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## Morphometry of *Culex pipiens pipiens* Linneus , 1758 (Dipterae;Culicidae) principal vector of West Nile Virus, harvested from 2 zones, humid, semiarid (East of Algeria).

F. Bendali-Saoudi\* , W. Oudainia\*\*, L. Benmalek\*, A.Tahar\*\*\* and N. Soltani\*

\*Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba23000-Annaba (Algeria)

\*\* Ecology Laboratory Terrestrial and aquatic systems. Badji Mokhtar University of Annaba23000-Annaba (Algeria)

\*\*\*Laboratory of Ecology and environment, Faculty of Sciences, Badji Mokhtar University of Annaba23000-Annaba (Algeria)

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### ABSTRACT

The morphometrical study concerned a species of medical and veterinarian interest, belonging to the Culicid family: *Culex pipens* Linnaeus 1758 collected from 2 types of ecosystems: Annaba, and Oum EL Bouaghi, situated respectively in The East and Northeast of Algeria. These 2 zones are known for their Mediterranean climate (Annaba) and semiarid climate (Oum El Bouaghi).The measurements have concerned 10 criteria for larvae of stage 4, and 32 for male and female adults. Themorphometrical data have undergone comparative analysis carried out by MINITAB 16 FR program. On one hand, the test of student disclosed the presence of significant differences, highly significant, on the other hand, the analysis of multivaried variance MANOVA, using 3 tests, Wilk 'S Lamba, Lawley \_Hotteling and Pillai's showed for all larval criteria highly significant differences, highly significant differences for females, and only significant for males.

**Key words:** Culicidae, *Culex pipens*, Morphometric, Algerian East.

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### INTRODUCTION

The mosquitoes are considered as the first group of vectors [1], during the last 20 years, Culicidain fauna of Algeria was the subject of big number of researches interesting particularly in systematic, morphometry, biology, biological and chemical control by growth inhibitors [2,3,4,5].The culicidae presents morphological characteristics generally distinct, leading to identify easily this family with its description. On the other hand, their grouping is sub families, genus, or in sub genus is much more difficult [6] .however the identification of twin species or sub species is so delicate to identify vectorial roles, control methods towards one of them. Many researchers have put in an obvious the diachotomical keys [7] and systematical identification as program [8]. Only many characteristics are to determine for the subspecies and twin species. The biometry is the only way to solve this problem. Some researches concerning length of wings for divers species of mosquitoes (genus *Aedes*, *Culex*) have been reported [9, 10, 11, 12, 13]. Van thiel, 1927 Confirms that the Anophels with short wings are considered as vectors of Malaria [14] has made measurements on male sexual organs and female ommatidies [15] uses biometry of wing to determine set of *Culex pipiens* [16], show that the length of wings is linked to the species, and may serve as discriminating character determining 3 sub species of *Anopheles maculipennis* : *atroparvus*, *maculipennis* and *melanoon*. This work consists in comparative morphometrical study of 10morphological criteria for 4<sup>th</sup> stage larvae, and 32 criteria concerning females and male adults of 20 individuals belonging to medical and veterinarian important species : *Culex pipiens*

*pipiens* Linnaeus, 1758, is cosmopolitan species found throughout the world and often associated with human habitation. *Culex pipiens* is a vector of filariasis in some region of the world. Moreover, *Culex pipiens* is a principal vector of West Nile fever which is emerged as arboviral disease. Since 10 years, about hundred epidemic cases have been reported in the Mediterranean basin [17], in Tunisia 173 cases have been recorded during 3 months, 8 of which are dead [18]. This species was collected from 2 ecosystems totally different, Annaba situated in the far North east, which characterized by damp Mediterranean climate, and Oum El Bouaghi located in semiarid region in the North east of Algeria.

## MATERIALS AND METHODS

### Presentation of the study region

**Annaba** : Annaba is situated at 600 Km from the capital Algiers, and at 100 Km from Tunisian border, for North east of Algeria. The area is about 1411, 98 km<sup>2</sup>. Annaba is limited from the North by Mediterranean Sea, from the south by Guelma, East by El Taref, west by skikda, the climate of this region is Mediterranean type with alternating of rainy and dry season, due to combined action of different climatic factors [19,20] (Fig.1).



Fig. 1: Presentation of the sampling sites (Annaba) [20].

**Oum El Bouaghi** : Oum El Bouaghi is located in the North East of highlands, with area of 6187.96 Km<sup>2</sup>, it is demarcated in the north by Constantine, in the South by Khenchela, in the East by Guelma and in the South by Ahras towns, in the West by Mila, Batna from the South-West, and Tebessa from the South-East. The up streams take up 63, 8% of the area, 19,80% by high plains. The mountains represent 17, 3% as for « Sidi Arghis » at 1635m altitude, knowing that the highest mountain is Djbal Gueryoun at altitude of 1729m. Oum El Bouaghi is known for its agriculture activity, the most agricultural lands are on the high lands of North-East region, with an existence of rocky zones. The wadis (rivers) of the town have often dried up for 6 to 10 months in the year; they are supplied essentially during rainy season, sometime they cause floods at the beginning and at the end of winter. The most important wadis are Boulefraï and Ouedkaouider (Oum El Bouaghi forest protection, 2011). The town of Oum El Bouaghi as urban site has been divided into 3 stations: the first situated in the north of the town, the second in the south, the third in the west side. The rural site corresponding to Ain Surabaya, belonging to Ain Zitoune district which constitutes a humid zone (Garat Taref) at 21 km in the south from the town capital, along the road linking Oum El Bouaghi to Khenchela. The rural site is divided into 5 stations, the three first stations are aquatic habitats and corresponding to the artificial shelter of rainwater temporary stagnations, the two other stations correspond to the stables [21,22].

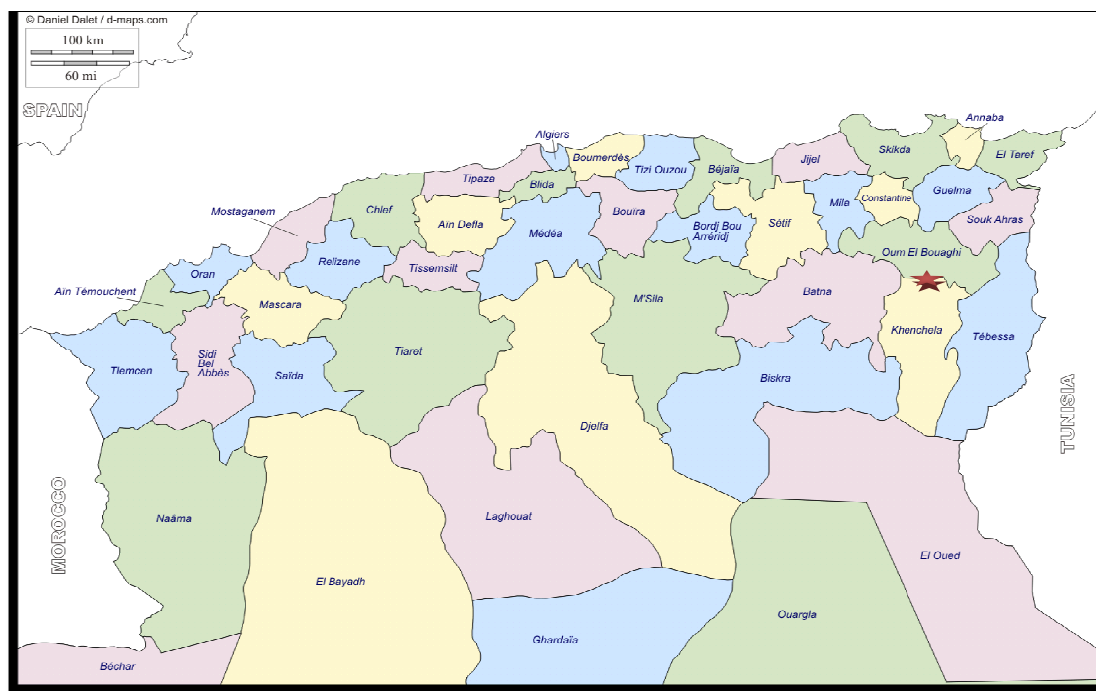


Fig. 2: Presentation of the sampling sites (Oum El Bouaghi) [22].

### Sampling method

The adults were sampled around buildings with specific glass tube, put delicately on the insect at rest. When the tube is moved, the insect will fly in the bottom, one of the tube ends is closed, and the other is opened leading mosquitoes to pass to the rearing cage [23]. The larvae are collected with ladle of 500 milliliters, which is put in the water, and moved with uniform movement to avoid the eddies. In the case of deep shelter (tanks, wells) we use a can or bucket, tied to rope during 10 months.

### Mounting and preservation of specimens

The adults of Culicidae are kept dry in Eppendorff tube, containing a chemical (Silica gel), cotton or absorber paper, after the adult mosquitoes are introduced inside. For the 3 first larval stages and nymphs their rearing is maintained in the shelter water until the emergence either for systematic identification or to start a new colonies. The larvae should stay 48 hours in 5% NaOH to become transparent and easy to be observed under optic microscope, then they are removed delicately with supple soft pliers in Petri dish, filled up of distilled water, finally the larvae are cleaned twice time to eliminate all product traces, the larvae will be ready to be examined. The larvae is put in its dorsal face, two drops of glycerin lead to the good larval conservation, the larvae will be put down delicately on the specimen [23, 4].

### Statistical analysis

The obtained results are represented by the mean, standard deviation followed with maximal and minimal values, we used test T of student [24, 25], we used MANOVA command of Minitab 16 program, with biometrical criteria of mosquitoes collected in 2 regions during 12 months.

## RESULTS AND DISCUSSION

### Morphometrics

Biometry is often used to well characterize species in their taxonomy and to give reliable systematical identification of species and subspecies. Many morphological parameters have been taken into consideration either for larval of 4<sup>th</sup> stage or for male and female adults of one important sub species listed in 2 study region. Table (1 and 2) represents biometrical values of 32 criteria considered as important in female and male taxonomy. The length and width of any criterion are represented by the mean value, standards deviation minimal and maximal values. These criteria were used either for detailed systematical identification of *Culex pipiens* or for sexual dimorphism. The results point out that the length of female palpe is lower than the lenth of gronotum. Contrary they are equals for the males; however the thorax is longer than width for the 2 sexes. On the other hand, the biometry shows for adults, the length of the 4<sup>th</sup>tasomer of the 1<sup>st</sup> scrawl is equal with the 5<sup>th</sup>tarsomer, length of the 1<sup>st</sup>tarsomer of the same scrawl is longer than tibia. Biometrical study of length and width larval head, thorax, abdomen, siphon, trachea are

presented in the table 3. Measurements point out, head and thorax are wider than length ,length of abdomen is lower than width, siphon of larvae is 2 to 3 times longer than with.

**Table 1: Measurements of 32 female morphometrical criteria of *Culex pipiens* collected from 2 study regions:**

N= 20; unity :  $\mu\text{m}$ . L : length; Q :width. h : head; Pa : Palpe ; Pr : Pronotum ;An: Antennae; Th : Thorax ; w:wing; Ab: Abdomen; F:Femur; T:Tibia; Tar: Tarsomer; S: scrawl; M :mean; S: Standard diviation; min V : minimal value; max V :maximal value.

Region Variable	Annaba				Oum El Bouaghi			
	M.	S.	V <sub>min.</sub>	V <sub>max.</sub>	M.	S.	V <sub>min.</sub>	V <sub>max.</sub>
S. L	1217,8	250,2	932,5	1865,0	1122,5	205,4	639,0	1420,0
S. Q	1161,9	259,9	746,0	1678,5	943,6	285,5	113,6	1405,8
Pa. L	712,4	128,6	559,5	932,5	542,4	131,8	255,6	752,6
Pa. Q	141,74	22,96	111,90	186,50	141,2	50,1	42,6	255,6
Pr. L	5134	721	4103	6901	5083	922	1775	6617
Pr. Q	158,5	46,7	74,6	261,1	178,9	49,3	113,6	284,0
An.L	4620	530	3730	5409	3565	966	1718	5481
An. Q	37,300	0,000	37,300	37,300	48,99	11,72	28,40	71,00
Th. L	3487,6	284,5	2797,5	3916,5	3678,5	437,8	2414,0	4189,0
Th. Q	3655,4	259,6	3170,5	4103,0	3346	853	341	4260
Al. L	10341	915	8766	11936	10217	1303	5609	11857
Al. Q	2736,0	294,0	2051,5	3170,5	2374	517	1193	2840
Ab. L	6798	546	5222	7460	6652	1117	4104	9372
Ab. Q	1337,2	134,9	1119,0	1678,5	1008,2	315,8	639,0	2115,8
F. P. 1 L	4653	471	3730	5595	4942	536	3195	5680
F. P. 1 Q	262,6	61,4	186,5	373,0	281,2	68,1	113,6	426,0
Ti. P. 1 L	4924	725	3730	6528	5314	678	3337	6177
Ti. P. 1 Q	169,71	22,56	111,90	186,50	200,2	55,3	113,6	340,8
Tar. P. 1 L	6043	1136	4103	8393	6594	999	5396	9145
Tar. P. 1 Q	136,15	21,90	111,90	186,50	151,42	21,58	115,96	208,26
F. P. 2 L	5119	455	4103	5968	5399	684	3337	7100
F. P. 2 Q	290,9	63,6	186,5	410,3	289,68	38,66	227,20	355,00
Ti. P. 2 L	5707	720	4663	7087	5538	1037	2031	6447
Ti. P. 2 Q	171,58	25,38	111,90	186,50	303,3	433,3	127,8	2134,0
Tar. P. 2 L	6611	1138	4103	9325	11029	4147	6063	17864
Tar. P. 2 Q	206,7	293,3	74,6	1449,2	139,26	20,24	93,72	164,72
F. P. 3 L	5061,6	296,5	4662,5	5445,8	5144	703	2840	6603
F. P. 3 Q	277,9	52,0	149,2	373,0	281,2	53,6	170,4	355,0
Ti. P. 3 L	5650,9	358,3	5035,5	6341,0	5586	662	3266	6390
Ti. P. 3 Q	158,53	23,82	111,90	186,50	220,0	61,5	113,6	340,8
Tar. P. 3 L	11444	2150	8020	14547	15031	6196	2707	27094
Tar. P.3Q	130,55	39,21	74,60	261,10	126,52	23,79	79,52	201,64

**Table 2 : Measurements of 32 criteria determining males of *Culex pipiens* caught from 2 region :**

N= 20; unity :  $\mu\text{m}$ . L :length; Q : width ; M :mean ; S :standard deviation ; min V : minimal value, max v : maximal value.

Region Variable	Annaba				Oum El Bouaghi			
	M.	S.	V <sub>min.</sub>	V <sub>max.</sub>	M.	S.	V <sub>min.</sub>	V <sub>max.</sub>
T. L	993,8	304,8	500,0	1500,0	1093	963	653	5140
T. Q	780,0	160,1	500,0	1250,0	652,5	143,6	454,4	979,8
P. L	5493	788	4500	8000	4373	915	2755	5964
P. Q	110,00	20,52	100,047	150,00	101,53	29,60	56,80	142,00
Pr. L	5203	558	250	7000	4175	1090	1860	6617
Pr. Q	137,50	27,51	100,0	200,00	112,18	29,10	42,60	170,40
An. L	3368	607	2500	4250	3018	838	1846	5084
An. Q	50,000	0,000044	50,00	50,000	48,28	8,50	42,60	71,00
Th. L	3026,3	1,7	2250,	4000,0	2605,7	416,2	1846,0	3280,2
Th. Q	3036,3	439,8	2500,	4250,0	2418	449	1633	3266
Al. L	7888	1062	6000	11250	6724	1107	4544	8520
Al. Q	1782,5	226,7	1500	2500,0	1370,3	308,6	653,2	1775,0
Ab. L	6505	910	5500	9500	8677	13726	3735	66874
Ab. Q	977,5	210,6	650,0	1550,0	679,5	360,4	269,8	1888,6
F. P. 1 L	4050,0	433,8	3250,	5500,0	3592	774	1690	4956
F. P. 1 Q	212,50	35,82	150,037	250,00	71,0	57,5	177,5	326,6
Ti. P. 1 L	4450	475	50	6000	3875	656	2840	5041
Ti. P. 1 Q	140,00	34,79	100,035	200,00	142,00	41,46	85,20	227,20

Tar. P. 1 L	4803	692	00	6500	5140	2033	3465	13348
Tar. P. 1 Q	122,50	41,28	100,0	250,00	107,92	15,28	88,04	133,48
F. P. 2 L	4523	815	1750	5750	4070	657	2840	504
F. P. 2 Q	237,5	45,5	150,0	300,0	178,9	48,0	85,2	284,0
Ti. P. 2 L	4853	570	3750	6250	4181	861	1889	5439
Ti. P. 2 Q	145,00	42,61	100,055	250,00	137,74	23,98	99,40	170,40
Tar. P. 2 L	6643	746	00	9250	7391	1435	5368	9613
Tar. P. 2 Q	100,00	0,0000	100,0	100,00	106,36	29,09	76,68	215,84
F. P. 3L	4350	473	3750	5500	4070	657	2840	5041
F. P. 3 Q	212,50	42,53	150,022	250,00	178,9	48,0	85,2	284,0
Ti. P. 3 L	4680	791	50	6500	4181	861	1889	5439
Ti. P. 3 Q	150,00	32,44	100,075	200,00	137,74	23,98	99,40	170,40
Tar. P. 3 L	10338	1710	00	15450	7391	1435	5368	9613
Tar. P. 3Q	102,50	11,18	100,0	150,00	106,36	29,09	76,68	215,84

Table 3:Measurements of 10 criteria determining larval of *Culex pipiens* caught from 2region :

N= 20; unity :  $\mu\text{m}$ . L :length; Q : width ; M :mean ; S :standard deviation ; min V : minimal value, max v : maximal value.

Region Variable	Annaba				Oum El Bouaghi			
	M.	S.	V.min.	V.max.	M.	S.	V.min.	V.max.
T. L	2532,7	272,5	1865,0	2909,4	2019,0	327,3	923,0	2424,5
T. Q	2551,3	296,0	1865,0	2984,0	2564,4	171,5	2275,3	2834,8
Th. L	2443	545	1679	3544	2301	579	1492	3544
Th. Q	3131	464	2425	4103	2833	716	634	3730
Ab. L	12603	5068	7274	22783	8609	2076	2387	11265
Ab. Q	19502	7474	10817	34167	2088,8	380,6	1492,0	3095,9
Sph. L	3202,0	320,6	2797,5	3916,5	3105	612	858	3730
Sph. Q	1018,3	184,0	857,9	1678,5	887,3	86,7	746,0	1081,7
Tra. L	3002,2	365,0	2461,8	3730,0	1025,8	144,5	820,6	1342,8
Tra. Q	619,2	332,8	373,0	1230,9	1113,4	112,4	895,2	1342,8

Table4: Results of test T of student, comparison between 2 regions, the mean of each 32 criteria determining females of *culex pipiens*.

Variable	Oum El Bouaghi	Annaba	t <sub>obs</sub>	P
T. L	1122,5	1217,8	1,32	0,196 N.S
T. Q	943,6	1161,9	2,53	0,016*
Pa. L	542,4	712,4	4,13	0,000***
Pa. Q	141,2	141,74	0,04	0,965 N .S
Pr. L	5083	5134	0,20	0,845 N .S
Pr. Q	178,9	158,5	1,34	0,187 N .S
An. L	3565	4620	4,28	0,000 ***
An. Q	48,99	37,300	4,26	0,000 ***
Th. L	3678,5	3487,6	1,64	0,110 N .S
Th. Q	3346	3655,4	1,55	0,129 N .S
Al. L	10217	10341	0,35	0,728 N .S
Al. Q	2374	2736,0	2,72	0,010*
Ab. L	6652	6798	0,52	0,603 N .S
Ab. Q	1008,2	1337,2	4,28	0,000***
F. p. 1 L	4942	4653	1,81	0,078 N .S
F. p. 1 Q	281,2	262,6	0,91	0,370 N .S
Ti. p 1 L	5314	4924	1,76	0,086 N .S
Ti. p 1 Q	200,2	169,71	2,29	0,028*
Tar patte1 L	6594	6043	1,63	0,111 N .S
Tar patte1 Q	151,42	136,15	2,22	0,032*
F patte2 L	5399	5119	1,52	0,137 N .S
F patte2 Q	289,68	290,9	0,08	0,940 N .S
Ti p2 L	5538	5707	0,60	0,553 N .S
Ti p2 Q	303,3	171,58	1,36	0,183 N .S
Tar p2 L	11029	6611	4,59	0,000***
Tar pa2 Q	139,26	206,7	1,03	0,311 N .S
F p 3L	5144	5061,6	0,48	0,634 N .S
Fp3 Q	281,2	277,9	0,20	0,846 N .S
TiP3 L	5586	5650,9	0,38	0,703 N .S
TiP3 Q	220,0	158,53	4,17	0,000***
Tar p3 L	15031	11444	2,45	0,019*
Tar P3Q	126,52	130,55	0,39	0,697 N .S

**Data statistical analysis****Test of student for independent sample**

The comparative statistical study with test T of student was done between measurements of females (table4) of 2 regions which show the existence of significant differences in the width of a head,wings,tarsomer,1st scrawl, and in the length of tarsomer of 3<sup>rd</sup> scrawl. Highly significant difference concerned the length of palpe antennae, the width of antennae, abdomen, tarsomer of 2<sup>nd</sup> scrawl and tibia of 3<sup>rd</sup> scrawl . For males differences are significant in the width of head, tarsomer of 2<sup>nd</sup> scrawl and tibia of 3<sup>rd</sup> scrawl. The length and width of pronotum ; length of thorax ,wings tibia of 1<sup>st</sup> scrawl ; and width of femur of 3<sup>rd</sup> scrawl show highly .significant differences (table 5) . For the comparison of larval measurement , the test points out highly significant differences in the length of abdomen , and width of siphon , and very highly significant difference in the length of head , width of abdomen ; and length , width of trachea (table 6) .

**Table 5: Results of test T of student, comparison between regions, the mean of 32 morphometrical criteria of culex pipiens males.**

Variables	Oum El Bouaghi	Annaba	t <sub>obs</sub>	P
T L	1093	993,8	0,44	0,664 N .S
T Q	652,5	780,0	2,65	0,012*
PaL	4373	5493	4,15	0,000***
P Q	101,53	110,00	1,05	0,300 N .S
Pr L	4175	5203	3,75	0,001**
Pr Q	112,18	137,50	2,83	0,007**
An L	3018	3368	1,51	0,139NS
An Q	48,28	50,000	0,13	0,901 NS
TH L	2605,7	3026,3	3,10	0,004**
TH Q	2418	3036,3	4,40	0,000***
Al L	6724	7888	3,39	0,002**
Al Q	1370,3	1782,5	4,81	0,000***
Ab L	8677	6505	0,71	0,484 N .S
Ab Q	679,5	977,5	3,19	0,003**
F p 1 L	3592	4050,0	2,31	0,026*
F p 1 Q	71,0	212,50	2,31	0,026*
T p 1 L	3875	4450	3,17	0,003**
T p 1 Q	142,00	140,00	0,17	0,870 N .S
Tar patte1 L	5140	4803	0,70	0,486 N .S
Tar patte1 Q	107,92	122,50	1,48	0,147 N .S
F patte2 L	4070	4523	1,93	0,061 N .S
F patte2 Q	178,9	237,5	3,96	0,000***
Ti p2 L	4181	4853	2,91	0,006**
Ti p2 Q	137,74	145,00	0,66	0,511 N .S
Tar p2 L	7391	6643	2,07	0,045*
Tar pa2 Q	106,36	100,00	1,04	0,313 NS
F p 3L	4070	4350	1,54	0,131 N .S
Fp3 Q	178,9	212,50	2,34	0,024**
TiP3 L	4181	4680	1,91	0,064*
TiP3 Q	137,74	150,00	1,36	0,182 N .S
Tar p3 L	7391	10338	5,90	0,000***
Tar P3Q	106,36	102,50	0,55	0,583 N .S

**Table 6 : Results of test T of student , comparison between regions ,mean of 10 morphological criteria of culex pipiens larval .**

Variables	Oum El Bouaghi	Annaba	t <sub>obs</sub>	P
T. L	2019,0	2532,7	5,35	0,000***
T. Q	2564,4	2551,3	0,17	0,865 N .S
Th. L	2301	2443	0,80	0,430 N .S
Th. Q	2833	3131	1,56	0,126 N .S
Ab. L	8609	12603	3,26	0,002**
Ab.Q	2088,8	19502	10,41	0,000***
Sph. L	3105	3202,0	0,63	0,535 N .S
Sph.Q	887,3	1018,3	2,88	0,006**
Tra. L	1025,8	3002,2	22,51	0,000***
Tra. Q	1113,4	619,2	6,29	0,000***

**Multivoried statical analysis**

Statistical analysis ( MANOVA ) shows significant difference between adult females (table 8) and very highly significant difference for larval and adult males (table 8.9 ) and this for all measurements of morphometrical criteria



Table 7: Test results of MANOVA applied to 32 biometrical criteria determining *Culex pipiens* females.

Test	The observed value of test	Fobs	P
Wilks'	0,01717	12,518	0,001**
Lawley-Hotelling	57,22653	12,518	0,001**
Pillai's	0,98283	12,518	0,001**

Table 8: Test results of MANOVA applied to 32 biometrical criteria determining *Culex pipiens* males .

Test	The observed value of test	Fobs	P
Wilks'	0,06056	3,393	0,049*
Lawley-Hotelling	15,51199	3,393	0,049*
Pillai's	0,93944	3,393	0,049*

Table 9: Test results of MANOVA applied to 32 biometrical criteria determining *Culex pipiens* larval.

Test	The observed value of test	Fobs	P
Wilks'	0,01958	145,183	0,000***
Lawley-Hotelling	50,06307	145,183	0,000***
Pillai's	0,93042	145,183	0,000***

### CONCLUSION

Biometrical study allow systematical identification of sub species , and allows the comparison between species collected from different biotopes' . In that capacity our study was devoted to measure 10 larval criteria and 32 criteria for male and female adults of *Culex pipiens* . On the other hand , comparative study of biometry between mosquitoes was carried out in 2 regions (Annaba , and Oum el Bouaghi ). The results point out , even though it's the same species ( *Culex pipiens* ) , caught from 2 different zones , there is highly and very highly significant difference.

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