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Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in Iraq

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Abstract

History of the study of *Artemia* in Iraq goes back to 1921. But there are many unfortunate misidentifications of the species. The work before 2004 emphasized the use of the scientific name *A. salina*, which is limited to the Mediterranean basin. Two species of *Artemia* were recognized in Iraq: a bisexual populations; *A. franciscana*, which, so far, have only been recorded from the south of Iraq, and possibly occur in the middle of the country, and a parthenogenetic populations which were reported from the south and middle of Iraq. However, *Artemia* has not yet been encountered in North Iraq. The invasion of *A. franciscana* to the country is thought to be intentional.

Key Words: *Artemia,* biogeography, Iraq

Introduction

The spectacular brine shrimp *Artemia* is unique in the animal kingdom. It inhabits about 6oo natural and artificial habitats with extreme salinity, in the tropical and subtropical regions, and producing cysts, which are embryos "preserved" in a shell, at a rate of about 100 cysts every four days, which can be dormant for 15 years and remained viable (Sorgeloos *et al*., 1986; Lavens *et al*., 2000; Sorgeloos *et al*., 2001).

The importance of the newly hatched nauplii of *Artemia* had been recognized by Seale (1933) as live food for growing and culturing larvae of marine fish, and were widely and intensively used in fish hatcheries for it's nutritional value and largely favoured by the larvae of cultured species (Dhert *et al*., 1993). Larvae and adult *Artemia* are considered as the best food because of their high protein and unsaturated fatty acid contents and have energy value much higher than other live food used in aquaculture, like rotifers and algae (Léger *et al*., 1986). The use of *Artemia* has largely increased in aquaculture not only being considered as the best food but, in most cases, as the only available natural food for the larvae of cultured species (Kinne & Rosenthal, 1977). Moreover, it can also be used to transfer certain compounds like hormones, fatty acids and vitamins to the larvae of carnivore species which is unlikely to be done otherwise (Sorgeloos *et al*., 1998).

At present, larvae of *Artemia* are considered as one of the most important live food used in fish and shellfish cultures and in stimulation of sexual maturity of the prawn *Penaeus vannamei* (Naessens *et al*., 1997), besides of the possibility of using the decapsulated cysts in feeding fish larvae and prawns for their ease of digestion (Lim *et al*., 2002). For these reasons (economic interest) and more others, *Artemia* was considered as one of the most test-object, in various fields of biological sciences like genetic, histology, radiobiology, toxicology, molecular biology and ecology (Sorgeloos, 1980; Ward-Booth & Reiss, 1988).

Since early time, the beneficial role of *Artemia* in solar salt work was known, what was worth to hear the name brineworm. This branchiopod was first reported from Urmia Lake in 982 by an unknown Iranian geographer (Asem, 2008). The first illustration of the species was by SchlÖsser in 1756, on specimens collected from salt lakes near Lymington in London (Kuenen & Baas-Becking, 1938). Earlier Linnaeus (1778) named it *Cancer salinus,* then Leach (1819) transferred it to the present genus and named it *Artemia salina* (see Artom, 1931), and remained for several years as the only bisexual species, but many morphological variations lead to the distinction of different entities and variations under the influence of salt concentrations (Artom, 1931). Later, a parthenogenetic mode of reproducetion was reported in Villeneuve, southern France (see Barigozzi, 1980). However, the study of chromosomal number had largely progressed the work of systematic of *Artemia*. *Artemia* is considered as a good example of polyploids. Brauer (1893) (In Barigozzi, 1980) found that parthenogenetic *Artemia* from Capodistria (Adriatic Sea) had 84 chromosomes, whereas most of the bisexual species had 42 chromosomes, except *A. persimilis,* from Argentina had 44 (Barigozzi *et al*., 1984; Triantaphyllidis *et al*., 1998). However, some of the parthenogenetic populations had 42 chromosomes, but based on cross breeding tests and electrophoresis of DNA, it was found that they are reproductively isolated, although having the same chromosomes numbers (Clark and Bowen, 1976). Moreover, bisexuality is very rare among polyploids, whereas parthenogenetic populations exhibit diploid, polyploid and heteroploid (Badaracco *et al*., 1991).

Artemia is widely distributed in the world, it is found on every continent except Antarctica (Browne and Bowen, 1991), it is found abundantly in athalassohaline and thalassohaline environments at salinity ranges from 10 - 340 g/L (Post and Youssef, 1977, Agh *et al*., 2007). The wide disperse of *Artemia* is due to their ability to survive in widely different habitats as it tolerates great variations in temperature, salinity, dissolved oxygen and food availability (Castro *et al*., 2000).

Artemia is considered as a complex genus formed of several species and superspecies (groups of species and subspecies that are physiologically distinct and ecologically isolated) (Bowen *et al*., 1985). This genus contains seven bisexual species and

some parthenogenetic populations. The bisexual species were divided into two groups (Triantaphyllidis *et al*., 1997), the New World species (NW) which include:

1. *Artemia franciscana* Kellogg, 1906: found in north, middle and South America, also it invaded most of the world because it is used as food in aquaculture.
2. *A. persimilis* Piccinelli and Prosdocimi, 1968: found in Argentina only.

The Old World species (OW) is formed of:

1. *A. salina* (L. 1758): found in Europe and the neighboring countries.
2. *A. urmiana* Günther, 1899: found in Urmia Lake, Iran.
3. *A. sinica* Cai, 1989: found in China and the neighboring countries.
4. *Artemia* sp.: found in Khazakhstan (Pilla & Beardmore, 1994).
5. *A. tibetiana* Abatzopoulos *et al*., 1998: limited in occurrence to highland Tibet at an altitude of 5040 m (Yuan *et al*., 2007).
6. parthenogenetic population(s) of *Artemia*, from the Mediterranean basin, Africa, Asia and Australia (see Abatzopoulos *et al*., 2002; Asem *et al*., 2010).

The dispersion of *Artemia* occurred in two ways: natural dispersion in which the cysts are transported either by water birds where cysts cling to the legs and feathers of the birds or passes out with their feaces. It may also be transport by winds due to their small size and light weight (Vanhaecke *et al*., 1987). The other way is accidental inoculations (through hatchery effluents) or by deliberate interference of man through designed transportation as in the case of *A. franciscana*, which is transported for aquaculture either for production of biomass or the collection of cysts to be used in hatcheries of aquatic animal, as in Brazil (Persoone & Sorgeloos, 1980), Australia (Geddes, 1980), China (Triantaphyllidis *et al*., 1995), Egypt and Portugal (Triantaphyllidis *et al*., 1998). At certain times, *Artemia* was introduced to improve salt production from ponds exposed to sunlight (Sorgeloos *et al*., 1986). Moreover, *A. franciscana* was introduced seasonally to some Southeast Asian countries, as these regions are subjected to dry hot atmosphere at certain seasons of the year; these conditions are good for *Artemia* production. However, due to the particular climatic conditions of these countries (high rates of rainfall which decreases salt concentration during the wet seasons), the *Artemia* populations are not permanent and annual inoculations are required (Trian-taphyllidis *et al*., 1998) as in the case of Philippines (De Los Santos *et al*., 1980), Thailand (Tarnchalanuki and Wongrat, 1987).

Furthermore, the habitat inhabited by *Artemia* environment varies greatly according to climatic conditions from humid-subhumid (Vanhaecke *et al*., 1987) to arid and Saharan (Ben Naceur *et al*., 2009). Other environmental factors had varied between *Artemia* sites, in anionic composition. This variability induced to the reproductive isolation and physiological distinction (Triantaphyllidis *et al*., 1998). For instance, *A. franciscana* Kellogg, 1906 lives in water dominated by chloride salts as in the Great Salt Lakes, Utah state, in the coast of North, Middle, and South America. *A. monica* Verrill, 1869 lives in habitats dominated by carbonate salts as in Mono Lake, USA. Whereas *A. franciscana* lives in lakes having low concentrations of chloride salts and high potassium salts as in Nebraska State, USA (Browne & Bowen, 1991), also *Artemia* was found in Chaplin Lake, Canada, which had sulphate waters (Hammer *et al*., 1975). According to Browne and Bowen (1991), the diversification among these populations is due mainly to differences in lake water chemistry, and thus intolerance for each other's natural habitat.

Recently great effort was made to report on new *Artemia* populations. Our knowledge on the biogeography of this Anostracan is still very limited for many Asian, African and Eastern European regions (Triantaphyllidis *et al*., 1998). The present article aims at reporting on the distribution of *Artemia* in Iraq. This would add to our knowledge important information on the biogeography of this poorly known region of Asia.

Artemia in Iraq

The distribution of *Artemia* in Iraq is shown in Figure 1. However, the study of *Artemia* in Iraq is quite puzzling, the first record of *Artemia* was from saline pools at the neighborhood of Al Amarah, south of Iraq (Table 1) made by Gurney (1921) who identified it as *A. salina* var. *arietina*, Fischer-Daday.

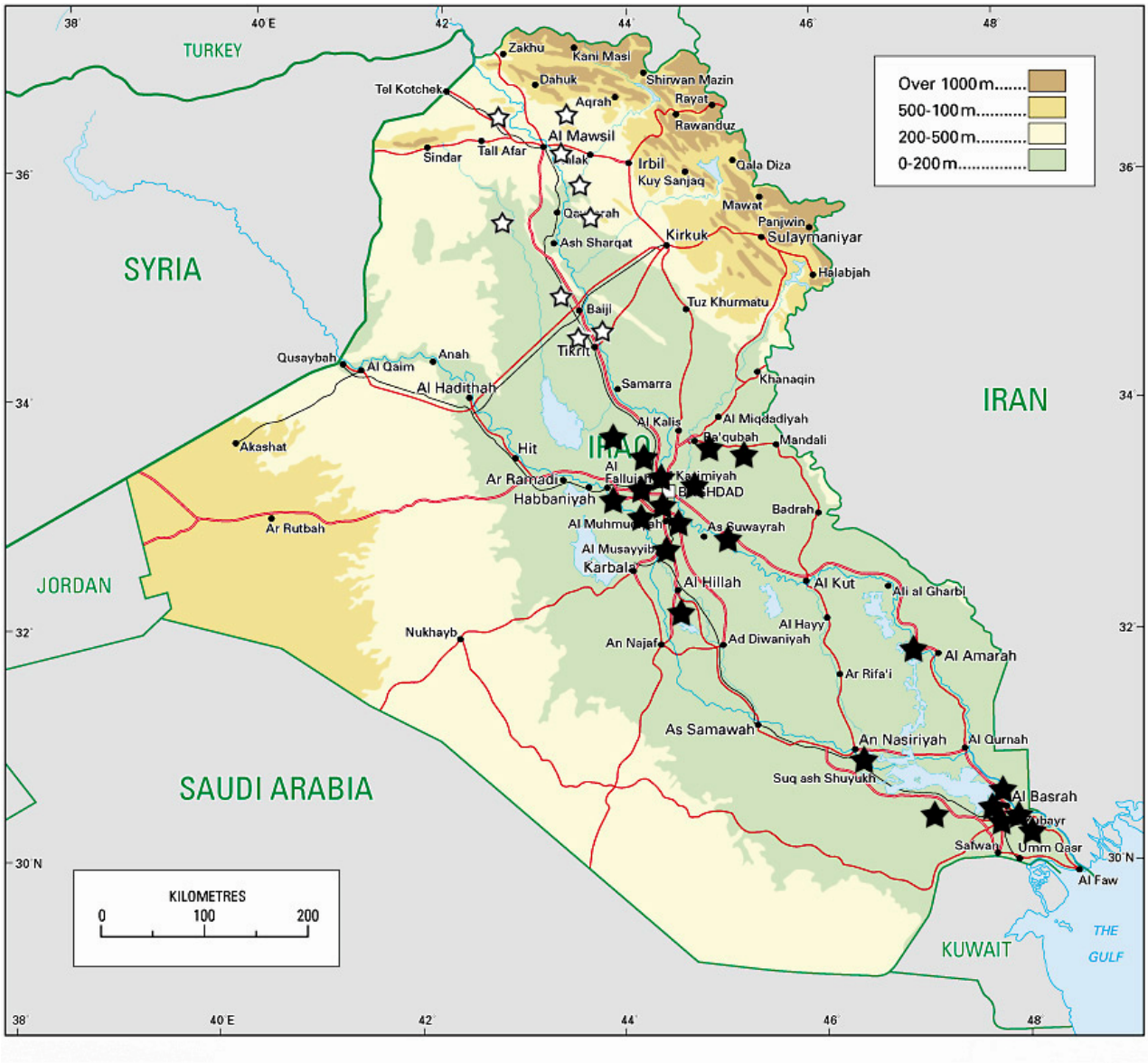

Fig. 1: Map of Iraq indicating the sites of the presence of *Artemia*, closed asterisks and the sites of the absence of *Artemia*, open asterisks.

Apparently he found only parthenogenetic females, but males were not encountered among the sampled specimens. Moreover, the same author reported that the number of setae on the furca branch is quite variable ranging from 12-2. Gurney's (1921) report of *A. salina* is questionable, although at that time this was the only known species of this genus. According to the recently accepted nomenclature, this specimen should be identified as parthenogenetic population of *Artemia* (see Asem *et al*., 2010).

AL-Uthman (1971) sampled the area between Baghdad in the north and Basrah in the south (33° 40 N; 33° 50; 47° 50 E; 44° W), in the period 1964-1968 and noticed the presence of *Artemia* during

Tab. 1: *Artemia* sites in Iraq

Locality	Latitude	Longitude	species	Ref.
Amara	31°49'37.71"N	47° 8'55.37"E	*A. salina*	1
Diayala Bridge	33°12'18.21"N	44°32'25.15"E	*A. salina*	2
Al-Mahmudiya	33° 31'33.78"N	44°22'15.62"E	*A. salina* P. population	2, 3
Al-Musayyab	32°46'37.92"N	44°18'7.18"E	*A. salina*	2
Al-Hillah	32°29'7.08"N	44°25'56.85"E	*A. salina* P. population	2, 3
Abu Ghraib	33°18'39.07"N	44° 9'26.66"E	*A. salina*	4
Garmat Ali	30°34'47.57"N	47°45'39.43"E	*A. salina* *A. salina* *Artemia* sp. *Artemia* sp. *Artemia* sp.	5 6 7 8 9
Garmat Ali	30°34'47.57"N	47°45'39.43"E	partheno. *Artemia*	3
Wanbi	30°30'22.09"N	47°47'13.94"E	*A. salina*	5
Saad Square	30°29'31.32"N	47°47'58.06"E	*Artemia* sp.	10
Al-Kibla	30°28'27.09"N	47°48'57.69"E	*A. salina*	11
Hamdan	30°27'52.10"N	47°54'10.12"E	*A. urmiana*	3
Al-Shuaibah	30°14'2.85"N	47°26'56.72"E	P. population	3
Al-Gazaizah	30°32'28.31"N	47°45'7.99"E	*A. urmiana* *A. franciscana*	3 12
Al-Nasiriah	31° 3'4.58"N	46°16'0.03"E	P. population	3
Al-Faluiah	33°20'42.72"N	43°45'25.19"E	P. population	3
Bany Saad	33°34'18.51"N	44°32'9.84"E	P. population	3
Baqubah	33°45'36.40"N	44°37'15.73"E	P. population	3
Rashdiya	33°24'53.19"N	44°21'32.21"E	P. population	12
Al-Latifiya	32°59'6.54"N	44°21'20.27"E	P. population	12
Suwairah	32° 48'17.03"N	45°4'46.85"E	P. population	12
Al-Toobah & Al-Nikhalah	30°32'44.54"N	47°44'29.61"E	*A. franciscana*	7

P. population: Parthenogenetic Population

Ref:. 1. Gurney (1921); 2. Al-Uthman (1971); 3. Maknoon (2001); 4. Khalaf *et al.* (1976); 5. Sarker *et al.* (1977); 6. Sarker & Salman (1986); 7. Abdullah (1995); 8. Ali & Abdullah (1995); 9. Ahmed & Salman (1999); 10. Sultan & Abdul-Sahib (1992); 11, Ahmed (1999); 12. Al-Obaydi (2005)

late November and early March. At Dayala Bridge and Al Muhmudiyah, large numbers of brine shrimp were collected in all seasons, except in 1967, when *Artemia* was absent. He called the species as *A. salina* (L.), yet he mentioned that the males were extremely rare and nearly absent. This indicates that *Artemia* population collected from this site should be identified as parthenogenetic population. He did not manage to get *Artemia* from Al Musayyib. From Babil(Al Hillah) few brine shrimp were collected during 1966 only. Moreover, no shrimps were encountered at Al Amarah in three trips during 1964-1968, whereas in Basrah, two males were obtained. Again he used the name *A. salina* to designate these brine shrimps. He concluded that because brine shrimp males were extremely rare, then *A. salina* in Iraq is mainly consists of parthenogenetic races with perhaps some geographical variations.

Khalef *et al*.(1976) carried out an ecological and biological study of a brine shrimp attributed to *A. salina* (L.) in two temporary saline ponds located on a road side about 10 km northwest to the college of Agriculture, Baghdad University, at Abu-Ghraib. They found that both ponds supported a good population of *Artemia*, at salinity of 30.2 ‰ and the population consisted of both adult parthenogenetic females and immature stages. On 1st July 1974, pond- I has no *Artemia*, whereas pond- II contained few specimen,

the salinity was 42 and 36‰ in the two ponds, respectively. On the 9th July 1974, only three specimens of *Artemia* were collected from pond- II (salinity 38 ‰). Afterward *Artemia* was completely disappeared. In the next year and on 26th February 1975 they collected few specimens of *Artemia* nauplii and metanauplii (salinity was 5.0 and 5.5 ‰ in the two ponds, respectively) and two weeks later they reported the presence of large number of females only from both ponds. Meanwhile, females bearing resting eggs were also collected during that period, and they concluded that the mean life span of *Artemia* was 50 and 78 days in ponds I and II, respectively. They reported the disappearance of *Artemia* from the two ponds when the salinity was 33.6 and 37.1 ‰, respectively, and noticed the presence of females bearing eggs and males of *Artemia* on 9th March 1975 which were only lasted for 2-3 weeks, unlike the parthenogenetic females. They emphasized that both parthenogenetic and sexual populations were observed during the period of investigation, mean-while, the sexual population lasted for only a short period when severe condition prevailed and both populations were termed as *A. salina*.

In an experiment on the eggs desiccation rate of the brine shrimp taken from the same pond mentioned above, at 10 km northwest of Abu-Ghraib, to the west of Baghdad, Khalaf *et al*. (1977) used the name *A. salina*. At the same time Zahid and AL-Haidary (1977 a, b) collected specimens of *Artemia* from the same ponds at Abu-Ghraib, and assigned them to *A. salina* but their work is rather histological. Moreover, in Basrah, Garmat-Ali (two ditches were sampled: 3.2 X 1.8m, depth 5-11cm and 13.8 X 6m, depth 4-9cm, both with muddy bottom) and Wanbi (a pool, 25X25m, depth 4-39cm, muddy substratum) and at nearly the same time, Sarker *et al*. (1977) designated their brine shrimp as *A. salina* without referring to the population structure, they noticed the availability of *Artemia* during winter (January-February) and found a direct relation between the abundance of brine shrimp and rainfall, and a reverse correlation with temperature and salinity, whereas the oxygen and pH had no relation with the abundance of the brine shrimp. However, in a laboratory experiment on brine shrimps (assigned to *A. salina*) collected from Garmat-Ali, Basrah, Sarker and Salman (1986) defined the lower salinity limit as 0- 7.5 ‰ and the upper limit as 350-375 ‰, with an optimum limit 57-157 ‰.

Later, Sultan and Abdul-Sahib (1992), while studying the growth and production of a population of the brine shrimp in Basrah recognized the problem inherent in the nomenclature of *Artemia* and assigned it to *Artemia* sp. They calculated the average life span of the species to be six weeks. They emphasized that most of the adults collected were females with only one male found during the whole sampling period, from 20th October 1990-12th January 1991, and they recognized six generations throughout the course of the study. Therefore, the population should be assigned to parthenogenetic *Artemia* population.

In an experimental work on the nutritional value of larvae and adult brine shrimp collected from Garmat-Ali, at the University campus, Abdullah (1995) also designated the species collected as *Artemia* sp., similarly the case in the study of Ali and Abdullah (1995) where the specimens (*Artemia* sp.) were taken from the same salt pond. In both studies the populations were bisexual (D. S. Mohammed, personal observation). It is suspected, therefore, that the species is *A. franciscana*. Ahmed (1999) in an experimental work on a bisexual population from Al-Kibla district, Basrah assigned the population to *A. salina*. At the same time Ahmed and Salman (1999) made a physiological study on a local strain of brine shrimp termed it as *Artemia* sp.

Later, Maknoon (2001) made an extensive survey of the zoogeography of the brine shrimp in the whole country. He divided Iraq into 3 sectors, the southern sector between latitudes 30° and 32°, the middle sector between 32° and 35°, and the northern sector between latitudes 35° and 38°.

I. The southern sector includes the following locations:

1. Hamdan
2. Al Shuaibah
3. Al Guzaizah
4. An Nasiriyah
5. Garmat-Ali

Monthly samples were taken from the first 3 sites

from March-May 2000, whereas in the last two sites, samples were taken once (adults and cysts), for comparison.

II. The middle sector includes the following locations:
6. Al Hillah (Babil)
7. Al Muhmudiyah
8. Al Fallujah
9. Badran
10. Bany-Saad
11. Ba'qubah

Biweekly samples were taken from the first three sites, whereas, the last 3 sites were sampled once for both adults and cysts for comparison.

III. The northern sector includes the following locations:
12. Baiji
13. Al Ojah
14. Perimeters of Tikrit City
15. Al Ayadhiah
16. Ba'doosh
17. Noran
18. Al Mawsil City
19. Shuabat Owanatt
20. Faidah and Teebah

In the southern sector, bisexual *Artemia* were present in Hamdan (site 1, a pool, 20 X 30m, depth 60cm, sandy substratum) and Al Guzaizah (site 3, a pool 20 X 20m, depth 75cm, sandy bottom). Parthenogenetic *Artemia* were apparently detected in Al Shuaibah (site 2, a pool, 10 X 18m, depth 15cm, muddy bottom), An Nasiriyah (site 4) and Garmat-Ali (site 5).

In the middle sector, parthenogenetic *Artemia* was found in Al Hillah (site 6, a pool, 8 X 35m, depth 130cm, sandy bottom), Al Muhmudiyah (site 7), Al Fallujah (site 8), Badran (site 9, a pool, 10 X 22m, depth 90cm, muddy substratum), Bany-Saad (site 10, a pool 6 X 20m, depth 120cm, sand bottom), and Ba'qubah (site 11).

However, in the northern sector (sites 12-20), Maknoon (2001) did not find *Artemia* there, and justified this to the scarcity of the temporary pools, and added that, if the pools are present they contain fresh water with salinities ranging from 0.2-0.5 ‰ which are not suitable for the existence of *Artemia.* Meanwhile, Maknoon (2001) reported that *Artemia* had appeared in sites 6 and 10 (middle sector) at the end of December and disappeared in May or early June, whereas in sites 2 and 3 (southern sector), the disappearance of *Artemia* was at the end of April and beginning of May. He recognized two species of *Artemia* in Iraq, the first was represented by two parthenogenetic strains; one diploid (2n=42) and the other is triploid (3n=63). Whereas the second species is formed of bisexual diploid populations. He followed the suggestion of Triantaphyllidis *et al*. (1998) designation of the parthenogenetic populations as: diploids parthenogenetic *Artemia* populations and triploids parthenogenetic *Artemia* populations but he called the bisexual populations as: *A. urmiana* Günther, 1899. Maknoon (2001) in designating the bisexual *Artemia* to *A. urmiana*, had referred to the statement of Abreu-Grobois & Beardmore (1982) in discussing the origin of the triploids from diploids and that the origin of parthenogenesis is morphometric and closely related to the line leading to *A. urmiana* (Beardmore & Abreu-Grobois, 1983). It is worth to mention here that *A. urmiana* is endemic to Lake Urmia, northwest Iran (Van Stappen, 2001), and lately it has only been recorded from Lake Koyashskoe, a hypersaline lake on the Black Sea, coast of the Crimean peninsula, Ukraine (Abatzopoulos *et al*., 2009). The specimens designated to *A. urmiana* by Maknoon (2001) were collected from Basrah: Hamdan and Guzaizah, the latter site is very close to the sampling site of Mohammed *et al*. (2010), which is less than 1 km across. In the meantime, *A. urmiana* is very much larger than the specimens collected by Maknoon; 15.48–17.22 mm for female *A. urmiana* (Triantaphyllidis *et al*., 1997), whereas females recorded by Maknoon (2001) were 9.30 ± 0.11 mm, and this size limit is closely similar to 8.44 ± 0.89 mm reported by Mohammed *et al*. (2010) for *A. franciscana.* Shortly after, Ahmed (2002) and Ahmed *et al*. (2004) attributed the bisexual *Artemia* collected from Basrah city and Al Kibla district, to *A. salina*.

Recently, Al-Obaydi (2005) in an experimental

approach, collected cysts from three sites around Baghdad, these were: Rashdiah- 20km northeast, Lattifia- 30km southwest and As Suwayrah- 50km southeast Baghdad. Samples were collected from late December 2000 to late December 2002. Most of the adults were parthenogenetic females; however, she encountered very few adult males and young individuals. Also she collected cysts from Basrah and assigned them to *A. franciscana* without any scientific basis.

Finally, in an experimental study, Abdullah (2007) collected specimens of brine shrimp from a saline pool (with an area of 30 X 50m, depth 100cm, with muddy substratum) close to the highway road, opposite to the University campus at Garmat-Ali, Basrah. The specimens were bisexual. Morphometric characters were measured according to Amat (1980) and Amat *et al*. (2004), and compared with features of *A. franciscana*, from various habitats in the World (Tables III and IV in Mohammed *et al*., 2010). The result indicated that there were clear cut differences between the different species. Moreover, there were variations in the morphological traits between the original description of *A. franciscana* from San Francisco Bay, and the population in Basrah and those of other populations in the World. These differences are most likely due to locality and culture conditions (Amat, 1980; Triantaphyllidis *et al*., 1997; Camargo *et al*., 2005). In order to clarify the identity of the bisexual *Artemia* from Basrah, the newly hatched nauplii were sent to CO: GENICS, a division of clinical Data Genome Express, France, for DNA sequencing, and comparing the present specimens with those from other parts of the World (Mohammed *et al*., 2010).

The result of DNA analysis showed that the bisexual population in Basarh is *A. franciscana*. Moreover, the relationship of the Basrah *Artemia* population with that of San Francisco Bay and with those of the same species from other localities in the World could be established (Figure 2). It is apparent from the cladogram that at least five groups of *Artemia* can be recognized, with extremely high bootstrap (98–100) between them. *A. salina* popul-ations from eleven habitats in the World were grouped together (bootstrap 100). *A. sinica* popula-tions from three localities in China were linked together (bootstrap 100). Whereas, *A. franciscana* population from the original locality of San Francisco Bay were clustered with that from Basrah and with those from two other regions with a bootstrap 100. However, *Artemia* sp. from Qi Xiang (Tibet, P.R. China) is forming a group linked to the main group of *A. urmiana* and to *Artemia* sp. (Pilla and Beardmore, 1994), from Kazakhstan (this is in support of the results of Hou *et al*., 2006) and to parthenogenetic *Artemia* populations. Furthermore, *A. persimilis* from Argentina is linked to *A. urmiana* and related species.

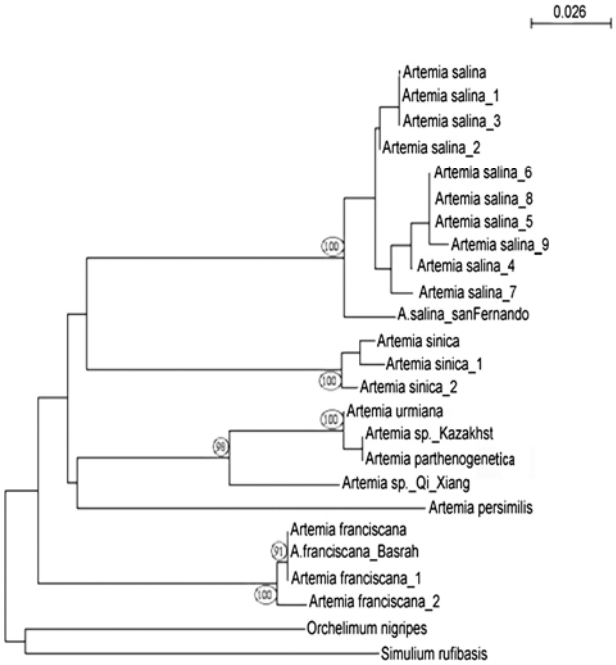

Fig. 2: A cladogram of *Artemia* species from 149 sites all over the world, based on DNA analysis carried out by CO:GENICS, a division of Clinical Data Genome Express, France.(reprinted with permission from Crustaceana, see Mohammed et al., 2010)

It can be concluded that the bisexual *Artemia* population present in Basrah are all should be assigned to *A. fransicana*. However, the identity of *Artemia* from other parts of Iraq still has to be confirmed and there are many places need further investigation to ensure the presence or absence of *Artemia* in them.

Regarding the process of invasion of *A. franciscana* to the south of Iraq and possibly to other places in the middle of the country, we think that the cysts of this species of brine shrimp have been

intentionally introduced to these areas, through human activity, by the practice of the ornamental fish trade, which was flourishing during the 1990s, as the countries bordering Iraq like Turkey, Iran, Syria, Jordan, Kuwait and Saudi Arabia have, so far, no reports on endemic populations of this species.

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