Morphological Differentiation of Seven Parthenogenetic *Artemia* (Crustacea: Branchiopoda) Populations from China, with Special Emphasis on Ploidy Degrees

ALIREZA ASEM AND SHI-CHUN SUN*
Institute of Evolution and Marine Biodiversity, Ocean University of China, 5 Yushan Road, Qingdao 266003, China

KEY WORDS parthenogenetic *Artemia*; scanning electron microscope; morphology; morphometric analysis; ploidy degree

ABSTRACT Parthenogenetic *Artemia* from seven Chinese locations with different elevations and various ploidies are characterized by phenotypic and morphometric analyses. Our findings show that the studied populations exhibit dissimilar patterns of ovisac. Four phenotypic patterns of furca are qualified and one of them is shared among di-, tetra- and pentaploid *Artemia*. Results of discriminant analysis based on morphometric data reveal that tetra- and pentaploid populations are grouped together, but the Aqqikkol Lake population is clearly differentiated. Previous hypothesis/conclusion that polyploid *Artemia* are larger than diploids is only partly supported by the present results, which show that pentaploid and tetraploid populations are larger than the mostly diploid populations in terms of the total length, but the body size of the Aibi Lake triploids has not significant difference with the sympatric diploids and the mostly diploid Aqqikkol population that inhabit in very high altitude has the largest body size among all parthenogenetic populations. The founding confirms that body size of *Artemia* is following with Bergmann's rule. *Microsc. Res. Tech. 79:258–266, 2016*.

INTRODUCTION

The genus *Artemia* Leach, 1819, which has been recorded from more than 600 sites in all continents except Antarctica (Van Stappen, 2002), consist of seven confirmed bisexual species and some undescribed taxa and a large number of parthenogenetic populations (Abatzopoulos et al., 2002a,b; Amat et al., 2007; Zheng and Sun, 2013). Though different nonmorphological methods, such as electrophoresis of allozymes, molecular phylogeny and cross-fertility test, have been employed to identify *Artemia* species (Abreu-Grobois and Beardmore, 1982; Baxevanis et al., 2006; Gajardo et al., 2001; Maccari et al., 2013; Maniatsi et al., 2011; Muñoz et al., 2010; Zheng and Sun, 2008), morphologic and morphometric studies have been providing useful data for the characterization of intra- and interspecific variability *Artemia* (Asem and Rstegar-Pouyani, 2008; Ben Naceur et al., 2011b; Gajardo and Mura, 2011; Torrentera and Dodson, 1995).

While some characters like gonopods and frontal knobs are useful characters in characterizing male *Artemia* (Gajardo and Mura, 2011; Mura, 1990; Torrentera and Dodson, 1995; Zheng and Sun, 2008), ovisac and furca are two characters used by some authors for characterizing adult females of *Artemia* (Amat, 1980; El-Gamal, 2010; Gajardo and Mura, 2011; Vetriselvan and Munuswamy, 2011; Vasudevan, 2012; Torrentera and Dodson, 1995). Tejeda (1987) showed that the morphological differentiation of ovisac and furca was obvious among bisexual *Artemia* populations from Dominica. Amat et al. (1995a) demonstrated that the ovisac of *Artemia salina* from South Africa, Spain, and Egypt were considerably similar in the morphological pattern.Studies on females of this species have shown that the Natruum (Egypt) population had a furca pattern (El-Gamal, 2010) significantly different from that of seven South Africa and Spanish populations (Amat, 1980; Amat et al., 1995a). Several other studies have shown that the ovisac of *Artemia franciscana* had a widely intra-specific variation (Gajardo and Mura, 2011; Torrentera and Dodson, 1995). With regard to parthenogenetic *Artemia*, similar observation was performed for handful populations, mostly from the areas surrounding the Mediterranean (Amat, 1980; El-Gamal, 2010; Vasudevan, 2012). Among these studies, only Amat (1980) compared the morphology of furca and ovisac among different ploidy levels and showed that Mediterranean di- and triploid populations both represented the same structure, while considerable differences existed among tetraploid populations. Given that there are numerous parthenogenetic *Artemia* populations and some of them contain strains of different ploidy levels, our knowledge about the variation of ovisac and furca of parthenogenetic *Artemia* is still limited.

Many studies have been performed for comparing the numerical characters of bisexual *Artemia* grown

*Correspondence to: Shi-Chun Sun, E-mail: sunsc@ouc.edu.cn

Received 9 November 2015; accepted in revised form 23 December 2015

REVIEW EDITOR: Prof. George Perry

Contract grant sponsor: National Natural Science Foundation of China; Contract grant number: 31172046.

DOI 10.1002/jemt.22625
Published online 18 January 2016 in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. List of parthenogenetic populations of Artemia and their summarized information

Locality	Abbreviation	Geographic coordinates	Sampling year	Altitude (m)	Ploidy degree	Ref.
Aibi Lake[a], Jinghe, Xinjiang	AB	44°53′N, 83°00′E	2000	194	$2n > 90\%$	1
Aqqikkol Lake, Ruoqiang, Xinjiang	AQK	37°04′N, 88°22′E	2005	4255	$2n = 80\%$, $4n = 20\%$	2, 3
Dongfanghong Saltern, Laizhou, Shandong	DFH	37°20′N, 119°55′E	1985	∼sea level	$2n > 98\%$	4
Ga Hai Lake, Delingha, Qinghai	GH	37°08′N, 97°33′E	2000	2870	$2n = 100\%$	2, 3
Barkol Lake[b], Barkol, Xinjiang	BRK	43°40′N, 92°44′E	1986	1617	$2n = 27.44\%$, $3n = 4.88\%$, $4n = 37.8\%$, $5n = 29.88\%$	1
Hoh Lake[c], Ulan, Qinghai	HOH	36°57′N, 98°17′E	1988	2995	$4n = 100\%$	5
Yinggehai Saltern, Ledong, Hainan	YGH	18°31′N, 108°44′E	1987	∼sea level	$5n > 97\%$	5

[a]Ebinur Hu or Ebinur Lake.
[b]Balikun Lake.
[c]Keke Salt Lake.
Refs: (1) Yang et al. (1995), (2) Wang and Sun (2007), (3) Wang (2009), (4) Pan et al. (1991), (5) Xu et al. (1993).

under laboratory conditions (Abatzopoulos et al., 2009; Amat et al., 1995b; Baxevanis et al., 2005; Ben Naceur et al., 2011a, 2013; Cohen et al., 1999; Camargo et al. 2003; Gajardo et al., 2001; Hontoria and Amat, 1992a,b; Zheng and Sun, 2008; Zhou et al. 2003a). These studies have confirmed that morphometry, particularly morphometry plus discriminant analysis, could be a useful approach in characterizing *Artemia* populations and species. For instance, the analysis of Hontoria and Amat (1992a) thoroughly characterized three different *Artemia* types (bisexual diploid, parthenogenetic diploid, and parthenogenetic tetraploid) from Mediterranean areas; that of Hontoria and Amat (1992b) distinguished the *Artemia* from Americas as three different clusters (North American athalassic inland populations, Caribbean coast area populations and Pacific Ocean shore populations); and Zheng and Sun (2008) showed that three North China populations (*Artemia sinica*) were divergent from four Tibetan populations (*Artemia tibetiana*), with the latter possessed a larger body size, longer gonopod, larger basal gonopod spine, and so on.

Parthenogenetic *Artemia* populations are a new divided group (Asem et al., in press; Baxevanis et al., 2006; Maccari et al., 2013, Maniatsi et al., 2011) that occupy a large number of localities in both the Old World and Australia. This group contain populations with variable degrees of ploidies (i.e., $2n$, $3n$, $4n$, $5n$, and heteroploids) (Amat, 1980; Pan et al., 1991; Sun et al., 1999; Wang and Sun, 2007; Wang, 2009; Xu et al., 1993; Yang et al., 1995) and are distributed in altitudes ranging from sea level to as high as 4255 m (Wang and Dou, 1998). Polyploidization is known as the main reason to grow larger adult body size in plants and invertebrates (Gregory and Mable, 2005; Otto, 2007; Otto and Whitton, 2000), but few studies have been conducted for *Artemia*. Wang (1988) demonstrated that pentaploid *Artemia* are larger than sympatric diploids. Triantaphyllidis et al. (1996) found that the Madagascan triploid population had a larger body size than the mainly diploid Namibian population. Although a significant correlation between the cyst size and habitat altitude could not be confirmed (Asem and Sun, 2014), it has been demonstrated that *Artemia* from very high habitat produced cysts significantly larger than those from lower habitat. For example, the parthenogenetic *Artemia* from Aqqikkol Lake (Ruoqiang, Xinjiang), which is located at 4255 m above the sea level, has the largest cysts among all parthenogenetic *Artemia* populations (Asem and Sun, 2014; Wang, 2009; Wang and Sun, 2007); the bisexual *Artemia tibetiana*, which are restricted to the Qinghai–Tibetan Plateau, have the largest cysts in *Artemia* (Abatzopoulos et al., 1998; Liu et al. 1998; Van Stapen et al., 2003; Wang, 2009; Wang and Sun, 2007; Zhou et al., 2003b), meanwhile other bisexual *Artemia* have smaller cysts than *A. tibetiana* (see Asem et al., 2007). Similarly, larger nauplii were documented for both bisexual and parthenogenetic *Artemia* from very high habitat (>4000 m above the sea level) than those from lower habitats (Abatzopoulos et al. 1998; Ma et al., 2003; Ma and Wang, 2003; Pan et al., 1991; Van Stappen et al. 2003; Zhou et al. 2003b). Studies on bisexual *Artemia* also revealed significant difference of adult sizes among different species/populations, with those of *Artemia tibetiana* and *Artemia urmiana* significantly large than the other species (Baxevanis et al., 2005; Zheng and Sun, 2008; Zhou et al. 2003a). Unfortunately, a similar comparison cannot be made for parthenogenetic *Artemia* populations because the lack of morphometric data, particularly for the populations from very high latitude habitats.

The present paper will present the morphological variations of ovisac and furca, and the morphometry of seven parthenogenetic *Artemia* populations from China. The aim of the study is to gain a better characterization of the morphologic diversity of parthenogenetic *Artemia* from different habitats of China, as well as the relationships between morphologic/mophometric characters and ploidy degrees.

MATERIALS AND METHODS

Cyst samples from the seven Chinese parthenogenetic *Artemia* populations were obtained from the Institute of Evolution and Marine Biodiversity, Ocean University China. Information about their habitats and ploidy composition is given in Table 1 and Figure 1.

The cysts were hatched in 0.2-μm filtered seawater (salinity 32–33 g l^{-1}) under optimal conditions (Sorgeloos et al., 1986). Instar I nauplii were raised according to the standardized laboratory method of Coutteau et al. (1992). The salinity was adjusted to 70 g l^{-1} by adding sea salt to seawater, temperature and photoperiod were 25 ± 1°C and 12 h L/12 h D, respectively. A mixed diet of the *Dunaliella salina* and LANSY ZM (INVE, Thailand) was supplied following the feeding schedule given by Triantaphyllidis et al. (1995).

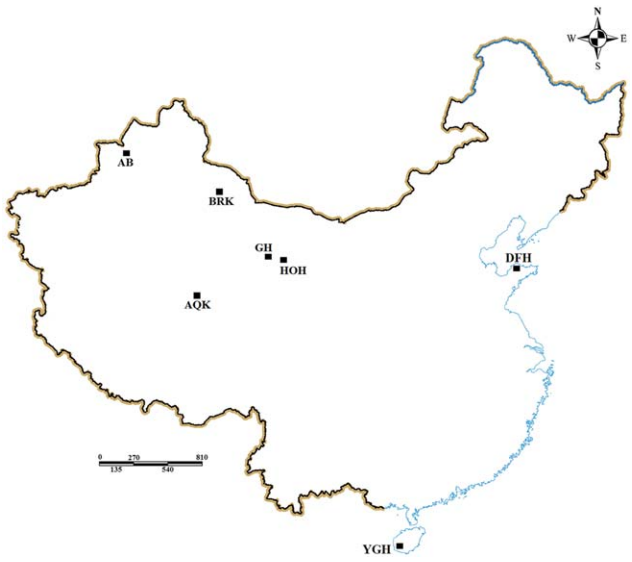

Fig. 1. Geographical distribution of the studied *Artemia* populations from China. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Random samples of exactly 100 individuals per population were examined for intra- and interpopulation phenotypic variations of the ovisac and furca using a light microscopy Nikon SMZ-800 stereomicroscope. Because the Aibi Lake *Artemia* has two different phenotypes of ovisac and furca (phenotypes A and B), both phenotypes were cloned to determine the ploidy degrees. For each population 3–4 specimens were studied for detailed morphology of the ovisac with a scanning electronic microscope. After washing the samples with phosphate buffer and redistilled water, they were dehydrated in 30, 50, 80, 90, and 100% ethanol at 30 min intervals. Afterwards, samples were transferred into isoamyl acetate: ethanol (1:3, 1:2, and 1:0 for 30 min, respectively). After being critical point dried in CO_2 and covered by gold, specimens were observed and photographed with a KYKY-2800B SEM.

For morphometric analysis, 30 adults were examined from each population with the exception of Aqqikkol Lake ($n = 29$) and Aibi Lake, type B ($n = 17$). The following 14 morphometric parameters were examined: total length, abdominal length, width of head, length of the first antenna, diameter of compound eye, distance between the eyes, width of ovisac, width of the third abdominal segment, length from the end of the eighth abdominal segment to the third abdominal segment, length of telson, length of left furca, length of right furca, number of setae on the left branch of furca, number of setae on the right branch of furca. Morphometrical characters were measured using a Nikon SMZ800 stereo-microscope equipped with an Eyepiece ruler. In addition, the length of the ovisac spine was estimated on images for three observed SEM samples by software of CellSens Standard.

Morphometric results were analyzed by ANOVA (Tukey's test). Significant differences between the lengths of right and left furca and also their numbers of setae were checked by pair sample t test for each population. Discriminant analysis and principal components analysis on the above 14 morphometric parameters were carried out to check the differentiation among the seven *Artemia* populations and also between two phenotypes of the Aibi Lake *Artemia*. The computer program SPSS 16 was performed for statistical analysis.

RESULTS

Phenotype of Ovisac and Furca

Ovisacs show unique appearance for each studied population (Fig. 2 and Table 2), while furca can be classified into four morphological types (Fig. 3 and Table 3). Aibi Lake *Artemia* presents two different patterns of ovisac and furca, type A and B, with 92 and 8% frequency respectively. Our cytological experiments confirm that all 17 studied specimens of type B are triploid and the samples of type A belong to diploid.

Morphometry

The mean values of morphometric and meristic characters are presented in Table 4. The ratio of the between groups to within groups mean square (F values) and their P level (sig.) are also exhibited. F values revealed significant differences among all populations in all studied characters (Sig. =0.000). Width of head and length of left furca show the highest between-group variability, while width of the third abdominal segment displays the lowest variability (Table 4).

In three of the 14 analyzed characters (i.e., total length, abdominal length, and length from the end of the eighth abdominal segment to the third abdominal segment), AQK population shows significantly larger values ($P < 0.05$) than the other populations. Additionally, *Artemia* of AQK have high values in the other seven characters (width of head, length of the first antenna, distance between eyes, width of ovisac, length of telson, width of the third abdominal segment, and diameter of compound eye) (Table 4). In contrast, Ga Hai *Artemia* show the smallest values in total length, abdominal length, length of the first antenna, length from the end of the eighth abdominal segment to the third abdominal segment and the length of telson ($P < 0.05$, Table 4). By the way, the ovisac spine of AQK is larger than that of the other populations (Table 2).

According to the results of Table 4, the population of DFH displays the longest furca and the most setae, while the AQK population has the shortest furca and the fewest setae. The linear regression and Pearson's correlations analyses confirm the positive correlation between the furca length and the number of setae ($y = 278.11x - 1.0073$, $r = 0.88$, Sig. = 0.000). Pair samples test for the length and seta number of left and right furca shows significant difference only for the seta number in YGH (pair t test, $P = 0.027$).

Differentiation Among Populations

Discriminant analysis classified the seven Chinese parthenogenetic *Artemia* populations into three separate groups. The first one contains AB (both phenotypes A and B), DFH, BRK and GH, the second includes HOH and YGH and the third holds only AQK (Fig. 4). The first and second discriminant functions describe 50.7 and 19.8% of the variance, respectively

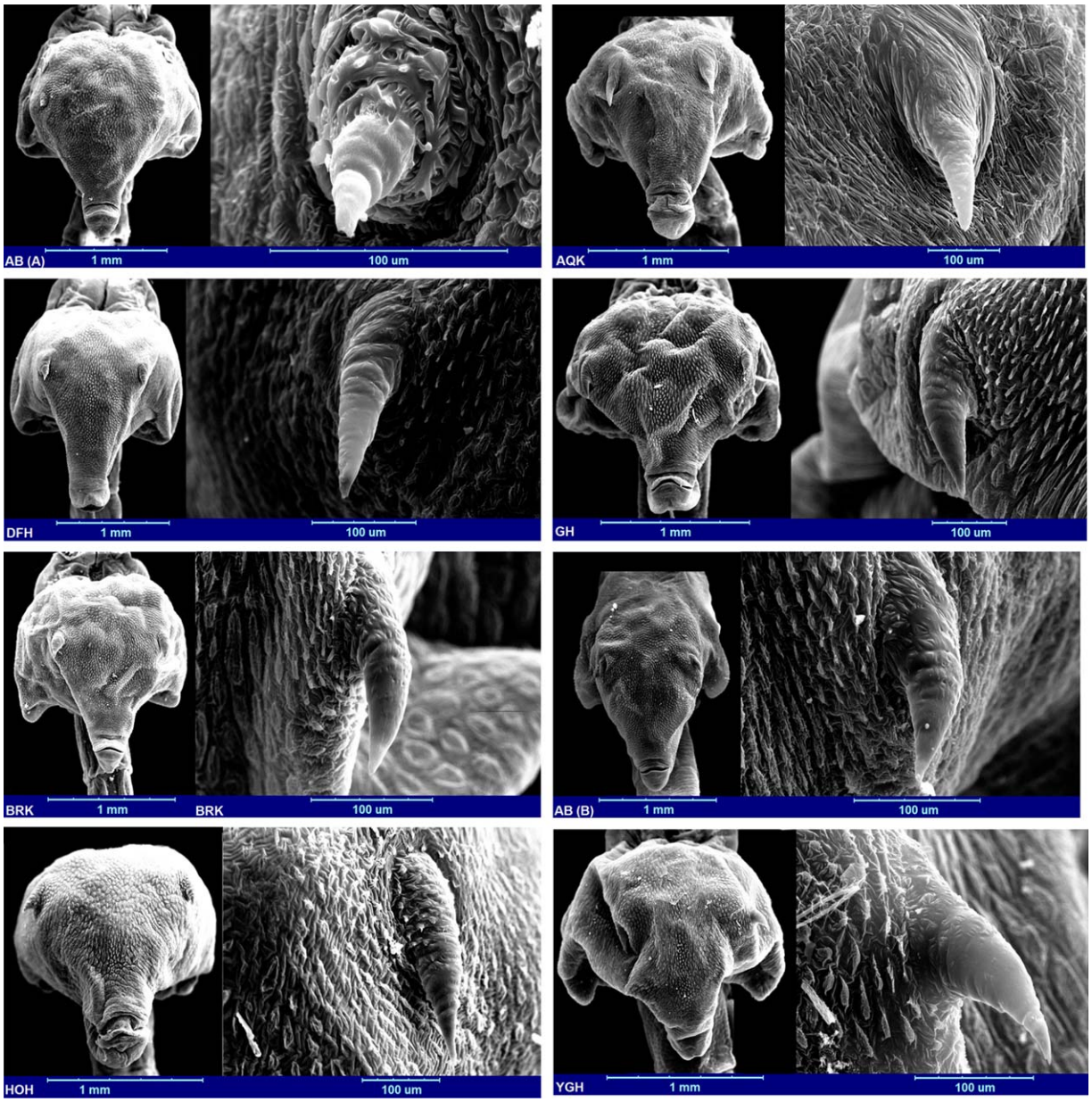

Fig. 2. SEM photographs of ovisac (left) and spine (right) of seven parthenogenetic *Artemia* populations from China. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(Table 5). The diameter of compound eye (0.780) and total length (0.621) are effective characters in the first function and the width of head (0.408) and diameter of compound eye (0.304) in second one as well. The least competent characters belong to the width of the third abdominal segment (0.154), the length of left furca (0.169) and the number of setae on the left furca (0.191) in the first function, and the length of right furca (0.074) in the second function (Table 5).

The highest and the lowest percentage of correctly classified individuals are observed in AQK (100%) and phenotype A of AB (33.3%), respectively. The whole percentage of individuals correctly classified into original groups is 81.0% (Table 6).

Two different phenotypes of Aibi Lake *Artemia*, A ($2n$) and B ($3n$), revealed significant difference ($P < 0.05$) for eight of the 14 studied characters (Table 4), with the phenotype A having larger means than the phenotype B. As mentioned above, the length of ovisac spine also shows significant difference between these two phenotypes, but phenotype A is smaller than phenotype B (Table 2). However, principal components analysis showed no significant differentiation between diploid and triploid Aibi Lake *Artemia* (Fig. 5). First

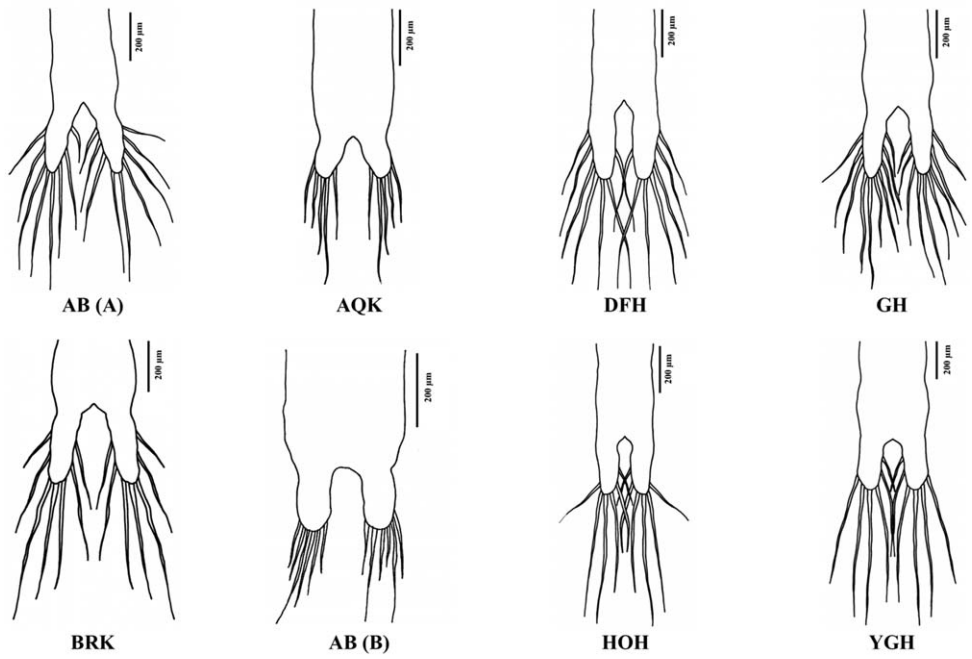

Fig. 3. Schematic illustrations of the furca of seven parthenogenetic *Artemia* populations from China.

TABLE 2. Description of ovisac in the SEM photographs shown in Fig. 2 (same letters in each column show nonsignificant difference; n = 3, ANOVA, Tukey's test, P > 0.05 (abbreviations of localities are defined in Table 1)

Population	General shape (length/wide)	Lateral lobes	Spines (length, μm)
AB (A)	Rounded/heart-shaped (1.7 ± 0.03^{a})	Developed, rounded distally	Tiny, relatively along axial plane (124.2 ± 12.4^{a})
AQK	Triangular (1.3 ± 0.1^{b})	Developed, sharp distally	Enormous, leaning proximally (350.7 ± 26.5^{e})
DFH	Pear-shaped (1.6 ± 0.1^{ab})	Developed, rounded distally	Medium, between axial plane and proximal (221.9 ± 4.0^{c})
GH	Rounded/ellipsoid (1.3 ± 0.2^{b})	Developed, rounded distally	Tiny, relatively along axial plane (169.2 ± 22.7^{ab})
BRK	Triangular (1.5 ± 0.2^{ab})	Developed, sharp distally	Medium, relatively along axial plane (216.5 ± 14.4^{bc})
AB (B)	Narrow pear-shaped (1.5 ± 0.1^{ab})	Developed, sharp distally	Small, relatively along axial plane (181.0 ± 17.6^{bc})
HOH	Ellipsoid (1.2 ± 0.1^{b})	Undeveloped/rudimentary	Large, leaning proximally (296.5 ± 9.1^{d})
YGH	Rounded (1.2 ± 0.2^{b})	Developed, sharp distally	Small, relatively along axial plane (209.2 ± 20.0^{bc})

TABLE 3. Description of furca in the schematic illustrations in Fig. 3 (abbreviations of localities are defined in Table 1)

Type	Pattern	Groove[a]	Distribution of setae	Shape of rectal part	Population(s)
I	Two parallel lobed: narrow, long	Deep	In lower two-thirds of furca	Angled	DFH, GH, HOH, YGH
II	Two divergent lobed: narrow, long	Deep	In lower two-thirds of furca	Angled	AB (A), BRK
III	Two parallel lobed: wide, short	Deep	Restricted to distal portion	Rounded	AB (B)
IV	Two divergent lobed: wide, short	Deep	Restricted to distal portion	Angled	AQK

[a]Deep groove: length of groove > width of groove; wide groove: width of groove ≥ length of groove.

two components produced by principal components analysis explain 70.9% of total variance.

DISCUSSION

Ovisac phenotypes are differential among the studied populations of parthenogenetic *Artemia* (Fig. 2 and Table 2) and show differences with those reported from Mediterranean area. While Mediterranean tetraploid *Artemia* were illustrated to have a triangular ovisac (Amat, 1980), the ovisac of the tetraploid HOH population is ellipsoid (Fig. 2). The Chinese diploid populations are variable in the general appearance of ovisac (rounded/heart-shaped, triangular, pear-shaped, and rounded/ellipsoid), but all of them are not similar to the semicircular ovisac of Mediterranean diploid parthenogenetic *Artemia* (see Amat, 1980). Moreover, different ploidy levels ($2n$ = 27.44%, $3n$ = 4.88%, $4n$ = 37.8%, $5n$ = 29.88%) coexist in Barkol Lake, but morphological differentiation of ovisac was not identified.

We found that the diploid and triploid strains of the AB population are different in the phenotypes of the furcae (type B of triploid vs. type A of diploid; Fig. 3 and Table 3), whereas similar relationship between furca pattern and ploidy level was not confirmed for other populations. For example, Amat (1980) reported

TABLE 4. Mean values (SD) of morphometric characters for the seven parthenogenetic populations of Artemia from China (same letters in each row show nonsignificant difference; ANOVA, Tukey's test, $P > 0.05$ (abbreviations of localities are defined in Table 1)

Characters	AB (A) ($n = 30$)	AQK ($n = 29$)	DFH ($n = 30$)	GH ($n = 30$)	BRK ($n = 30$)	AB (B) ($n = 17$)	HOH ($n = 30$)	YGH ($n = 30$)	*F* value	Sig.
A	9.39^{a} (0.99)	11.56^{d} (0.53)	9.31^{a} (0.51)	8.71^{b} (0.48)	8.94^{ab} (0.49)	8.96^{ab} (0.34)	9.98^{c} (0.60)	10.08^{c} (0.57)	45.0	.000
B	5.06^{ab} (0.77)	6.48^{e} (0.49)	4.95^{ab} (0.36)	4.71^{a} (0.41)	4.92^{ab} (0.39)	5.11^{bc} (0.13)	5.46^{cd} (0.43)	5.78^{d} (0.40)	32.3	.000
C	0.91^{a} (0.08)	0.96^{d} (0.06)	0.91^{a} (0.07)	0.81^{bc} (0.04)	0.80^{b} (0.04)	0.85^{c} (0.04)	0.98^{d} (0.05)	0.96^{d} (0.05)	99.4	.000
D	1.11^{ac} (0.11)	1.22^{d} (0.06)	1.07^{ac} (0.08)	0.99^{b} (0.06)	1.13^{c} (0.09)	1.05^{ab} (0.02)	1.23^{d} (0.09)	1.24^{d} (0.11)	43.3	.000
E	0.27^{a} (0.02)	0.30^{e} (0.02)	0.25^{c} (0.01)	0.24^{c} (0.01)	0.24^{bc} (0.01)	0.23^{b} (0.01)	0.28^{d} (0.02)	0.32^{e} (0.02)	66.9	.000
F	1.59^{a} (0.13)	1.71^{c} (0.14)	1.48^{b} (0.08)	1.49^{b} (0.08)	1.44^{b} (0.09)	1.41^{b} (0.08)	1.68^{c} (0.07)	1.71^{c} (0.08)	45.3	.000
G	1.83^{ad} (0.24)	2.08^{e} (0.18)	1.74^{ac} (0.14)	1.74^{ac} (0.15)	1.62^{bc} (0.15)	1.57^{b} (0.13)	1.73^{ac} (0.17)	1.96^{de} (0.15)	27.0	.000
H	0.66^{a} (0.06)	0.66^{a} (0.06)	0.64^{a} (0.05)	0.58^{b} (0.04)	0.57^{b} (0.07)	0.63^{a} (0.03)	0.64^{a} (0.05)	0.64^{a} (0.05)	12.7	.000
I	3.91^{abc} (0.71)	4.86^{e} (0.56)	3.81^{ab} (0.19)	3.57^{a} (0.26)	3.82^{ab} (0.39)	$4.12b^{cd}$ (0.16)	4.32^{d} (0.36)	4.24^{cd} (0.31)	34.5	.000
J	1.10^{a} (0.21)	1.33^{c} (0.12)	1.07^{ab} (0.06)	0.99^{b} (0.08)	1.07^{ab} (0.07)	1.11^{a} (0.06)	1.24^{c} (0.09)	1.27^{c} (0.10)	26.1	.000
K	0.29^{a} (0.05)	0.24^{bc} (0.04)	0.34^{e} (0.06)	0.28^{ad} (0.04)	0.26^{cd} (0.04)	0.22^{b} (0.05)	0.26^{acd} (0.03)	0.28^{ad} (0.03)	20.1	.000
L	0.30^{a} (0.05)	0.24^{bc} (0.03)	0.34^{e} (0.06)	0.28^{ad} (0.04)	0.26^{cd} (0.04)	0.22^{b} (0.04)	0.26^{cd} (0.03)	0.27^{ad} (0.03)	24.6	.000
M	6.86^{acd} (1.46)	5.03^{b} (1.45)	8.28^{e} (1.17)	$7.60d^{e}$ (1.33)	5.93^{ab} (1.62)	5.18^{b} (1.55)	6.07^{abc} (0.87)	7.12^{cd} (1.00)	20.8	.000
N	6.87^{a} (1.46)	4.83^{b} (1.28)	8.13^{d} (1.12)	7.97^{d} (0.96)	6.33^{ac} (1.77)	5.41^{bc} (1.28)	6.23^{ac} (1.16)	6.74^{a} (1.11)	21.5	.000

A: total length; B: abdominal length; C: width of head; D: length of the first antenna; E: diameter of compound eye; F: distance between the eyes; G: width of ovisac; H: width of the third abdominal segment; I: length from the end of the eighth abdominal segment to the third abdominal segment; J: length of telson; K: length of left furca; L: length of right furca;, M: number of setae on the left branch of furca; N: number of setae on the right branch of furca (parameters A to L are in mm).

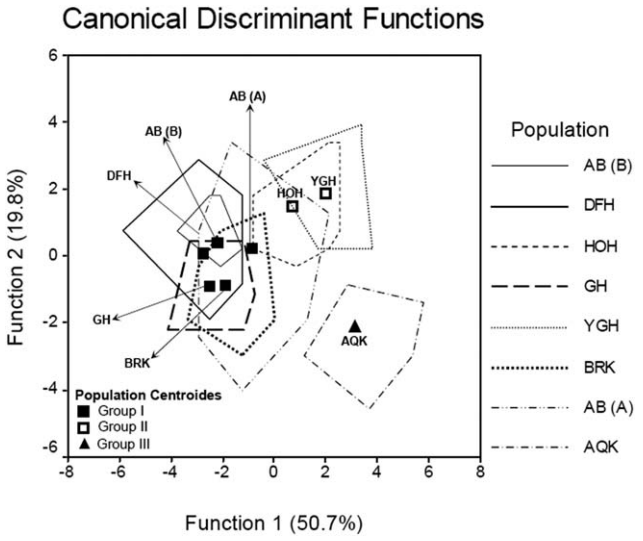

Fig. 4. Scatterplot of the first two canonical discriminant functions (group centroids) resulting from the discriminant analysis on seven parthenogenetic *Artemia* populations from China, using populations as separating factor.

that di- and triploid populations of parthenogenetic *Artemia* (from Spain, Italy, Portugal, and Moracco) represented the same furca structure; and in the present study, no intrapopulation variation was detected in the BRK population with a complex ploidy composition, and apparent difference was not found among populations with different ploidies like DFH ($2n > 98\%$), GH ($2n = 100\%$), HOH ($4n = 100\%$), and YGH ($5n > 97\%$). Several other studies have shown that parthenogenetic populations from Egypt and India had multiple furcal morphologies, but the ploidy levels were not considered (El-Gamal, 2010; Vetriselvan and Munuswamy, 2011; Vasudevan, 2012).

Studies have shown that morphometrical and statistical analyses are capable to characterize *Artemia* species and populations. For example, Amat (1980) proved that morphometric analysis can separate *A. franciscana* (SFB) from Spanish and Mediterranean bisexual and parthenogenetic *Artemia*. Multivariate statistical procedures could clearly separate parthenogenetic populations from bisexual *Artemia* (see Amat et al., 2005; Agh et al., 2009; El-Gamal, 2010; Mura et al., 2005, 2006; Vasudevan, 2012); discriminant analysis classified Mediterranean *Artemia* populations into three groups, bisexual, diploid and tetraploid parthenogenetic *Artemia* (Hontoria and Amat, 1992a; Amat et al., 1995b); and morphometric results of three Greek tetraploid populations showed high similarity with other Mediterranean tetraploids (Abatzopoulos et al. 1987, 1989). These results are basically supported by the present discriminant analysis, which classified the seven Chinese parthenogenetic *Artemia* populations into three separate groups. Among them, two populations with high ploidy levels (HOH and YGH) were clustered to a group, and those containing diploids or mixed ploids formed another group, with the exception of AQK population (Fig. 4). The fact that AQK population could not be clustered together with the other diploid populations is apparently related to the high altitude of its habitat, which might have brought about

TABLE 5. Discriminant analysis of morphometric variables for the seven parthenogenetic populations of Artemia from China

Variable	Function						
	1	2	3	4	5	6	7
E	0.780	0.304	−0.067	0.443	0.043	−0.008	0.109
A	0.621	−0.196	0.466	0.075	−0.210	0.211	−0.194
B	0.532	−0.112	0.205	−0.054	−0.323	0.348	−0.206
J	0.463	0.104	0.210	−0.153	−0.068	0.234	−0.186
I	0.387	−0.074	0.273	−0.235	−0.245	0.140	−0.178
C	0.415	0.408	0.510	0.167	−0.163	−0.184	−0.141
L	−0.201	0.169	0.244	0.697	0.202	0.209	0.252
M	−0.191	0.219	−0.017	0.656	0.043	0.059	−0.215
K	−0.169	0.179	0.217	0.639	0.170	0.190	0.191
N	−0.256	0.175	−0.065	0.570	0.167	−0.095	−0.313
G	0.355	−0.103	0.063	0.446	−0.328	0.004	0.326
D	0.414	0.224	0.118	−0.161	0.556	0.262	0.173
F	0.504	0.196	0.115	0.249	0.088	−0.518	0.102
H	0.154	0.166	0.351	0.063	−0.465	−0.141	0.580
Eigenvalue	4.658	1.813	1.140	0.934	0.294	0.235	0.105
% of variance	50.7[a]	19.8[a]	12.4	10.2	3.2	2.6	1.1

[a]First two functions produced by discriminant analysis explain a cumulative 70.5% of total variance.

TABLE 6. Classification results of discriminant analysis for the seven parthenogenetic populations of Artemia showing the percentage of individuals classified in each group

	Population	Predicted group membership								Total
		AQK	AB (A)	BRK	YGH	GH	HOH	DFH	AB (B)	
Original count	AQK	29	0	0	0	0	0	0	0	29
	AB (A)	2	10	2	1	3	6	4	2	30
	BRK	0	2	23	0	3	0	0	2	30
	YGH	0	0	0	27	0	3	0	0	30
	GH	0	3	2	0	25	0	0	0	30
	HOH	0	1	0	0	0	29	0	0	30
	DFH	0	2	1	0	2	0	25	0	30
	AB (B)	0	0	0	0	1	0	1	15	17
%	AQK	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
	AB (A)	6.7	33.3	6.7	3.3	10.0	20.0	13.3	6.7	100
	BRK	0.0	6.7	76.7	0.0	10.0	0.0	0.0	6.7	100
	YGH	0.0	0.0	0.0	90.0	0.0	10.0	0.0	0.0	100
	GH	0.0	10.0	6.7	0.0	83.3	0.0	0.0	0.0	100
	HOH	0.0	3.3	0.0	0.0	0.0	96.7	0.0	0.0	100
	DFH	0.0	6.7	3.3	0.0	6.7	0.0	83.3	0.0	100
	AB (B)	0.0	0.0	0.0	0.0	5.9	0.0	5.9	88.2	100

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 (abbreviations of localities are defined in Table 1).

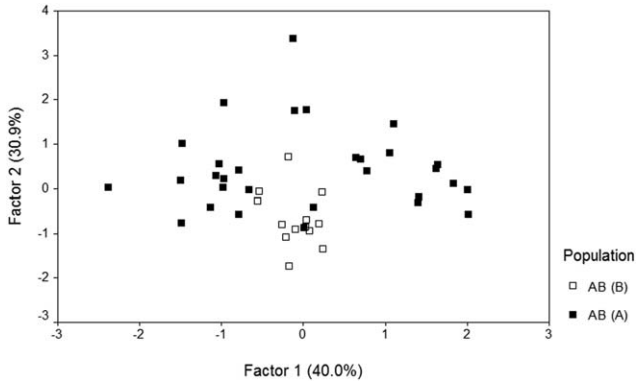

Fig. 5. Scatterplot of the first two factors resulting from the principal components analysis on two phenotypes of AB population.

some adaptive morphometric futures considerably different from *Artemia* from lower habitats (see further). Though diploid Namibian (Swakopmund Saltwork) and triploid Madagascan (Ankiembe) parthenogenetic *Artemia* presented indicative morphometrical differentiations (Triantaphyllidis et al. 1996), morphometric diversification was not detected between the diploid and triploid types of AB population. In another study, the triploid Madagascan population was clustered with European diploid parthenogenetic *Artemia* (Mura et al., 2006). These suggests that the morphometric differentiation between diploid and triploid *Artemia* is not as significant as that between diploids/triploids and tetraploids/pentaploids.

Previous studies have demonstrated that polyploid parthenogenetic *Artemia* are larger than diploids (Wang, 1988; Triantaphyllidis et al., 1996). This is partly supported by the present results, which show that tetraploid and pentaploid populations are usually larger in body size than populations with lower ploidy levels (Table 4). However, the mostly diploid AQK population ($2n$ = 80%, $4n$ = 20%) has the largest body size rather than the populations with the highest ploidy level (HOH, $4n$ = 100%; YGH, $5n$ > 97%) (see also Asem and Sun, 2014); and significant differences were not detected when comparing the AB triploid (type B)

with the sympatric diploid (type A) and the diploid GH population (Table 4). Studies on other animals also revealed that polyploids were not necessarily larger than diploids of the same species. For example, *Poeciliopsis monachalucida* (Chordata: Actinopterygii) triploids have a smaller body size than sympatric diploids (Schultz, 1982; Walsh and Zhang, 1992); and in *Gasterosteus aculeatus* (Chordata: Actinopterygii) the body size of triploids is similar to that of diploids (Swarup, 1959).

The AQK population which living at 4255 m above the sea level, has the largest body size among all parthenogenetic *Artemia* populations (Table 4), Also studies on bisexual *Artemia tibetiana* that inhabit in very high altitude showed often the largest body size (Zheng and Sun, 2008; Zhou et al., 2003a). Such large body size, also large sizes of resting eggs and nauplii (see Abatzopoulos et al. 1998; Van Stappen et al. 2003; Wang and Sun, 2007; Zhou et al. 2003b) is likely a result of convergent evolution for adapting the unique environmental condition of high altitude habitat e.g. low temperature, low-pressure oxygen. Past observations have proved that marine crustaceans are following with Bergmann's rule which means the populations and species with larger size are inhabited in colder environments (Broyer, 1977; Mauchline, 1980; Reaka, 1986; Timofeev, 1992, 2001), so the large body of AQK parthenogenetic population and also *A. tibetiana* can attribute to pursuing with Bergmann's rule.

In conclusion, ovisac and furca show high variations among parthenogenetic *Artemia* populations. Their morphology is more likely to be population specific than to be related to the ploidy level of certain population. Tetra- and pentaploid parthenogenetic *Artemia* are usually distinguished from di- and triploid *Artemia* by morphometric analyses, whereas such differentiation is not so obvious between the latter two forms. The body size of parthenogenetic *Artemia* is not closely related to ploidy levels. Similar to that has been documented for bisexual species, parthenogenetic *Artemia* from very high habitat (the Aqqikkol Lake) have significantly larger body size than those from lower habitats, which might be an adaptation feature for living in high altitude habitats. Comparative studies on cloned laboratory populations and collected samples from natural habitats are suggested to understand the roles of genetic feature and environmental factors in determining the morphological and morphometrical characters.

ACKNOWLEDGMENTS

Our thanks are to Dr. Wei Liu (Institute of Oceanology, Chinese Academy of Sciences) for his assistance in SEM work. The help of Dr. William D. Shepard (University of California, Berkeley, USA) with the English text is highly appreciated.

REFERENCES

Abatzopoulos TJ, Triantaphyllidisand CD, Kastritsis CD. 1987. Preliminary studies on some *Artemia* population from northern Greece. In: Sorgeloos P, Bengtson DA, Decleir W, Jaspers E. editors. *Artemia* research and its applications, Vol. 1. Wetteren: Universa Press. pp. 107–113.

Abatzopoulos TJ, Karamanlidis G, Leger P, Sorgeloos P. 1989. Further characterization of two *Artemia* populations from northern Greece: Biometry, hatching characteristics, caloric content and fatty acid profiles. Hydrobiologia 179:211–222.

Abatzopoulos TJ, 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). Int J Salt Lake Res 7:41–44.

Abatzopoulos TJ, Kappas I, Bossier P, Sorgeloos P, Beardmore JA. 2002a. Genetic characterization of *Artemia tibetiana* (Crustacea: Anostraca). Biol J Linn Soc 75:333–344.

Abatzopoulos TJ, Beardmore JA, Clegg JS, Sorgeloos P. 2002b. *Artemia*: Basic and applied biology. Dordrecht, The Netherlands: Kluwer Academic Publishers. pp. 286.

Abatzopoulos TJ, Amat F, Baxevanis AD, Belmonte G, Hontoria F, Maniasti S, Moscatello S, Mura G, Shadrin NV. 2009. Updating geographic distribution of *Artemia urmiana* Günther, 1890 (Branhiopoda: Anostraca) in Europe: An integrated and interdisciplinary approach. Int Rev Hydrobiol 94:560–579.

Abreu-Grobois, FA, Beardmore JA. 1982. Genetic differentiation and speciation in the brine shrimp *Artemia*. In: Barigozzi C, editor. Mechanisms of speciation. New York: Alan R. Liss Inc. pp. 345–376.

Agh N, Bossier P, Abatzopoulos TJ, Beardmore J, Van Stappen G, Mohamadyari A, Rahimian H, Sorgeloos P. 2009. Morphometric and preliminary genetic characteristics of *Artemia* populations from Iran. Int Rev Hydrobiol 94:194–207.

Amat F. 1980. Differentiation on *Artemia* strains from Spain. In: Persoone G, Sorgeloos P, Roels O, Jaspers E, editors. The brine shrimp *Artemia*, Vol. 1. Wetteren: Universa Press. pp. 19–39.

Amat F, Barata C, Hontoria F. 1995a. A Mediterranean origin for the Veldrif (South Africa) *Artemia* Leach population. J Biogeogr 22:49–59.

Amat F, Barata C, Hontoria F, Vavarro JC, Varo I. 1995b. Biogeography of the genus *Artemia* (Crustacea, Branchiopoda, Anostraca) in Spain. Int J Salt Lake Res 3:175–190.

Amat F, Cohen R, Hontoria F, Navarro J. 2004. Further evidence and characterization of *Artemia franciscana* (Kellogg, 1906) populations in Argentina. J Biogeogr 31:1735–1749.

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

Amat F, Hontaria F, Navarro JC, Vieira N, Mura G. 2007. Biodiversity loss in the genus *Artemia* in the Western Mediterranean Region. Limnetica 26:177–194.

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

Asem A, Rastegar-Pouyani N. 2008. Morphological differentiation of *Artemia urmiana* Günther, 1899 (Crustacea: Anostraca) in different geographical stations from the Urmia Lake-Iran. Res J Biol Sci 3:222–228.

Asem A, Sun S-C. 2014. Biometric characterization of Chinese parthenogenetic *Artemia* (Crustacea: Anostraca) cysts, focusing on its relationship with ploidy and habitat altitude. North-Western J Zool 10:149–157.

Asem A, Eimanifar E, Sun S-C. in press. Genetic variation and evolutionary origins of parthenogenetic *Artemia* (Crustacea: Anostraca) with different ploidies. Zool Scr., doi:10.1111/zsc.12162.

Baxevanis AD, Triantaphyllidis GV, Kappas I, Triantafyllidis A, Triantaphyllidis CD, Abatzopoulos TJ. 2005. Evolutionary assessment of *Artemia tibetiana* (Crustacea, Anostraca) based on morphometry and 16S rRNA RFLP analysis. J Zool Sys Evol Res 43:189–198.

Baxevanis AD, Kappas I, Abatzopoulos TJ. 2006. Molecular phylogenetics and asexuality in the brine shrimp *Artemia*. Mol Phylogenet E 40:724–738.

Ben Naceur H, Jenhani A, Romdhane M. 2011a. Preliminary characteristics (biometry, sexual dimorphism and fatty acid profile) of the brine shrimp *Artemia salina* (L., 1758) (Crustacea: Anostraca) from Sabkhet Boujmal, Tunisia. Int J Artemia Biol 1:29–40.

Ben Naceur H, Ben Rejeb Jenhani A, Romdhane MS. 2011b. Taxonomical study of the brine shrimp *Artemia* populations (Crustacea: Branchiopoda) from Tunisia based on morphological characterization. Mar Life 17:55–65.

Ben Naceur H, Ben Rejeb Jenhani A, Romdhane MS. 2013. Morphometric characterization of adult *Artemia* (Crustacea: Branchiopoda) populations from costal and inland Tunisian salt lakes. Afr Invertebr 54:543–555.

Broyer C. 1977. Analysis of the gigantism and dwarfness of Antarctic and Subantarctic Gammaridean Amphipoda. In: Llano GA, editor. Adaptations within antarctic ecosystems. Washington DC: Smithsonian Institute. pp. 327–334.

Camargo WN, Ely JS, Sorgeloos P. 2003. Morphometric characterization of thalassohaline *Artemia franciscana* populations from the Colombian Caribbean. J Biogeogr 30:697–702.

Cohen RG, Amat F, Hontoria F, Navarro JC. 1999. Preliminary characterization of some Argentinean *Artemia* population from La Pampa and Buenos Aires provinces. Int J Salt Lake Res 8:329–340.
Coutteau P, Brendonck L, Lavens P, Sorgeloos P. 1992. The use of manipulated baker's yeast as an algal substitute for the laboratory culture of Anostraca. Hydrobiologia 234:25–32.
El-Bermawi N, Baxevanis AD, Abatzopoulos TJ, Van Stappen G, Sorgeloos P. 2004. Salinity effects on survival, growth and morphometry of four Egyptian *Artemia* populations (International study on *Artemia*. LXVII). Hydrobiologia 523:175–188.
El-Gamal M. 2010. Morphometric and molecular variability of three *Artemia* strains (El-Max and Wadi El-Natrun, Egypt and San Francisco Bay, USA). J Am Sci 6:98–107.
Gajardo G, Colihueque N, Parraguez M, Sorgeloos P. 1998. International study on *Artemia*: 58. Morphologic differentiation and reproductive isolation of *Artemia* populations from South America. Int J Salt Lake Res 7:133–151.
Gajardo G, Parraguéz M, Beardmore JA, Sorgeloos P. 2001. Reproduction in the brine shrimp *Artemia*: Evolutionary relevance of laboratory cross-fertility tests. J Zool 253:25–32.
Gajardo G, Mura G. 2011. The highly divergent New World *Artemia* species (Branchiopoda, Anostraca), *A. franciscana* and *A. persimilis*, show subtle differences in morphological traits involved in mating. Zootaxa 2912:37–48.
Gregory TR, Mable BK. 2005. Polyploidy in animals, In: Gregory TR, editor. The evolution of the genome. San Diego: Elsevier. pp. 427–517.
Hontoria F, Amat F. 1992a. Morphological characterization of adult *Artemia* (Crustacea, Branchiopoda) from different geographical origins. Mediterranean population. J Plankton Res 14:949–956.
Hontoria F, Amat F. 1992b. Morphological characterization of adult *Artemia* (Crustacea, Branchiopoda) from different geographical origins. American populations. J Plankton Res 14:1461–1471.
Liu Z, Zheng M, Luo J. 1998. In: Chinese with English Summary. A study of *Artemia* in Lagkor Co, Tibet, I: Biological feature. J Lake Scis 10:92–96.
Ma L, Wang Q, Wang C. 2003. Comparative studies on the biological characteristics of three *Artemia* strains from northwestern China. J Ocean Uni Qingdao 33:865–870.
Ma L, Wang W. 2003. Comparison of the biological characteristics of two *Atermia* populations from Aqikekule Lake and Gahai Lake. Mar Scis 27:34–37 (in Chinese with English summary).
Maccari M, Amat F, Gómez A. 2013. Origin and genetic diversity of diploid parthenogenetic *Artemia* in Eurasia. PLoS One 8:e83348. doi:10.1371/journal.pone.0083348
Maniatsi S, Baxevanis AD, Kappas I, Deligiannidis P, Triantafyllidis A, Papakostas S, Bougiouklis D, Abatzopoulos TJ. 2011. Is polyploidy a persevering accident or an adaptive evolutionary pattern? The case of the brine shrimp *Artemi*a. Mol Phylogenet E 58:353–364.
Mauchline J. 1980. The biology of Mysids and Euphausiids. Adv Mar Biol 18:1–681.
Muñoz J, Gómez A, Green AJ, Figuerola J, Amat F, Rico C. 2010. Evolutionary origin and phylogeography of the diploid obligate Parthenogen *Artemia parthenogenetica* (Branchiopoda: Anostraca). PLoS One 5:e11932. doi:10.1371/journal.pone.0011932
Mura G. 1990. *Artemia salina* (Linnaeus, 1758) from Lymington, England: Frontal knob morphology by scanning electron microscopy. J Crust Biol 10:364–368.
Mura G, Baxevanis AD, Lopez GM, Hontoria F, Kappas I, Moscatello S, Fancello G, Amat F, Abatzopoulos TJ. 2005. The use of a multidisciplinary approach for the characterization of a diploid parthenogenetic *Artemia* population from Torre Colimena (Apulia, Italy). J Plankton Res 27:895–907.
Mura GI, Kappas AD, Baxevanis S, Moscatello Q, D'Amico GM, Lopez F, Hontoria F Amat TJ, Abatzopoulos. 2006. Morphological and molecular data reveal the presence of the invasive *Artemia franciscana* in Margherita di Savoia Salterns (Italy). Int Rev Hydrobiol 91:539–554.
Otto SP, Whitton J. 2000. Polyploid incidence and evolution. Annu Rev Genet 34:401–437.
Otto SP. 2007. The evolutionary consequences of polyploidy. Cell 131: 452–462.
Pan Z, Sun J, Bian B, Li M. 1991. The biometrics of *Artemia parthenogenetica* from different localities in Shandong and Xinjiang. Trans Oceano Limnolo 2:62–69 (in Chinese with English summary).
Reaka ML. 1986. Biogeographic patterns of body size in stomatopod Crustacea: Ecological and evolutionary consequences. In: Gore RH, Heck KL, editors. Biogeography of the Crustacea. Rotterdam: Balkema Press. pp. 209–235.
Schultz RJ. 1982. Competition and adaptation among diploid and polyploid clones of unisexual fishes, In: Dingle H, Hegmann JP, editors. Evolution and genetics of life histories. New York: Springer. pp. 103–119.
Sorgeloos P, Lavens P, Leger P, Tackaert W, Versichele D. 1986. Manual for the Culture and Use of Brine Shrimp *Artemia* in Aquaculture. Belgium: Ghent University Press. pp. 295.
Sun Y, Zhong YC, Song WQ, Zhang RS, Chen RY. 1999. Detection of genetic relationships among four *Artemia* species using randomly amplified polymorphic DNA (RAPD). Int J Salt Lake Res 8:139–147.
Swarup H. 1959. Effect of triploidy on the body size, general organization and cellular structure in *Gasterosteus aculeatus* (L). J Genet 56:143–155.
Tejeda NLD. 1987. Preliminary characterization of four populations of *Artemia* from the Dominican Republic. In: Sorgeloos P, Bengtson DA, Decleir W, Jaspers E, editors. *Artemia* research and its applications, Vol.1. Wetteren: Universa Press. pp. 221–226.
Timofeev SF. 1992. Phylogenetic changes of body length in Euphausiacea (Crustacea, Malacostraca, Eucarida). Zool Zh 71:25–31.
Timofeev SF. 2001. Bergmann's Principle and Deep-Water Gigantism in Marine Crustaceans. Biol Bull 28:646–650.
Torrentera L, Dodson SI. 1995. Morphological diversity of populations of *Artemia* (Branchiopoda) in Yucatan. J Crust Biol 15:86–102.
Torrentera L, Belk D. 2002. New penis characters to distinguish between two American *Artemia* species. Hydrobiologia 470:149–156.
Triantaphyllidis GV, Poulopoulou K, Abatzopoulos TJ, Perez CAP, Sorgeloos P. 1995. International study on *Artemia*. XLIX. Salinity effects on survival, maturity, growth, biometrics, reproductive and lifespan characteristics of a bisexual and a parthenogenetic population of *Artemia*. Hydrobiologia 302:215–227.
Triantaphyllidis GV, Abatzopoulos TJ, Miasa E, Sorgeloos P. 1996. International study on *Artemia* population from Namibia and Madagascar; cytogenetics, biometry, hatching characteristics and fatty acid profiles. Hydrobiologia 335:97–106.
Van Stappen G. 2002. Zoogeography. In: Abatzopoulos TJ, Beardmore JA, Clegg JS, Sorgeloos P, editors. *Artemia* basic and applied biology. Dordrecht: Kluwer. pp. 171–224.
Van Stappen G, Sui L, Xin N, Sorgeloos P. 2003. Characterization of highaltitude *Artemia* populations from the Qinghai–Tibet Plateau, PR China. Hydrobiologia 500:179–192.
Vasudevan S. 2012. Biometrical, morphological and biochemical characterization of three *Artemia* (Crustacea: Anostraca) populations from South India. Int J Artemia Biol 2:7–29.
Vetriselvan M, Munuswamy N. 2011. Morphological and molecular characterization of various *Artemia* strains from tropical saltpans in South East Coast of India. J Am Sci 7:689–695.
Walsh EJ, Zhang L. 1992. Polyploidy and body size variation in a natural population of the rotifer *Euchlanis dilatata*. J Evol Biol 5:345–353.
Wang RX, Cai YN, Li JY. 1991. Clonal and chromosome study of parthenogenetic brine shrimp (*Artemia parthenogenetica*) from North China. Oceano Limnolo Sin 22:1–7 (in Chinese with English summary).
Wang SM, Dou HS. 1998. Lakes in China. China, Beijing: Science Press. pp. 503 (in Chinese).
Wang SC, Sun SC. 2007. Comparative observations on the cyst shells of seven *Artemia* strains from China. Microsc Res Tech 70:663–670.
Wang SC. 2009. Biometrics of *Artemia* resting eggs from seven localities of China. Mar Sci Bull 28:62–67 (in Chinese with English summary).
Wang RX. 1988. An Analysis of *Artemia* Clones from the Salterns of Northeast China. Unpublished Master, Thesis Shandong College of Oceanography (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. J Ocean Uni Qingdao 23:109–118 (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. Trans Oceano Limnolo 3:39–47 (in Chinese with English summary).
Zheng B, Sun SC. 2008. Taxonomic consideration of eight Chinese bisexual *Artemia* populations, based on the morphology of frontal knob and gonopod and the result of cross-breeding tests. Zootaxa 1919:25–44.
Zheng B, Sun SC. 2013. Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in China. Int J Artemia Biol 3: 20–50.
Zhou KX, Xu MQ, Yin XC. 2003a. Morphological characterization of sexual *Artemia* (Branchiopoda) from China. Crustaceana 76:1331–1346.
Zhou KX, Xu MQ, Guan YQ, Yin XC. 2003b. Numeric taxonomy using cysts and nauplii of bisexual *Artemia* from China. J Lake Sci 15: 153–159 (in Chinese with English summary).