# Effects of Certain Natural Breeding Site Characteristics on the Distribution of Culicidae (Diptera) Mosquito Speci....

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# Effects of certain natural breeding site characteristics on the distribution of Culicidae (Diptera) mosquito species in southeast Algeria

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This study aimed to characterise mosquito species distribution based on the nature and type of breeding habitats and the influence of the vegetation that occupies these sites. Knowledge of variation in breeding site types can help in the design of optimal vector control strategies. In different areas of Biskra (southeast Algeria), mosquito immature stages were collected using standard dippers. Twenty-four breeding sites were found in four areas of Biskra (Chetma, Bbordj Ben Azouz, Sidi Okba and Biskra). With a total of 44 677 individuals, the inventory of Culicidae revealed 22 species distributed through six genera from 2009 to 2013. Three species – *Aedes caspius*, *Culex pipiens* and *Culiseta longiareolata* – were the most predominant, occupying several different types of permanent and temporary breeding sites. In addition, analysis of the distribution of species based on the flora occupying these sites revealed that *Culex laticinctus*, *Aedes annulipes*, *Anopheles sergentii*, *Culiseta longiareolata*, *Culex pipiens* and *Aedes caspius* were species that had a strong ability to cohabit.

**Key words**: mosquitoes, breeding sites, Biskra, species diversity.

## INTRODUCTION

In recent years, the expanded global distribution of vector-borne diseases, such as yellow fever, malaria and recently zika virus, has generated a renewed interest in the biology and control of these vectors. One of the most important factors is vegetation, which allows remote sensing and geographic information system technologies to monitor and predict larval densities (Savage *et al*. 1990; Rejmankova *et al*. 1991; Rodriguez *et al*. 1993).

Several surveys have been done in Algeria to list the mosquito species of this territory. More than 60 species have been collected since 1930 (Senevet & Andarelli 1960; Bruhnes *et al*. 1999; Merabti & Ouakid 2011; Bebba & Berchi 2004; Rioux (1958); Bendali 2006; Soltani 2015). Medically important species include *An. sergentii, An. multicolor* and *An. hispaniola*.

An understanding of the environmental factors that affect the mosquitoes' larval abundance is a key factor for their control. Knowledge of the relationships among habitats, environmental factors and the occurrence of mosquito larvae are essential for an efficient application of mosquito control methods. In Algeria, particularly in the region of Biskra, an agricultural production centre, a new irrigation project aimed at agricultural development as well as the system of irrigation in the oases, which is based on water from dams, wells and drilling, offers an environment conducive to the emergence of different mosquito species.

The aim of this project was to carry out field surveys of possible mosquito breeding sites in order to describe their characteristics and to classify the mosquito species collected in them based on the similarity of their habitats.

## MATERIAL AND METHODS

### *Study area*

The region of Biskra is situated in the southeast part of Algeria (34°51′N 5°44′E) and has a surface area of 21 671 km$^2$ (Fig. 1). The climatic characteristics show that it is situated in the dry bioclimatic zone and receives 138.13 mm of precipitation annually (O.N.M. 2016). Preliminary investigations were conducted at four stations – Chetma, Bordj Ben Azouz, Sidi Okba and Biskra. Six breeding sites were identified at each station. A fortnightly sample of mosquito larvae was taken from each of the 24 targeted breeding sites (Table 1). Using the standard dipping method

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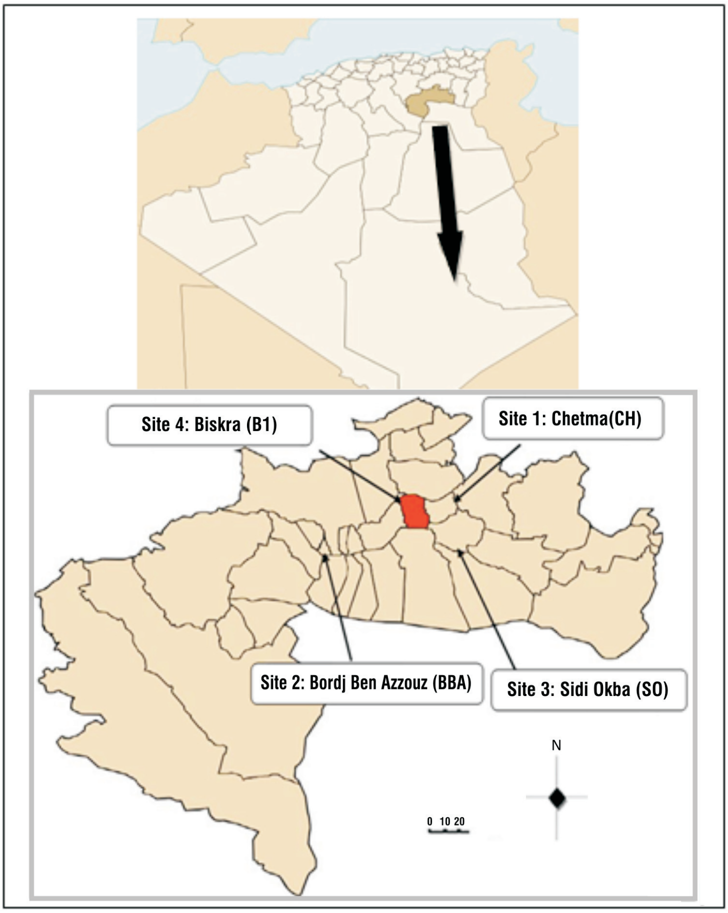


**Fig. 1**. Geographic location of the Biskra region, Algeria.

(Papierok *et al*. 1975), which consists of sampling larvae and pupae without repetition in several places at each breeding site using a 1-litre ladle. The breeding sites were classified using the following criteria: nature (temporary or permanent), type (waste-water canal, tyre, dam water, valley[1] grassy puddle, discharge, irrigation basin, drainage ditch, marsh, lagoon depression, traditional basin water, faucet water, well water). The vials into which the larvae were poured from the ladle were labelled by date, site and number of larvae collected. The collections were carried out from January 2009 to December 2013.

### *Mosquito identification*

The specimens were identified using the Mediterranean Africa software (Bruhnes *et al*. 1999). Identifications were based on the external morphological characteristics of fourth instar larvae and adults, *i.e*. the eggs, first to third instar larvae and pupae that were collected were reared to the fourth instar or adult stage for identification. Identifications were made using a microscope at ×10 magnification after following a special mounting protocol (Rioux 1958).

### *Vegetation sampling*

A botanical survey was conducted to identify the plant species in the habitats that were sampled, using identification keys from Chopra *et al*. (1960) and Chehma (2006).

### *Data analysis*

The harvest points of the immature stages (eggs, larvae and pupae) of the mosquitoes were grouped together. The input data were the list of

[1]Waters of valley come from crop irrigation and also from rain of a secondary valley that feeds into the sampled valley.

**Table 1**. Natural characteristics of the 24 mosquito breeding habitats from the four study stations, Biskra region, Algeria.

| BS[1] | Alt (m) | Lat (N) | Long (E) | BSN | NBS[2] | W[3] |
|---|---|---|---|---|---|---|
| Bs1.CH[4] | 112 | 34°51′49.00″N | 5°47′26.17″E | Valley | T | R/Ro |
| Bs2.CH | 109 | 34°51′29.91″N | 5°47′36.94″E | Valley | T | R/Ro |
| Bs3.CH | 109 | 34°51′17.89″N | 5°48′18.42″E | Irrigation basin | P | Wl |
| Bs4.CH | 102 | 34°50′16.11″N | 5°47′40.23″E | Valley | P | R/Ro |
| Bs5.CH | 100 | 34°50′03.71″N | 5°47′47.89″E | Valley | P | R/Ro |
| Bs6.CH | 99.5 | 34°49′51.10″N | 5°48′08.98″E | Irrigation basin | T | Wl |
| Bs1.BBA | 115 | 34°41′08.42″N | 5°22′31.49″E | Drainage ditch | P | R |
| Bs2.BBA | 115 | 34°41′11.33″N | 5°22′09.73″E | Drainage ditch | T | R |
| Bs3.BBA | 116 | 34°42′20.94″N | 5°20′59.56″E | Lagoon depression | T | R |
| Bs4.BBA | 119 | 34°49′51.10″N | 5°48′08.98″E | Traditional basin water | T | Wl |
| Bs5.BBA | 121 | 34°49′51.10″N | 5°48′08.98″E | Lagoon depression | P | R |
| Bs6.BBA | 121 | 34°49′51.10″N | 5°48′08.98″E | Irrigation basin | P | Wl |
| Bs1.SO | 55 | 34°44′58.17″N | 5°53′46.29″E | Traditional basin water | T | Dw |
| Bs2.SO | 55 | 34° 45′ 01.12″N | 5°53′ 43.61″E | Traditional basin water | T | Dw |
| Bs3.SO | 56 | 34°45′03.81″N | 5°53′38.65″E | Traditional basin water | T | Dw |
| Bs4.SO | 57 | 34°45′15.82″N | 5°53′41.09″E | Irrigation basin | P | Wl |
| Bs5.SO | 57 | 34°45′31.68″N | 5°53′52.62″E | Irrigation basin | P | Wl |
| Bs6.SO | 61 | 34°45′46.76″N | 5°53′56.96″E | Irrigation basin | T | Wl |
| Bs1.BI | 123 | 34°51′05.49″N | 5°40′18.34″E | Valley | T | R/Ro |
| Bs2.BI | 120.5 | 34°50′33.31″N | 5°44′31.17″E | Valley | T | R/Ro |
| Bs3.BI | 117 | 34°51′23.99″N | 5°44′14.06″E | Valley | T | R/Ro |
| Bs4.BI | 116 | 34°49′08.49″N | 5°44′55.21″E | Valley | P | R/Ro |
| Bs5.BI | 15 | 34°50′29.09″N | 5°45′00.75″E | Irrigation basin | P | Fw |
| Bs6.BI | 113 | 34°50′44.21″N | 5°44′52.66″E | Irrigation basin | T | Fw |

[1]BS: breeding site, Alt: altitude, Lat: latitude, Long: longitude, BSN: breeding site nature.

[2]NBS: nature of breeding site,T: temporary, P: permanent.

[3]W: water, R: rain, Ro: rolling, Wl: well, Ba: barrage, Dw: dam water, Fw: faucet water.

[4]CH: Chetma region, BBA: Bordj Benn Azouz region, SO: Sidi Okba region, BI: Biskra region.

species identified in a cross-matrix between 'species/breeding site nature' and 'species/breeding site type' for factorial analysis of correspondence and a cross-matrix for hierarchical descending classification (CHA) by species of mosquito and vegetation cover. Statistix version 8.0 software was used for this analysis.

## RESULTS

With a total of 44 677 individuals, the inventory of Culicidae revealed 22 species distributed through six genera from 2009 to 2013. Among these, 16 were identified at the larval stage from the 24 larval breeding habitats, and six species were found at the adult stage (*Aedes* sp. 1, *Aedes* sp. 2, *Anopheles* sp., *Culiseta* sp., *Uranotaenia unguiculata* and *Orthopodomyia pulcripalpis*). Based on the centesimal frequency rates of all species, *Culex pipiens*, *Culiseta longiareolata* and *Aedes caspius* were ranked as the three most common species with rates of 28 %, 25 % and 21 %, respectively. They were followed by *Culex modestus* (7 %), *Anopheles multicolour* (6 %), *Culex hortensis* (2 %), and *Anopheles sergentii* (1 %). For the other species, the rates were very low, ranging between 0 and 1 % (Table 2).

### Distribution of species according to the nature of breeding site

The factorial correspondence analysis aimed to describe the gatherings based on two objects. The nature of the breeding site (temporary and permanent) was the parameter that was used to study the distribution of the species. The contribution to total inertia for the construction of axis 1 was 100 %, and that for axis 2 was 0 %, for a total of 100 %. This percentage difference was greater

**Table 2**. List of species caught by absolute numbers (*N*) and relative proportions (% *N*), Biskra region, Algeria.

| Species | *N* | % *N* |
|---|---|---|
| *Aedes ochleratatus caspius* | 1029 | 12.97 |
| *Aedes ochleratatus annulipes* | 146 | 1.84 |
| *Aedes vexans* | 24 | 0.30 |
| *Aedes dorsalis* | 99 | 1.24 |
| *Aedes* sp. 1 | 132 | 1.66 |
| *Aedes* sp. 2 | 85 | 1.07 |
| *Anopheles multicolor* | 573 | 7.22 |
| *Anophles sergentii* | 226 | 2.84 |
| *Anopheles* sp. | 14 | 0.18 |
| *Culex hortensis hortensis* | 118 | 1.48 |
| *Culex pipiens* | 2275 | 28.65 |
| *Culex modestus* | 395 | 4.97 |
| *Culex theileri* | 71 | 0.89 |
| *Culex laticinctus* | 9 | 0.19 |
| *Culex torrentium* | 9 | 0.19 |
| *Culiseta longiareolata* | 2190 | 27.59 |
| *Culiseta annulata* | 61 | 0.77 |
| *Culiseta subochrea* | 122 | 1.54 |
| *Culiseta ochroptera* | 9 | 0.19 |
| *Culiseta* sp. | 9 | 0.19 |
| *Uranautenia unguiculata* | 259 | 3.26 |
| *Orthopodomya pulcirtarsis* | 61 | 1.07 |
| Total | 7913 | 100 |

than 50 %, so there was a significant difference between the two groups.

This analysis distinguished the species inventoried into two groups: the first was a common group representing 11 species (*Ae. caspius, Ae. dorsalis, Ae. vexans, An. sergentii, An. multicolor, Cx. hortensis, Cx. laticinctus, Cx. modestus, Cx. pipiens, Cs. annulata, Cs. longiareolata*) that were found at the two breeding sites of different natures (permanent and temporary). The second group comprised species listed only in the temporary breeding sites (*Cx. theileri, Cs. ochroptera, Ae. annulipes, Cx. torrentium, Cs. subochrea*) (Fig. 2).

## Distribution of species according to the breeding site type

From the graphical representation (Fig. 3) of the factorial correspondence analysis, the contribution to total inertia for the construction of axis 1 was 57.65 % and of axis 2 20.14 % for a total of 77.79 %. This rate is higher than 50 %; therefore, the results of the factorial correspondence analysis could be used only from axes 1 and 2.

The different species were distributed in the four quadrants based on the breeding site types occupied by these species (Q1 is represented by seven species, Q2 by four species, Q3 by two species and Q4 by three species). Simple observation suggested the presence of three groups. Group 1 represents five species (*Cx. modestus, Cs. ochroptera, Ae. vexans, Ae. dorsalis* and *An. multicolor*) that were harvested from dam water (Daw), valley (Val) and grassy marsh (Grp). The second group represents five species (*Cx. torrentium, Cx. theileri, Cx. hortensis, Cs. subochrea* and *Cs. annulata*) that were inventoried from canal wastewater (Wwc) and tyre water (Tyr) sites. The third group is presented by six species (*Cs. longiareolata, Ae. caspius, Cx. pipiens, An. sergentii, Cx. laticinctus* and *Ae. annulipes*) inventoried in lagoon depression (Lad), grassy marsh (Mar), drainage ditch (Drd), traditional basin water (Tbw), irrigation basin (Irb), marsh (Mar) and rejects (Rej) sites

## Distribution of species based on vegetation surrounding breeding site

The hierarchical ascending classification presented in Fig. 4 takes into account an inventory of vegetation that occupies the 24 sampling sites, combining them in a matrix with the 22 mosquito species listed. To distinguish the presence of three distinctive groups of these species, the tree branch of the similarity index from Phi Pearson's point of view is split to almost 60 %.

Analysis of the matrix (species) reveals three groups of species. The first group encompasses *Ae. caspius*, *Cx. pipiens* and *Cs. longiareolata,* which were harvested in almost all types of breeding sites with different localised plant species, with a strong or weak distribution among plant species (*Phragmites comunis, Phoenicia dactylifora, Ampelodesma mauritanica, Tamarix gallica* L., *Nerium oleander, Zizyphus lotus, Cynodon dactylon, Sueda fructicosa, Echinops spinosus, Anabasis articulate, Atriplex halimus, Algues verte* and *Zygophyllum album*). However, *Ae. caspius* was absent among three plant species *Pituranthos chloranthus, Arthrocnemum macrostachyum* and *Halocnemum strobilaceum*.

A second group was composed of 16 mosquito species: *Cs. annulata, Cx. laticinctus, Uranotaenia unguiculata, Cs. ochroptera, Cx. torrentium, Cx. theileri, Cx. modestus, Orthopodomyia pulcirtarsis, Cx. hortensis, Ae. annulipes, Anopheles* sp., *Ae. dorsalis, Ae. vexans,* and *Cs.* sp. These species have

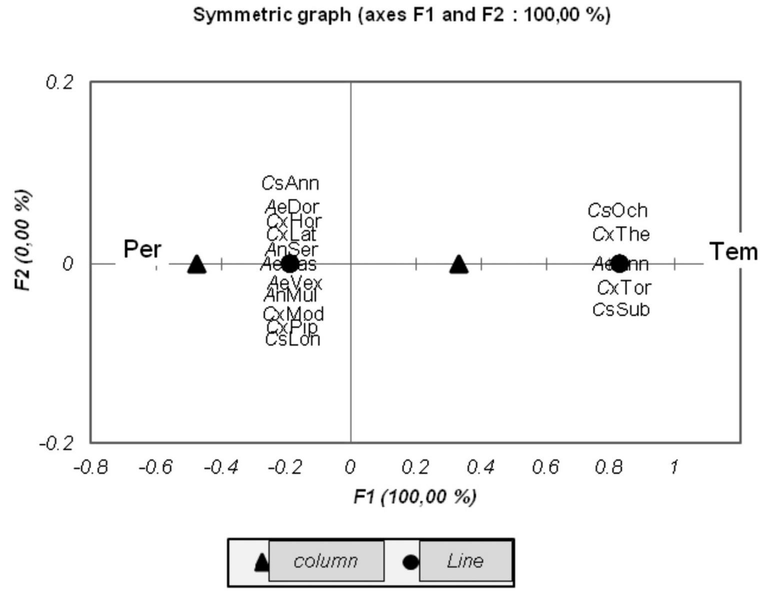


**Fig. 2**. Projection of mosquito species based on the nature of the breeding sites surveyed, Biskra, Algeria.

an average distribution based on the number of plants that occupy their environment, such as *Phragmites comunis, Phoenicia dactylifora* and *Nerium oleander*. The third group, which includes three mosquito species, *Cs. subochrea, An. multicolour* and *An. sergentii,* was found in the presence of three plants: *Anabasis articulate, Ampelodesma mauritanica* and *Phragmites comunis*.

## DISCUSSION

The results obtained from this work fall within a global framework concerning the ecological study of mosquito species in Algeria and constitute data toward a baseline survey of potential disease vector species in the region. The presence of at least 22 species belonging to the two subfamilies of

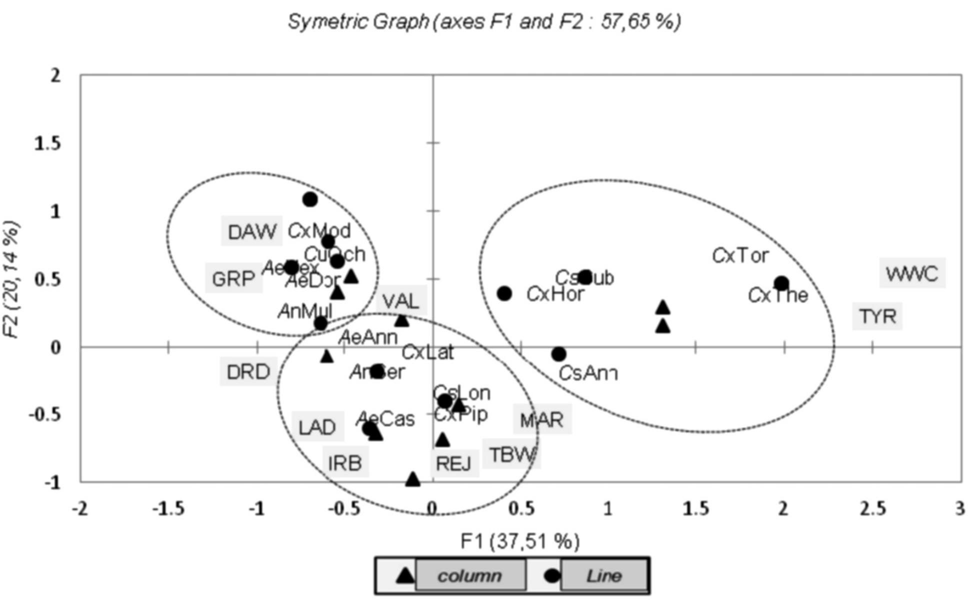


**Fig. 3**. Factor analysis of correspondences between mosquito species and type of breeding site, Biskra, Algeria.

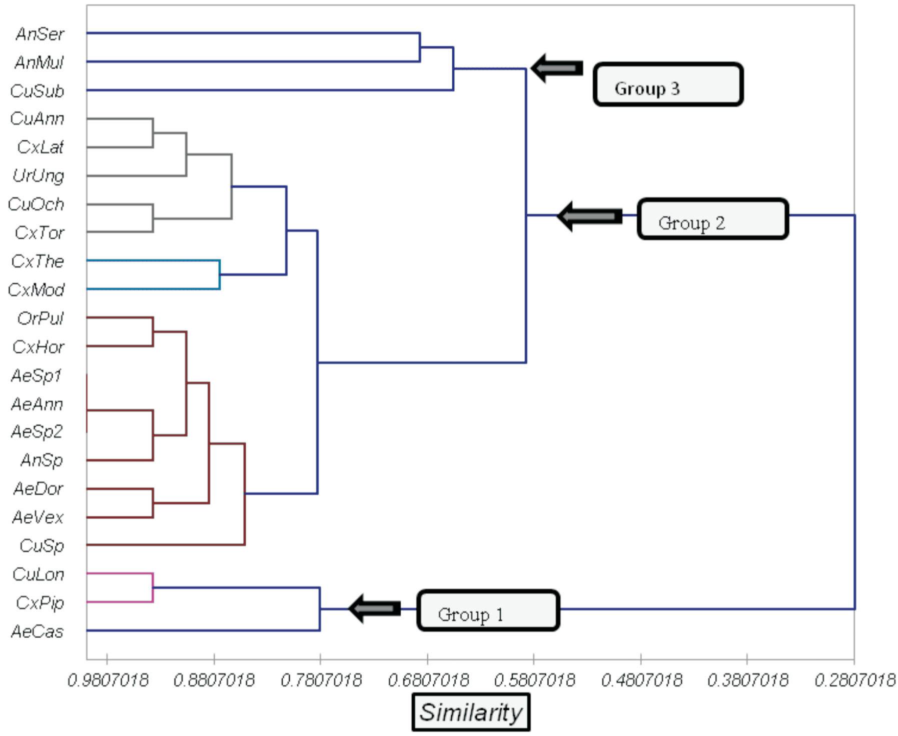


**Fig. 4**. Dendrogram of the Hierarchical Ascending Classification applied to the listed mosquito species (breeding sites / plants). *AeCas: Aedes ochleratatus caspius*, ***AeAnn**: Aedes ochleratatus annulipes*, ***AeVex**: Aedes vexans*, ***AeDor**: Aedes dorsalis*, ***AeSp1**: Aedes* sp. 1, ***AeSp2**: Aedes* sp. 2, ***AnMul**: Anopheles multicolour*, ***AnSer**: Anophles sergentii*, ***AnSp**: Anopheles* sp., ***CxHor**: Culex hortensis hortensis*, ***CxPip**: Culex pipiens*, ***CxMod**: Culex modestus*, ***CxThe**: Culex theileri*, ***CxLat**: Culex laticinctus*, ***CxTor**: Culex torrentium*, ***CuLon**: Culiseta longiareolata*, ***CuAnn**: Culiseta annulata*, ***CuSub**: Culiseta subochrea*, ***CuOch**: Culiseta ochroptera*, ***CuSp**: Culiseta* sp., ***UrUng**: Uranautenia unguiculata*, ***OrPul**: Orthopodomya pulcirtarsis*.

Culicidae: Culicinae and Anophelinae (Merabti & Ouakid 2011), precipitated the necessity to study their ecology and distribution stratified by breeding site characteristics.

The choice of sites for egg deposition in mosquitoes is a discriminating factor in the behaviour of female oviposition (Bently & Day 1989). The heterogeneity of species behaviour and environmental factors may influence the oviposition preferences of the females (Obsomer *et al*. 2007). Many shallow aquatic ecosystems such as wetlands (constructed and natural, permanent and temporary) have the potential to provide such habitats for a variety of mosquito species (Knight *et al*. 2003; Gingrich *et al*. 2006; Rey *et al*. 2006).

Several species belonging to four mosquito genera (*Aedes*, *Anopheles, Culex* and *Culiseta*) were collected from different natural habitats (temporary and permanent). These included three *Aedes* species (*Ae. caspius, Ae. dorsalis*, and *Ae. vexans*) and two *Anopheles* species (*An. multicolour* and *An. sergentii*), corroborating previous surveys (Sinegre 1974; Bebba & Berchi 2004; Brunhes *et al.* 1999). Adityaa *et al.* (2008) studied the different temporary larval habitats and species composition of mosquitoes in Darjeeling, Himalayas, India. They recorded mosquitoes belonging to four genera, *Aedes*, *Armigeres*, *Culex* and *Toxorhynchites*, of which the *Aedes* and *Culex* are congruent with the current study. Hayes *et al.* (1985) noted that some species of *Culex* and *Aedes* occupy submerged biotopes, which are conducive to the construction of permanent larval breeding habitats (roadside ditches, waste lagoons, the margins of ponds, excess irrigation water, marshes and wetlands).

Tadesse *et al*. (2011) noted in their study in Ethiopia (2005/2006) that small water bodies associated with dams (such as small ponds, hoof prints of cattle, or irrigation channels) rather than the dams themselves are likely to host most of the mosquito larvae. This result is consistent with the data presented here. Bruhnes *et al.* (1999) and Rioux & Arnold (1955) noted that species of the genera *Culex*, *Anopheles* and, in particular, *Aedes* develop in ponds and swamps with brackish or saline waters that are generally shallow. These habitats are mostly near coastlines but also occur in areas where salty lands are exposed. Rageau *et al.* (1970) confirmed these results.

Bruhnes *et al*. (1999), Louah (1995) and El-djoubari *et al.* (2014) reported that the genus *Culiseta*, particularly *Cs. longiareolata* and *Cs. subochrea,* which are species of high ecological valence, are able to withstand the abiotic conditions of environments that can be soft and brackish (such as wells, marshes, reject wastewater, or valleys). Trari (1991) and Schachter (1950) reported halophilic characteristics of *Cs. longiareolata*. In Ghana and Kenya many studies have shown that some species of *Culex* and *Anopheles* can inhabit many breeding habitats (lagoon, dam water, valley and grassy marsh), unpolluted or polluted by the influence of anthropogenic effects (Chinery 1984; Klinkenberg *et al*. 2008; Keating *et al*. 2004)

Three genera, *Aedes*, *Culex* and *Anopheles*, were collected from wastewater (Mukhtar *et al*. 2014; Rohi *et al.* 2014). Similar findings are reported in Kumar & Vam (1992), who studied the breeding habitats of mosquitoes in nine categories including fountains, tanks, tyres, barrels and tins in Panaji, India.

The study of the distribution of the species of mosquitoes based on the floristic cortege that surrounds their breeding habitat showed that three species – *Ae. caspius, Cx. pipiens* and *Cs.longiareolata* – were distributed in different habitats. Bruhnes *et al.* (1999) showed that *Ae. vexans* was also collected from different habitats, with or without vegetation. *Anopheles multicolor* has been found in clean waters under the roots of plants in riverbeds (Senevet & Andarelli 1960). Kenea *et al*. (2011) showed that *Anopheles* species were most abundant in habitats with vegetation. The early findings of Theobald (1901) stated that mosquito larvae seem to prefer shallow water, especially when there is a good growth of green algae. This same observation was made by Hopkins (1936). Abd-El-Maguid (1987) recorded the presence of Culicinae mosquito larvae in drainage channels, cesspools and seepage pools with turbid and slightly turbid stagnant water, with shallow depths ranging between 5 and 20 cm, widths between 0.3 and 5 m, and emergent vegetation in semi-shaded sites.

The presence of vegetation is an important predictor of the presence and abundance of *Anopheles* and *Culex* larvae (Mwangangia *et al*. 2007). Tadesse *et al*. (2011) showed that the presence of vegetation can help larvae to hide from their predators. Thus, such factors should be considered when designing an integrated vector control programme. The strong concentration of organic and mineral matter in decomposition would also explain the presence of Culicinae in spring water dams or valleys (Bruce-Chwatt 1983). Vegetation has been shown to alter the aquatic chemistry of the larval environments in naturally occurring and artificial container habitats, such as tree holes and used tyres (Walker *et al*. 1991).

Therefore, vegetation may have a potential effect on larval abundance because trees and shrubs may offer resting habitats and sugar sources to larvae and adult mosquitoes as well as their avian blood meal hosts, thus encouraging the mosquitoes to oviposit in these specific sites. These factors were significant during the collection of several species that have ecological preferences for these habitats, according to Allison *et al*. (2013). The nonexistence of some species in some habitats may be explained by the presence of plants containing natural compounds that can deter females from laying eggs. It is evident that several factors, such as precipitation, the nature of the substrate, or the physico-chemical characteristics of the breeding habitat water, affect the distribution of mosquitoes during the year.

## CONCLUSION

This study is the first to describe the ecology of the Culicidae species of the Sahara of Algeria. Three species – *Aedes caspius*, *Culex pipiens* and *Culiseta longiareolata* – were the most predominant, occupying several different types of permanent and temporary breeding sites. In addition, analysis of the distribution of species based on the flora occupying these sites reveals that *Culex laticinctus*, *Aedes annulipes*, *Anopheles sergentii*, *Culiseta longiareolata*, *Culex pipiens* and *Aedes caspius* are species that have a strong ability to cohabit. Understanding the breeding habitats of mosquito species, especially those of medical significance, assists in the design of control strategies, particularly the potential usefulness of larval source management.

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