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Systematic and Biotypical study of the family Culicidae (Diptera-Nematocera) in the region of Tebessa (Algeria)

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Abstract
The study on the biodiversity of the culicidian fauna that in Tebessa (north-eastern Algeria) was conducted from July 2009 to April 2011 allowed us identifying 17 species of Culicidae belonging to two subfamilies Anophelinae and Culicinae with five genera (*Culex*, *Culiseta*, *Aedes*, *Uranotaenia* and *Anopheles*). The statistical study of the Culicidae population revealed that the species *Culiseta longiareolata* was the most abundant followed by the *Culex pipiens* species. The study of specific diversity indicated a poorly diversified and moderately balanced population. Through the distribution analysis of the Culicidian species according to the altitude by the PCA on one side and the study of the distribution of Culicidian species according to the type of the deposit on the other side. We found that most of the culicidian fauna tend to live in less than 800 meters high places and prefer colonizing the permanent rural biotopes rich in vegetation.

Keywords: Culicidae, inventory, altitude, type of deposit, Tebessa, Northeastern Algeria

1. Introduction
The conservation of biodiversity necessarily requires a perfect knowledge of the distribution of flora and fauna [33]. Insects constitute the most numerous fauna in the world; they constitute 9/10 of Arthropods [3]. Many species play an active role in the transmission of diseases that may be mild, severe or even fatal, and their knowledge and identification are essential for physicians, ecologists, zoologists and entomologists [29].
Medical and veterinary entomology consider that Diptera, particularly hematophages, are the main vectors of infectious diseases to humans and animals. Mosquitoes are considered as the primary concern in the environment, first because of their dual ecological role in aquatic and terrestrial ecosystems, and second due to their impact on public health. Several mosquito species, mainly belonging to the genera Anopheles, Aedes and Culex, are vectors of pathogens (arboviruses, protozoa) and indirect cause of the greatest morbidity and mortality for humans compared to other organisms [30, 34].
The distribution of mosquito adults is related to immature stage habitat preferences. The study of the distribution and the ecological requirements of the larvae are crucial for an effective management of the potential deposits. The relevance of an environment for a mosquito depends on the complex interactions of abiotic factors such as precipitation, temperature, humidity and type of soil [7], and biotic factors such as competition, predation and food availability [22, 13].
The bioecological study of Culicidae larvae in this study aims at presenting the inventory of Cuicidae species in the region of Tebessa and highlighting the relationship between culicidae fauna and their habitat.

2. Materials and Methods
2.1 Study area
Tebessa is located in the northeast of Algeria (35°20' N, 8°6' E, Altitude: 960 m). It is 13878 km2 large, limited to the north by the province Souk-Ahras, to the south by the province of El Oued, to the west by the province of Oum El Bouaghi and Khenchla, and to the east by the Algerian-Tunisian borders. It belongs to a semi-arid bioclimatic stage.

2.2 Study site
During this study, 9 ecologically different breeding sites located in nine stations have been selected. To facilitate work, prospecting deposits have been named by codes formed of letter T followed by a number. The characteristics of these deposits are mentioned in Table 1.

2.3 Sampling Technique
The mosquito larvae sampling periods were spread over a 9-month period from September 2014 to May 2015. Samples were taken monthly. For the mosquito larvae sampling, "dipping" method has been used [14, 26 32]. This method involves harvesting in several locations of the deposit and without repetition a 1-litre ladle of water (*c*). Using this method, we performed a series of 5 samples and then calculated the average number (*n*) of larvae per sample. This number is an estimate of the average larval density per liter. The Culicidae larvae were identified using Rioux [24], and confirmation was made using the Mediterranean Africa's mosquito identification software of Brunhes *et al.* [9].

2.4 Statistical analysis
To better understand the obtained results, ecological indices of composition (species richness, relative abundance and frequency of occurrence), ecological structural indices (Shannon index and equitability index) and principal component analysis PCA have been used.

Table 1: Principal characteristics of the prospect larval biotopes.

Station name	Code of deposit	Type of deposit	Nature of the Deposit	Density of vegetation	Altitude
Méridj	T1	Natural source	permanent	Scanty vegetation	500m
Ain chania	T2	Source	permanent	Very abundant vegetation	600m
Ain zerga	T3	Source	permanent	Without vegetation	800m
Youkous	T4	Detention	Temporary	Abundant vegetation	975m
Ain Ghorab	T5	OuedGhorab extension	Temporary	Scanty vegetation	1130m
Kouif	T6	Detention	Temporary	Veryabundant vegetation	1140m
Ouenza	T7	Sewer restraint	permanent	Without vegetation	534m
Boulhef-Dyre	T8	Sewer	permanent	Veryabundant vegetation	759m
Merdja	T9	Sewer	permanent	little vegetation	813m

3. Results
3.1 Inventory of Culicidian species in the region of Tebessa
During the prospection period in the region of Tebessa, 17 species belonging to two sub-families: the subfamily Anophelinae with four species belonging to the genera *Anopheles*, and 13 species of the Culicinae subfamily belonging to four genera (*Culex*, *Culiseta*, *Aedes* and *Uranotaenia*) have been inventoried.

Table 2: Inventory of the species of Culicidae at stations in the region of Tebessa.

Family	Subfamily	General	Species
Culicidae	Culicinae	*Culex*	*Culex* (*Culex*) *pipiens* Linné, 1758 *Culex* (*Culex*) *theileri* Theobald, 1903 *Culex* (*Culex*) *perexiguus* Theobald, 1903 *Culex* (*Neoculex*) *deserticola* kirkpatrick, 1924 *Culex* (*Culex*) *antennatus* Becker, 1903 *Culex* (*Barraudius*) *modestus* Ficalbi, 1890 *Culex* (*Culex*) *laticinctus* Edwards, 1913 *Culex* (*Neoculex*) *territans* Waker, 1856 *Culex* (*Neoculex*) *hortensis* Ficalbi, 1924
		Culiseta	*Culiseta* (*Allotheobaldia*) *longiareolata* Maquart, 1828 *Culiseta* (*Ciliseta*) *Subochrea* Eswards, 1921
		Aedes	*Aedes* (*Ochlerotatus*) *caspius* Pallas, 1771
		Uranotaenia	*Uranotaenia*(*Uranotaenia*)*unguiculata* Edwards, 1913
		Anopheles	*Anopheles* (*Anopheles*) *labranchiae* falleroni, 1926 *Anopheles* (*Mysomyia*) *Sergentii sergentii* Theobald, 1907 *Anopheles* (*Anopheles*) *Claviger* Meigen, 1804 *Anopheles* (*Mysomyia*) *multicolor* Caamboliu, 1902

3.2 Statistical study of the results
3.2.1 Study of the results by ecological index of composition
The relative abundance and occurrence frequency of species of Culicidae are shown in Table 3

Table 3: Relative Abundances and Occurrence Frequencies Applied to the Species of Culicidae Harvested from Selected biotopes in the Region of Tebessa.

Species	Ni	C %	N	F %	Category
Culiseta longiareolata	1498	74.9	43	23,5	Accid
Culiseta subochrea	69	3.45	8	4,371	Accid
Anopheles labranchiae	9	0.45	3	1,639	Accid
Anopheles sergentii sergentii	110	5.5	10	5,464	Accid
Anopheles claviger	43	2.15	5	2,732	Accid
Anopheles multicolor	57	2.85	9	4,918	Accid
Aedes caspius	1	0.05	1	0,546	Accid
Culex pipiens	1278	63.9	32	17,49	Accid
Culex theleri	105	5.25	28	15,3	Accid
Culex perexiguus	23	1.15	11	6,01	Accid
Culex deserticola	5	0.25	3	1,639	Accid
Culex antennatus	146	7.3	18	9,83	Accid
Culex modestus	4	0.2	2	1,092	Accid
Culex laticinctus	9	0.45	6	3,278	Accid
Culex territans	3	0.15	1	0,546	Accid
Culex hortensis	3	0.15	1	0,546	Accid
Uranotaenia unguiculata	7	0	2	1,092	Accid
TOT	3370				

Ni: number of species, C: relative abundance, F: frequency of occurrence.

The study of the relative abundances recorded in Table 3 shows that the abundant and most frequent species are represented by *Culiseta longiareolata* with 74.9% and 23.5% of relative abundance and frequency of occurrence respectively, followed by *Culex pipiens* (63.9% abundance and 17.49% frequency of occurrence). Other species have very low abundances and frequencies, ranging from 0.05% to 7.3% relative abundance of *Aedes caspius* and *Culex. antennatus* respectively and 0.546% to 15.3% frequency of occurrence for species *Culex territans*, *Culex hortensis* and *Culex theileri* respectively.

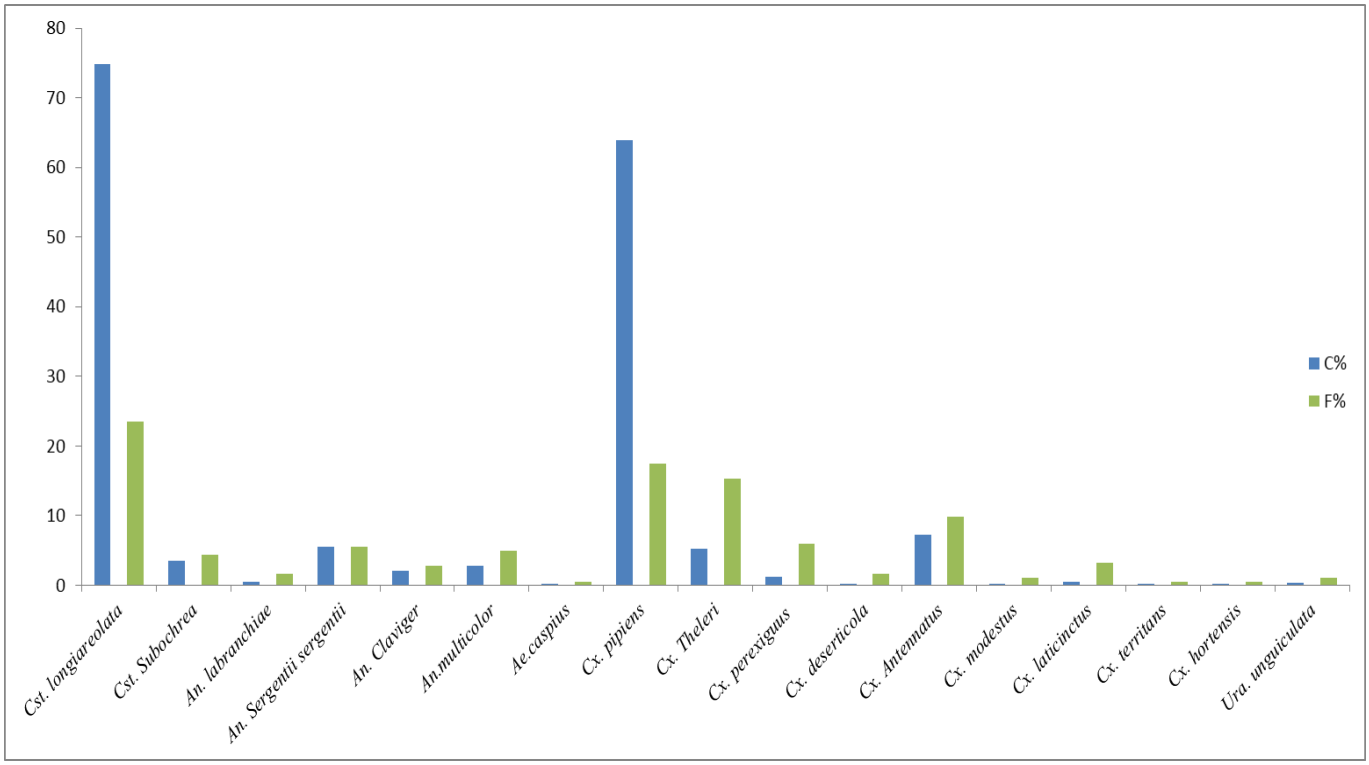

Fig 1: Relative Abundances and Occurrence Frequencies applied to the species of Culicidae in deposits at the Region of Tébessa.

3.2.2 Study of the results through the ecological index of structure.

The values of the Shannon-Weaver diversity index, maximum diversity and equidistribution for species of Culicidae harvested in the Region of Tebessa are shown in Table 4.

Table 4: Values of the Shannon-Weaver diversity index and equidistribution applied to species of culicidae in the region of Tebessa.

Species	Ni	Pi	log_2pi	pilog2pi
Culiseta longiareolata	1498	0,445	-1,170	-0,520
Culiseta subochrea	69	0,020	-5,610	-0,115
Anopheles labranchiae	9	0,003	-8,549	-0,023
Anopheles sergentii sergentii	110	0,033	-4,937	-0,161
Anopheles claviger	43	0,013	-6,292	-0,080
Anopheles multicolor	57	0,017	-5,886	-0,100
Aedes caspius	1	0,000	-11,719	-0,003
Culex pipiens	1278	0,379	-1,399	-0,530
Culex theleri	105	0,031	-5,004	-0,156
Culex perexiguus	23	0,007	-7,195	-0,049
Culex deserticola	5	0,001	-9,397	-0,014
Culex antennatus	146	0,043	-4,529	-0,196
Culex modestus	4	0,001	-9,719	-0,012
Culex laticinctus	9	0,003	-8,549	-0,023
Culex territans	3	0,001	-10,134	-0,009
Culex hortensis	3	0,001	-10,134	-0,009
Uranotaenia unguiculata	7	0,002	-8,911	-0,019
Tot	3370	1		-2,019
H’				2,02
H'max				4,0875
E				-0,4939

Ni: number of species, Pi: abundance of species, H': Shannon-Weaver index, H'max: maximum diversity, E: equitability

The specific diversity corresponding to the Schannon-Weaver index (H') is equal to 2.02 bits, while the maximum diversity (H'max) is 4.08, which indicates that this population is little diversified. On another hand, equitability is about 49.38% so; species of Culicidae found in the deposits at the region of Tebessa are moderately balanced.

3.3 Distribution of the culicidian Species based on altitude.

For the distribution study of culicidian species according to the altitude, six stations located at different altitudes have been chosen: T1, T2, T3, T4 and T6 whose altitudes are 500m, 600m, 800m, 975m and 1140m, the results are shown in Table 4 and Figure 2.

Table 5: Distribution of the culicidian species based on the altitude of the deposits in the region of Tebessa.

Altitude/Species	1140m	800m	500m	600m	975m
Culiseta longiareolata (Sp1)	5,75	9,5	7,95	4,25	5
Culiseta subochrea (Sp2)	0	0	3,15	0	0
Anopheles labranchiae (Sp3)	0	0	0	0,45	0
Anopheles sergentii sergentii (Sp4)	0	0	5,4	0	0
Anopheles claviger (Sp5)	0	0	0	2,15	0
Anopheles multicolor (Sp6)	0	0	2,85	0	0
Aedes caspius (Sp7)	0	0	0,05	0	0
Culex pipiens (Sp8)	0,4	0	10,6	9,55	0
Culex theleri (Sp9)	0	0	3,05	0,8	0,1
Culex perexiguus (Sp10)	0,2	0	0,15	0,7	0,05
Culex deserticola (Sp11)	0	0	0,05	0	0,2
Culex antennatus (Sp12)	0	0	0,05	7,2	0
Culex modestus (Sp13)	0	0	0	0,1	0
Culex laticinctus (Sp14)	0	0,05	0,15	0,05	0,15
Culex territans (Sp15)	0	0	0	0,15	0
Culex hortensis (Sp16)	0	0	0	0,15	0
Uranotaenia unguiculata (Sp17)	0	0	0	0,35	0

The analysis of the data given by the PCA (Fig. 2) shows 92.60% of the information collected on the first two axes (F1 X F2) and gives a better representation of the projections of the culicidian Species’s distribution according to the altitude of the stations: T1, T2, T3, T4 and T6 which shows a great variability taken by the first two axes F1 and F2 that summarize respectively 69.44% and 23.24% of the total information. The F3 axis has a low percentage of inertia (7.26%) and has not been retained for the interpretations.

The variables positively correlated with the F1 axis are the altitudes of the T3 stations (Ain zerga station), T4 (Youkous station), T6 (Kouif station) and the *Culiseta longiareolata* species. The negative correlation is expressed by Sp2 (*Culiseta subochrea*), Sp5 (*Anopheles claviger*), Sp6 (*Anopheles multicolor*) and Sp9 (*Culex theileri*). Thus according to this correlation, the axis F1 opposes the species found with zero densities in the T3, T4 and T6 stations of the species *Culiseta longiareolata* which was found with high densities in the same stations.

The F2 axis is positively correlated with the two deposits T1 (Meridj) T2 (Ain chania) and the species *Anopheles Sergentii sergentii*. (Sp4), *Culex pipiens* (Sp8) and *Culex antennatus*

(Sp12) and negatively correlated with other species *Anopheles labranchiae* (Sp3), *Aedes caspius* (Sp7), *Culex perexiguus* (Sp10), *Culex deserticola* (Sp11), *Culex modestus* (Sp13)), *Culex laticinctus* (Sp14), *Culex territans* (Sp15), *Culex hortensis* (Sp16) and *Uranotaenia unguiculata* (Sp17). Thus, the F2 axis differentiates the species that live in the T1 and T2 stations with a high larval density are *Anopheles Sergentii sergentii* (Sp4), *Culex pipiens* (Sp8) and *Culex antennatus* (Sp12) species found in the same stations but with low densities. (Fig. 2a and 2b). The factorial design (F1xF2) brings out additional information that separates two station categories and three groups of species:

1st category: contains the stations whose altitude is higher than 800 m which are those of T1 and T2

2nd category: contains stations that are at altitudes below 800 m: T3, T4 and T6.

1st group: species found at all altitudes represented by *Cst. Longiareolata.*

2nd group: species living only at altitudes less than 1000 m: *Culiseta subochrea, Anopheles labranchia, Anopheles Sergentii sergentii, Anopheles Claviger, Anopheles multicolor, Aedes caspius, Culex antennatus, Culex modestus, Culex territans, Culex hortensis* and *Uranotaenia unguiculata*

3rd group: species existing in the two categories of stations which are: *Culex pipiens, Culex Theileri, Culex perexiguus, Culex deserticola* and *Culex laticinctus.*

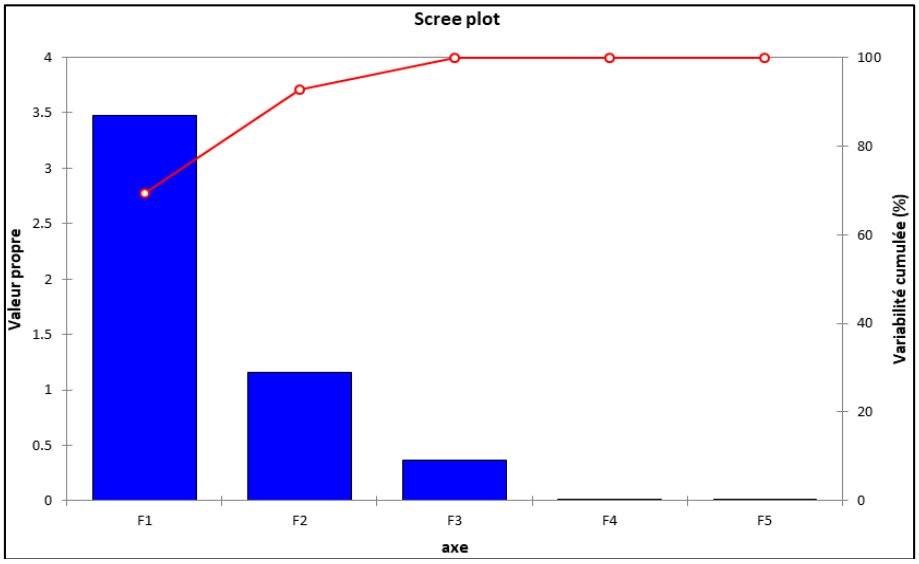

Fig 2a: Values of the densities of the culicidian species according to the altitudes of the deposits.

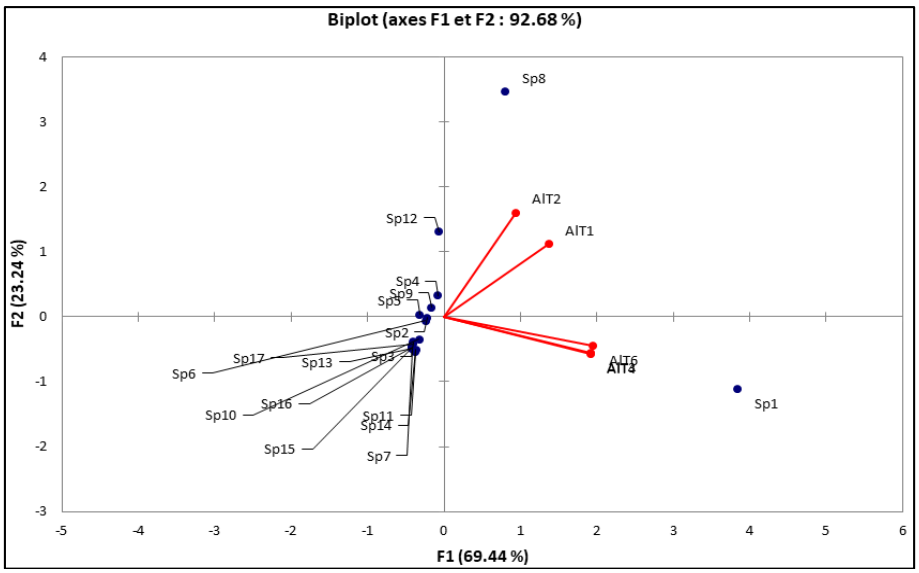

Fig 2b: Factorial map F1xF2 representing the distribution of culicidian species according to the altitude of the deposits in the region of Tebessa.

3.4 Organization of the Culicidian population according to the type of biotopes

The study of the culicidian population presents according to their ecological tolerances to varied biotopes. Indeed, different types of prospected deposits have been grouped into six categories: temporary deposits, permanent deposits, rural deposits, urban deposits, rich and no-vegetation deposits.

Table 6: Distribution of the culicidian species according to the type of deposit.

Species/Deposit	GAvgt	GSvgt	GPr	GTp	GRr	GUr
Culiseta longiareolata	4,25	9,5	55,3	19,65	42,1	32,9
Culiseta subochrea	0	0	3,45	0	3,15	0,3
Anppheles labranchiae	0,45	0	0,45	0	0,45	0
Anopheles sergentii serdentii	0	0	5,5	0	5,5	0
Anopheles claviger	2,15	0	2,15	0	2,15	0
Anopheles multicolor	0	0	2,85	0	2,85	0
Aedes caspius	0	0	0,05	0	0,05	0
Culex pipiens	9,55	0	63,4	0,5	24,7	39,3
Culex theileri	0,8	0	5	0,25	4,9	0,35
Culex perexiguus	0,7	0	0,9	0,25	1,1	0,05
Culex deserticola	0	0	0,05	0,2	0,25	0
Culex antennatus	7,2	0	7,3	0	7,25	0,05
Culex modestus	0,1	0	0,2	0	0,2	0
Culex laticinctus	0,05	0,05	0,25	0,2	0,45	0
Culex territans	0,15	0	0,15	0	0,15	0
Culex hortensias	0,15	0	0,15	0	0,15	0
Uranotaenia unguiculata	0,35	0	0,35	0	0,35	0

GAvgt: deposit with vegetation, GSvgt: deposit without vegetation, GPr: permanent deposit, GTp: temporary deposit, GUr: urban deposit, GRr: rural deposit.

According to the results given in Table 6 and Figure 3, we note that the permanent, rural, vegetation-rich deposits contain many more species than the temporary, urban and low-lying deposits, which contain only a few species of both genera *Culex* and *Culiseta* such as *Culex laticinctis* found in temporary and non-vegetated deposits and *Culex antennatus* and *Culiseta subochrea* species that have been reported in urban deposits, as well as species: *Culex pipiens*, *Culex theileri* and *Culex perexiguus* that were collected from temporary and urban deposits.

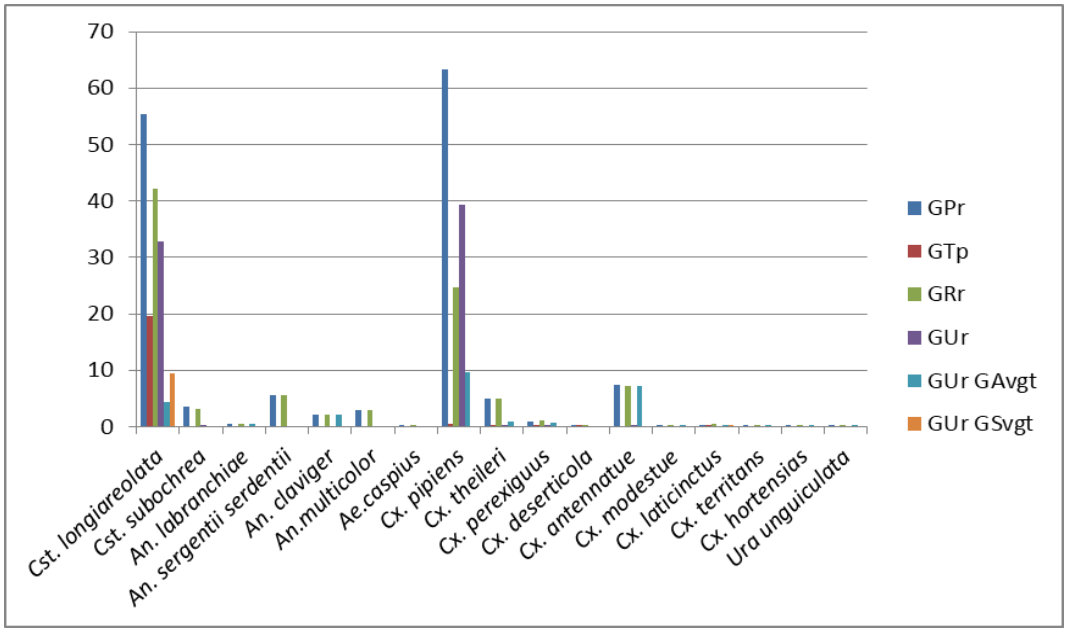

Fig 3: Distribution of the culicidian species according to the type of deposit.

4. Discussion

The study of the Culicidian inventory carried out in the regions of Tebessa during the period from July 2009 to April 2011 revealed the existence of 17 Culicidian species belonging to five genera and two sub-families represented in Algeria, namely: Anophelinae and Culicinae. It appears from the results that there was a predominance of the Culicinae subfamily. In the Smir marsh in Morocco [16] identified 14 Culicidian species (5 *Culiseta* species, 2 *Culex* species, 5 *Ochlerotatus* species and 2 *Anopheles* species) and in the region of Constantine in Algeria Berchi *et al*. [4] collected 6 species, belonging to the four genera, *Culex*, *Culiseta*, *Anopheles* and *Uranotaenia*.

In the subfamily Anophelinae, four species have been identified: *Anopheles labranchiae*, *Anopheles claviger*, *Anopheles muliticolor* and *Anopheles sergentii sergentii*, the species *An. Labranchiae* and *An. Clavigerr* are considered as major vectors of malaria [23], although the vector role of *An. claviger* is neglected in the Maghreb [8]. The subfamily Anophelinae is represented by a single species in the eastern region of Algiers, which is *An. Labranchiae* [21].

For the subfamily Culicinae, 13 species have been sampled

and distributed in four tribes: Culicini, Culisetini, Aedini and Uranotaeniini for the latter, one species was identified as: *Uranotaenia unguiculata*; this species was inventoried in Tebessa by Bouabida [5] while the Cilistini tribe is composed of two species (*Culiseta longiareolata* and *Culiseta subochrae*); *Culiseta longiareolata* was reported in the region of Oum El Bouaghi by Abbed [1]. The tribe of Aedini is composed of a single species (*Aedes caspius*).

In the northern region of Aures Andarelli [2] noted the absence of the *Aedes* genera, while he reported it in the southern region of Aures. The Culicini tribe is composed of nine species; *Culex pipiens*, *Culex theileri*, *Culex laticinctus*, *Culex antennatus*, *Culex hortensis*, *Culex perexiguus*, *Culex modestus*, *Culex territans* and *Culex deserticola*. After a statistical study of the results, all the inventoried species were found are accidental, whose frequency varies between 23.5% for the species *Culiseta longiareolata* and 0.54% for the species *Aedes caspius*, *Culex territans* and *Culex hortensis*, while the most abundant species is *Culiseta longiareolata* with 79.7% followed by *Culex pipiens* with 63.9% relative abundance. The value of the Shannon-Weaver (H') index is 2.02 bits, it is lower than the maximum diversity (Hmax) which equals 4.08bits, and the value of equitability at 49.93% which shows that the culicidian population is little diversified and moderately balanced.

The study of the Culicidin species distribution according to the altitude shows us that the stations at altitude below 800 m contain more species than the stations at altitude higher than 800 m. Our results agree with those of Hassain [19] who showed that the specific richness of Culicidae of Mediterranean Africa is a function of altitude; the author reported the existence of 48 species for altitudes between 0 and 100 m and 20 species between 100 and 1500 m. The most abundant species is *Cst. Longiareolata*, it has been found in the stations at altitude: 500m, 600m, 800m, 975m and 1140m, this species was found by Gilot *et al*. [18] with a great frequency in the plain, it does not seem to exceed 500 m altitude, but Ruffi [28] harvested it at 850 m altitude. While the species *Cst. subochrea* was encountered at 500 m. Our results indicate in an equivalent way that species of the genera *Anopheles* are found exclusively at altitudes below 800 m (500 m, 600 m), the same observation is noted for the results of Roman and Moler [27] who founded the *Anopheles* genera at 700 m and 800 m. However Rioux [24] reported the presence of the species *An. claviger* at more than 1500 m.

Species of the genera *Culex* were seen at different altitudes: 500 m, 600 m, 800 m, 975 m, and 1040 m. Various authors have mentioned the presence of the species *Culex pipiens* at high altitudes such as Doby [15] who found it at 2000 m. Callot [10] and Sicart [31] who also reported the presence of the species of *Cx. pipiens* at 1000 m. Hassain [19] reported that the species *Cx. pipiens* can reach 2500 m altitude, and it was also found by Doby [15] at 180 m, 400 m, 590 m, 938 m, 1300 m and 1500 m. According to Callot [11] the species *Cx. territans* was found in none of the studies conducted by French researchers at an altitude above 900 m, but Gilot *et al*. [18] found it at 214 m and 360 m, as this research indicates the presence of this species with low density at 600m.

In general, the nature of the deposit greatly influences the distribution of Culicidian species. The nature of the larval deposit favors the presence of one or the other species depending on whether the deposit is urban or rural, temporary or permanent, lacking or rich in vegetation [24. Regarding the distribution of Culicidian species according to the type of deposit, our results revealed that the majority of the species prefer much more the permanent rural deposits, rich in vegetation because, the vegetal cover plays a primordial role in the constitution of the larval deposits, because the screen that it forms decreases evaporation, which is favorable for the egg-laying and the conservation of the eggs of certain Culicidian species [20]. Except some species with special ecological preferences such as *An. Sergentii sergentii*, *An. Multicolor*, *Cst. Subochrea*, *Cx. deserticola* and *Ae. Caspius*. We also noted the existence of the species *Cst. Longiareolata* in all types of deposits, according to Hassain [19] this species presents a great aptitude to colonize very varied natural and artificial biotopes. Rioux [24] also reported the existence of the same species in permanent and temporary deposits, rich or poor in vegetation. Callot [12] found the species *Cst. longiareolata* in polluted deposits. The species *Cx. pipiens* is the most common; it shows remarkable accessibility in all larval deposits. Larvae of this species grow in rural and urban deposits and in waters rich in vegetal matter [25]. They are also found by Boulknafeth [6] in the permanent and temporary deposits rich in vegetation.

5. Conclusion

The study of the systematic and biotypology of Culicidian species in the region of Tebessa allowed concluding, on the one hand, the presence of two sub-families existing in Algeria: Anophelinae and Culicinae with the predominance of *Culiseta longiareolata* species, and on the other hand, the tendency of Culicidian species to colonize rural larval deposits, permanent water deposits, and deposits rich in vegetation. These deposits are always located at altitudes less than 800m.

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