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**Morphometric comparison of two bisexual species of *Artemia*: *Artemia franciscana* Kellogg, 1906 from Mexico and *Artemia urmiana* Günther, 1899 from Lake Urmia**

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**Abstract:** Inland waters *Artemia* populations have specific biological characteristics, due to isolation pattern in their habitat environment. These populations mainly have different ionic compositions in the living environment which is actually described their biological characteristics. Owing to this reason, two bisexual species of *Artemia* from different geographical locations were selected in order to determine morphological variabilities within and among populations. The two species are *A. franciscana* from Mexico containing four populations and *A. urmiana* from Lake Urmia, Iran. Morphological variations were carried out based on cyst and decapsulated cyst diameter, nauplii and adults (males and females) length. Biometry of *A. urmiana* cysts showed bigger size than Mexican cysts. *A. urmiana* nauplii length showed significant outcome with all Mexican populations with the exception of Cuatro Ciénegas. Morphometric analysis of adult *Artemia* did not show significant differences in some variable parameters. Analysis of males and females gave principally discriminant variables in three parameters including head width, distances between compound eyes and length of furca.

**Key Words:** Inland waters, *A. franciscana*, *A. urmiana*, morphometry, discriminant Analysis

## **Introduction**

*Artemia* was described at 18th century and has been extensively studied since the 19th century (Sorgeloos, 1980). When ecological

conditions are adverse in their habitat, they produce forms of resistance eggs called cysts. *Artemia* cysts are hatched under standard

incubation procedure and eventually after 24-24 hours free-swimming nauplii are produced. Nauplii are used as a live food for different aquatic organisms. This cosmopolitan crustacean has a discontinuous distribution around the world and populations are located in isolated habitats from temperate to tropical climates (Stella, 1933). Consequently the ecological, physical and chemical characteristics are widely differed in each habitat (Cole and Browne, 1967; Persoone and Sorgeloos, 1980; Correa and Bückle, 1993). *Artemia* is found in wide distribution areas almost 600 habitat regions throughout the world (Van Stappen, 2002).

*Artemia* populations were restricted at hypersaline coastal and inland water bodies (Persoone and Sorgeloos, 1980), and show great plasticity differences in their life cycle (Lenz and Browne, 1991), morphology (Amat, 1980; Schrehardt, 1987), and biochemical characteristics (Léger *et al.*, 1986). Differences are attributed to different degree of intra population characters and the gene "pool" for each species (Abreu-Grobois, 1987; Gajardo and Beardmore, 1993). Other researchers like Gilchrist (1960), Baid (1963), Vanhaecke and Sorgeloos (1980), Lenz and Dana (1987), (Correa *et al.*, 1993), indicated that geographical isolation and habitat characteristics are formed different *Artemia* phenotypes, with different biological, chemical and physiological characteristics (Erhardt *et al.*, 1971; Amat, 1980; Castritsi and Christodo-

ulopoulou, 1987; Lysenko, 1987; Yaneng, 1987; Castro *et al.*, 1989 and Correa and Bückle, 1993). The geographical distribution of *Artemia* has a correlation with climate condition by which 97% of *Artemia* habitat were classified as an extremely aridity areas (Vanhaecke *et al.*, 1987). In America, there are three bisexual species which including: *Artemia franciscana* Kellogg, 1906; *Artemia persimilis* Piccinelli and Prosdocimi, 1968; and *Artemia monica* Verrill, 1869. Four species are existed in Eurasia including *Artemia urmiana* Günther, 1899; *Artemia sinica* Cai, 1989; *Artemia tibetiana* Abatzopoulos *et al.*, 1998 and parthenogenetic population(s) of *Artemia* (see Asem *et al.*, 2010a).

Inland waters *Artemia* have specific biological characteristics, due to isolation pattern in their habitat areas. These populations mainly have different ionic compositions in the living environment which is actually described their biological characteristics. They are differentiated because of evolutionary, morphology and reproductive performance which is consequently caused speciation process for each species (Asem *et al.*, 2007; Castro *et al.*, 2011).

Therefore, the main goal of current study is to determine morphological variation of two bisexual species, which are isolated by geographical barriers.

## Material and Methods

### Sampling localities

*Artemia* cyst samples used in the present study was provided by *Artemia* cysts bank of Autonoma Metropolitana-Xochimilco University.

The geographical localities and coordinates for each population are shown in Tab. 1.

**Tab. 1: Geographical localities and coordinates of inland water *Artemia* used in the present study.**

Species	Localities	Country	Geographical coordinates
<i>Artemia urmiana</i>	Lake Urmia	Iran	37°36' N-45°30' E
<i>Artemia franciscana</i>	Cuatro Ciénegas de Carranza, Coahuila (CCI)	Mexico	26°56' N-102°05' W
<i>Artemia franciscana</i>	Santo Domingo, Zacatecas (ZAC)	México	23°19' N-101°43' W
<i>Artemia franciscana</i>	Las Salinas de Hidalgo, San Luis Potosí (LSLP)	México	22°37' N-101°43' W
<i>Artemia franciscana</i>	Texcoco, Edo. de México (TEX)	México	19°33' N-99°00' W

### Experimental conditions

For each *Artemia* strain, 0.2 g cyst was taken, hydrated during one hour with tap water and decapsulated in 100 mL of decapsulated solution (50 mL of sodium hypochlorite and 50 mL of salt water at 100 gL<sup>-1</sup> salinity). The decapsulated cysts were collected and transferred to 4 L conical container with water salinity of 35 gL<sup>-1</sup>, light (40 watts white solar light) and continuous aeration during 24 hours. The hatched nauplii for each population were collected and introduced to 200 L container plastic beaker with 160 L of water at 60 gL<sup>-1</sup> salinity. The organisms were fed *ad libitum* with 200 mL of rice bran solution (300 g of rice brine, blended in 4 L of water at 100 gL<sup>-1</sup> salinity and 2 L of *Tetraselmis suecica* culture medium (500,000 cell mL<sup>-1</sup>). The 200 L containers were maintained at 25±2°C temperature, pH 8-10, light (40 watts white solar

tubular focus) and continuous aeration for 21 days of experimental periods.

### Cysts, decapsulated and nauplii morphology

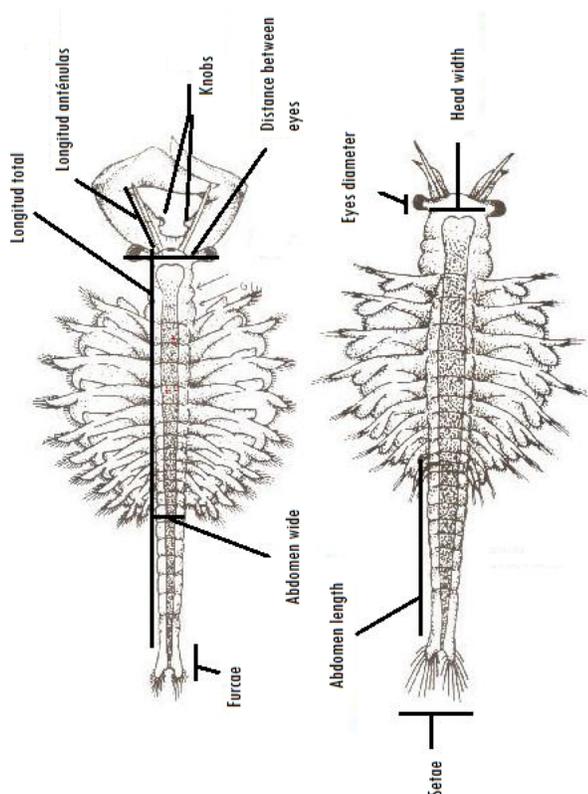
For each population, 0.05 g of cysts hydrated with tap water for one hour and 100 individual cysts were measured. The counted cysts were decapsulated with 10 mL decapsulated solution and measured again. For total length nauplii, 100 nauplii were fixed with Lugol solution (5%) and measured under the microscope.

### Adult (male and female) morphometry

After 21 days of culturing period, 100 females and 100 males were separated and fixed with two droops of acetic acid (CH<sub>3</sub>COOH). The morphometry variables were: total body, abdomen, furca and antennule length, head and ovisac (in females) width, distance and

diameter of compound eyes (Fig.1).

All data for each stage (cyst, decapsulated, nauplii and adults) were taken using an optical microscope SZX12 Olympus® equipped with a camera and Pro-Plus 7.0 (Media Cybernetics®) image software program.



**Fig.1: Morphometry measurements considered for *Artemia* adults (male and female)**

### Statistical analysis

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 all data set. Analyses of variance (ANOVA) were also performed to determine significant differences between populations (Tatsuoka 1970, Kachigan 1991). The least significant differences (LSD) and pair-wise comparison (Tukey method;  $P < 0.05$ ) test were used to compare significant differences between populations. Type classifications were based on population and grouped for biometric analysis according to the specific salinity of their culture medium (Sokal and Rohlf 1981, Kachigan 1991). Statistical analyses were carried out using SYSTAT 13 software package (Systat Software Inc., Calif. EEUU).

## Results

### Morphometry of cyst, decapsulated and nauplii length

*A. urmiana* showed the highest mean value of cyst diameter in comparison to four Mexican populations. The diameter of cyst and decapsulated cyst of *A. urmiana* provided significant outcome with four Mexican populations (Tab. 2). The mean value of nauplii length of *A. urmiana* gave significant outcome with all Mexican populations except CCI ( $P=1.000$ ). The mean values of cyst diameter, decapsulated cyst diameter and nauplii length of all tested populations are depicted in Table 2.

Tab. 2: Mean values ( $\pm$ S.D.) of cysts diameter and nauplii length for all examined populations

Populations	Cyst diameter ( $\mu$ m)	Decapsulated cyst Diameter ( $\mu$ m)	Nauplii length ( $\mu$ m)
Lake Urmia, Iran	259.11 a ( $\pm$ 8.02)	249.14 a ( $\pm$ 10.75)	469.17 a ( $\pm$ 12.07)
Cuatro Ciénegas, Coahuila (CCI)	230.36 b ( $\pm$ 2.52)	213.21 b ( $\pm$ 4.50)	468.45 a ( $\pm$ 13.78)
Santo Domingo, Zacatecas (ZAC)	230.58 b ( $\pm$ 2.20)	226.36 b ( $\pm$ 4.01)	430.62 b ( $\pm$ 10.26)
Las Salinas, San Luis Potosí (LSLP)	231.29 b ( $\pm$ 6.07)	217.59 b ( $\pm$ 5.35)	425.84 b ( $\pm$ 7.26)
Texcoco, State of México (TEX)	230.86 b ( $\pm$ 2.33)	212.54 b ( $\pm$ 2.49)	423.62 b ( $\pm$ 18.45)

-Same letters shows non-significant values with respect to *A. urmiana*

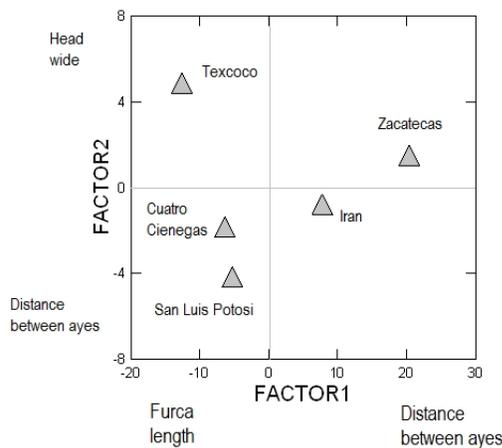


Fig. 2: Discriminant analysis of adult female *Artemia*

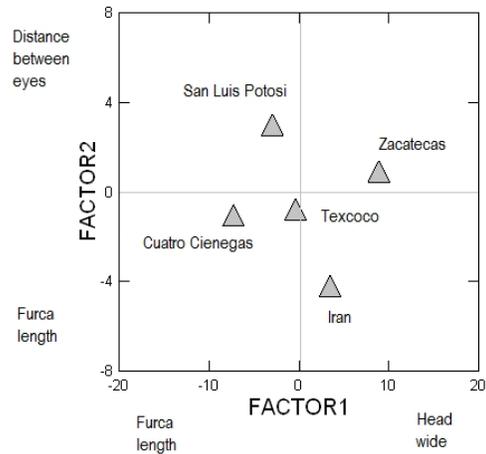


Fig. 3: Discriminant analysis of adult male *Artemia*

**Morphometry of adult *Artemia* (female)**

According to statistical analysis, *A. urmiana* showed non-significant values with CCI in abdomen length and width. There is no significant value with ZAC in total length body, antennules length and diameter of compound

eyes. *A. urmiana* showed significant differences with Mexican populations in head width and furca length ( $P < 0.001$ ). *A. urmiana* did not show significant differences with LSLP only in distance between compound eyes and ovisac width. Finally, there is significant value with TEX

**Tab. 3: Morphometry means values ( $\pm$ S.D.) of adult female *Artemia* for all examined populations**

Population	TL	A L	A W	AnL	DE	CE	HE	OW	FL
Lake Urmia, Iran	9.229 a ( $\pm$ 1.19)	3.658 a ( $\pm$ 0.37)	0.631 a ( $\pm$ 0.03)	0.575 a ( $\pm$ 0.04)	1.263 a ( $\pm$ 0.15)	0.251 a ( $\pm$ 0.03)	0.814 ( $\pm$ 0.14)	1.424 a ( $\pm$ 0.31)	0.216 a ( $\pm$ 0.01)
Cuatro Ciénegas, Coahuila (CCIEN)	7.885 b ( $\pm$ 1.50)	3.649 a ( $\pm$ 0.94)	0.601 a ( $\pm$ 0.11)	0.616 b ( $\pm$ 0.09)	1.243 b ( $\pm$ 0.18)	0.268 b ( $\pm$ 0.03)	0.566 ( $\pm$ 0.09)	1.592 b ( $\pm$ 0.54)	0.490 b ( $\pm$ 0.12)
Zacatecas (ZAC)	10.093 a ( $\pm$ 0.33)	3.727 b ( $\pm$ 0.27)	0.643 b ( $\pm$ 0.01)	0.579 a ( $\pm$ 0.04)	1.384 b ( $\pm$ 0.04)	0.246 a ( $\pm$ 0.04)	0.932 ( $\pm$ 0.04)	1.640 b ( $\pm$ 0.10)	0.156 a ( $\pm$ 0.01)
Las Salinas, San Luis Potosí (LSLP)	7.499 b ( $\pm$ 0.76)	3.685 b ( $\pm$ 0.64)	0.496 b ( $\pm$ 0.05)	0.606 b ( $\pm$ 0.08)	1.114 a ( $\pm$ 0.15)	0.227 b ( $\pm$ 0.03)	0.461 ( $\pm$ 0.09)	1.186 a ( $\pm$ 0.25)	0.344 b ( $\pm$ 0.08)
Texcoco, México State (TEX)	6.567 b ( $\pm$ 0.88)	3.159 a ( $\pm$ 0.63)	0.424 b ( $\pm$ 0.07)	0.513 b ( $\pm$ 0.08)	0.846 b ( $\pm$ 0.21)	0.220 b ( $\pm$ 0.03)	0.594 ( $\pm$ 0.12)	0.870 b ( $\pm$ 0.23)	0.456 b ( $\pm$ 0.12)

-TL = Total length, AL = Abdomen Length, AW = Abdomen Width, AnL = Antennule length, DE = Distance between compound eyes, CE = Compound eyes Diameter, HW = Head Width, OW = Ovisac width, FL = Furca length

-Same letters shows non-significant values with respect to *A. urmiana*

**Tab. 4: Canonical discriminant functions standardized for female *Artemia***

	Function 1	Function2
Eigen values	151.680	10.557
Canonical correlations	0.997	0.956
Cumulative proportion of total dispersion	0.898	0.960
Morphometry variables		
Total length	1.281	0.825
Abdomen length	-1.016	-0.768
Abdomen width	1.082	-0.502
Antennule length	-0.492	-1.075
Distance between compound eyes	1.756	-1.638
Eye diameter	0.038	0.009
Head width	1.476	1.943
Ovisac width	0.324	-0.182
Furca length	-4.250	1.364

in all parameters except abdomen length. Table 3 gives mean value of all parameters for all examined populations.

Regarding to discriminant analysis, the

Jackknifed classification matrix showed 100 % correct values in all populations. Canonical discriminant functions standardized by all parameters are shown Table 4. Three principal-

ly morphometric parameters were able to discriminate all populations: distance between compound eyes, head width and furca length. Canonical discriminant functions using these parameters were shown in Fig. 2.

### **Morphometry of adult *Artemia* (male)**

According to statistical analysis, *A. urmiana* showed significant differences with all Mexican populations in total body ( $P < 0.001$ ). *A. urmiana* showed non-significant differences with ZAC in abdomen length and width, as well as head width. There is no significant difference with LSLP in abdomen and furca length. *A. urmiana* showed non-significant values with TEX in antennule length, distance between compound eyes and diameter. Table 5 gives mean value of all parameters for all examined populations.

Regarding to discriminant analysis, the Jackknifed classification matrix showed 100 % correct classification for Mexican and 86 % for *A. urmiana* population. Some values are similar with TEX and ZAC populations. Canonical discriminant functions standardized by all parameters are shown Table 6. Three principally morphometric parameters were able to discriminate all populations: distance between compound eyes, head width and furca length. Canonical discriminant functions using these parameters were shown in Fig. 3.

### **Discussion**

The genus *Artemia* shows wide variations of ecological effects on its biological processes which are depends on physicochemical conditions of the saline lake. The variable characters are salinity level and concentration, temperature, light intensity or quantity and quality of food. Due to this reason, laboratory studies are carried out in order to minimize the effects of variables, allowing them possible to do biometric comparison with and between populations.

This is not contrary with the statements of Asem and Rastegar-Pouyani (2008), where they mentioned that samples for biometric evaluation must be taken from their natural habitats, because the ecological speciation of salty composition and concentration can modify not only biometry characteristics but also morphology of adult *Artemia* (Bowen *et al.*, 1985, 1988; Hontoria and Amat, 1992). Laboratory values cannot deviate from the correct knowledge of biometry comparison studies. We agree about deviation knowledge of ecological speciation process in different population of *Artemia*. Concerning to significant differences in morphology and biometry, comparative studies are considered for minimizing the differences.

With respect to cyst and nauplii size, *A. urmiana*, have the biggest diameter of cyst and nauplii size, but it can be observed that Cuatro Ciénegas nauplii size has no significant

**Tab. 5: Morphometric mean values ( $\pm$ S.D.) of adult male *Artemia* for all examined populations**

Population	TL	AL	AW	AnL	DE	CE	HW	FL
Lake Urmia, Iran	7.922 ( $\pm$ 1.10)	3.133 a ( $\pm$ 0.34)	0.543 a ( $\pm$ 0.05)	0.878 a ( $\pm$ 0.11)	1.422 a ( $\pm$ 0.22)	0.273 a ( $\pm$ 0.03)	0.811 a ( $\pm$ 0.08)	0.195 a ( $\pm$ 0.04)
Cuatro Cienegas, Coahuila (CCI)	6.400 ( $\pm$ 0.82)	2.677 b ( $\pm$ 0.50)	0.478 b ( $\pm$ 0.05)	1.198 b ( $\pm$ 0.24)	1.501 b ( $\pm$ 0.23)	0.360 b ( $\pm$ 0.06)	0.527 b ( $\pm$ 0.11)	0.345 b ( $\pm$ 0.07)
Zacatecas (ZAC)	7.932 ( $\pm$ 1.10)	2.851 a ( $\pm$ 0.55)	0.501 a ( $\pm$ 0.07)	0.741 b ( $\pm$ 0.11)	1.469 b ( $\pm$ 0.18)	0.242 b ( $\pm$ 0.03)	0.795a ( $\pm$ 0.09)	0.139 b ( $\pm$ 0.02)
Las Salinas, San Luis Potosí (LSLP)	6.252 ( $\pm$ 0.73)	2.791 a ( $\pm$ 0.46)	0.455 b ( $\pm$ 0.04)	1.094 b ( $\pm$ 0.18)	1.421 b ( $\pm$ 0.31)	0.319 b ( $\pm$ 0.06)	0.511 b ( $\pm$ 0.15)	0.230 a ( $\pm$ 0.05)
Texcoco, México State (TEX)	6.454 ( $\pm$ 0.53)	2.652 b ( $\pm$ 0.52)	0.403 b ( $\pm$ 0.06)	0.880 a ( $\pm$ 0.16)	1.187 a ( $\pm$ 0.22)	0.278 a ( $\pm$ 0.04)	0.567 b ( $\pm$ 0.10)	0.210 b ( $\pm$ 0.04)

-TL = Total length, AL = Abdomen Length, AW = Abdomen Width, AnL = Antennule length, DE = Distance between compound eyes, CE = Compound eyes Diameter, HW = Head Width, FL = Furca length

-Same letters shows non-significant values with respect to *A. urmiana*

**Tab. 6: Canonical discriminant functions standardized for male *Artemia***

	Function 1	Function2
Eigen values	32.787	4.557
Canonical correlations	0.985	0.906
Cumulative proportion of total dispersion	0.790	0.900
Morphometry variables		
Total length	0.707	0.101
Abdomen length	-0.295	0.025
Abdomen width	0.324	-0.380
Antennule length	-0.062	1.347
Distance between compound eyes	0.026	2.426
Compound eye diameter	-1.016	0.330
Head width	2.102	-1.539
Furca length	-1.756	-2.381

differences with *A. urmiana* nauplii. Cysts diameter values of *A. urmiana* collected from natural habitat shows a variation of  $247.6 \pm 14.5 \mu\text{m}$  to  $259.3 \pm 11.3 \mu\text{m}$  (Asem *et al.*, 2007). Our results are compatible with those samples taken by Asem *et al.* (2007) at N3-1

geographical region in Lake Urmia. The cysts diameter and nauplii length values are congruent with Pilla and Beardmore (1994) and Asem *et al.* (2007). *A. urmiana* also has the widest range of variation in the genus *Artemia* (Asem *et al.*, 2007). These differences can be

attributed to seasonal fluctuations in physico-chemical parameters and food availability in different sites or stations or regions of Lake Urmia that cysts were taken (Abatzopoulos *et al.*, 2006; Asem *et al.*, 2007; Asem *et al.*, 2010b).

With respect to adult males and females biometry, we agree with Asem and Rastegar-Pouyani (2007, 2010c), who displayed that it is important to analyze them separately because they are sexually dimorphic. Studies of sexual dimorphism in *Artemia* both in natural habitats and laboratory conditions are very important to understand the morphological differentiation of *Artemia* population or specie (Asem and Rastegar-Pouyani, 2007). This sexually dimorphic characteristic can also be observed in *A. franciscana* populations (Camargo *et al.*, 2003). The females *Artemia* gave bigger values than males *Artemia*. Asem *et al.* (2007, 2010c) shown that in most animals, the males are bigger than females, and this dimorphism is a sexual selection consequence for competitive advantages to mating. In the reversed sexual dimorphism, it is considered as a mating advantage because the female carries the male during copulation. That's why female body is larger and is an advantage for surviving the mating process (Asem and Rastegar-Pouyani, 2007; Asem *et al.*, 2010c).

With respect to discriminant analysis, in both cases (male and female), two discriminant function explain more than 90% of the

information and all populations were 100% correctly classified in their original grouped cases. This result was also confirmed by results of Camargo *et al.* (2003) and Asem and Rastegar-Pouyani (2008), indicating classification based on male characters provides better group membership than females. The biometric comparison of different species at the same salinity, allows discriminate populations using furca length, head width and distance between compound eyes. These characters are studied by genetic expression of population. Biometry variables such as total length, abdomen length and width, can be modified by ionic composition or salt concentration in culture medium and cause an increasing or decreasing in growth biometry in populations or *Artemia* species, but these changes were made at same rate when the populations or species were cultivated at same ionic composition or salt concentration. That's why it is possible we can found biometrical variables with no significant differences between these two studied species.

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