

-Scientific note-

## New survey of *Artemia* Leach, 1819 (Crustacea: Anostraca) in Tunisia: Report of three new populations

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**Abstract:** The presence or absence of *Artemia* in some natural habitat can have several explanations. *Artemia* cysts can be naturally dispersed over long distances by becoming attached to feathers or after surviving passage through digestive system of wading birds or being carried by wind. They can be also deliberately inoculated into salt pans for salt production improvement or for aquaculture purpose. Tunisian wetlands are composed by 254 sites, where temporary saline lakes: sabkha and chott represent 30% of totals surface of wetland. In last *Artemia* biogeographical study in Tunisia, this branchiopod was reported from 23 sites. However, in this work *Artemia* is reported from three new sites composed by two natural salt lakes (Sabkhat Kouria and Sabkhet Nadhour) in Kuriat Island and one artificial pond of seawater desalination plant in Ben-Guerdane. Reproduction mode study revealed that *Artemia* from Sabkhat Kouria and Sabkhet Nadhour are bisexual, while *Artemia* from Ben-Guerdane is parthenogenetic.

**Keywords:** *Artemia*, distribution, reproduction mode, Tunisia

### Introduction

The genus *Artemia* (Crustacea: Anostraca) consists of six bisexual species including *Artemia salina* (Linnaeus, 1758); *Artemia franciscana* Kellogg, 1906; *Artemia sinica* Cai, 1989; *Artemia. persimilis* Piccinelli and Prosdocimi, 1968; *Artemia urmiana* Günther, 1899; *Artemia tibetiana* Abatzopoulos *et al.*, 1998 and variety of parthenogenetic populations (diploid  $2n=42$ , triploid  $3n=63$ , tetraploid  $4n=84$  and pentaploid  $5n=105$ ) that are morphologically similar.

This crustacean is largely distributed, around the world, in inland and coastal natural or artificial salt lake. *Artemia* may inhabit chloride, sulphate, potassium or carbonate waters (Van Stappen, 1996) and combinations of more than two anions (Lenz, 1987). The distribution of *Artemia* is discontinuous, not all highly saline biotopes are populated with *Artemia*. Indeed, as *Artemia* is incapable of active dispersion, wind and waterfowl are most important natural dispersion vectors; floating cysts adhere to feet and feathers of birds, and when ingested they remain intact for at least a couple of days in digestive tract of birds. Consequently, absence of migrating birds is probably the reason why certain areas that are suitable for *Artemia* are not naturally inhabited by brine shrimp (Van Stappen, 1996). However, besides

natural dispersion of cysts, deliberate inoculation of *Artemia* in solar man made salters has been done (Van Stappen, 2002).

Brine shrimp *Artemia* was reported from Urmia Lake (Iran) in the first half of the 10th century AD (Asem and Eimanifar, 2016). However, first drawing of *Artemia salina* was illustrated in French edition (1756) by Schlosser's concerning *Artemia* samples harvested from Lymington Lake (destroyed today), England (Kuenen and Bass-Becking, 1938). Since then, many *Artemia* sites have been recorded and different taxa and morphological characteristics were attributed to genus. The first trial to list all known *Artemia* sites dates to 1922 when Artom reported 18 of them (Artom, 1922). Later, Stella (1933) and Barigozzi (1946) reported occurrence of *Artemia* populations in 28 and 29 sites respectively, spread over five continents. Number of *Artemia* sites has steadily increased and Persoone and Sorgeloos (1980) record 80 *Artemia* sites. The most recent investigations on *Artemia* biogeography have been published by Triantaphyllidis *et al.* (1998) with 505 *Artemia* sites and Van Stappen (2002) with 598 *Artemia* sites, distributed all over the world.

## **Artemia biotope in Tunisia**

Tunisian climate is characterized by long dry summers and short rainy winters, which generally implies a lack of a well-developed permanent surface hydrographic network. For this reason, ephemeral and temporary habitats are most common and representative natural superficial waters (Ben Naceur *et al.*, 2009b).

The brine shrimp *Artemia* is a cosmopolitan organism inhabiting hypersaline natural ecosystems (salt lakes and lagoons), as well as manmade solar salters built for commercial salt exploitation. Tunisian wetlands are composed by 254 sites, where temporary saline lakes: sabkha and chott represent 30% of total surface of wetland (Hughes *et al.*, 1997). Sabkha is a local Gulf Arabic word for a salt flat and its geological usage implies intra-sediment evaporate growth beneath a flat geomorphic surface with an elevation that is dictated by top of capillary fringe (Warren and Kendall, 1985); chott is a geological equivalent to continental sabkha in south of Tunisia. However, chott encompasses other related settings, including saline pans, distal alluvial fans, sand flats and dune fields (Warren, 2006).

## **Distribution of Artemia in Tunisia**

The presence of *Artemia* in Tunisia was first reported by Seurat (1921) and Gauthier (1928) in Sabkhet Ariana and Sabkhet Sidi El Hani, respectively. Later, Ben Abdelkader (1985), Sorgeloos *et al.* (1986), Romdhane (1994), Triantaphyllidis *et al.* (1998) and Romdhane *et al.* (2001) announced occurrence of *Artemia* populations in 10 other sites. More recently, after investigation of 49 saline lakes in Tunisia, Ben Naceur *et al.* (2009a, 2010) reported occurrence of brine shrimp *Artemia* in 22 sites located at different hydrogeographic zones. Last *Artemia* distribution list in Tunisia has been realised by Ben Naceur *et al.* (2012) and reported *Artemia* from 23 sites.

In this work, *Artemia* is reported from three new sites composed by two natural salt lakes (Sabkhat Kouria and Sabkhet Nadhour) in Kuriat Island and one artificial pond of seawater desalination plant in Benguerdan (Tab. 1, Fig. 1).

## **New Artemia sites description**

### **Kuriat Island**

The Kuriat (Qûrya) Archipelago lies in Khnis Bay, 16 km off Cape of Monastir, and includes two islets: Great Kuriat or Qûrya El Kabira (35°47'49"N, 11°02'01"E) and Small Kuriat or Qûrya Essaghira,

also called Conigliera (35°46'06"N, 11°00'26"E). Surface is 2.7 and 0.7 km<sup>2</sup> respectively. Both are characterized by a flat morphology, with a maximum elevation of less than 5 m a.s.l., and are formed by limestone substrate overlain by calcareous and sandstone crusts (Oueslati, 1995). In these islands *Artemia* was observed only in Great Kuriat Island in two sabkha:

- Sabkhet Kouria: situated in east part of island. Its length is about 0.5 km and its width is approximately 0.35 km. The most important source of water feeding Sabkha is rain water. During our visit, no other meso-zooplanktonic organism was observed in this site with *Artemia*.
- Sabkhet Nadhour: located in west part of island. Maximum length and width are 0.4 and 0.2 km, respectively. The most important source of water feeding Sabkha is rain water. During our visit, no other meso-zooplanktonic organism was observed in this site with *Artemia*.

### **Artificial pond of seawater desalination plant**

This pond is situated 7 km North of Ben-Guerdane city. The maximum length and width are approximately 0.42 km and 0.28 km, respectively. *Artemia* seems the only meso-zooplanktonic organisms living in this site.

At this three new *Artemia* sites, cysts were harvested from sabkha shore or pond, and adult specimens were collected with a plankton net (150 µm mesh size). Mating behaviour, presence or absence of males was recorded *in situ* and after laboratory culturing. For this purpose, nauplii obtained by cyst hatching were made to grow up in 2 L plastic containers, with 90 g L<sup>-1</sup> filtered and autoclaved sea water plus crude sea salt (Amat *et al.*, 2005). Temperature was maintained at 24°C, with a photoperiod of 16 h light/8 h dark. Animals were fed with unicellular algae *Dunaliella salina*. Results showed that *Artemia* from Sabkhet Kouria and Sabkhet Nadhour was composed by male and female individuals, with bisexual reproduction mode; however, *Artemia* harvested from artificial pond of seawater desalination plant of Ben-Guerdane city was only formed by females, with a parthenogenetic reproduction mode (Tab. 1).

Tab. 1: Site description, reproduction mode and taxonomical status of *Artemia* was recorded in Tunisia

Governorate	Site	Geographical Coordinates	Hydro-geographical zone	Type of habitat	Approximate surface (km <sup>2</sup> )	Reproduction mode	Taxonomical status	Identification Methods	Ref.
Tunis	Chott Ariana	36°55'38"N, 10°15'22"E	upper semi-arid	Coastal Salt Lake	39	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet Sijoumi	36°46'49"N, 10°07'16"E		Inland salt lake	28-30	B/P	<i>A. salina</i> Parthenogenetic pop. (4n)	Mc and Mm Mm and Al	1-5
Zaghuan	Megrine Saltwork	36°47'N, 10°14'E		Coastal Salt Lake	10	B	<i>A. salina</i>	Mc and Mm	1-3
Sousse	Sabkhet Korzia	36°24'47"N, 09°47'10"E		Inland salt lake	12	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet Assa Jriba	36°14'09"N, 10°26'20"E	lower semi-arid	Coastal Salt Lake	60	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet Halk El Menzel	36°00'40"N, 10°27'30"E		Coastal Salt Lake	13-19.5	B	<i>A. franciscana</i>	Mc and Mm	1-3, 6
	Sabkhet Sidi El Hani	35°37'43"N, 10°22'46"E		Inland salt lake	350	B	<i>A. salina</i>	Mc, Mm and Al	1-3
Monastir	Sahline Saltwork	35°45'58"N, 10°46'58"E		Coastal Salt Lake	12	B	<i>A. salina</i>	Mc, Mm, Al and DNA	1-3, 7
	Bkalta Saltwork	35°34'19"N, 11°01'39"E		Coastal Salt Lake	1.2	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet Moknine	35°36'20"N, 10°55'37"E		Inland salt lake	40	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet Kouria	35°47'47"N, 11°02'32"E		Coastal Salt Lake	0.17	B	?	-	-
	Sabkhet Nadhour	35°47'51"N, 11°01'35"E		Coastal Salt Lake	0.07	B	?	-	-
Mahdia	Sabkhet El Jam	35°09'29"N, 10°43'48"E	upper arid	Inland salt lake	30	B	<i>A. salina</i>	Mc, Mm and Al	1-3
Sfax	Sfax Saltwork	35°45'N, 10°43'E		Coastal Salt Lake	15	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet Boujmal	34°57'53"N, 10°24'04"E		Inland salt lake	50	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet Mcheguig	34°57'16"N, 10°02'28"E		Inland salt lake	24	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet Wadrane	34°26'03"N, 10°15'48"E		Coastal Salt Lake	40	B	?	-	-
Sidi Bouzid	Sabkhet Noueiel	34°27'28"N, 09°54'51"E	lower arid	Inland salt lake	110	B	<i>A. salina</i>	Mc and Mm	1-3
Mednine	Mhabau Saltwork	33°24'35"N, 10°51'20"E		Inland salt lake	3	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet El Melah	32°21'34"N, 10°55'22"E		Inland salt lake	150	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Zarzis Saltwork	33°24'48"N, 11°03'43"E		Coastal Salt Lake	1.7	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet Mnikhra	33°08'59"N, 11°20'09"E		Inland salt lake	17	B	<i>A. salina</i>	Mc, Mm and Al	1-3
	Sabkhet El Adhibet	33°05'42"N, 11°24'29"E		Inland salt lake	125	B	<i>A. salina</i>	Mc, Mm, Al and DNA	1-3, 7
	Ben-Guerdane*	33°11'52"N, 11°12'41"E		Artificial pond	0.12	P	Parthenogenetic pop. (?n)	-	-
Tozeur	Chott El Jerid	33°56'21"N, 08°26'50"E	upper Saharian	Inland salt lake	5000	B	<i>A. salina</i>	Mc and Mm	1-3
	Chott El Gharsa	34°09'07"N, 08°04'07"E		Inland salt lake	320	B	<i>A. salina</i>	Mc and Mm	1-3

\*Artificial pond, Mc: Morphologic study, Mm: Morphometric study, Al: Allozymic study and DNA: mitochondrial Cytochrome c Oxidase Subunit gene study, B: Bisexual; P: Parthenogenetic; pop: population; ?: Unknown. Ref.: 1: Ben Naceur et al. (2011); 2: Ben Naceur et al. (2013); 3: Ben Naceur (2010); 4: Ghiala and Charfi-cheikhrouha (2008); 5: Ghomari et al. (2012); 6: Ben Naceur et al. (2010); 7: Muñoz et al. (2008).

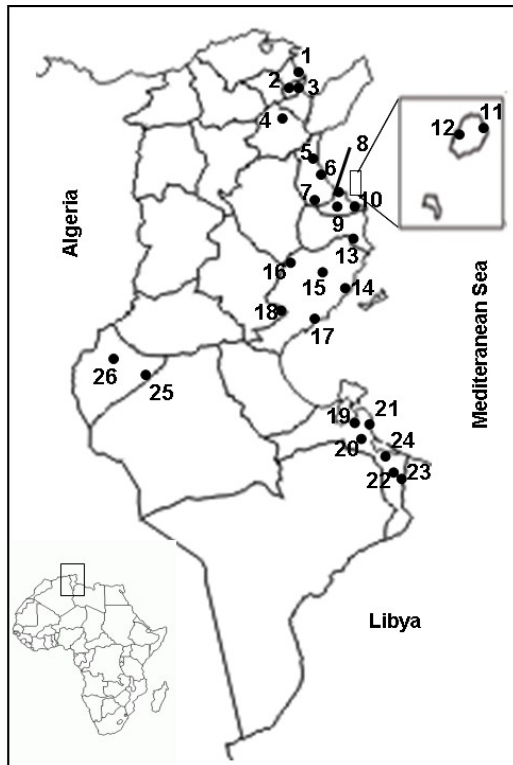


Fig. 1. *Artemia* site location in Tunisia. 1, Chott Ariana; 2, Sabkhet Sijoumi; 3, Megrine saltwork; 4, Sabkhet Korzia; 5, Sabkhet Assa Jriba; 6, Sabkhet Halk El Menzel; 7, Sabkhet Sidi El Hani; 8, Sahline Saltwork; 9, Sabkhet Moknine; 10, Bkalta Saltwork; 11, Sabkhet Kouria; 12, Sabkhet Nadhour; 13, Sabkhet El Jam; 14, Sfax Saltwork; 15, Sabkhet Boujmal; 16, Sabkhet Mcheguig; 17, Sabkhet Wadrane; 18, Sabkhet Noueiel; 19, Mhabeul Saltwork; 20, Sabkhet El Melah; 21, Zarzis Saltwork; 22, Sabkhet Mnikhra; 23, Sabkhet El Adhibet; 24, Artificial pond of Ben-Guerdane; 25, Chott El Jerid; 26, Chott El Gharsa.

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