COMPARISON OF THE DIAMETER OF THE FRONTAL KNOBS IN *ARTEMIA URMIANA* GÜNTHER, 1899 (ANOSTRACA)

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ABSTRACT

In four samples of *Artemia urmiana* Günther, 1899 from Urmia Lake, Iran, the following parameters were compared: the diameter of the frontal knobs (right and left), and the respective ratios of that diameter with total body length and head width. The results do not denote significant differences in frontal knob diameter that would agree with similar results observed in frontal knob diameter in populations of *A. franciscana* Kellogg, 1906 and *A. persimilis* Piccinelli & Prosdocimi, 1968. However, we report differences in the other parameters studied for each of the populations studied, that could be explained by the environmental heterogeneity of the studied site. Ecological, biogeographical, and systematic issues related with the results are discussed.

RESUMEN

Para cuatro poblaciones de *Artemia urmiana* Günther, 1899 del lago Urmia, Irán, se compararon los siguientes parámetros: diámetro de los lóbulos frontales (derecho e izquierdo), y las razones respectivas de los diámetros con el largo total del cuerpo y el ancho de la cabeza. Los resultados no mostraron diferencias significativas entre los diámetros del lóbulo frontal, lo cual sería similar a resultados observados para poblaciones de *A. franciscana* Kellogg, 1906 y *A. persimilis* Piccinelli & Prosdocimi, 1968. Por otro lado, hubo diferencias significativas para los otros parámetros estudiados para las poblaciones estudiadas, lo cual se debería a la heterogeneidad ambiental del sitio estudiado. Se discutieron tópicos de ecología, biogeografía y sistemática relacionados con los resultados obtenidos en el presente estudio.

INTRODUCTION

The genus *Artemia* Leach, 1819 is cosmopolitan and includes seven species, with various geographically widespread strains (Triantaphyllidis et al., 1998). The

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origin of populations can be natural through cyst dispersal by water birds or wind, or artificial through inoculation of cysts in salt works (Sorgeloos et al., 1986). *Artemia* strains can be sexual, such as in the American and in some European and Asiatic populations, and there are parthenogenetic populations, distributed mainly throughout Asia and Europe (Triantaphyllidis et al., 1998). *Artemia* has a remarkable genetic variability that can be expressed in various phenotypic characteristics, such as morphometry, growth rate, or molecular composition (Gajardo et al., 1995, 1998, 2004; Triantaphyllidis et al.,1997a, b). Such variations constitute a first step in the geographical isolation of strains, and the allopatry is often associated with strong environmental heterogeneity that consequently generates genetic isolation concurrent with differences in the expression of phenotypes, which may eventually lead to reproductive isolation, i.e., the origin of potentially new species (Gajardo et al., 1995, 1998, 2004; De los Ríos & Zúñiga, 2000).

In part, reproductive isolation is associated with mechanisms relating to reproduction, such as the frontal knobs in *Artemia* males, two protuberances that are introduced into a pair of cavities located in the posterior zone of the female ovisac, similar to a specific "key-and-lock" mechanism (Wolfe, 1980; Wilson et al., 1993). Considering the importance of reproductive isolation in *Artemia* the frontal knob can be used for species discrimination (De los Ríos & Zúñiga, 2000). The first comparative study of frontal knob morphometry for *Artemia* strains mainly involved Chilean and Argentinean populations, and established significant differences between populations of *A. franciscana* Kellogg, 1906 and *A. persimilis* Piccinelli & Prosdocimi, 1968 (cf. De los Ríos & Zúñiga, 2000). Also, the descriptions by Mura et al. (1989a, b), Mura (1990), and Mura & Brecciaroli (2004) indicate that American *Artemia* populations and bisexual species from Asia, including *A. urmiana*, have subspherical frontal knobs.

The present study was carried out with strains of *A. urmiana* Günther, 1899, which is a sexual species endemic to Urmia Lake, Iran, a permanent, saline lake. Recently, this lake underwent salinity changes due to climate change and human intervention (Abatzopoulos et al., 2006). The aim of this study is to compare the diameter of the frontal knobs in four samples of *A. urmiana*, in order to determine possible dissimilarities between the samples.

MATERIAL AND METHODS

Site description. — Urmia Lake is a large, shallow lake (approx. 140 × 80 km area, on average ca. 16 m deep) located in the north of Iran (45°40′N 37°20′E, fig. 1), it is hypersaline and oligotrophic, and located at 1250 m above sea level. Recently, human intervention has caused alterations in the lake's morphometry,

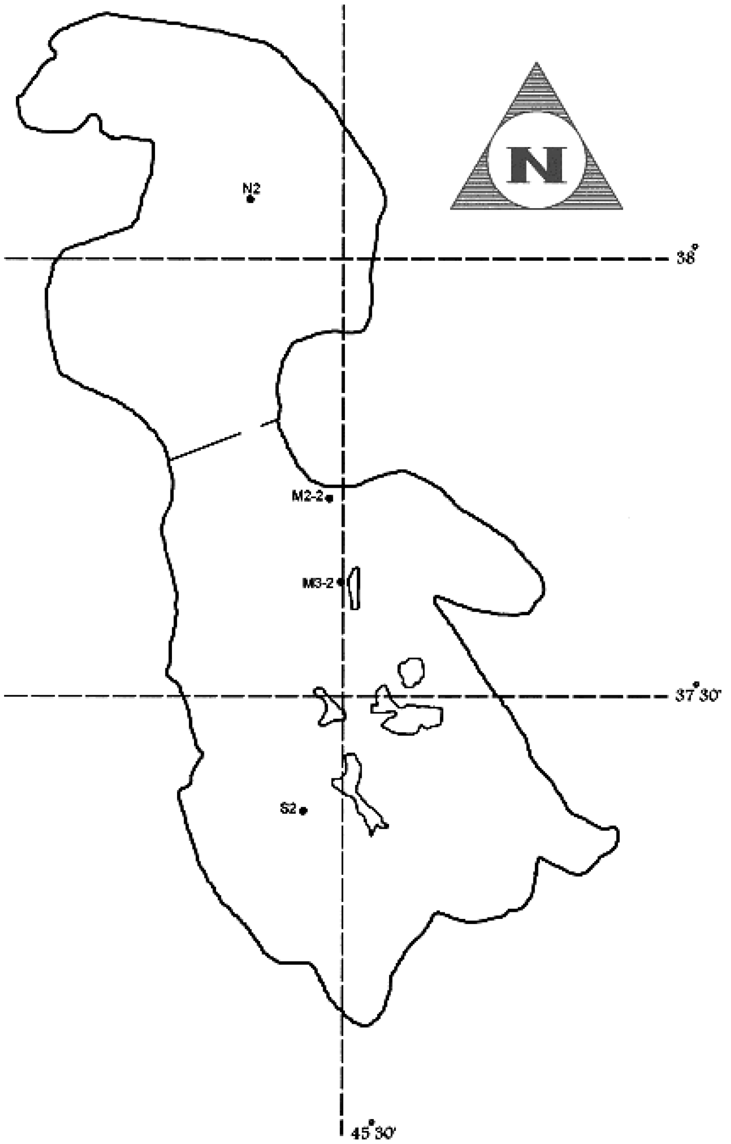

Fig. 1. Geographical location of the sites studied in the present paper, in Urmia Lake, Iran: N2, M2-2, M3-2, and S2.

and in that of its surrounding effluent rivers. Four samples of *Artemia* were collected at different sites, and salinity and pH were recorded. The sites are marked (from north to south), as N2, M2-2, M3-2, and S2, respectively. Adult specimens were collected and fixed with formaldehyde, and 30 male specimens of *A. urmiana* were selected from each site (Asem et al., 2007). For each specimen, measurements of total body length (TL), and head width (HW) were taken (cf. Amat, 1980;

Hontoria & Amat, 1992), as well as the left and right frontal knob diameter (LKD and RKD, respectively) (cf. De los Ríos & Zúñiga, 2000), and of each frontal knob (left and right), the ratios with head width (LKD/HW and RKD/HW) and total length (LKD/TL and RKD/TL), were determined.

The information available on the frontal knobs was analysed in the following steps: the homogeneity of variances was verified using a Levene test for determining the prerequisite condition for a parametric test (Anderson & Underwood, 1997). Then a Principal Component Analysis (PCA) was applied to determine the most important variables to discriminate the populations studied. All statistical analyses were done with the software Xlstat.

RESULTS

The ecological parameters observed when the wild specimens of *Artemia urmiana* were collected, invariably indicated hypersaline waters, and in general low differences in pH, salinity, depth, and transparency values for each station sampled (table I). The results revealed variation in the homogeneity of all parameters studied (table I). The results of the correlation matrix denoted significant, direct correlations of LKD/HW with LKD, LKD/HW with RKD, RKD/HW with LKD, RKD/HW with RKD, and RKD/HW with LKD/HW (table II). A significant, inverse relation was observed of LKD/TL with TL, RKD/TL with TL, LKD with HW, RKD with HW, LKD/HW with HW, and RKD/HW with HW (table II). The results of the PCA revealed a contribution of 97.34% of the variables studied, the first axis contributing for 63.45%, and the main contributing factors being HW, LKD, RKD, LKD/HW, and RKD/HW, with a contribution between 15.199 and 19.112% (table II). The second axis contributed for 33.890%, and the main contributing variables were TL with 25.100%, LKD/TL with 30.948%, and RKD/TL with 30.444% (table II). The results of the PCA indicate that the populations studied are markedly different: the N2 population has high values of RKD, LKD, LKD/HW and RKD/LW and a low value of HW; the M3-2 population has high values of RKD/TL and LKD/TL and a low value of TL; the M2-2 has a high value of TL and low values of LKD/TL and RKD/TL; finally, the S2 population has a high value of HW, and low values of RKD, LKD, LKD/HW, and RKD/LW (fig. 2).

DISCUSSION

The results obtained agree with similar results for American brine shrimp species that have a wide range in frontal knob diameters (De los Ríos & Zúñiga, 2000). On this basis, the frontal knobs would provide a powerful tool to study

TABLE I

Environmental characteristics for stations sampled in Urmia Lake, morphometric parameters (average ± standard error; in mm), and results of statistical analysis ("*p*" values lower than 0.05 denote significant differences) for *Artemia urmiana* Günther

	N2	M3-2	M2-2	S2
Salinity (g/l)*	260	260	274	245
Salinity (g/l)**	268	260	240	256
pH*	7.34	7.26	7.23	7.30
pH**	7.34	7.30	7.30	7.35
Depth (m)	2.2	4.1	2.7	2.7
Transparency (m)	1.8	2.5	0.5	0.5

Morphometric information and results of homocedasticity test and ANOVA (previous to discriminant analysis)

	N2	M3-2	M2-2	S2	Levene statistic
TL	10.3 ± 0.2	9.0 ± 0.2	13.2 ± 0.2	9.6 ± 0.1	0.785; $p < 0.504$
HW	0.650 ± 0.011	0.670 ± 0.009	0.662 ± 0.011	0.710 ± 0.009	0.306; $p < 0.821$
LKD	0.202 ± 0.003	0.202 ± 0.005	0.202 ± 0.004	0.195 ± 0.004	1.527; $p < 0.211$
RKD	0.202 ± 0.003	0.195 ± 0.005	0.195 ± 0.004	0.188 ± 0.004	1.566; $p < 0.201$
LKD/TL	0.020 ± 0.001	0.023 ± 0.001	0.015 ± 0.001	0.020 ± 0.001	2.243; $p < 0.087$
RKD/TL	0.020 ± 0.001	0.022 ± 0.001	0.015 ± 0.001	0.020 ± 0.001	1.920; $p < 0.130$
LKD/HW	0.312 ± 0.006	0.302 ± 0.007	0.309 ± 0.009	0.275 ± 0.006	1.291; $p < 0.281$
RKD/HW	0.313 ± 0.006	0.292 ± 0.007	0.297 ± 0.008	0.266 ± 0.006	1.113; $p < 0.347$

Abbreviations: TL, total length; HW, head width; LKD, left frontal knob diameter; RKD, right frontal knob diameter; LKD/TL, ratio left frontal knob diameter/total length; RKD/TL, ratio right frontal knob diameter/total length; LKD/HW, ratio left frontal knob diameter/head width; RKD/HW, ratio right frontal knob diameter/head width.

and discriminate *Artemia* populations, considering the role of these structures in mechanisms of reproductive isolation (De los Ríos & Zúñiga, 2000). If we considerer the results obtained in the present study, the *A. urmiana* samples show morphological differences (Asem et al., 2007) (table I; fig. 2) that could be explained by the existence of environmental heterogeneity in the habitats of the studied populations (Abatzopoulos et al., 2006). *A. urmiana* only occurs in Urmia Lake (Abatzopoulos et al., 2006; Baxevanis et al., 2006), and it can coexist there with *A. parthenogenetica* Bowen & Sterling, 1978 (cf. Abatzopoulos et al., 2006; Agh et al., 2007).

If we considerer that Urmia Lake is a relatively heterogeneous habitat due to its natural conditions and due to human intervention (Abatzopoulos et al., 2006), those conditions could probably be the cause of the variation observed in the morphometric parameters studied as noted in the present study, between the four widely separated sites sampled. The results indicate that the frontal knob diameter as a method to study population heterogeneity and potential reproductive isolation

TABLE II

A, Correlation matrix of parameters of *Artemia urmiana* Günther morphometry considered in the present study; values in **bold** denote significant correlations ($p < 0.05$); and, B, percentages of importance of the morphometric parameters considered in the present study

A

Variables	HW	LKD	RKD	LKD/TL	RKD/TL	LKD/HW	RKD/HW
TL	−0.371	0.331	0.154	**−0.970**	**−0.985**	0.431	0.300
HW		**−0.949**	**−0.942**	0.178	0.210	**−0.996**	**−0.986**
LKD			0.816	−0.101	−0.167	**0.964**	**0.888**
RKD				0.000	0.000	**0.906**	**0.983**
LKD/TI					**0.993**	−0.232	−0.129
RKD/TL						−0.272	−0.143
LKD/HW							**0.966**

B

	F1	F2
TL	6.260	25.100
HW	18.769	1.740
LKD	16.514	2.125
RKD	15.199	5.931
LKD/TL	2.951	30.948
RKD/TL	3.436	30.444
LKD/HW	19.112	0.873
RKD/HW	17.758	2.839

Abbreviations: TL, total length; HW, head width; LKD, left frontal knob diameter; RKD, right frontal knob diameter; LKD/TL, ratio left frontal knob diameter/total length; RKD/TL, ratio right frontal knob diameter/total length; LKD/HW, ratio left frontal knob diameter/head width; RKD/HW, ratio right frontal knob diameter/head width.

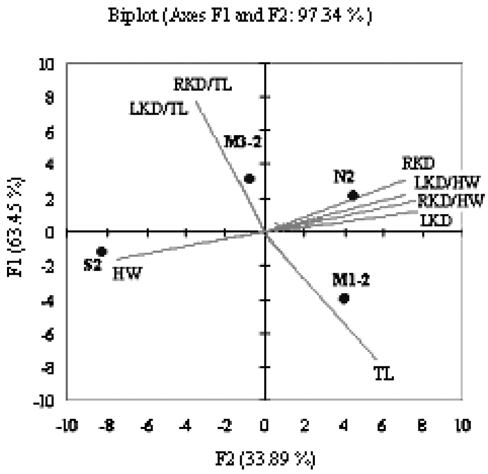

Fig. 2. Principal Component Analysis of the *Artemia urmiana* Günther, 1899 populations studied. For explanation see text.

of *Artemia* populations as suggested by De los Ríos & Zúñiga (2000), can be used for *A. urmiana* samples, but as a complement to morphological characters, cyst biometry (Abatzopoulos et al., 2006; Asem et al., 2007), and genetic information (Yarmohammadi & Pourkazemi, 2004; Baxevanis et al., 2006).

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