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International study on *Artemia*. LIV. Morphological study of *Artemia* with emphasis to Old World strains. II. Parthenogenetic populations

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Abstract

Eleven morphometric and one meristic character in 15 parthenogenetic *Artemia* populations have been studied by using discriminant and cluster analysis as well as scanning electron microscopy. Discriminant analysis revealed five main groups of morphological patterns: (i) the coastal Chinese populations together with a population from Kazakhstan, (ii) the inland Chinese salt lake populations, (iii) the Greek populations, (iv) one African population from Namibia and (v) a Chinese population from Xuyu (Jiangsu province). Cluster analysis was not always in agreement with discriminant analysis and these results are discussed.

Introduction

The genus *Artemia* comprises bisexual species, which are found on all continents except Antarctica, and parthenogenetic populations, which are endemic in Europe, Asia and Australia. *Artemia* is known for two phenomena which are rare in the animal kingdom: obligatory parthenogenesis and polyploidy. Polyploidy is common among parthenogenetic populations which are either diploid ($2n = 42$), triploid ($3n = 63$), tetraploid ($4n = 84$) or pentaploid ($5n = 105$) with diploidy and tetraploidy being the most frequently observed (for a review see Barigozzi, 1974; Abreu-Grobois, 1987). Mixed ploidy levels often occur in natural populations (Abreu-Grobois & Beardmore, 1982; 1991; Abatzopoulos et al., 1986; Pilla, 1992; Zhang & King, 1993). Amat (1980) and Hontoria & Amat (1992a) studying parthenogenetic populations from the Western Mediterranean basin demonstrated that ploidy level affects the morphology of *Artemia*.

Ionic composition of the habitat can produce ecological isolation (Bowen et al., 1985, 1988) and can result in morphological differences (Hontoria & Amat, 1992b).

In this paper we study parthenogenetic populations living in geographically isolated habitats and we try to analyze their morphological characteristics using multivariate methods.

Materials and methods

Fifteen parthenogenetic populations were evaluated in this study. Table 1 summarizes the origin of each population and the abbreviations that are used. For the Chinese populations we refer also to the code number

Table 1. List of the studied populations, *Artemia* Reference Center (ARC) cyst bank code number and abbreviations. For the Chinese populations the code numbers proposed by Xin et al. (1994) are given as well

Population	ARC cyst bank number	Code number of Chinese populations	Abbreviation
Megalon Embolon, Greece	1279		MEM
Citros, Greece	1280		CIT
Polychnitos, Greece	1281		POL
Kalloni, Greece	1282		KAL
Aibi Lake, Xinjiang, P. R. China	1198	11010891	AIL
Balikun Lake, Xinjiang, P. R. China	1235	11030991	BAL
Kazakhstan (unknown source)*	1060		CAT
Chengkou, Shandong, P. R. China	1210	04010991	CHK
Dongjiagou, Liaoning, P. R. China	1216	01030991	DOJ
Hangu, Tianjin, P. R. China	1212	03010991	HAN
Swakopmund, Namibia	1186		NAM
Pulandian, Liaoning, P. R. China	1217	01040991	PUL
Xuyu, Jiangsu, P. R. China	1283	05010992	XUW
Huanghua, Hebei, P. R. China	1233	02041091	HUA
Daban Lake, Xinjiang, P. R. China	1197	11020891	DAL

* Sample received from Catvis bv, Netherlands.

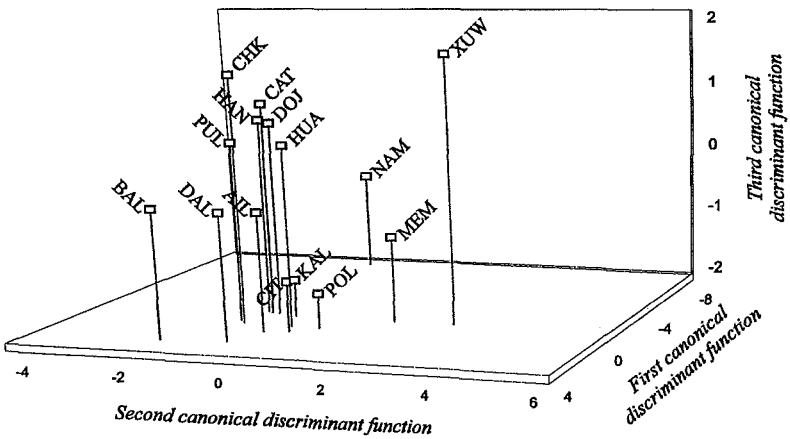

Figure 1. Scatterplot of the first three canonical discriminant functions (group centroids) resulting from the discriminant analysis when using the origin of each population as separation factor.

proposed by Xin et al. (1994) in order to better describe the different strains.

The methodology for studying the morphology of the adults, the culture conditions, the methodology for scanning electron microscopy as well as the statistical analysis have been described in detail in a previous study (Triantaphyllidis et al., 1997). For each population at least 28 individuals have been measured. Survival ranged between 56 ± 12% and 86 ± 9% for the populations studied.

Table 2. Mean values (standard deviation in parenthesis) of morphometric and meristic characters of different parthenogenetic *Artemia* populations. n = number of animals analysed. Abbreviations of populations in Table 1. A: total length, B: abdominal length, C: length from the third abdominal segment to the end of the abdomen, D: length of the eighth abdominal segment, E: width of third abdominal segment, F: length of furca, G: width of head, H: length of first antenna, I: distance between eyes, J: diameter of complex eye, K: number of setae on the left branch of the furca, L: number of setae on the right branch of the furca, M: width of ovisac. Wilks' lambda (λ) is given by the equation $\lambda = 1 - \eta^2$, where η^2 is the ratio of the between-groups sum of squares to the total sum of squares and represents the proportion of the total variance attributable to differences among the groups (Norusis, 1993)

	MEM $n=31$	CIT $n=31$	POL $n=31$	KAL $n=33$	AIL $n=30$	BAL $n=30$	CAT $n=30$	CHK $n=30$	DOJ $n=30$	HAN $n=30$	NAM $n=32$	PUL $n=30$	XUW $n=30$	HUA $n=30$	DAL $n=28$	*F* ratio	Wilks' lambda	*F* probability
A	13.90	13.66	13.77	12.76	13.11	12.55	12.98	11.71	12.23	12.00	9.68	12.45	13.73	12.99	14.10	62.6978	0.33440	< 0.00005
	(0.74)	(0.60)	(0.73)	(1.29)	(0.69)	(0.90)	(0.95)	(0.65)	(0.73)	(0.89)	(0.51)	(0.76)	(0.66)	(1.03)	(1.20)			
B	7.45	7.46	7.40	6.96	7.08	7.07	6.93	6.26	6.50	6.20	4.63	6.61	7.34	6.96	8.15	77.8490	0.28807	< 0.00005
	(0.48)	(0.45)	(0.50)	(0.70)	(0.44)	(0.63)	(0.62)	(0.40)	(0.58)	(0.59)	(0.35)	(0.52)	(0.34)	(0.74)	(0.84)			
C	5.76	6.00	5.92	5.44	5.71	5.87	5.40	5.07	5.13	4.83	3.41	5.24	5.77	5.61	6.58	98.6835	0.24197	< 0.00005
	(0.40)	(0.38)	(0.47)	(0.58)	(0.36)	(0.49)	(0.49)	(0.33)	(0.48)	(0.47)	(0.25)	(0.45)	(0.29)	(0.66)	(0.53)			
D	1.53	1.60	1.65	1.53	1.55	1.54	1.40	1.38	1.41	1.32	0.90	1.47	1.53	1.55	1.69	62.2272	0.33608	< 0.00005
	(0.14)	(0.15)	(0.17)	(0.22)	(0.13)	(0.14)	(0.21)	(0.11)	(0.17)	(0.13)	(0.09)	(0.16)	(0.12)	(0.20)	(0.11)			
E	0.84	0.75	0.79	0.82	0.77	0.68	0.79	0.66	0.74	0.72	0.77	0.71	0.82	0.74	0.76	23.8616	0.56899	< 0.00005
	(0.05)	(0.05)	(0.06)	(0.06)	(0.06)	(0.06)	(0.08)	(0.03)	(0.04)	(0.07)	(0.05)	(0.05)	(0.06)	(0.05)	(0.06)			
F	0.35	0.34	0.34	0.31	0.28	0.25	0.49	0.34	0.37	0.36	0.49	0.36	0.35	0.35	0.33	39.0559	0.44645	< 0.00005
	(0.04)	(0.03)	(0.04)	(0.04)	(0.09)	(0.04)	(0.06)	(0.04)	(0.06)	(0.04)	(0.04)	(0.05)	(0.05)	(0.05)	(0.04)			
G	1.13	1.07	1.10	1.03	1.07	0.98	1.01	0.99	0.96	0.94	0.92	0.96	1.07	1.05	1.04	27.6189	0.53282	< 0.00005
	(0.04)	(0.05)	(0.06)	(0.08)	(0.06)	(0.06)	(0.08)	(0.08)	(0.06)	(0.07)	(0.04)	(0.05)	(0.06)	(0.07)	(0.09)			
H	1.52	1.45	1.46	1.31	1.35	1.24	1.27	1.31	1.36	1.25	1.20	1.32	1.58	1.46	1.35	34.0843	0.48030	< 0.00005
	(0.10)	(0.07)	(0.09)	(0.13)	(0.11)	(0.09)	(0.11)	(0.13)	(0.07)	(0.12)	(0.05)	(0.08)	(0.09)	(0.15)	(0.08)			
I	2.13	1.95	2.04	1.87	1.96	1.80	1.85	1.88	1.79	1.77	1.63	1.80	2.24	1.97	1.93	43.8081	0.41828	< 0.00005
	(0.09)	(0.09)	(0.10)	(0.17)	(0.13)	(0.09)	(0.14)	(0.15)	(0.09)	(0.18)	(0.06)	(0.12)	(0.10)	(0.16)	(0.13)			
J	0.33	0.30	0.30	0.30	0.32	0.28	0.31	0.31	0.30	0.29	0.27	0.29	0.37	0.32	0.30	27.3055	0.53566	< 0.00005
	(0.02)	(0.02)	(0.03)	(0.02)	(0.03)	(0.02)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.02)			
K	5.19	5.13	5.26	6.06	6.27	7.07	9.53	8.33	8.77	8.63	8.84	8.73	6.50	7.03	6.68	24.9799	0.55772	< 0.00005
	(0.98)	(1.06)	(1.15)	(1.39)	(2.36)	(1.11)	(1.63)	(2.69)	(1.73)	(1.99)	(1.17)	(1.96)	(1.55)	(2.01)	(1.09)			
L	5.26	5.06	5.48	5.93	6.20	7.03	9.70	8.80	8.73	8.47	9.31	8.80	6.33	7.50	6.89	27.9311	0.53003	< 0.00005
	(0.96)	(0.85)	(1.18)	(1.17)	(2.38)	(0.99)	(1.62)	(2.37)	(1.74)	(1.81)	(1.53)	(2.29)	(1.40)	(2.33)	(1.16)			
M	2.43	2.06	2.24	2.30	2.03	1.87	2.07	1.72	2.07	2.00	2.15	1.97	2.54	1.96	2.10	27.0707	0.53781	< 0.00005
	(0.19)	(0.21)	(0.21)	(0.21)	(0.25)	(0.20)	(0.38)	(0.12)	(0.23)	(0.25)	(0.11)	(0.16)	(0.23)	(0.15)	(0.26)			

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Results

Chi-square and Kolmogorov-Smirnov tests showed that the transformed morphological characters fit to the normal distribution ($P > 0.05$). Two different analysis of variance tests were performed for each of the thirteen characters listed in Table 2. The first was a standard one-way analysis of variance (Anova), where variances are assumed to be homogeneous (Sokal & Rohlf, 1981). However, Levene's test (Norusis, 1993) showed that the variances were significantly different and since logarithmic or square root transformations did not eliminate the problem, we conducted an approximate test of equality of means according to the procedure of Games & Howell (1976).

The mean values of morphometric and meristic characters are presented in Table 2. Anova resulted in F ratios, the significance of which revealed highly statistical significant differences ($P < 0.00005$). The variables that present the larger F ratios are the length from the third abdominal segment to the end of the abdomen and the abdominal length. Table 3 summarizes the significant differences of the means after applying the test of Games & Howell (1976).

Discriminant analysis based on the origin of each population as a separation criterion resulted in 13 canonical discriminant functions after 26 steps. The first nine discriminant functions are highly statistically significant ($P < 0.0001$) and obtain a cumulative separation percentage of 99.42% while the last four are not statistically significant ($P > 0.05$). The first three functions that appear in Table 4 resulted in a separation percentage of 78.23%. In Table 4 the unstandardized and standardized canonical discriminant function coefficients are also presented. Using morphometric and meristic characters each population can be classified correctly in one of the fifteen groups with an overall accuracy of 93.20% (Table 5).

Figure 1 depicts the plot of the discriminant analysis, while Figure 2 summarizes the results of hierarchical cluster analysis using the single linkage method (nearest neighbor technique) in a dendrogram. NAM population is clustered separately from the other populations suggesting that it has unique morphological features. The Greek populations CIT, KAL and POL are grouped very close while MEM appears to be clustered a little bit further from the 'Greek core'. XUW population is discriminated from all the other populations. The Chinese populations CHK, HAN, PUL and DOJ (all located in coastal areas) exhibited great morphological similarities. Close to them appeared the CAT

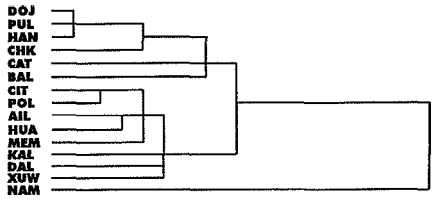

Figure 2. Dendrogram of hierarchical cluster analysis using the single linkage method to join clusters (nearest neighbor technique).

population which inhabits an inland salt lake. BAL, DAL and AIL are close in the three dimensional space of Figure 1, suggesting that these inland salt lake populations from Xinjiang province (P. R. China) exhibited morphological similarities. AIL is closer to DAL while BAL is further discriminated by the first two functions. Although in Figure 1 discriminant analysis separates the Chinese inland-lake populations BAL, DAL and AIL and groups them closely, cluster analysis classifies BAL population separately, close to the coastal habitat group (see Figure 2).

Summarizing the above data, discriminant analysis revealed five main groups of morphological patterns.

(i) The coastal Chinese populations CHK, PUL, HAN, DOJ and HUA together with the CAT population from Kazakhstan,
(ii) the inland Chinese salt lake populations BAL, DAL and AIL,
(iii) the Greek polyploid populations CIT, KAL, POL and MEM,
(iv) the African NAM population and
(v) the Chinese XUW population.

Scanning electron microscopy of the head and uterus showed the same difficulties of quantification as in the bisexual animals (Triantaphyllidis et al., 1995a). The scanning electron micrographs of Figure 3 depict an example of the differences observed in the morphology of the furca.

Discussion

The coastal Chinese populations are all diploid (Pilla, 1992; Triantaphyllidis et al., unpublished data) with the exception of HAN for which few tetraploid individuals appeared. Because the habitat of these populations is coastal they all experience an environment with similar ionic composition (chloride is the prevailing anion). CAT population, from Kazakhstan, seem

Table 3. Significant differences for the mean values of morphometric and meristic characters of parthenogenetic females as determined by the approximate test of equality of means of Games & Howell (1976). Populations that share the same letter (a, b, ..., h) per row, are not significantly different (a = 0.05). The codes (A, B, ..., M) for the various characters are the same as in Table 2. The abbreviations of populations can be found in Table 1

	MEM	CIT	POL	KAL	AIL	BAL	CAT	CHK	DOJ	HAN	NAM	PUL	XUW	HJA	DAL
A	e,f,g,h	e,f,g,h	g,h	b,c,d,e	e,f,g	b,c,d,e	c,d,e,f	b	b,c,d	b,c	a	b,c,d,e	e,f,g,h	c,d,e,f	h
B	e,f,g	e,f,g,h	e,f,g	b,c,d,e	c,d,e,f,g	c,d,e,f,g	b,c,d,e	b	b,c	b	a	b,c,d	e,f,g	b,c,d,e,f	h
C	d,e,f	e,f,g	e,f	b,c,d,e	e,f	d,e,f	b,c,d,e	b,c	b,c	b	a	b,c,d	e,f	c,d,e,f	g
D	b,c,d,e,f,g	d,e,f,g,h	f,g,h	b,c,d,e,f,g	d,e,f,g,h	d,e,f,g,h	b,c,d	b,c	b,c,d,e	b	a	b,c,d,e,f	d,e,f,g	d,e,f,g,h	g,h
E	f,g,h	b,c,d,e,f	d,e,f,g,h	f,g,h	c,d,e,f	a,b	c,d,e,f,g,h	a	b,c,d,e,f	a,b,c,d	c,d,e,f,g	a,b,c	f,g,h	b,c,d,e	b,c,d,e,f
F	b,c,d	b,c,d	b,c,d	a,b,c	a,b	a	e	b,c,d	d	d	e	b,c,d	b,c,d	b,c,d	b,c,d
G	h	e,f,g	f,g,h	b,c,d,e,f	e,f,g,h	a,b,c,d	b,c,d,e	a,b,c,d,e	a,b,c	a,b	a	a,b,c	e,f,g	d,e,f,g	b,c,d,e,f
H	e,f	d,e	e,f	a,b,c,d	b,c,d	a,b	a,b,c	a,b,c,d	b,c,d	a,b	a	b,c,d	f	e	b,c,d
I	f,g	c,d,e	d,e,f	b,c,d	c,d,e	b	b,c	b,c,d	b	a,b	a	b	g	c,d,e,f	b,c,d,e
J	g	c,d,e,f	c,d,e	a,b,c,d	d,e,f,g	a,b,c	c,d,e,f,g	c,d,e,f,g	c,d	a,b,c,d	a	a,b,c	h	d,e,f,g	a,b,c,d
K	a,b	a	a,b	a,b,c	a,b,c	c,d,e	f	c,d,e,f	e,f	c,d,e,f	f	c,d,e,f	a,b,c	b,c,d,e	b,c,d
L	a	a	a	a,b	a,b,c	b,c,d,e	f	c,d,e,f	e,f	d,e,f	f	e,f	a,b,c	c,d,e,f	b,c,d,e
M	g,h	b,c,d,e	d,e,f,g	d,e,f,g,h	b,c,d	a,b	b,c,d,e	a	b,c,d,e	b,c,d	d,e,f	b,c,d	h	b,c	b,c,d,e

Table 4. Results of the discriminant analysis. The classification was based on the origin of each population

Variable	Unstandardized canonical discriminant function coefficients*			Standardized canonical discriminant function coefficients*		
	1	2	3	1	2	3
Total length	20.5503828	−1.9919531	−0.9182448	0.59319	−0.05750	−0.02651
Abdominal length	−8.7883584	2.8049734	3.3432993	−0.31134	0.09937	0.11844
Length up to third ab.s.	26.6589978	−4.1719837	2.1435532	0.96804	−0.15149	0.07784
Length of the 8th abd.s	1.9909037	−2.0349200	−3.1557132	0.09085	−0.09286	−0.14400
Width of 3rd abd. segm.	−8.4306974	−1.5479552	−8.6719410	−0.27365	−0.05025	−0.28148
Length of furca	−7.3195161	2.2719251	−0.1865445	−0.48763	0.15136	−0.01243
Width of head	−4.2482949	−16.0326828	−29.3804486	−0.11865	−0.44778	−0.82057
Length of 1st antenna	−5.6265499	7.7990300	0.1409685	−0.18562	0.25729	0.00465
Distance between eyes	7.6838676	26.1122522	24.1504402	0.22133	0.75214	0.69563
Diameter of eyes	−3.4419944	6.4880032	18.3382265	−0.11871	0.22376	0.63246
No. of setae left branch	0.5978158	−0.5033924	1.3742926	0.18628	−0.15686	0.42822
No. of setae right branch	0.1405275	−0.7587268	1.2522186	0.04300	−0.23218	0.38320
Width of the ovisac	−11.9591987	7.5841354	−2.5473703	−0.54556	0.34598	−0.11621
Constant	−40.8168018	0.2035977	−7.2071804			

	Eigenvalue**	Percentage of variance	Cumulative percentage	Canonical correlation	Wilks' lambda***	Chi-square	DF	*P*
Function 1	5.8768	53.78	53.78	0.9244	0.003982	2436.967	182	< 0.0001
Function 2	1.4922	13.66	67.44	0.7738	0.027383	1586.651	156	< 0.0001
Function 3	1.1786	10.79	78.23	0.7355	0.068242	1183.948	132	< 0.0001

* The unstandardized coefficients are the multipliers of the variables when they are expressed in the original units, while the standardized coefficients are used when the variables are standardized to a mean of 0 and a standard deviation of 1, just like in multiple regression (Norusis, 1993).

** Eigenvalue is the ratio of the between groups to within groups sums of squares. Large eigenvalues are associated with 'good' functions (Norusis, 1993).

*** Wilks' lambda (λ) is given by the equation $\lambda = 1 - \eta^2$, where η^2 is the ratio of the between-groups sum of squares to the total sum of squares and represents the proportion of the total variance attributable to differences among the groups (Norusis, 1993).

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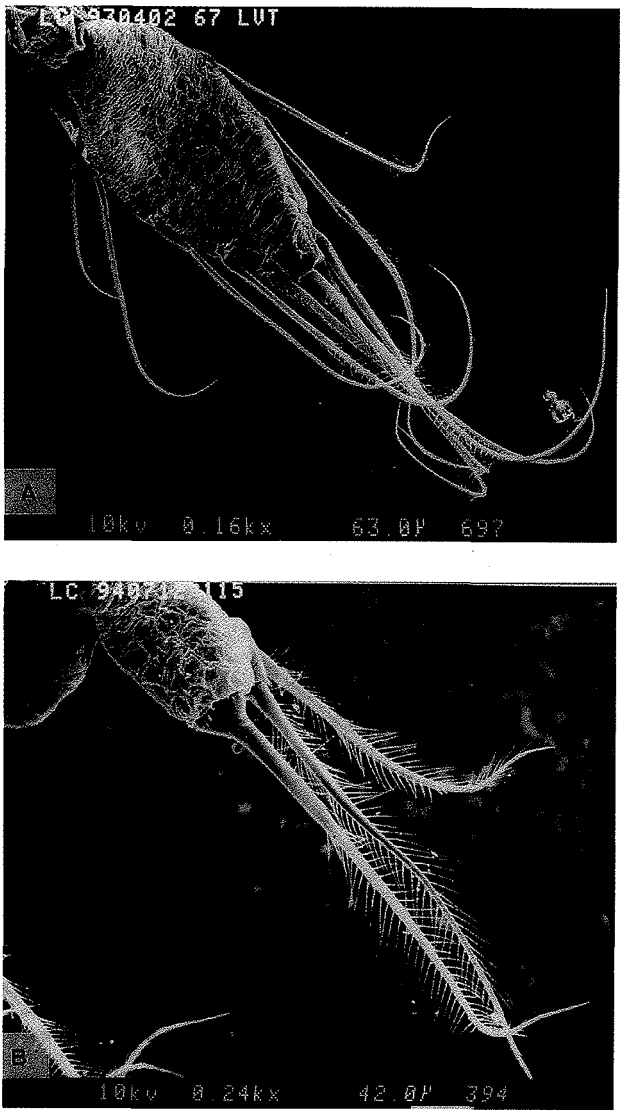

Figure 3. Scanning electron micrographs of the differences observed in the length of the furca and number of setae. Plate A: NAM population, plate B: CIT population.

Table 5. Classification results of discriminant analysis showing the percentage of individuals classified in each group. The diagonal elements are the number of cases classified correctly into the groups and serve as an indicator of the effectiveness of the discriminant analysis. The percent of 'grouped' cases correctly classified is 93.20%. The abbreviations of populations can be found in Table 1

Actual	No of	Predicted Group Membership (%)														
group	Cases	MEM	CIT	POL	KAL	AIL	BAL	CAT	CHK	DOJ	HAN	NAM	PUL	XUW	HUA	DAL
MEM	31	93.5	0.0	3.2	0.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CIT	31	0.0	90.3	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2
POL	31	3.2	6.5	83.9	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2
KAL	33	9.1	0.0	3.0	87.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AIL	30	3.3	0.0	0.0	0.0	90.0	3.3	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0
BAL	30	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CAT	30	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CHK	30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	93.3	0.0	0.0	0.0	3.3	0.0	3.3	0.0
DOJ	30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	93.3	0.0	0.0	6.7	0.0	0.0	0.0
HAN	30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	93.3	0.0	3.3	0.0	0.0	0.0
NAM	32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
PUL	30	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	93.3	0.0	0.0	3.3
XUW	30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
HUA	30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	10.0	0.0	83.3	0.0
DAL	28	0.0	0.0	0.0	0.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	96.4

to exhibit great morphological similarities to the previous populations. It is known that CAT population is diploid (Pilla, 1992) but we lack information of the ionic composition and of the exact geographical location of this population. Hontoria & Amat (1992a) showed that parthenogenetic diploid and tetraploid populations, from the Western Mediterranean basin, can be thoroughly recognized by their morphological characteristics. The same ploidy level of the populations that form the first group seemed to result in similar morphological patterns.

BAL, DAL and AIL populations are in geographically close inland salt lakes where sulphate is the prevailing anion. They presented different ploidy levels: AIL is diploid, BAL tetraploid and DAL a mixture of diploids and tetraploids (Pilla, 1992; K. Thomas, personal communication). The differences in the ploidies could be the reason why DAL population appears between AIL and BAL.

The third group is comprised by the Greek populations. They are polyploid (Abatzopoulos et al., 1986; Triantaphyllidis et al., 1993) and they all exist in coastal saltworks. Although MEM population appears to diverge from the 'core' of the Greek cluster, the Greek populations are clearly discriminated from all the other parthenogenetic populations.

NAM population is a mixture of different ploidies (Barigozzi, 1986). However, the studied batch is mainly diploid (Triantaphyllidis et al., unpublished data) and exhibits some characteristics that allow 100% discrimination from all the other populations studied here. The telson is very long (the longest among the populations studied here) and the abdomen is very short, characteristics that allow the NAM population to form a group alone, far discriminated from all the other groups.

Due to lack of information about the ploidy level of the XUW population it is rather difficult to understand the reasons that contributed to such distinct discrimination. Discriminant analysis classifies it with 100% prediction and this suggests that the overall morphology of this population differs not only from the other Chinese populations but from all the other populations considered in this study. Cluster analysis groups it close to the Greek populations and this could possibly mean that it is a polyploid population. Further study needs to be done on this population.

Geographically-isolated populations, even if they share the same ploidy level, do not necessarily present similar morphological patterns. For instance, although BAL population and the four Greek populations are all tetraploid, they do not cluster together; this may be due to the fact that BAL population inhabits a sulphate environment while the Greek populations live in coastal salinas where chloride is the prevailing anion. Despite their similarities in their ploidy level the latter populations experience environments with different ionic composition and it is possible that natural selec-

tion favours genotypes (clones) which are better adapted to that particular environment. This selection could eventually lead to slight morphological changes that become apparent after applying multivariate statistical methods. It is known that ecological isolation exists in *Artemia* and that there are differences in tolerance of anion concentrations between populations (Bowen et al., 1985, 1988). Ecological isolation or environments with different ionic composition can lead to morphological differentiation (Hontoria & Amat, 1992b). Thus, ploidy level is possible to dictate a morphological pattern only in geographically-close populations provided that the environmental or physical traits of their ecosystems are also quite identical. As more populations are made available for study, we see that the possible morphological repertoires are enriched.

Parthenogenetic populations can be discriminated from females of bisexual populations as has already been demonstrated by Triantaphyllidis et al. (1995) for an *A. franciscana* and a parthenogenetic population from Tanggu (Tianjin, People's Republic of China). By applying discriminant analysis the morphological differences between parthenogenetic and bisexual females allowed allocation to the correct species as of day 14 with almost 100% accuracy and in salinities ranging from 35 to 180 ppt (Triantaphyllidis et al., 1995b). The same resulted from merging the data of this study with the data of the earlier study of Triantaphyllidis et al. (1995). In all cases parthenogenetic females could be discriminated from the bisexual females with 100% accuracy.

The results from the discriminant analysis presented in Figure 1 seem to contradict the results of cluster analysis presented in Figure 2, i.e. BAL population in discriminant analysis seems to cluster closer to the coastal Chinese group rather than to the Chinese inland lake group. This partial conflict between the results of discriminant and cluster analysis was not observed in the case of bisexual populations (Triantaphyllidis et al., 1997). The potential problems of cluster analysis occur at every level of the strategy (Pimentel, 1979). A given set of cluster analyses can be subjected to more than one interpretation. The hierarchical feature limits the method to extracting a one dimensional portrayal of relationships among individuals or populations in our case. Farris (1977) has demonstrated that the single dimension extracted by cluster analysis need not be the most parsimonious representation of the operational taxonomic units (OTU's). Whenever relationships among OTU's require more dimensions for clear protrayal, cluster analysis will be imprecise. Since different strategies can result in drastically different dendrograms, various interpretations of a given study are possible. Choosing the 'best' strategy should be approached very carefully and must be accomplished on a non-statistical basis (Pimentel, 1979); different researchers, most probably, would select different dendrograms as being most biologically meaningful. 'In spite of the above difficulties, there is little doubt that cluster analysis can and has produced useful results. This would imply that in given circumstances (as in the case of bisexual populations described in a previous study) the potential pitfalls either do not exist, or methodology is sufficiently robust to circumvent the pitfalls' (Pimentel, 1979).

Conclusions

Application of multivariate methods in the morphologic study of *Artemia* populations seems to be a useful tool. If information of the chromosome number, ionic composition of the water as well as climatic conditions will be combined with genetic data (i.e. isozymes) then we can obtain a very precise picture of the studied populations. In agreement with Hontoria & Amat (1992a,b) great care should be given to the standardization of the culture conditions and we repeat here the proposal for using standard laboratory conditions for all the studies that will take place in the future.

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International Study on *Artemia*. LVII. Morphological and molecular characters suggest conspecificity of all bisexual European and North African *Artemia* populations

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Abstract A scanning electron microscopy (SEM) study of bisexual *Artemia* populations revealed that populations representing the species *A. franciscana*, *A. persimilis*, *A. urmiana*, *A. sinica* and a recently described species from Kazakhstan have a pair of spine-like outgrowths at the basal parts of their penes, whereas populations from southern Europe and North Africa (i.e. Mediterranean populations) lack these spine-like outgrowths. Allozyme and DNA polymorphisms, detected by allozyme starch gel electrophoresis and AFLP fingerprinting, respectively, suggested conspecificity of the studied populations from the broader Mediterranean basin. Male specimens from the collection of the Natural History Museum of London (UK) of the extinct *A. salina* population from Lymington lack spine-like outgrowths at the basal parts of the penes. This finding, based on a taxonomic character which is quite reliable, suggests conspecificity of *A. salina* from Lymington and the present bisexual *Artemia* populations from the Mediterranean basin, grouped under the binomen *A. tunisiana*.

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Introduction

The brine shrimp *Artemia* (Crustacea: Branchiopoda) is a well-studied organism, however, taxonomists are still puzzled about the evolution and the phylogenetic relationship of the populations that comprise the genus (for a review of the confusing names in the genus *Artemia* see Belk and Brtek 1995). Schlösser made the first description of the brine shrimp in 1755 on material collected from solar saltworks near Lymington, England, UK (Kuenen and Baas-Becking 1938). Linnaeus described the brine shrimp as *Cancer salinus* in 1758 and Leach renamed it as *A. salina* in 1819 (Artom 1931). In the following years many populations have been identified, and nowadays the genus *Artemia* is comprised of a complex of bisexual species and superspecies as well as parthenogenetic populations with various degrees of ploidy (Browne and Bowen 1991).

Artom (1905, 1906, 1907), Stella (1933), Stefani (1963), Stefani and Cadeddu (1967), Halfer Cervini et al. (1968), Piccinelli et al. (1968) and Barigozzi (1974), studying the *Artemia* populations in Italy, used the binomen *A. salina* for the bisexual populations that had 42 chromosomes. Ever since 1910 (Barigozzi 1974) and even recently (Sorgeloos and Beardmore 1995), most scientific papers referred to all brine shrimps as *A. salina* although in the meantime it was known that two distinct modes of reproduction occurred (parthenogenetic or zygogenetic) and that new bisexual species had been described: *A. franciscana* (Kellogg 1906) in the New World and *A. urmiana* (Günther 1890) in Lake Urmia, Iran. Piccinelli and Prosdocimi (1968) described a new species, *A. persimilis*, from the saltworks of San Bartolomeo, Cagliari (Sardinia) and Hidalgo (Argentina). This species has 44 chromosomes and a different adult morphology. It was considered to be sympatric with *A. salina*. Clark and Bowen (1976) showed that the