NORTH-WESTERN JOURNAL OF ZOOLOGY 10 (1): 149-157 ©NwjZ, Oradea, Romania, 2014
Article No.: 131306 http://biozoojournals.ro/nwjz/index.html

Biometric characterization of Chinese parthenogenetic *Artemia* (Crustacea: Anostraca) cysts, focusing on its relationship with ploidy and habitat altitude

Alireza ASEM and Shi-Chun SUN*

Institute of Evolution and Marine Biodiversity, Ocean University of China, 5 Yushan Road, Qingdao 266003, China
* Corresponding author, S.-C. Sun, E-mail: sunsc@ouc.edu.cn

Received: 26. August 2013 / Accepted: 7. November 2013 / Available online: 28. December 2013 / Printed: June 2014

Abstract. This paper reports on biometrical variation of parthenogenetic *Artemia* cysts from 12 saline lakes and salterns of China. The results showed that the largest diameter of untreated and decapsulated cysts of the studied populations belong to Aqqikkol Lake (325.4 ± 13.7 µm; 301.4 ± 14.2 µm, respectively) and the smallest from Xiaotan Saltern (258.7 ± 10.8 µm) and Gaodao Saltern (244.3 ± 15.5 µm). The thickest and the thinnest chorion layers are from Yinggehai Saltern (12.2 µm) and Aibi Lake (3.5 µm), respectively. Our results confirm that the studied biometrical parameters (the diameters of untreated and decapsulated cysts as well as chorion thickness) present a significant correlations ($p < 0.05$) and also that the populations at very high-altitude locations have the biggest cysts. However, the regression analysis showed that the correlations between altitude and biometrical parameters of cysts were not significant. The relationship between degree of ploidy and cyst size is not clear, so size is more likely due to the combined effect of ploidy degree, physico-chemical conditions, food availability and population-specific parameters. The present results also suggest that the high standard deviations (SD) and coefficients of variation (CV) in cysts of Barkol population may be related to their complex ploidy composition (2n: 27.4%; 3n: 4.9%; 4n: 37.8% and 5n: 29.9%). Cluster analysis and principal components analysis do not reveal correlation between biogeographical distribution and cyst biometrics.

Key words: *Artemia*, parthenogenetic, cyst biometry, ploidy, altitude.

Introduction

The brine shrimp *Artemia* is a crustacean genus with a wide distribution over all continents except Antarctica (Van Stappen 2002). It has become adapted to widely different ecological conditions, such as temperature and altitude (Abatzopoulos et al. 1998, Triantaphyllidis et al. 1998) and various ionic compositions (Bowen et al. 1985, Lenz 1987, Browne et al. 1988), as well as a very broad range of salinity between 7.0 $g.l^{-1}$ to 340 $g.l^{-1}$ (Liu & Zheng 1990, Post & Yossef 1977). These specializations have led to the genus becoming a cosmopolitan taxon, being naturally distributed or artificially introduced to tropic, temperate and cold regions.

Artemia is well known as an invaluable live food for aquaculture (Sorgeloos et al. 2001, John et al. 2004, Ben Naceur et al. 2012a). Although more than 600 locations have been reported for resident populations of *Artemia* around the world (Van Stappen 2002), their harvest or production from natural locations are generally not economical except for a few locations. On the other hand, *Artemia franciscana*, an American native species, because of its high biological productivity has been introduced and cultured in several African, European, Asian and Australian locations, so a number of habitats have been invaded by *A. franciscana* (Zheng et al. 2004, Amat et al. 2005, Abatzopoulos et al. 2006a, Mura et al. 2006, Ruebhart et al 2008, Vikas et al. 2012, Ben Naceur et al. 2012b, Salman et al. 2012, Pinto et al. 2013, Zheng & Sun, 2013). Even if this approach has been justified by the economic benefits, it can also lead to the extermination of native populations (Zhou et al. 2006). In the long run this will cause irreparable losses to *Artemia* biodiversity. Consequently, description of biological characteristics of native *Artemia* populations, and the careful choice of suitable locations and populations for *Artemia* aquaculture can support commercial enterprise as well as safeguarding *Artemia* biodiversity.

Biometrical characterization of cysts represents an important and useful criterion in the study of each *Artemia* population. Such information can show the variability in the size of cysts and be of value in aquaculture. For example, the smallest cysts have high quality with regard to economical and biological view points (Sorgeloos et al. 1978, Asem et al. 2007).

Generally, *Artemia* is divided into two reproductive groups: bisexual and parthenogenetic populations. Parthenogenetic populations are widely distributed in the Old World and in oce-

Table 1. List of parthenogenetic populations of *Artemia* and their summarized information.

Locality	Abbreviation	Geographic coordinates	Sampling year	Altitude (m)
Aibi Lake[1] , Jinghe, Xinjiang	AB	44°53'N, 83°00'E	2000	194
Barkol Lake[2], Barkol, Xinjiang	BRK	43°40'N, 92°44'E	1986	1617
Chengkou Saltern, Wudi, Shandong	CK	38°03'N, 117°55'E	1985	~ sea level
Dongfanghong Saltern, Laizhou, Shandong	DFH	37°20'N, 119°55'E	1985	~ sea level
Gaodao Saltern, Wendeng, Shandong	GD	36°59'N, 122°03'E	1985	~ sea level
Xiaotan Saltern, Haiyang, Shandong	XT	36°41'N, 121°08'E	1985	~ sea level
Aqqikkol Lake, Ruoqiang, Xinjiang	AQK	37°04'N, 88°22'E	2005	4255
Hoh Lake[3], Ulan, Qinghai	HOH	36°57'N, 98°17'E	1988	2995
Ga Hai Lake, Delingha, Qinghai	GH	37°08'N, 97°33'E	2000	2870
Xuwen Saltern, Xuwen, Guangdong	XW	20°15'N, 109°56'E	1987	~ sea level
Dongfang Saltern, Dongfang. Hainan	DF	19°11'N, 108°41'E	1988	~ sea level
Yinggehai Saltern, Ledong, Hainan	YGH	18°31'N, 108°44'E	1987	~ sea level

1) = Ebinur Hu or Ebinur Lake; 2) = Balikun Lake; 3) = Keke Salt Lake.

anic land masses (Van Stappen 2002), and can be of different ploidy degrees (Vanhaecke & Sorgeloos 1980, Triantaphyllidis et al. 1996). Interestingly, coexistence of parthenogenetic populations with different ploidy levels can occur in the same habitat (Wang et al. 1991, Pan et al. 1991, Xu et al. 1993, Yang et al. 1995, Wang & Sun 2007, Wang 2009). These can be viewed as interesting models for a variety of biological studies. In this sense, China, where *Artemia* populations thrive in a big variety of climates, reliefs and saline wetlands, could be considered as a suitable region for diversity studies.

The main aim of this study is to present the biometrical variation for cysts of parthenogenetic populations, examine statistical correlations between biometric parameters and their correlations with altitude and ploidy degrees.

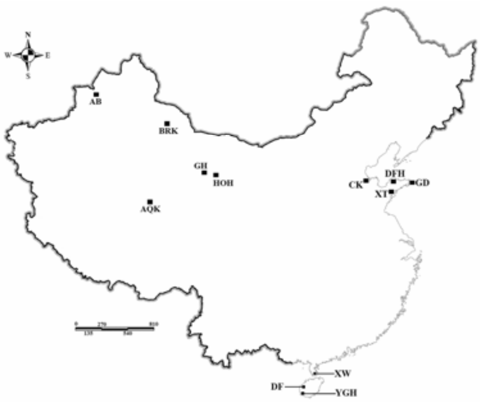

Figure 1. Geographical distribution of the studied *Artemia* sites.

Materials and Methods

Biometrical characterizations of cysts of parthenogenetic *Artemia* were studied in 12 localities from China (Table 1). Figure 1 shows the geographical distributions of studied populations.

The cysts of each population were hydrated and decapsulated with standard methods (see Asem et al. 2007). Diameters of untreated (fully hydrated) and decapsulated cysts were measured using a Nikon eclipse 600 microscope equipped with an Olympus DP72 camera and software of CellSens Standard. Chorion thickness of cysts was calculated as follows: "Chorion thickness = (Mean diameter of untreated cyst - Mean diameter of decapsulated cyst)/2". This value is reported without standard deviations (see Vanhaecke & Sorgeloos 1980, Asem et al. 2007).

Significant differences between means were determined by One-Way ANOVA (Tukey, $p < 0.05$). Three characters (diameter of untreated cysts, diameter of decapsulated cysts and chorion thickness) were used for the grouping of populations using the hierarchical cluster analysis and principal components analysis (PCA). The linear regression and Pearson's correlation were computed between the biometrical parameters of cysts and also between biometrical parameters and habitat altitude. The computer program SPSS 16 was used for statistical analysis.

Results

Cyst Biometry

Biometrical information on cysts is given in Table 2. The largest diameter of untreated cysts belongs to AQK (325.4 ± 13.7 µm) that is significantly different from all other cyst diameters. (ANOVA, Tukey; $p < 0.05$). XT yields the smallest size of hydrated cysts (258.8 ± 10.8 µm), followed by DFH (259.1 ± 11.3 µm), GD (260.4 ± 14.0 µm), BRK (263.5 ± 20.4 µm), GH (264.3 ± 11.7 µm) and AB (264.9 ± 19.7 µm), with none of these being significantly different (Table 2; ANOVA, Tukey; $p > 0.05$). The highest coefficient of variation (C.V) is reported

Table 2. Biometric values for cysts of parthenogenetic Artemia from 12 localities in China. Abbreviations of *Artemia* populations are defined in Table 1. Same letters in each column show non-significant difference (ANOVA, Tukey test, p > 0.05).

Site	Untreated cyst (µm)		Decapsulated cyst (µm)		Chorion	Degree of ploidy (%)				Ref.
	Mean ± SD	C.V	Mean ± SD	C.V	thickness (µm)	2n	3n	4n	5n	
AB	264.9 ± 19.7 ^{abc}	7.4	257.8 ± 17.4^{c}	6.8	3.5	91.03	-	8.28	-	1
BRK	263.5 ± 20.4^{abc}	7.7	247.4 ± 18.5^{ab}	7.5	8.1	27.44	4.88	37.8	29.88	1
CK	266.6 ± 15.0^{bc}	5.6	254.0 ± 14.0^{c}	5.5	6.3	93	-	7	-	2, 3
DFH	259.1 ± 11.3^{a}	4.3	247.3 ± 11.3^{ab}	4.6	5.9	98.55	-	1.40	-	4
GD	260.4 ± 14.0^{ab}	5.4	244.3 ± 15.5^{a}	6.3	8.1	72.88	-	6.78	20.84	4
XT	258.8 ± 10.8^{a}	4.2	248.1 ± 10.0^{ab}	4.0	5.4	92.42	-	7.58	-	4
AQK	325.4 ± 13.7^{f}	4.2	301.4 ± 14.2^{g}	4.7	12.0	80	-	20	-	2, 3
HOH	278.6 ± 14.0^{d}	5.0	264.8 ± 12.1^{d}	4.6	6.9	-	-	100	-	5
GH	264.3 ± 11.7^{abc}	4.4	252.9 ± 13.0^{bc}	5.1	5.7	100	-	-	-	2, 3
XW	295.4 ± 14.7^{e}	5.0	281.5 ± 12.4^{f}	4.4	7.0	not analyzed				
DF	268.9 ± 17.0^{c}	6.3	255.2 ± 14.5^{c}	5.7	7.0	not analyzed				
YGH	295.4 ± 14.7^{e}	5.0	271.1 ± 13.1^{e}	4.8	12.2	-	-	2.7	97.3	5

SD: standard deviations, CV: coefficient of variation
Ref. 1: Yang et al. (1995); 2: Wang & Sun (2007); 3: Wang (2009); 4: Pan et al. (1991); 5: Xu et al. (1993)

for diameters of untreated cysts from BRK (7.7) and the lowest for those from XT and AQK (4.2).

AQK presents the biggest size of decapsulated cyst (301.4 ± 14.2 µm) with statistical differences in comparison with other populations. The smallest decapsulated cyst is observed in GD (244.3 ± 15.5 µm), without significant differences with DFH (247.3 ± 11.3 µm), BRK (247.4 ± 18.5 µm) and XT (248.1 ± 10.0 µm) (ANOVA, Tukey; p > 0.05). The maximum and minimum coefficients of variation appear in BRK (7.5) and XT (4.0), respectively. According to the ANOVA (Tukey test) 42 pairs of means of untreated cysts among the 12 populations show significant differences, whereas 51 pairs of means of decapsulated cysts show statistically significant differences (see Table. 2).

The thickest chorion is observed in cysts from YGH (12.2 µm), followed by those from AQK (12.0 µm), and the thinnest chorion belongs to the cysts from AB (3.5 µm). We also note that cysts from Aqqikkol Lake (AQK) exhibit dark brown shells as compared to the light brown shells of other populations.

<u>Hierarchical cluster analysis</u>

According to hierarchical cluster analysis, which used three biometrical characters of cysts, 12 parthenogenetic populations of *Artemia* are separated into two clusters (Fig. 2). Cluster I contains three divided groups at intervals of approximately 4. Group I includes two sub-groups. Sub-group I holds five populations: DFH, XT, GH, CK, DF; and sub-group II comprises only two populations: BRK and GD. Groups II and III consist of a single population, HOH and AB, respectively.

Cluster II is divided into two sub-clusters at the interval of 10-15 (Fig. 2). Sub-cluster I is cut up into two categories at a distance of approximately 8, both containing only a single population. Category I includes XW and another includes YGH po-

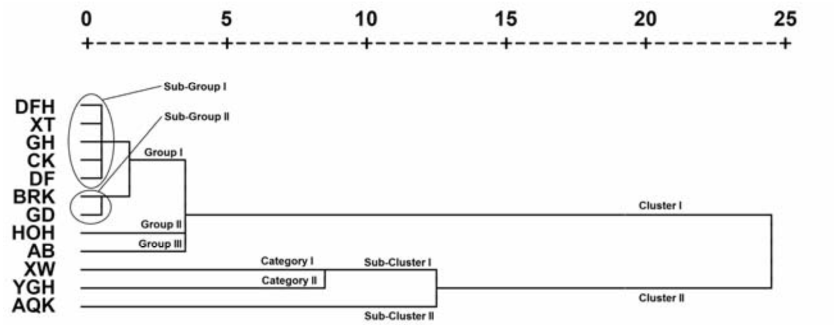

Figure 2. Diagram of hierarchical cluster analysis based on three biometrical characters of cysts (see text for further details).

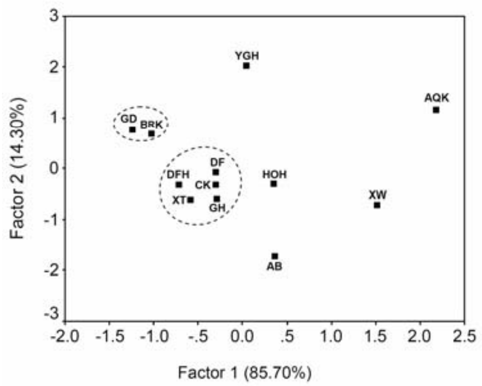

Figure 3. Scatterplot of principal component analysis based on three biometrical characters of cysts.

pulation. AQK belongs to sub-cluster II.

Principal components analysis (PCA)

With regard to PCA, the first and second components showed 85.70% and 14.30% of variation and, on the whole, the two components involved 100% of separation (Fig. 3). In the first component, the means of decapsulated cysts (0.957), untreated cysts (0.880) and chorion thickness (0.350) have a major influence on classification of populations and in the second one, the chorion thickness (0.554), the means of decapsulated cysts (-0.335) and untreated cysts (-0.142) show the main impact on grouping, respectively. PCA could group seven populations into two separated collections, one consisting of BRK and GD, and another of DF, CK, GH, DFH and XT. However, the other populations are separately located in PCA scatter (Fig. 3).

Regression and correlation

The equations of linear regression and Pearson's correlations are presented in Figure 4. The results show that there is a positive and significant correlation between diameters of untreated cyst and decapsulated cysts, as well as between diameters of untreated cysts and chorion thickness and between diameters of decapsulated cyst and chorion thickness. Linear regressions between altitude and biometrical characters of cysts indicates a positive relationship (Fig. 5), whereas significant correlations between altitude and diameter of untreated cysts (r = 0.50, Sig. = 0.098). Moreover there are no significant correlations between altitude and diameter of decapsulated cysts (r = 0.50, Sig. = 0.095) and also between altitude and chorion thickness (r = 0.33, Sig. = 0.298).

Discussion

The first comprehensive study of size variation of cysts was done by Vanhaecke & Sorgeloos (1980), who divided all cysts into three groups: i) small cysts, ii) large cysts, and iii) cysts with intermediate sizes. Although Vanhaecke & Sorgeloos (1980) documented that the large cyst size was a character of parthenogenetic populations, a study of 11 Iranian *Artemia* populations has established that cysts of the bisexual *Artemia urmiana* had larger diameters and thicker chorions than some parthenogenetic populations (Abatzopoulos et al. 2006a). Triantaphyllidis et al. (1996) characterized cysts of parthenogenetic *Artemia* from Ankiembe saltworks in Madagascar (3n = 63) and Swakopmund saltworks in Namibia (2n = 42). They showed that the diameters of untreated and decapsulated Madagascan cysts (258.9 ± 11.6 µm; 246.2 ± 11.7 µm) were larger than Namibian cysts (246.7 ± 11.0 µm; 233.1 ± 9.8 µm), probably related with their ploidy levels, but no significant difference between chorion thicknesses were observed.

Yu & Xin (2006) recorded the largest mean size of cysts for a population of an unidentified *Artemia* (untreated cyst: 358.78 µm, decapsulated cyst: 338.73 µm and chorion thickness: 10.02 µm) from an ambiguous site "Gaize Lake", which was recently clarified to be Lagkor Co (see Zheng & Sun 2013), the type locality of *A. tibetiana*. Wang & Sun (2007) reported similar results for the cysts from Lagkor Co (untreated cyst: 344.5 µm, decapsulated cyst: 320.5 µm and chorion thickness: 12.0 µm). An earlier study also reported big size of cysts from "Gaize Lake" (Wang, 2002), but whether Wang's "Gaize Lake" represents the same locality could not be determined (see Zheng & Sun 2013). Although Castro et al. (2006) reported the diameter of 386.3 µm for untreated cysts of *A. franciscana* from El Marquez (Oaxaca: Mexico), a corrected report put the value at 253.77 µm (Jorge M. Castro, pers. comm. 2012). Ma & Wang (2003) found the largest cysts of *Artemia* belonging to parthenogenetic population of Aqqikkol Lake (China) (315.8 µm and 303.1 µm for untreated and decapsulated cysts, respectively), which was supported by the results of Wang & Sun (2007), Wang (2009) and also of the present study (Table 2). Therefore, it is most likely that *Artemia* from Aqqikkol Lake has the largest cysts among all parthenogenetic populations. We found that cysts from Aqqikkol Lake, a habitat at high altitude (4255 m), exhibit shells that have a much darker brown color than the other

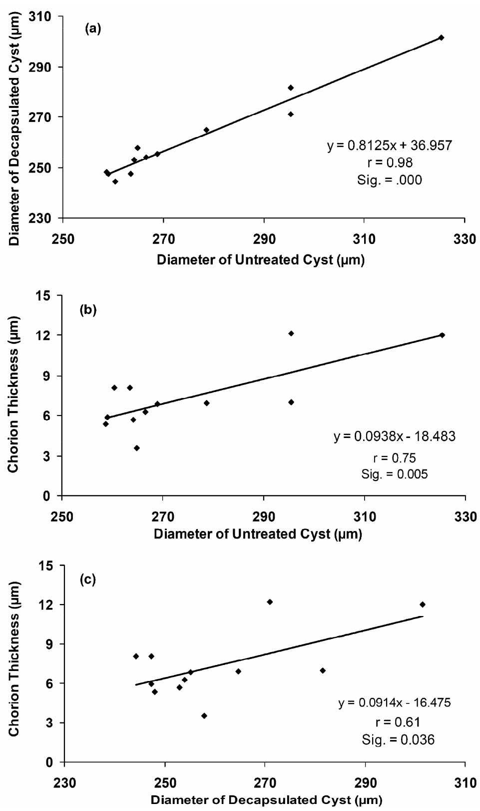

Figure 4. Regression lines and equations: a) The relationship between diameters of untreated cyst and decapsulated cyst; b) The relationship between diameter of untreated cyst and chorion thickness; c) The relationship between diameter of decapsulated cyst and chorion thickness.

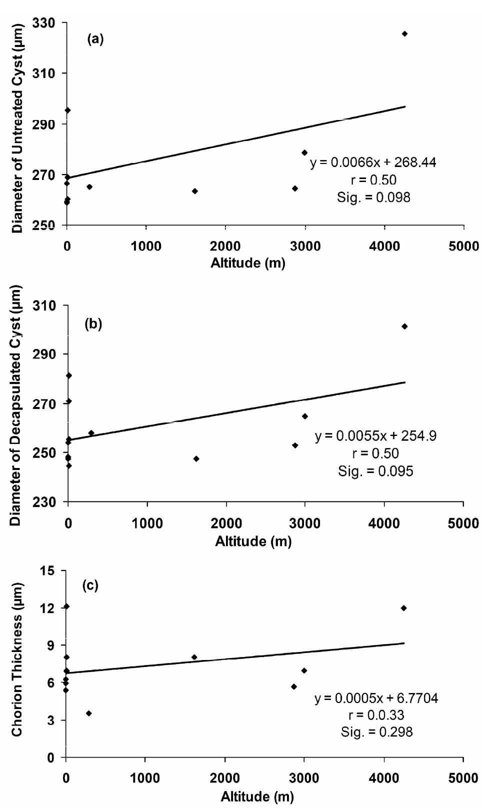

Figure 5. Regression lines and equations: a) The relationship between altitude and diameter of untreated cyst; b) The relationship between altitude and diameter of decapsulated cyst, c) The relationship between altitude and chorion thickness.

samples of the present study, which was also mentioned for some bisexual populations from Qinghai-Xizang Plateau by Wang (2009).

Ma et al. (1994) have described the smallest Chinese parthenogenetic untreated cysts from Ga Hai Lake (244.6 µm) and that result has been verified by Wang (2002) who reported a value of 246 µm for cysts from this lake. But our study indicates bigger cyst size for this population (264.3 µm) and this result is in agreement with those of others (Liu et al. 1998, Zhang et al. 1998, Ma et al. 2003, Wang & Sun 2007, Wang 2009). Yang et al. (1995) have reported that the smallest decapsulated cysts of Chinese parthenogenetic *Artemia* are from Ga Hai Lake (233.06 µm) and Aibi Lake (233.57 µm), and Ma & Wang (2003) reported a value of 239.2 µm for decapsulated cysts from Ga Hai. In contrast, others have obtained significantly higher values (Ga Hai Lake: 248.9-255.1 µm, Aibi Lake: 251.4-267.3 µm; see Pan et al. 1991, Liu et al. 1998, Zhang et al. 1998, Ma et al. 2003, Yang et al. 2005, Wang & Sun 2007, Wang 2009). In the present study, Ga Hai Lake exhibited larger untreated cyst diameters, (264.3 µm) (Table 2), in comparison with previous reports.

The thickest chorion among all parthenogenetic populations and bisexual *Artemia* species has been reported for parthenogenetic cysts from Chengkou Saltern with a thickness of 17.3 µm (Xu 1996). In contrast, our results on this population indicate a chorion thickness of only 6.3 µm. In addition, values of 10.2 µm (Wang & Sun 2007, Wang 2009) and 8.7 µm (Liu et al. 1998) have also been reported in this saltern. According to our observations, the thinnest chorion of Chinese parthenogenetic *Artemia* belongs to cysts from Aibi Lake, being 3.5 µm, although other authors have reported values between 6.6 to 10.61 µm (Zhang et al. 1990, Pan et al. 1991, Yang et al. 1995, Liu et al. 1998, Zhang et al. 1998, Ma et al. 2003, Yang et al. 2005).

The biometrical diversifications of parthenogenetic *Artemia* cysts have been imputed to degrees of populations' ploidy by Vanhaecke & Sorgeloos (1980). Wang et al. (1991) cloned three populations with a complex of diploid (2n) and pentaploid (5n) chromosome numbers in the laboratory and measured their cyst diameters. Their results confirmed that, in all three populations, cysts of pentaploid specimens were larger than diploid ones. Also, Triantaphyllidis et al. (1996) attributed the large diameters of Madagascan cysts (3n = 63) and the small sizes of Namibian cysts (2n = 42) to their different ploidy levels. In the present study, the YGH population with 97.3% pentaploid (5n) has bigger cysts than those from the HOH (4n: 100%) and the both are larger than cysts of AB, CK, DFH, XT and GH (in all these populations, 2n>90%; see Table 2). Furthermore Barkol Lake population show high standard deviations (SD) and coefficients of variation (CV) for untreated and decapsulated cysts (Table 2). This population has been noted as complex in ploidy composition (2n: 27.4%; 3n: 4.9%; 4n: 37.8% and 5n: 29.9%) (Yang et al, 1995), so the high values of SD and CV likely result from the variation in ploidy. Thus the present results basically support the previous results mentioned above. However, AQK (2n: 80%; 4n: 20%) has the biggest diameter in all the studied populations, including those populations with high percentages of diploids and pentaploids such as GD, of diploids, tetraploids and pentaploids like BRK, or 100% of tetraploids as HOH; also cysts from populations with similar ploidy composition may have significant difference in cyst sizes (e.g. CK shows significantly larger cyst than XT). Moreover, Xu (1996) documented the cyst size of 283.7 ± 16.0 µm for CK population (2n>90%), apparently bigger than the present value (266.6 ± 15.0 µm) of the same population and even larger than the mean value of the tetraploid HOH sample (278.6 ± 14.0) (Table 2). According to Abatzopoulos et al. (2006b) it is possible that the biometrical variation of parthenogenetic cysts can be related to multiple factors such as ploidy level, nutrition and physico-chemical characteristics of habitats, and even to cyst batches from the same population.

Zhou et al. (2003) studied the numeric taxonomy of 15 Chinese populations of *Artemia* using biometrical traits of cysts and nauplii. In accordance with cluster analysis, all 11 populations of *Artemia sinica* and one of *A. tibetiana* have been arranged in a collection while the rest three populations of *A. tibetiana* in the other one (see Zheng & Sun, 2013). Wang's (2009) analyses on the biometrical parameters of cysts and shell layers of seven *Artemia* populations in this country also showed that populations of *A. sinica* and *A. tibetiana* are located in separate clusters, however, parthenogenetic strains were found without particular separation. The present study reveals that the populations in cluster I show lower biometrical variation than do the members of cluster II (Fig. 2). This could be because all nine populations in the first cluster separate at the 5-0 interval but the three populations in the second cluster have been disjoined at farther (at the 15-5 interval). Also, two sub-groups have been clearly separated in cluster I. DFH, XT, GH, CK and DF are located in the same collection, and BRK and GD in another one.

In our case, the results of principal components analysis reveal that the changes in decapsulated cyst diameter are more effective in grouping Chinese parthenogenetic *Artemia* populations than the other two biometrical characters. In contrast, Asem et al. (2010) revealed that untreated cyst size was the main parameter for the intra-specific variation in *A. urmiana* cysts between rainy and drought periods at Urmia Lake. The present results of cluster analysis are not completely confirmed by PCA, although BRK, GD, DFH, XT, GH, CK and DF populations have the same classification patterns by two statistical analyses. In contrast to hierarchical cluster analysis, PCA showed a high heterogeneity among HOH, AB, XW, YGH and AQK. Aqqikkol Lake (AQK) population is located separately from the others in both analyses. This finding can be justified that AQK cysts have one of the thickest chorions and display significant differences from other populations in diameters of untreated and decapsulated cyst (Table 2). When comparing the geographical distribution with the arrangement of populations by PCA, it displays a significant heterogeneity in some habitats. Hoh Lake and Ga Hai Lake are neighboring habitats in Qinghai Province but they have not been located in the same group. Instead, three populations from Shandong Province (DFH, CK and XT) made a uniform collection with Ga Hai Lake (Qinghai Province) and Dongfang Saltern (Hainan Province). Barkol Lake (Xinjiang Autonomous Region) and Gaodao Saltern (Shandong Province) are habitats separated by great geographical distance, but they have displayed similar biometrical characteristics and are located in the same group (Fig. 3). Although two neighboring habitats of Xuwen Saltern (Guangdong Province) and Yinggehai Saltern (Hainan Province) were collected in a sub-cluster by hierarchical cluster analysis, they do not exhibit such a relationship by PCA (Figs 2, 3). As a hypothesis none coordination or integration of geographical distribution with biometrical characterizations can be attributed to specific causes for each population, like diet, difference or similarity of phisico-chemical conditions, as well as existence or lack of gene flow.

Moraiti-Ioannidou et al. (2007) have noted a highly significant correlation between untreated and decapsulated cyst diameters (r = 0.96) of five Greek parthenogenetic populations. In the present study, the result of regression analysis shows that there are positive and significant relationships between all biometrical parameters, which means that populations with large diameters of untreated cysts also have big decapsulated cysts and thick chorions (Fig. 4). Although the correlations between altitude and three biometrical parameters of cysts were not significant, the comparison of biometrical outputs with altitude of habitats showed interesting tendencies (Fig. 5). For example, Aqqikkol Lake, located at 4255 m asl, has the biggest untreated (325.4 µm) and decapsulated cysts size (301.4 µm) as well as thicker chorions (12.0 µm). Zhou et al. (2003) showed that a population of *A. sinica* located in Xiaochaidan (Xiao Qaidam), Qinghai Province (3172 m asl) has the largest untreated cyst (274.65 µm), decapsulated cyst (251.60 µm) and the thickest chorion (11.53 µm) compared to all the other studied cysts of *A. sinica*. There is evidence for a possibly causal relationship between altitude and cyst biometry; all *Artemia* populations from very high habitats (e.g., >4000 m), regardless the reproductive mode, possess large cysts and thick chorions (see Zhou et al. 2003, Van Stappen et al. 2003, Wang & Sun 2007, Wang 2009). However, such relationship doesn't hold for all cases since there are known examples where cysts at low altitudes also have large diameters and thick chorions such as YGH in the present work (Tables 1, 2).

Conclusion

Our results and literature reviews of biometrical characterizations of Chinese parthenogenetic *Artemia* cysts have revealed high inter-population and intra-population variability. The degree of ploidy in parthenogenetic populations can not be taken to be the only one cause of size variation; others could be specific of each population, adaptations to biotic and abiotic parameters. The parthenogenetic population of Aqqikkol Lake and also bisexual *Artemia* living at very high altitudes may have large cysts and thick chorions, as well as dark shells. However, the correlation of the biometrical characteristics of cysts with altitude is not significant, since certain populations at low altitudes can display comparatively larger cysts and thick chorions compared to others at similar altitudes. Hierarchical cluster analysis and principal components analysis display an irregular pattern of groupings for the studied populations, the groupings are not always congruent with geographical distribution pattern.

Acknowledgements. This study was carried out with the financial support of the China Scholarship Council (CSC: 2012GZX795) and the Fundamental Research Funds for the Central Universities (201122005). The critical help of Prof. Jim Clegg (University of California, USA) in English editing is greatly acknowledged.

References

Abatzopoulos, T.J., Zhang, B., Sorgeloos, P. (1998): International study on *Artemia*: LIX. *Artemia tibetiana*: preliminary characterization of a new *Artemia* species found in Tibet (People's Republic of China). International Journal of Salt Lake Research 7: 41-44.

Abatzopoulos, T.J., Agh, N., Van Stappen, G., Razavi-Rouhani, S.M., Sorgeloos, P. (2006a): *Artemia* sites in Iran. Journal of the Marine Biological Association of the United Kingdom 86: 299-307.

Abatzopoulos, T.J., Baxevanis, A.D., Triantaphyllidis, G.V., Criel, G., Pador, E.L., Van Stappen, G., Razavi Rouhani, S.M., Sorgeloos, P. (2006b): Quality evaluation of *Artemia urmiana* Gunther (Urmia Lake, Iran) with special emphasis on its particular cyst characteristics (International Study on Artemia LXIX). Aquaculture 254: 442-454.

Amat, F., Hontoria, F., Ruiz, O., Green, A., Sánchez, M., Figuerola, J., Hortas, F. (2005): The American brine shrimp as an exotic invasive species in the western Mediterranean. Biological Invasions 7: 37-47.

Asem, A., Rastegar-Pouyani, N., Agh, N. (2007): Biometrical study of *Artemia urmiana* (Anostraca: Artemiidae) cysts harvested from Lake Urmia (West Azerbaijan, Iran). Turkish Journal of Zoology 31: 171-180.

Asem, A, Rastegar-Pouyani, N., De Los Rios, P., Manaffar, R., Mohebbi, F. (2010): Biometrical comparison of *Artemia urmiana* Günther, 1899 (Crustacea: Anostraca) cysts between rainy and drought years (1994-2003/4) from Urmia Lake, Iran. International Journal of Biological and Life Sciences 6: 100-106.

Ben Naceur, H., Ben Rejeb, J.A., Romdhane, M.S. (2012a): Quality characterization of cysts of the brine shrimp *Artemia salina* from Tunisia focusing on their potential use in aquaculture. Journal of Biological Research-Thessaloniki 17: 16-25.

Ben Naceur, H., Ben Rejeb, J.A., Romdhane, M.S. (2012b): Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in Tunisia. International Journal of Artemia Biology 2: 24-39.

Bowen, S.T., Fogarino, E.A., Hitchner, K.N., Dana, G.L., Chow, V.H.S., Buoncristiani, M.R., Carl, J.R. (1985): Ecological isolation in *Artemia*: population differences in tolerance of anion concentrations. Journal of Crustacean Biology 5: 106-129.

Browne, R.A., Davis, L.E., Sallee, S.E. (1988): Temperature effects on life history traits and relative fitness sexual and asexual *Artemia*. Journal of Experimental Marine Biology and Ecology 124: 1-20.

Castro, B.T., Gajardo, G., Castro, J.M., Castro, G.M. (2006): A biometric and ecologic comparison between *Artemia* from Mexico and Chile. Saline Systems 2: 13.

John, C.J.A., Abatzopoulos, T.J., Marian, P.M. (2004): Characterization of a new parthenogenetic *Artemia* population from Thamaraikulam, India. Journal of Biological Research 2: 63-74.

Lenz, P.H. (1987): Ecological studies on *Artemia*: a review. pp. 5-18. In: Sorgeloos, P., Bengtson, D.A., Decleir, W., Jaspers, E. (eds), *Artemia* Research and its Applications. Volume 3. Ecology, Culturing, Use in Aquaculture. Wetteren, Belgium, Universa Press.

Liu, J.Y., Zheng, M.P. (1990): Geographic distribution of *Artemia* in Chinese interior salt lakes. Larviculture and Artemia Newsletter 17: 22-24.

Liu, Z., Zheng, M., Luo, J. (1998): A study of *Artemia* in Lagkor Co. Tibet, I. Biological feature. Journal of Lake Sciences 10: 92-96. [in Chinese with English summary]

Ma, L., Wang, W. (2003): Comparison of the biological characteristics of two *Artemia* populations from Aqikekule Lake and Ga Hai Lake. Marine Sciences 27: 34-37. [in Chinese with English summary]

Ma, L., Wang, Q., Wang, C. (2003): Comparative studies on the biological characteristics of three *Artemia* strains from northwestern China. Journal of Ocean University of Qingdao 33: 865-870. [in Chinese with English summary]

Ma, Z., Chen, H., Wu, Z. (1994): Studies on biological characteristics of brine shrimps (*Artemia* spp.) from saline lakes of China and the nauplii as a feed for penaeid shrimp, *Penaeus chinensis* Osbeck larvae. Modern Fisheries Information 9: 14-19. [in Chinese with English summary]

Moraiti-Ioannidou, M., Castritsi-Catharios, J., Miliou, H., Kotzamanis, Y.P. (2007): Fatty acid composition and biometry of five Greek *Artemia* populations suitable for aquaculture purposes. Aquaculture Research 38: 644-1672.

Mura, G., Kappas, I., Baxevanis, A.D., Moscatello, S., D'Amico, Q., Lopez, G.M., Hontoria, F., Amat, F., Abatzopoulos, T.J. (2006): Morphological and molecular data reveal the presence of the invasive *Artemia franciscana* in Margherita di Savoia Salterns (Italy). International Review of Hydrobiology 91: 539-554.

Pan, Z., Sun, J., Bian, B., Li, M. (1991): The biometrics of *Artemia parthenogenetica* from different localities in Shandong and Xinjiang. Transactions of Oceanology and Limnology 2: 62-69. [in Chinese with English summary]

Pinto, P.M., Amat, F., Almeida, V.D., Vieira, N. (2013): Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in Portugal. International Journal of Artemia Biology 3: 51-56

Post, F.J., Youssef, N.N. (1977): A prokaryotic intracellular symbiont of the Great Salt Lake brine shrimp *Artemia salina* (L.). Canadian Journal of Microbiology 23: 1232-1236.

Ruebhart, D.R., Cock, I.E., Shaw, G.R. (2008): Invasive character of the brine shrimp *Artemia franciscana* Kellogg, 1906 (Branchiopoda: Anostraca) and its potential impact on Australian inland hypersaline waters. Marine and Freshwater Research 59: 587-595.

Salman, D.S., Mohammed, D.S., Ali, M.H. (2012): Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in Iraq. International Journal of Artemia Biology 2: 62-73.

Sorgeloos, P., Persoone, G., Baeza-Mesa, M., Bossuyt, E., Bruggeman, E. (1978): The use of *Artemia* cysts in aquaculture: the concept of "hatching efficiency" and description of a new method for cyst processing. pp. 715-721. In: Avault, J.W. (ed), Proceedings of the 9th Annual Meeting, World Mariculture Society, Louisiana State University, Baton Rouge, Louisiana.

Sorgeloos, P., Dhert, P., Candreva, P. (2001): Use of the brine shrimp, *Artemia* spp. in marine fish larviculture. Aquaculture 200: 147-159.

Triantaphyllidis, G.V., Abatzopoulos, T.J., Miasa, E., Sorgeloos, P. (1996): International study on *Artemia*. LVI. Characterization of two *Artemia* populations from Namibia and Madagascar; cytogenetics, biometry, hatching characteristics and fatty acid profiles. Hydrobiologia 335: 97-106.

Triantaphyllidis, G.V., Abatzopoulos, T.J., Sorgeloos, P. (1998): Review of the biogeography of the genus *Artemia* (Crustacea, Anostraca). Journal of Biogeography 25: 213-226.

Van Stappen, G. (2002): Zoogeography. pp. 171-224. In: Abatzopoulos, T.J., Beardmore, J.A., Clegg, J.S., Sorgeloos, P. (eds), *Artemia* Basic and Applied Biology. Dordrecht, Kluwer Academic Publishers.

Van Stappen, G., Sui, L., Xin, N., Sorgeloos, P. (2003): Characterisation of high-altitude *Artemia* populations from the Qinghai-Tibet Plateau, PR China. Hydrobiologia 500: 179-192.

Vanhaecke, P., Sorgeloos, P. (1980): International study on *Artemia* IV, The biometrics of *Artemia* strains from different geographical origin. pp. 393-405. In: Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (eds), The Brine Shrimp *Artemia*. Vol. 3. Wettern, Belgium, Universa press.

Vikas, P.A., Sajeshkumar, N.K., Thomas, P.C., Chakraborty, K., Vijayan, K.K. (2012): Aquaculture related invasion of the exotic *Artemia franciscana* and displacement of the autochthonous *Artemia* populations from the hypersaline habitats of India. Hydrobiologia 684: 129-142.

Wang, J.L. (2002): Sustainable development of *Artemia salina* (Linnaeus) in salt lake and quality of its egg. Modern Fisheries Information 17: 11-13. [in Chinese with English title]

Wang, R., Cai, Y., Li, J. (1991): Clonal and chromosome study of parthenogenetic brine shrimp (*Artemia parthenogenetica*) from North of China. Oceanologia et Limnologia Sinica 22: 1-7. [in Chinese with English summary]

Wang, S., Sun, S. (2007): Comparative observations on the cyst shells of seven *Artemia* strains from China. Microscopy Research and Technique 70: 663-670.

Wang, S. (2009): Biometrics of *Artemia* resting eggs from seven localities of China. Marine Science Bulletin 28: 62-67. [in Chinese with English summary)

Xu, J., Bian, B., Li, M. (1993): Preliminary studies on the biology of *Artemia salina* from different localities of P. R. China. Journal of Ocean University of Qingdao, 23: 109-118. [in Chinese with English summary]

Xu, J. (1996): Biometrics of *Artemia parthenogenetica* from four localities of China. Journal of Oceanography in Taiwan Strait 15: 11-15. [in Chinese with English summary]

Yang, G., Cai H., Hou L. (1995): Study on the biological characteristics of *Artemia* populations from six salt lakes in China. Transactions of Oceanology and Limnology 3: 39-47. [in Chinese with English summary]

Yang, Z., Yao, D., Wei, W. (2005): A study on the biological characters of six *Artemia* populations. Journal of Yangzhou University (Agricultural and Life Science Edition) 26: 93-94. [in Chinese with English summary]

Yu, X., Xin N. (2006): The characterization of *Artemia* cysts from 4 salt lakes of Tibet. Journal of Salt and Chemical Industry 35: 26-26. [in Chinese with English summary)

Zhang, B., Xin, N.H., Yu, X.L., Sui, L.Y. (1998): Strain characterization of *Artemia* from 9 inland salt lakes of China. Journal of Lake Sciences 10: 19-24. [in Chinese with English summary]

Zheng, B. Sun, S. Ma, L. (2004): The occurrence of an exotic bisexual *Artemia* species, *Artemia franciscana*, in two coastal salterns of

Shandong Province, China. Journal of Ocean University of China 3: 171-174.
Zheng, B., Sun, S. (2013): Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in China. International Journal of Artemia Biology 3: 20-50.
Zhou, K., Xu, M., Guan, Y., Yin, X. (2003): Numeric taxonomy using cysts and nauplii of bisexual *Artemia* from China. Journal of Lake Sciences 15: 153-159. [in Chinese with English summary]
Zhou, K., Xu, M., Yin, X. (2006): Constitution of *Artemia* strains in coastal salterns of Hebei Province. Chinese Journal of Zoology 41: 1-5. [in Chinese with English summary]
Zhang, R., Liu, F., Zhao, X., Zheng, J. (1990): Studies on the chromosomal ploidy composition of the brine shrimp, *Artemia* spp. Acta Zoologica Sinica 36: 412-419. [in Chinese with English summary]