

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 ($51,2 \pm 28,5$). En outre, nous avons révélé une corrélation élevée et positive entre *Culex theileri* (Theobald 1903) et *An labranchia*

($r_s=0,89$, $p>0,001$). 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 504km², the human population density is approximately 230 inhabitants/km². 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**.

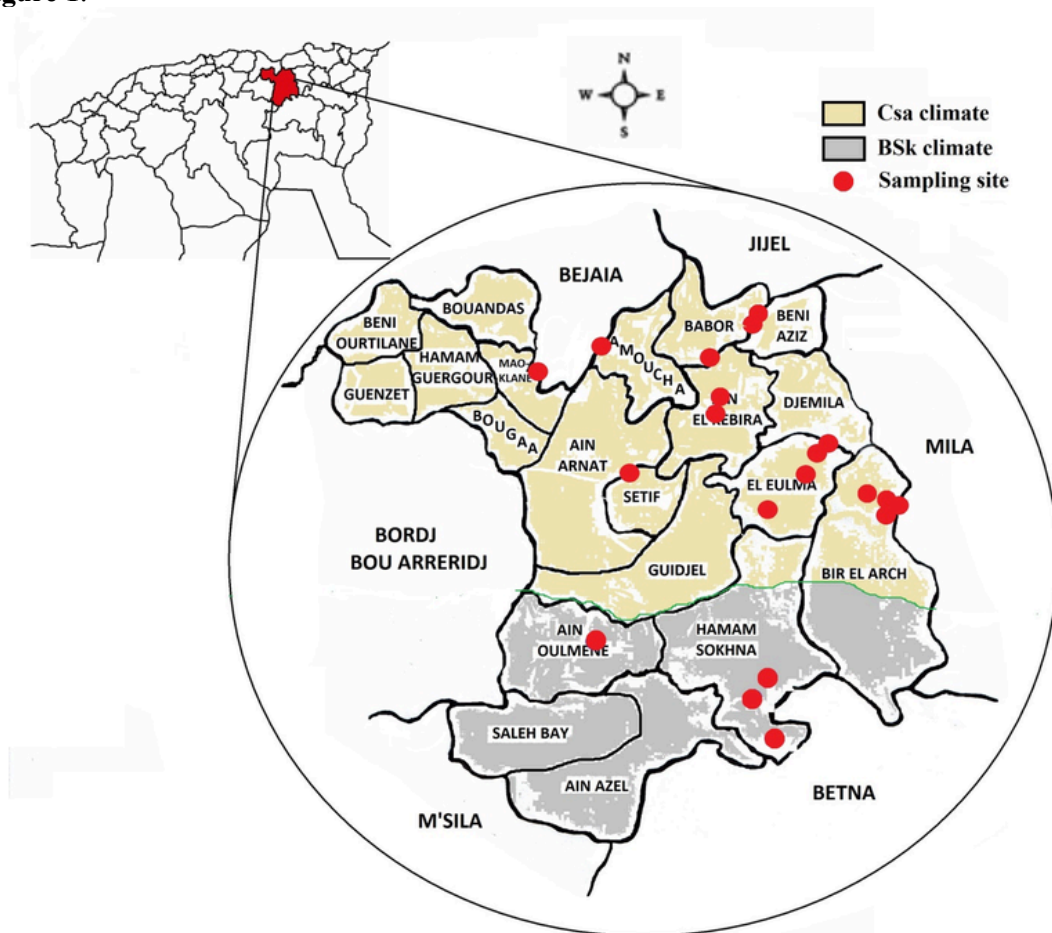


Figure 1: The geographical localization of Setif region (Algeria) and the distribution of the sampling sites (n=20) in two types of climate zones: Csa (Mediterranean climate) and BSk (steppe climate).

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 MgCl₂; 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 $0 < r_s \leq 0.4$, moderate if $0.4 < r_s \leq 0.7$ 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: Comparison 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	Identity %	Accession	Country
<i>Culex Pipiens s.l.</i> (Linnaeus 1758)	MK047302 MK047304 MK047308 MK047309 MK047311 MK047314 MK047315	<i>Cx pipiens</i>	100	JQ958370	Iran
<i>Culex theileri</i> (Theobald 1903)	MK047307	<i>Cx theileri</i>	99.42	HE610459	Portugal
<i>Culex hortensis</i> (Ficalbi 1889)	MK047303 MK047305	<i>Cx hortensis</i>	99.10	MH807266	Austria
<i>Anopheles labranchiae</i> (Falleroni 1926)	MK047310	<i>An labranchiae</i>	99.54	HQ860410	UK
<i>Anopheles cinereus hispaniola</i> (Theobald 1901)	MK047312	<i>An cinereus</i>	99.93	Private sequences (BOLD system)	Morocco
<i>Ochlerotatus caspius</i> (Pallas 1771)	MK047306 MK047313	<i>Oc caspius</i>	99.85	KM258354	Belgium

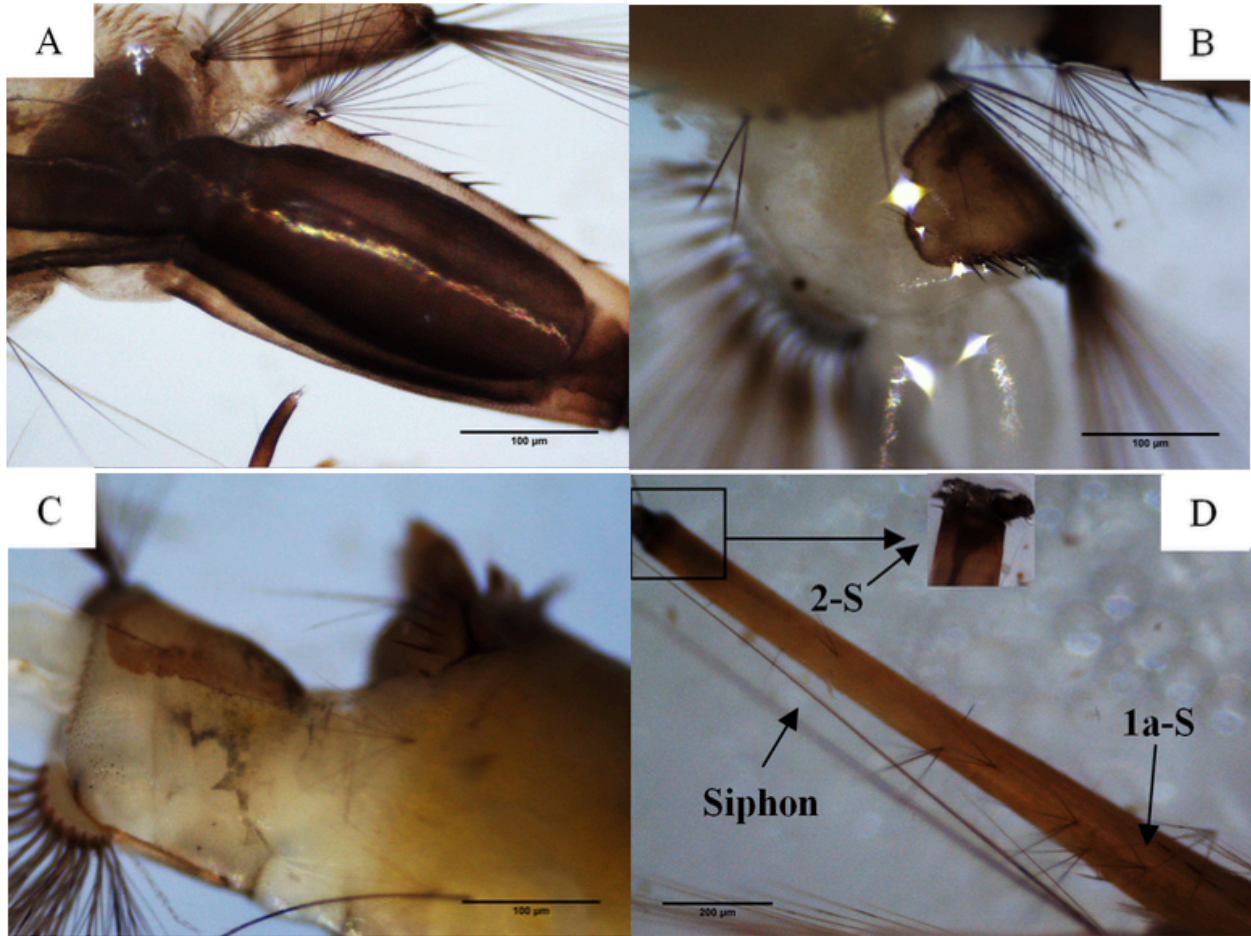


Figure 2: Morphological characters of three mosquito species: (A) siphon of *Culiseta longiareolata* (Macquart 1838) larvae. (B) Incomplete saddle in *Cs longiareolata* larvae. (C) Saddle without tuffs in *Coquillettidia richiardii* (Ficalbi 1889). (D) Long siphon in *Culex simpsoni* (Theobald 1905)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

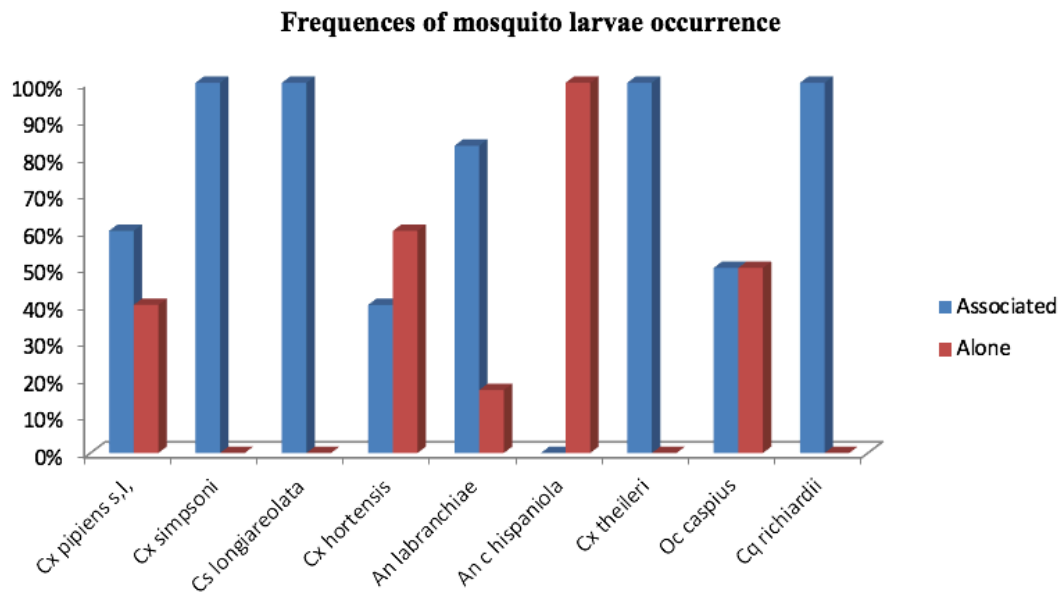


Figure 3: Frequencies of association and non-association in larvae occurrence for the sampled mosquito species (*Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889), *Culex theileri* (Theobald 1903), *Culex simpsoni* (Theobald 1905), *Culiseta longiareolata* (Macquart 1838), *Ochlerotatus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

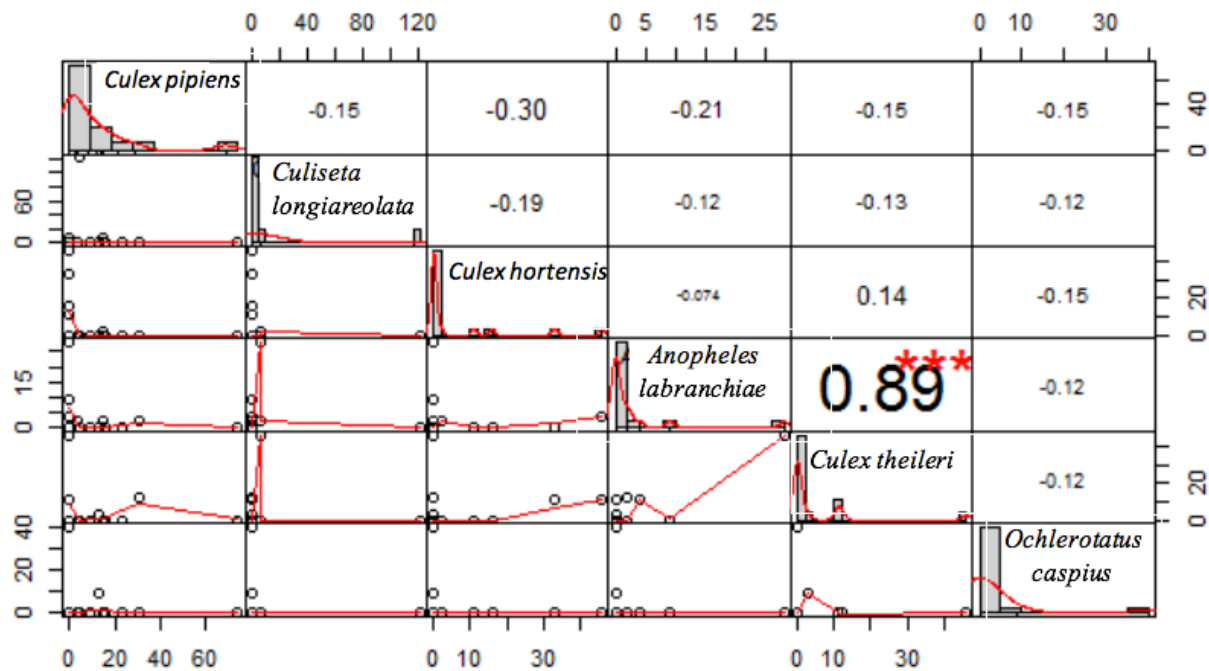


Figure 4: Correlogram of mosquito larvae co-occurrence within Setif region, Algeria (*Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889), *Culex theileri* (Theobald 1903), *Culiseta*

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.

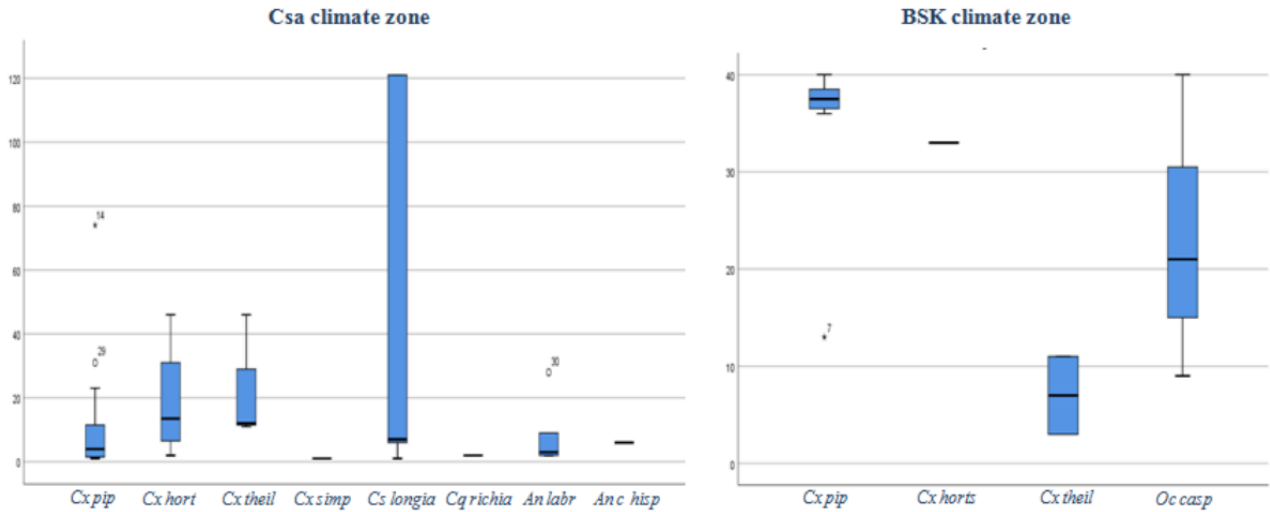


Figure 5: Box and whisker plot showing mean density of the mosquito species (*Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889), *Culex theileri* (Theobald 1903), *Culex simpsoni* (Theobald 1905), *Culiseta longiareolata* (Macquart 1838), *Ochlerotatus caspius* (Pallas 1771), *Coquillettidia 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).

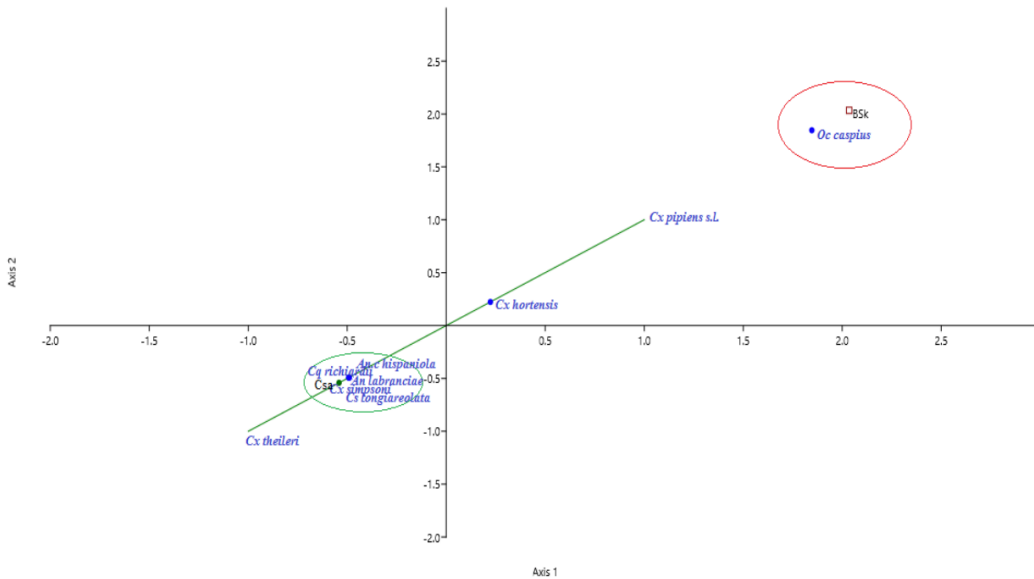


Figure 6: Canonical Correspondence Analysis (CCA) ordination biplot of species distribution in Setif region by climate zones (Csa, BSk). Csa and BSk climate zone with species constituted two distinct clusters. *Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889) and *Culex theileri* (Theobald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

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