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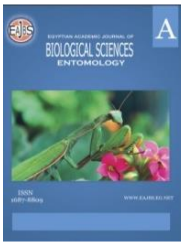

A biometric Study of The Two Forms of Calliptamus barbarus (Costa, 1836) (Orthoptera: Calliptaminae) Living in Algeria and Spain

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ABSTRACT

The aim of this study is to know whether the populations of the two forms of *C. barbarus* in Algeria and Spain are homogeneous or not. For that, a morphometric study was performed in order to compare the specimens collected in five different stations in Algeria with those of the collection of *C. barbarus* in the laboratory of Orthopterology, the University of Murcia in Spain collected from different stations in the Iberian Peninsula. For the two countries, each specimen of both sexes (male and female) has been studied using a different morphometric index of body size in both forms (one and three femoral spots) and both sexes (male and female) as follows: total length of the body TL, length of elytra EL, length of femora FL, the width of femora FW, the width of head HW, and ratio EL/FL, FL/FW. The results obtained were statistically analyzed with analysis of variance (ANOVA). From the results obtained, all the parameters gave significant differences, except for the FL/FW couple in females with one spot. Where the difference was not significant. We can conclude that there is a very highly significant difference between the populations of Algeria and Spain.

INTRODUCTION

Among the locust pest of cultivated plants, species of the genus *Calliptamus* have a considerable interest in the world. The genus *Calliptamus* belongs to the family of Acrididae, and the subfamily of Calliptaminae. The systematics of this genus has been confused, until the revision of Jago (1963). In this revision, the author listed thirteen species, four of which are found in North Africa and the South West of the Mediterranean Sea (Dirsh, 1965; Blanchet, 2009). These are *C. italicus*, *C. siciliae*, *C. wattenwylianus* and *C. barbarus*. The last two are the only species present in Algeria (Chara, 1987).

Among these two species, *C. barbarus* is the most important one, it was the subject of several studies related to different aspects: morphology (Chopard, 1943; Clemente *et al.*, 1987, Rouibah *et al.*, 2020), diet (Benzara *et al.*, 2003; Mesli *et al.*, 2005; Rouibah *et al.*, 2018), dispersal ability (Louveaux, 1991; Termier, 1991), egg development (Fabry *et

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al., 1987), population dynamics (Rouibah,1994, 2018), sound production (Larrosa *et al.*, 2008), ecology (Monrad,1986; Tekkouk, 2012) and molecular biology (Blanchet *et al.*, 2012; Sofrane *et al.*, 2015; Rouibah *et al.*, 2016).

Morphologically, the male length varies between 15 and 17 mm, that of females between 24 and 31 mm (Chopard, 1943). According to the latter, the cerci of the male have a median lobe slightly longer than the lower lobe. The sub-genital plate is short and rounded at the top. The pallium is blunt (Bellman & Luquet, 1995). It is inserted near the apex of the subgenital plate and standing vertically (Defaut, 1988).

Biologically, In Algeria, copulation of *C. barbarus* begins in mid-July. The oviposition is held in July and continues until September. Therefore, this species has an embryonic diapause. The hatching of eggs begins in mid-May and extends until June. The juveniles appear in mid-June. In the Mediterranean region, *C. barbarus* has one generation per year (Monard, 1986). According to Hugueny and Louveaux (1986), the number of ovarioles varies with latitude. According to them the size of individuals is correlated there, the number of ovarioles is all the more important as the size of the individual is large. According to Louveaux (1991), *C. barbarus* has an average of 38 ovarioles while *C. italicus* accounts 50. Chara (1987) noted that *C. wattenwylianus* has more ovarioles and eggs than *C. barbarus*. According to Larrosa *et al.* (2008), the sound of this species is produced by the friction of mandibles in males as in females. It is an intraspecific recognition system during mating.

Ecologically, *C. barbarus* has the ability to adapt to different ecological environments. It was reported on the dunes near the sea, in wastelands, steppe**,** open and semi-open scrubland at different altitudes (Claridge and Singhrao, 1978). *C. barbarus* is also found in lowlands and at medium altitude in fallow land and open scrubland surrounded by fallows (Chara, 1987; Defaut, 1988; Benzara, 2004). It is widely distributed, in almost all the western Mediterranean regions, North Africa, Southern Europe, and the Middle East (Jago, 1963; COPR 1982; Luquet, 1992). Its range also extends to the countries bordering the eastern part of the Mediterranean and penetrates eastward to central Asia: Kazakhstan, Turkestan, Pakistan, Afghanistan and Iran (Hugueny and Louveaux, 1986; Fabry *et al.*, 1987). It has also been reported in southern Russia and eastern Siberia (Stolyarov, 2000).

C. barbarus presents a very marked polymorphism, described by Jago (1963) as having three black spots separated (3S) or completely fused spots (1S) in the inner side of the posterior femora ranging from pale yellow, orange and red (Fig.1). The hind tibia is red on the inner side, yellow on the outside.

Fig.1. *Calliptamus barbarus* female with 3 spots (left) and 1 spot (right)(Rouibah *et al,*2016).

According to Larosa *et al.* (2004), the form with one femoral spot is larger than the form with three spots, for both males and females. In their study on inter and intrasexual behavior in captive males of *C. barbarus* with 1 and 3 femoral spots, Larrosa *et al.* (2007)

established eleven behavioral units before mating related to hind legs (femurs, shins) and wings.

By comparing the form with 1S with that at 3S in Spain, Clemente *et al.* (1987), observed a clear separation, especially between the males. For them, the hypothesis considering the two size groups in relation to the color of the internal surface of the posterior femur is not to be discounted. They add that only in special and very limited cases, it is possible to observe the two bioforms simultaneously. On the other hand, the temporal characteristics of the acoustic emission of the two forms of the male of *C. barbarus* (with one and three femoral spots) are significantly different. Some differences were also detected in females (Larrosa et al., 2008).

According to Oueldelhadj (2004), *C. barbarus* is one of the non-gregarious locust species observed in Algeria. This country, by its geographical location and its territory's extension, occupies an important place in the habitat area of this insect. The one femoral spot form occurs in semi-arid regions like Djelfa and Medea. Form with three femoral spots occupies the humid and sub-humid regions as Tizi Ouzou, Boumerdes and Jijel. In this last region, the population living in Jijel is homogeneous and belongs to the form with three femoral spots (Rouibah *et al.,* 2020).

According to Benzara (2004), in Algeria, females of *C. barbarus* belonging to the sub-humid bioclimate (North) have a size between 25 mm and 30 mm that of the males ranged from 13 mm to 16 mm. The population of semi-arid bioclimate (South) which contain individuals having 1 black spot, their size varied from 33 mm to 44 mm for females and 19 mm to 29 mm for males that show heterogeneity between individuals.

According to Rouibah *et al.* (2020), This difference in size between individuals explains that in Algeria there is a geographical polymorphism in *C. barbarus* between the population of semi-arid characterized by a large size and the population of humid and sub-humid both characterized by a smaller size. *C. barbarus* polymorphism is mostly related to environmental conditions (Jago, 1963). According to the latter, pigmentation of the inner face of the femur can be reduced, expanded, or fused in relation to environmental conditions.

The objective of this work is to know if the populations of Spain are homogeneous from the point of view of morphometric parameters with the populations of Algeria for both 1S form and 3S form and confirm the hypothesis according to which those southern populations of *C. barbarus* differ from the northern population by the large size of the adult. This will help us to know more about the status of this locust species.

MATERIALS AND METHODS

Five stations were chosen in Algeria, three of them in the Jijel region, one in Medea and one in Djelfa (Fig.2). The station selection is based on several parameters: relief, altitude, latitude and especially the type of environment: wasteland, scrubland, dune of coast and steppes. The first station is located in Texenna: 36° 39' 38'' N; 5° 47' 28'' E, 18 km South of Jijel. It rises to 728 m with a slope of 7% and a southeast exposure. There is a scrubland installed on a clay and limestone soil type. In this station, the vegetation is divided into two levels: herbaceous layer mainly composed of grasses and shrub *Cistus monspeliensis*. The second station Kissir: 36°48' 00'' N; 540' 60'' E is located near the Taza National Park, 12 km in West of Jijel. This is a wasteland. Its altitude is only 20m; the ground is flat and slightly acidic sandy-loam texture. Floristically, 40 plants species were identified. They are divided into three levels: herb layer, shrub layer and tree stratum. The third station is located near the city of Jijel: 36°49' 00'' N and 5°46' 00'' E. This is a fixed dune off the coast. The soil is sandy type. The fourth and fifth stations are located respectively in the region of Medea: 35° 53' 20" N; 2° 44' 56" E, altitude: 623 m and Djelfa: 34°40′22″ N; 3°15′46″ E, altitude: 1138 m. These stations are installed on flat ground. The soil has a sandy texture. The vegetation is scattered, consists mainly of Wormwood, Harmal and Alfa.

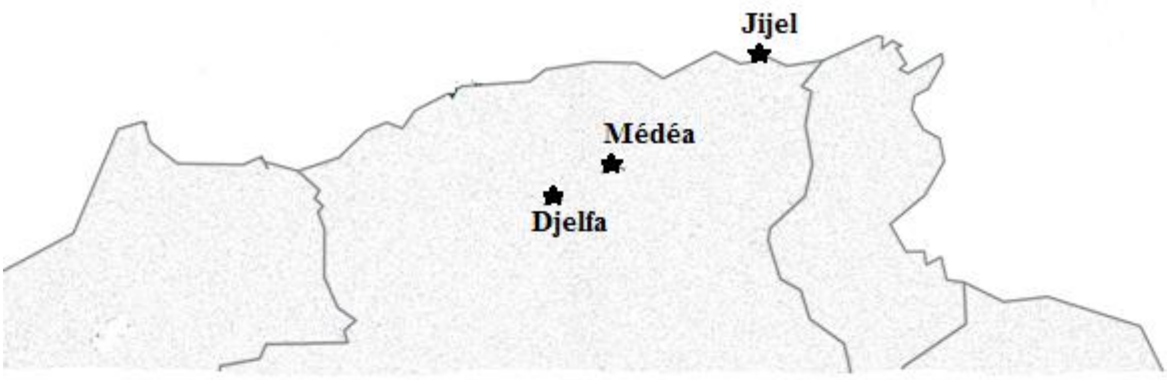

Fig. 2 Sampling localities in Algeria.

In Spain, we studied the collection preserved in the laboratory of Orthopterology in the University of Murcia (South-Eastern Spain). Preserved samples were collected between 1987 and 1999, from different localities especially Sierra Espuña (Murcia) (fig.3) but from other regions as Albacete, Alicante, Almeria, and also from Portugal.

Fig.3. Sierra Espuña (Murcia) mountain in South-Eastern Spain.

Specimens were collected by mowing net and plastic bags, altitude was measured by an altimeter and geographic coordinated by a GPS, and some photos were taken in each locality.

In the laboratory, a caliper (with a precision of 0.01 mm) for body dimensions was used. A sampling of insects was carried out between October 2011 and August 2014. The morphometric study is made in both laboratory of biology at the University of Jijel (Algeria) and the laboratory of Orthopterology at the University of Murcia in Spain.

In systematic studies, morphological characters have long been the only criteria used to differentiate species. They are still useful today as a first approach and in addition to molecular biology techniques (Benzara, 2004). The classic morphometric aims to highlight the possible relations between the sizes of the different organs of the body of the insect and to emphasize the degree of variability between populations. The joint analysis of body dimensions of form and color as systematic criteria is an effective method to reduce the risk of appreciation of differences and similarities and to define individual variations (Blondin, 1980).

A total of 256 individuals in Algeria (61 one Spot: 36 males; 25 females and 195 tree Spots: 74 males; 121 females), and 913 individuals belonging to the two forms for

Spain (69 one Spot and 844 tree Spot: 549 males; 295 females) were measured.
From each specimen, seven measurements were taken: Total Length of the body (TL), Elytra Length (EL), Femora Length (FL), Femora Width (FW), Head Width (HW) and the ratio EL/FL, FL/FW. These parameters were used by Jago (1963), Clemente et al. (1987); Benzara (2004) and recently by Rouibah et al. (2020) on *C. barbarus*. It should also be remembered that these parameters have already been used by Defaut (2005); Benfekih (2006); El Ghadraoui et al. (2003) on *Locusta migratoria*.
Statistical analysis

In this work, we used analysis of variance (1-way ANOVA) to compare the different parameters between them. The program of statistics that was used is SPSS (version 21). To calculate if the results were or not significant, we used the value of the Fisher statistic F. According to Scherrer (1984), ANOVA makes it possible to verify the meaning of variable of interest between all combinations of modalities. On the other hand, the morphometric parameters in the two forms of *C. barbarus* were studied by the PCA Principal Component Analysis in order to establish the typology of the two countries and to clarify and discriminate all the data.

RESULTS

Measures taken on males and females with one spot and three spots from Algeria and Spain and results of ANOVA as well as PCA on morphometric parameters are summarized in tables one to eight and figures four to seven.

Male with One Spot:

The results for males with 1S of Algeria and Spain are shown in tables 1, 2 and figure 4.

Table1. Measurements and descriptive Analysis of variance of different parameters in population males 1S between Algeria and Spain

Source		N	Mean	Standard deviation	Standard error	Confidence interval at 95% for the mean		Min	Max
						Lower limit	Upper limit		
TL	Alg	36	22,7100	1,76004	,29334	22,1145	23,3055	20,26	27,06
	Spain	52	20,5250	2,13782	,29646	19,9298	21,1202	14,77	25,36
	Total	88	21,4189	2,25670	,24057	20,9407	21,8970	14,77	27,06
EL	Alg	36	17,2433	1,63097	,27183	16,6915	17,7952	14,22	21,31
	Spain	52	15,1106	1,88795	,26181	14,5850	15,6362	10,03	19,93
	Total	88	15,9831	2,06684	,22033	15,5451	16,4210	10,03	21,31
FL	Alg	36	11,9389	,65180	,10863	11,7184	12,1594	10,82	13,32
	Spain	52	11,3858	,98136	,13609	11,1126	11,6590	8,57	13,34
	Total	88	11,6120	,90015	,09596	11,4213	11,8028	8,57	13,34
FW	Alg	36	4,0931	,22075	,03679	4,0184	4,1677	3,60	4,53
	Spain	52	3,7908	,37901	,05256	3,6853	3,8963	2,73	4,39
	Total	88	3,9144	,35518	,03786	3,8392	3,9897	2,73	4,53
HW	Alg	36	3,8497	,16708	,02785	3,7932	3,9063	3,50	4,22
	Spain	52	3,7115	,25163	,03490	3,6415	3,7816	3,01	4,22
	Total	88	3,7681	,23026	,02455	3,7193	3,8169	3,01	4,22
EL/FL	Alg	36	1,4430	,08999	,01500	1,4125	1,4734	1,28	1,63
	Spain	52	1,3247	,09465	,01313	1,2983	1,3510	1,06	1,53
	Total	88	1,3731	,10923	,01164	1,3499	1,3962	1,06	1,63
FL/FW	Alg	36	2,9195	,12663	,02111	2,8767	2,9624	2,63	3,16
	Spain	52	3,0120	,15633	,02168	2,9685	3,0556	2,69	3,48
	Total	88	2,9742	,15123	,01612	2,9421	3,0062	2,63	3,48

Total: Total number of individuals measured for all 2 countries
N: Total number of individuals measured for each country
Mean: Average of measured individuals

Table2. Analysis of variance of different parameters in population males 1S between Algeria and Spain

Source		Sumsq	Df	MeanSq	F value	Pr (>F)
TL	Modeling	101,561	1	101,561	25,576	,000
	Residue	341,504	86	3,971		
	Total	443,065	87			
EL	Modeling	96,762	1	96,762	30,273	,000
	Residue	274,885	86	3,196		
	Total	371,648	87			
FL	Modeling	6,508	1	6,508	8,747	,004
	Residue	63,986	86	,744		
	Total	70,494	87			
FW	Modeling	1,944	1	1,944	18,510	,000
	Residue	9,032	86	,105		
	Total	10,975	87			
HW	Modeling	,406	1	,406	8,305	,005
	Residue	4,206	86	,049		
	Total	4,613	87			
EL/FL	Modeling	,298	1	,298	34,576	,000
	Residue	,740	86	,009		
	Total	1,038	87			
FL/FW	Modeling	,182	1	,182	8,663	,004
	Residue	1,808	86	,021		
	Total	1,990	87			

Df: degree of freedom, Sq' Sum: sum of squares, Sq'mean: mean of squares = Sum Sq/ Df, F value: the value of the Fisher statistic, Pr (> F): the probability that we can meet the calculated value of statistics.

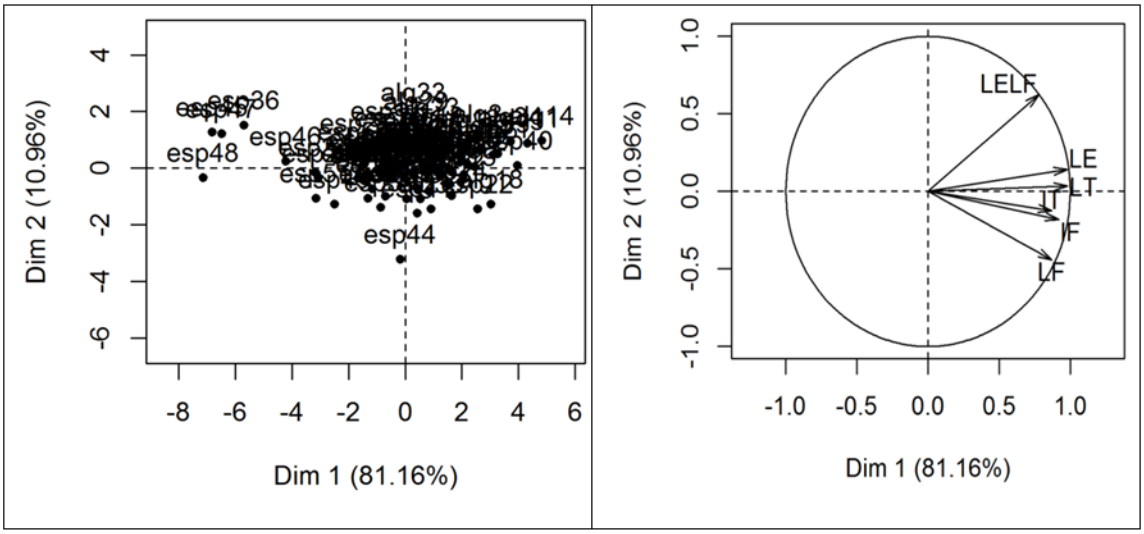

Fig.4. Factor map of Principal Component Analysis for males 1S (First axis: Dim 1 and Second axis: Dim 2). up: individuals; down: morphometric parameters.

Female with One Spot:

The results concerning females with 1S for the 2 countries are represented in tables 3, 4 and figure 5.

Table3. Measurements and descriptive Analysis of variance of different parameters in population females 1S between Algeria and Spain.

Source		N	Mean	Standard deviation	Standard error	Confidence interval at 95% for the mean		Min	Max
						Lower limit	Upper limit		
TL	Alg	25	35,0312	2,67144	,53429	33,9285	36,1339	30,03	40,12
	Spain	17	29,2882	2,84195	,68928	27,8270	30,7494	23,87	33,98
	Total	42	32,7067	3,93313	,60689	31,4810	33,9323	23,87	40,12
EL	Alg	25	26,8476	2,16628	,43326	25,9534	27,7418	21,99	30,80
	Spain	17	21,4606	2,84173	,68922	19,9995	22,9217	15,98	26,48
	Total	42	24,6671	3,61395	,55764	23,5410	25,7933	15,98	30,80
FL	Alg	25	18,9984	1,24839	,24968	18,4831	19,5137	16,41	20,90
	Spain	17	16,5406	1,35374	,32833	15,8446	17,2366	13,71	18,79
	Total	42	18,0036	1,76589	,27248	17,4533	18,5539	13,71	20,90
FW	Alg	25	5,8716	,42215	,08443	5,6973	6,0459	5,09	6,63
	Spain	17	5,1982	,55459	,13451	4,9131	5,4834	4,05	5,83
	Total	42	5,5990	,57987	,08948	5,4183	5,7797	4,05	6,63
HW	Alg	25	5,4748	,32796	,06559	5,3394	5,6102	4,79	6,00
	Spain	17	4,9594	,34919	,08469	4,7799	5,1389	4,36	5,60
	Total	42	5,2662	,41965	,06475	5,1354	5,3970	4,36	6,00
EL/FL	Alg	25	1,4127	,05628	,01126	1,3894	1,4359	1,29	1,52
	Spain	17	1,2937	,09658	,02343	1,2441	1,3434	1,05	1,50
	Total	42	1,3645	,09479	,01463	1,3350	1,3941	1,05	1,52
FL/FW	Alg	25	3,2397	,13950	,02790	3,1822	3,2973	3,03	3,66
	Spain	17	3,1956	,20172	,04892	3,0919	3,2994	2,94	3,66
	Total	42	3,2219	,16658	,02570	3,1700	3,2738	2,94	3,66

Table4. Analysis of variance of different parameters in population females 1S between Algeria and Spain.

Source		Sumsq	Df	MeanSq	F value	Pr (>F)
TL	Modeling	333,743	1	333,743	44,424	,000
	Residue	300,506	40	7,513		
	Total	634,249	41			
EL	Modeling	293,654	1	293,654	48,571	,000
	Residue	241,833	40	6,046		
	Total	535,486	41			
FL	Modeling	61,128	1	61,128	36,644	,000
	Residue	66,725	40	1,668		
	Total	127,853	41			
FW	Modeling	4,588	1	4,588	19,953	,000
	Residue	9,198	40	,230		
	Total	13,786	41			
HW	Modeling	2,688	1	2,688	23,722	,000
	Residue	4,532	40	,113		
	Total	7,220	41			
EL/FL	Modeling	,143	1	,143	25,411	,000
	Residue	,225	40	,006		
	Total	,368	41			
FL/FW	Modeling	,020	1	,020	,704	,407
	Residue	1,118	40	,028		
	Total	1,138	41			

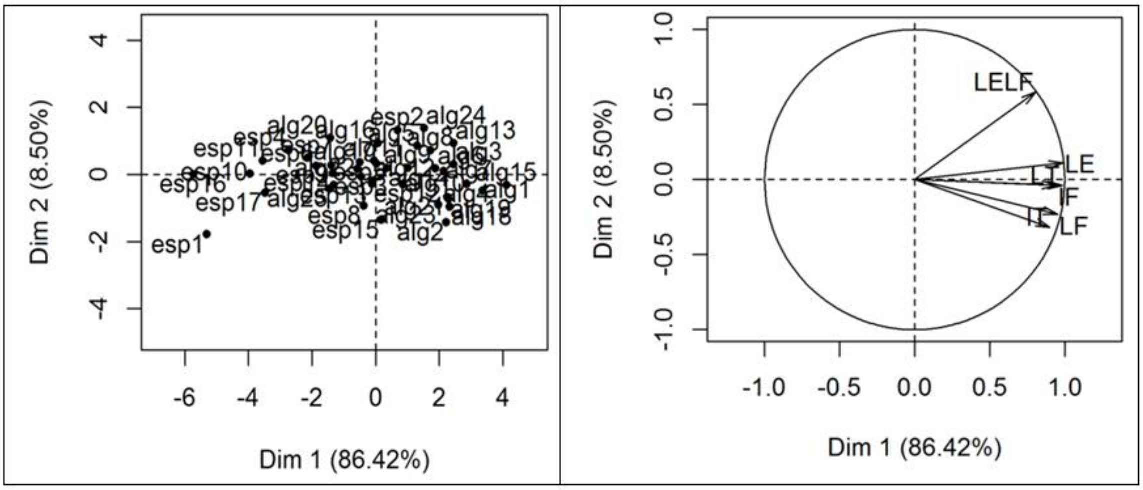

Fig.5. Factor map of Principal Component Analysis for females 1S

Male with Three Spot:

The results concerning the male population with 1S are grouped in tables 5, 6 and figure 6.

Table 5. Measurements and descriptive Analysis of variance of different parameters in population males 3S between Algeria and Spain.

Source		N	Mean	Standard deviation	Standard error	Confidence interval at 95% for the mean		Min	Max
						Lower limit	Upper limit		
TL	Alg	74	17,2605	1,19692	,13914	16,9832	17,5378	14,46	19,90
	Spain	549	16,0782	1,34101	,05723	15,9658	16,1907	12,57	20,29
	Total	623	16,2187	1,37806	,05521	16,1102	16,3271	12,57	20,29
EL	Alg	74	12,0319	1,09521	,12732	11,7782	12,2856	9,00	14,30
	Spain	549	11,3032	1,21005	,05164	11,2018	11,4046	8,50	14,95
	Total	623	11,3898	1,21921	,04885	11,2938	11,4857	8,50	14,95
FL	Alg	74	9,5973	,68519	,07965	9,4386	9,7560	8,10	11,00
	Spain	546	9,2297	,71339	,03053	9,1698	9,2897	7,38	11,50
	Total	620	9,2736	,71949	,02890	9,2169	9,3303	7,38	11,50
FW	Alg	74	3,3726	,39809	,04628	3,2803	3,4648	2,62	4,00
	Spain	546	2,9622	,29395	,01258	2,9375	2,9869	,00	3,82
	Total	620	3,0112	,33540	,01347	2,9848	3,0377	,00	4,00
HW	Alg	74	3,4685	,36147	,04202	3,3848	3,5523	2,90	4,10
	Spain	549	3,1590	,19512	,00833	3,1426	3,1753	2,53	3,84
	Total	623	3,1957	,24274	,00973	3,1766	3,2148	2,53	4,10
EL/FL	Alg	74	1,2564	,10886	,01265	1,2312	1,2817	,89	1,51
	Spain	549	1,2175	,12171	,00519	1,2073	1,2277	,00	1,55
	Total	623	1,2221	,12084	,00484	1,2126	1,2316	,00	1,55
FL/FW	Alg	74	2,8717	,27716	,03222	2,8075	2,9359	2,03	3,47
	Spain	549	3,0977	,34075	,01454	3,0691	3,1263	,00	4,05
	Total	623	3,0708	,34156	,01368	3,0440	3,0977	,00	4,05

Table 6. Analysis of variance of different parameters in population males 3S between Algeria and Spain.

Source		Sum Sq	Df	Mean Sq	F value	Pr (>F)
TL	Modeling	91,154	1	91,154	51,930	,000
	Residue	1090,054	621	1,755		
	Total	1181,208	622			
EL	Modeling	34,626	1	34,626	24,161	,000
	Residue	889,956	621	1,433		
	Total	924,582	622			
FL	Modeling	8,805	1	8,805	17,461	,000
	Residue	311,634	618	,504		
	Total	320,438	619			
FW	Modeling	10,973	1	10,973	115,597	,000
	Residue	58,661	618	,095		
	Total	69,633	619			
HW	Modeling	6,249	1	6,249	127,636	,000
	Residue	30,402	621	,049		
	Total	36,650	622			
EL/FL	Modeling	,099	1	,099	6,851	,009
	Residue	8,983	621	,014		
	Total	9,082	622			
FL/FW	Modeling	3,330	1	3,330	29,865	,000
	Residue	69,236	621	,111		
	Total	72,565	622			

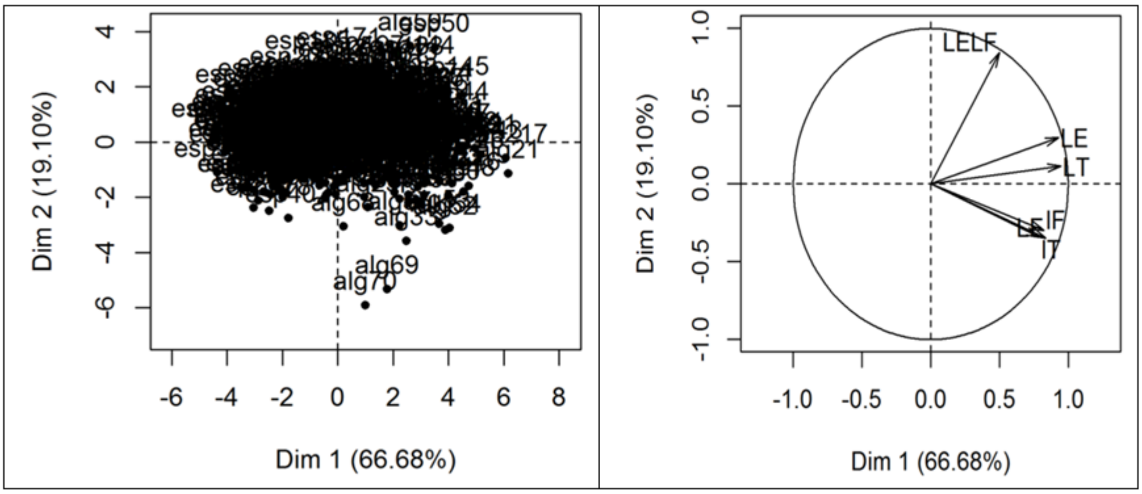

Fig.6. Factor map of Principal Component Analysis for males 3S

Female with Three Spot:

The results concerning the female population with 3S are grouped in tables 7, 8 and figure 7.

Table7. Descriptive Analysis of variance of different parameters in population females 3S between Algeria and Spain.

Source		N	Mean	Standard deviation	Standard error	Confidence interval at 95% for the mean		Min	Max
						Lower limit	Upper limit		
TL	Alg	121	29,0950	2,51775	,22889	28,6419	29,5482	22,00	34,50
	Spain	295	26,6795	2,10419	,12251	26,4383	26,9206	21,92	33,40
	Total	416	27,3821	2,48518	,12185	27,1426	27,6216	21,92	34,50
EL	Alg	121	20,9579	2,02450	,18405	20,5935	21,3223	16,00	25,50
	Spain	295	18,5304	1,80817	,10528	18,3232	18,7376	14,40	24,06
	Total	416	19,2365	2,17250	,10652	19,0271	19,4459	14,40	25,50
FL	Alg	121	16,4642	1,51353	,13759	16,1918	16,7366	11,29	19,00
	Spain	295	15,1038	1,06518	,06202	14,9818	15,2259	12,64	18,09
	Total	416	15,4995	1,35972	,06667	15,3685	15,6306	11,29	19,00
FW	Alg	121	5,4070	,71513	,06501	5,2783	5,5357	3,57	7,30
	Spain	295	4,6085	,40791	,02375	4,5618	4,6552	3,47	5,83
	Total	416	4,8408	,63055	,03092	4,7800	4,9015	3,47	7,30
HW	Alg	121	5,0782	,47148	,04286	4,9933	5,1630	3,96	6,10
	Spain	295	4,5656	,26159	,01523	4,5357	4,5956	3,88	5,43
	Total	416	4,7147	,40875	,02004	4,6753	4,7541	3,88	6,10
EL/FL	Alg	121	1,2765	,09935	,00903	1,2586	1,2944	,95	1,47
	Spain	295	1,2261	,06977	,00406	1,2181	1,2341	1,03	1,42
	Total	416	1,2408	,08263	,00405	1,2328	1,2487	,95	1,47
FL/FW	Alg	121	3,0688	,25382	,02307	3,0231	3,1145	2,55	3,78
	Spain	295	3,2872	,18983	,01105	3,2654	3,3089	2,77	4,04
	Total	416	3,2237	,23241	,01139	3,2013	3,2461	2,55	4,04

Table 8. Analysis of variance of different parameters in population females 3S between Algeria and Spain.

Source		Sum Sq	Df	MeanS q	F value	Pr (>F)
TL	Modeling	500,678	1	500,678	100,504	,000
	Residue	2062,415	414	4,982		
	Total	2563,092	415			
EL	Modeling	505,641	1	505,641	144,065	,000
	Residue	1453,058	414	3,510		
	Total	1958,699	415			
FL	Modeling	158,795	1	158,795	108,044	,000
	Residue	608,470	414	1,470		
	Total	767,265	415			
FW	Modeling	54,712	1	54,712	205,379	,000
	Residue	110,287	414	,266		
	Total	164,999	415			
HW	Modeling	22,542	1	22,542	199,436	,000
	Residue	46,794	414	,113		
	Total	69,336	415			
EL/FL	Modeling	,218	1	,218	34,536	,000
	Residue	2,616	414	,006		
	Total	2,834	415			
FL/FW	Modeling	4,090	1	4,090	92,408	,000
	Residue	18,326	414	,044		
	Total	22,416	415			

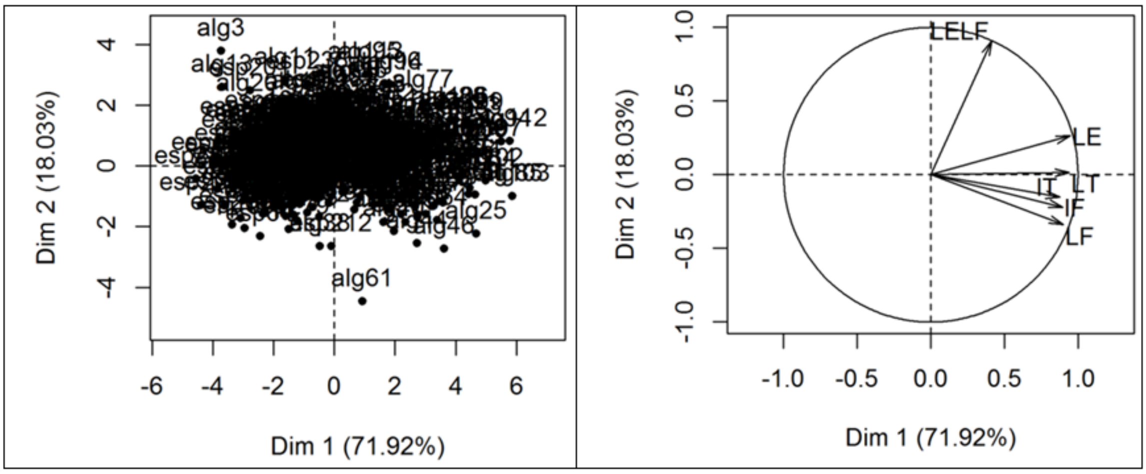

Fig.7. Factor map of Principal Component Analysis for Females 3S

Firstly, we highlight that specimen from Algeria (both males and females) resulted bigger than those from Spain, both those with 1Spot and 3Spots. Indeed, males and females with 1S from Algeria vary from 20.26 to 27.06 mm and from 30.03 to 40.12 mm respectively, while those from Spain vary from 14.77 to 25, 36 mm and 23.87 to 33.98 mm respectively. Regarding the 3S form, the body size of Algerian males and females resulted to be between 14.46 and 19.9 mm and between 22 and 34.5 mm respectively, while those from Spain were 12. 57 to 20.29 mm and 21.92 to 33.4 mm respectively.

Furthermore, the analysis of variance showed, that there is a very highly significant difference between the two populations of the two countries concerning all the parameters studied in both male and female individuals in the two bioforms with one and three femoral spots, except for the couple FL/FW in females with 1S where we recorded a not significant probability equal to 0.407.

Moreover, the application of PCA on the results of the morphometric parameters in the 1S males form reveals that the contributions to the total variance are 81.16 and 10.96% for axe 1 and axe 2 respectively (fig.4). Since the first two axes explain 94.92 % of the variance, they will be used for the factorial analysis. Concerning the 3S males form, the contributions are 66.68 and 19.10% for axe 1 and axe 2 respectively (fig.5). For the females, those contributions are 86.42 and 8.50% (1S form); 71.92 and 18.03% (3S form) for axe 1 and axe 2 respectively (Fig. 6 and 7). The representation of the orientations on the plane formed by axes 1 and 2 indicates that all the morphometric parameters studied are positively correlated and contribute significantly with axis 1 except LE / LF couple for the 3S individuals. This explains that individuals from Algeria differ from those from Spain in terms of size. These results confirm those obtained by ANOVA and therefore suggest that specimens of *C. barbarus* from Spain and Algeria belong to two different subpopulations.

DISCUSSION

According to Vieira and Pureauint (1993), the analysis of morphometric data makes it possible to evaluate the degree of variability of the different populations. Previously, Clemente et al. (1987) used the same morphometric data to compare the two bioforms of *C. barbarus*. They based the work on the total length of the body, tegmina and the posterior femur, as well as the width of the head.

The Total Length of the insect (TL), Elytra Length (EL), Femora Length (FL), Femora Width (FW), Head Width (HW) and the ratio EL/FL, FL/FW of the two populations are heterogeneous from the point of view of these parameters. Effectively, from the results obtained, all the parameters gave significant differences, except for the FL/FW couple in females with 1S where the difference was not significant (F-value equal to 0,704 and probability P equal to 0,407 there for > 0.05).

Analysis of the variance of total body length in males with 1S population between the two countries gives a calculated F-value equal to 25,576 and a probability P equal to 0.000 (< 0.05), (Table 2). The difference is therefore very highly significant. On the other hand, analysis of the total length variance in the female with 1S population gives a calculated F-value equal to 44,424 and a probability P equal to 0.000 (< 0.05), (Table 4). The difference is also very highly significant. For its part, analysis of the variance of total body length in males with 3S population gives a calculated F-value equal to 51,930 and a probability P equal to 0.000 (< 0.05), (Table 6). The difference is also very highly significant.

Finally, concerning females with 3S, analysis of the variance of the same parameter gives a calculated F-value equal to 100,504 and a probability P equal to 0.000 (< 0.05), (Table 8). The difference is very highly significant too. We can conclude that there is a very highly significant difference between the populations of Algeria and Spain.
The reason for the big difference observed during this study is probably due to the difference in climate, altitude and vegetation that characterize each country. According to Rouibah *et al.* (2020), it is probable that food and altitude are two important factors in the life of an insect; they can change the morphometric parameters a little, but there are also other important ecological (and possibly ethological) factors involved.

The South of Spain is a semi-arid region with a poor flora consisting mainly of *Pinus halepensis.* The matorral vegetation is mostly composed of shrubs and perennial grasses, the most representative being *Rosmarinus officinalis, Juniperus oxycedrus, Juniperus phoenicea, Rhamnus lycioides, Thymus vulgaris* and *Brachypodium retusum* (Lopez-Bermudez,1998). This region is located up to 800 m altitude is located up to 800 m altitude, where there is a semi-arid Mediterranean climate characterized by mild winters (an average of 11 °C in December and January) and warm summers where the daily maximum regularly exceeds 40 °C, with little precipitation of about 300 to 350 mm. It is therefore different from northeast Algeria which is characterized by a humid climate with big precipitation of about 1000 mm and very dense vegetation constituted mainly by Mediterranean scrub, so this will generate even big differences in size between the individuals of the two countries. We conclude that there is a big geographical polymorphism between the populations of Spain and Algeria. Since the body dimensions are closely correlated, growth is done in the same proportions for all body parts and larger individuals are isometric to smaller of them (Hugueny and Louveaux, 1986).

Indeed, it clearly appears that the size of the specimens increases as we move from the north (Spain) to the south (Algeria) for both the 1S and the 3S form, for males as well as for females. This hypothesis can be confirmed by Hugueny and Louveaux (1986). Indeed, according to these last authors, it is well established that southern populations of *C. barbarus* differ from northern populations on the one hand by the large size of the adult and on the other by a supernumerary stage of development. The size varies from single to double between latitude30 ° and 48 ° (Hugueny and Louveaux, 1986).

In addition, comparing the 1S form with the 3S form, we see that the first is larger than the second in both Algeria and Spain. Effectively, it is clear that most of morphometric differences are very much stronger in the males between the 1S form and the 3S form in Spain, or in Algeria than the morphometric differences between the 1S form, or the 3S form in Spain and in Algeria too. For example, in the case of the mean values for EL in males we observe that the difference is stronger between 15.11 and 11.30 mm than between 15.11

and 17.24 mm, and stronger between 12.03 and 17.24 mm than between 12.03 and 11.30 mm. It occurs the same with TL, FL, FW, HW, and partially with EL/FL in the males. In the opposite, the finding is a little different in the female populations for TL, EL, FL and EL/FL the differences between 1S and 3S are stronger in Algeria, but they are weaker in Spain. It may be due to the acoustic emissions being "significantly different" as reported by Larrosa et al. (2008). According to the latter, the temporal characteristics of the acoustic emission of the two forms of the male of *C. barbarus* are significantly different.

Clemente *et al.* (1985) argue that in Portugal as well as in central and northern Spain, the form with a red tibia and separate femoral spots (3S) are small and live in the wetlands, while the orange tibia shape pale with only a large femoral spot (1S) occupies the arid southern regions. This could therefore be explained by an adaptation to ecological conditions.

According to Hewitt (1985), two forms that have developed adaptations to different environments, considered as their ecological niches, may diverge or acquire a prezygotic isolation mechanism. They probably involve the partner recognition system. Pigmentation of the inner surface of the femur is also subject to varying degrees of variation. This clearly implies a distinct geographical distribution of the ecotypes (Benzara, 2004). But despite apparent differences in size between the two forms, it is difficult to discuss their status because of the geographical polymorphism of the species (Clemente *et al.*, 1987).

CONCLUSION

This morphometric analysis shows that there is a big geographical polymorphism between the populations of *C. barbarus* living in Algeria and Spain. Whether for the 1S living in semi-arid bioclimatic or 3S form belonging to humid climate. Indeed, we have noticed through this study that the size of individuals in *C. barbarus* increases as we move from the north (Spain to the south (Algeria). This is in addition to the great polymorphism that this locust species has known in different aspects: geometric morphometry, the number of ovarioles, sound production, protein and enzyme system, reproduction.

On the basis of all the works carried out on this species for several years and in particular that of Rouibah *et al.* (2016) in which it was shown that the 2 forms of C. barbarus were phylogenetically homogeneous, we can say that the speciation process that started in this insect is not yet complete. We think that the forms with 1S and 3S are none other than subspecies pending further studies which may determine the true status of this highly controversial species.

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