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Biometric comparison of four populations of *Artemia franciscana* Kellogg, 1906 (Crustacea: Anostraca) from Mexico and Peru

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Abstract

The biometric characteristics of the cysts, nauplii and adult stages (male and female) of four populations of *Artemia franciscana* from Mexico (Yavaros and Cuatro Cienegas) and Peru (Virrrila and Chilca) were compared. All organisms used in the study were cultured under identical laboratory conditions. The data showed that the smallest cysts were those of the Virrila population (206.07 ± 4.96 μm). In addition, this population had the lowest values of chorion thickness (5.59 μm) and nauplii length (374.61 ± 9.58 μm). The adult females were discriminated by furcae length, antennule length and head width, which are considered a reflection of the population genetics, while males organisms are discriminated by head width, furcae length and abdomen width and length. Head width, antennule and furcae length are determinated by genetics; while the abdomen length and width are determined by the environmental characteristics. Mexican and Peruvian populations indicated significant differences in total and abdomen length. These differences may provoke interbreeding problems between these *Artemia* populations in the long term. That's why it is necessary to make interbreeding crosses experi-ments with these *Artemia* populations.

Key Words: *Artemia*, biometry, Coastal waters, Inland waters, Mexico, Peru

Introduction

The crustacean *Artemia* lives and reproduces in continental and coastal hyper saline environments around the world, except in the Antarctica (Vanhaecke *et al.* 1987; Triantaphyllidis *et al*. 1998; Van Stappen, 2002). Its wide distribution is mainly due to natural dispersion produced by the wind, migrating birds or introduction made by man (Lenz and Brown, 1991). The habitat where this crustacean develops has physicochemical, climatic and ecological characteristics, which are reflected in the biometric, phenotypic plasticity and/or genetic heterogeneity (Abreu-Grobois, 1987; Beardmore *et al.* 1996; Naceur *et al*., 2011).

In Latin America *Artemia* populations do not have a continuous distribution, actually they are found in isolated biotopes, with temperate to tropical climates (Stella, 1933). Correa and Bückle (1993) have showed that the geographic isolation and the habitat condition induce different morphological changes on *Artemia*, as well as other biological, chemical and physiological characteristics, that allow them to respond to particular environments (Castro *et al.* 1989; Correa and Bückle, 1993; Litvinenko *et al*., 2007; Asem and Restergar-Pouyani, 2008).

Mexico and Peru host different ecosystems where *Artemia* populations inhabit. In Peru, there are nine places registered along the coastal line (Vinatea, 1995). In Mexico, Castro *et al.* (2000, 2001) mentioned that there are 17 places registered with *Artemia*, three in continental water and the rest in coastal zones, both in the Pacific Ocean and in the Gulf of Mexico.

The main objective of the present study was to determine the biometric variation between two *Artemia franciscana* populations collected in Mexico and two from Peru, with thalassohaline and athalassohaline habitat characteristics. Biometric differences were also assessed by comparing hydrated and decapsulated cysts diameter, nauplii length and nine biometrical variables in adult female and eight biometrical variables in adult male.

Materials and methods

The geographical localization of the populations of *Artemia franciscana* considered in this study is shown in Table 1 and Fig. 1. The cysts were collected in 1998, with exception of those from Cuatro Cienegas (Mexico) that were collected in 1995. Before use, all cysts were cleaned by salt density process (Castro *et al.,* 2001) and dried in an oven at 25ºC during 24 hrs. Dried cysts were sieved with metallic sieve with a mesh size of 500 μm. Then the cysts were kept in a freezer at -10°C in the Live Food Laboratory (Universidad Autonoma Metropolitana, campus Xochimilco).

To determine the diameter of the cysts, 0.2 g of dehydrated cysts of each population were hydrated in tap water for an hour, and then, the diameter of 100 cysts was measured with an Olympus microscope model BX50 equiped with an integrated digital camera, and an image analyzer (Olympus Inc., Tokyo, Japan). The same cysts were placed in 0.5 mL of sodium hypochlorite to remove chorion, decapsulating the embryos (Bruggeman *et al.,* 1979) and their diameters measured with the microscope.

To determine the size of the nauplii, 0.2 g of dehydrated cysts of each population were hatched in a 3 L glass beaker with tap water prepared at 40 gL^{-1} salinity with sodium chloride (NaCl), in 24°C, continuous light and aeration. The newly hatched nauplii were fixed by a 5% Lugol´s solution and then their lengths were measured by the microscope.

To determine the biometric characteristics of adult stages (male and female), 0.2 g of dehydrated cysts of each population were hatched. The newly hatched nauplii were put in 200 L plastic cylinders with 160 L of tap water (60 gL^{-1} salinity with NaCl), in 20 ± 2°C and constant aeration. The cylinders were supplied with a mix of two microalgae (*Tetraselmis suecica* and *Chaetoceros müllueri*, in a 1:1 ratio) as food. The organisms were kept under these conditions for 21 days and then, the *Artemia* was filtered through a sieve with mesh size of 2.0 mm. The males and females were separated and 100 organisms were randomly selected from each group, to be photographed and measured. The following biometrical characteristics were detrmined: total abdomen, furcae, antennules lengths, eye diameter and distance between eyes for male and female adults respectively. Also ovisac width in

Tab. 1: Geographical location of *Artemia franciscana* studied sites.

Country	Site	Abreviation	Habitat	Geographic Location
Peru	Chilca	CHIL	Athalassohaline	12º31'S; 76º43'W
	Virrila	VIRR	Thalassohaline	05º52'S; 80º50'W
Mexico	Cuatro Cienegas	CCIEN	Athalassohaline	29°36'N; 99°20'W
	Yavaros	YAV	Thalassohaline	26°40'N; 109°35'W

Fig. 1: Geographical localization of studied *Artemia franciscana* habitats.

females was measured.

A data base was created with the software Microsoft Excel 2010 (Microsoft Corp., Washington, USA). Stem and leaf displays and Box Plot were performed to ensure that the assumption of normality was being met for each data set. A descriptive statistical analysis was made to obtain mean values and standard deviation for each biometrical characteristic. One-way analyses of variance (ANOVA) were used too, to determine if there were significant differences among means (Tatsuoka 1970; Kachigan 1991). The Least Significant Differences (LSD) pairwise comparison Tukey method ($P<0.05$) was used to compare pairs of samples means after having generated ANOVAs for all biometric characters studied. Forward stepwise discriminant analysis was used to assign group membership among male and female *Artemia* individuals by the type of population to which they belong (Mexico or Peru). Type classifications were based on population biometric analysis and classified by their country origin. The SYSTAT 10.2 (Systat Software Inc., California USA) software package was used for statistical analysis.

Results

Cysts, embryos and nauplii

The mean values of the hydrated cysts diameter, decapsulated embryos diameter, chorion thickness of the cysts and nauplii length are presented in Table 2. VIRR population showed the smallest size of the hydrated cysts and decapsulated embryos, as well as the chorion thickness and nauplii size, while the CCIEN population, showed the higher values (Table 2). All populations showed significant differences in hydrated cyst diameter and

Tab. 2: Mean (µm) ± S.D of hydrated cyst and decapsulated cyst diameter, chorion thickness and nauplii length (n = 100).

Population	Hydrated cyst	decapsulated cyst	Chorion thickness	Nauplii length
YAV	229.20^{a} (±8.97)	211.67^{a} (±11.38)	8.76	389.60^{a} (±15.30)
CCIEN	231.21^{b} (±4.40)	212.87^{a} (±6.95)	9.11	472.93^{b} (±26.60)
CHIL	227.08^{c} (±3.39)	212.14^{a} (±5.37)	7.47	453.57^{c} (±29.99)
VIRR	206.07^{d} (±4.96)	194.90^{b} (±3.14)	5.59	374.61^{d} (±9.58)

same letter (in column) shows no significant differences (P<0.05).

Tab. 3: Mean (mm) ± S.D of biometrical characteristics of female *Artemia*.

population	Biometrical Characteristics								
	A	B	C	D	E	F	G	H	I
YAV	7.09^{ab} (±0.37)	3.37^{a} (±0.05)	0.27^{a} (±0.08)	0.62^{a} (±0.04)	0.50^{a} (±0.03)	1.11^{a} (±0.07)	0.53^{a} (±0.03)	0.25^{a} (±0.14)	1.08^{a} (±0.14)
CCIEN	7.89^{c} (±1.50)	3.65^{b} (±0.94)	0.49^{b} (±0.12)	0.62^{a} (±0.16)	0.60^{b} (±0.11)	1.59^{b} (±0.55)	0.57^{b} (±0.09)	0.27^{b} (±0.03)	1.24^{b} (±0.19)
CHIL	7.37^{a} (±0.58)	2.83^{c} (±0.47)	0.19^{c} (±0.03)	0.59^{a} (±0.07)	1.25^{a} (±0.15)	0.46^{c} (±0.03)	0.76^{c} (±0.05)	0.23^{c} (±0.03)	1.17^{c} (±0.08)
VIRR	7.17^{ab} (±0.41)	3.68^{b} (±0.68)	0.19^{c} (±0.02)	0.59^{a} (±0.04)	1.14^{a} (±0.17)	0.61^{b} (±0.09)	0.75^{c} (±0.06)	0.25^{a} (±0.02)	1.18^{c} (±0.07)

A=Total length; B= Abdomen length; C=Furcae length; D=Antennule length; E=Ovisac width; F=Abdomen width; G=Head width; H=Eyes diameter; I=Distance between eyes.
- same letter in column, shows no significant differences (P<0.05).

nauplii lengths (P<0.001). Only in hydrated decapsulated embryo diameter, the populations of YAV, CCIEN and CHIL showed no significant differences (P = 0.996; 0.692 and 0.816 respectively).

The populations that showed the lowest and the highest chorion thickness were VIRR and CCIEN respectively (Table 2).

Adult female

The mean values of the biometric measures of four populations are shown in Table 3. The highest and the lowest values of total length were observed in the Mexican populations (CCIEN = 7.89 mm; YAV = 7.09 mm). In this respect, YAV population did not showed significant difference with VIRR population. There was no significant difference between CCIEN and VIRR populations regarding abdomen length. Peruvian population showed bigger mean values in ovisac width. Mexican populations showed bigger mean values in abdomen width.

In all biometrical values, the ANOVA test showed no significant differences (P>0.05) between Peruvian populations. In contrast, Mexican populations showed significant differences among themselves in all biometrical data. Between Peruvian and Mexican populations, YAV and VIRR populations showed no significant differences in total length and antennule length, ovisac width and eyes diameter. On the other hand, antennule length and abdomen width did not showed significant differences between VIRR and CCIEN populations. Besides, total length and antennule length did not showed significant differences between CHIL and YAV populations; and antennule length did not showed significant differences between CHIL and CCIEN populations.

The discriminant analysis indicated a classification matrix with 100% correctly values in all female populations. With only two discriminate canonical functions 97% of total values were explained. Mexican and Peruvian populations were

discriminated principally with head width, furcae length and antennule length as the biometric variables (Table 4 and Fig. 2).

Tab. 4: Results of the discriminate analysis on the biometric variables measured in *Artemia franciscana* female grouped by geographical origin to which they belong (Mexico and Peru).

Variable	Function 1	Function 2
Total length	3.089	1.148
Abdomen length	-2.869	1.329
Furcae length	-4.504	-2.567
Antennule length	-1.350	3.564
Abdomen width	-0.080	-0.386
Ovisac width	2.270	0.502
Head width	4.646	-2.311
Eyes diameter	-1.345	-1.417
Distance between eyes	Not considered	Not considered
Eigen values	195.050	10.579
Canonical correlation	0.997	0.956

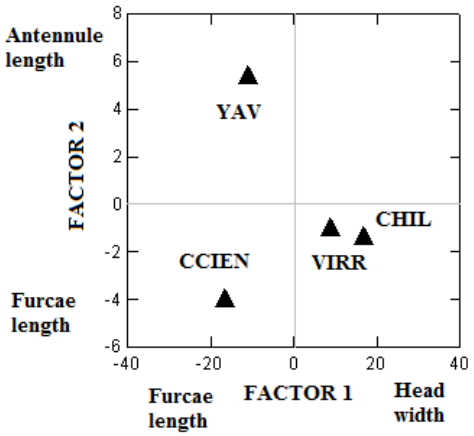

Fig. 2: Group centroids solved by the first two discriminant functions for *Artemia franciscana* females grouped by their geographical origin.

Adult males

In Table 5 are presented the mean and significance values of the biometric variables considered for the adult males.

CCIEN and CHIL populations had the highest and the lowest total length values with 6.40 ± 0.82 mm and 5.30 ± 0.38 mm respectively. The Peruvian populations comparison (CHIL and VIRR) did not showed significant differences in total length, furcae length, head width, eye diameter and distance between eyes. The Mexican populations comparison (YAV and CCIEN) did not showed significant differences in abdomen width variable too. In population biometrical comparison between Peruvian and Mexican populations, VIRR and YAV populations did not show significant differences in abdomen length and width, antennule length and eye diameter.

The results of the discriminant analysis indicated that the classification matrix was 100% correctly values in all male populations. With only two discriminate canonical functions 97% of total values were explained. Mexican and Peruvian populations were discriminated by furcae length, head width, abdomen width and length (Table 6 and Fig. 3).

Discussion

According with Triantaphyllidis *et al.* (1997) SFB population 224.37 ± 0.44 µm of hydrated cyst diameter, 209 ± 7.47µm of decapsulated hydrated cyst diameter, 7.69 µm of chorion thickness and, 426.19 ± 13.91µm of nauplius length. VIRR population has the lower values than SFB population and CHIL, YAV and CCIEN have higher values than SFB.

The Peruvian CHIL population had a higher similarity to that of San Francisco Bay, because both populations live in coastal environments; while VIRR population is from inland waters that would be intervening in the inherent biometric characteristics of this population (Naceur *et al*., 2011; Litvinenko *et al*., 2007; Asem and Rastegar-Pouyani, 2008).

It should be pointed out that our values on the hydrated cysts of the VIRR and CHIL populations, differ from those reported by Salgado (2001) (i.e. 210.5 ± 12.5 µm and of 245.3 ± 1.12 µm, respectively). As well, the same author mentioned that the mean nauplii length of CHIL was 439.00 ± 1.08 µm and VIRR 403.00 ± 0.203µm, showing variances with the data obtained in our research

Tab. 5: Mean (mm) ± S.D of biometrical characteristics of male *Artemia*.

population	Biometrical Characteristics							
	A	B	C	D	E	F	G	H
YAV	5.36^{a} (±0.30)	2.10^{a} (±0.18)	0.25^{a} (±0.05)	0.77^{a} (±0.11)	0.47^{a} (±0.06)	1.35^{a} (±0.86)	0.27^{a} (±0.04)	1.07^{a} (±0.13)
CCIEN	6.40^{b} (±0.82)	2.68^{b} (±0.51)	0.34^{b} (±0.07)	1.20^{b} (±0.24)	0.48^{a} (±0.06)	0.53^{b} (±0.12)	0.36^{c} (±0.07)	1.50^{b} (±0.24)
CHIL	5.30^{c} (±0.38)	2.24^{c} (±0.40)	0.23^{c} (±0.03)	0.88^{c} (±0.08)	0.37^{b} (±0.05)	0.64^{c} (±0.03)	0.29^{b} (±0.03)	1.26^{c} (±.06)
VIRR	5.70^{c} (±0.31)	2.11^{a} (±0.22)	0.23^{c} (±0.02)	0.82^{a} (±0.06)	0.50^{c} (±0.02)	0.65^{c} (±0.04)	0.28^{ab} (±0.01)	1.24^{c} (±0.04)

A=Total length; B= Abdomen length; C= Furcae length; D= Antennule length; E= Abdomen width; F= Head width; G= Eyes diameter; H= Distance between eyes.
- same letter (in column) shows no significant differences ($p<0.05$).

(18.31 µm for the nauplii of CHIL and 25.0 µm for the VIRR). These differences might be possible because of the different equipments used to perform the measurements.

Tab. 6: Results of the discriminate analysis on the biometric variables measured in *Artemia franciscana* male grouped by geographical origin to which they belong (Mexico and Peru).

Variable	Function 1	Function 2
Total length	1.330	2.416
Abdomen length	0.481	-2.293
Furcae length	4.013	-0.971
Antennule length	0.998	-1.136
Abdomen width	-1.754	2.479
Head width	-4.436	-0.354
Eyes diameter	-0.065	0.516
Distance between eyes	-0.637	-0.708
Eigen values	99.517	14.313
Canonical correlation	0.995	0.967

Regarding the relation between the Mexican and Peruvian populations, it is possible to observe significant differences, regardless they are from a coastal environment or an inland water. According to Triantaphyllidis *et al.* (1998) and Van Stappen (2002), these differences should be explained by the distance between the places and the environmental characteristics of each habitat.

Respect the chorion thickness, the Peruvian populations showed lower values, which allow the nauplii to hatch easily, but decreasing the protection against changes in temperatures, UV radiation and continuous hydration and dehydration (Van Stappen, 2002). In addition, there is a problem when sodium hypochlorite is added to decapsulation process, as the eliminator of this layer, because of the low thickness, the decapsulation mechanism may be too fast and the sodium hypochlorite may damage the embryo.

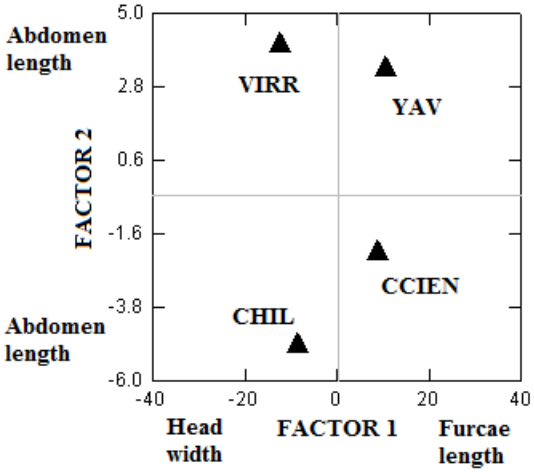

Fig. 3: Group centroids solved by the first two discriminant functions for *Artemia franciscana* males grouped by their geographical origin.

Regarding the adult biometry, the differences between the population of San Francisco Bay and those populations was clearly observed. According

to Triantaphyllidis *et al*. (1997), SFB population showed higher size in all biometric measurements considered in this research. These differences, as mentioned by Browne *et al.* (1984),Vanhaecke *et al.* (1984), Wear and Haslett (1987), Wear *et al.* (1986), Vanhaecke and Sorgeloos (1989), Abatzopoulos *et al.* (1993), Triantaphyllidis *et al.* (1995), Browne and Wanigasekera (2000), Naceur *et al*. (2011), Camargo *et al*. (2004), Naceur *et al*. 2009 and Asem and Rastegar-Pouyani, 2008), is due to the phenotypic and genetic responses of the populations of this crustacean presents to the salinity and the ionic composition of the water, affecting not only the biometric characteristics, but also the maturation, reproduction and life span of the different populations of *Artemia*.

When the discriminate analysis was considered for the females to observe the separation between the populations, these were discriminated mainly by the furcae length, antennule length and head width, which was considered as a reflection of the population genetic (Castro, 2004).

Regarding the male adult populations, the variation among biometric values was less than that of the females. However, the variables than discriminate the populations are the head width, furcae length and abdomen width and length. According to Castro (2004); Naceur *et al*. (2011) and Triantaphyllidis *et al.* (1995), the habitat or culture media salinity has a great influence on these morphometric parameters that most contributed to the discrimination between samples.

Bowen *et al.* (1985) and Triantaphyllidis *et al.* (1998) mentioned that the *Artemia* species from the American Continent are derived from *Artemia franciscana*, however, the four populations studied here presented biometric values significantly different from the population of San Francisco Bay (SFB) and same results were obtained between the populations of Mexico and Peru. According to Gajardo *et al*. (1998), the morphologic differences found in the populations of *Artemia* is a consequence of the natural selection over the species, as a result of the adaptation to different environmental conditions, as individuals with reproductive patterns with disposition to a better adaptation to the environment, always will be favored. As well, Abreu-Grobois and Beardmore (1982); Browne *et al.* (2002) and Gajardo *et al.* (2001) mentioned that the dynamic of the hypersaline environments promotes the genetic differentiation of *Artemia* populations, by restricting the genetic flow and selecting local forms of the population. Then, the differences on the biometry may provoke interbreeding problems between these *Artemia* populations in the long term and that may leading to reproductive isolation.

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