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Biometrical Study of *Artemia urmiana* (Anostraca: Artemiidae) Cysts Harvested from Lake Urmia (West Azerbaijan, Iran)

Alireza ASEM[1,*], Nasrullah RASTEGAR-POUYANI[1], Naser AGH[2]

[1]Department of Biology, Faculty of Science, Razi University, 67149 Kermanshah, IRAN

[2]*Artemia* and Aquatic Animals Research Institute, Urmia University, Urmia, IRAN

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Abstract: *Artemia urmiana* was reported from Lake Urmia by Günther in 1899. The objectives of the present study were to investigate the diameter of untreated and decapsulated cysts and chorion thickness at 26 stations in Lake Urmia. One-way ANOVA (Tukey test, $P < 0.05$) was used for analyzing the data. Only 31 pair means of untreated cysts among 26 stations showed significant differences, but, on the other hand, 157 pair means of decapsulated cysts among stations showed significant differences. There was a high variation in cyst diameter among the different localities in Lake Urmia. It was shown that this lake consists of recognizable sections producing cysts with different hatching characteristics, which can be used for diverse aquacultural purposes.

Key Words: *Artemia urmiana*, Lake Urmia, Iran, untreated cyst, decapsulated cysts, chorion thickness, hatching efficiency

Introduction

The different populations of the Branchiopod *Artemia*, scattered over all temperate and tropical areas of the world, have become one of the most interesting models for systematic, taxonomic, and evolutionary studies (Hontoria and Amat, 1992; Gajardo et al., 2002). The complex of species in the genus *Artemia* have a wide variety of adaptive processes, mainly related to survival in extremely hyperhaline environments (Abreu-Grobois and Beardmore, 1982; Browne and Bowen, 1991; Hontoria and Amat, 1992). Van Stappen (2002) published an updated list, covering 600 *Artemia* sites, and completed it with geographical coordinates and information about reproduction mode and species name. Bisexual populations of *Artemia* are distributed in both the New World (*A. franciscana* Kellogg, 1906, *A. persimilis* Piccinelli and Prosdocimi, 1968) and the Old World (*A. urmiana* Günther, 1899, *A. sinica* Cai, 1989, *A. tibetiana* Abatzopoulos et al., 1998, *A. salina* (Linnaeus 1758)). Schlösser wrote the first description of the brine shrimp, *Artemia,* in 1755 from Lymington, UK (Kuenen and Bass-Becking, 1938). Günther (1899) described *Artemia urmiana* from Lake Urmia as a bisexual species. This species is endemic to Urmia.

Taxonomists are still puzzled about the evolution and phylogenetic relationship of the *Artemia* species (Triantaphyllidis et al., 1997). Different methods have been used in order to characterize the species of the genus *Artemia*. The most relevant methods are comparison of biometrical and morphological characteristics, electrophoretic patterns of different allozymes and cross-fertility test and electron microscopic survey of the morphology such as frontal knob and penis (Abreu-Grobois and Beardmore, 1982; Mura, 1990; Hontoria and Amat, 1992; Triantaphyllidis et al., 1997; Torrentera and Belk, 2002).

Ionic 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). In this paper we study biometrical variation in the cyst from 26 different sampling sites in Lake Urmia. We analyze their biometrical characteristics (diameter of untreated and decapsulated cysts and chorion thickness) using multivariate analysis.

* E-mail: alireza_1218@yahoo.com

Materials and Methods

Field study:

We chose 26 stations in the middle, northern, and southern parts of Lake Urmia (Figure 1). Four primary ecological factors were measured for each station: salinity (0.5 m from the surface and 0.5 m from the bottom), pH (0.5 m from the surface and 0.5 m from the bottom), depth and transparency, as well as the date of sampling (Agh, 2004).

Diameter of Untreated cysts:

Sample preparation

Prior to analysis, cyst samples containing a considerable amount of debris, which might interfere with the analysis, should be washed and cleaned. In any case, all salt or salty water should be removed before hydration.

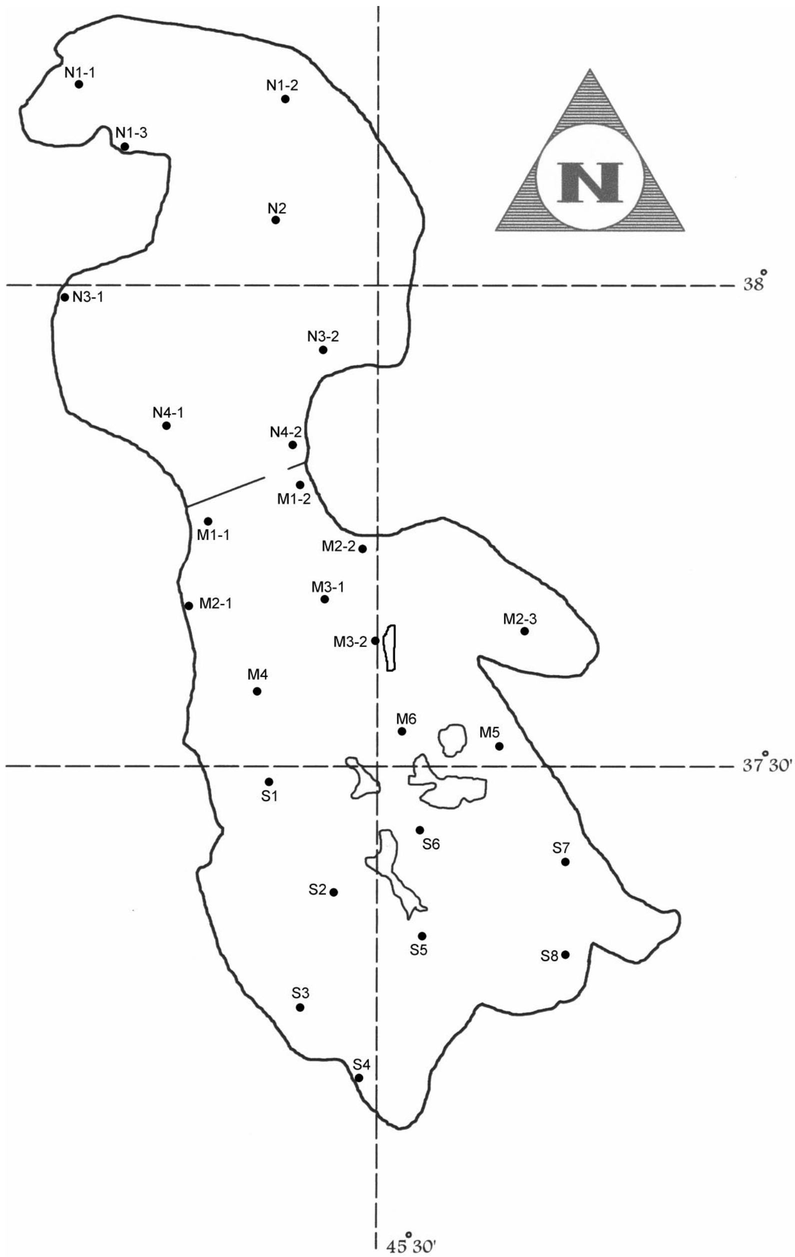

Figure 1. Geographical location of the study area, Lake Urmia, Iran.

Sample hydration

The cyst diameter is normally determined on fully hydrated cysts, when the embryos become spherical. Generally cysts are fully hydrated after incubation at room temperature for at least 2 h (Lavens and Sorgeloos, 1987). Nevertheless, some strains or batches may require longer periods to reach maximal hydration (Vanhaecke et al., 1980). Therefore, a small but representative cyst sample (a few hundred individual cysts are sufficient) is incubated in a small vial for at least 12 h at room temperature (20-25 °C). Freshwater can be used as incubation medium. In order to inhibit metabolic activity within the cysts, a few drops of Lugol's solution are added. Neither the addition of the Lugol's solution nor the storage temperature has a significant influence on the final volume of the cysts (Vanhaecke et al., 1980).

Measurements and calculation

The maximum diameter of at least 100 cysts is determined under a microscope equipped with a calibrated eyepiece. The cysts are measured at random to avoid biased results. The cysts that are measured are taken at random, so that no bias occurs towards e.g. bigger cysts. Cracked or hatching cysts, empty cyst shells, obviously not fully hydrated cysts etc. are not taken into account. Average value and standard deviation are calculated, and the data are expressed in micrometers to 1 decimal point.

Diameter of decapsulated cysts:

- Cysts are decapsulated according to the following procedure (Bruggeman et al., 1980): Wash and purify sample, if necessary (see above)
- Hydrate cysts by incubating them for 2 h in fresh water in a tube with aeration from the bottom at room temperature (20-25 °C)
- Bring hydrated cysts into a tube with a minimum of fresh water, equal volume of liquid bleach (NaOCl) and a few drops of NaOH (to increase pH and obtain a fast chemical reaction). The gradual decapsulation of the cysts can be followed under a stereoscopic microscope.
- When decapsulation is completed (removal of cyst shell after 3-15 min, depending on concentration of chemicals and on ambient temperature), rinse thoroughly over a 100-125 μm sieve to remove decapsulating chemicals. Prolonged exposure of decapsulated embryos to a high concentration of NaOCl may result in gradual degradation of embryos (and hence problematic measurement) and should therefore be avoided. When the decapsulated cysts are uniquely used for biometrical measurement, the hatchability of the embryos is not an issue; when the batch of decapsulated cysts is also used for hatching, a stricter modus operandi has to be followed in order to maintain maximal viability of the embryos (Lavens and Sorgeloos, 1996).
- Take samples to measure a minimum of 100 embryos (see "Diameter of untreated cysts"). Atypical embryos (i.e. not fully hydrated cysts; hatched cysts, embryos with hatching membrane not completely filled with yolk mass) should not be taken into account.

Chorion thickness:

This value is calculated as follows:

chorion thickness = (Mean diameter of untreated cyst – Mean diameter of decapsulated cyst)/2

Consequently, this value is presented without standard deviation (Vanhaecke and Sorgeloos, 1980).

Statistical analysis:

Biometrical variation of cysts from different locations in Lake Urmia was determined by one-way ANOVA (Tukey test, $P < 0.05$) and hierarchical cluster analysis using the computer program SPSS 11.5.

Results

Some physico-chemical parameters for the 26 sampling locations are shown in Table 1. The diameter of the untreated and decapsulated cysts and chorion thickness are summarized in Table 2. These results are also shown in Figures 2 and 3. The statistical comparisons of the results are given in Tables 3 and 4. The biggest untreated cysts were found at N3-1 station (259.34 ± 11.36 μm) and the smallest at M3-2 station (247.63 ± 11.47 μm). The biggest decapsulated cysts were found at M2-2 (251.6 ± 11.24μm) and the smallest at N1-2 (231.29 ± 10.43 μm). The thickest chorion was observed at N4-1 (9.37 μm) and the thinnest in the cyst chorion belongs to the cysts harvested from M3-2 (1.31

Table 1. The primary ecological factors for each sampling station.

Station	Date	Salinity (ppt)		pH		Depth (m)	Transparency (m)
		surface	depth	surface	depth		
N1-1	Aug 2004	290	282	7.22	7.23	6.3	3.5
N1-2	Aug 2004	285	285	7.24	7.23	5.2	4.5
N1-3*	Jun 2003						
N2	Jun 2004	260	268	7.34	7.34	2.2	1.8
N3-1*	Jun 2003						
N3-2	Jun 2004	260	275	7.26	7.26	1.1	0.9
N4-1	Jun 2004	260	255	7.22	7.25	4.8	3.5
N4-2	Jun 2004	240	285	7.36	7.37	3.1	2.7
M1-1	Jul 2004	256	250	7.37	7.38	3.2	2
M1-2	Jul 2004	274	240	7.23	7.3	2.7	0.5
M2-1*	Jun 2003						
M2-2	Aug 2004	285	283	7.23	7.23	3.3	2.4
M2-3	Jul 2004	280	284	7.2	7.15	2	1
M3-1	Jul 2004	280	280	7.18	7.14	4.1	1.9
M3-2	Jun 2004	260	260	7.26	7.3	4.1	2.5
M4	Jul 2004	242	265	7.55	7.47	3.7	1
M5	May 2004	275	280	7.2	7.18	2.7	1.5
M6	Jul 2004	275	285	7.19	7.12	3.4	1.5
S1	May 2004	245	270	7.54	7.44	3.9	0.8
S2	May 2004	245	256	7.3	7.35	2.7	0.5
S3	May 2004	255	270	7.51	7.45	3.1	0.85
S4*	Jun 2003						
S5	Jul 2004	255	265	7.49	7.46	3.3	0.95
S6	Jul 2004	280	285	7.1	7.2	3	2.3
S7	Jul 2004	280	280	7.23	7.23	2	1.4
S8	Jul 2004	283	280	7.11	7.14	1.6	1.1

* Cyst samples from these stations were provided by West Azerbaijan Fisheries office and data have not been recorded.

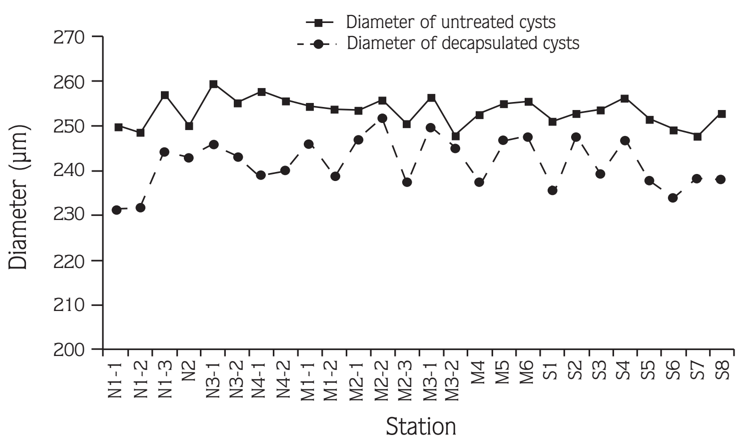

Figure 2. Mean of untreated and decapsulated cysts of *A. urmiana* from 26 stations, Lake Urmia, Iran.

Table 2. Biometrical data for 3 traits of *A. urmiana* cysts from 26 stations, Lake Urmia, Iran.

Station	Diameter of untreated cysts (μm)		Diameter of decapsulated cysts (μm)		Chorion thickness (μm)
	Mean ± S.D.	C.V.	Mean ± S.D.	C.V.	
N1-1	249.88 ± 12.11	4.84	231.28 ± 10.43	4.51	9.29
N1-2	248.32 ± 11.56	4.65	231.66 ± 11.84	5.11	8.33
N1-3	256.81 ± 10.57	4.11	244.18 ± 10.64	4.36	6.31
N2	249.79 ± 25.83	10.3	242.73 ± 14.36	5.91	3.53
N3-1	259.34 ± 11.36	4.38	245.58 ± 10.60	4.31	6.88
N3-2	255.04 ± 12.58	4.93	243.00 ± 11.76	4.84	6.02
N4-1	257.51 ± 11.88	4.61	238.75 ± 10.02	4.19	9.37
N4-2	255.42 ± 15.25	5.97	240.00 ± 10.62	4.42	7.70
M1-1	254.29 ± 13.87	5.45	246.01 ± 15.18	6.17	4.13
M1-2	253.53 ± 13.98	5.51	238.48 ± 11.11	4.66	7.52
M2-1	253.27 ± 11.94	4.71	246.76 ± 12.10	4.90	3.25
M2-2	255.68 ± 13.79	5.39	251.60 ± 11.24	4.46	2.04
M2-3	250.36 ± 15.95	6.37	237.19 ± 11.70	4.93	6.58
M3-1	256.17 ± 13.05	5.09	249.50 ± 11.48	4.60	3.33
M3-2	247.62 ± 11.74	4.74	244.99 ± 12.05	4.92	1.31
M4	252.24 ± 13.67	5.41	237.19 ± 10.82	4.56	7.52
M5	254.93 ± 16.60	6.51	246.71 ± 11.97	4.85	4.11
M6	255.36 ± 16.39	6.41	247.46 ± 15.04	6.08	3.95
S1	250.85 ± 13.45	5.36	235.26 ± 11.27	4.79	7.79
S2	252.62 ± 13.53	5.35	247.47 ± 12.89	5.20	2.57
S3	253.37 ± 15.84	6.25	239.13 ± 11.39	4.76	7.12
S4	256.01 ± 11.80	4.61	246.65 ± 12.80	5.19	4.67
S5	251.28 ± 13.75	5.47	237.62 ± 13.01	5.47	6.82
S6	249.02 ± 14.25	5.72	233.75 ± 15.09	6.45	7.63
S7	247.62 ± 14.54	5.87	238.22 ± 13.42	5.63	4.70
S8	252.73 ± 15.82	6.26	237.84 ± 13.48	5.66	7.44

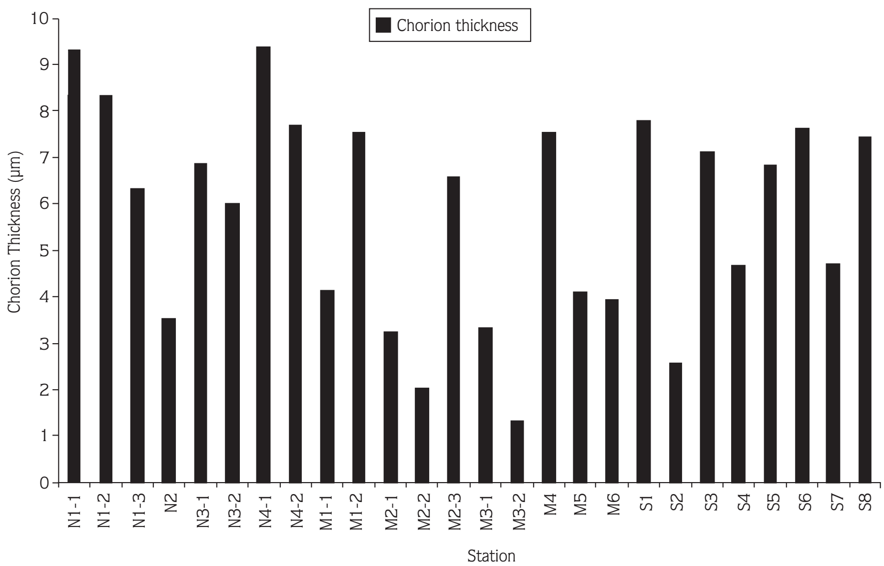

Figure 3. Cysts chorion thickness of *A. urmiana* from 26 stations, Lake Urmia, Iran.

Table 3. Statistical comparison (ANOVA, $P < 0.05$) of the untreated cysts of *A. urmiana* from 26 different geographical origins from Lake Urmia, Iran (symbol **+** shows significant differences between means).

N1-2	N1-3	N2	N3-1	N3-2	N4-1	N4-2	M1-1	M1-2	M2-1	M2-2	M2-3	M3-1	M3-2	M4	M5	M6	S1	S2	S3	S4	S5	S6	S7	S8	Station
-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N1-1
	+	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	N1-2
		-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	N1-3
			+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N2
				-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	+	+	+	-	N3-1
					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N3-2
						-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	N4-1
							-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	N4-2
								-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M1-1
									-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M1-2
										-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M2-1
											-	-	+	-	-	-	-	-	-	-	-	-	+	-	M2-2
												-	-	-	-	-	-	-	-	-	-	-	-	-	M2-3
													+	-	-	-	-	-	-	-	-	-	+	-	M3-1
														-	-	+	-	-	-	+	-	-	-	-	M3-2
															-	-	-	-	-	-	-	-	-	-	M4
																-	-	-	-	-	-	-	-	-	M5
																	-	-	-	-	-	-	+	-	M6
																		-	-	-	-	-	-	-	S1
																			-	-	-	-	-	-	S2
																				-	-	-	-	-	S3
																					-	-	+	-	S4
																						-	-	-	S5
																							-	-	S6
																								-	S7

μm). According to Tables 3 and 4, only 31 pair means of untreated cysts among the 26 stations showed significant differences, whereas, on the other hand, 157 pair means of decapsulated cysts showed significant differences.

With regard to hierarchical cluster analysis, which used means of untreated and decapsulated cysts and chorion thickness, the 26 stations are divided into 6 main groups. These groups form 2 clusters. The first cluster has 2 categories isolated in 20-25 span. The second cluster also has 2 categories divided in 15 span (Figure 4).

Discussion

This study offers an opportunity to expand our knowledge on biometrical variations in *A. urmiana* from Lake Urmia. Vanhaecke and Sorgeloos (1980) performed a comprehensive study on biometrical variations in *Artemia* strains from different geographical origins. According to their findings, *Artemia* cysts could be categorized into 3 groups:

Table 4. Statistical comparison (ANOVA, $P < 0.05$) of the decapsulated cysts of *A. urmiana* from 26 different geographical origins from Lake Urmia, Iran (symbol + shows significant differences between means).

N1-2	N1-3	N2	N3-1	N3-2	N4-1	N4-2	M1-1	M1-2	M2-1	M2-2	M2-3	M3-1	M3-2	M4	M5	M6	S1	S2	S3	S4	S5	S6	S7	S8	Station
-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	-	-	+	+	N1-1
	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	-	-	+	-	N1-2
		-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	+	+	-	-	N1-3
			-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	N2
				-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-	+	+	+	+	N3-1
					-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	N3-2
						-	+	-	+	+	-	+	-	-	+	+	-	+	-	+	-	-	-	-	N4-1
							-	-	+	+	-	+	-	-	+	+	-	+	-	+	-	-	-	-	N4-2
								+	-	-	+	-	-	+	-	-	+	-	+	-	+	+	+	+	M1-1
									+	+	-	+	+	-	+	+	-	+	-	+	-	-	-	-	M1-2
										-	+	-	-	+	-	-	+	-	+	-	+	+	+	+	M2-1
											+	-	+	+	-	-	+	-	+	-	+	+	+	+	M2-2
												+	+	-	+	+	-	+	-	+	-	-	-	-	M2-3
													-	+	-	-	+	-	+	-	+	+	+	+	M3-1
														+	-	-	+	-	-	-	+	+	+	+	M3-2
															+	+	-	+	-	+	-	-	-	-	M4
																-	+	-	+	-	+	+	+	+	M5
																	+	-	-	-	+	+	+	+	M6
																		+	-	+	-	-	-	-	S1
																			+	-	+	+	+	+	S2
																				+	-	-	-	-	S3
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																						-	-	-	S5
																							-	-	S6
																								-	S7

1. The smallest cysts belong to the Adelaid strain and *Artemia* from the San Francisco Bay area, including the SFB inoculated strain from Macau and Barotac Nuevo.
2. The parthenogenetic strains from China, France, Italy, and India are characterized by large cysts.
3. Strains with cysts of intermediate size but with very thin chorion, which is characteristic for *A. franciscana* from Chaplin Lake and Great Salt Lake.

Hontoria (1990), studying 14 *A. franciscana* populations, found a diameter ranging between 217 and 230 µm, with the exception of Great Salt Lake (Utah, USA) and Galera Zamba (Colombia) cysts, with 242 and 245 µm diameter. Zhenqiu et al. (1991) collected cysts from Xinjiana Uighur and Shandong. They showed that all of these samples belong to parthenogenetic populations and their cyst size is larger than those of bisexual species. Pilla and Beardmore (1994) reported the diameter of untreated cysts for *A. urmiana*, *A. sinica*, and *Artemia* sp.

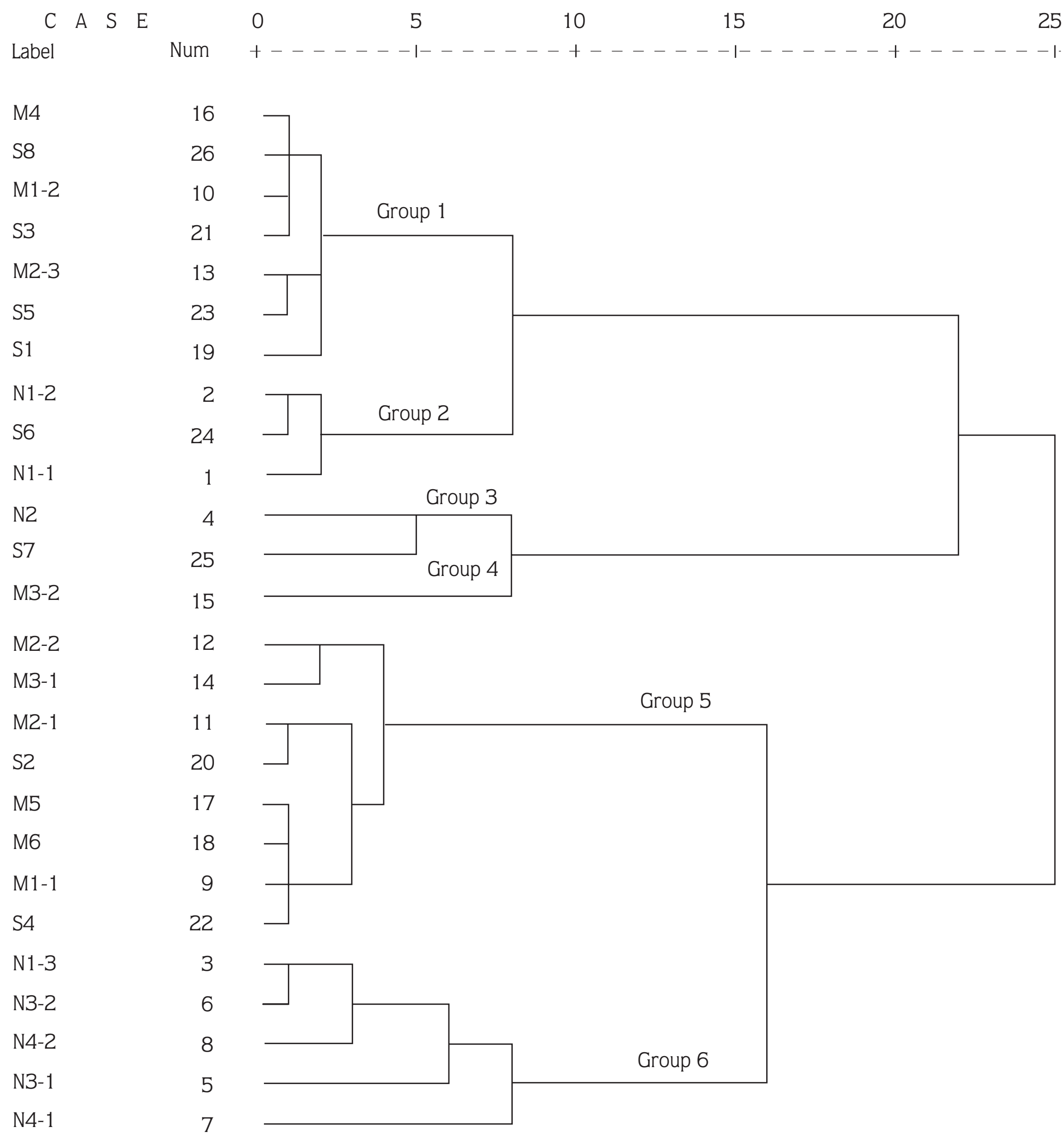

Figure 4. Classification of stations by hierarchical cluster analysis based on 3 cyst characters (diameter of untreated cyst, diameter of decapsulated cyst, and chorion thickness).

(RUS). Mean cyst size differed significantly between the 3 populations ($P < 0.001$). Cysts of *A. urmiana* are bigger (265.82 ± 15.85 µm). Duncan's test for multiple comparisons of the mean indicates that cyst diameters of *Artemia* sp. and *A. sinica* (230.05 ± 15.14 and 232.75 ± 11.22 µm) are also significantly different ($P < 0.01$) from each other. Triantaphyllidis et al. (1996) characterized 2 *Artemia* populations from Namibia and Madagascar. They showed that the diameters of untreated cysts from these localities were 247.7 ± 11 and 285.9 ± 11.6 µm and for decapsulated cysts were 233.1 ± 9.8 and 246.2 ± 11.7 µm, respectively. They suggested that the cysts from Namibia were significantly smaller compared with those from Madagascar. According to the results reported by Abatzopoulos et al. (1998) *A. tibetiana* is the biggest ever recorded for both bisexual and parthenogenetic species (323 ± 11.2 and 230 ± 14.6 µm). Cohen et al. (1999) found a diameter ranging between 246.1 ± 21 and 230.3 ± 1 µm from 4 Argentinean *Artemia* populations.

Comparing our results with those published by other researchers, it could be said that the cyst diameter of *A. urmiana* exhibits the widest range in the genus *Artemia*. Moreover, our results were different from those reported by Pilla and Beardmore (1994) with regard to the biometry of untreated cysts of *A. urmiana*. Abatzopoulos et al. (2006a, 2006b) reported the diameter of untreated and decapsulated cysts and their thickest chorion for *A. urmiana* from the Urmia Lake as follows:

A. urmiana cysts were collected from 7 sites in Lake Urmia. The mean value of the diameter of the untreated cysts ranged from 262.7 to 286.6 µm, decapsulated cysts from 258.6 to 274.4 µm, and the thickest chorion ranged from 1.2 to 9.3 µm (Abatzopoulos et al., 2006a).

In the other study *A. urmiana* cysts were harvested at 3 sites in Lake Urmia. The mean value of the diameter of the untreated cysts ranged from 249.8 to 280.7 µm, decapsulated cysts from 218.4 to 259.8 µm, and the thickest chorion ranged from 2.7 to 15.6 µm (Abatzopoulos et al., 2006b).

These 9 sites that have recently been studied conform to our stations, but the data do not correspond in terms of some traits.

These differences can be attributed to seasonal fluctuations in physico-chemical parameters and food availability in different regions of Lake Urmia (Abatzopoulos et al., 2006b) and the differences can be observed at different times in a single site. However, it should be noted that the pattern of population/species genetics determines the ranges of the biometry of the cysts.

Our results suggest that all 3 cyst traits of *A. urmiana* show variation between the 26 stations but a high variation was observed in the diameter of decapsulated cysts and chorion thickness. Hatching efficiency (H.E.) refers to the number of nauplii hatched per gram dry weight of cysts. Typically high H.E. is possible due to the small diameter of cysts (high number per gram); therefore a small diameter of cysts shows the high quality of cysts (Sorgeloos et al., 1978). According to the results, Lake Urmia has high variation in the diameter of cysts in different localities, and so nowadays the brine shrimp of *Artemia* is used as a useful aquatic animal in aquaculture and it may be proved of some economical value. It is suggested that proper management in *Artemia* cyst harvesting should be focused on selecting localities that produce small cysts with high H.E.

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