 2/3 of the siphon length; medium siphon (2 < L <= 4) (Photo 2.3).
- Smooth antennal integument; short antennal seta and seta 1-A hardly noticeable (Photo 2.4).

Photo 2.3. Siphon of *Culiseta longiareolata* (personal photo magnification 10x).

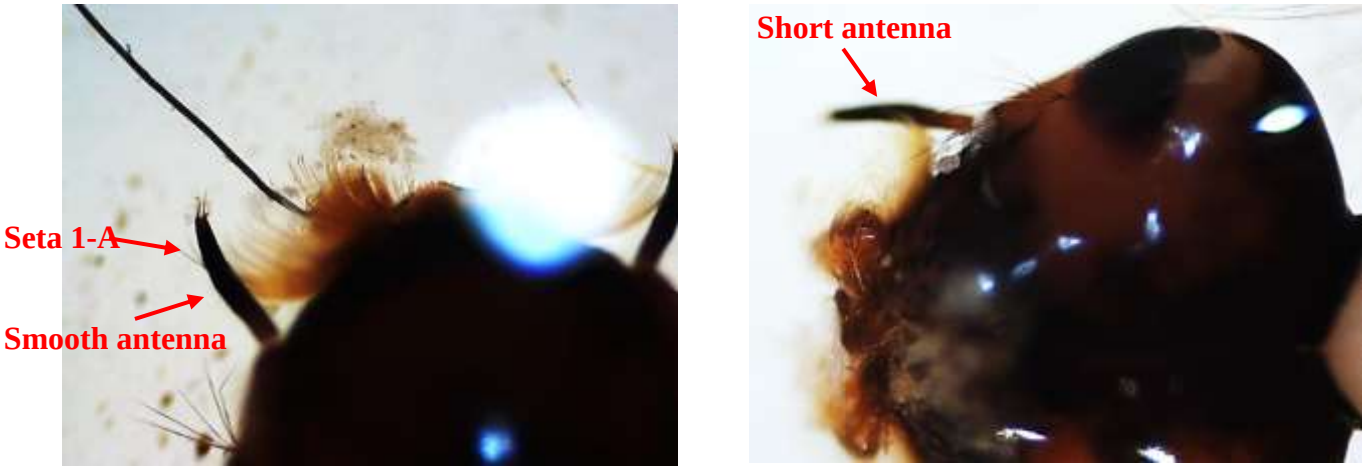


Photo 2.4. *Culiseta longiareolata* larvae: smooth antenna and cephalic setae 1-A hardly noticeable (personal photo magnification 10x).

2.1.1.2.2 *Culiseta longiareolata* adult

- Maxillary palpus clearly shorter than proboscis (Photo 2.5);
- Tergite with creamy white and dark scales (Photo 2.6);
- Scutum's ornamentation with continuous bands (Photo 2.6);
- Scutum's continuous bands dark with pale bands (Photo 2.6);
- The color of proboscis is entirely dark.

Photo 2.5. *Culiseta longiar*eolata: a) pinned adult, b) mouthpart: maxillary palpus clearly shorter than proboscis (personal photo).

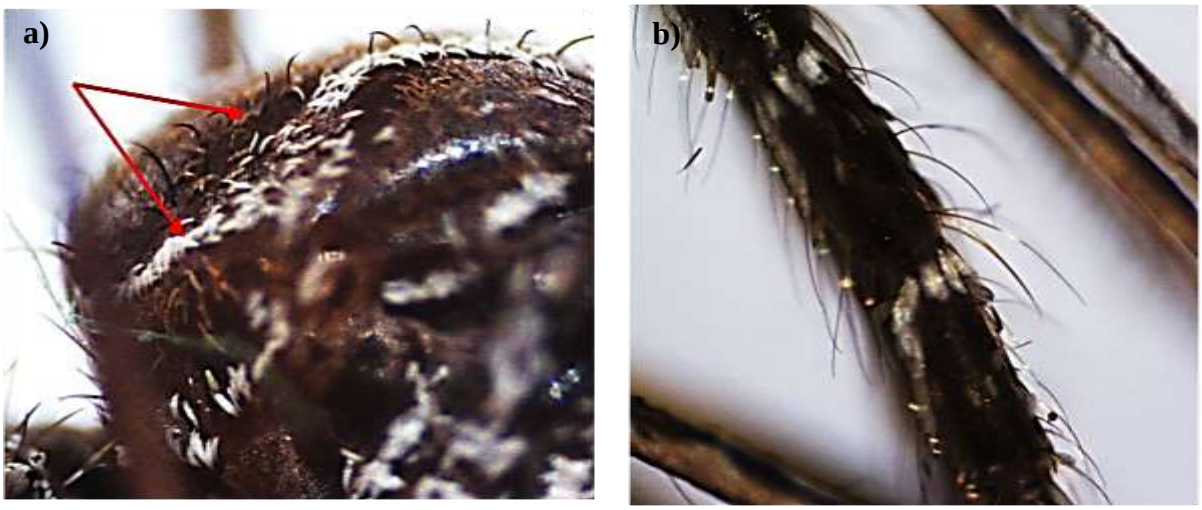


Photo 2.6. *Culiseta longiareolata*: a) Scutum ornamentation with pale continuous bands; b) Tergite: with creamy and dark scales (personal photo magnification 10x).

2.1.1.3 *Ochlerotatus caspius*

2.1.1.3.1 *Ochlerotatus caspius* larvae

- Siphon constitue a cylindrical tube;
- Abdominal plates absent;
- One pair of siphonal tuft inserted close to the middle or near the apex of the siphon;
- Insertion of the seta 1-S is beyond the last pecten tooth;
- Arrangement of the pecten teeth on the siphon is without any clearly isolated teeth;
- Insertion of the last pecten tooth is at the siphon's middle part;
- Location of the antennal seta 1-A is on the basal half (Photo 2.7);
- Number of branches on the antennal seta 1-A is more than 3 branches (Photo 2.8).

Photo 2.7. Siphon of *Ochlerotatus caspius* (personal photo magnification 10x).

Photo 2.8. Antenna of *Ochlerotatus caspius* larvae: number of branches and position of seta 1-A (personal photo magnification 10x).

2.1.1.3.2 *Ochlerotatus caspius* adult

- Maxillary palpus is clearly shorter than proboscis;
- The tergite color: is almost completely covered with pale scales (Photo 2.9);
- The tergite ornamentation is almost completely covered with pale scales (Photo 2.9);
- Mixture of pale and dark scales on the wing veins (Photo 2.10);
- Scutum with continuous bands (Photo 2.11);
- The tarsomere 5 of the leg III is entirely white (Photo 2.11).

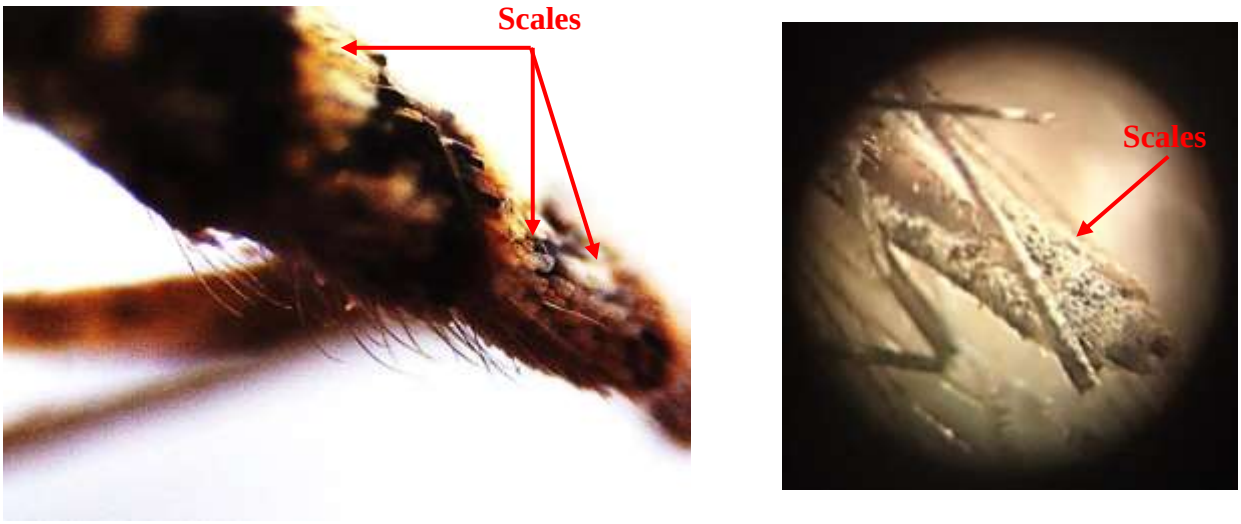


Photo 2.9. *Ochlerotatus caspius*: tergite covered with pale scale scales (personal photos magnification 10x).

Photo 2.10. *Ochlerotatus caspius*: Dark and pale scales on the wing (personal photos magnification 10x).

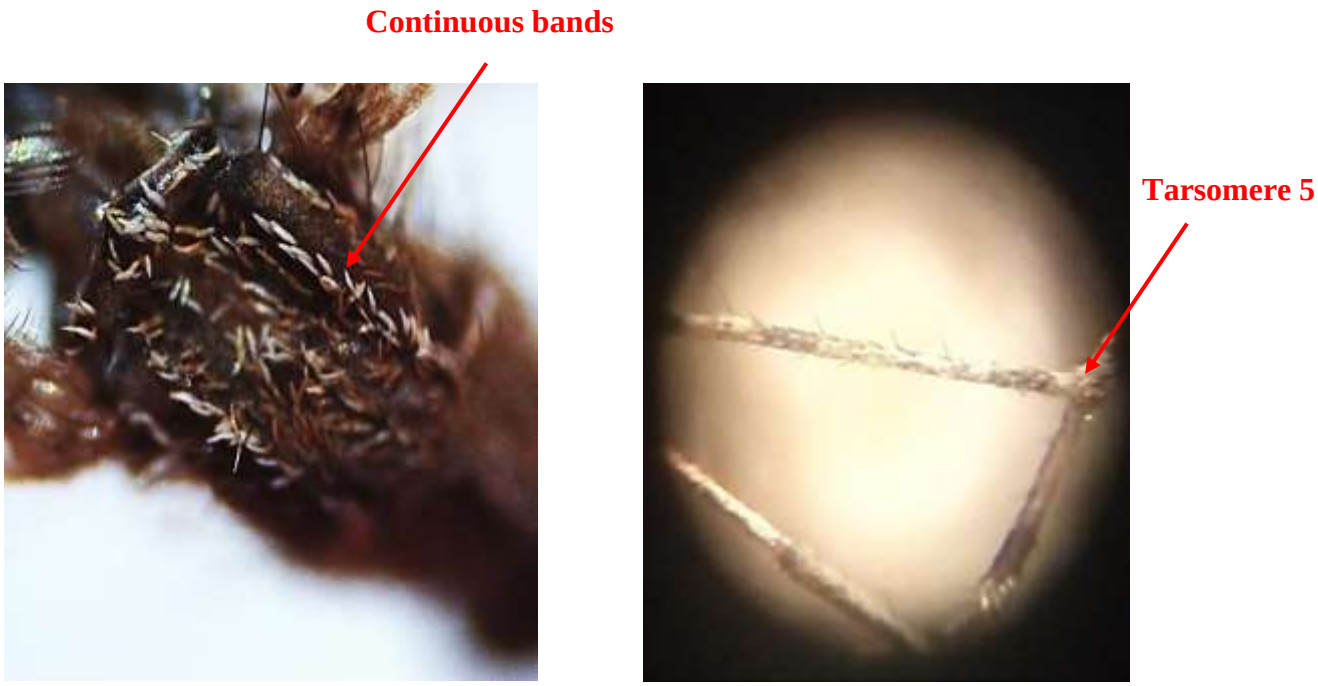


Photo 2.11. *Ochlerotatus caspius*: a) Torax, b) leg (personal photos magnification 10x).

2.1.1.4 *Culex theileri*

2.1.1.4.1 ***Culex theileri* larvae**

- The siphon constitute a cylindrical tube;
- Abdominal plates absent;
- Several pairs of siphonal tufts (Photo 2.13);
- Insertion of the seta 1a-Sis beyond the last pecten tooth (Photo 2.13);
- Arrangement of the siphon setae 1-S is ventral and lateral setae (Photo 2.13);
- The sub-apical spine 2-S is short (Photo 2.13);
- The comb scales are all with a median spine (Photo 2.12);

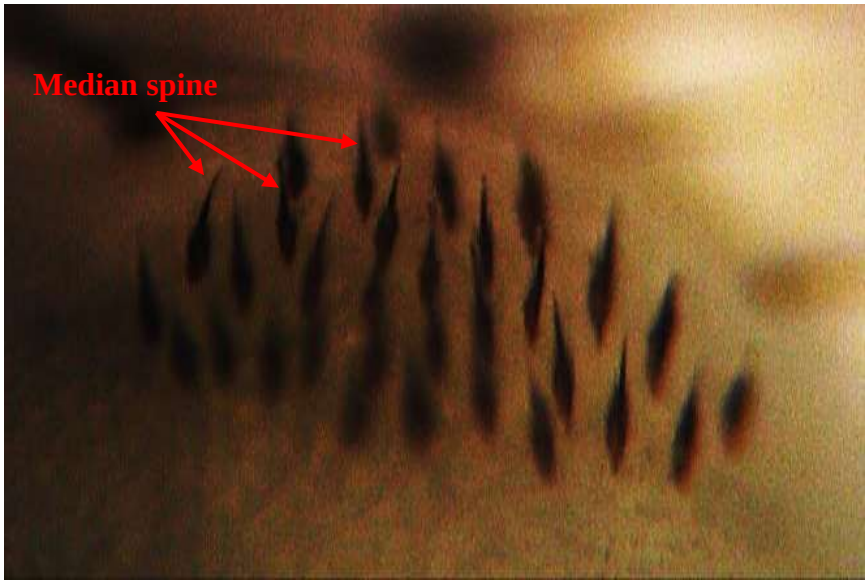


Photo 2.12. Comb scales with median spine (personal photo magnification 40x).

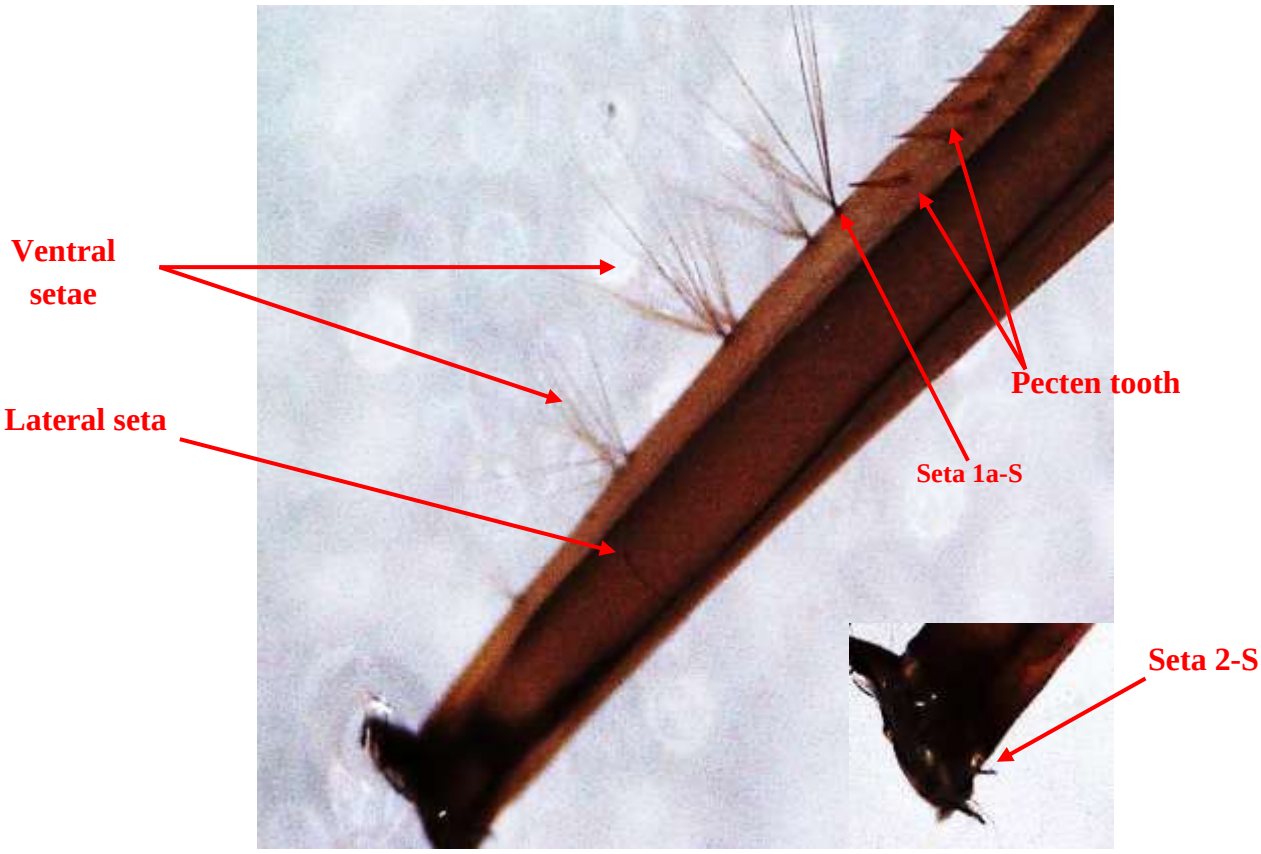


Photo 2.13. Siphon of *Culex theileri* larvae: ventral and lateral tuffs, 1a-S beyond the pecten, seta 2-S is short (personal photo magnification 10x).

2.1.1.4.2 ***Culex theileri* adult**

- Maxillary palpus clearly are shorter than proboscis;
- The proboscis is entirely dark;
- The maxillary palpus is entirely dark;
- Prespiracular and postspiracular setae absent (Photo 2.14);
- The tergite is with creamy white and dark scales (Photo 2.15);
- The tergite III is with a basal pale band extending backwards in a median triangle (Photo Photo 2.15);
- Male genitalia: ventral arm of aedeagus with 3 lateral teeth (Photo 2.16).

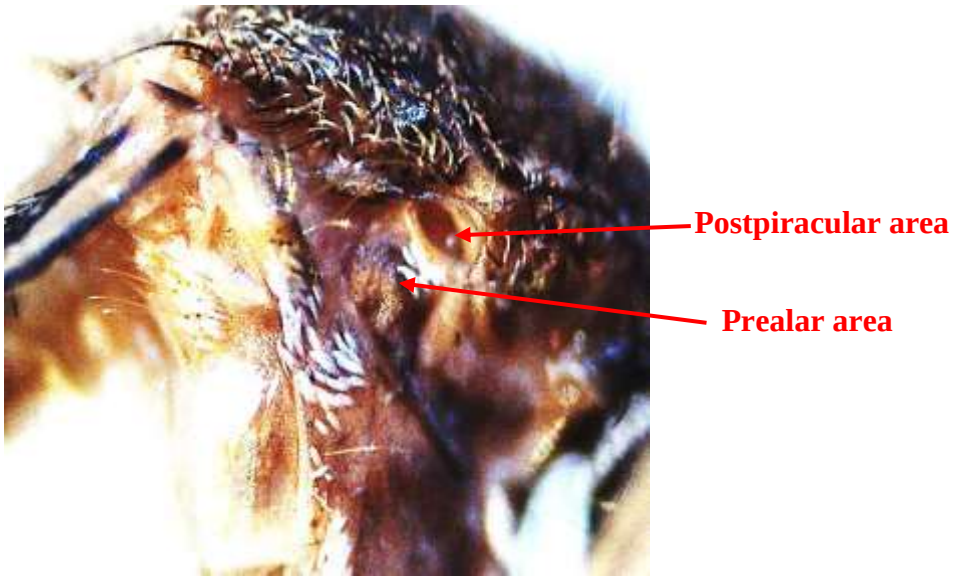


Photo 2.14. *Culex theileri*: prespiracular and postspiracular setae absent (personal photo magnification 10x).

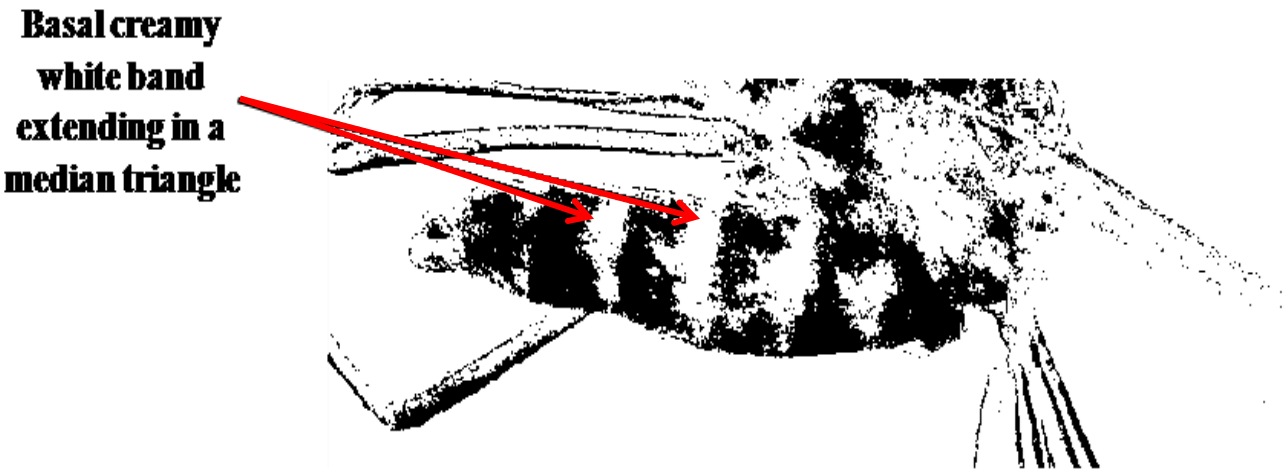


Photo 2.15. *Culex theileri* larvae: the tergite with a basal pale band extending backwards in a median triangle (personal photo).

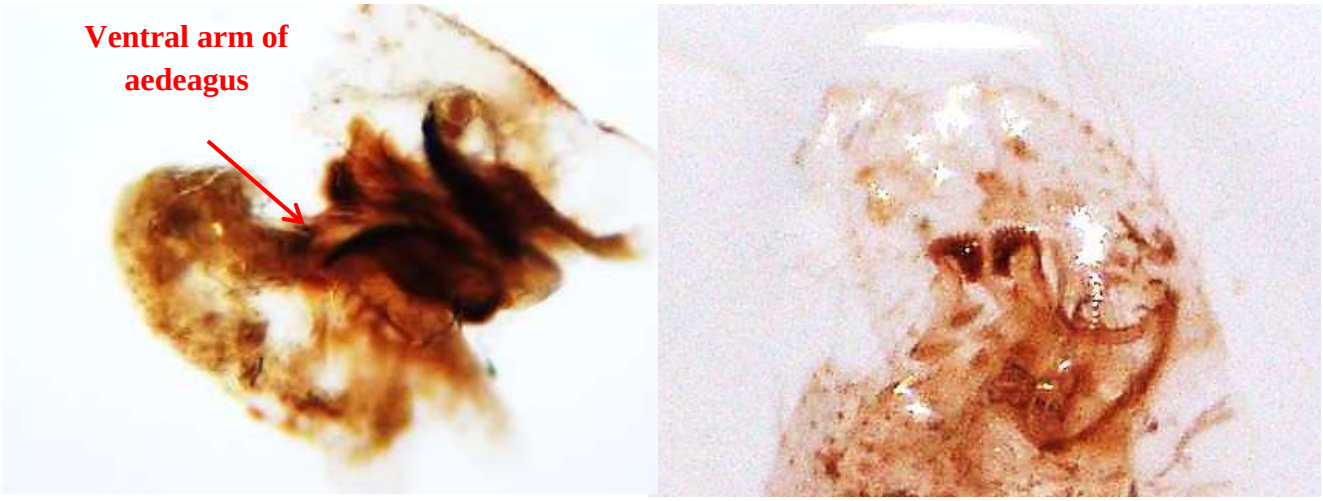


Photo 2.16. *Culex theileri*: male hypopygium (personal photo magnification 10x).

2.1.1.5 *Culex simpsoni*

2.1.1.5.1 *Culex simpsoni* larvae

- Siphon constitue a cylindrical tube;
- Abdominal plates absent;
- Several pairs of siphonal tufts (Photo 2.17);
- Seta 1a-S is longer than the diameter (Photo 2.17);
- 1a-S is 3 branched (Photo 2.17);
- Arrangement of the siphon setae 1-S is ventral and lateral;
- Siphon index is greater than 6 (Photo 2.17);
- The sub-apical spine 2-S is short (Photo 2.17);
- Distal tooth with 3 basal denticles.

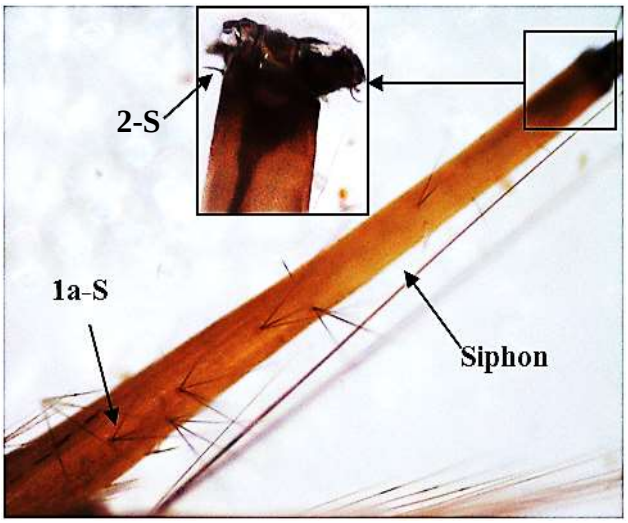


Photo 2.17. *Culex simpsoni*: insertion and number of branches of seta 1a-S, and shape of 2-S (personal photo magnification 4x).

2.1.1.5.2 *Culex simpsoni* adult

- Maxillary palpus clearly shorter than proboscis;
- The tergite is covered with creamy white and dark scales;
- Ornametation of tergite III with a basal pale band;
- Color of tarsomere 5 is entirely dark;
- Scutum's ornamentation is without well-marked patterns;
- Color of maxillary palpus is entirely dark (Photo 2.18);
- Color of proboscis is entirely dark (Photo 2.18).

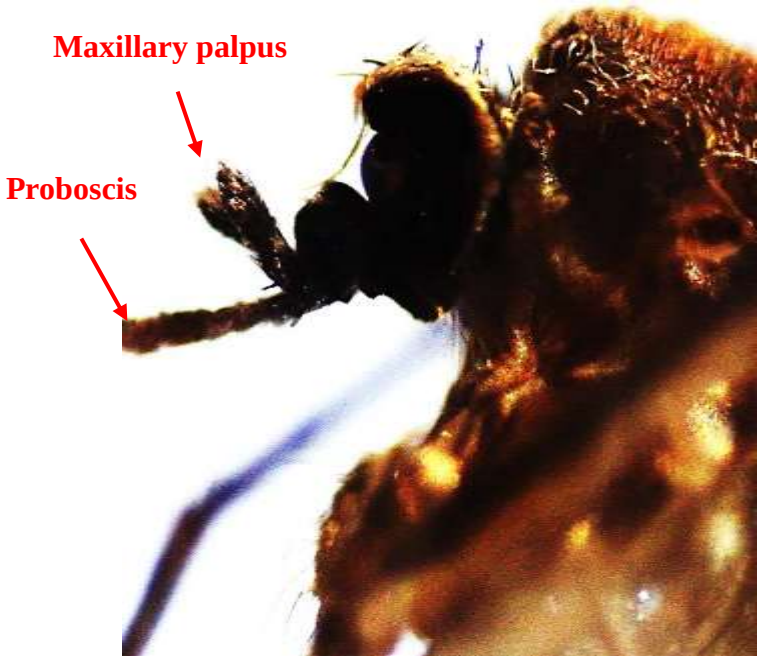


Photo 2.18. *Culex simpsoni* proboscis and maxillary palpus (personal photo magnification 10x).

2.1.1.6 *Culex hortensis* larvae

- Siphon constitue a cylindrical tube;
- Abdominal plates absent;
- Several pairs of siphonal tufts (Photo 2.19);
- Arrangement of the siphon setae 1-S is ventral and lateral (Photo 2.19);
- Siphon index is greater than 6;
- The sub-apical spine 2-S is long and hooked (Photo 2.19).

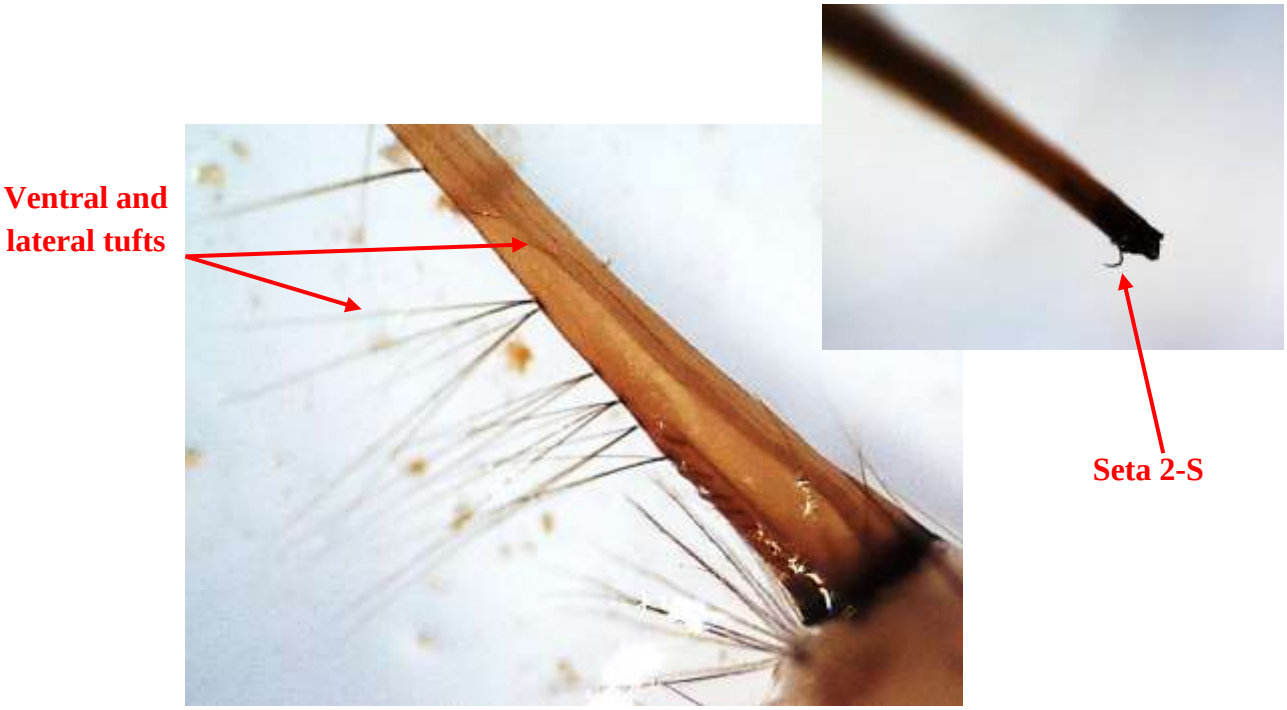


Photo 2.19. *Culex hortensis* larvae: siphon with ventral and lateral tufts and seta 2-S long and hooked (personal photos magnification 10x).

2.1.1.7 *Culex pipiens* s.l.

2.1.1.7.1 *Culex pipiens* s.l. larvae

- Siphon constitue a cylindrical tube;
- Abdominal plates absent;
- Several pairs of siphonal tufts;
- Arrangement of the siphon setae 1-S is ventral and lateral;
- Number of branches of the seta 14-C is 1 branch (Photo 2.21);
- Number of branches of the seta 6-C is 5 branches (Photo 2.21);
- Number of branches of the saddle seta 1-X is 1 branch (Photo 2.20);
- The siphon is with straight sides (Photo 2.20).

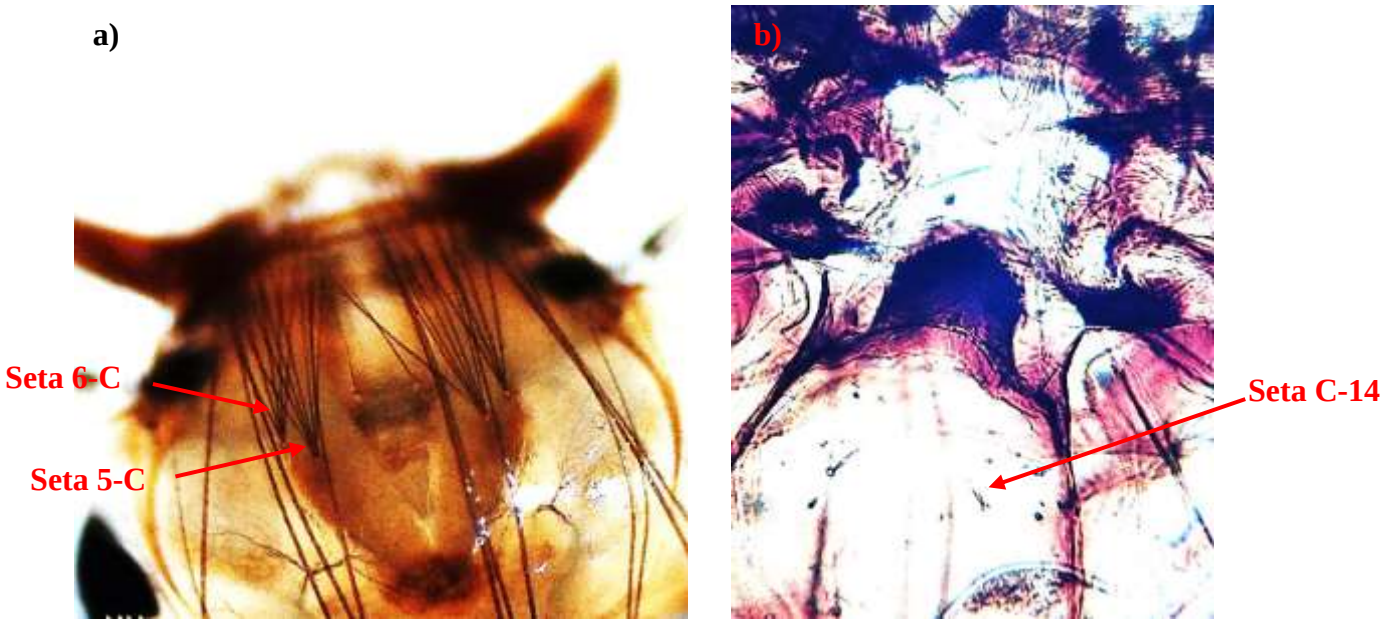


Photo 2.21. *Culex pipiens*, cephalic setae: a) 6-C and 5-C, b) 14-C (personal photo magnification 10x, 40x).

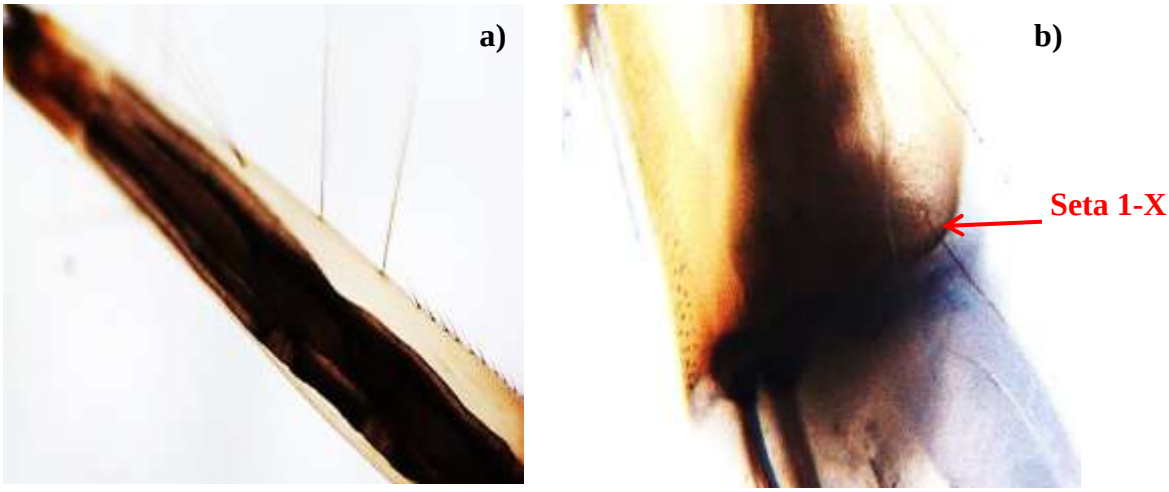


Photo 2.20. *Culex pipiens* larvae: a) siphon with straight sides, b) saddle seta 1-X (personal photo magnification 10x).

2.1.1.7.2 ***Culex pipiens* adult**

- Maxillary palpus clearly shorter than proboscis;
- The base wing with a fringe of scales (Photo 2.22);
- The wing is entirely dark (Photo 2.22);
- LEG III: the tarsomere 1 is entirely dark (Photo 2.22);
- LEG III: the tarsomere 5 is entirely dark (Photo 2.22);
- LEG III: the tibia is entirely dark;
- The maxillary palpus is entirely dark;
- The proboscis is entirely dark;

- Scutum is without well-marked patterns(Photo 2.23);
- The prespiracular and postspiracular setae are absent;
- The tergite is covered with creamy white and dark scales (Photo 2.23);
- The tergite III is with a basal pale band (Photo 2.23).

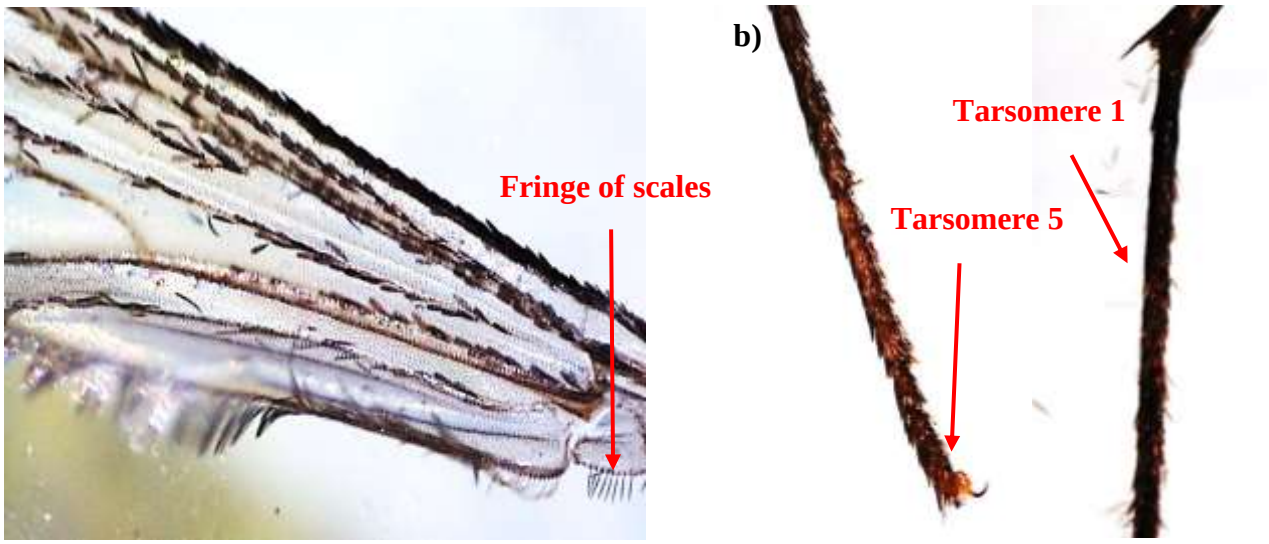


Photo 2.22. *Culex pipiens*: a) wings with scale in the base, b) leg III, tarsomere 1 and 5 (personal photos magnification 10x).

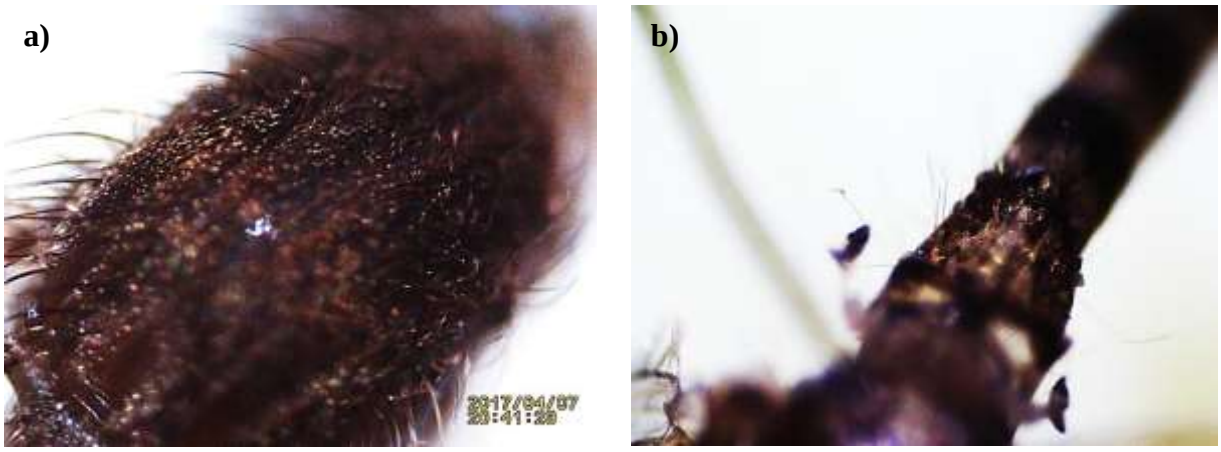


Photo 2.23. *Culex pipiens*: a) thorax, b) tergite with base pale band (personal photos magnification 10x).

2.1.1.8 *Anopheles labranchiae*

2.1.1.8.1 *Anopheles labranchiae* larvae

- Siphon directly opening on the segment VIII;
- Palate hair distributed from segment III to segment VII;
- Seta 8-C six branched (Photo 2.24);
- Seta 2-C aciculate at the apex (Photo 2.24).

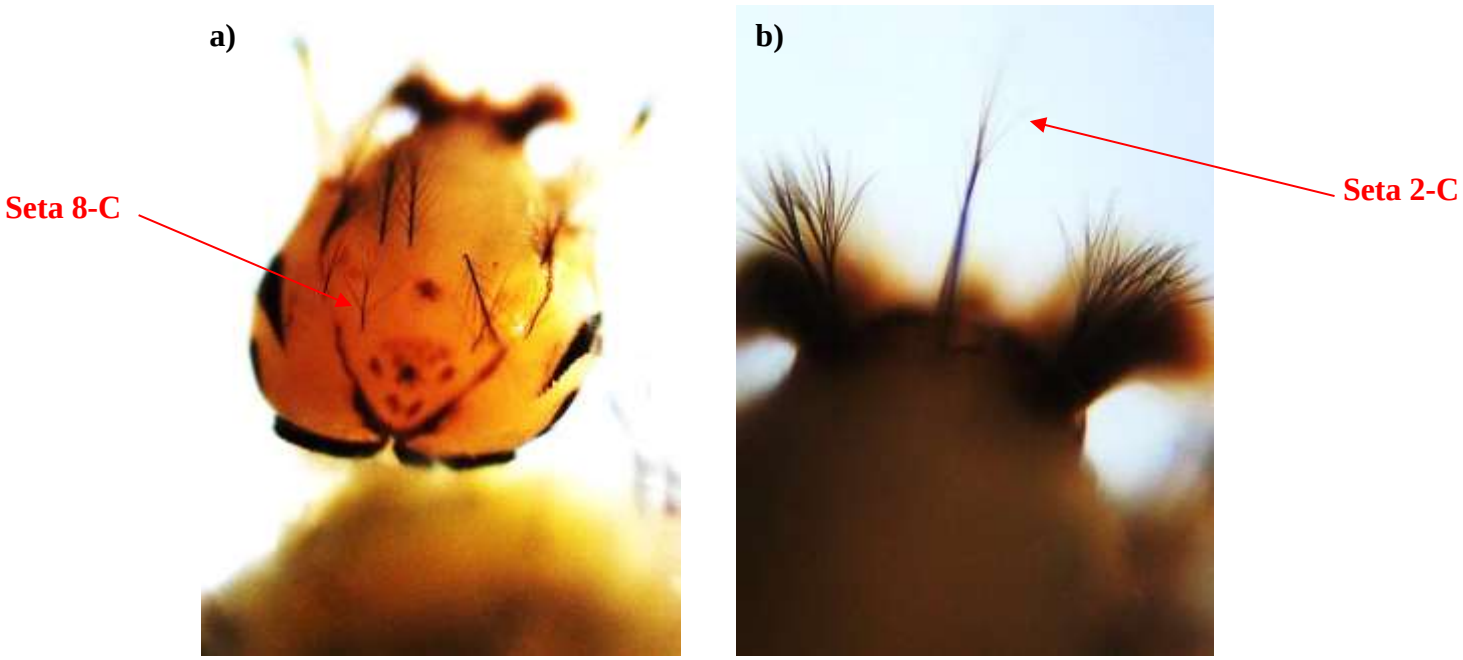


Photo 2.24. Cephalic setae of *Anopheles labranchiae* larvae: a) seta 8-C six branched, b) seta 2-C aciculate at the apex (personal photos magnification 10x, 4x).

2.1.1.8.2 ***Anopheles labranchiae*** **adult**

Female:

- Maxillary palpus nearly equal to proboscis (Photo 2.25);
- Dark wings with black spots (Photo 2.26);
- Scutum's ornamentation with only one median band (Photo 2.27);

Male:

- Gonocoxite without lobes (Photo 2.28);
- Gonostylus about as long as gonocoxite (Photo 2.28);
- Two parabasal setae (Photo 2.28);

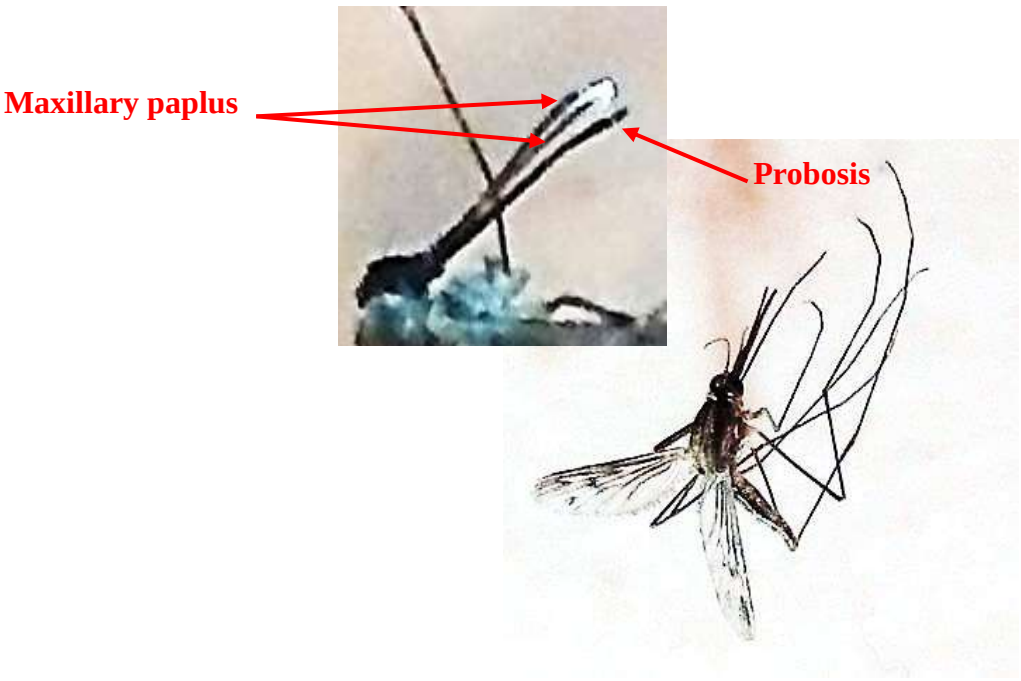


Photo 2.25. *Anopheles labranchiae* female: Maxillary palpus nearly equal to proboscis (personal photos).

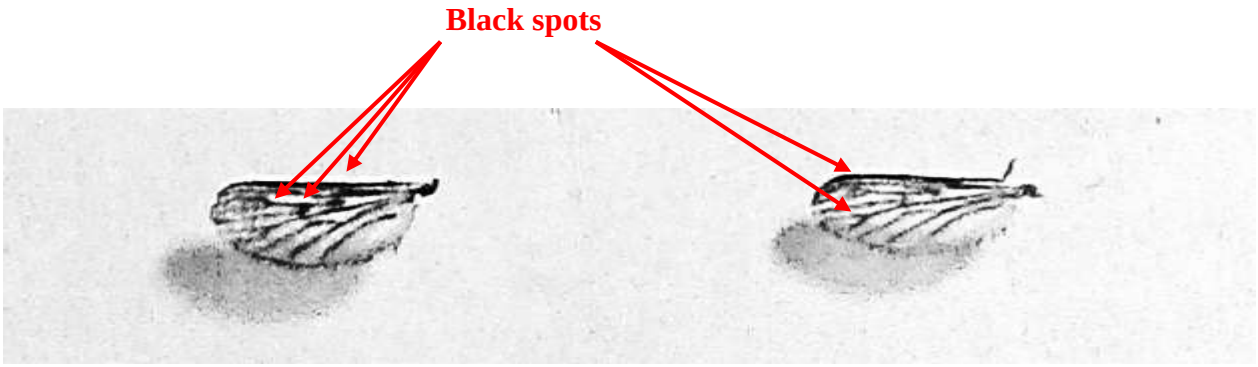


Photo 2.26. *Anopheles labranchiae* female: dark wings with black spots (personal photo).

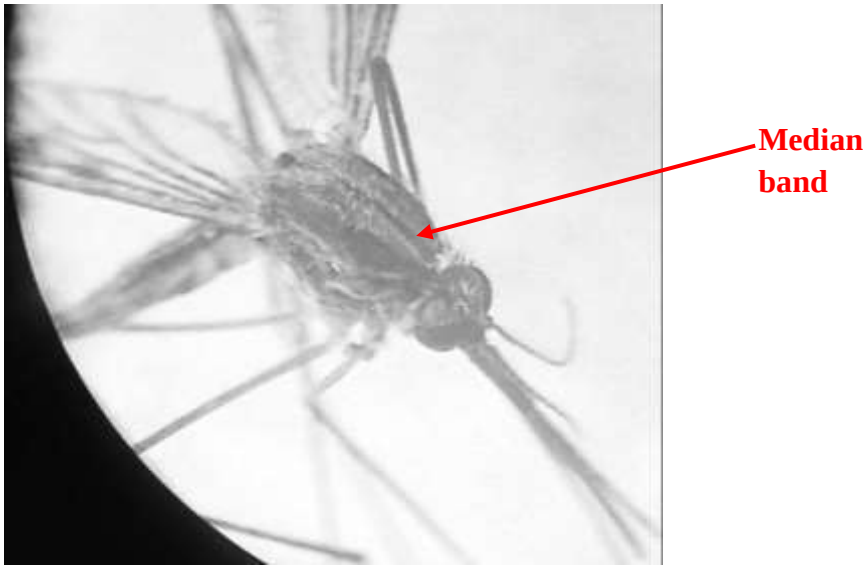


Photo 2.27. *Anopheles labranchiae* female: scutum with one median band (personal photo).

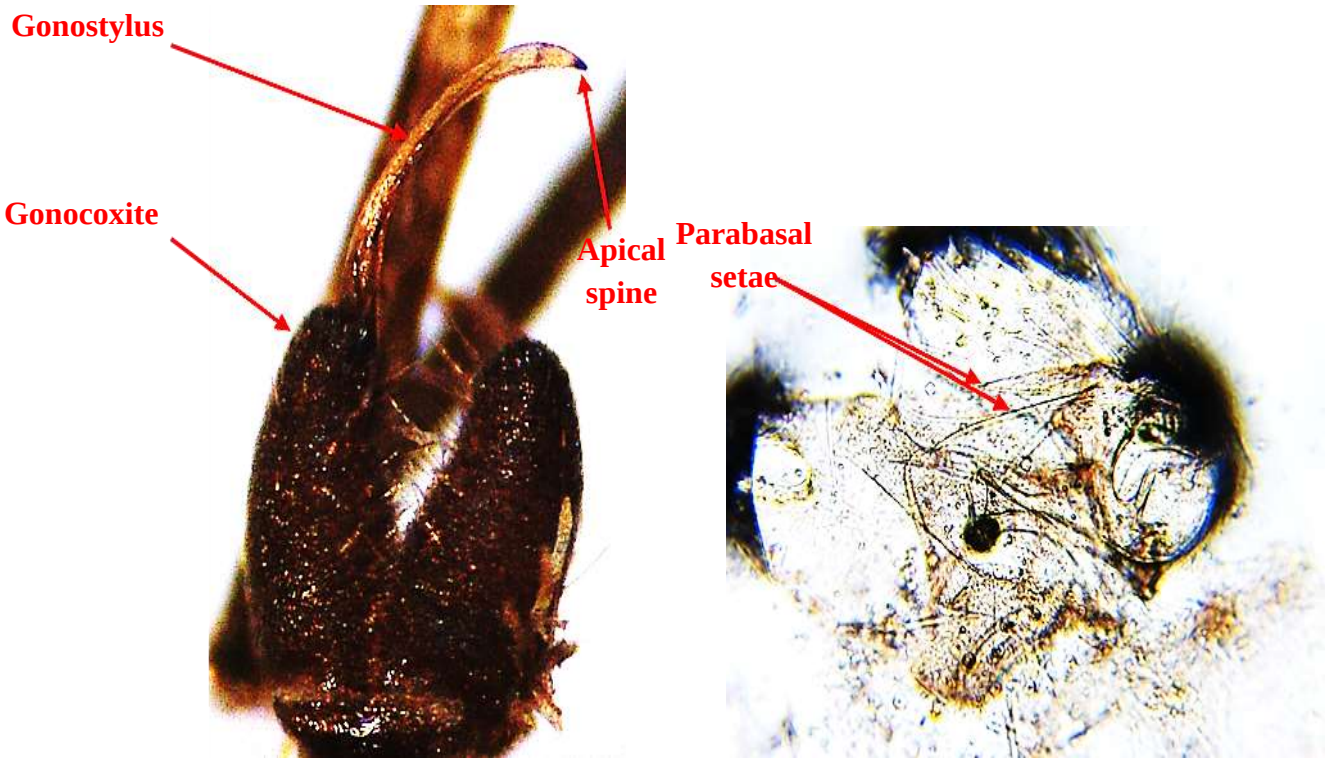


Photo 2.28. *Anopheles labranchiae* hypopygium: a) Gonostylus about as long as gonocoxite b) Two parabasal setae (personal photos magnification 10x).

2.1.2 **Morphological variation noted on *Culex pipiens* s.l larvae**

A total of 581 *Culex pipiens* larvae were sampled from the study area, only fourth-instar larvae were used in the morphological examination. The data of the branch number of head setae (5-C, 6-C), abdominal setae (7-I, 6-I, 1-III, 1-IV) and siphonal seta (1-S) as well as the number of pecten teeth, the siphon shape, the arrangement of seta 1-S and the insertion of the first siphonal seta 1a-S are mentioned in Table 2.1.

Our observations during this study, revealed inconstant morphological characters even within larvae collected from the same breeding site, especially in seta 1-S. However, in the most often, the dorsal anal papillae were longer than saddle and the ventral papillae were smaller (Photo 2.30); the mental plate showed 11 spines on the left side of the apical median tooth and 10 on the right side (Photo 2.30).

Notably, the abdominal setae 1-III and 1-IV were registered because of their importance in the differentiation of the members of *Culex pipiens* complex, they were respectively 1-3 and 1-2 branched and their arrangement together was as follows: 1-2, 2-1, 2-2, 3-2 respectively. Additionally, the branch number of the abdominal seta 7-I and 6-I were also noted, they were normally 2-3 branched except for one group were 7-I was 4 branched.

The siphon had a narrowing shape with a straight side (Photo 2.31); however, larvae from Beni Fouda and Beni Aziz had a siphon with a more wide shape (Photo 2.31). 57% of larvae with straight siphon side had siphon index less than 5 where the total of larvae with wide siphon represented siphon index less than 4.62. The siphon index measured was 3.1-5.3.

The siphonal seta 1a-S was 4 branched in 411 larvae and 5 branched in 143 larvae. Moreover, the alignment of seta 1-S showed a variation; in larvae with seta 1a-S 4 branched, seta 1c-S was out of line with the other siphonal hair tufts (Photo 2.32) in 365 larvae but in 24 larvae 1d-S was out of line (Photo 2.32). In the other hand, larvae with seta 1a-S 5 branched divided into two groups, a group of larvae that showed the same arrangement of seta 1-S in the both sides of the siphon n=93, where 1d-S was out of line with the other siphonal hair tufts (Photo 2.32); and a second group where the arrangement of seta 1-S had changed from a side to another n=50, where 1c-S was out of line in the right side of the siphon and 1d-S was out of line in the left side (Photo 2.31).

The first and second siphonal seta 1a-S and 1b-S was 2 to 5 branched. Seta 1a-S was inserted above and near to the last pecten tooth (Photo 2.29) or above and far from the last pecten tooth (Photo 2.29); except for larvae collected from Beni Fouda n=74, where 1a-S was inserted next to the last pecten tooth: in the both sides in 24 larvae (Photo 2.29), and only in the left side in 50 larvae (Photo 2.29). This group had generally, siphon with straight side and 1-S 5 arranged and exceptionally siphon with wide shape and 1-S 4 arranged. We noted as well a variation in the number of the pecten teeth within this group (13-16).

Table 2.1. Morphological characteristics of *Culex pipiens* s.l populations sampled from Setif region

Sites	N	Number of branches											P	A	Mean siphon index	Pecten tooth
		5-C	6-C	7-I	6-I	1-III	1-IV	1a-S	1b-S	1c-S	1d-S	1e-S				
Belaa	138	-	5	2	2	2	2	4	5	3	3		P1	A1	3.6	10-13
Beni Fouda	50	6	5	3	2	2	2	3	3	2	2	2	P3	A2	3.3	14
	24	5	4	2-3	2-3	2	1-2	4	4	3	-		P2	A3	4	12-16
Beni Aziz	93	5	4	3	3	1	2	3	3	3	3	1	P1	A3	3.2	14
	31	6	5	2	3	2	2	2	3	4	-		P1		4.3	9-13
Ain Oulmen	43	5	5	2	3	1	2	4	5	2	2		P1	A1	4.2	10
El Eulma	140	5	-	4	2	2	2	2	2	2	3		P1	A1	5 .2	10-13
Mawklen	35	-	-	3	2	2	2	3	3	4	2		P1	A1	4.1	11

P: 1a-S position;

P1: 1a-S above the last pecten tooth;

P2: 1a-S next the last pecten tooth;

P3: 1a-S above the last pecten tooth in the right side and next to the last pecten tooth in the left side.

A: arrangement of siphonal setae

A1: 1c-S out of line with the others;

A2: 1c-S out of line with the others in the right side and 1d-S in the left;

A3: 1d-S out of line with the others.

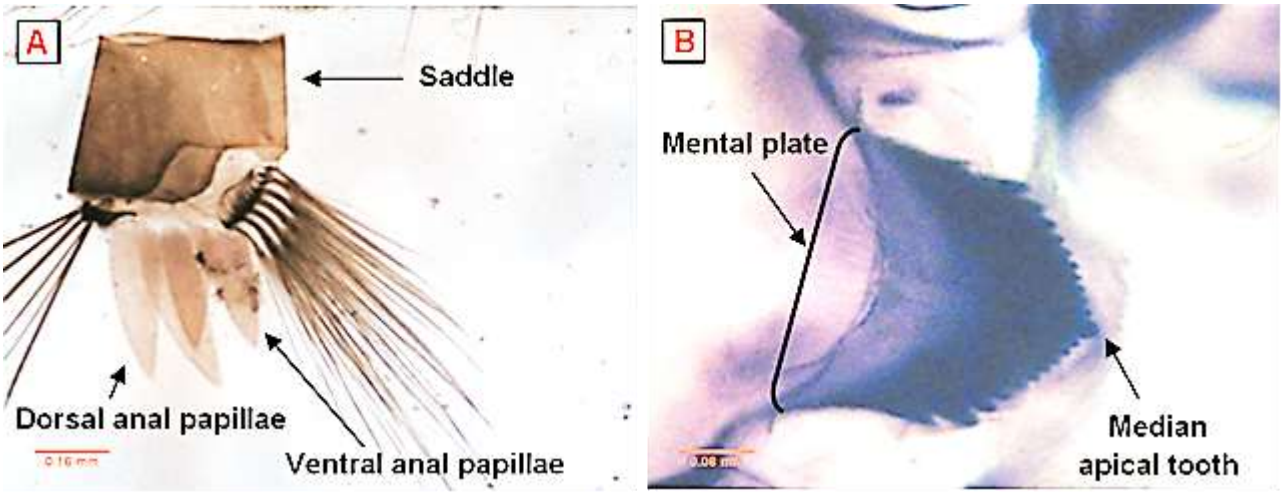


Photo 2.30. Shared characters in the sampled larvae: A) dorsal anal papillae equal to the saddle and ventral anal papillae smaller than saddle, B) mantle plate with 11 teeth on the left side of the median apical tooth and 10 on the right side (personal photos magnification 10x, 4x).

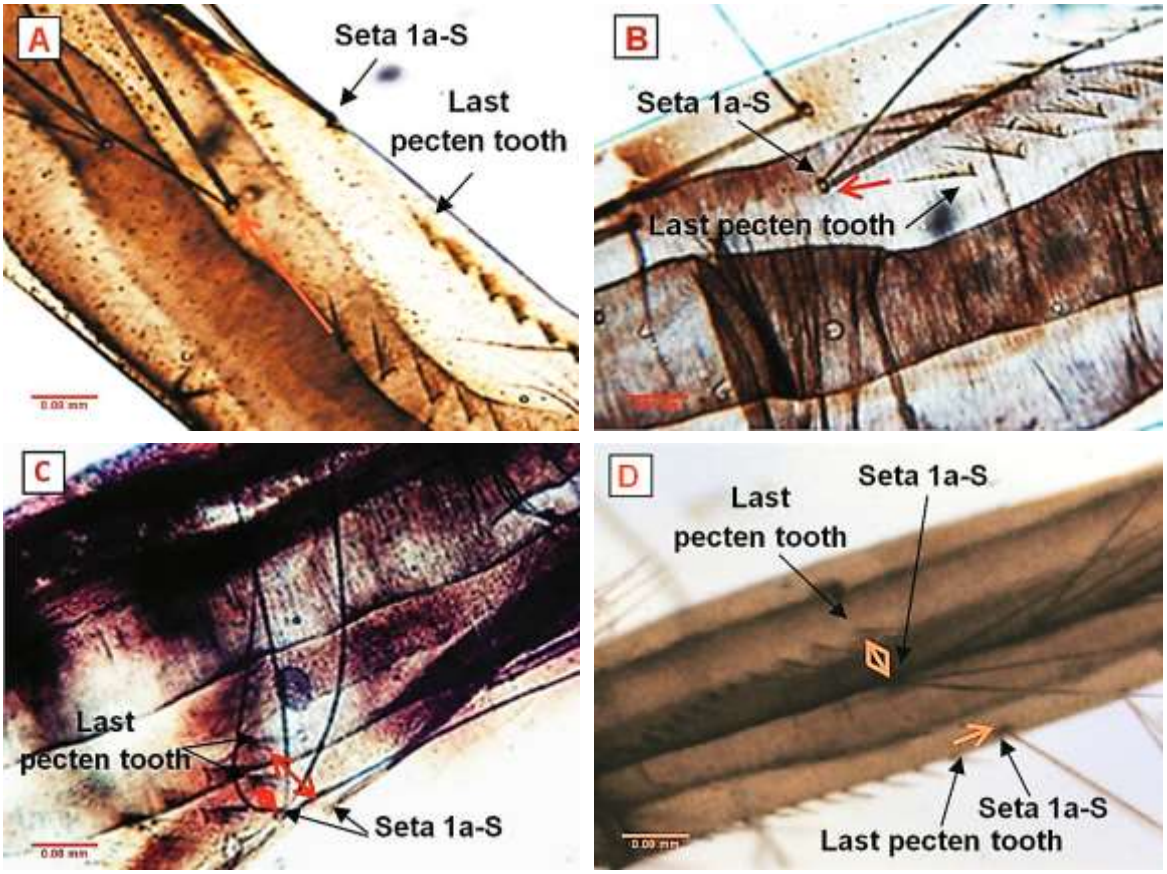


Photo 2.29. Variation in the position of siphonal seta 1a-S: A) 1a-S above and far from the most distal pecten tooth B) la-S above and very near to the most distal pecten tooth, C) 1a-S next to the most distal pecten tooth in the both sides, D) 1a-S next to the most distal pecten tooth in the left side and 1a-S above the most pecten tooth in the right side (personal photos magnification 4x).

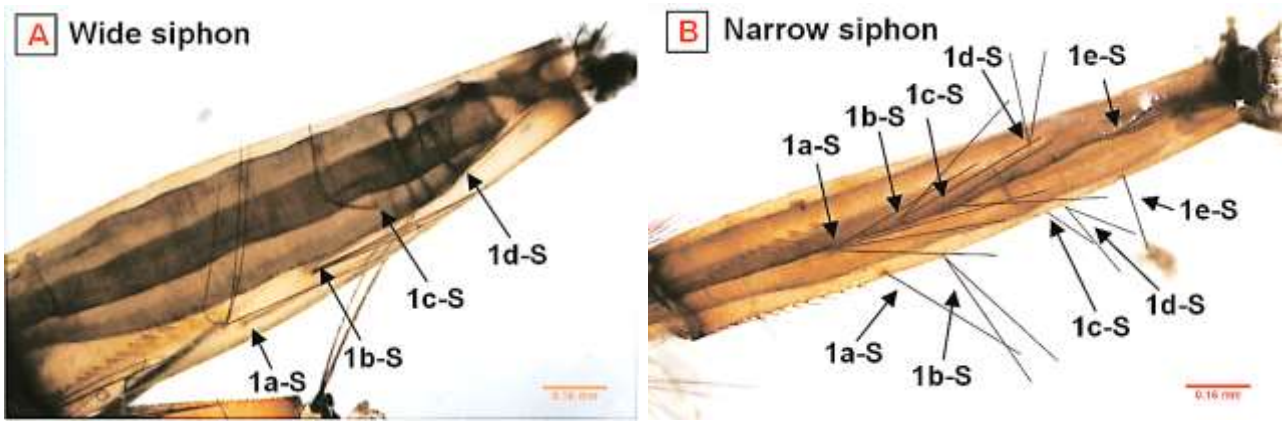


Photo 2.31. Shape of siphon and Arrangement of siphonal setae in larvae collected from Beni Fouda : A) Wide siphon with setae 4 arranged and 1c-S out of line, B) Narrow siphon with setae 5 arranged and 1d-S out of line in the left side of the siphon and 1c-S out of line in the right side (personal photos magnification 10x).

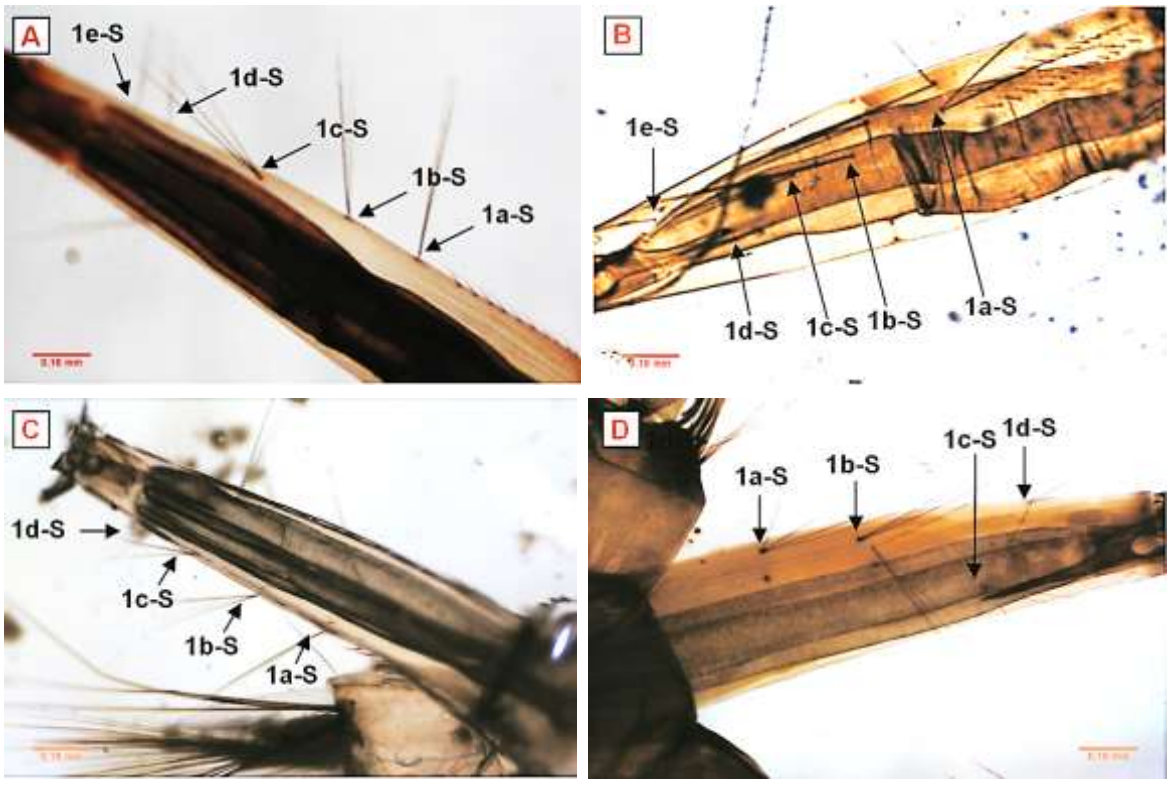


Photo 2.32. Arrangement of siphon setae in larvae with 1a-S inserted above the last pecten tooth : A) 1-S 5 arranged and 1c-S is out of line, B) 1-S 5 arranged and 1d-S is out of line, C) 1-S 4 arranged and 1d-S is out of line, D) 1-S 4 arranged and 1c-S is out of line (personal photos magnification 10x).

2.1.3 **Molecular identification**

The molecular analyses were conducted in the institute of parasitology and tropical pathology, Strasbourg, France. The blast of COI locus extracted from the sampled species showed a range of 99% to 100% closest matching to sequences from Genbank and BOLD. The generated sequences and the similarities obtained are illustrated in Table 2.2.

We noted higher similarity for *Culex pipiens* s.l. to *Cx. p pipiens* from Iran (JQ958370) and Germany (HF562662) (identy 99% to 100%, query covrage 92% to 100%) and *Cx. p quinquifaciatus* from Brasil (KF919190) (identy 100%, query covrage 91% to 95%) ; lower matching was noted to only one sequence *Cx. pmolestus* from Russia (FN395171) (identy 99%, query covrage 99%). Likewise, *Cx. theileri* from Spain (JN051388), Portugal (HE610459), USA (KJ012182) and United Kingdom (FJ210898) revealed high similarity to our *Cx. theileri* sequences (99% identy), while *Cx. hortensis* only sequences from USA showed closest matching (99% identy) with our *Cx. hortensis* sequences. We noted also higher similarity of *An labranchiae* with *An labranchiae* from United Kingdom (identy 99%, query covrage 95%), while other members of the *maculipennis* complex presents lower similarity: 98% identy for *An atroparvus* and *An maculipennis* s.s., and 97% identy for *An messea.* For *Oc. caspius*, the blast showed closest matching (99% identy) to sequences: from USA MG242478 (query coverage 95%), Belgium KM258357 (query coverage 92%), Spain LC090050 (query coverage 91%) and United Kingdom JQ246394 (query coverage 89%), while the closely species *Oc. dorsalis* from Sweden (KP942726) showed 98% identy with our *Oc. caspius* sequences. In contrast, the closest matching sequences for *An. c hispaniola* in Genbank were *An. darlingi* from USA (DQ076236) and Brazil (JF923693) with 93% identy then *An. cinereus* (KM068089) from Saudi Arabia with 92% identy and its homologous *An. turkhudi* (KM389467) from Iran with 92% identy. However, *An. cinereus* sequences from Morocco provided in BOLD system (private sequences) showed high level of similarity 99.93% with our *An. c hispaniola* sequence.

Culex torrentium presented 2.8% minimum divergence with sequences from the sampled *Cx. pipiens* sl. The genetic distance among *An. labranchiae* sequences from Genbank including our sequences averaged between 0,5% and 1.6%; while the divergence between *An. labranchiae* and the other maculipennis members *An. maculipennis s.s., An. atroparvus, An. beklemishevi, An. melanoon, An. sacharovi, An. subalpinus* and *An. messea* varied from 2.3% to 82.2%. Further, we noted divergence of 8.9% between *Anopheles cinereus hispaniola* and

An. c cinereus from Saudi Arabia, and divergence of 73% between our *An. c hispaniola* sequence and *An. turkhudi* sequences from Iran (KM389467). The genetic distance between *Oc. caspius* and *Oc. dorsalis* from Genbank varied from 2.2% to 3.7%, and the pairwise divergence within *Oc. caspius* sequences varied from 0.05% to 0.26%. The phylogenetic tree in Figure 3.1 represents the evolutionary relationship among the sampled species and sequences from Genbank.

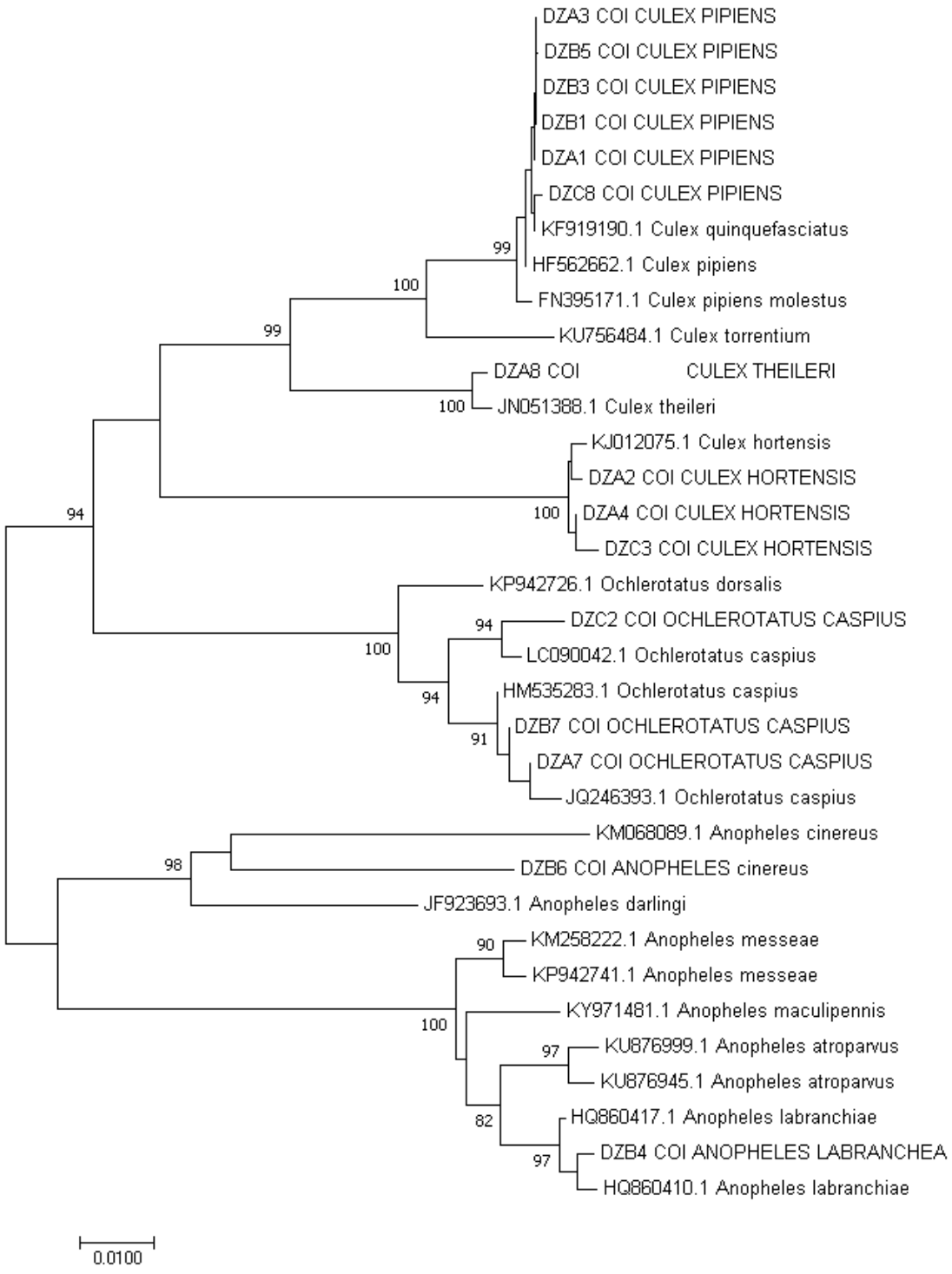


Figure 3.1. Phylogenetic tree represents the evolutionary relationship among the sampled species and sequences from Genbank.

Table 2.2. The blast results of the sampled species sequences with Genbank accessions.

Sampled species		Compared sequences			
Species name	Accession	Species name	Identy %	Accession	Country
Cx. pipiens s.l.	(MK047302, MK047304, MK047308, MK047309, MK047311, MK047314, MK047315)	*Cx. pipiens pipiens*	100	HF562662	Germany
			99	JQ958370	Iran
		Cx. pipiens quinquifaciatus	100	KF919190	Brasil
		Cx. pipiens molestus	99	FN395171	Russia
Cx. theileri	(MK047307)	*Cx. theileri*	99	JN051388	Spain
			99	HE610459	Portugal
			99	KJ012182	USA
			99	FJ210898	United Kingdom (UK)
Cx. hortensis	(MK047303, MK047305)	*Cx. hortensis*	99	KJ012075	USA
An. labranchiae	(MK047310)	*An. labranchiae*	99	HQ860410	UK
		An. atroparvus	98	KU876999	UK
		An. messea	97	KM258222	Belgium
An. c hispaniola	(MK047312)	*An. cinereus*	99.93	Private sequences (BOLD system)	Morocco
		An. darlingi	93	DQ076236	USA
			93	JF923693	Brazil
		An. cinereus	92	KM068089	Saudi Arabia
		An. turkhudi	92	KM389467	Iran
Oc. caspius	(MK047306, MK047313)	*Oc. caspius*	99	MG242478	USA
			99	KM258357	Belgium
			99	LC090050	Spain
			99	JQ246394	UK

2.2 **Sampling data**

A total of 1165 mosquitoes have been sampled during the years 2016, 2017, 2018, and 2019. 800 larvae and 365 adults were sampled from 21 sites in Setif region. The sampling data including sites, date of sampling, Nature of sites, type of site, presence or absence of algae, stage of sampling and number of specimens is provided in Table 2.3. The ecological indices indicated Taxa_S= 9, Simpson_ 1-D= 0.7, Shannon_H= 1.5, Evenness E= 1.5.

Table 2.3. Sampling data of mosquitoes in Setif, Northeastern Algeria, from 2016 to 2019.

No. Site	Date	Nature	Type	Algae	Stage	Species	No.
Not considered	29/04/2016	Rural	Temporary	Presence	Larvae	Not identified	13
Not considered	18/05/2016				Larvae	Not identified	17
Not considered	25/06/2016				Larvae	Not identified	4
Site 1	04/05/2016	Rural	Permanent	Presence	Larvae	*An. labranchiae*	2
	04/05/2016				Larvae	Not identified	11
	12/08/2016				Larvae	*Cx. pipiens* s.l.	3
Site 2	12/08/2016	Rural	Permanent	Absence	Larvae	*Cx. pipiens* s.l.	9
	02/04/2017				Larvae	*Cx. pipiens* s.l.	16
Site 3	03/11/2018	Rural	Temporary	Absence	Larvae	*An. labranchiae*	2
	03/11/2018				Larvae	*Cx. theileri*	12
	03/11/2018				Larvae	*Cx. pipiens* s.l.	31
Site 4	03/11/2018	Rural	Permanent	Presence	Larvae	*An. labranchiae*	28
	03/11/2018				Larvae	*Cs. longiareolata*	6
	03/11/2018				Larvae	*Cx. theileri*	46
	03/11/2018				Larvae	*Cx. simpsoni*	1
Site 6	02/05/2016	Rural	Temporary	Presence	Larvae	*Cx. hortensis*	16
Site 7	03/11/2018	Rural	Permanent	Presence	Larvae	*Cx. hortensis*	11
Site 8	11/08/2016	Rural	Temporary	Absence	Larvae	*Cx. pipiens* s.l.	3
	11/08/2016				Larvae	*Cs. longiareolata*	117
Site 9	11/08/2016				Larvae	*Cx. pipiens* s.l.	5

	11/08/2016				Larvae	*Cs. longiareolata*	125
Site 17	16/05/2017	Rural	Permanent	Presence	Larvae	*An. labranchiae*	4
	16/05/2017				Larvae	*Cx. hortensis*	46
	16/05/2017				Larvae	*Cx. theileri*	11
Not considered	07/05/2016	Rural	Temporary	Presence	Larvae	Not identified	18
Site 18	17/05/2017	Rural	Permanent	Presence	Larvae	*Cx. hortensis*	33
	17/05/2017				Larvae	*Cx. theileri*	11
Site 13	27/03/2017	Rural	Temporary	Absence	Larvae	*Cx. pipiens* s.l.	74
Site 14	03/11/2018	Rural	Permanent	Absence	Larvae	*An. labranchiae*	2
	03/11/2018				Larvae	*Cs. longiareolata*	7
	03/11/2018				Larvae	*Cx. pipiens* s.l.	15
	03/11/2018				Larvae	*Cq. richiardii*	2
	03/11/2018				Larvae	*Cx. hortensis*	2
Site 20	14/08/2016	Urban	Permanent	Presence	Larvae	*Oc. caspius*	40
Site 21	02/11/2018	Urban	Temporary	Absence	Larvae	*Oc. caspius*	8
	02/11/2018				Larvae	*Cx. theileri*	3
	02/11/2018				Larvae	*Cx. pipiens* s.l.	13
	02/11/2018				Larvae	*Oc. caspius*	1
Site 15	18/05/2017	Rural	Permanent	Presence	Larvae	*An. labranchiae*	9
Site 16	18/05/2017	Urban	Permanent	Absence	Larvae	*Cx. pipiens* s.l.	23
Site 5	29/11/2018	Rural	Beside house		Adult	*Cs. longiareolata*	1
	29/11/2018				Adult	*Cx. pipiens* s.l.	4
	03/12/2018				Adult	*Cx. pipiens* s.l.	3
	12/12/2018				Adult	*Cx. pipiens* s.l.	5
	20/12/2018				Adult	*Cx. pipiens* s.l.	1
Site 10	08/08/2016	Urban	Inside house		Adults	*Cx. pipiens* s.l.	1
	10/08/2016				Adults	*Cx. pipiens* s.l.	1
	03/11/2018				Adult	*Cx. pipiens* s.l.	5
	03/11/2018				Adult	*Cx. pipiens* s.l.	1
	02/12/2018				Adult	*Cx. pipiens* s.l.	1
	07/12/2018				Adult	*Cx. pipiens* s.l.	17

Site 11	18/08/2016	Urban	Inside house		Adults	*Cx. pipiens* s.l.	7
	13/08/2016				Adults	*Cx. pipiens* s.l.	1
	08/11/2018				Adult	*Cx. pipiens* s.l.	1
	09/11/2018				Adult	*Cx. pipiens* s.l.	1
	18/11/2018				Adult	*Cx. pipiens* s.l.	2
	20/11/2018				Adult	*Cx. pipiens* s.l.	1
	23/11/2018				Adult	*Cx. pipiens* s.l.	1
	26/11/2018				Adult	*Cx. pipiens* s.l.	2
	27/11/2018				Adult	*Cx. pipiens* s.l.	2
	01/12/2018				Adult	*Cx. pipiens* s.l.	4
	23/12/2018				Adult	*Cx. pipiens* s.l.	2
	26/12/2018				Adult	*Cx. pipiens* s.l.	4
	29/12/2018				Adult	*Cx. pipiens* s.l.	1
	11/02/2019				Adult	*Cx. pipiens* s.l.	1
	25/02/2019				Adult	*Cx. pipiens* s.l.	1
	17/02/2019				Adult	*Cx. pipiens* s.l.	2
Site 12	10/08/2016	Rural	Livestock		Adults	*An. c hispaniola*	6
Site 19	15/08/2016	Urban	School courtyard		Adult	*Oc. caspius*	21
	26/11/2018				Adult	*Cx. pipiens* s.l.	37
	03/12/2018				Adult	*Cx. pipiens* s.l.	38
	10/12/2018				Adult	*Cx. pipiens* s.l.	36
	17/12/2018				Adult	*Cx. pipiens* s.l.	39
	24/12/2018				Adult	*Cx. pipiens* s.l.	40
	31/12/2018				Adult	*Cx. pipiens* s.l.	37
	07/01/2019				Adult	*Cx. pipiens* s.l.	38

2.3 **Mosquito descriptive parameters**

2.3.1 **Total descriptive parameters**

The total mean density of mosquitoes in the study area was 20.8±3.8 (AM±SE) per 56 samplings, the standard deviation SD= 28.2 refers to a remarkable difference in the number of species between the sites (Table 2.4). This is confirmed also by the test of normality of Kolmogorov Smirnov (P<0.05, non-normality distribution of data).

Table 2.4. Descriptive statistics of mosquitoes in the study area.

	N	Minimum	Maximum	Mean	Std. Deviation	Std. Error
Nb mosquito	56	1	130	20.80	28.211	3.770

When we calculated the mean density of larvae and adults separately, the larvae mean density was 40 larvae by site and the adults mean density was 10.14 adult per site (Table 2.5).

Table 2.5. Descriptive statistics of larvae and adults in the study area.

	N	Minimum	Maximum	Mean	Std. Deviation	Std. Error
Nb Larvae	20	3	130	40,0	36.281	8,113
Nb Adults	36	1	40	10.14	14.44	2.407

2.3.2 **Species descriptive parameters**

The species densities with the other descriptive parameters for the total species are illustrated in Table 2.6, and the frequency percentages are demonstrated in Figure 3.2.

For the total species, *Culiseta longiareolata* showed the highest density (51.2±28.5) in the total of the sampled species, however, it was found 5 times (*f*=7%). Simultaneously, *Cx. pipiens* s.l. was the most frequent (*f*=61%), however, its density was low (12.3±2.5) comparing to the other species. *An. labranchiae* was the second frequent species *f*=9% with low density (7.8±4.2). *Cq. richiardii* (n=2), *Cx. simpsoni* (n=1), and *An. c hispaniola* (n=6) were found one time during the sampling.

Table 2.6. Descriptive statistics of species in the study area.

Species	AM	SE	SD	N
Cx. pipiens s.l.	12.30	2.551	16.728	43
Cx. hortensis	21.60	7.916	17.700	5
Cx. theileri	16.60	7.527	16.832	5
Cs. longiareolata	51.20	28.542	63.822	5
Oc. caspius	17.50	8.568	17.137	4
An. labranchiae	7.83	4.183	10.245	6

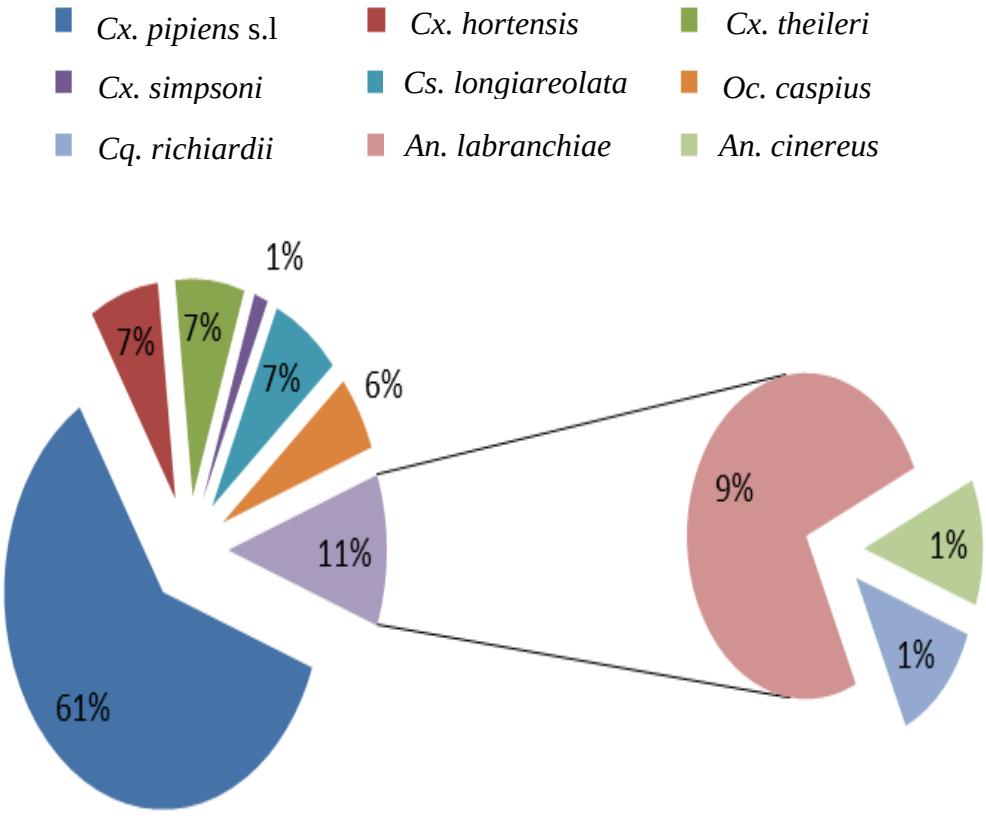


Figure 3.2. Frequency percentages of mosquito species sampled from Setif region from 2016 to 2019.

For the larvae sampling, *Culiseta longiareolata* showed likewise the highest density (6.7±33.1), Followed by *Cx. hortensis* (Table 2.7). Further, *Cx. pipiens* was the most frequent in the breeding sites (*f*=31.2%) followed by *An. labranchiae* (*f*=7.9%).

Table 2.7. Descriptive statistics of species in the breeding sites.

Species	AM	SE	SD	N	*f* (%)
Cx. pipiens s.l.	19,20	21,223	6,711	10	13,2
Cx. hortensis	21,60	7,916	17,700	5	6,6
Cx. theileri	16,60	7,527	16,832	5	6,6
Cx. simpsoni	-	-	-	1	1,3
Cs. longiareolata	63,75	33,094	66,188	4	5,3
Oc. caspius	16,33	12,005	20,793	3	3,9
Cq. richiardii	-	-	-	1	1.3
An. labranchiae	7,83	4,183	10,245	6	7.9

For the adults sampling, *Cx. pipiens* was the most frequently found (*f*=43.4%) with a mean density of 10.2±2.6, *Oc. caspius* (n=21), *Cs. longiareolata* (n=1), and *An. c hispaniola* (n=6) were found one time with a 1.3% frequency.

2.4 Co-occurrence frequency

The co-occurrence of the sampled mosquito species was evaluated in order to measure the tendency of species to occur alone or associated and the degree of correlation between the sampled species. The species associations obtained by crosstabs are illustrated in Table 2.8.

2.4.1 Co-occurrence in captured adults

We calculated the frequency of each species to occur alone or associated using the function frequency in SPSS.

The results obtained are illustrated in Figure 3.3. *Culex hortensis, Cx. theileri, Cx. simpsoni, Cq. richiardii, An. labranchiae* were not captured in adult stage. *Cx. pipiens* was captured in 94.3% of samples and was found alone in a high percentage (*f*=91.4%). *Cs. longiareolata* was captured one time with *Cx. pipiens, Oc. caspius* was found one time alone.

Table 2.8. Species associations observed during mosquito sampling in Setif region 2016-2019.

		Species2							
		An. labranchiae	*Cq. richiardii*	*Cs. longiareolata*	*Cx. hortensis*	*Cx. pipiens s.l.*	*Oc. caspius*	*Cx. simpsoni*	*Cx. theileri*
Species1 larvae	*An. labranchiae*		1	2	2	2	0	1	3
	Cq. richiardii			0	1	0	0	0	0
	Cs. longiareolata				1	1	0	1	1
	Cx. hortensis					1	0	0	2
	Cx. pipiens s.l.						1	0	1
	Oc. caspius							0	1
	Cx. simpsoni								1
	Cx. theileri								
Species1 adult	*Cs. longiareolata*	0	0		0	1	0	0	0
	Cx. pipiens s.l.	0	0	1	0	0	0	0	0

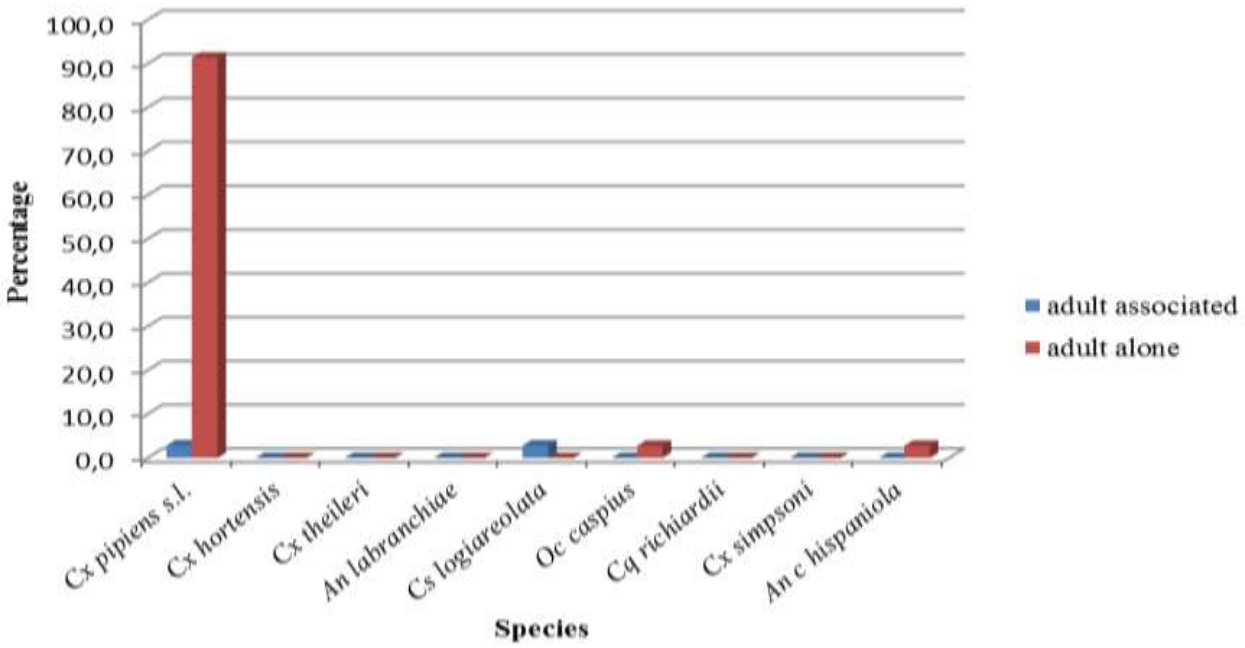


Figure 3.3. Frequency of adult mosquitoes sampled from Setif region 2016-2019 to occur alone or associated.

2.4.2 **Larvae co-occurrence in breeding sites**

Anopheles cinereus hispaniola was not sampled in the larvae stage. *Cx. hortensis* and *An. labranchiae* were found more frequently associated with other mosquito larvae, while *Cx. pipiens* s.l. and *Oc. caspius* were found equally alone and associated. *Cx. theileri, Cx. simpsoni, Cs. longiareolata,* and *Cq. richiardii* were always found associated (Figure 3.4).

The Figure 3.5 constitutes a corrgram measured in order to estimate the level of correlation between the larvae found co-occurred. The spearman's rho test revealed a positive correlations between *Cx. hortensis-Cx. theileri,* and *Cx. theileri-An. labranchiae,* however, the association between *Cx. theileri* and *An. labranchiae* is the only strong and highly significant correlation (r_s=0.89, p<0.001).

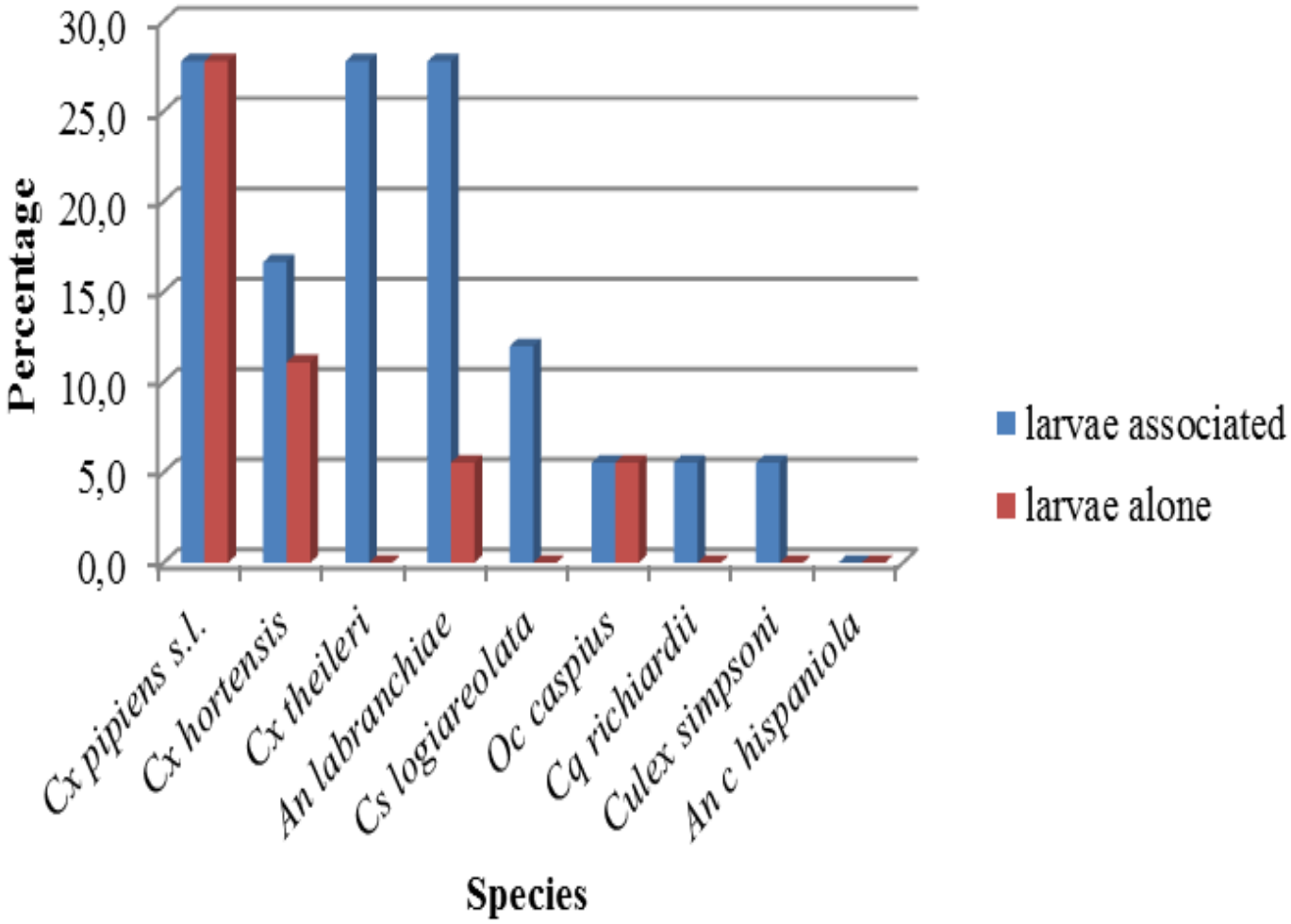


Figure 3.4. Frequency of mosquito larvae sampled from Setif region 2016-2019 to occur alone or associated.

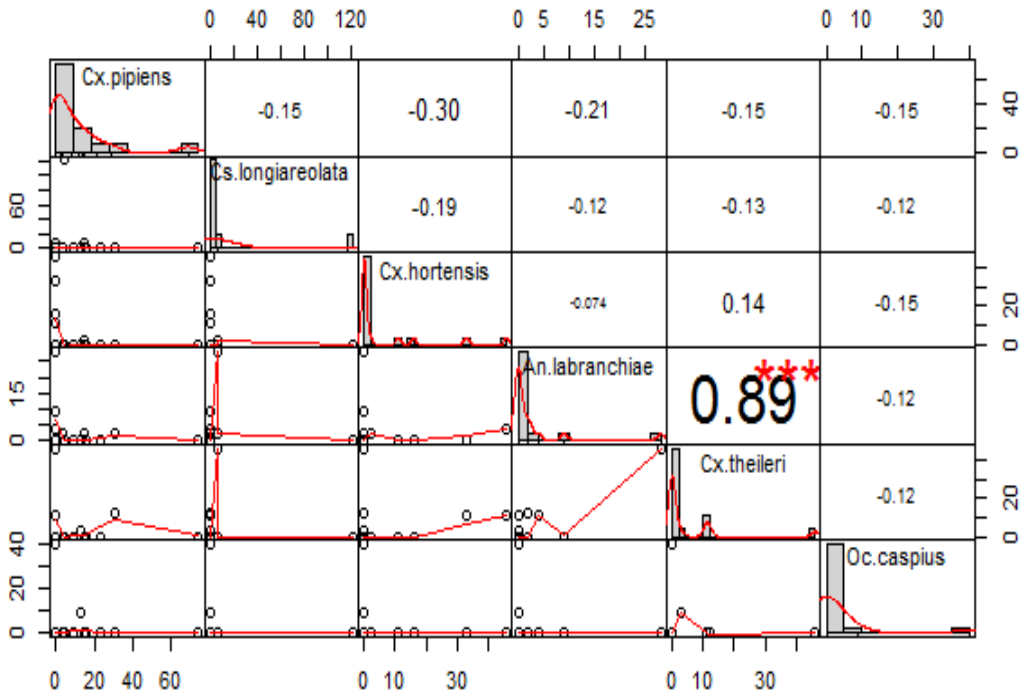


Figure 3.5. Corrgram estimated of the level of correlation between mosquito larvae found co-occurred in breeding sites.

2.5 Characterization of larvae habitat preferences

2.5.1 Frequency and crosstabs

The characteristics of breeding sites observed during the sampling were analyzed in order to recognize the preferences of mosquito population relatively to their reproduction mechanism (Figure 3.6). The breeding sites found during the sampling were mostly situated in rural areas (f=85%), surrounding the cities or far in nature. Further, the predominant sites were permanent (f=57.5%), while the sites were equally characterized by presence or absence of algae (f=50%).According to the crosstabs results, the rural sites were mainly permanent represented in rivers and stream retentions; they were characterized by the predominant of algae (56% of sites with presence of algae). The urban site are mostly temporary (f=67%) represented in small pools, without presence of algae.

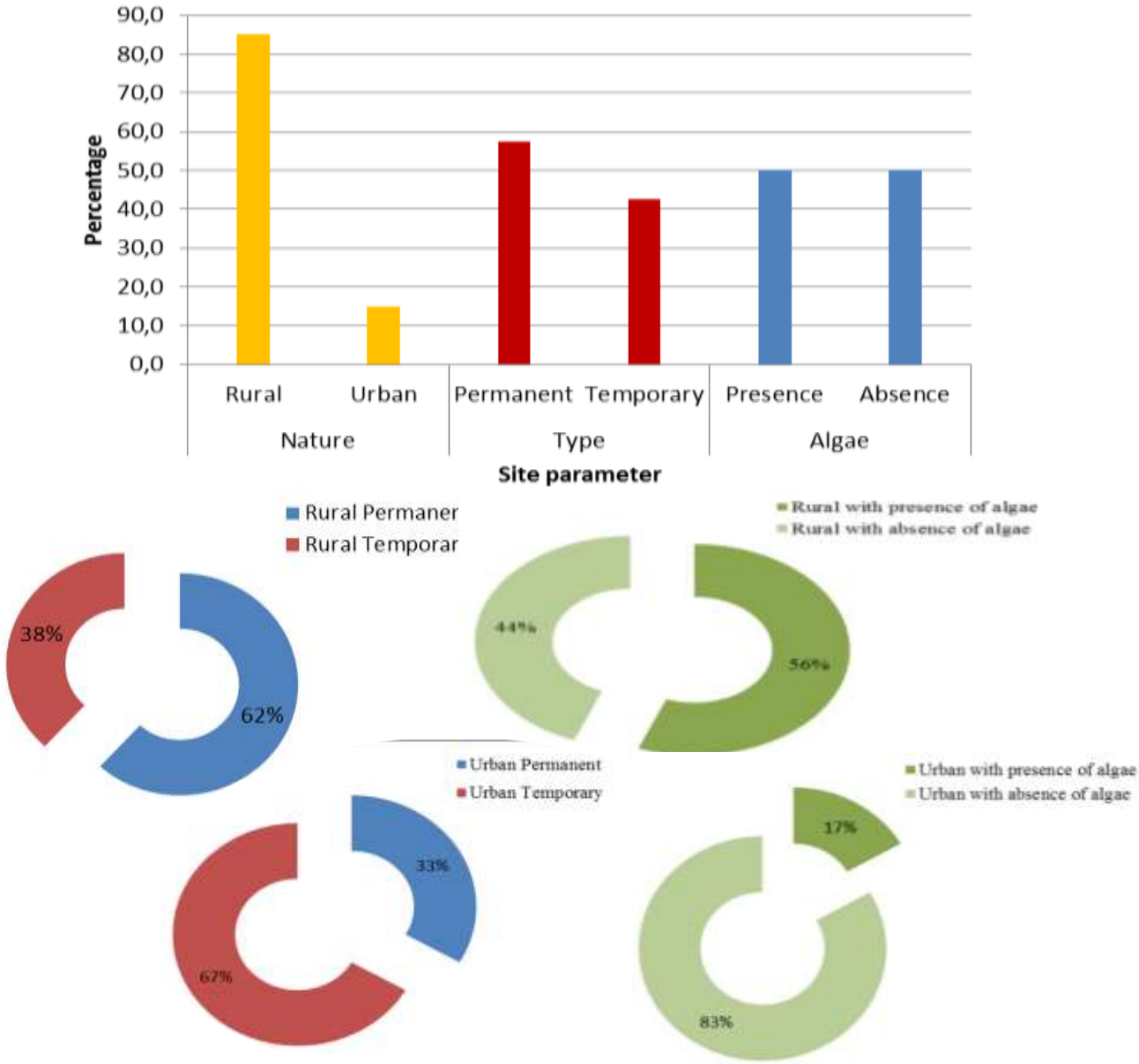


Figure 3.6. Frequency percentages of the general parameters of the sampling sites, with crosstabs frequencies.

Table 2.9. Descriptive statistics of species in the study area.

Site parameters		AM	SE	SD
Nature	Rural	44.44	8.444	35.827
	Urban	29.33	5.364	9.292
Type	Permanent	46.8	11.224	35.493
	Temporary	41.5	13.6	38.467
Algae	Presence	36.42	6.436	22.293
	Absence	60.5	21.628	52.978

2.5.2 **Density according to breeding sites parameters**

The distribution of density data for nature and type of sites were not normal (P<0.05), the difference in the larvae density between rural and urban sites were not significant (M-W U= 18.5, P>0.05), however, the mean density was higher in rural sites (44.4±8.4). Likewise, the difference in the larvae density between permanent and temporary sites was not significant (M-W U= 27, P>0.05), however, the density was higher in permanent sites.

The distribution of density data for presence or absence of algae in the sites was normal (P>0.05). The larvae density in sites characterized by absence of algae were significantly higher than their density in sites characterized by presence of algae (F=5.3, P<0.05) (Table 2.9).

2.6 **Distribution patterns**

2.6.1 **Mosquito distribution across months**

The data collected from 2016, 2017, 2018, and 2019 were assembled and analyzed in order to estimate mosquito fluctuation during the year. In general, the mosquito sampling was positive over the year but not in the same frequency. Three peaks were observed in May, August, and December, the mosquito density starts to increase in May and it reached its top density in August (Figure 3.7).

If we analyze the data in terms of species (Figure 3.8), before May and after November only *Culex pipiens* was found, it was the only species fluctuated during all the year. However, the density of *Cx. pipiens* increased in November and December. *Cx. theileri*, *Cx. hortensis*, and *An. labranchiae* were sampled in May and November; while *Cs. longiareolata* and *Oc. caspius* occurred in August and November.

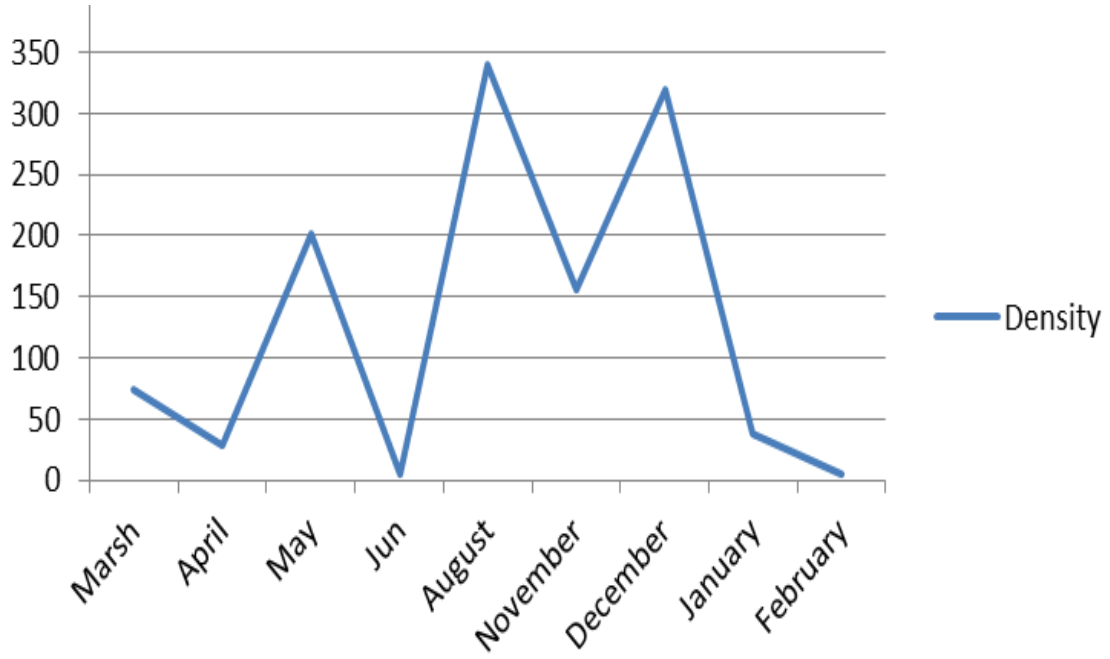


Figure 3.7. Mosquito density fluctuation during the year shows three peaks in May, August, and December.

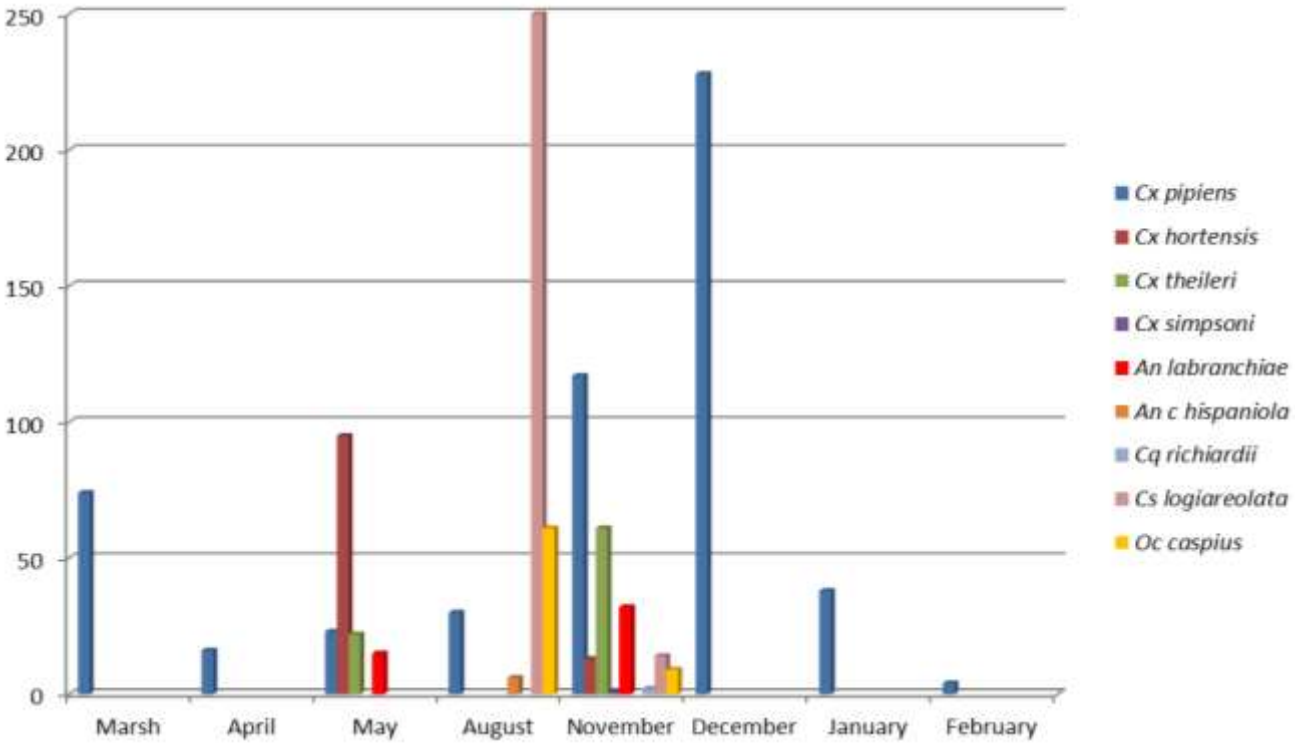


Figure 3.8. Mosquito species fluctuation during the year according to the data sampling from Setif region 2016-2019

2.6.2 **Mosquito distribution across climate zone**

In order to evaluate the effect of climate on mosquito distribution in Setif region, we analyzed the distribution patterns in terms of density and taxa across Csa and BSk zones.

2.6.2.1 Comparing densities

The data distribution is not normal ($p<0.05$). The descriptive parameters of mosquitoes sampled from Setif region across climate zone is illustrated in Figure 3.9.

It exist a significant difference in mosquito mean density between Csa and BSk zones (M-W U=192, $p<0.05$). The mean mosquito density was higher in BSk zone (26.3±3.9) comparing to the mean density in Csa zone (12.6±3.1).

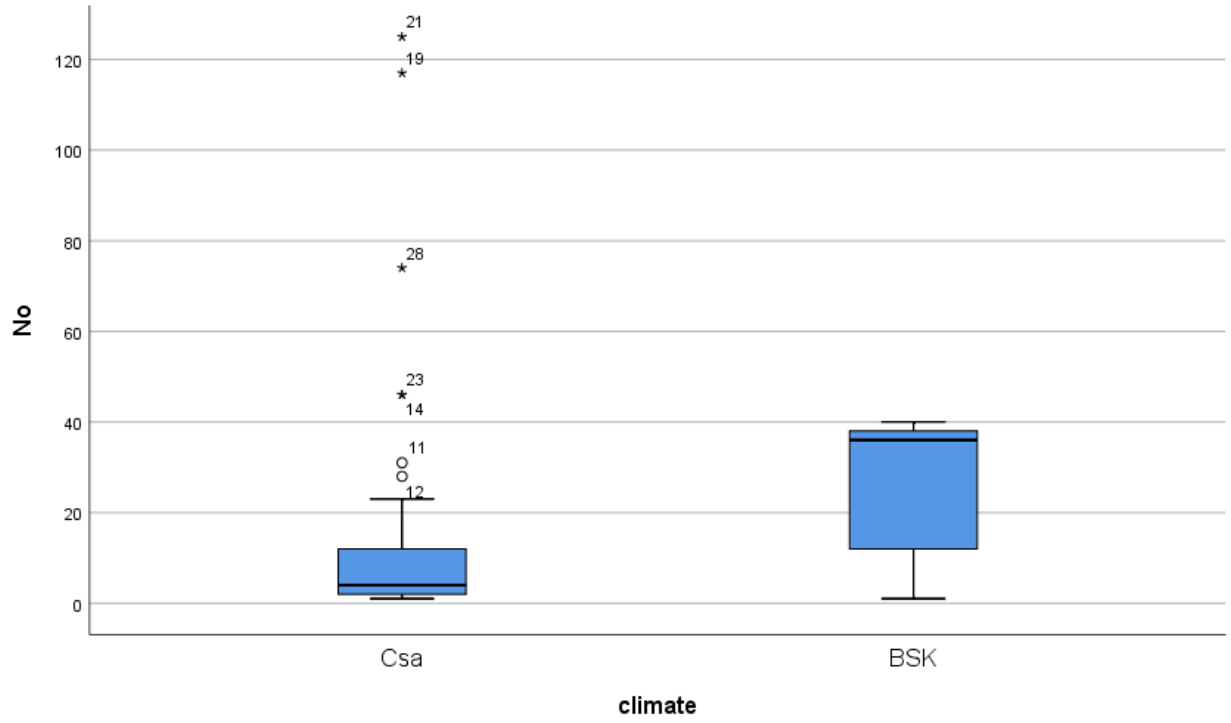


Figure 3.9. Mosquito descriptive parameters across climate zone Bsk and Csa in Setif region.

Culex pipiens showed the highest mean density in BSk zone (34.7±3.1), it was followed by *Oc. caspius* (17.5±8.6). The other descriptive parameters are illustrated in Figure 3.10. In Csa zone, *Cs. longiareolata* showed the highest mean density (51.2±28.5), followed by *Cx. theileri* (23±11.5) and *Cx. hortensis* (18.7±9.5). *An. labranchiae* (7.8±4.2) and *Cx. pipiens* (7.1±2.3) showed the lowest density. The other descriptive parameters are illustrated Figure 3.11.

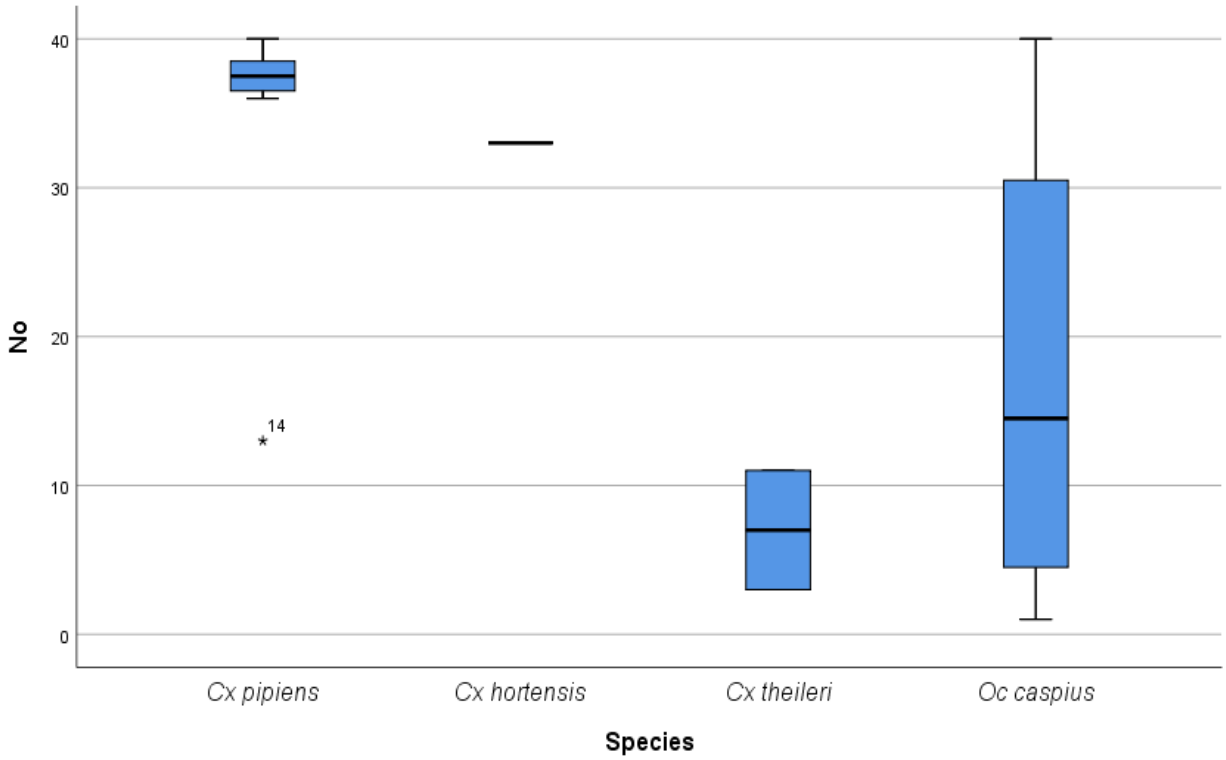


Figure 3.10. Mosquito descriptive parameters in BSk zone in Setif region.

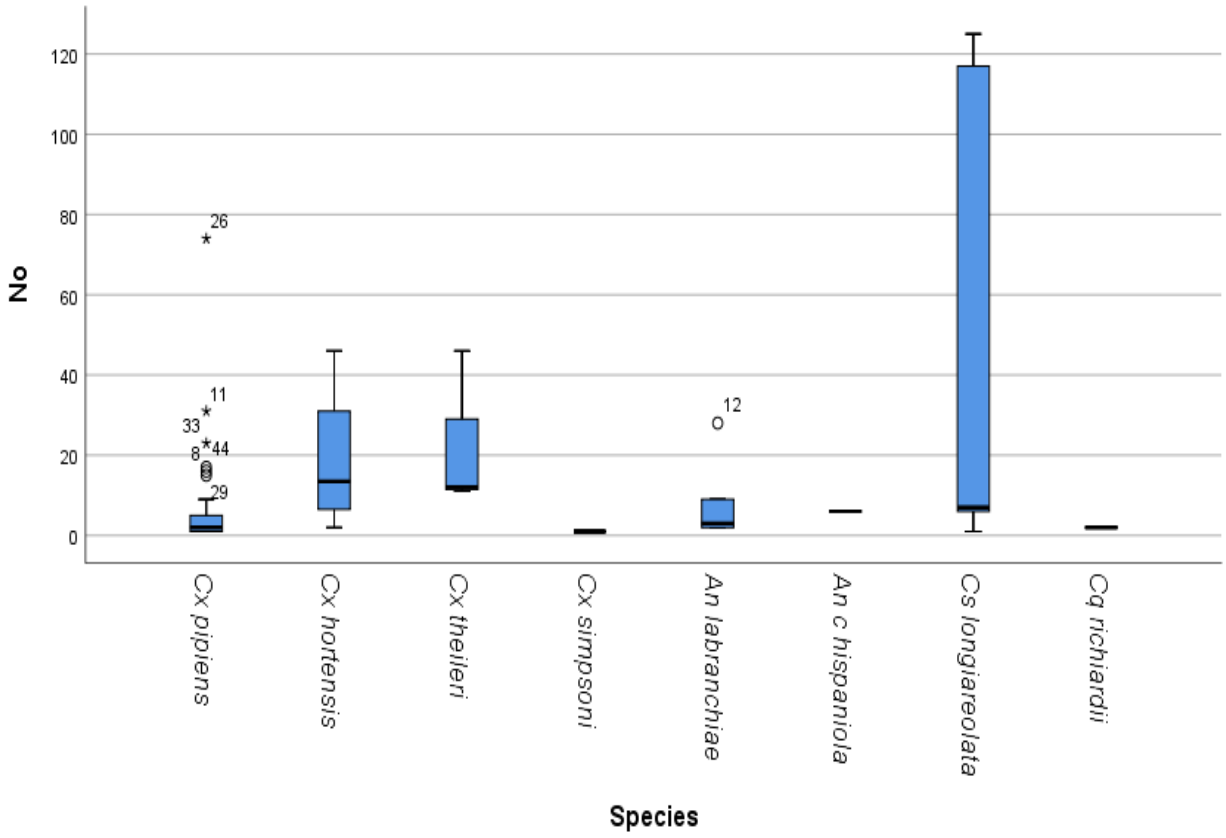


Figure 3.11. Mosquito descriptive parameters in Csa zone in Setif region.

2.6.2.2 Comparing species distribution

The most frequent species in BSk zone is *Culex pipiens* with a frequency percentage of 53.3%, the second frequent species is *Oc. caspius* (*f*=26.7%). The frequency of the rest of species is presented in Figure 3.12. The most frequent species in Csa zone is likewise *Cx. pipiens* with a frequency percentage of 62.5%; the second frequent species is *An. labranchiae* (*f*=10.7%). The frequencies of the rest of species in Csa climate zone were presented inFigure 3.13.

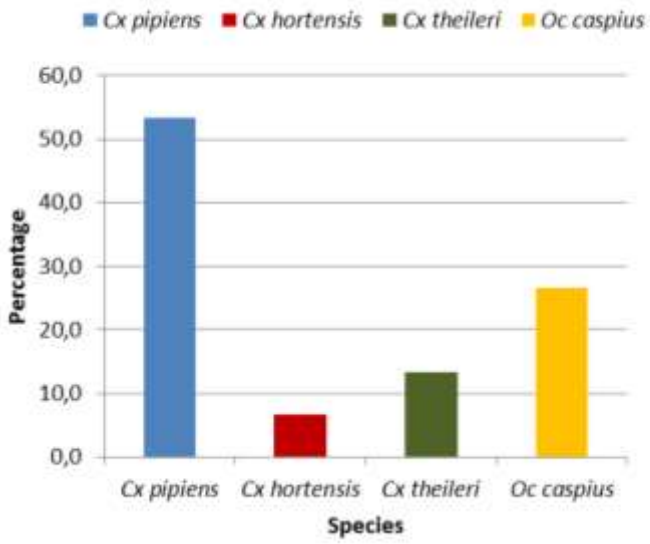


Figure 3.12. Mosquito species frequency in BSk climate zone

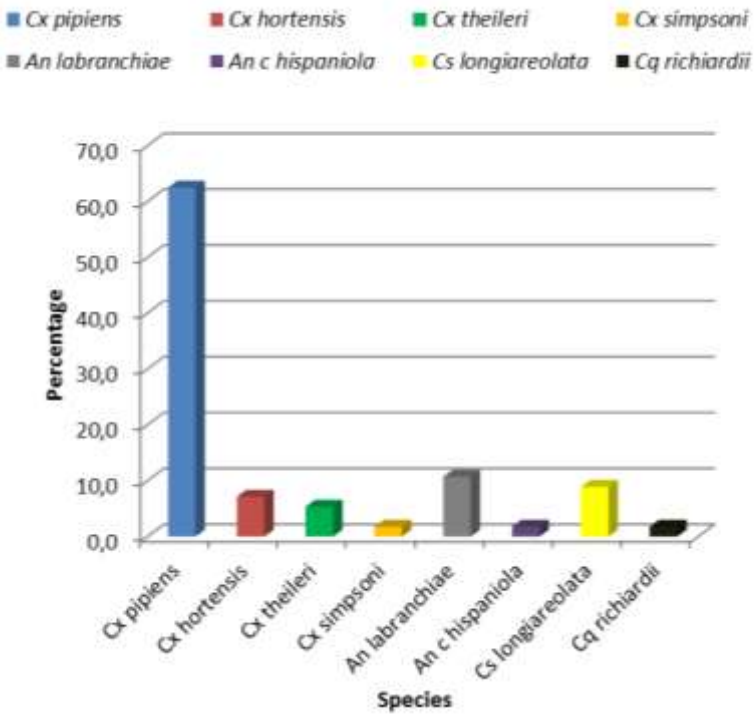


Figure 3.13. Mosquito species frequency in Csa climate zone

The CCA was estimated in order to analyze the species distribution in the study area in terms of climate zone. The analysis showed the existence of two separate clusters marked in circles: *An labranchiae, An c hispaniola, Cs. longiareolata, Cx. simpsoni, Cq. richiardii* /Csa and *Oc. caspius*/BSk, the third cluster is constituted by three species *Cx. pipiens s.l, Cx. hortensis* and *Cx. theileri*, which appear less associated to a specific climate zone (Figure 3.14).

The cartography of mosquito distribution across Csa and BSk climate zones is designed and presented in Figure 3.15.

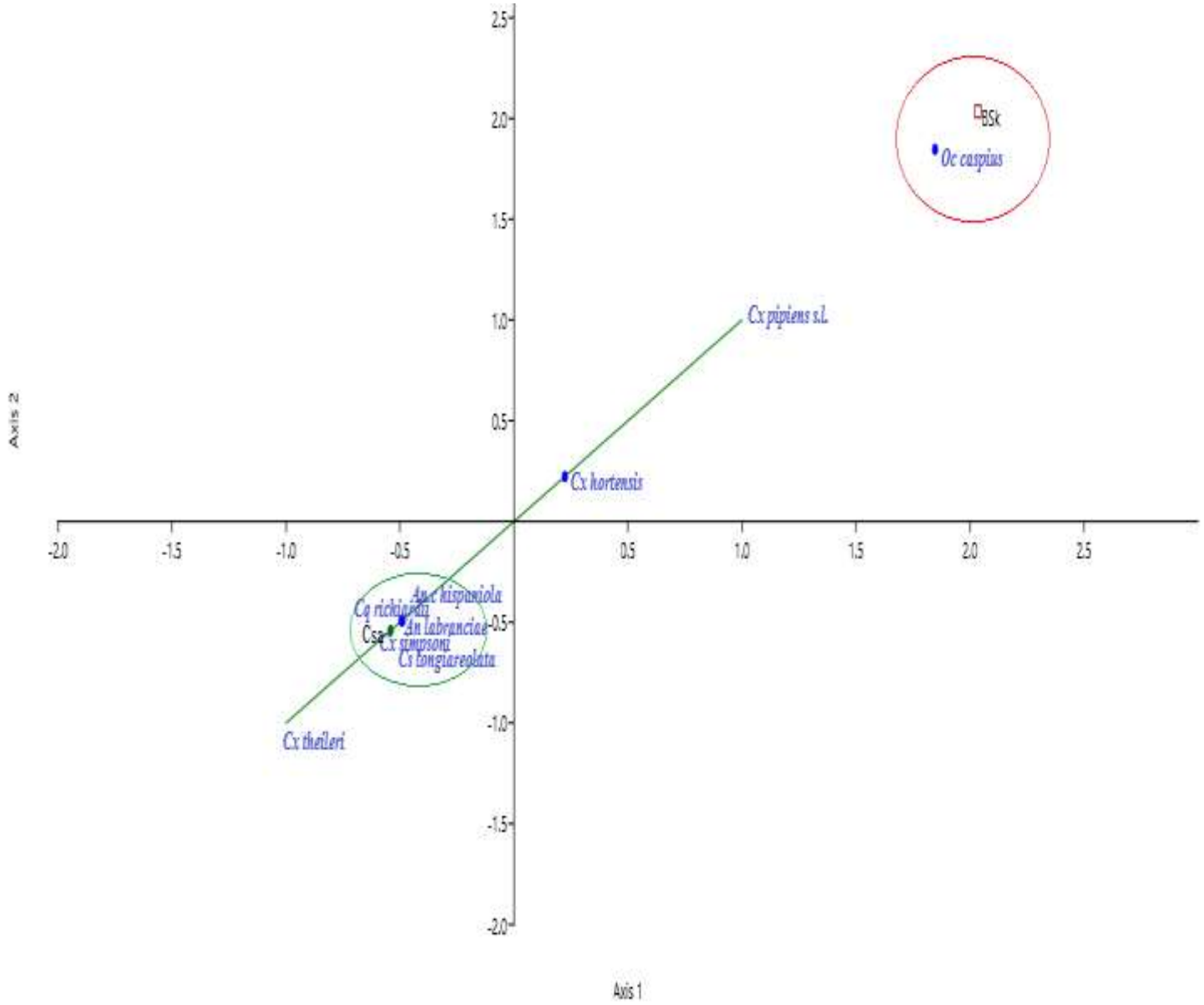


Figure 3.14. CCA analysis show three clusters, two clusters related to climate zone Csa and BSk marked in circles, and a third cluster not related to any of the climate zones (*Culex pipiens, Cx theileri, Cx hortensis*).

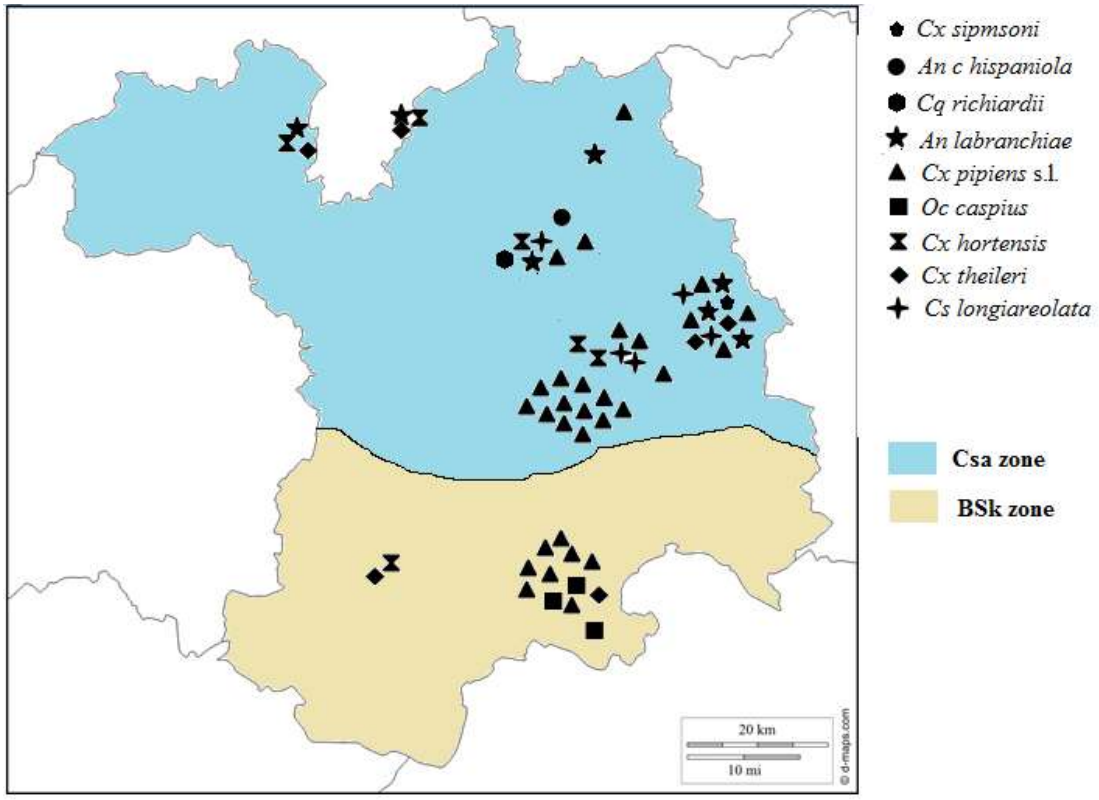


Figure 3.15. Mosquito species distribution card of Setif region.

3 Discussion

The collected information including the description of the breeding sites were noted in order to provide a complete description of the studied species. We used interactive taxonomy approach to enhance biodiversity inventory results (Schlick-Steiner *et al.*, 2010).

3.1 Mosquito biodiversity in Setif region

The investigation of Setif region has yielded the identification of nine mosquito species *Culex pipiens, Cx. theileri, Cx. hortensis, Cx. simpsoni, Cs. longiareolata, Oc. caspius, Cq. richiardii, An labranchiae* and *An c hispaniola*.

Culex simpsoni

This is the first declaration of *Culex simpsoni* in Algeria; *Cx. simpsoni* was identified in Morocco and it is usually distributed in south Africa and southwestern Asia (Army Public

Health Center, 2019). The larvae of this species is close to those of *Cx. antennatus, Cx. sinaiticus* and *Cx. theileri* (Gunay *et al.*, 2018); for this reason, we have adopted pictorial keys in the discrimination of this species. Seta 5-C was 2 branched while it is more branched in *Cx. theileri* (3-4 branches), seta 1a-S was 3 branched and longer than the siphon diameter while it is shorter than the diameter of the siphon in *Cx. antennatus* and the pecten was on less than one third of the siphon, while the pecten is longer in *Cx. sinaiticus* (Harbach, 1985). Though, the morphological keys were not sufficient to insure the identification especially that only one specimen was sampled, thus, a molecular analysis was fundamental to confirm the species.

***Culex pipiens* s.l.**

According to the obtained results, *Culex pipiens* s.l was the most frequent species in the Setif region. The assemblage *Cx. pipiens* s.l in Algeria is represented by *Cx. pipiens pipiens* (14.9 %), *Cx. pipiens* biotope *molestus* (48.3 %) and their hybrids (36.8 %) (Korba *et al.*, 2016). According to the results, the Algerian *Cx. pipiens* had maintained the common characters that characterize them from the other mosquitoes: head setae 5-C and 6-C were 4 to 6 branched. In the other hand, the abdominal setae 1-III and 1-IV were the most often double and less frequently single; Dehghan *et al.* (2016) have considered these two keys as valuable characters to differentiate *Cx. pipiens* (1-III and 1-IV usually double branched) from *Cx. quinquifasciatus* (1-III and 1-IV usually single). Furthermore, seta 1a-S did not exceed 5 branches, this key is used as well as diagnostic character where 1a-S is more branched in *Cx. quinquifasciatus* (6-9 branches) than in *Cx. pipiens* (2-6 branches) according to (Dehghan *et al.*, 2013) and the neotype description of these two subspecies (Sirivanakarn and White, 1978; Harbach *et al.*, 1985).

Likewise, the siphon index was 3.3–5.2 with an average of 4.25. This index comprises in the interval reported for *Cx. pipiens* in similar researches, where the minimum siphon index reported was 2.43 and the maximum was 5.8 (Vinogradova and Shaikevich, 2007; Dehghan *et al.*, 2010) Dehghan et al. 2010). However, the siphon had appeared in two distinct shapes: despite the most often, with a slightly narrowing shape with a straight side (Fig. 4B), but even less frequently with a wide shape rather than narrow. The second shape was more similar to the siphon shape in the neotype description of *Cx. molestus* designated by Harbach *et al.* (1984).

However, the comparison of the siphon characters of the sampled larvae with the neotype description designated by Harbach *et al.* (1985) demonstrated a differentiation in the

arrangement of seta 1-S and the position of the insertion of seta 1a-S. Seta 1-S was more frequently (74.2%) 4 arranged while it was 5 arranged in 25.8% of specimens. Simultaneously, the alignment of hair tufts 1-S varied largely. The position of seta 1c-S in relation with the other siphonal hair tufts was the most often out of line (on the left side of the siphon in 64.2% of specimens and on the right side in 73.3% of specimens). Less frequently, 1d-S was out of line on the left side of the siphon in 30.9% of specimens and on the right side in 21.1% of specimens. (Larvae from Ain Oulmen were not counted because the non-certitude of the character). Resulting, the percentage of the larvae finding with 1-S 4 arranged (72.4%) rather than 5 arranged (27.6%) and 1d-S out of line (26%) rather than 1c-S out of line (68.5%) is acceptable in some extent to be used in discriminating the local *Cx. pipiens*.

Moreover, among larvae collected from Beni Fouda n=74, we registered a variation in the alignment of the siphonal hair tufts from a side to another within the same siphon, 32.4% of these larvae showed different characters, they had 4 pairs of siphonal setae where 1c-S was out of line and 1a-S inserted next to the last pecten tooth. Therefore, larvae collected from this region (Beni Faouda) that presented 13.3% of the total of the sampled larvae were the only ones that have showed 1a-S next to the last pecten tooth rather than beyond. However, previous researches that have described *Cx. pipiens* larvae have not mentioned similar characters (Harbach, 1985; Vinogradova and Shaikevich, 2007; Dehghan *et al.*, 2016).

The number of branches of setae 1-III, 1-IV and 1a-S is considered as constant character to discriminate the local *Cx. pipiens* larvae; the morphological variations noted during this study on the siphonal characters had confused the identification of the specie: the insertion of seta 1a-S next to the last pecten tooth was an unusual character in identification of *Cx. pipiens* larvae; furthermore, the observation of the both sides of the siphon was occasionally indispensable to provide a complete description. Nevertheless, the variations can indicate possibly a subspecies hybridization especially that previous researches conducted in Algeria have confirmed the existence of the *molestus* form of *Cx. pipiens* with high proportion comparing with *pipiens* form and hybrids (Benallal *et al.*, 2015; Korba *et al.*, 2016).

Molecularly, as in the previous study conducted by Batovska *et al.* (2016), the COI barcode did not help us to define the sub-species for *Culex pipiens* sequences where the results were not significant, except for *Cx. torrentium* that presented 2.8% as minimum divergence, particularly when we take into account the barcode gap proposed by Rubinoff to separate vertebrate (2%) and invertebrate (3%) species based on COI sequences (Barcodes,

2006; Chan *et al.*, 2014). Although, according to the study conducted in Italy on the molecular genetic structure of the complex *Culex pipiens* (Luca et al. 2016), both COI barcode and CQ11 locus separated the two forms *pipien*s and *molestus*. However, CQ11 locus as a diagnostic marker showed better results in separating the *Culex pipiens* complex members (Di Luca et al. 2016).

Culex pipiens s.l is a competent vector that can transmit West Nile (WN) Virus (Andreadis *et al.*, 2001; Hamer *et al.*, 2008), a human and animal neurophathogen worldwide disease that can range in severity from uncomplicated WN fever to fatal meningoencephalitis (Campbell *et al.*, 2002; Kaleemullah and Sill, 2019). *Cx. pipiens* is also a vector of Rift Valley fever virus (Moutailler *et al.*, 2008), an emergence disease that can cause important livestock industry losses, and moderate human morbidity and mortality (Pepin *et al.*, 2010; Hartman *et al.*, 2019).

Anopheles labranchiae

Anopheles labranchiae, the only representative member of the *An. maculipennis* s.l. complex in North Africa (Tabbabi *et al.*, 2017), has been reported recently in Mila (Messai *et al.*, 2010), Tizi-Ouzou (Lounaci *et al.*, 2016) and the Atlas Mountains western Algeria (Laboudi *et al.*, 2011). In the majority of these studies, *An. labranchiae* females had a tendency to lay their eggs in stagnant water. However, during our field inspection, it was clear that the fresh pure running water is the main habitat for *An. labranchiae* larvae within the Algerian High plains. The pattern on the eggs is the separating character in *An. maculipennis* subspecies, nevertheless, the ornamentation of the wings can also be used to separate slightly certain members (Kirti and Kaur, 2004; Vicente *et al.*, 2011). The females have wings with several spots varying in number, position and darkness intensity; the common spots that characterized the complex are situated on the base of Rs likewise on the bifurcation of R_{2+3} and M. however, the *An. labranchiae* females sampled during this field inspection appeared with six dark spots where their positions described previously in the morphological identification results, show a possibility of using this parameter to identify the Algerian *An. labranchiae* females.

DNA barcoding of the nuclear rDNA ITS2 is usually used as a better approach in separating *Anopheles maculipennis* members (Sevgili and Simsek, 2012). However, our PCR results showed that the COI barcode was useful to differentiate *An. labranchiae* from the other *maculipennis* species. Low genetic distance (0,5% to 1.6%) were detected among *An.*

labranchiae population, while the divergence between *An. labranchiae* and the other maculipennis members *An. maculipennis s.s., An. atroparvus, An. beklemishevi, An. melanoon, An. sacharovi, An. subalpinus* and *An. messea* was more than 2%.

This species as a member of *Anopheles maculipennis* s.l. Complex is the primary malaria vector in Europe and North Africa and more particularly in Algeria (Marinucci et al, 1999; Kuhn, 2002) (Marinucci *et al.*, 1999; Kuhn *et al.*, 2002; Boubidi *et al.*, 2010; Snow *et al.*, 2012; WHO-Algeria, 2014). According to the obtained ecological data, *An. labranchiae* is a frequent species in the Setif region that occur in a low density, this low density may returns into the program of eradication applied by the Algerian authorities, however, the species is always exist and in a high frequency, which poses the risk of disease transmission in the study area.

Anopheles cinereus hispaniola

During the current study, *Anopheles cinereus hispaniola* was captured from a livestock, which is homogeneous with the information provided by Faraj *et al.* (2009) about the zoophilic tendency characterizes this species. The morphological identification of the captured *An. c hispaniola* females was slightly confusing, the thorax ornamentation was not very clear which compounds the possibility of misidentification with the other *Cellia* Anopheles *An. multicolor*. However, the wing size (4.6mm) was a conclusive parameter to differentiate *An. c hispaniola* (wing length more than 4.1mm) from *An. multicolor* (wing length 3 to 4 mm) (Gunay *et al.*, 2018). *An. c hispaniola* has been described for the first time from a material collected in Spain Theobold 1903, then in Algeria Theobold 1907 (Ramsdale, 1998). It represents a member of the complex *An. cinereus.* Although the close morphology of the two subspecies, *An. c hispaniola* is distinguishable by its darker tarsomeres and its distribution (Hervy *et al.*, 1998); *An. c hispaniola* is usually distributed in the Arab Maghreb (Trari *et al.*, 2002; Tabbabi *et al.*, 2017) and the other Mediterranean regions (Samanidou-Voyadjoglou and Darsie Jr, 1993; Bueno Marí and Jiménez Peydró, 2010); in the other hand, *An. c cinereus* is distributed in Eastern Africa and Arabian Peninsula (Amr *et al.*, 1997; Alahmed, 2012), South and Central Africa (Animut *et al.*, 2012). Regardless, as far as we know, *An. c hispaniola* was not reported in Algeria since 1983 by Ramsdale (1983).

The phylogenic test showed significant divergence of 8.9% between *Anopheles cinereus hispaniola* and *An. c cinereus* from Saudi Arabia. Moreover, we noted significant divergence of 73% between our *An. c hispaniola* sequence and *An. turkhudi* sequences from Iran

(KM389467). In contrast, the blast in BOLD showed highest similarity of 99.93% with *An. cinereus* from Morocco (The sequences are private thus we could not calculate the genetic distance). The previous results confirmed that COI barcode is a robust diagnostic method for the identification of *An. cinereus* sub-species.

Anopheles cinereus hispaniola is considered as a potential malaria vector, it was found infected by *P. falciparum* in Eritrea (Shililu *et al.*, 2003). However, *An. c hispaniola* was found one time during the sampling, thus, it could be considered as a sporadic species.

Ochlerotatus caspius

The genus *Ochlerotatus* regroups several species that are morphologically similar and almost undistinguishable including *Ochlerotatus caspius* and *Oc. dorsalis* (Milankov *et al.*, 2000). *Oc. caspius* is able to transport *Tahyna* Virus (Lu *et al.*, 2009) and Rift Valley fever virus (Chevalier *et al.*, 2010); it is designated into two forms A and B morphologically undistinguishable (Wassim *et al.*, 2013). However, the ultrastructure of *Oc. caspius* eggs was used as tool to separate the two forms. Females have a susceptibility to lay their first egg batches without a blood meal (autogeny) and larvae are mostly halophilic (Metge and Hassaine, 1998; Alahmed, 2012). The larvae in Setif region were harvested from freshwater of flooded grounds and streams in low depth sites. The adults were captured with a simple CDC miniature light trap in a grassy area; this type of traps was valuable to catch *Oc. caspius* adults (40 adults in one night). The morphological identification of *Oc. caspius* larvae was based on the number of branches of the setae 1-S (7 branches) and 3-VIII (10 branches) which can be more branched in *Oc. caspius* versus to *Oc. dorsalis* (Gunay *et al.*, 2018). Moreover, it is likely important to mention an heterogeneous shape of the comb scales that we observed on the eighth abdominal segment of *Oc. caspius* larvae, which makes it unuseful key tool to separate morphologically *Oc. caspius* from *Oc. dorsalis*.

Although morphologically similar, COI barcodes was useful to separate to confirm the identification of *Ochlerotatus caspius*, and separate the sequences from the similar species *Oc. dorsalis*, we noted 2.2% to 3.7% genetic distance between the two species. Further, we noted pairwise divergence varied from 0.05% to 0.26% within *Oc. caspius* sequences, which is considers lower comparing with the pairwise divergence between sequences collected from Iran (Azari-Hamidian *et al.*, 2010). Nevertheless, COI barcode did not help to separate the *Oc. caspius* forms A and B, thus acetylcholinesterase gene can be used as support test to provide more accurate results (Wassim *et al.*, 2013).

Culiseta longiareolata

Culiseta longiareolata is considered as a primary vector of *Plasmodium* (*Giovannolaia*) *circumflexum* (Kikuth, 1931), *P. relictum* (modified from Garnham, 1966) and *P. polare* (Manwell 1934) in birds, and its capacity to transmit *P. relictum* in Algeria was proved experimentally (Valkiunas, 2004; Santiago-Alarcon *et al.*, 2012). In the current study, *Cs. longiareolata* has been reported as the mosquito species that showed the highest density in Setif region. Simultaneously, it was reported in previous studies as one of the most abundant and frequent species in Algeria (Bouabida *et al.*, 2012; Lafri *et al.*, 2014). Thus, *Cs. longiareolata* can represents a disease vector in Algeria including Setif region. *Cs. longiareolata* pools are usually found beside human habitations; this species can bite humans, however, according to Al-Jaran and Katbeh-Bader (2001) the females prefer bird's blood and more exactly pigeon blood. It has a uniquely adaptive and survivor features; Kiflawi *et al.* (2003) have confirmed that the females of this species showed an adaptive response against the risk of predation and negative density effects. In Setif region *Cs. longiareolata* has also find in pools behind habitation but in rural areas, females avoid lying their eggs in predator pools, the pools of *Cs. longiareolata* are often free of other life organisms which exclude the risk of toxicity of other organisms in case of using larvicides.

3.2 **Breeding sites preferences**

The characteristic of the breeding sites is of crucial importance for mosquito females, thus we can estimate their preferences according to the breeding site parameters. The obtained results confirm that mosquitoes prefer rural sites or sites surrounding urban areas with presence of algae (food availability) and low water turbidity. According to an experience conducted by blaustein and kotler (1993); *Culiseta longiareolata* females prefer to lay their eggs in sites characterized by food availability; in another study, *Cx. restuans* (Theobald 1901) showed an oviposition preference to sites rich of sod and grass, while *Cx. pipiens* prefer sites rich of rabbit chow (Lampman and Novak, 1996). The existence of source of food is than an important oviposition parameter, this explain the predominance of sites rich on algae.

Moreover, some mosquito species as *Coquillettidia* species need the availability of host plants in the water to breath; therefore, the existence for *Coquillettidia* species is always associated with the presence of larval host plants. Further, Sérandour *et al.* (2010) confirmed that also the water quality affect the habitat selection in *Coquillettidia* species where they

prefer water with low salt concentration and neutral pH; which is possible more in low turbid sites. Likewise, *Anopheles* larvae are known to prefer presence in clear water (Tabbabi *et al.*, 2017; Dom, 2019). More specifically, *Anopheles labranchiae* and *An arabiensis* breed more in natural breeding sites and their larvae found more in natural and rural areas (Animut *et al.*, 2012; Boccolini *et al.*, 2012).

The results showed in another side the influence of the presence of temporary sites on the high and rapid reproduction of mosquito populations. It is known that urban *Culex* and *Aedes* species reproduce highly in urban sites which are mostly temporary sites, as well, floodwater mosquitoes breed in temporary sites as pools and usage tires. Speaking in particular about tiger mosquito, *Aedes albopictus* was previously considered as a rural species (Higa, 2011); whereas it has adapted subsequently well with the urban and suburban environment (Wu *et al.*, 2010; Caputo *et al.*, 2012). *Ae. albopictus* females lay their eggs frequently in tires, brick holes, abandoned plastic containers, rock pools and tree holes (Simard *et al.*, 2005). In contrast, another study confirmed that the number of *Ae. albopictus* breeding sites was higher in urban than in rural areas (Li *et al.*, 2014). Likewise, studies on diversity of temporary pools confirmed the abundantly presence of mosquito species (*Culex* species) (Ogbeibu, 2001a; Ogbeibu, 2001b).

Larvae of *Culex simpsoni* were found breeding profusely in rock pools in the river bed of the Tamarin Gorges, they were sharing habitat with larvae of *An. gambiae*, *An. maculipalpis* s.l. and *Cx. tritaeniorhyncus* (Halcrow, 1954). We found *Cx. simpsoni* in a small lack in grassy area beside livestock and a river retention sharing habitat with *Cs. longiareolata, An. labranchiae,* and *Cx. theileri*.

On the other hand, mosquitoes were used in previous researches as environmental bioindicators (Montes, 2005; da Rocha *et al.*, 2010), which confirm the fact that mosquitoes can affect or be affected by their habitat parameters. Wherefore, the presence of relationships between species and their habitat characteristics is an indisputable mater. Nevertheless, the degree of association between species and their habitat can be discussed. According to our results, the level of associations of mosquito species to the breeding site characteristics has been varied across species. The mosquito population stability is important to control the popular health situation; this stability is significantly associated with the relationships between conspecific individuals (Porretta *et al.*, 2016). The species sampled in the study area showed a tendency to co-occur with other mosquito species; however, the spearman's rho test

confirmed only one real correlation. *Cx. theileri* and *An. labranchiae* larvae were strongly correlated, the level of the correlation purpose the possibility of considering *Cx. theileri* as a species indicator for *An. labranchiae,* however, the measurement of species co-occurrence for choosing indicator species needs detailed studies (De Cáceres *et al.*, 2012; Neeson and Mandelik, 2014) and this subject is not well developed in the entomological field.

3.3 **Distribution patterns**

According to the estimation of mosquito fluctuation during the sampling, and if we compare them with the temperature and precipitation fluctuations, we will conclude that mosquitoes start their first hatch in May after the second precipitation peak in April where the temperature is relatively adequate. The second peak in mosquito density was in August, after the temperature peak in July. And the third density peak was noted in December after the third precipitation peak although the low temperature levels. *Culex pipiens* was the only species fluctuated during almost all the year. However, the density of *Cx. pipiens* increased in November and December.

Here, we can speak about the effect of precipitation and temperature fluctuations on the mosquito density and distribution discussed in previous researches. Wang *et al.* (2016) confirmed that the mosquito abundance is depended on temperature and the distribution of precipitation during the year, and they suggested clearing water breeding sites or spraying insecticides between April and August. Beck-Johnson *et al.* (2017) examined the details of the impact of diurnal and annual temperature fluctuations on mosquito population dynamics using stage-structured, temperature-dependent delay-differential equations, the obtained results showed the importance level of temperature and precipitation diagrams to explain mosquito disease fluctuations, which are related directly to mosquito population dynamics. The water temperature of 30.5°C±3.09°C is important for *An. labranchiae* larvae according to Tabbabi and Daaboub (2017). Further, according to another study conducted by Alto and Juliano (2001) that investigated how temperature and precipitation affects *Aedes albopictus* populations, "*Ae. albopictus* populations occurring in warmer regions are likely to produce more adults as long as containers do not dry completely". This relationship between density-temperature-precipitation is then indispensable to manipulate mosquito populations.

Furthermore, the results of the statistical analyses comparison of the mosquito ecological descriptives across climate zones Csa and BSk supports the fact of the influence of climate

not only on mosquito density but also on species distribution. The mosquito mean density was higher in BSk sites, this climate zone features hot and dry summer and cold wet winter; the temperature in this zone tend to feature major swings between day and night. Simultaneously, CCA analyses confirmed that the Csa sites are more diversified and the majority of the sampled species are related into the Csa climate zone. This zone is characterized by the disponibility of various water surface sites, a more humid climate and longer wet season with hot and dry summer. The majority of the mosquito-born-diseases are related to mosquito density (Churcher *et al.*, 2015; Bradley *et al.*, 2018) and are sensitive to climate features (Reiter, 2001; Li *et al.*, 2019); further, it exists a direct and clear association between mosquito dynamics and climate variations (Beck-Johnson *et al.*, 2013; Wilke *et al.*, 2017). Therefore, the significant difference noted in the mosquito mean densities between the climate zones, and the density and distribution patterns related to a particular zone, explained the importance of surveying mosquito populations according to a defined climate zone as best strategy to control outbreaks.

CHAPTER 3: LARVICIDAL ACTIVITY TEST

1 Material and methods

1.1 Essential oils

EOs are primary material extracted from plants, they present a larvicidal activity, they can affect the survival, larvae duration, pupation and emergence of insects (Bessah and Benyoussef, 2015). The tested essential oils were extracted by stream distillation of five autochthon medicinal plants: *Thymus vulgaris* L., *Artemisia herba-alba* Asso, *Juniperus phoenicea* L., *Rosmarinus officinali* Linn and *Eucalyptus globulus* L.. The plants were harvested from Setif region and its environs.

1.1.1 *Thymus vulgaris*

Thymus vulgaris is a spicy herb belongs to the genus *thymus*, family of Lamiaceae; it is a flowering and highly aromatic plant. *T. vulgaris* is a medicinal plant that can be cultivated, it is worldwide distributed and used (Hosseinzadeh *et al.*, 2015). Applied to the skin, thyme is reported to relieve bites. The collection of young thyme plants before the end of the vegetative cycle provide the best essential oil in term of quality and quantity (Hudaib *et al.*, 2002)

In previous study, Park *et al.* (2005) *have* examined the repellency of thyme essential oil compounds against *Culex pipiens* mosquito, the results showed higher repellent efficacy of α-terpinene and Carvacrol than the commercial formulation diethyltoluamide (DEET) and an equal efficacy of Thymol component to the DEET; the thyme essential oil larvicidal activity was likewise assessed against *Ochlerotatus caspius* (Knio *et al.*, 2008). A model of chemical composition of *T. vulgaris* harvested from northeastern Algeria is illustrated in Table 1.1.

1.1.2 *Artemisia herba-alba*

Artemisia herba-alba original of dry steps of the Mediterranean regions, belongs to the genus *Artemisia*, family of Asteraceae. This plant has an aromatic leaves, the essential oil extracted from *A. herba-alba* was found to be Antioxidant and Antibacterial active (ez zoubi *et al.*, 2018); moreover, it showed as well an efficient insecticide activity (Derwich *et al.*, 2009). The chemical composition of *A. herba-alba* Essential oil is illustrated in Table 1.2.

1.1.3 *Juniperus phoenicea*

Juniperus phoenicea is an evergreen shrub belongs to the genus *Juniperus*, family of Cupressaceae. It occurs in the whole Mediterranean region, the fruits of this small tree have been used in cosmetics and traditional medicine (Caudullo, 2016). The essential oil of *J. phoenicea* harvested from Algeria showed a moderate antimicrobial and antioxidant activity, and its chemical composition is illustrated in Table 1.3 (Mazari *et al.*, 2010). A larvicidal activity of the essential oil extracted from *J. phoenicea* was noted against *Aedes aegypti*, *Ae albopictus* and *Cx pipiens* (Lee, 2006; Giatropoulos *et al.*, 2013).

1.1.4 *Rosmarinus officinalis*

R. officinalis commonly known as rosemary is an aromatic evergreen shrub belongs to the genus *Rosmarinus*, family of Lamiaceae; native to the Mediterranean and Asia. *R. officinalis* is known as a medicinal plant since long time ago, its oil chemical composition was studied many times in Algeria (Boutekedjiret *et al.*, 1998; Touafek *et al.*, 2004; Giordani *et al.*, 2008), the chemical composition of *R. officinalis* Essential Oil is illustrated in table 3.1.4. the antimicrobial and antioxidant activity of *R. officinalis* essential oil were proved (Djeddi *et al.*, 2007); further, its larvicidal activity was assessed against *Aedes aegypti, Ae albopictus, An stephenis, Cx p quinquifaciatus* (Prajapati *et al.*, 2005; Conti *et al.*, 2010; Duarte *et al.*, 2015).

Table 1.1. Chemical composition of the essential oils *Thymus vulgaris* (Algeria) (Giordani *et al.*, 2008).

Components	Concentrations (%)
p-Cymene	26.36
Thymol	25.57
α-Pinene	12.1
Carvacrol	11.41
Thymoquinone	10.5
Linalool	2.71
β-Caryophyllene	2.34
α -Terpinene	1.5
Limonene	1.24
Thymol methyl ether	1.11
α-Thujene	0.99
β-Bisabolene	0.72
Γ-Muurolene	0.46
Valencene	0.45
γ-Cadinene	0.36
trans-Sabinene hydrate	0.34
Camphene	0.3
Sabinene	0.27
δ-Cadinene	0.2

Table 1.2. Chemical composition of *Artemisia herba-alba* Essential oil harvested from M'sila, Algeria (Dob and Benabdelkader, 2006).

Components	**C (%)**	**Components**	**C (%)**	**Components**	**C (%)**
α-pinene	Tr	verbenone	0.5	spathulenol	0.1
camphene	Tr	*trans*-piperitol	0.4	caryophyllene oxide	0.9
sabinene	Tr	*trans*-carveol	Tr	viridiflorold,e	0.7
β-pinene	Tr	nerald	0.1	capillined,e	0.3
yomogi alcohold	Tr	carvoned	0.1	humulene epoxide Id,e	0.1
α-phellandrene	Tr	piperitone	0.2	1-epi-cubenold,e	0.7
δ-3-carened,e	Tr	geraniold,e	Tr	cubenold,e	0.8
α-terpinene	Tr	perillaldehyded,e	0.4	β-eudesmol	0.1
p-cymene	Tr	geraniald,e	0.7	α-bisabolol oxide Bd,e	Tr
limonene	Tr	lavandulyl acetated	2.2	γ-cadinened,e	Tr
1,8-cineole	Tr	thymold	0.1	δ-cadinene	0.1
(E)-β-ocimened	Tr	carvacrold	0.1	(Z)-nerolidold,e	Tr
γ-terpinene	22.8	α-cubebene	Tr	(E)-nerolidold	0.1
artemisia alcohold	0.1	α-ylangene	2.6	*trans*-calamenened,e	0.1
fenchoned,e	Tr	α-copaene	0.2	borneol	Tr
terpinolene	0.1	β-cubebened	0.1	lavandulold	0.8
α-thujone	1.5	β-elemened,e	Tr	terpinen-4-ol	2.7
β-thujone	15.0	cyperened,e	0.1	myrtenal	0.9
chrysanthenone	15.8	β-caryophyllene	Tr	myrtenol	0.1
trans-pinocarveol	16.9	(Z)-β-farnesened,e	0.1	germacrene D	0.7
camphor	19.4	α-humulene	0.1		
cis-β-terpineold	0.6	allo-aromadendrened,e	0.3		
isoborneold,e	0.1	β-santalened,e	0.2		
cis-chrysanthenol	1.2	ethyl (E)-cinnamated,e	2.8		

Table 1.3. Chemical composition of *Juniperus phoenicea* essential oil (Algeria). (Mazari *et al.*, 2010)

Components	**Concentration (%)**	**Components**	**Concentration (%)**
α-Pinene	34.5	Linalol	0.1
β-Phellandrene	22.4	α-Terpineol	0.1
α-Terpinyl acetate	14.7	γ-Muurolene	0.1
Myrcene	5.6	γ-Cadinene	0.1
δ-3-carene	4.7	α-Eudesmol	0.2
Terpinolene	1.9	Manoyl Oxide	0.2
β-Pinene	1.8	β-Bourbonene	Tr
Germacrene D	1.5	β-Selinene	Tr
Limonene	1.2	β-Eudesmol	Tr
Citronellol	1.2		
β-Caryophyllene	1		
Sabinene	0.7		
α-Phellandrene	0.6		
Camphene	0.5		
Bornyl acetate	0.5		
δ-Cadinene	0.5		
α-Humulene	0.4		
Elemol	0.4		
Linalyl acetate	0.3		
Tricyclene	0.2		
α-Terpinene	0.2		
Borneol	0.2		
β-Elemene	0.2		
α-Muurolene	0.2		
γ-Elemene	0.2		
α-Thujene	0.1		
γ-Terpinene	0.1		

Table 1.4. Composition of *Rosmarinus officinalis* Essential Oil (Algeria). (Djeddi *et al.*, 2007)

Components	**C (%)**	**Components**	**C (%)**
α -Pinene	5.4	γ -Cadinene	1.1
Camphene	7.2	δ -Cadinene	2
β -Pinene	8.5	Caryophyllene oxide	3.1
1-Octen-3-ol	0.6	*cis*-a-Bisabolene	Tr
1,8-Cineole	12.2	Caryophylla-4(12),8(13)-dien-5b-ol	0.1
γ -Terpinene	1.4	α -Bisabolol	Tr
Camphor	14.6	α -Muurolene	0.4
Borneol	10.6		
Terpinolene	0.7		
Linalool	2.2		
α -Terpineol	5.2		
cis-Piperitol	0.1		
Citronellol	0.1		
Bornyl acetate	5.3		
Carvacrol	0.2		
α -Cubebene	0.3		
α -Copaene	1.3		
β -Bourbonene	0.1		
α -Cubebene	0.1		
α -Cadinene	0.2		
β -Funebrene	0.1		
β -Caryophyllene	10.9		
Aromadendrene	0.3		
α -Humulene	3		
α -Amorphene	1.3		
β -Selinene	0.2		
α -Zingiberene	0.5		

1.1.5 **Eucalyptus globulus**

E. globulus, an evergreen aromatic tree native to Australia, belongs to the genus *Eucalyptus*, family of Mytraceae. According to Batish *et al.* (2008), the Eucalyptus essential oil is a natural pesticide that possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal, insect repellent, herbicidal, acaricidal and nematicidal. Further, the larvicidal activity of *Eucalyptus* essential oils was previously assessed against *Aedes aegypti* and *Ae albopictus*, and had proved for its efficacy against the tested larvae (Cheng *et al.*, 2009; Alvarez Costa *et al.*, 2017).

Table 1.5. Chemical composition of *Eucalyptus globulus* essential oil from Algeria (Samir *et al.*, 2001)

Components	**Concentration (%)**
1,8-cineole	71.3
α -pinene	8.8
trans-pinocarveol	3.3
limonene	2.7
α -terpineol	2.7
globulol	1.6
aromadendrene	1.5
pinocarvone	0.7
allo-aromadendrene	0.4
epi-globulol	0.4
terpinen -4-ol	0.3
β-pinene	0.3
myrcene	0.2
camphene	0.1
sabinene	Tr

1.2 **Mosquito collection**

Culiseta longiareolata larvae were collected regularly from three fixed pools (Figure 3.16), larvae of third and fourth instar were used directly in the test; eggs, first and second instar larvae were reared in room temperature (27°C±2°C), in a 12 h light: 12 h dark photoperiod, until reached the fourth instar.

Figure 3.16. *Culiseta longiareolata* pools (personal photos).

1.3 **Larvicidal bioassays**

According to WHO guidelines for laboratory and field testing of mosquito larvicides (Organization, 2005), we have tested the larvicidal activity of EOs extracted from the leaves of five aromatic medicinal plants *Thymus vulgaris*, *Artemisia herba-alba*, *Juniperus Phoenicia*, *Rosmarinus officinalis*, *Eucalyptus globulus* against *Culiseta longiareolata* larvae under laboratory conditions. The EOs were extracted by steam distillation, they were then serially diluted in ethanol to obtain 10%, 1%, 0.1% and 0.01% of stock solution (Table 1.6), 0.1-1ml of the previous dilutions were added to 100ml of water to obtain final concentrations (Table 1.7). A series of concentration and control were applied each on 25 mosquito larvae distributed in five cups containing 100ml of water. We started the test by the lowest concentrations, the concentrations that showed less than 10% of mortality were excluded, concentrations that showed 10% of mortality or more were replicated 4 times, each test was run three times. After 24h of exposure, moribund and dead larvae were counted. We have chosen four concentrations which caused between 10% and 90% of mortality to determine the

LC_{50} and LC_{90} values, the data obtained from the four replicates in the three tests were pooled for analysis.

Table 1.6. Dilution of essential oil volumes to obtain initial concentrations.

IC (%)	Essential oil (ml)	Alcohol 90% (ml)	Obtained Concentration (%)
100	2	18	10
10	2	18	1
1	2	18	0.1
0.1	2	18	0.01

Table 1.7. Aliquots added to obtain final concentrations in ppm.

IC %	Aliquot (ml)	FC(ppm)
0.01	0.1	0.1
	0.5	0.5
	1	1
0,1	0.1	1
	0.5	5
	1	10
1	0,1	10
	0.2	20
	0.4	40
	0.5	50
	0.6	60
	0.7	70
	0.8	80
	0.9	90
	1	100
10	0,1	100
	0,2	200

1.4 **Statistical analyses**

Data were subjected to probit analysis using SPSS software V25 (Using probit model because of the normal distribution of data); final concentrations were transformed to log10. Lethal concentration LC_{50} and LC_{90} with 95% confidence limit (CL) suspected to kill 50% and 90% of the population respectively were calculated and presented with the regression equations (Y= b +ax) and regression coefficients (R^2).

2 **Results**

Five plant oils were tested to evaluate their larvicidal activity, all the mortality data obtained by testing the larvicidal activity of the five essential oils on *Culiseta longiareolata* larvae are mentioned in Table 2.1, Table 3.2, Table 3.3, Table 3.4, Table 3.5. The majority of the tested oils showed 100% of mortality at 200ppm final concentration except for *T. vulgaris* that showed 100% of mortality at 80ppm. Further, the oils started to cause 10% of mortality at different levels of concentration; *T. vulgaris* oil caused more than 10% of mortality at 20ppm, *J.* Phoenicia at 40ppm, *A. herba-alba* and *R. officinalis* at 50ppm and *E. globulus* oil at 70ppm (Table 2.6). The 24h LC_{50} and LC_{90} estimate, upper and lower values obtained from the larvicidal activity test of EOs extracted from the five plants are presented in table 3.2.7, *T. vulgaris* was the most efficient with 25.64 (16.58-32.03) LC_{50} and 50.53 (40.15-82.43) LC_{90}, while *A. herba-alba* was the less efficient. The probit transformed responses for the five tested oils with the regression equations and regression coefficients are illustrated in Figure 3.17, Figure 3.18, Figure 3.19, Figure 3.20, Figure 3.21. The increase of the concentration by one unit will increase the mortality by 4.36 times in *T. vulgaris*, by 3.66 in *J. phoeniciea*, by 6.08 in *A. herba-alba*, by 7.16 times in *R. officinalis* and by 5.28 times in *E. globulus*. The R^2 was close to 1 in all probit analysis, the minimal residuals obtained between the observed and expected values was in *E. globulus* EO (R^2=0.99) (Figure 3.21).

Table 2.1. Mortality data obtained through testing the larvicidal activity of *T. vulgaris* on *Culiseta longiareolata* larvae

IC		0,10%			1%						
Aliquot		0,1	0,5	1	0,1	0,2	0,4	0,5	0,6	0,8	1
FC		0,1	0,5	1	10	20	40	50	60	80	100
test1	R1	1	0	0	0	5	21	21	25	25	25
	R2	0	0	2	2	8	20	15	20	25	25
	R3			4	0	1	0	13	24	25	25
	R4			0	0	10	23	25	24	25	25
test2	R1					17	25	10	25	25	25
	R2					4	25	25	25	25	25
	R3					8	23	24	23	25	25
	R4					1	22	25	25	25	25
Test3	R1					10	24	25	24	25	25
	R2					17	25	24	25	25	25
	R3					4	22	25	23	25	25
	R4					8	23	25	23	25	25

Table 2.2. Mortality data obtained through testing the larvicidal activity of *J. Phoenicea* on *Culiseta longiareolata* larvae.

IC		1%							10%
Aliquot		0,1	0,2	0,4	0,5	0,6	0,8	1	0,2
FC		10	20	40	50	60	80	100	100
test1	R1	2	1	14	13	19	23	24	25
	R2	0	0	7	4	19	23	21	25
	R3			9	5	14	17	22	25
	R4			0	4	22	0	15	25
test2	R1			15	13	11	14	25	25
	R2			9	11	11	20	24	25
	R3			9	16	11	20	20	25
	R4			2	10	12	12	22	25
Test3	R1			7	9	13	12	7	25
	R2			9	4	7	12	25	25
	R3			5	4	15	15	25	25
	R4			8	10	2	8	25	25

Table 2.3. Mortality data obtained through testing the larvicidal activity of *A. herba-alba* on *Culiseta longiareolata* larvae.

IC		1%							10%
Aliquot		0,1	0,2	0,4	0,5	0,6	0,8	1	0,2
FC		10	20	40	50	60	80	100	200
test1	R1	0	0	0	1	4	11	9	25
	R2	0	0	0	2	3	8	5	25
	R3				4	2	21	20	25
	R4				0	13	11	18	25
test2	R1				9	17	5	24	25
	R2				4	7	5	22	25
	R3				1	1	5	22	25
	R4				0	0	8	23	25
Test3	R1				1	3	4	9	25
	R2				1	2	5	14	25
	R3				1	0	6	25	25
	R4				0	5	9	25	25

Table 2.4. Mortality data obtained through testing the larvicidal activity of *R. officinalis* on *Culiseta longiareolata* larvae.

IC		1%							10%
Aliquot		0,1	0,2	0,4	0,5	0,6	0,8	1	0,2
FC		10	20	40	50	60	80	100	200
test1	R1	0	0	0	4	12	19	13	25
	R2	0	0	0	0	5	13	25	25
	R3				0	14	18	15	25
	R4				3	16	21	25	25
test2	R1				7	2	22	24	25
	R2				15	3	20	25	25
	R3				5	9	20	24	25
	R4				5	14	20	23	25
Test3	R1				2	6	16	25	25
	R2				6	2	21	25	25
	R3				15	7	22	25	25
	R4				13	16	24	25	25

Table 2.5. Mortality data through testing the larvicidal activity of *E. globulus* on *Culiseta longiareolata* larvae.

IC		1%							10%
Aliquot		0,1	0,5	0,6	0,7	0,8	0,9	1	0,5
FC		10	50	60	70	80	90	100	200
test1	R1	0	0	1	4	10	4	7	25
	R2	0	2	0	5	12	15	4	25
	R3				3	15	10	23	25
	R4				2	8	6	20	25
test2	R1				2	14	9	17	25
	R2				7	6	5	12	25
	R3				2	2	14	14	25
	R4				3	8	4	8	25
Test3	R1				10	8	14	17	25
	R2				14	8	16	7	25
	R3				7	9	20	13	25
	R4				9	7	17	17	25

Table 2.6. Mortality percentages of *Culiseta longiareolata* larvae caused by the tested essential oils at different concentrations, with the arthemitric mean (AM) and standard error (SE).

IC (%)	Aliquot (ml)	FC (ppm)	Dead in a total of 300 larvae (AM±SE)				
			Thymus vulgaris	*Juniperus phoenicea*	*Artemisia herba-alba*	*Rosmarinus officinalis*	*Eucalyptus globulus*
1	0,2	20	93 (7.75±1.53)	-	-	-	-
	0,4	40	253 (21.08±1.97)	94 (7.83±1.23)	-	-	-
	0,5	50	257 (21.42±1.59)	103 (8.58±1.23)	24 (2±0.75)	75 (6.25±1.54)	-
	0,6	60	286 (23.83±0.42)	156 (13±1.58)	57 (4.75±1.52)	106 (8.83±1.56)	-
	0,7	70	-	-	-	-	68 (5.67±1.1)
	0,8	80	300 (7.75±1.53)	176 (14.67±1.91)	89 (8.17±1.36)	236 (19.67±0.85)	107 (8.92±1.02)
	0,9	90	-	-	-	-	134 (11.17±1.6)
	1	100	300 (25±0.0)	255 (21.25±1.55)	216 (18±2.03)	274 (22.83±1.21)	159 (13.25±1.69)
10	0,2	200	300 (25±0.0)	300 (25±0.0)	300 (25±0.0)	300 (25±0.0)	300 (25±0.0)

IC (initial concentration), FC (final concentration)

Table 2.7. The LC_{50} and LC_{90} values of essential oils extracted from *T. vulgaris*, *A. herba-alba*, *J. phoenicea*, *R. officinalis* and *E. globulus* against 3rd and 4th instars larvae of *Culiseta longiareolata* after 24 hours exposure period.

Essential oils	LC_{50} (ppm) 95% CI			LC_{90} (ppm) 95% CI			Sig (df)
	Estimate	Lower	Upper	Estimate	Lower	Upper	
Thymus vulgaris	25.64	16.58	32.03	50.53	40,15	82.43	p<0,05 (2)
Juniperus phoenicea	59.83	45.36	75.81	137.68	97.21	>250	p<0,05 (3)
Artemisia herba-alba	86.67	66.59	>250	139.55	98.03	>250	p<0,05 (2)
Rosmarinus officinalis	64.18	55.41	72.56	96.55	82.73	139.84	p<0,05 (2)
Eucalyptus globulus	95.83	92.27	101.09	168.25	146.59	201.87	p<0,05 (2)

Sig (significance level), df (degrees of freedom)

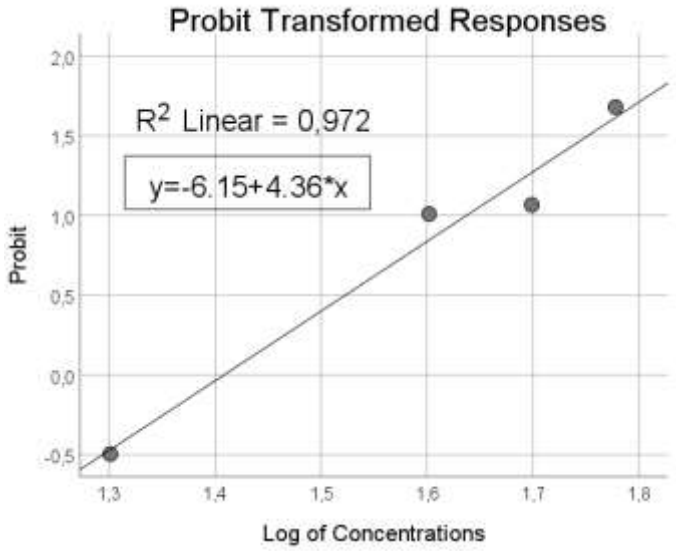


Figure 3.17. Probit transformed responses with equation regression and coefficient of determination R^2 for *T. vulgaris* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

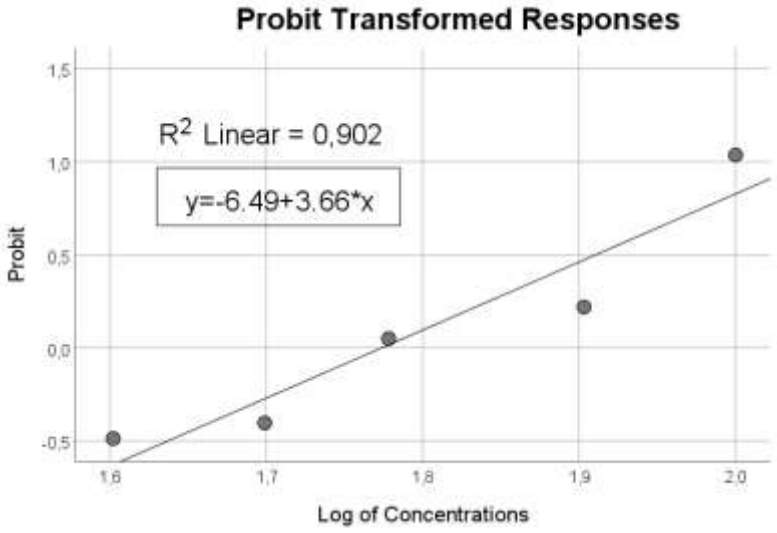


Figure 3.18. Probit transformed responses with equation regression and coefficient of determination R^2 for *J. phoenicea* tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

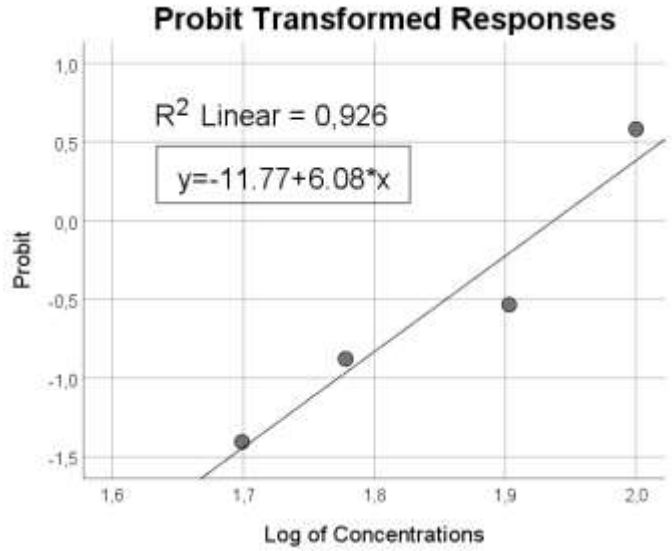


Figure 3.19. Probit transformed responses with equation regression and coefficient of determination R^2 for *A. herba-alba* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h

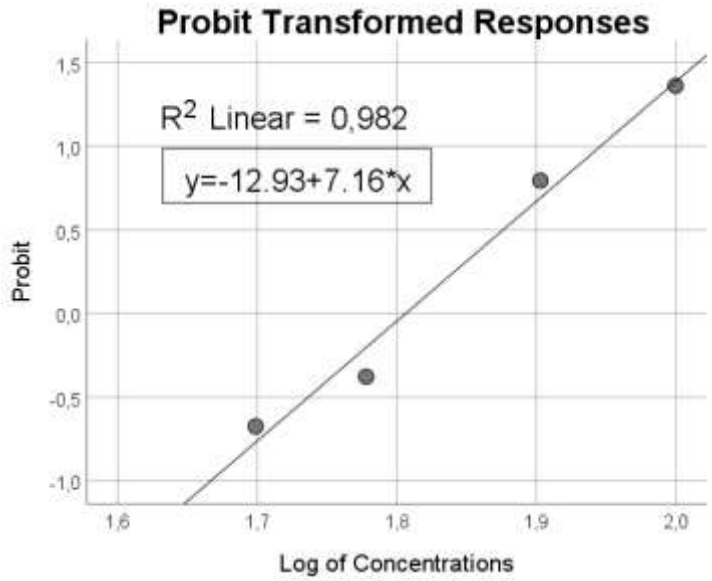


Figure 3.20. Probit transformed responses with equation regression and coefficient of determination R^2 for *R. officinalis* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

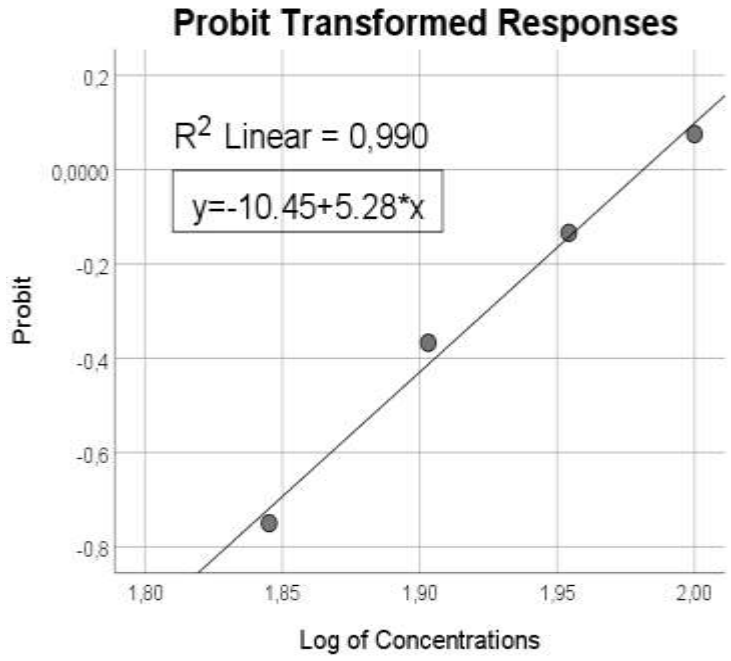


Figure 3.21. Probit transformed responses with equation regression and coefficient of determination R^2 for and *E. globulus* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

3 Discussion

The current study has confirmed that the EOs extracted from the aromatic medicinal plants *T. vulgaris*, *A. herba-alba*, *J. phoenicea*, *R. officinalis* and *E. globulus* present an efficient larvicidal activity against *Culiseta longiareolata* larvae; however, the mortality responses obtained were varying.

T. vulgaris is a flowering herb that has a worldwide distribution (Hosseinzadeh *et al.*, 2015). From the total of the tested oils, the *T. vulgaris* EO was the most efficient. This EO was previously assessed by Knio *et al.* (2008) against *Ochlerotatus caspius* (Pallas 1771) larvae; however, its toxicity against *Oc. caspius* (LC_{50}=33.65ppm; LC_{90} =50.85ppm) was less than that shown by our *T. vulgaris* EO. Likewise, the larvicidal activity of the EOs extracted from *Juniperus* species was tested in previous studies: *J. phoenicea* against *Aedes albopictus* (Skuse 1894) (LC_{50}= 55.5ppm; LC90= 77ppm), *J. virginiana* L. against *Ae. aegypti* (Linnaeus 1762) and *Cx. pipiens* (Lee, 2006; Giatropoulos *et al.*, 2013). Comparing to our results, our *J. phoenicea* EO showed lower larvicidal activity against *Cs. longiareolata*. Moreover, the larvicidal activity of *R. officinalis* EO was assessed against *Ae albopictus* (LC_{50}>250ppm), *Cx. tritaeniorhynchus* (Giles 1901) (*LC_{50}= 115.38ppm; LC90= 211.53ppm) and *Anopheles*

subpictus Grassi (LC_{50}= 64.5ppm; LC90= 113.74ppm) (Conti *et al.*, 2010; Govindarajan, 2011); the *R. officinalis* EOs tested against *Ae. albopictus*, *Cx. tritaeniorhynchus* and *An. subpictus* in the previous researches showed lower values than the toxicity results that we obtained by testing the same EO against *Cs. longiareolata.*

The other EOs *E. globulus* and *A. herba-alba* were less efficient; however, their lethal concentrations were notable. *E. grandis* L. EO and its major components were assessed for their larvicidal activity against *Aedes aegypti* by Lucia *et al.* (2007), the EO showed 32.4ppm LC_{50} and the principal components α-pinene (52.71%) and 1,8-cineole (18.38%) showed 15.4ppm and 57.2ppm LC_{50} respectively. The principal leaf oil components of *E. globulus* harvested from Algeria are α-pinene and 1,8-cineole, according to Samir *et al.* (2001); however, our *E. globulus* EO tested against *Cs. longiareolata* was less efficient (LC_{50}= 95.83ppm). Furthermore, EOs extracted from *Artemisia* genus were assessed for their larvicidal activity against various mosquito species. Our *A. herba-alba* EO tested against *Cs longiareolata* larvae was more efficient (LC_{50}= 86.67ppm) than *A. vulgaris* L. that was tested by Ilahi and Ullah (2013) against *Cx. quinquefasciatus* (LC_{50}= 803.2ppm), but less efficient than *A. absinthium L. tested* by Govindarajan and Benelli (2016) *against An. stephensi (*Liston 1901), *An. subpictus*, *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus* (Say 1823), and *Cx. tritaeniorhynchus* (LC_{50}=41.85, 52.02, 46.33, 57.57, 50.57, and 62.16 ppm respectively). Various mosquito species were targeted in the previous researches to assess the larvicidal activity of EOs. However, *Cs. longiareolata* was not previously targeted by EOs, but by the lichen metabolites evaluated by Cetin *et al.* (2012) that showed high larvicidal activity against *Cs. longiareolata*.

The obtained results confirm the previous studies; the use of EOs can serve as an eco-friendly method to control mosquito larvae. However, the noted variability in the efficacy level of the tested oils may be due to their chemical composition and the percentages of their principal components as α-Pinene, Camphor and 1,8-Cineole (Samir *et al.*, 2001; Dob and Benabdelkader, 2006; Djeddi *et al.*, 2007; Giordani *et al.*, 2008, Mazari *et al.*, 2010); whereas, the direct use of the principal components of EOs may produce higher efficacy in mosquito control. This hypothesis was proven in the study conducted by Lucia *et al.* (2007), where the principal components of Turpentine and *E. grandis* EO showed lower LC_{50} than that obtained by the use of the entire *E. grandis* EO. Moreover, the repellency effect of the thyme EO compounds against *Culex pipiens* mosquito evaluated by Park *et al.* (2005) showed higher

repellent efficacy of α-Terpinene and Carvacrol than the commercial formulation diethyltoluamide (DEET) and an equal efficacy between the Thymol component to the DEET.

GENERAL CONCLUSION

Humanity has been afflicted with the nuisance and disease transmitted by mosquitoes for a long time. These insects are cosmopolitan, adaptive, and dangerous; this is why they play an indisputable role in the schematization of human life. Mosquitoes transmit dangerous and deadly diseases that can affect both humans and animals, we can mention: malaria, West Nile Virus, dengue fever, Zika, encephalitis, and other Arbovirus and plasmodium diseases. Due to the importance of these insects, the study of mosquito biodiversity became an obligation for better control. In order to provide an integrated study on mosquito biodiversity in the Setif region that may be used as a comprehensive control program, we investigated species list, the descriptive parameters, the breeding sites characterization, the seasonal fluctuations, and the distribution patterns of the mosquito population that occupy the study area.

Setif region has never been investigated before; for this reason, we were interested to provide a precise mosquito species list. Therefore, we used an integrative taxonomy approach which focalizes on the simultaneous use of both morphological and molecular identification. The determination results of mosquito species has yielded the identification of nine mosquito species, *Culex pipiens* s.l., *Cx. theileri, Cx. hortensis, Cx. simpsoni, Cs. longiareolata, Oc. caspius, Cq. richiardii, An. labranchiae, An. c hispaniola*; and have provided mosquito sequences published in Genbank under the accession numbers MK047302-MK047315.

Culex simpsoni is recorded for the first time in Algeria. Moreover, the molecular analyses helped us to report *An. c hispaniola* that was not reported in Algeria since 1983, where the morphological identification was very difficult and uncertain. The high genetic divergence detected between our *An. c hispaniola* sequences and *An. cinereus* sequences from Saudi Arabia supported the use of geographic distribution to identify *An. cinereus* members and confirms the ability of COI barcode to differentiate them. We insured likewise through the molecular analysis, that *An. labranchiae* is the only representative of the *An maculipennis* complex members in Algeria.

From the issues that we have arisen, the morphological variation noted on *Culex pipiens* s.l.. The insertion of seta 1a-S next the last pecten tooth perturbed the morphological identification, and only after the molecular analyses conducted we insured the species. This is why we provided a morphological characterization of *Culex pipiens* s.l. populations occupy Setif region in order to facilitate their recognition in further studies.

GENERAL CONCLUSION

The descriptive study of mosquito population represented *Culex pipiens* as the most frequent species, and *Cs. longiareolata* as the most abundant; like in the rest of Algerian regions. We noted *An. labranchiae*, the malaria vector, as the second frequent species in Setif; however, it was found at low density. The mosquito population in Setif region is thus constituted mainly of vector species; this is why the analysis of their characteristics, fluctuation and distribution patterns were successive objectives of the current study.

We analyzed the mosquito breeding sites in Setif region as abiotic factor that may provide utile information about mosquito population and their female breeding preferences. The breeding sites were mainly rural, permanent, characterized by the presence of algae. Although mosquitoes in Setif use permanent sites in rural areas as primary breeding sites for long and non-intensive reproduction, they use on the other hand the temporary rural pools surrounding human habitation for rapid and intensive reproduction. These pools are formed indirectly from rain agglomerations or consequently to human activities.

On the other side, we broached species co-occurrence as a biotic factor that can explain females' mechanisms in the selection of breeding sites. *Anopheles labranchiae* and *An theileri* are the species the most found associated with other mosquito species and together noted a highly significant positive correlation. Therefore we can open the possibility of using *An theileri* as an indicator species of the presence of the malaria vector *An labranchiae*.

The importance of breeding sites for mosquito reproduction is something intuitive, the elimination of breeding sites is then a primary tool in mosquito control; therefore, the determination of density fluctuation peaks will facilitate the process. During the sampling we noted three density fluctuation peaks in May, August, and December; in return, three precipitation peaks were noted during the sampling period in January, April, and November. In January the temperature is very low thereby preventing the eggs hatch, whereas, after April and November pecks the mosquito density reaches its maximum. Thus the elimination of breeding sites in April and December, or the use of insecticides in these periods will definitely decrease mosquito density.

We have seen the effect of climate on mosquito fluctuation, but the analyze of mosquito population descriptives we have conducted across the climates zones Csa and BSk in Setif confirmed equally the effect of climate on mosquito density and species distribution. We observed higher diversity in Csa because of the disponibility of various water surface types, and higher density in BSk because of the longer temperature periods over the year.

From all previous findings, we conclude the necessity of an effective tool to control mosquitoes in Setif region beside the biodiversity management. For this reason, we tried to

assess the efficiency of an eco-friendly method to control mosquito larvae. The essential oils extracted from five aromatic medicinal plants *Thymus vulgaris*, *Artemisia* herba-alba, *Juniperus phoenicea, Rosmarinus officinalis* and *Eucalyptus globulus* assessed against *Culiseta longiareolata* larvae showed positive larvicidal activities. *T. vulgaris* and *J. phoenicea* were the most efficient and noted the lowest LC_{50} and LC_{90} values; which means that lower concentrations of these essential oils will serve to eliminate mosquito larvae. We tried also to provide previous studies about the composition of the tested essential oils harvested from the same regions to highlight their principal components which granted the larvicidal efficacy to the essential oils. Consequently, the tested essential oils and their principal components may serve as safe products to control *Culiseta longiareolata* larvae and likely the other mosquito species.

PERSPECTIVES

The study we conducted on mosquito biodiversity in Setif region is considered as a platform for further researches on mosquitoes where we mention:

- The molecular characterization of *Culex pipiens* s.l. populations in Setif region using CQ11 genetic structure.
- The evaluation of the vector role of mosquito species in Setif region especially for *Culex pipiens* s.l. and *Cs longiareolata* because of the fluctuation of West Nile Virus disease in Algeria.
- The investigation of the underground mosquito population.
- The evaluation of the possibility of using mosquito species as indicator of other vector species.
- The field application of the tested essential oils and the evaluation of their negative effects on non-targeted organisms.

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Annex

ISTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE

Eur J Biol 2019; 78(2)

Larvicidal Activities of Essential Oils Extracted from Five Algerian Medicinal Plants against *Culiseta longiareolata* Macquart. Larvae (Diptera: Culicidae).

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Please cite this article as: Nabti I, Bounechada M. Larvicidal Activities of Essential Oils Extracted from Five Algerian Medicinal Plants against *Culiseta longiareolata* Macquart. Larvae (Diptera: Culicidae). Eur J Biol 2019; 78(2). Advanced Online Publication. DOI: 10.26650/EurJBiol.2019.0015

ABSTRACT

Objective: The use of essential oils in mosquito control is considered as a potential alternative of synthetic insecticides. The current study aimed to assess the larvicidal activity of the essential oils extracted from five medicinal plants collected from northeastern Algeria against the *Culiseta longiareolata* larvae, a vector of the *Plasmodium* species in birds and one of the most abundant mosquito species in Algeria.

Materials and Methods: The essential oils extracted from: *Thymus vulgaris*, *Artemisia herba-alba, Juniperus phoenicea, Rosmarinus officinalis*, and *Eucalyptus globulus* were tested against the 3rd and 4th instar *Culiseta longiareolata* larvae. The larvae were exposed to a series of concentrations of the tested essential oils for 24h. The concentrations that caused between 10% and 90% mortality were replicated four times, and the entire test was repeated three times. The collected data were used to determine the LC_{50} and LC_{90} values,

Results: The tested oils revealed an efficient larvicidal activity. *T. vulgaris* showed 100% mortality at 80ppm final concentration, while the other tested oils showed 100% mortality at 200ppm. Furthermore, the lethal concentrations that caused 50% and 90% mortality (LC_{50} and LC_{90}) were varying. *T. vulgaris* was the most efficient essential oil (LC_{50}=25.64ppm, LC_{90}=50.53ppm), followed by *J. Phoenicea* (LC_{50}=59.83ppm, LC_{90}=137.68ppm), *R. officinalis* (LC_{50}= 64.18ppm, LC_{90}= 96.55ppm), *A. herba-alba* (LC_{50}=86.67ppm, LC_{90}=139.55ppm), then *E. globules* (LC_{50}=95.83ppm, LC_{90}= 168.25ppm).

Conclusion: The use of essential oils or their principal active components as α-pinene, 1,8-cineole and Camphor may serve as an eco-friendly method to control mosquito larvae. Nevertheless, the field application of essential oils and their principal components remains a fundamental step to evaluate the field efficacy of these botanic extracts and to note their possible secondary effects on non-targeted organisms.

Keywords: Aromatic medicinal plants, *Culiseta longiareolata*, Essential oil, Larvicidal activity, Mosquitoes

INTRODUCTION

Culicidae, or mosquitoes as commonly known, is a family of Diptera insects that reproduce quickly and abundantly. Simultaneously, this family includes major vectors for many deadly and dangerous diseases. Therefore, the importance of the mosquito family in terms of public health makes mosquito control an important initiative to minimize the negative effects of mosquito-born-diseases. Mosquito control may depend on various strategies; the most common in the past decades was the use of synthetic insecticides as inexpensive and available products. However, the use of synthetic insecticides has over time created environment pollution and resistance problems (1, 2).

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Submitted: 17.09.2019 • **Revision Requested:** 01.10.2019 • **Last Revision Received:** 06.10.2019 • **Accepted:** 16.10.2019

Recently, eco-friendly methods were developed to control mosquitoes. For instance, the enhancement of behavior-based control tools and the development of repellent and toxic products based on botanic components can target different mosquito life stages (3, 4). Essential oils (EOs) extracted from different parts of plants were frequently tested for their mosquitocidal activity (5). These primary botanic materials present various biological activities. They can act as insecticides where they can affect the oviposition, survival, larval duration, pupation and insect emergence (6, 7). However, the larvae stage appears to be more appropriate to control mosquito populations because of the high reproduction rates and larvae food mechanisms that allow a high number of mosquito individuals to be targeted simultaneously. Therefore, the assessment of the larvicidal efficacy of various plant derivatives was the main objective of many research papers (8-11).

Culiseta longiareolata (Macquart 1838) constitutes with the *Culex pipiens* (Linnaeus 1758) complex the most abundant species in Algeria. It usually breeds near human habitations, however, the females prefer to feed on bird blood (12). *Cs longiareolata* has uniquely adaptive and survivor features. Kiflawi et al. (13) have confirmed that the females of this species showed an adaptive response against the risk of predation and negative density effects where they avoid laying their eggs in predator pools. Further, *Cs longiareolata* is considered as a primary vector of *Plasmodium* (*Giovannolaia*) *circumflexum* (Kikuth 1931), *Plasmodium relictum* (modified from Garnham 1966) and *Plasmodium polare* (Manwell 1934) in birds, and its capacity to transmit *P. relictum* in Algeria was proven experimentally (14, 15). In this context, we have assessed the larvicidal activity of EOs extracted from five aromatic medicinal plants, harvested from Northeastern Algeria, against *Cs longiareolata* larvae. The efficacy of the tested EOs will be evaluated by calculating the LC_{50} and LC_{90} values and by comparing them with the LC_{50} and LC_{90} values of the same EOs tested previously against other targeted mosquito species.

MATERIALS AND METHODS

Mosquito Collection

Culiseta longiareolata larvae were collected regularly from three clean fixed and controlled pools in Algeria, where the mosquitoes were not exposed to any insecticides. Larvae of the third and fourth instar were used directly in the test; eggs, first and second instar larvae were reared in room temperature (27°C±2°C), in a 12 h light: 12 h dark photoperiod, until the fourth instar was reached.

Essential Oils Extraction

The aerial parts of the tested plants were collected from different regions in the Mediterranean and semi-arid climate northeastern Algeria: *Thymus vulgaris* L. from Guelma, *Artemisia herba-alba* Asso from M'Sila, *Juniperus phoenicea* L. from Jijel, *Rosmarinus officinalis* Linn from Bouira and *Eucalyptus globules* L. from Batna. The plants' collection started at the beginning of the summer (June) in 2018. The samples were air-dried at room temperature. The dried plants were submitted to classical steam distillation for 3-6 h. The samples were exposed to the water vapor produced in the flask crosses, the vapor was charged with the EO, and then was condensed in the condenser. The EO floated on the water surface was then recuperated. The yield of the EOs was between 0.8 and 1.5%.

Larvicidal Bioassay

According to WHO guidelines for laboratory and field testing of mosquito larvicides (16), we tested the larvicidal activity of EOs extracted from the leaves of five aromatic medicinal plants *T. vulgaris*, *A. herba-alba*, *J. phoenicea, R. officinalis ,E. globulus* against *Culiseta longiareolata* larvae under laboratory conditions. The EOs were extracted by steam distillation, they were next serially diluted in ethanol to obtain 10%, 1%, 0.1% and 0.01% of stock solution, and 0.1-1ml of the previous dilutions were added to 100ml of water to obtain the final concentrations. A series of concentrations and controls were applied on 25 mosquito larvae distributed in five cups containing 100ml of water. A total of 8925 larvae were tested. We started the test with the lowest concentrations. The concentrations that showed less than 10% mortality were excluded. Concentrations that showed 10% mortality or more were replicated 4 times, and each test was run three times. After 24h of exposure, moribund and dead larvae were counted. We have chosen four concentrations which caused between 10% and 90% mortality to determine the LC_{50} and LC_{90} values. The data obtained from the four replicates in the three tests were pooled for analysis.

Statistical Analyses

Data were subjected to probit analysis using SPSS software V25 (Using probit model because of the normal distribution of data); and final concentrations were transformed to log10. Lethal concentration LC_{50} and LC_{90} with a 95% confidence limit (CL) suspected of killing 50% and 90% of the population respectively, were calculated and presented with the regression equations ($Y= a +b*x$) and regression coefficients (R^2).

RESULTS

Five plant EOs were tested to evaluate their larvicidal activity, and the tested oils revealed various mortality percentages at different concentrations (Table 1). The majority of the tested oils showed 100% mortality at 200ppm final concentration, except for *T. vulgaris* that showed 100% mortality at 80ppm. Further, the oils started to affect the larvae life at different concentrations; the lowest concentration that caused equal or more than 10% mortality was 20ppm for *T. vulgaris* , 40ppm for *J. phoenicea*, 50ppm for *A. herba-alba* and *R. officinalis* and 70ppm for *E. globules* (Table1). The 24h LC_{50} and LC_{90} estimate, upper and lower values obtained from the larvicidal activity test of EOs extracted from the five plants in addition to the regression equations and regression coefficients are presented in Table 2. *T. vulgaris* was the most efficient with 25.64 (16.58-32.03) LC_{50} and 50.53 (40.15-82.43) LC_{90}, while *A. herba-alba* was the least efficient. Likewise, the influence degree of increasing one unit of EOs concentration on their larvicidal activity was different. Among the tested EOs, the augmentation of one unit

Table 1: The mortality observed to the *Culiseta longiareolata* larvae, caused by the application of the tested essential oils at different concentrations, with the arithmetic mean (AM) and standard error (SE).

			Dead in a total of 300 larvae (AM±SE)				
IC (%)	**Aliquot (ml)**	**FC (ppm)**	***Thymus vulgaris***	***Juniperus phoenicea***	***Artemisia herba-alba***	***Rosmarinus officinalis***	***Eucalyptus globules***
1	0,2	20	93 (7.75±1.53)	-	-	-	-
	0,4	40	253 (21.08±1.97)	94 (7.83±1.23)	-	-	-
	0,5	50	257 (21.42±1.59)	103 (8.58±1.23)	24 (2±0.75)	75 (6.25±1.54)	-
	0,6	60	286 (23.83±0.42)	156 (13±1.58)	57 (4.75±1.52)	106 (8.83±1.56)	-
	0,7	70	-	-	-	-	68 (5.67±1.1)
	0,8	80	300 (7.75±1.53)	176 (14.67±1.91)	89 (8.17±1.36)	236 (19.67±0.85)	107 (8.92±1.02)
	0,9	90	-	-	-	-	134 (11.17±1.6)
	1	100	300 (25±0.0)	255 (21.25±1.55)	216 (18±2.03)	274 (22.83±1.21)	159 (13.25±1.69)
10	0,2	200	300 (25±0.0)	300 (25±0.0)	300 (25±0.0)	300 (25±0.0)	300 (25±0.0)

IC(initial concentration), FC (final concentration)

Table 2: The LC_{50} and LC_{90} values of essential oils extracted from *T. vulgaris, A. herba-alba, J. phoenicea, R. officinalis* and *E. globules* against the 3rd and 4th instar larvae of the *Culiseta longiareolata*, after 24 hours exposure period; with regression equations and regression coefficients (R2).

	LC50 (ppm) 95% CI			**LC90 (ppm) 95% CI**					
Essentialoils	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**	**Sig (df)**	**Regression equation**	**R2**
Thymus vulgaris	25.64	16.58	32.03	50.53	40,15	82.43	p>0.05 (2)	y=-6.15+4.36*x	0.97
Juniperus phoenicea	59.83	45.36	75.81	137.68	97.21	<250	p>0.05 (3)	y=-6.49+3.66*x	0.9
Artemisia herba-alba	86.67	66.59	<250	139.55	98.03	<250	p>0.05 (2)	y=-11.77+6.08*x	0.93
Rosmarinus officinalis	64.18	55.41	72.56	96.55	82.73	139.84	p>0.05 (2)	y=-12.93+7.16*x	0.98
Eucalyptus globules	95.83	92.27	101.09	168.25	146.59	201.87	p>0.05 (2)	y=-10.45+5.28*x	0.99

Sig (significance level), df (degrees of freedom)

of *R. officinalis* concentration showed the highest influence in increasing the LC_{50} and LC_{90} (b=7.16). The R^2 was close to 1 in all probit analysis, the minimal residuals obtained between the observed and expected values was shown by *E. globulus* EO (R^2=0.99) (Table 2).

DISCUSSION

The current study has confirmed that the EOs extracted from the aromatic medicinal plants *T. vulgaris*, *A. herba-alba*, *J. phoenicea, R. officinalis* and *E. globulus* present an efficient larvicidal activity against the *Culiseta longiareolata* larvae; however, the mortality responses obtained were varying.

T. vulgaris is a flowering herb that has a worldwide distribution (17). From the total of the tested oils, the *T. vulgaris* EO was the most efficient. This EO was previously assessed by Knio et al. (18) against the *Ochlerotatus caspius* (Pallas 1771) larvae; however, its toxicity against *Oc caspius* (LC_{50}=33.65ppm; LC_{90} =50.85ppm) was less than that shown by our *T. vulgaris* EO. Likewise, the larvicidal activity of the EOs extracted from the *Juniperus* species was tested in previous studies: *J. Phoenicea* against *Aede salbopictus* (Skuse 1894) (LC_{50}= 55.5ppm; LC90= 77ppm), and *J. virginiana* L. against *Ae aegypti* (Linnaeus 1762) and *Cx pipiens* (19, 20). Comparing our results, our *J. phoenicea* EO showed lower larvicidal activity against *Cs longiareolata*. Moreover, the larvicidal activity of *R. officinalis* EO was assessed against

Ae albopictus (LC_{50}<250ppm), *Cx tritaeniorhynchus* (Giles 1901) (*LC_{50}*= 115.38ppm; LC90= 211.53ppm) and *Anopheles subpictus* Grassi (*LC_{50}*= 64.5ppm; LC90= 113.74ppm) (21, 22). The *R. officinalis* EOs tested against *Ae albopictus, Cx tritaeniorhynchus* and *An subpictus* in the previous researches showed lower values than the toxicity results that we obtained by testing the same EO against *Cs longiareolata.*

The other EOs *E. globules* and *A. herba-alba* were less efficient; however, their lethal concentrations were notable. *E. grandis* L. EO and its major components were assessed for their larvicidal activity against *Aedes aegypti* by Lucia et al. (23). The EO showed 32.4ppm LC_{50} and the principal components α-pinene (52.71%) and 1,8-cineole (18.38%) showed 15.4ppm and 57.2ppm LC_{50} respectively. The principal leaf oil components of *E. globules* harvested from Algeria are α-pinene and 1,8-cineole, according to Samir et al. (24). However, our *E. globules* EO tested against *Cs longiareolata* was less efficient (LC_{50}= 95.83ppm). Furthermore, EOs extracted from *Artemisia* genus were assessed for their larvicidal activity against various mosquito species. Our *A. herba-alba* EO tested against *Cs longiareolata* larvae was more efficient (LC_{50}= 86.67ppm) than *A. vulgaris* L. that was tested by Ilahi and Ullah (25) against *Cx quinquefasciatus* (LC_{50}= 803.2ppm), but less efficient than *A. absinthium* L. tested by Govindarajan and Benelli (26) against *An stephensi (*Liston 1901), *An subpictus*, *Ae aegypti*, *Ae albopictus*, *Cx quinquefasciatus* (Say 1823), and *Cx tritaeniorhynchus* (LC_{50}=41.85, 52.02, 46.33, 57.57, 50.57, and 62.16 ppm respectively). Various mosquito species

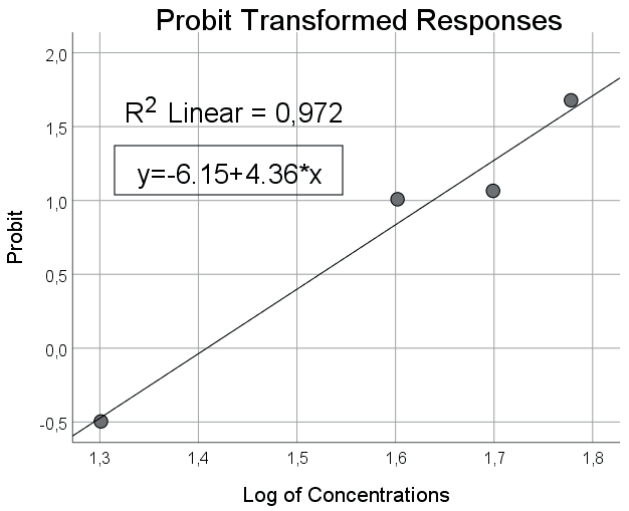

Figure 1. Probit transformed responses with equation regression and coefficient of determination R^2 for *Thymus vulgaris* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

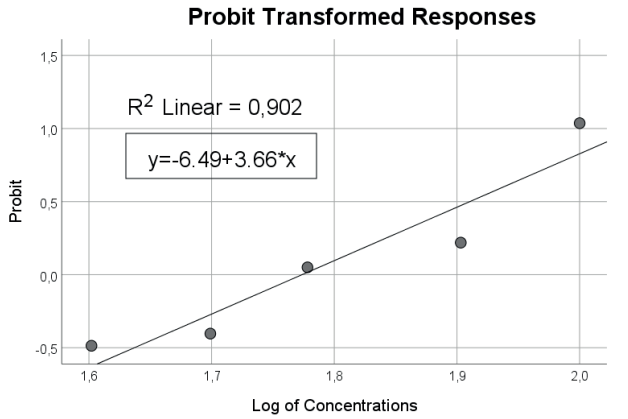

Figure 2. Probit transformed responses with equation regression and coefficient of determination R^2 for *Juniperus Phoenicia* tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

Table 3: Principal component percentages of *T. vulgaris, A. herba-alba, J. Phoenicea, R. officinalis and E. globules* harvested from Algeria, according to previous works.

Principalcomponents	***T. vulgaris (29)***	***J. phoenicea (30)***	***A. herba-alba (31)***	***R. officinalis (32)***	***E. globules (24)***
Carvacrol	11.41	-	-	-	-
Thymol	25.57	-	-	-	-
α-Pinene	12.1	34.5	Tr	5.4	8.8
α-Terpinylacetate	-	14.7	-	-	-
p-Cymene	26.36	-	-	-	-
Thymoquinone	10.5	-	-	-	-
β-Phellandrene	-	22.4	-	-	-
Camphor	-	-	19.4	14.6	-
1,8-Cineole	-	-	Tr	12.2	71.3
β –Caryophyllene	-	-	-	10.9	-
Borneol	-	-	-	10.6	-
γ-terpinene	-	-	23.8	-	-
β-thujone	-	-	15.0	-	-
chrysanthenone	-	-	15.8	-	-
trans-pinocarveol	-	-	16.9	-	-

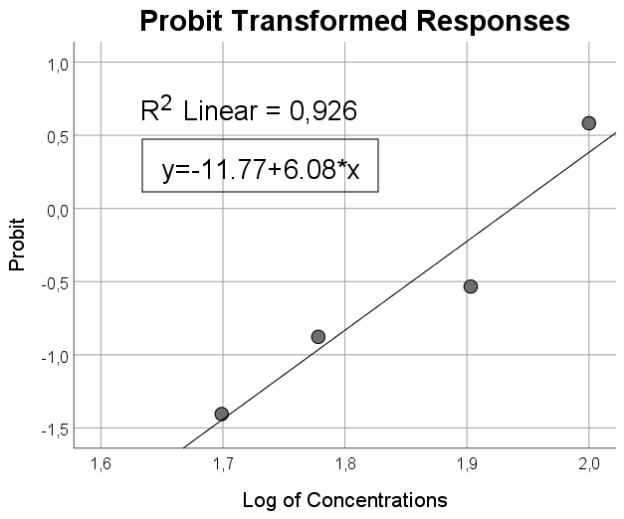

Figure 3. Probit transformed responses with equation regression and coefficient of determination R^2, for *Artemisia* herba-alba essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

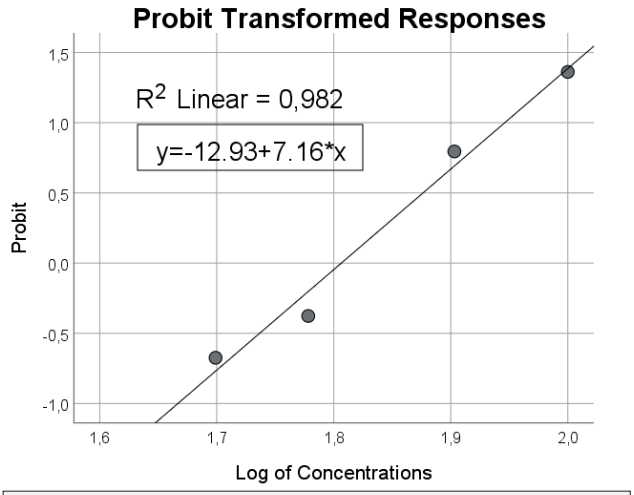

Figure 4. Probit transformed responses with equation regression and coefficient of determination R^2 for *Rosmarinus officinalis* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

were targeted in the previous researches to assess the larvicidal activity of EOs. However, *Cs longiareolata* was not previously targeted by EOs, but by the lichen metabolites evaluated by Cetin et al. (27), that showed high larvicidal activity against *Cs longiareolata*.

The results obtained confirm the previous studies; the use of EOs can serve as an eco-friendly method to control mosquito larvae. However, the noted variability in the efficacy level of the tested oils may be due to their chemical composition and the percentages of their principal components as α-Pinene, Camphor and 1,8-Cineole (Table 3); whereas, the direct use of the principal components of EOs may produce a higher efficacy in mosquito control. This hypothesis was proven in the study conducted by Lucia, Gonzalez-Audino (23), where the principal components of Turpentineand *E. grandis* EO showed lower LC_{50} than that obtained by the use of the entire *E. grandis* EO. Moreover, the repellency effect of the thyme EO compounds against *Culex pipiens* mosquito evaluated by *Park et al. (28)* showed higher repellent efficacy of α-Terpinene and Carvacrol than the commercial formulation diethyltoluamide (DEET), and an equal efficacy between the Thymol component and the DEET.

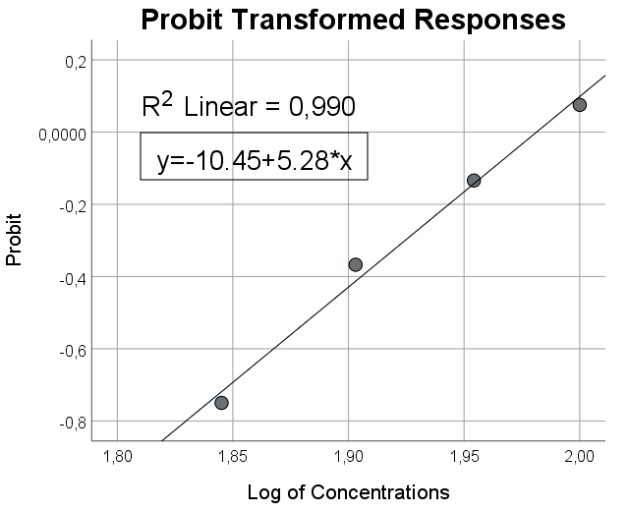

Figure 5. Probit transformed responses with equation regression and coefficient of determination R^2 for and *Eucalyptus globulus* essential oil tested on 3rd and 4th instars larvae of *Culiseta longiareolata* for 24 h.

CONCLUSION

The EOs extracted from the aromatic medicinal plants and their principal components may serve as safe products to control the *Culiseta longiareolata* larvae in Algeria; nevertheless, their practical application remains a fundamental step to evaluate their field efficacy and to note their possible secondary effects on non-targeted organisms.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of study: I.N., M.B.; Data Acquisition: I.N.; Data Analysis/Interpretation: I.N.; Drafting Manuscript: I.N.; Critical Revision of Manuscript: I.N., M.B.; Final Approval and Accountability: I.N., M.B.; Supervision: M.B.

Conflict of Interest: The authors declare that they have no conflicts of interest.

Financial Disclosure: There are no funders to report for this submission.

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Mosquito biodiversity in Setif region (Algerian High Plains), density and species distribution across climate zones

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Algeria has experienced outbreaks related to mosquitoes; additionally, it is exposed at the present to the installation of the invasive species *Aedes albopictus* (Theobald 1907). In this context, we performed a mosquito inventory in the Algerian high plains (Setif region) from 2016 to 2018, in order to provide the list of mosquitoes in the study area and analyze their diversity, density and species distribution across two climate zones (Mediterranean Csa and steppe BSk Zones) using biostatistical tests. The identification of species was done using a combination of morphological and molecular approaches (COI barcoding). The sampling yielded the identification of nine mosquito species including the malaria vectors *Anopheles labranchiae* (Falleroni 1926) and *Anopheles cinereus hispaniola* (Theobald 1901). A new species *Culex simpsoni* (Theobald 1905) is also recorded. The COI sequences of six species are provided in Genbank (MK047302-MK047315). From the total sampled mosquitoes, *Culex pipiens s.l* (Linnaeus 1758) showed the highest density in BSk zone (34.7±8.9), while *Culiseta logiareolata* (Macquart 1838) showed the highest density (51.2±28.5) in Csa. Further, we have revealed a high and positive correlation between *Culex theileri* (Theobald 1903) and *An labranchiae* (r_s=0.89, $p<0.001$). Moreover, the pairwise comparison and Ordination Corresponding Analyses ascertained the presence of a significant association between species distribution/density and climate zones in the study area (K-W U=51, $p<0.01$), and confirm the effect of the climate changes on the mosquito population. The results provided will hopefully accentuate our knowledge about mosquito population dynamics and facilitate the installation of an effective control program.
Keywords: Mosquito; COI barcode; biodiversity; ecology Algeria.

L'Algérie a connu des épidémies liées aux moustiques. De plus, ce pays est actuellement exposé à l'installation de l'espèce invasive *Aedes albopictus* (Theobald 1907). Dans ce contexte, nous avons réalisé un inventaire des moustiques dans la région de Sétif de 2016 à 2018, afin de fournir une liste de moustiques dans la zone d'étude et d'analyser leur diversité, densité et répartition dans deux zones climatiques (méditerranéenne Csa et haute plaine BSK) en utilisant des tests biostatistiques. L'identification a été réalisée à l'aide d'une combinaison d'approches morphologiques et moléculaires (COI barcode). Nous avons identifié neuf espèces, dont les vecteurs du paludisme *Anopheles labranchiae* (Falleroni 1926) et *Anopheles cinereus hispaniola* (Theobald 1901). Nous signalons aussi la présence d'une nouvelle espèce *Culex simpsoni*

(Theobald 1905). Les séquences COI de six espèces publiés sur Genbank sont fournies (MK047302-MK047315). *Culex pipiens s.l* (Linnaeus 1758) présente la densité la plus élevée dans la zone BSK, tandis que *Culiseta logiareolata* (Macquart 1838) présente la densité la plus élevée dans Csa (51,2±63,7). En outre, nous avons révélé une corrélation élevée et positive entre *Culex theileri* (Theobald 1903) et *An labranchia* (rs=0,89, p>0,001). La comparaison par paires et les analyses d'ordination correspondantes ont permis d'établir la présence d'une association significative entre la répartition/densité des espèces et les zones climatiques (KWU=51, p>0,01), ce qui confirme l'effet des changements climatiques sur les populations de moustique. Nous espérons que les résultats fournis renforceront nos connaissances sur la dynamique des populations de moustique et faciliteront la mise en place d'un programme de contrôle efficace.
Mots clés : Moustique, COI barcode, biodiversité, écologie, Algérie.

1 INTRODUCTION

The members of the Culicidae or mosquito family are considered from the most important cosmopolitan Diptera insects in the world. Mosquitoes constitute effective and active members when they occupy an ecosystem since they enter into the food chain as prey or even as predators. However, many mosquito species can act as vectors of dangerous and deadly diseases and threaten the public health (Schaffner *et al.*, 2013; Guarner & Hale, 2019). Therefore, mosquito-borne-diseases pose a huge impact on human affairs which makes mosquito control a priority (Greisman *et al.*, 2019; Petersen *et al.*, 2019). Thus, effective mosquito control requires a good knowledge of the mosquito population in terms of species diversity and ecological characteristics (Manguin & Boëte, 2011; Li *et al.*, 2019).

Algeria has experienced, in the last decades, fluctuations of mosquito-borne-diseases (Boubidi *et al.*, 2010; Lafri *et al.*, 2017). *Anopheles sergentii* (Theobald 1907) and *Anopheles cinereus hispaniola* (Theobald 1901) were involved in malaria transmission (Sinka *et al.*, 2010; Snow *et al.*, 2012); in addition, *Aedes albopictus* (Skuse 1894), the vector of Zika virus, has invaded lately the North part of the country (Izri *et al.*, 2011; Benallal *et al.*, 2016). Mosquito surveys have been conducted lately in Algeria and a total of 27 mosquito species has been reported (Bouabida *et al.*, 2012; Boudemagh *et al.*, 2013; Lafri *et al.*, 2014). The conducted studies focused more on beta biodiversity, whereas intraspecific interactions, species density, and distribution patterns were poorly explored.

Furthermore, mosquito inventories conducted previously in Algeria were based on the morphological identification. The invasion of new species (*Aedes albopictus*) in addition to the existence of members of complexes *Culex pipiens* (Linnaeus 1758), *Anopheles labranchiae* (Falleroni 1926) and *An c hispaniola*, made the morphological identification insufficient because of the difficulty of separating close species (Harbach, 2007; Werblow *et al.*, 2016). The similar morphology of close species, complex species and hybrids led to a major problem particularly to the non-experienced researchers, not only in Algeria but in the entire world. Since the morphological identification is sometimes insufficient or even useless for the separation of mosquito species, the integrative taxonomy approach becomes a more suitable method consisting of the combination of morphological and molecular identification. The remarkable progress in molecular researches served the mosquito identification; the DNA-based identification was adopted as a more accurate identification method to support mosquito inventories using the sequence divergence at cytochrome c oxidase subunit 1 (COI) (Werblow *et al.*, 2016); likewise, COI barcoding is seen as a useful, precise, and time-effective approach in mosquito species separation (Laboudi *et al.*, 2011; Engdahl *et al.*, 2014; Chan *et al.*, 2014; Afizah *et al.*, 2019).

In this study, we investigated the Setif region which is one of the most populated provinces in Algeria in order to renew our knowledge about mosquito biodiversity. We collected larvae and adult mosquitoes during 2016-2018 and identified them: morphologically, using diagnostic keys; and molecularly by sequencing the COI genes of the harvested specimens, using the PCR-PFLP approach. Furthermore, we used the collected data to adopt a better description of mosquito biodiversity in the study area and to analyze the density and species distribution patterns across climate zones. The results will likely provide information crucial for

mosquito control in the study area and highlight the effect of global climate change on mosquito populations. The ecological data was analyzed using bio-statistical analyses.

2 MATERIALS AND METHODS

2.1 Study area

Setif region of high plains Northeastern Algeria (36°03'N 5°31'E) stretches over a surface of 6 504km^2, the human population density is approximately 230 inhabitants/km^2. The human population is distributed in the different landscape structures according to the nature of their life activities. The agriculture constitutes an important sector in the study area due to the availability of farmlands and water surfaces (dams and rivers) (Rouabhi *et al.*, 2012; Rouabhi *et al.*, 2016). Setif region is characterized by heterogeneity of climate according to Köppen climate classification (Köppen *et al.*, 2011). We can differentiate two sectors: a north part of Csa climate, and a south part of BSk climate (semi-arid; cold and dry). Therefore, the sampling sites in the study area were regrouped within two groups:

- The first group: includes 16 sites within the region characterized by a Csa climate;
- The second group: regroups 4 sites within the region characterized by a BSk climate.

The distribution of the sampling sites in the study area and the limitation of the climate zones are illustrated in **Figure 1**.

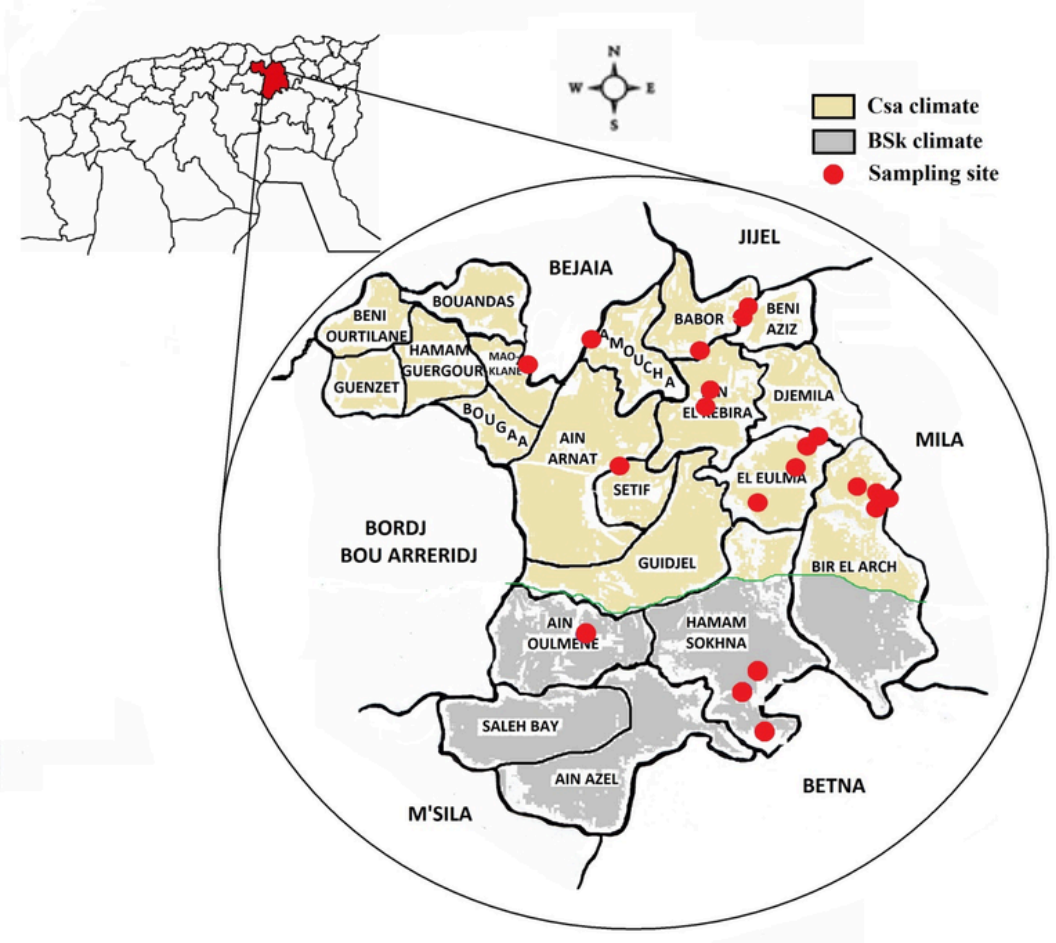

Figure 1: The geographical localization of Setif region (Algeria) and the distribution of the sampling sites (n=20) in two types of climate zones: Csa (Mediterranean climate) and BSk (steppe climate).

2.2 Sampling and morphological identification

The sampling was conducted during 2016-2018 and targeted larval and adult forms. The larvae sampling occurred using a standard dipper (1L) while adult sampling was done using simple CDC miniature light traps (Handmade: yellow light lamp and fan "12VDC").

The sampled specimens were first identified morphologically, 3rd and 4th instar larvae were identified alive (this operation preserve the setae that can be lost easily with the intense manipulation), or after being mounted for permanent preparations (Becker *et al.*, 2003). First and second instar larvae were reared in the breeding site water until they reached the fourth instar. The larvae identification was carried out using a microscope (Browser LCD MICRO 5MP microscope) with a camera built (5MP CNOS 1/2.5", 2560 x 1920 pixel array). Adults were analyzed using binocular microscope loupe. The morphological identification (larvae and adults) was done based on characters described by (Becker *et al.*, 2003) and the last version of interactive keys provided by the French National Research Institute for Sustainable Development (IRD) (Gunay *et al.*, 2018).

2.3 Molecular identification

DNA of the harvested and reared adults (n=24) was extracted from the legs (one of each specimen) using the DNeasy blood & tissue kit (Qiagen, Hilden, Germany) by following the handbook instructions. The PCR amplification of the COI barcode was performed in a total volume of 35 µl consisting of 10x reaction buffer; 2.5 mM MgCl 2; 200 µM of dNTPs; 28 pmol each primer LCO1490 and HCO2191 (Vrijenhoek, 1994); 2.5 U of TaqDNA polymerase. A volume of 3µl of genomic DNA was added to each PCR reaction and samples without DNA were included to exclude carryover contamination. The PCR procedure was as follows: initial denaturation stage and activation of the enzyme at 95°C for 2 minutes; 40 cycles at 94°C for 40 seconds, 50°C for 40 seconds and 72°C for 1 minute, followed by a final extension phase at 72 °C for 7 minutes. PCR products were examined on 1% agarose gel and the band's intensity was noted using a gel imaging system (ChemiDoc™ XRS+ System with Image Lab™ Software #1708265); both strands of the successful amplifications were sequenced at GATC Biotech (Konstanz, Germany). Sequencing results were analyzed using Geneious 10.2.3 software (https://www.geneious.com/) (Kearse *et al.*, 2012). The data of positive sequences were edited using BioEdit (Hall, 1999) and compared with sequences deposited in GenBank and Bold using BLASTn (Zhao & Chu, 2014). COI sequences were deposited in GenBank with the accession numbers from MK047302 to MK047315.

2.4 Data analysis

We analyzed the total mosquito population by calculating the abundance (the number of species specimens divided by the total number of samples), frequency in percentage (*f*) and mean density (Mean±Standard deviation). Only identified specimens were included.

The majority of the sampled larvae co-occurred; thus, we calculated the frequency of the species association and non-association, and the level of correlation between the co-occurred species using the *cor.test* (method=Spearman's" non-normality of data") and the *corrgram* package (Wright & Wright, 2018) in R studio Version 2.1.1335 (Team, 2018). The Spearman's correlation (r_s) was considered as weak if $0<r_s\leq0.4$, moderate if $0.4<r_s\leq0.7$ and as strong if $0.7<r_s<1$. Only species found more than one time was included in the analyses. Species found only one time were excluded for the correlation test.

Next, the difference in mosquito density between climate zones was analyzed using the non-parametric test Mann-Whitney U (non-normality and heteroscedasticity of data). Further, the mean density of the sampled mosquitoes was calculated by climate zone. The analysis performed using SPSS version 25.

Simultaneously, Alpha diversity within Csa and BSk climate zones was evaluated using species richness (S), Simpson index (1-D) (Simpson, 1949), Shannon index (H') (Shannon & Weaver, 1949) and Evenness (E") indices (Hill, 1973). Moreover, a canonical correspondence analysis (CCA) was performed to evaluate the effect of the variable 'climate zone' on mosquito distribution in the study area where sites and species constituted the axes 1 and 2. The diversity and multivariate analyses were conducted using PAST3 (Hammer *et al.*, 2001).

3 RESULTS

From 42 samples distributed in 20 sites, a total of 1144 specimens were harvested (921 larvae and 223 adults), of which 94.5% of specimens were identified. The sampling yielded nine mosquito species of which six were confirmed by molecular analysis. BLAST analysis of the COI gene of our samples displayed an identity of 99% and 100% on the nucleotide level with a null error value (**Table 1**). The rest three species were confirmed by morphological identification using diagnostic keys. *Culiseta longiareolata* (Macquart 1838) was easy to distinguish in its larval stage, the siphon was short and the saddle was incomplete (**Figure 2**). *Coquillettidia richiardii* (Ficalbi 1889) was identified in the larval stage; the saddle was without tuffs (**Figure 2**). Finally, a new record of *Culex simpsoni* (Theobald 1905) (n=2) was noted, the larvae had a long siphon (siphon index=9.6), the sub-apical spine S-2 was short, and the siphonal seta 1a-S was longer than the diameter of the siphon (**Figure 2**).
The majority of the sampled species showed a tendency to co-occur (**Figure 3**). However, the spearman's rho test revealed only one significant high and positive correlation between *Culex theileri* (Theobald 1903) and *An labranchia* (r_s=0.89, p<0.001). Along similar lines, the test rejects the presence of a real correlation in the other association cases (**Figure 4**).

As the study area is characterized by heterogeneity of climate, we have analyzed the distribution and the density of mosquito species by climate zones (Csa and BSk). The mean mosquito density has varied across climate zones (K-W U=108, p<0.01). The highest density was observed in the BSk (28.2±13.6) comparing to Csa (20.1±42.3). Further, the difference in the mean density of *Culex pipiens s.l* between Csa and BSk zones was statistically highly significant (K-W U=13, p<0.001); the density of *Cx pipiens s.l* was higher in BSK zone (34.7±8.9), it was followed by *Ochlerotatus caspius* (Pallas 1771) (23.3±15.9). Further, *Cs longiareolata* showed the higher mean density in Csa zone (51.2±28.5), it was followed by *Culex hortensis* (Ficalbi 1889) (18.7±19); while *Cx pipiens s.l* (9.7±15.7) and *An labranchiae* (7.8±10.2) showed the lowest mean density (**Figure 5**).

Likewise, we noted higher biodiversity indices in the Csa climate zone (1-D=0.7, H'=1.5) comparing to BSk (1-D=0.5, H'=0.9). However, the species frequencies in the BSk zone were more similar (E"=0.6). On the other hand, the species abundance was not the same across climate zones. In Csa sites, *Culiseta longiareolata* (38.1%) was the most abundant, followed by *Cx pipiens s.l* (33.3%), *Cx hortensis* (11%), *Cx theileri* (10.1%) and *An labranchiae* (6.9%); while, *Cx simpsoni* (0.1%), *Cq richiardii* (0.3%) and *An c hispaniola* (0.9%) was noted as sporadic. In BSk sites, *Cx pipiens s.l* (70.4%) was the most abundant, followed by *Oc caspius* (17.7%). Moreover, the CCA analysis displayed 'climate zone' as a variable that may explain the species distribution in the study area. The results indicated the existence of two separate species/climate zone clusters: *An labranchiae, An c hispaniola, Cs logiareolata, Cx simpsoni, Cq richiardii* /Csa and *Oc caspius*/BSk. Another cluster constituted by three species *Cx pipiens s.l, Cx hortensis* and *Cx theileri* was noted, and appeared less associated to a specific climate zone (**Figure. 6**)

Table 1: Comparaison of BLASTn results of the sampled species sequences with Genbank and BOLD accessions displayed an identity between 99% and 100% on the nucleotide level with a null error value.

Sampled species		Compared sequences			
Species	Accession	Species	Identy %	Accession	Country
Culex Pipiens s.l. (Linnaeus 1758)	MK047302 MK047304 MK047308 MK047309 MK047311 MK047314 MK047315	*Cx pipiens*	100	JQ958370	Iran
Culex theileri (Theobald 1903)	MK047307	*Cx theileri*	99.42	HE610459	Portugal
Culex hortensis (Ficalbi 1889)	MK047303 MK047305	*Cx hortensis*	99.10	MH807266	Austria
Anopheles labranchiae (Falleroni 1926)	MK047310	*An labranchiae*	99.54	HQ860410	UK
Anopheles cinereus hispaniola (Theobald 1901)	MK047312	*An cinereus*	99.93	Private sequences (BOLD system)	Morocco
Ochlerotatus caspius (Pallas 1771)	MK047306 MK047313	*Oc caspius*	99.85	KM258354	Belgium

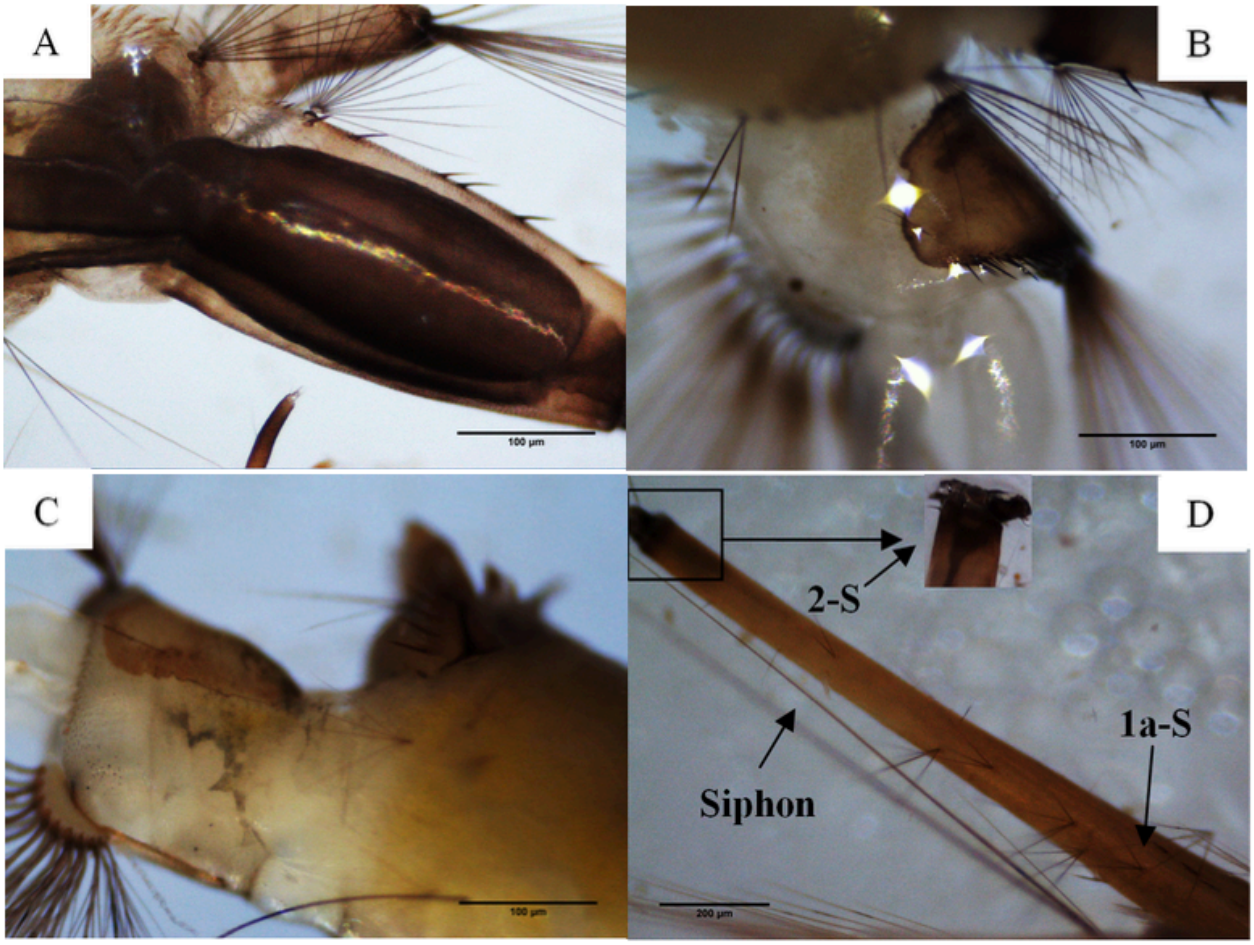

Figure 2: Morphological characters of three mosquito species: (A) siphon of *Culiseta longiareolata* (Macquart 1838) larvae. (B) Incomplete saddle in *Cs longiareolata* larvae. (C) Saddle without tuffs in *Coquillettidia richiardii* (Ficalbi 1889). (D) Long siphon in *Culex simpsoni* (Theobald 1905) with 1a-S longer than the siphon diameter and 2-S short.

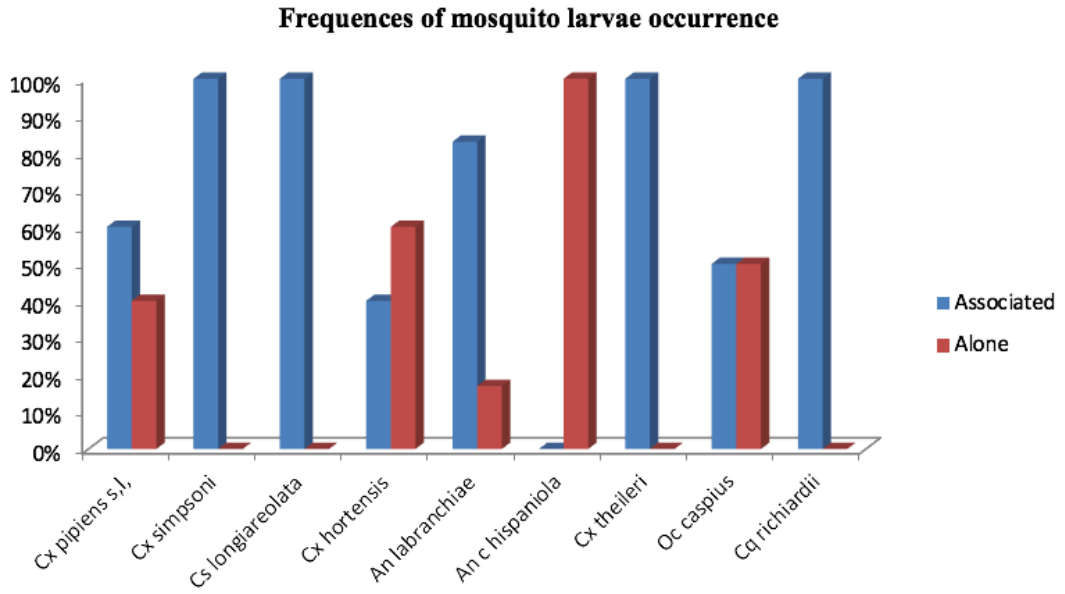

Figure 3: Frequencies of association and non-association in larvae occurrence for the sampled mosquito species (*Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889), *Culex theileri* (Theobald 1903), *Culex simpsoni* (Theobald 1905), *Culiseta longiareolata* (Macquart 1838), *Ochlerotatus caspius* (Pallas 1771), *Coquillettidia richiardii* (Ficalbi 1889), *Anopheles labranchiae* (Falleroni 1926), *Anopheles cinereus hispaniola* (Theobald 1901)).

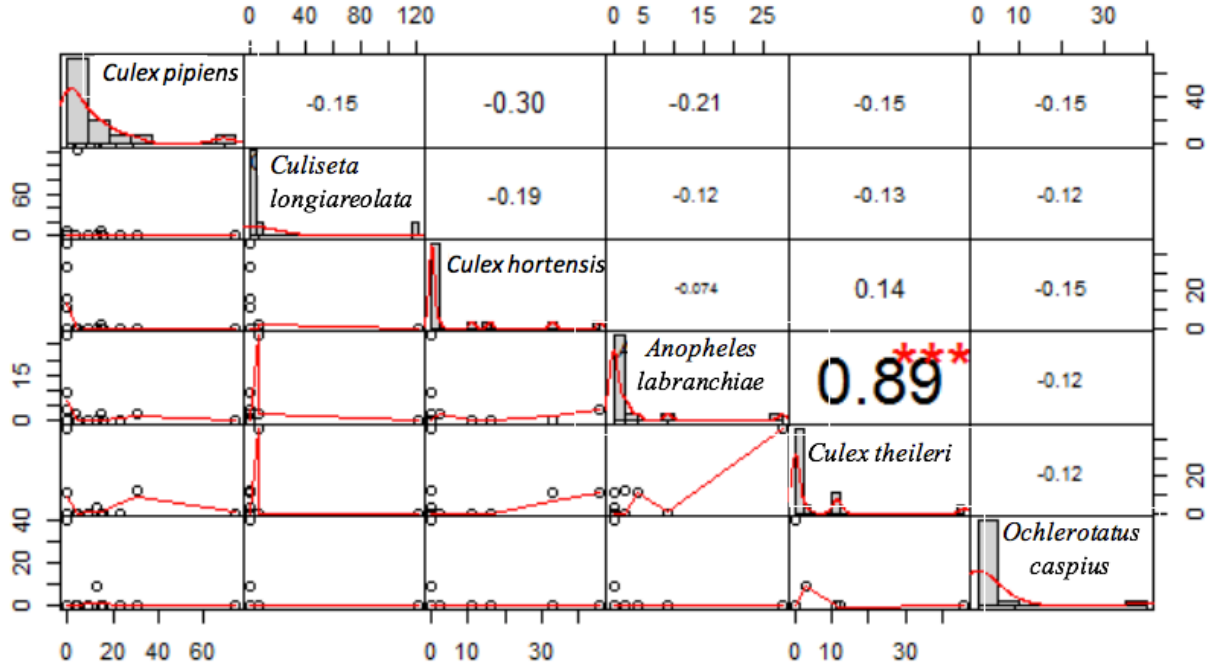

Figure 4: Correlogram of mosquito larvae co-occurrence within Setif region, Algeria (*Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889), *Culex theileri* (Theobald 1903), *Culiseta*

longiareolata (Macquart 1838), *Ochlerotatus caspius* (Pallas 1771), *Anopheles labranchiae* (Falleroni 1926)). Upper are the correlation coefficients (rho) with the significance level (***=p-value<0.001). Lower are scatter plots with tendency curves. Values with no significant level refer to the absence of correlation.

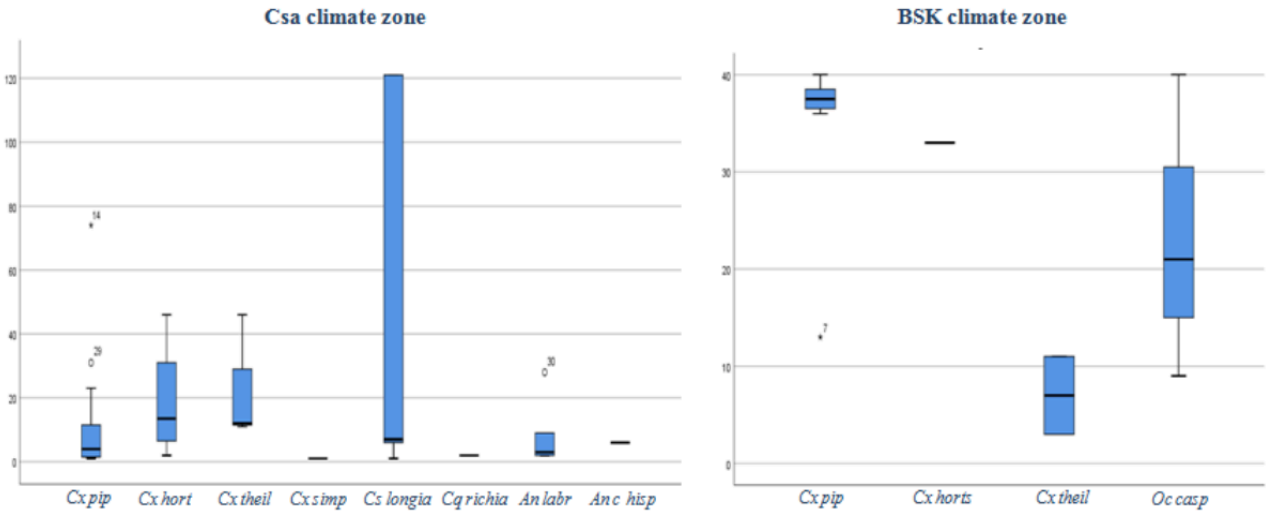

Figure 5: Box and whisker plot showing mean density of the mosquito species (*Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889), *Culex theileri* (Theobald 1903), *Culex simpsoni* (Theobald 1905), *Culiseta longiareolata* (Macquart 1838), *Ochlerotatus caspius* (Pallas 1771), *Coquillettidia richiardii* (Ficalbi 1889), *Anopheles labranchiae* (Falleroni 1926), *Anopheles cinereus hispaniola* (Theobald 1901)) sampled from Setif province, from 2016 to 2018, in two different climate zones (BSk and Csa).

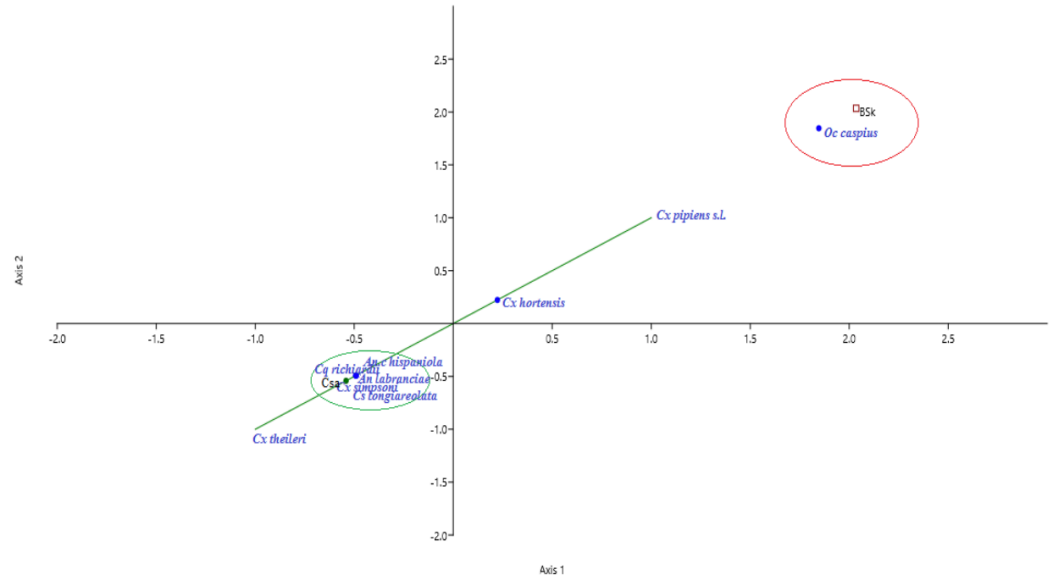

Figure 6: Canonical Correspondence Analysis (CCA) ordination biplot of species distribution in Setif region by climate zones (Csa, BSk). Csa and BSk climate zone with species constituted two distinct clusters. *Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889) and *Culex theileri* (Theobald 1903) are widely distributed.

4 DISCUSSION

The invasion of *Aedes albopictus* in the Algerian territory requires a renovation of the list of mosquito species; the current work was carried out in order to inspect a new region Northeastern Algeria. The inventory of Setif province has yielded the identification of nine mosquito species. As in the rest of the inspected Algerian regions (Lafri *et al.*, 2014), *Cx pipiens* s.l was the most abundant and the most frequent species in the study area; this species is a competent vector that can transmit West Nile Virus (Andreadis *et al.*, 2001; Hamer *et al.*, 2008), a human and animal neurophathogen worldwide disease that can range in severity from uncomplicated West Nile fever to a fatal meningoencephalitis (Campbell *et al.*, 2002; Kaleemullah & Sill, 2019). *Cx pipiens* s.l. is also a vector of Rift Valley fever virus (Moutailler *et al.*, 2008), an emerging disease that can cause important livestock industry losses, and moderate human morbidity and mortality (Pepin *et al.*, 2010; Hartman *et al.*, 2019). On the other hand, two important members of the *Anopheles* subfamily, *An labranchiae* and *An c hispaniola,* were identified molecularly to ensure the morphological identification results. For *An labranchiae*, the comparison of our sequences revealed a 99.54% of similarity with *An labranchiae* sequences from UK (it was the higher matching for the *maculipennis* complex species sequences provided in Genbank and BOLD). Likewise, an inventory conducted by Laboudi *et al.*, (2011) confirmed that *An labranchiae* is the only representative member of *An maculipennis s.l.* Meigen complex in North Africa. *An labranchiae* constituted with *An sergentii* and *Anopheles gambiae* (Giles 1902) the malaria vectors in Algeria (Boubidi *et al.*, 2010; Snow *et al.*, 2012; WHO-Algeria, 2014), the frequent presence of this species in the study area, even at a low density, poses the risk of outbreaks in the region. The other *Anopheles* species *An c hispaniola* was not recorded in Algeria since 1983 (Ramsdale, 1983). The comparison with sequences from the BOLD platform revealed a 99.93% of similarity with *An c hispaniola* sequences from Morocco. *An c hispaniola* is a member of the complex *An cinereus*; it is usually distributed in the Arab Maghreb and other Mediterranean regions (Samanidou-Voyadjoglou & Darsie Jr, 1993; Trari *et al.*, 2002; Bueno Marí & Jiménez Peydró, 2010; Tabbabi & Daaboub, 2017); while the other *An cinereus* member *An c cinereus* is distributed in Arabian Peninsula, and Eastern, South and Central Africa (Amr *et al.*, 1997; Alahmed, 2012; Animut *et al.*, 2012). *An c hispaniola* is as well considered as a potential malaria vector, it was found infected by *Plasmodium falciparum* (Trager and Jensen 1976) in Eritrea (Shililu *et al.*, 2003). However, *An c hispaniola* was found one time during the sampling, thus, it could be considered as a sporadic species. The sampling yielded likewise the identification of *Cx simpsoni*; as far as we know, this species is reported for the first time in Algeria. *Cx simpsoni* was identified in Morocco and it is usually distributed in south Africa and southwestern Asia (Army Public Health Center, 2019). The larvae of this species are close to those of *Cx antennatus*, *Cx sinaiticus* and *Cx theileri* (Gunay *et al.*, 2018); for this reason, we have adopted pictorial keys for its discrimination; Seta 5-C was 2 branched while it is more branched in *Cx theileri* (3-4 branches), seta 1a-S was 3 branched and longer than the siphon diameter while it is shorter than the diameter of the siphon in *Cx antennatus* and the pecten was on less than one third of the siphon, while the pecten is longer in *Cx sinaiticus* (Harbach, 1985). However, the morphological identification was not sufficient to ensure the species; especially that only two specimens were sampled. Further, the sampling results confirmed the absence of the invasion species *Ae albopictus* in the Algerian high plains and limit its presence in the far North of the country.

The presence of vector species in the study area makes a reason for the importance of the evaluation of the species interactions, habitat preferences and distribution patterns. The mosquito population stability is important to control the popular health situation; this stability is significantly associated with the relationships between conspecific individuals (Porretta *et al.*, 2016). The species sampled in the study area showed a tendency to co-occur with other mosquito species. However, the spearman's rho test confirmed only one real

correlation. *Cx theileri* and *An labranchiae* larvae were strongly correlated, the level of the correlation purpose the possibility of considering *Cx theileri* as a species indicator for *An labranchiae,* however, the measurement of species co-occurrence for choosing indicator species needs detailed studies (De Cáceres *et al.*, 2012; Neeson & Mandelik, 2014) and this subject is not well developed in the entomological field.
Better control of mosquito population stability is related as well to the knowledge of the density and the distribution patterns in the study area. The comparative statistical analyses of the mosquito densities between climate zones showed a significant difference between BSk and Csa. The mosquito mean density was higher in BSk sites, this climate zone features hot and dry summer and cold wet winter; the temperature in this zone tend to feature major swings between day and night. Simultaneously, the biodiversity indices and CCA analyses confirmed that the Csa sites are more diversified and the majority of the sampled species are related to the Csa climate zone. This zone is characterized by a more humid climate and longer wet season with hot and dry summer. The majority of the mosquito-born-diseases are related to mosquito density (Churcher *et al.*, 2015; Bradley *et al.*, 2018) and are sensitive to climate features (Reiter, 2001; Li *et al.*, 2019). Further, there is a direct and clear association between mosquito dynamics and climate variations (Beck-Johnson *et al.*, 2013; Wilke *et al.*, 2017). Therefore, the significant difference noted in the mosquito mean densities between climate zones, and the density and distribution patterns that were related to a particular zone, explain the importance of surveying mosquito populations according to a defined climate zone as the best strategy to control outbreaks.

5 CONCLUSION

Overall, the current study has provided a list of mosquito species occurred in the study area with COI sequences of six species provided in Genbank under the accession numbers from MK047302 to MK047315. Likewise, the study analyzed the data collected during the sampling and provided information about the density and distribution patterns of mosquito populations. Further, the existence of a high and positive correlation between two species poses the possibility of using mosquito species as species indicators. Moreover, a strong relationship between mosquito population and climate zones was confirmed, thus, the climate changes can affect the mosquito population density and distribution. Finally, the obtained results will hopefully constitute a database for the installation of an effective mosquito control program.

6 ACKNOWLEDGEMENT

The authors are grateful to Dr. Bruno Mathieu for the help in the realization of Molecular analyses and the Institute of Parasitology and Tropical Pathology, Strasbourg, France (IPPTS) for funding the PCR tests.

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