Morphometric Characterization of Adult Artemia (Crustacea: Branchiopoda) Populations from Costal and Inland Tunisian Salt Lakes

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Morphometric characterization of adult *Artemia* (Crustacea: Branchiopoda) populations from costal and inland Tunisian salt lakes

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ABSTRACT

Tunisia has many *Artemia* populations, but little information is available concerning their taxonomy, biometry and morphology. This work is an updated systematic inventory of *Artemia* populations in Tunisia, based on the comparison of different morphometric parameters measured in cultured adult individuals. Sixteen Tunisian *Artemia* populations were examined. The study included reference samples of two *Artemia franciscana* (San Francisco Bay, California, USA; and Great Salt Lake, Utah, USA) populations. The variability among the diverse populations was studied by statistical treatment of data through the analysis of variance. This analysis did not unveil any particular similarity among the *Artemia* populations studied, apart from the width of the head of male specimens (F=1.088, P=0.360). Biometrical analysis of these data was performed via multivariate discriminant analysis, and using the origin of each population as a separation factor. Results revealed that all the Tunisian *Artemia* populations studied can be classified as *Artemia salina*, with the exception of the *Artemia* population from Sabkhet Halk El Menzel, which belongs to *Artemia franciscana*.

KEY WORDS: Crustacea, Anostraca, *Artemia*, brine shrimps, bisexual, culture, morphometrics, Tunisia.

INTRODUCTION

The brine shrimp *Artemia* Leach, 1819 (Branchiopoda: Anostraca) comprises a number of bisexual species and parthenogenetic strains that are morphologically similar. Brine shrimps are found in inland and coastal saline and hypersaline waters throughout the world, with the exception of Antarctica (Triantaphyllidis *et al*. 1998; Van Stappen 2002). The present *Artemia* distribution is not only due to natural means (high dispersal potential of diapausing cysts) but also to human activity. *Artemia* species and strains have a remarkable genetic variability that can be expressed in various phenotypic characteristics such as morphometry, life history, breeding mechanisms and growth rate, according to their genetic differentiation and population structure (Gajardo *et al*. 2004; Muñoz *et al*. 2008).

The taxonomic study of *Artemia* populations in the Western Old World, including Italy, the South of France and the Iberian Peninsula (Spain and Portugal), together with North Africa, is interesting owing to the presence of the Mediterranean bisexual *Artemia salina* (L., 1758) and at least two different parthenogenetic forms, diploid and tetraploid (Gilchrist 1960; Vieira & Amat 1985; Vanhaecke *et al*. 1987; Amat *et al*. 1995). This Mediterranean region also holds the recent invader species, *Artemia franciscana* Kellogg, 1906 (Narciso 1989; Amat *et al*. 1995; Amat *et al*. 2005; Mura *et al*. 2004; Amat *et al*. 2007; Scalone & Rabet 2013). In Tunisia, *Artemia* has been reported in 23 different locations, characterized as temporal or ephemeral catchments, and is distributed from semi-arid to Saharan hydrogeographical zones (Ben Naceur *et al*. 2009, 2010, 2012*a*). However, there is a paucity of data regarding their taxonomy, biogeography and genetic characteristics. Kaiser *et al*. (2006) published the most recent checklist of

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the zoogeography of *Artemia* and included eight Tunisian *Artemia* populations, three of which were cited as being of unknown specific identity.

The most relevant methods used for *Artemia* species characterization are the comparison of morphometric traits for specimens cultured under standard conditions (Hontoria & Amat 1992*a*, *b*; Gajardo *et al*. 1998; Amat *et al*. 2005; Mura *et al*. 2005), their genetic characterization (Zhang & King 1992; Abatzopoulos *et al*. 2002; Camargo *et al*. 2002; Kappas *et al*. 2004; Van Stappen *et al*. 2007; Muñoz *et al*. 2008; Tizol-Correa *et al*. 2009), electron microscopic examination of some morphological traits (Criel & Macrae 2002; Mura & Nagorskaya 2005; Mura *et al*. 2005; Ben Naceur *et al*. 2011*a*), and cross-fertility tests (Gajardo *et al*. 2001; Abatzopoulos *et al*. 2002).

Although modern methods rely on genetic characterization through molecular markers for the differentiation of anostracan populations, it is well known that among *Artemia*, morphometrical traits have been the basis for describing species and strains (e.g. Hontoria & Amat 1992*a*, *b*; Triantaphyllidis *et al*. 1997; Camargo *et al*. 2003). Work on morphometrics of *Artemia* (Schmankewitsch 1873 in Litvinenko *et al*. 2007; Gaevskaya 1916; Gilchrist 1960; Ben Naceur *et al*. 2011*b*, 2012*b*) has shown that *Artemia* individuals exhibit morphological changes in accordance with environmental conditions. Moreover, Hontoria and Amat (1992*a*) reported that individuals from different *Artemia* populations, although similar in body shape, show morphological traits that enable morphometric differentiation when they are cultured under standard laboratory conditions.

In the present work, variation in different morphometric variables measured on adult *Artemia* specimens from Tunisian populations, and cultured under laboratory conditions, has been studied. Multivariate discriminant analysis has been used in order to support current knowledge on the taxonomic status of *Artemia* in these populations.

MATERIAL AND METHODS

Specimens from 16 Tunisian *Artemia* populations (Table 1) were cultured in the laboratory, characterized morphometrically, and compared with cultured specimens from two *Artemia franciscana* populations (San Francisco Bay, California, USA; and Great Salt Lake, Utah, USA).

Culture conditions

Experimental *Artemia* populations were reared under standardized culture conditions in order to minimize the strong environmental influences on body morphometry. Nauplii hatched from original cysts were reared up in 2-L plastic containers filled with 90 ± 10 g/L filtered and autoclaved brine (seawater plus crude sea salt) (Amat *et al*. 2005). Temperature was maintained at 24 °C during a 16 h light / 8 h dark cycle. Shrimps were fed the unicellular alga *Chlorella* sp. at a density of 100–200×10^3 cells/mL. The medium was renewed twice a week with a new microalgal culture.

Adult morphometry

As soon as specimens reached reproductive maturity (i.e. well-developed antennae on males and a well-developed brood pouch on females), a random sample of 20 individuals was removed from the culture (for each sex and population), anaesthetized with a few droplets of water saturated with chloroform and measured under a microscope using a calibrated micrometer eye piece. The following were measured for each male and

TABLE 1

Sources of Tunisian *Artemia* populations used for morphometrical characterization and literature references for autochthonous populations with verified species status.

Site	Habitat	Abbreviation	Co-ordinates	Year	Species	References
Sabkhet Sijoumi	Inland salt lake	SIJ	36°55'38"N 10°15'22"E	2003	*A. salina*	Romdhane *et al.* 2004
Megrine saltwork	Coastal saltwork	MEG	36°47'N 10°14'E	1998	*A. salina*	Romdhane *et al.* 2004
Sabkhet Korzia	Inland salt lake	KOR	36°24'47"N 09°47'10"E	2006	*A. salina*	Ben Naceur *et al.* 2011*a*
Sabkhet Halk El Menzel	Coastal salt lake	HM	36°00'40"N 10°27'30"E	2009	Species status unknown	–
Sabkhet Sidi El Hani	Inland salt lake	SH	35°37'43"N 10°22'46"E	2006	Species status unknown	–
Sahline saltwork	Coastal saltwork	SAH 03	35°45'58"N 10°46'58"E	2003	*A. salina*	Romdhane *et al.* 2004 Muñoz *et al.* 2008
		SAH 06		2006	Species status unknown	–
Bkalta saltwork	Coastal saltwork	BK	35°34'19"N 11°01'39"E	2007	Species status unknown	–
Sabkhet Moknine	Inland salt lake	MOK	35°36'20"N 10°55'37"E	2006	Species status unknown	–
Sfax saltwork	Coastal saltwork	SFX	34°42'24"N 10°44'14"E		*A. salina*	
Sabkhet Boujmal	Inland salt lake	BJ	34°57'53"N 10°24'04"E	2008	*A. salina*	Ben Naceur *et al.* 2011*a*
Sabkhet Mcheguig	Inland salt lake	MCH	34°57'16"N 10°02'28"E	2006	Species status unknown	–
Sabkhet El Adhibet	Inland saltwork	ADH 03	33°05'42"N 11°24'29"E	2003	*A. salina*	Romdhane *et al.* 2004 Muñoz *et al.* 2008
		ADH 07		2007	*A. salina*	Ben Naceur *et al.* 2011*b*
Sabkhet Mnikhra	Inland salt lake	MNK	33°08'59"N 11°20'09"E	2007	*A. salina*	Ben Naceur *et al.* 2011*a*
Mhabeul saltwork	Inland saltwork	MHB	33°24'35"N 10°51'20"E	2006	Species status unknown	–
Sabkhet El Melah	Inland salt lake	MEL	32°21'34"N 10°55'22"E	2006	*A. salina*	Ben Naceur *et al.* 2011*a*
Zarzis saltwork	Inland saltwork	ZAR	33°24'48"N 11°03'43"E	2006	Species status unknown	–
San Francisco Bay	–	SFB	–	–	*A. franciscana*	Commercial cysts
Great Salt Lake	–	GSL	–	–	*A. franciscana*	Commercial cysts

female: total length (*tl*), abdominal length (*al*), width of third abdominal segment (*wts*), length of cercopods (*lc*), number of setae on left cercopod (*nlc*), number of setae on right cercopod (*nrc*), width of head (*wh*), diameter of compound eyes (*dy*), maximal distance between compound eyes (*dby*), length of first antenna (*la*), width of brood pouch (*wb*) (for females), width of second abdominal segment (*wss*) and width of frontal knob (*fk*) (for males); abdomen length : total length ratio (*ra, %*) was also calculated for both sexes (Amat *et al*. 2005). The morphometric variability among the diverse populations was established by statistical treatment of data through one-way ANOVA (Tukey, P<0.05). The homogeneity of variances was tested using the Leven's test (Norušis 1993). Biometrical analysis of these data was performed via multivariate discriminant analysis using the statistical package SPSS for Windows, version 10.0 (Norušis 2000).

RESULTS

The statistically significant inter-population differences in morphometrics have been observed for the male and female specimens (Tables 2, 3). Results of variance demonstrate different degrees of variation and do not show any particular similarity among the *Artemia* populations studied, apart from the width of the head of male specimens (F=1.088, P=0.360).

When the origin of populations was used as a separation factor for the multivariate discriminant analysis applied to morphometric data, this procedure showed 11 discriminant functions for males and 10 discriminant functions for females (Fig. 1, Table 4). The first five of these functions gave cumulative variance percentages of 85.7 % and 89.8 % for males and females, respectively. Morphometric characteristics showed a clear differentiation between Tunisian populations (except that from Sabkhet Halk El Menzel) and the two reference populations.

The centroids (Fig. 1) demonstrate that populations belonging to the same species tend to be grouped. In the case of males (Table 5), the analysis shows that morphometric

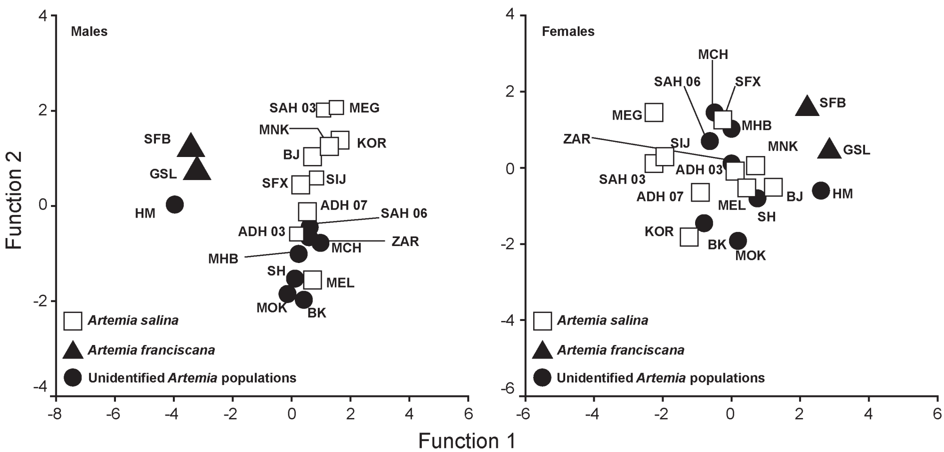

Fig. 1. Scatterplot of the first two canonical discriminant functions (group centroids) resulting from the discriminant analysis applied to morphological traits and morphometric data for the studied populations, using the origin of each population as a separation factor. Abbreviations for populations are explained in Table 1.

characters correlated with the first discriminant function are total length, length of cercopod, width of second abdominal segment and abdominal length. Those related to the second function are total length, abdominal length and total length and abdomen length/total body length ratio. For females, total length and abdominal length are morphometric traits correlated with the first discriminant function, while abdominal length, total length and abdomen length / total body length ratio, are related with the second function.

Tunisian *Artemia* populations, except for Sabkhet Halk El Menzel, were evidently grouped and separated from the two *A. franciscana* reference data sets (Fig. 1). Tunisian populations previously identified as *A. salina* (Table 1) were well integrated among unidentified Tunisian populations studied here, supporting their taxonomic assignment to the bisexual *A. salina* species. The *Artemia* population from Sabkhet Halk El Menzel was grouped with the two American populations and can be considered as belonging to the invasive *A. franciscana* species.

DISCUSSION

Morphometrical differentiation has been one of the most useful methods for *Artemia* taxonomy and biosystematics (Asem & Rastegar-Pouyani 2008). The results obtained here with multivariate discriminant analysis for 16 Tunisian *Artemia* populations and two American *A. franciscana* populations, using the origin of each population as a separation factor, provided two different phenotypic groups (the first group formed by populations identified as *A. salina* and unknown *Artemia* and the second group composed of the two American *A. franciscana* populations, together with the Tunisian population found in Sabkhet Halk El Menzel). Ben Naceur *et al*. (2011*a*, *b*) studied morphological characteristics (ovisac morphology in females and the frontal knob morphology and ornamentation (number of spines and mechanoreceptors), as well as the basal part of the penis, in males) of some Tunisian *Artemia* populations. Their results show that the male *Artemia* specimens from the Sabkhet Halk El Menzel population have a sub-spherical frontal knob shape, and spine-like projections present in the non-retractile basal part of the penis, leading to the conclusion that this population belongs to a species other than *A. salina*. In fact, based on the presence or absence of a spine on the basal part of penis, *A. salina* can be distinguished from all the other bisexual *Artemia* populations, since all non-Mediterranean bisexual *Artemia* (from both the New and the Old World) have spines basally on the penis (Triantaphyllidis *et al.* 1997; Mura & Nagorskaya 2005).

Several publications recently reported the presence of the invasive *A. franciscana* in different countries around Tunisia. This event was initially described in Portugal (Hontoria *et al*. 1987, in Amat *et al*. 2007), France (Thiery & Robert 1992; Scalone & Rabet 2013), Italy (Mura *et al*. 2004) and in Spain and Morocco (Amat *et al*. 2005; Green *et al*. 2005; Amat *et al*. 2007). The occurrence of *A. franciscana* in Portuguese hypersaline water bodies might date from the 1980s, and although the time and the place of the original introduction remain unknown, the introduction could have been intentional (Amat *et al*. 2007), especially as the beneficial effect of the branchiopod *Artemia* in salt production and their use in aquaculture as a food source were well known (Dhont & Sorgeloos 2002). This species was probably then dispersed via waterfowl northwards along the East Atlantic flyway (Green *et al*. 2005) and eastwards, reaching the Spanish, Moroccan and Italian sites where it was found (Amat *et al*. 2007). This hypothesis about

TABLE 2

Mean values 'mm' (standard deviation in parentheses) of morphometric characters measured in males of different Tunisian *Artemia* populations. Total length (*tl*), abdominal length (*al*), width of second abdominal segment (*wss*), width of third abdominal segment (*wts*), length of cercopods (*lc*), number of setae inserted on left cercopod (*nlc*), number of setae inserted on right cercopod (*nrc*), width of head (*wh*), maximal distance between compound eyes (*dby*), diameter for compound eyes (*dy*), length of first antenna (*la*), width of frontal knob (*fk*) and abdomen length : total length ratio (*ra, %*). Abbreviations for populations are explained in Table 1. Same letters show non-significant differences between mean in each row of main column (P=0.05).

	tl	*al*	*wss*	*wts*	*lc*	*nlc*	*nrc*	*wh*	*dby*	*dy*	*la*	*fk*	*ra (%)*
SIJ	8.17^{abcd} (0.77)	4.11^{defghi} (0.34)	0.47^{cde} (0.05)	0.40^{cdefg} (0.04)	0.32^{abc} (0.07)	8.95^{abcd} (3.39)	9.25^{abc} (3.46)	0.73^{a} (0.04)	1.46^{bcdef} (0.15)	0.33^{bcdefg} (0.03)	0.97^{bcde} (0.12)	0.16^{cde} (0.02)	50.46^{gh} (2.93)
MEG	7.98^{abf} (0.78)	3.78^{bcdefg} (0.37)	0.52^{ef} (0.09)	0.38^{efgh} (0.06)	0.38^{abcde} (0.07)	10.75^{abcd} (4.12)	10.60^{abcde} (3.80)	0.66^{a} (0.20)	1.55^{efg} (0.13)	0.35^{fgh} (0.04)	1.03^{def} (0.11)	0.19^{ghi} (0.02)	47.49^{cdefg} (3.82)
KOR	9.24^{g} (0.55)	4.42^{hij} (0.27)	0.54^{f} (0.05)	0.43^{i} (0.05)	0.47^{ab} (0.1)	7.90^{a} (5.39)	8.20^{ab} (5.61)	0.72^{a} (0.26)	1.58^{fg} (0.18)	0.36^{gh} (0.04)	1.05^{ef} (0.17)	0.19^{hi} (0.02)	47.94^{defg} (2.03)
HM	8.10^{abc} (0.63)	3.41^{ab} (0.39)	0.50^{ef} (0.07)	0.43^{a} (0.03)	0.16^{abcd} (0.05)	9.15^{abc} (3.32)	9.05^{abcd} (3.83)	0.60^{a} (0.07)	1.27^{a} (0.17)	0.28^{a} (0.06)	0.69^{a} (0.13)	0.1^{ab} (0.03)	42.06^{ab} (3.25)
SH	8.88^{cdefg} (1.15)	4.17^{efghi} (0.49)	0.41^{abc} (0.05)	0.40^{defgh} (0.06)	0.37^{def} (0.1)	13.00^{cde} (5.52)	13.00^{def} (5.34)	0.96^{a} (1.23)	1.49^{bcdef} (0.27)	0.34^{defgh} (0.05)	0.98^{bcde} (0.14)	0.15^{bcd} (0.02)	47.11^{cdef} (2.11)
SAH 03	8.90^{cdefg} (0.62)	4.19^{fghi} (0.23)	0.54^{f} (0.05)	0.46^{fghi} (0.03)	0.40^{abcde} (0.06)	9.25^{abc} (1.80)	9.05^{abcde} (1.76)	0.81^{a} (0.04)	1.71^{g} (0.09)	0.38^{h} (0.02)	1.13^{f} (0.10)	0.20^{i} (0.02)	47.14^{cdef} (2.15)
SAH 06	8.52^{abcdef} (0.47)	3.96^{cdefgh} (0.33)	0.44^{abcd} (0.03)	0.42^{defgh} (0.04)	0.35^{bcdef} (0.08)	11.60^{abcd} (4.18)	11.35^{bcdef} (3.95)	0.73^{a} (0.03)	1.52^{cdef} (0.07)	0.33^{bcdefg} (0.02)	0.94^{bcde} (0.1)	0.17^{cdefg} (0.02)	46.50^{cdef} (2.32)
BK	$8.71^{abcdefg}$ (0.58)	4.24^{ghi} (0.33)	0.39^{ab} (0.03)	0.36^{bcd} (0.04)	0.29^{ab} (0.05)	8.05^{a} (2.25)	8.10^{ab} (2.29)	0.70^{a} (0.04)	1.32^{ab} (0.10)	0.29^{ab} (0.02)	0.87^{bc} (0.09)	0.15^{abcd} (0.01)	48.67^{efg} (1.60)
MOK	8.53^{abcdef} (0.66)	$4.03^{cdefghi}$ (0.67)	0.38^{a} (0.05)	0.36^{ab} (0.04)	0.22^{abcde} (0.1)	10.05^{abcd} (3.63)	10.50^{abcde} (4.00)	0.62^{a} (0.06)	1.36^{abcd} (0.11)	0.31^{abcdef} (0.03)	0.91^{bcd} (0.11)	0.16^{cdef} (0.03)	47.22^{cdef} (6.23)
SFX	9.42^{g} (0.88)	4.18^{fghi} (0.42)	0.52^{ef} (0.07)	0.46^{ghi} (0.05)	0.41^{abcde} (0.07)	10.55^{abcd} (2.56)	10.85^{abcde} (2.62)	0.82^{a} (0.07)	1.55^{fg} (0.14)	0.34^{efgh} (0.05)	1.07^{ef} (0.13)	0.20^{i} (0.03)	44.38^{bc} (1.96)
BJ	9.13^{efg} (0.77)	4.50^{ij} (0.43)	0.50^{def} (0.05)	0.44^{bcde} (0.04)	0.30^{ab} (0.08)	8.40^{ab} (2.43)	8.40^{ab} (2.60)	0.78^{a} (0.06)	1.53^{defg} (0.15)	0.33^{bcdefg} (0.03)	1.14^{f} (0.12)	0.19^{fghi} (0.02)	49.28^{fgh} (3.00)

TABLE 2 (*continued*)

Mean values 'mm' (standard deviation in parentheses) of morphometric characters measured in males of different Tunisian *Artemia* populations. Total length (*tl*), abdominal length (*al*), width of second abdominal segment (*wss*), width of third abdominal segment (*wts*), length of cercopods (*lc*), number of setae inserted on left cercopod (*nlc*), number of setae inserted on right cercopod (*nrc*), width of head (*wh*), maximal distance between compound eyes (*dby*), diameter for compound eyes (*dy*), length of first antenna (*la*), width of frontal knob (*fk*) and abdomen length : total length ratio (*ra, %*). Abbreviations for populations are explained in Table 1. Same letters show non-significant differences between mean in each row of main column (P=0.05).

	tl	*al*	*wss*	*wts*	*lc*	*nlc*	*nrc*	*wh*	*dby*	*dy*	*la*	*fk*	*ra (%)*
MCH	8.17^{abcd} (0.74)	3.71^{bcde} (0.37)	0.41^{abc} (0.05)	0.40^{hi} (0.04)	0.44^{f} (0.06)	15.05^{e} (3.08)	15.50^{f} (3.25)	0.72^{a} (0.06)	1.45^{bcdef} (0.16)	0.32^{bcdefg} (0.04)	1.03^{def} (0.09)	0.16^{bcde} (0.02)	45.45^{cd} (2.81)
ADH 03	9.03^{defg} (0.72)	$4.06^{cdefghi}$ (0.36)	0.44^{bcd} (0.04)	0.44^{cdef} (0.04)	0.32^{abcd} (0.07)	9.10^{abc} (1.97)	9.05^{abcd} (1.82)	0.73^{a} (0.07)	1.51^{cdef} (0.18)	0.34^{defgh} (0.05)	1.06^{ef} (0.10)	0.18^{efghi} (0.02)	45.05^{bcd} (2.18)
ADH 07	7.89^{a} (0.65)	3.73^{bcdef} (0.33)	0.41^{abc} (0.03)	0.39^{abc} (0.03)	0.24^{a} (0.06)	7.35^{a} (1.87)	7.40^{a} (1.78)	0.68^{a} (0.05)	1.47^{bcdef} (0.1)	0.34^{cdefgh} (0.02)	1.01^{cdef} (0.08)	0.16^{bcde} (0.01)	47.29^{cdefg} (2.58)
MNK	9.13^{efg} (0.77)	4.79^{j} (0.42)	0.50^{def} (0.05)	0.44^{bcde} (0.05)	0.30^{ab} (0.08)	8.40^{ab} (2.43)	8.40^{ab} (2.60)	0.78^{a} (0.06)	1.53^{defg} (0.15)	0.33^{bcdef}g (0.03)	1.14^{f} (0.12)	0.19^{fghi} (0.02)	52.40^{h} (1.15)
MHB	7.95^{ab} (0.73)	3.67^{abcd} (0.40)	0.41^{abc} (0.03)	0.40^{fghi} (0.03)	0.39^{ef} (0.1)	13.15^{de} (3.77)	13.05^{ef} (3.85)	0.68^{a} (0.05)	1.344^{abc} (0.13)	0.30^{abcd} (0.03)	0.95^{bcde} (0.12)	0.15^{abcd} (0.02)	46.17^{cdef} (1.78)
MEL	8.37^{abcde} (0.93)	3.95^{cdefg} (0.45)	0.40^{ab} (0.04)	0.38^{defgh} (0.04)	0.35^{cdef} (0.08)	12.35^{bcde} (5.00)	12.25^{cdef} (4.66)	0.94^{a} (1.2)	1.42^{abcdef} (0.22)	0.33^{bcdefg} (0.04)	0.95^{bcde} (0.11)	0.14^{abc} (0.02)	47.26^{cdefg} (2.19)
ZAR	8.79^{bcdefg} (1.21)	3.94^{cdefg} (0.54)	0.44^{bcd} (0.06)	0.40^{defg} (0.05)	0.34^{abcde} (0.1)	10.10^{abcd} (3.49)	10.10^{abcde} (3.47)	0.73^{a} (0.08)	1.48^{bcdef} (0.19)	0.32^{abcdef} (0.06)	1.03^{def} (0.19)	0.17^{defgh} (0.03)	44.87^{bcd} (2.12)
GSL	8.09^{abc} (0.88)	3.22^{a} (0.53)	0.51^{ef} (0.04)	0.45^{a} (0.05)	0.20^{abcde} (0.1)	9.70^{abcd} (3.43)	9.55^{abcde} (3.91)	0.67^{a} (0.07)	1.37^{abcde} (0.16)	0.31^{abcde} (0.04)	0.93^{bcde} (0.17)	0.15^{bcd} (0.03)	39.68^{a} (2.81)
SFB	7.89^{a} (0.45)	3.63^{abc} (0.34)	0.54^{f} (0.06)	0.47^{ab} (0.06)	0.21^{abcde} (0.04)	11.10^{abcd} (3.30)	11.00^{abcde} (3.35)	0.67^{a} (0.03)	1.32^{ab} (0.14)	0.30^{abc} (0.06)	0.85^{b} (0.11)	0.12^{a} (0.02)	46.08^{cde} (3.99)
F-value	8.316	15.967	22.416	10.825	22.827	6.575	6.538	1.088	8.981	8.531	14.432	19.128	18.642
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.360	0.000	0.000	0.000	0.000	0.000

TABLE 3

Mean values 'mm' (standard deviation in parentheses) of morphometric characters measured in females of different Tunisian *Artemia* populations. Total length (*tl*), abdominal length (*al*), width of brood pouch (*wb*), width of third abdominal segment (*wts*), length of cercopods (*lc*), number of setae inserted on left cercopod (*nlc*), number of setae inserted on right cercopod (*nrc*), width of head (*wh*), maximal distance between compound eyes (*dby*), diameter for compound eyes (*dy*), length of first antenna (*la*), and abdomen length : total length ratio (*ra, %*). Abbreviations for populations are explained in Table 1. Same letters show non-significant differences between mean in each row of main column (P=0.05).

	tl	*al*	*wb*	*wts*	*lc*	*nlc*	*nrc*	*wh*	*dby*	*dy*	*la*	*ra (%)*
SIJ	8.80^{ab}	4.68^{abcde}	1.35^{abcdef}	0.43^{ab}	0.28^{cd}	7.20^{abcde}	7.10^{abcdef}	0.77^{bcdef}	1.26^{abc}	0.22^{abc}	0.63^{abc}	53.29^{gh}
	(0.79)	(0.39)	(0.17)	(0.03)	(0.08)	(2.56)	(2.46)	(0.04)	(0.1)	(0.02)	(0.10)	(3.64)
MEG	8.74^{a}	4.59^{abcd}	1.52^{cdefg}	0.45^{ab}	0.36^{ef}	10.50^{efgh}	10.65^{ghij}	0.76^{bcdef}	1.26^{abc}	0.23^{bcdef}	0.63^{abc}	52.55^{fgh}
	(0.46)	(0.35)	(0.23)	(0.04)	(0.09)	(4.68)	(4.82)	(0.06)	(0.08)	(0.01)	(0.06)	(3.57)
KOR	9.37^{abcde}	5.03^{cdef}	1.30^{abcd}	0.43^{ab}	0.21^{abc}	4.20^{a}	4.20^{a}	0.69^{a}	1.16^{a}	0.21^{a}	0.62^{abc}	53.70^{gh}
	(0.69)	(0.49)	(0.18)	(0.04)	(0.08)	(3.30)	(3.23)	(0.08)	(0.09)	(0.01)	(0.05)	(2.31)
HM	9.84^{cdefgh}	4.59^{abcd}	1.21^{ab}	0.47^{ab}	0.17^{a}	9.25^{defg}	9.30^{defghi}	0.71^{abcd}	1.23^{ab}	0.23^{abcd}	0.64^{abcd}	46.52^{ab}
	(0.65)	(0.56)	(0.11)	(0.05)	(0.05)	(3.25)	(3.55)	(0.05)	(0.08)	(0.02)	(0.05)	(2.91)
SH	10.28^{efgh}	5.26^{f}	1.32^{abcd}	0.45^{ab}	0.26^{bcd}	7.85^{bcdefg}	7.90^{bcdefghi}	0.74^{abcd}	1.32^{bcde}	0.24^{def}	0.67^{abcd}	51.18^{defg}
	(1.37)	(0.74)	(0.34)	(0.07)	(0.05)	(3.32)	(3.32)	(0.05)	(0.13)	(0.02)	(0.06)	(1.98)
SAH 03	8.95^{abc}	4.88^{abcdef}	1.61^{efg}	0.52^{abc}	0.24^{bcd}	5.10^{abc}	5.15^{abc}	0.75^{abcdef}	1.25^{abc}	0.24^{cdef}	0.65^{abcd}	54.46^{h}
	(0.63)	(0.53)	(0.32)	(0.08)	(0.05)	(1.74)	(1.72)	(0.06)	(0.14)	(0.02)	(0.09)	(3.30)
SAH 06	8.76^{ab}	4.44^{ab}	1.31^{abcd}	0.44^{ab}	0.28^{cd}	10.50^{efgh}	10.45^{fghij}	0.73^{abcd}	1.26^{abc}	0.23^{bcd}	0.62^{abc}	50.68^{cdefg}
	(0.68)	(0.41)	(0.14)	(0.05)	(0.06)	(2.52)	(2.68)	(0.04)	(0.08)	(0.01)	(0.07)	(2.06)
BK	10.13^{efgh}	5.30^{f}	1.34^{abcde}	0.42^{a}	0.26^{bcd}	6.65^{abcd}	6.80^{abcde}	0.76^{abcdef}	1.22^{ab}	0.22^{ab}	0.67^{abcd}	52.40^{fgh}
	(0.63)	(0.32)	(0.20)	(0.03)	(0.05)	(2.47)	(2.37)	(0.04)	(0.06)	(0.01)	(0.08)	(1.16)
MOK	9.91^{defgh}	5.13^{def}	1.28^{abc}	0.47^{ab}	0.24^{bcd}	7.75^{bcdef}	7.75^{bcdefgh}	0.70^{abc}	1.27^{abc}	0.22^{abc}	0.75^{d}	51.69^{efgh}
	(0.86)	(0.67)	(0.20)	(0.07)	(0.08)	(4.10)	(4.25)	(0.09)	(0.19)	(0.02)	(0.24)	(3.94)
SFX	10.43^{gh}	5.21^{ef}	1.56^{defg}	0.51^{abc}	0.38^{f}	11.35^{gh}	11.40^{ij}	0.85^{g}	1.37^{cde}	0.25^{efg}	0.72^{cd}	49.97^{cdef}
	(0.64)	(0.43)	(0.19)	(0.05)	(0.07)	(3.29)	(3.31)	(0.05)	(0.09)	(0.02)	(0.08)	(2.38)

TABLE 3 (*continued*)

Mean values 'mm' (standard deviation in parentheses) of morphometric characters measured in females of different Tunisian *Artemia* populations. Total length (*tl*), abdominal length (*al*), width of brood pouch (*wb*), width of third abdominal segment (*wts*), length of cercopods (*lc*), number of setae inserted on left cercopod (*nlc*), number of setae inserted on right cercopod (*nrc*), width of head (*wh*), maximal distance between compound eyes (*dby*), diameter for compound eyes (*dy*), length of first antenna (*la*), and abdomen length : total length ratio (*ra, %*). Abbreviations for populations are explained in Table 1. Same letters show non-significant differences between mean in each row of main column (P=0.05).

	tl	*al*	*wb*	*wts*	*lc*	*nlc*	*nrc*	*wh*	*dby*	*dy*	*la*	*ra (%)*
BJ	10.06^{efgh} (0.82)	4.94^{bcdef} (0.34)	1.17^{a} (0.25)	0.54^{abc} (0.08)	0.23^{abc} (0.06)	8.35^{cdefg} (2.05)	8.50^{cdefghi} (1.93)	0.78^{cdefg} (0.05)	1.27^{abcd} (0.08)	0.23^{bcd} (0.01)	0.65^{abcd} (0.08)	49.18^{bcde} (2.27)
MCH	8.99^{abcd}	4.35^{a}	1.21^{ab}	0.44^{ab}	0.39^{f}	13.10^{h}	13.15^{j}	0.74^{abcd}	1.23^{ab}	0.23^{bcd}	0.65^{abcd}	48.33^{bcd}
ADH 03	9.49^{abcdef} (0.80)	4.64^{abcd} (0.45)	1.39^{abcdefg} (0.24)	0.47^{ab} (0.05)	0.26^{bcd} (0.08)	6.10^{abcd} (2.51)	6.15^{abcd} (2.51)	0.74^{abcde} (0.07)	1.29^{bcde} (0.12)	0.24^{cdef} (0.02)	0.69^{abcd} (0.09)	48.94^{bcde} (2.22)
ADH 07	8.95^{abc} (0.51)	4.79^{abcdef} (0.39)	1.37^{abcdefg} (0.22)	0.45^{ab} (0.03)	0.19^{ab} (0.04)	4.70^{ab} (1.45)	4.85^{ab} (1.38)	0.70^{ab} (0.03)	1.25^{abc} (0.07)	0.23^{abcd} (0.02)	0.59^{a} (0.06)	53.51^{gh} (1.86)
MNK	10.40^{fgh} (0.86)	5.13^{def} (0.49)	1.61^{fg} (0.35)	0.55^{bc} (0.09)	0.25^{bcd} (0.05)	7.15^{abcde} (3.04)	7.25^{abcdefg} (3.27)	0.82^{fg} (0.07)	1.40^{de} (0.12)	0.25^{fg} (0.02)	0.75^{d} (0.08)	49.28^{bcde} (1.78)
MHB	8.99^{abc} (0.88)	4.49^{abc} (0.52)	1.29^{abcd} (0.28)	0.46^{ab} (0.06)	0.30^{de} (0.09)	11.200^{fgh} (5.33)	11.15^{hij} (5.31)	0.73^{abcd} (0.11)	1.25^{abc} (0.17)	0.24^{cdef} (0.03)	0.62^{abc} (0.10)	49.97^{cdef} (2.37)
MEL	9.96^{efgh} (0.95)	5.10^{def} (0.53)	1.39^{abcdefg} (0.25)	0.46^{ab} (0.06)	0.26^{bcd} (0.05)	7.85^{bcdefg} (3.32)	7.90^{bcdefghi} (3.32)	0.74^{abcd} (0.05)	1.32^{bcde} (0.13)	0.24^{def} (0.02)	0.67^{abcd} (0.06)	51.21^{defg} (2.07)
ZAR	9.67^{bcdefg} (1.01)	4.76^{abcdef} (0.64)	1.46^{bcdefg} (0.31)	0.49^{abc} (0.05)	0.29^{de} (0.07)	8.90^{defg} (2.98)	8.85^{defghi} (3.09)	0.73^{abcd} (0.09)	1.25^{abc} (0.14)	0.23^{bcde} (0.03)	0.69^{abcd} (0.10)	49.29^{bcde} (5.32)
GSL	10.30^{fgh} (1.15)	4.62^{abcd} (0.59)	1.55^{cdefg} (0.28)	0.61^{c} (0.08)	0.20^{ab} (0.04)	10.25^{efgh} (2.31)	10.05^{efghij} (2.21)	0.79^{defg} (0.08)	1.36^{cde} (0.10)	0.24^{cdef} (0.02)	0.70^{bcd} (0.07)	44.82^{a} (1.66)
SFB	10.60^{h} (0.84)	5.11^{def} (0.51)	1.62^{g} (0.18)	0.61^{c} (0.04)	0.24^{abc} (0.05)	10.90^{fgh} (1.99)	10.75^{ghij} (1.88)	0.81^{efg} (0.05)	1.41^{e} (0.09)	0.27^{g} (0.02)	0.60^{ab} (0.07)	48.13^{bc} (1.78)
F-value	12.217	6.936	6.745	4.712	15.932	12.039	11.649	7.976	10.698	6.310	4.347	17.211
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 4

Results for discriminant analysis based on morphometrical traits for *Artemia* populations from Tunisia, with the two commercial *A. franciscana* populations used as reference.

Function	Eigen-value	Percent of variance	Cumulative percent	Canonical correlation	Wilks' Lambda	Chi-square
Males						
1	2.512	34.3	34.3	0.846	0.009	1825.874
2	1.556	21.3	55.6	0.780	0.030	1344.078
3	0.996	13.6	69.2	0.706	0.077	984.162
4	0.645	8.8	78.1	0.626	0.153	719.041
5	0.556	7.6	85.7	0.598	0.252	528.109
6	0.417	5.7	91.4	0.543	0.393	358.567
7	0.242	3.3	94.7	0.441	0.556	224.829
8	0.143	1.9	96.6	0.353	0.691	141.824
9	0.109	1.5	98.1	0.313	0.789	90.731
10	0.092	1.3	99.4	0.290	0.875	51.202
11	0.047	0.6	100.0	0.212	0.955	17.580
Females						
1	2.077	39.9	39.9	0.822	0.028	1369.788
2	1.062	20.4	60.3	0.718	0.087	938.212
3	0.733	14.1	74.3	0.650	0.179	660.234
4	0.464	8.9	83.3	0.563	0.311	449.100
5	0.340	6.5	89.8	0.504	0.455	302.719
6	0.238	4.6	94.3	0.438	0.609	190.273
7	0.117	2.2	96.6	0.324	0.754	108.424
8	0.087	1.7	98.3	0.283	0.842	65.946
9	0.065	1.3	99.5	0.248	0.915	33.920
10	0.025	0.5	100.0	0.157	0.975	9.573

the natural dispersion of *A. franciscana* via water birds should not be dismissed in the light of intentional inoculations carried out in industrial salt pans exploited around the Mediterranean basin (Amat *et al*. 2007). Concerning the Tunisian *Artemia* populations, the results obtained herein suggest that *Artemia* harvested from Sabkhet Halk El Menzel belongs to the invasive *A. franciscana*. Ben Naceur *et al*. (2009) prepared a previous check list on the distribution of *Artemia* in Tunisia and signalled the presence of this branchiopod in 21 sites, with the exception of the Sabkhet Halk El Menzel population. Furthermore, Sabkhet Halk El Menzel has the main marine aquaculture (fish farm) in Tunisia, in its southeast part. This private farm, concerned with the intensive production of sea bass and sea bream, includes a hatchery where commercial *Artemia* cysts are used to obtain brine shrimp nauplii for feeding fish larval stages.

TABLE 5

Standardized coefficients for the first two discriminant functions and correlated morphometrical traits. Abbreviations for morphometric characters are explained in Tables 2 and 3.

Males			Females		
Morphometrical variables	First function	Second function	Morphometrical variables	First function	Second function
tl	-0.656	-1.914	*tl*	2.673	1.592
al	0.612	1.251	*al*	-2.349	-2.621
wss	-0.452	0.984	*wb*	-0.432	0.308
wts	-0.328	-0.023	*lc*	-0.673	0.297
lc	0.695	-0.058	*nlc*	0.482	0.315
nrc	-0.395	-0.157	*wh*	-0.470	0.471
dby	0.217	0.389	*dby*	0.361	-0.218
dy	0.099	-0.232	*dy*	0.272	0.525
la	0.358	0.305	*la*	-0.071	-0.468
fk	0.348	0.228	*ra* (%)	0.550	1.003
ra (%)	0.072	-0.555			

In conclusion, all Tunisian *Artemia* populations, except that from Sabkhet Halk El Menzel, were identified as *A. salina*. The new *Artemia* population from Sabkhet Halk El Menzel can be considered to be the invasive *A. franciscana*. However, other methods must be used (such as monitoring of reproduction and genetic characterization) to confirm the taxonomic status of the *Artemia* population from Sabkhet Halk el Menzel. The time and origin of the introduction there of this exotic species are unknown but aquaculture activities should be suspected as having been responsible.

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