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Morphological Differentiation of *Artemia urmiana* Günther, 1899 (Crustacea: Anostraca) in Different Geographical Stations from the Urmia Lake, Iran

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Abstract: The main objective of this study was to compare morphological differentiation between samples of *Artemia urmiana* in different stations from Urmia Lake. Male and female samples were analyzed separately because they are sexually dimorphic. Principal Components Analysis shows that male samples were almost clustered in two groups and these groups were separated. This finding was also confirmed by Discriminant Function Analysis. According to Principal Components Analysis the female samples were almost clustered in one group in all sites and it was not possible to make any separation among them. But Discriminant Function Analysis shows 2 separated groups. Therefore, according to morphological characters, there are minimum two populations of *Artemia urmiana* in the Urmia Lake.

Key words: *Artemia urmiana*, Urmia Lake, Iran, morphological differentiation, Principal Components Analysis, Discriminant Function Analysis

INTRODUCTION

The genus *Artemia* (Crustacea: Anostraca) is a complex of bisexual and parthenogenetic species, which have very similar morphological characters. *Artemia* has widely distribution on the 5 continents in many salt lakes, coastal lagoons and solar salt-works (Van staapen, 2002). The Urmia Lake is the second large lake (average total surface 5000 km2) in Iran, which is located in West Azerbaijan Province. It is the habitat of the endemic *Artemia urmiana* Günther, 1899. This characteristic was confirmed by Clark and Bowen (1976) who demonstrated the reproductive isolation of the species from other bisexual strains. Van Stappen (2002) prepared last check list of distribution and zoogeography of *Artemia*, in this manuscript many of specimens introduced as unknown population and labeled by "?" symbol. This shows that taxonomy and systematics of *Artemia* is still puzzled The most relevant methods are comparison of biometrical and morphological characteristics, electrophoretic patterns of different isozymes, cross-fertility tests and electron microscopic survey of morphology such as frontal knob and penis (Hontoria and Amat, 1992; Abreu-Grobois and Beardmore, 1982; Mura, 1990; Triantaphyllidis *et al*., 1997a,b; Torrentera and Belk, 2002).

Brine shrimp *Artemia* is an economical taxon which the main application of its have been shown in aquaculture industry (Sorgeloos, 1980; Sorgeloos *et al*., 1998, 2001; Bengtson *et al*., 1991). According to base concepts of biosystematics, species and populations have special characters therefore, biosystematical studies can lead to useful economical approach.

In this study, our objective is to study morphological differentiation of *A. urmiana* in different geographical locations and to investigate if there are isolated populations of *A. urmiana* in the Urmai Lake.

MATERIALS AND METHODS

Field study: In this study 4 stations were selected in the middle, northern and southern parts of the Urmia Lake (Fig. 1). Four primary ecological factors were measured for each station: Salinity (0.5 m from surface and 0.5 m from depth), pH (0.5 m from surface and 0.5 m from depth), depth and transparency. Table 1 shows chemical comparison of the Urmia Lake water, Dead Sea, Great Salt Lake and oceans.

Morphological study: Male and female samples were analyzed separately because they are sexually dimorphic. Study of sexual dimorphism is very important in stydy of morphological differentiation (Asem *et al*., 2005).

Thirty male and female specimens of *Artemia urmiana* were randomly collected from each of the four different harvesting sites in north, south and in the middle of the Urmia Lake: N2; M3-2; M1-2; S2 (Fig. 1). Twelve characters for males and thirteen for females were measured (total length, abdominal length,morphological

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Table 1: Chemical comparison of Urmia Lake water with Dead Sea, Great Salt Lake and oceans (Daneshvar and Ashasi sorkhabi, 1997)

	Concentration of compositionaccording to weight percent				g100/g soluble composition			
Chemical Compositions	G.S.L*	Dead Sea	Urmia Lake	Oceans	G.S.L*	Dead Sea	Urmia Lake	Oceans
Chloride	14.1	17.5	12.40	1.94	55.2	65.1	57.6	55.7
Sulfate	2.0	0.7	0.98	0.27	7.8	2.6	4.5	7.8
Sodium	7.6	3.3	7.43	1.06	29.8	12.3	34.5	30.6
Magnesium	1.1	3.4	0.54	0.13	4.6	12.6	2.5	3.7
Potassium	0.7	0.6	0.12	0.04	2.7	2.2	0.6	1.1
Calcium	0.016	1.4	0.06	0.04	0.06	5.2	0.3	1.1

*G.S.L: Great Salt Lake2

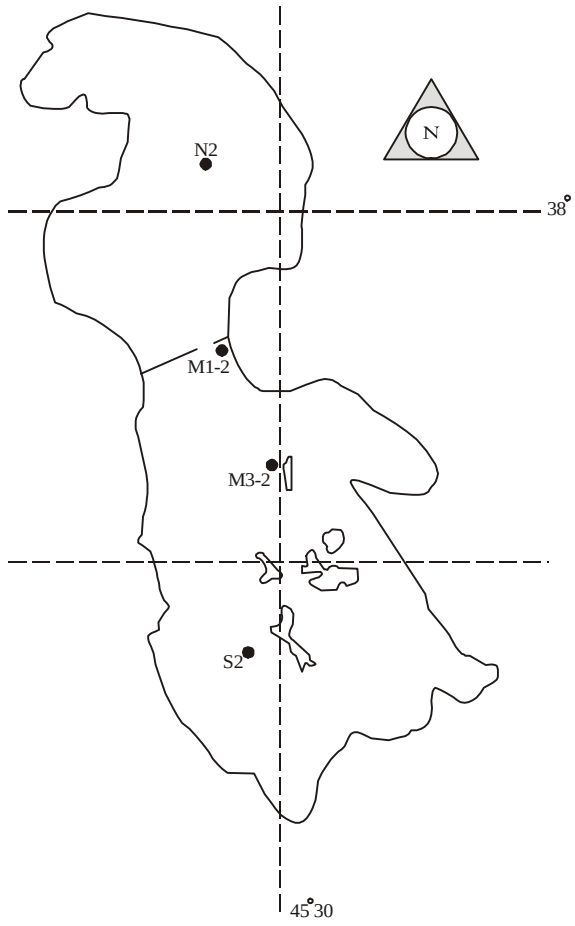

Fig. 1: Geographical location of the studied area, Urmia Lake-Iran

head width, distance between compound eyes, diameter of left eye, diameter of right eye, abdominal width, distance between 3rd abdominal segment to 8th abdominal segment, length of telson, length of furca, length of right antenna, length of left antenna, ovisac width) and number of setae per furca were counted. (Hontoria and Amat 1992, Pilla and Beardmore, 1994; Triantaphyllidis et al., 1997b; Cohen et al., 1999; Zhou et al., 2003; Camargo *et al.,* 2003; Amat *et al.,* 2005). Morphometric and meristic characters used in this study are shown in Fig. 2 and Table 2.

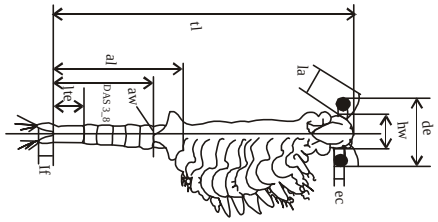

Fig. 2:Body measurements in *Artemia* (Version of Gajardo *et al.*, 1998)

Table 2: The metric and meristic character use in this study

Characters	Definition
TL	Total Length
AL	Abdominal Length
HW	Head Width
DE	Distance between compound Eyes
ED_LE	eye diameter (left)
ED_RI	eye diameter (right)
AW	abdominal width
DAS3_8	distance between 3rd abdominal segment to 8th abdominal segment
LTE	length of telson
LF	length of furca
LA_RI	length of antenna (right)
LA_LE	length of antenna (left)
OW	ovisac width
SF_RI	number of setae per furca (right branch)
SF_LE	number of setae per furca (left branch)

Statistical analysis: Morphological differentiation between samples of *A. urmiana* from four different geographical locations in the Urmia Lake was investigated by ANOVA (Tukey, $p<0.05$), Principal Components Analysis (PCA) and Discriminant Function Analysis (DA). In addition, DA used to appoint percent of corrected classification among stations. This was achieved by 2 methods: Calculated percent of overall correct classification as well as percent of correct classification between each pair stations. All statistical analysis was done by SPSS 11.5.

RESULTS

Primary physico-chimical parameters for 4 sampling locations are shown in Table 3 and morphometric and meristic characters as well as statistical comparisons of the results are summarized in Table 4.

According to Table 4, morphometric characters show different degrees of variation among stations. TL, AL and

Table 3: The primary ecological factors for each station

		Salinity (ppt)		pH			
Station	Date	Surface	Depth	Surface	Depth	Depth (m)	Transparency (m)
N2	Jun.2004	260	268	7.34	7.34	2.2	1.8
M1-2	Jul.2004	274	240	7.23	7.3	2.7	0.5
M3-2	Jun.2004	260	260	7.26	7.3	4.1	2.5
S2	May.2004	245	256	7.3	7.35	2.7	0.5

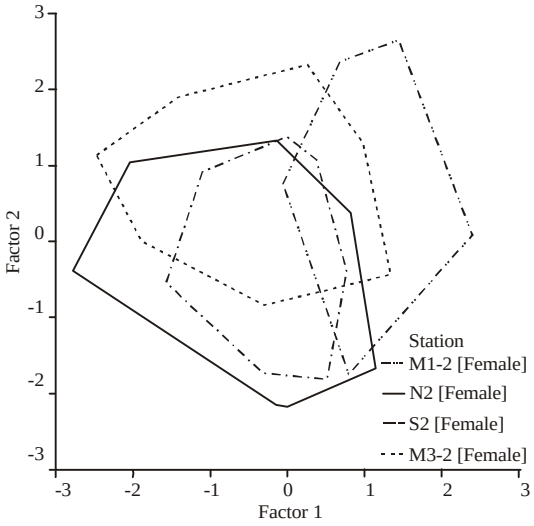

Fig. 3: Principal Component Analysis for female samples

LF are three characters that have high variation among male samples so they are significantly different ($p<0.05$) in all the male samples. Meristic characters don't show significant difference among all the male and female samples ($p<0.05$).

Differentiation among female samples: PCA shows that according to factor 1, station M1-2 comparatively was separated from other stations. Station S2 and N2 created one mixed group that with regard to factor 2, this collection fairly was separated from station M3-2 (Fig. 3).

In component 1, ED_RI, LA_RI, LA_LE and ED_LE characters and in component 2, TL and AL characters are the most important characters for morphological differentiation among female samples (Table 5) The first 2 factors show 66.88 and 12.07% of variety and altogether 2 components show 78.95% of variation (Table 5) DA comparatively confirmed PCA finding. According to DA, any of station didn't completely divide together (Fig. 4). But stations M1-2 and M3-2 show most separation in comparison with other stations. Stations S2 and N2 mixed in one group that with regard to function 2 this collection absolutely was separated from station M3-2. In function 1, TL, AL, LF and LTE characters and in function 2, ED_RI and LA_RI characters are the most important characters for morphological differentiation among female samples (Table 5). The first second function show 85.9 and 14.10% of variation and on the whole the 2 functions show 100% of diversification (Table 5) Correctly classified between each pair stations were tested by DA:

- Correctly classified between M1-2 and N2 equal 91.7%.
- Correctly classified between M1-2 and S2 equal 86.7%.
- Correctly classified between M1-2 and M3-2 equal 98.3%.
- Correctly classified between M3-2 and N2 equal 78%.
- Correctly classified between M3-2 and S2 equal 73.3%.
- Correctly classified between N2 and S2 equal 66.7%.

In sum, correctly classified for four female samples equal 60%; so station M3-2, 66.7%, station S2, 33.3%, station N2, 46.7 and station M1-2, 93.3% pleased in your group (Table 6).

Differentiation among male samples: In PCA, according to factor 1, station M1-2 was divided from other stations. Station S2 and M3-2 made one mixed group that with regard to factor 2 this collection positively was divided from station N2 (Fig. 5). In component 1, TL, AL, LTE and LF characters and in component 2, ED_LE, LA_LE, ED_R and AL_R characters are the most important traits for morphological differentiation in male samples (Table 5) The first 2 factors show 52.65 and 27.98% of variation and in total, 2 components show 80.64% of variety (Table 5).

According to DA, station M1-2 completely divided to other three stations. Stations S2 and M3-2 mixed in one group that didn't show most separation from station M3-2 (Fig. 6). In function 1, LTE, LF, AL and TL characters and in function 2, ED_RI, AL_RI, AL_LE and ED_LE characters are the most important characters for morphological differentiation among male samples (Table 5) The first and second functions show 83.1 and 13.5% of the total variation and in sum the 2 functions show 96.7% of variation (Table 5) Correctly classified between each pair stations were tested by DA:

- Correctly classified between M1-2 and N2 equal 100%.
- Correctly classified between M1-2 and S2 equal 100%.
- Correctly classified between M1-2 and M3-2 equal 100%.
- Correctly classified between M3-2 and N2 equal 90%.
- Correctly classified between M3-2 and S2 equal 73.3%.
- Correctly classified between N2 and S2 equal 88.3%.

In all correctly classified for four males sample equal 78.3% in the other side station M3-2, 53.3%; station, S2 70%; N2, 90 and station M1-2 100% pleased in your group (Table 6).

Table 4: Mean (mm) ±S.D of morphometric and meristic characters for male and female samples from four stations (same letters shows non-significant differences between means in each row of main column, p>0.05)

	Female				Male			
	N2	M1-2	M3-2	S2	N2	M1-2	M3-2	S2
TL	12.72±0.91a	15.24±0.95	12.63±1.10a	12.71±0.76a	10.25±1.01	13.20±0.93	8.96±0.88	9.62±081
AL	8.01±0.57a	9.59±0.59	7.95±0.69a	8.00±0.48a	5.98±0.59	7.70±0.54	5.23±0.51	5.61±0.47
HW	0.65±0.07a	0.76±0.09	0.69±0.07a	0.66±0.06a	0.65±0.059a	0.66±0.057a	0.67±0.051a	0.71±0.048
DE	1.32±0.13a	1.53±0.14	1.33±0.12a	1.33±0.09a	1.84±0.13c	1.81±0.14bc	1.70±0.14a	1.74±0.12ab
ED_LE	0.26±0.025a	0.31±0.032	0.28±0.026	0.26±0.020a	0.42±0.041b	0.41±0.043ab	0.39±0.033a	0.39±0.026a
ED_RI	0.26±0.023b	0.30±0.037a	0.28±0.024a	0.26±0.021b	0.43±0.041b	0.41±0.043ab	0.39±0.037a	0.39±0.025a
AW	0.36±0.039ab	0.40±0.050c	0.38±0.057ac	0.35±0.034b	0.36±0.037bc	0.34±0.040ac	0.34±0.047ab	0.33±0.035a
DAS3_8	5.52±0.46a	6.35±0.59	5.41±0.75a	5.37±0.50a	4.40±0.61a	5.27±0.58	3.35±0.79	3.98±0.58a
LTE	1.65±0.11a	1.97±0.12	1.64±0.15a	1.63±0.10a	1.53±0.15a	2.00±1.14	1.36±0.13	1.46±0.12a
LF	0.13±0.009a	0.15±0.011	0.13±0.011a	0.13±0.007a	0.16±0.016	0.21±0.015	0.14±0.014	0.15±0.013
LA_RI	0.91±0.08b	1.07±0.12a	1.01±0.08a	0.93±0.07b	1.50±0.14b	1.45±0.15ab	1.38±0.13a	1.39±0.08a
LA_LE	0.92±0.08a	1.08±0.11	1.00±0.99	0.92±0.06a	1.50±0.14b	1.42±0.15ab	1.38±0.11a	1.37±0.09a
OW	2.00 ± 0.34a	2.58 ± 0.38	2.05 ± 0.23a	1.99 ± 0.19a	-	-	-	-
SF_RI	2.40±0.67a	2.43±0.67a	2.46±0.62a	2.30±0.53a	2.69±0.89a	2.73±0.94a	2.83±1.11a	2.80±0.96a
SF_LE	2.36±0.61a	2.26±0.58a	2.33±0.54a	2.33±0.71a	2.76±1.04a	2.70±0.91a	2.70±1.11a	2.83±1.05a

Table 5: Portion of each trait in two first components and functions for morphological differentiation

	Female		Male		Female		Male		
	Component		Component		Function		Function		
traits	1	2	1	2	1	2	1	2	3
AL	0.417	0.901	0.988	0.118	0.996	0.065	0.907	-0.051	0.321
AW	0.457	0.244	-0.118	0.536	0.248	0.232	-0.004	0.179	0.147
DAS 3_8	0.146	0.876	0.877	0.152	0.747	-0.134	0.510	0.275	0.809
DE	0.713	0.439	0.265	0.780	0.444	0.439	0.077	0.155	0.252
ED_LE	0.879	0.298	0.190	0.952	0.402	0.662	0.087	0.419	0.123
ED_RI	0.909	0.251	0.188	0.948	0.445	0.895	0.080	0.442	0.173
HW	0.553	0.317	-0.048	0.453	0.252	0.242	-0.081	-0.392	0.566
LA_LE	0.885	0.285	0.190	0.952	0.413	0.653	0.087	0.419	0.123
LA_RI	0.897	0.255	0.188	0.948	0.458	0.876	0.080	0.442	0.173
LF	0.429	0.879	0.988	0.118	0.946	0.104	0.907	-0.051	0.321
LTE	0.444	0.877	0.988	0.103	0.943	0.111	0.908	-0.047	0.318
TL	0.416	0.901	0.988	0.118	0.997	0.066	0.907	-0.051	0.321
OW	0.708	0.468	-	-	0.433	0.477	-	-	-
	Total variance explained				Eigenvalues				
% of Variance	66.885	12.070	52.658	27.986	85.9	14.1	83.1	13.5	3.3
Cumulative %	66.885	78.955	52.658	80.644	85.9	100.0	83.1	96.7	100.0
Extraction Method: Principal Component Analysis.					Extraction Method: Discriminant Function Analysis.				

Table 6: Percentage of complete original female and male grouped classification by discriminant function Analysis (a) classification results

			Predicted Group Membership				
Station (female samples)			M3-2	S2	N2	M1-2	Total
Original	Count	M3-2	20	5	2	3	30
		S2	8	10	12	0	30
		N2	7	7	14	2	30
		M1-2	1	0	1	28	30
	%	M3-2	66.7				100.0
		S2		33.3			100.0
		N2			46.7		100.0
		M1-2				93.3	100.0
a 60.0% of original grouped cases correctly classified.							
Station (male samples)		Predicted Group Membership					Total
Original	Count	M3-2	16	11	3	0	30
		S2	6	21	3	0	30
		N2	2	0	27	1	30
		M1-2	0	0	0	30	30
	%	M3-2	53.3				100.0
		S2		70.0			100.0
		N2			90.0		100.0
		M1-2				100.0	100.0
a 78.3% of original grouped cases correctly classified.2222							

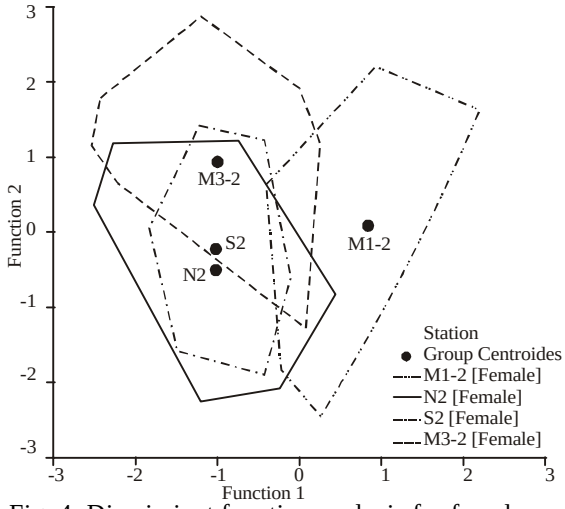

Fig. 4: Discriminat function analysis for female samples

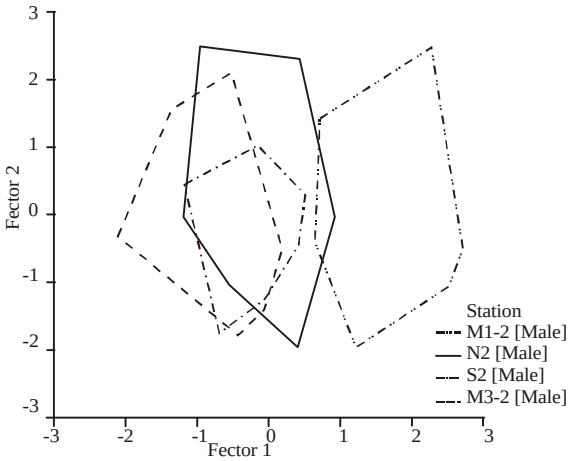

Fig.5: Principal component analysis for male samples

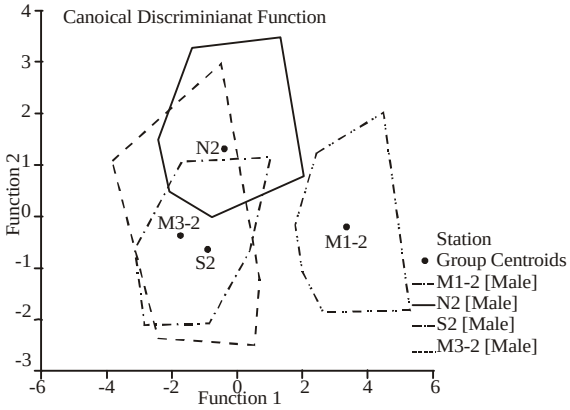

Fig. 6: Discriminat function analysis for male samples

DISCUSSION

Study of morphological differentiation is one of the most useful methods in *Artemia* taxonomy and biosystematics.

Hontoria and Amat (1992) studied adult female *Artemia* belonging to 25 populations from all over the America. They were able to clearly separate the populations of 2 different species, *A. franciscana* and *A. persimilis*. Discrimination based on morphometric characters within the Eastern Old World group clearly separates *A. urmiana* from the other populations (Pilla and Beardmore, 1994). Gajardo *et al*., (1998) studied 6 populations from Chile, the analysis showed that *A. franciscana* and *A. persimilis* are morphologically divergent. They analysed the data combining both males and females for the individuals grouped by the type of population to which they belong. Cohen *et al*., (1999) showed that adult females studied by multivariate discriminant analysis, provided evidence that populations from La Pampa and Buenos Aires provinces (Argentina) belonged to the species *A. persimilis*. Triantaphyllidis *et al.* (1997a) studied eleven bisexual populations according to morphological characters. These populations divided into four different groups: the *A. franciscana* group, the *A. tunisiana* group, the *A. urmiana* group and a broader group which includes Eastern Old World populations. They carried out same study about Morphological differentiation of 15 parthenogenetic *Artemia* populations. Discriminant analysis revealed five main groups of morphological patterns for 15 populations (Triantaphyllidis *et al*., 1997b). Morphometric characterization of *A. franciscana* populations from the Colomsbian Caribbean shown that Male and female Colombian Caribbean populations were separated from the North American populations Camargo et al. (2003).

With regard to biosystematics and taxonomic concepts, if species show sexual dimorphism, separate analyses for male and female data must be performed (Fowler *et al*., 1998; Manley, 1996). Male and female samples were analyzed separately because the *Artemia urmiana* populations from these four stations were shown sexually dimorphic (Asem *et al*., 2005).

In the PCA, the female samples were almost clustered in one group in all sites and it was not possible to make any separation among them. But in the DA, the station M1-2 was relatively separated from the other groups. In this analysis: 98.3% of original groups were correctly classified between M1-2/M3-2, 91.7% of original groups

were correctly classified between M1-2/N2, 86.7% of original groups were correctly classified between M1-2/S2.

On the other hand, the PCA shows that male samples in stations N2, S2 and M2-3 were almost clustered in one group and this mixed group was separated from station M1-2 with regard to the factor1. This finding was also confirmed by DA. In the latter analysis 100% of original groups were correctly classified between M1-2/M3-2, M1-2/N2 and M1-2/S2 stations. Male morphometric characters separated population groups more clearly than the female characters. This result was also confirmed by result of (Camargo et al. (2003) according to which classification based on male characters provides better group membership than females.

Therefore, based on the results, there are minimum 2 ecological populations of *Artemia urmiana* in the Urmia Lake. With regard to these results it isn't possible to make taxonomic decisions on these 2 populations and further investigation is needed to make the final decision.

With regard to the PCA and DA Scatterplots, there is no geographical arrangement between stations. Among the female samples, stations N2 and S2 assembled as a mixed group but these stations are geographically distant and among the male samples M3-2 and S2 mixed in one group and stations M3-2 and M1-2 were separated but these 2 stations are geographically very close. If we accept that Ionic composition of the habitat can produce ecological isolation and can result in morphological and biometrical differences (Bowen *et al*., 1985, 1988; Hantoria and Amat, 1992) therefore we can suggest, speciation of *A. urmiana* in the Urmia Lake goes by ecological speciation.

According to previous studies (Hontoria and Amat 1992, Pilla and Beardmore 1994, Triantaphyllidis *et al*., 1997b, Gajardo *et al*., 1998, Cohen *et al*., 1999, Camargo *et al*., 2003, Amat *et al*., 2005) *Artemia* materials have been harvested from the samples that grow in the same laboratory conditions. If we accept that salty composition of the habitat can produce ecological isolation and can result in morphological and biometrical differences (Bowen *et al*., 1985, 1988; Hontoria and Amat, 1992), accordingly we can suggest that the main mechanism of evolution in the genus *Artemia* is a kind of ecological speciation. Therefore study of effects of ecological processes in morphological differentiation and evolution of *Artemia* is of paramount importance. According to this scenario, collecting *Artemia* samples from their natural habitat has priority to the materials growing in the same conditions in laboratory. The use of the horizontal conditions for all species and populations of *Artemia* deviate us from correct knowledge of the ecological speciation process. In addition, maybe 2 populations have significant differences in morphology, but same laboratory conditions make limitation and disadvantageous conditions with ionic concentration and composition. Therefore, in these conditions, 2 populations couldn't disclose their differentiation. This hypothesis is completely acceptable with regard to ecological concepts. But provide of *Artemia* field sample is difficult because *Artemia* has short life cycle in natural habitat, also most habitats are temporary ponds and lagoons and if collecting of samples are difficult, therefore each population must grow in general ionic conditions according to natural habitat with different salinity treatments. These 2 methods associate main evolution and speciation processes in nature.

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