ARTEMIA BIODIVERSITY IN ALGERIAN SEBKHAS

BY

MOHAMMED.S.GHOMARI¹), GHALEM. S. SELSELET¹), FRANCISCO HONTORIA²), and
FRANCISCO AMAT^{2,3})

¹) Laboratoire de Technologie et Nutrition. Université de Mostaganem. BP 300 27000
Mostaganem, Algeria

²) Instituto de Acuicultura de Torre de la Sal (IATS-CSIC). 12595 Ribera de Cabanes, Castellón,
Spain

³) Corresponding author; e-mail: amat@iats.csic.es

ABSTRACT

This paper summarizes the results of a study conducted to establish the survey and the morphological characterization of Artemia populations from seven sebkhas, one chott, and an inland salt lake in Algeria. Sebkhas and chotts are two different types of closed inland basins. Morphological characters together with a multivariate discriminant analysis applied to morphometric data obtained from adult specimens cultured under standard conditions were used to differentiate the species and strains. The bisexual species A. salina (Leach, 1819) is present in seven of these saltmarshes, but frequently co-occurs with diploid and tetraploid parthenogenetic strains. The biodiversity pattern of the populations of Artemia in Algeria appears to be similar to that found in the rest of the western Mediterranean.

RESUME

Le présent document résume les recherches visant la localisation et la caractérisation morphologique des populations d'Artemia trouvées dans sept sebkhas, un chott et dans un lac salé en Algérie. Sebkhas et chotts sont deux différentes classes de bassins fermés d'intérieur. Une analyse discriminante multivariée appliquée aux paramètres morphométriques obtenus des individus adultes cultivés sous conditions standardisées a permis de différencier les espèces et souches. L'espèce bisexuelle A. salina est trouvée dans sept des marais salants, mais fréquemment en présence des souches parthénogénétiques diploïdes et tétraploïdes. Le profil de la diversité biologique des populations d'Artemia d'Algérie est similaire à celui retrouvé au niveau de la Méditerranée ouest.

INTRODUCTION

The brine shrimp Artemia (Branchiopoda, Anostraca) is cosmopolitan and includes extreme halophilic species and strains. Brine shrimps live in several hundreds of locations (Van Stappen, 2002) on the five continents where they, thrive in coastal solar salterns as well as in inland salt lakes (Triantaphyllidis et al., 1998; Muñoz & Pacios, 2010). Brine shrimp is a complex of both bisexual species and parthenogenetic strains. There are six bisexual species and a variety of parthenogenetic strains of diverse ploidy, as follows:

Two of these bisexual species are autochthonous in the Americas, with A. franciscana (Kellogg, 1906) spreading in the entire American continent (Hontoria & Amat, 1992; Triantaphyllidis et al., 1998; Abatzopoulos et al., 2002a), and A. persimilis (Piccinelli and Prosdocimi, 1968) present only in the American South Cone (Cohen et al., 1999; Gajardo et al., 2004). The bisexual species A. salina is present in the Mediterranean basin, being common in the African continent from the north to the south (Muñoz et al., 2008) where it is supposed to co-occur with parthenogenetic strains (diploid and tetraploid) (Amat, 1983). The rest of the other bisexual species native in the Old World are distributed in Eurasia. A. urmiana (Günther, 1890)

is present in Lake Urmia (Iran) and in Lake Koyashskoe (Crimea, Ukraine) (Abatzopoulos et al., 2009), A. sinica (Cai, 1989) is mostly present in China (Van Stappen et al., 2002), and A. tibetiana (Abatzopoulos et al., 1998) is located in several Tibetan plateau salt lakes (Abatzopoulos et al., 2002b).

All Artemia species and strains can reproduce in two ways, ovoviviparously, producing live nauplii, and oviparously, producing encysted cryptobiotic embryos, this mode operating under unfavourable environmental conditions. The oviparous mode assures the continuity of the populations in the form of floating cysts, either in the brine or stranded along the banks of the hypersaline ecosystem. Later on, when the environment becomes appropriate again, the cysts allow resume embryonic development and a living population starts again.

Algeria is endowed with a climate suited for the presence of plenty of biotopes fit for the development of Artemia populations (Vanhaecke et al., 1987). Among these biotopes, we must consider the sebkhas and chotts. According to the terminology accepted by North African geomorphologists a sebkha is a closed inland basin, with impermeable bottom, which accumulates soluble and insoluble products provided by water inflows from surrounding large hydrological areas. It can be filled up and flooded during short heavy rain periods, but becomes dry shortly after because of evaporation. A chott usually does not become completely dry because relies on underground water inflow. Their aquatic fauna is poorly known for a variety of reasons, usually the difficulty of access and drought.

Between the northern Algerian High Plains and the Mediterranean shore there is a vast complex of saltwater sebkhas. The Oran - Relizane complex in the west, and diverse sebkhas in the localities of Oum Bouagui, Batna and Khenchela to the east, including Setif, Garaet et Tarf and Ezzemoul, are the most important. Going to the south, the Goléa Mnea salt lake, located in the wilaya (province) of Ghardaia deserves special attention.

Sebkhas sometimes are exploited for salt production. During the winter rainy season,

mainly on November and December, usual rains cause some flooding due to their great hydrological catching area. The upper salt bed dissolves producing brines that allow the development of Artemia populations. These brines attain high salinities during the spring. In April salinities are higher than 190 gL^{-1} and salt is harvested in several crystallising ponds during the early summer period (May - June). From June to September the sebkha shows a complete dry salt surface without water (ENASEL, Algerian National Salt Enterprise, personal communication). Living Artemia populations are rarely found during this period until the next rainy season when water provokes cyst hatching and a provision of brines fit for the recruitment and growth of a new population.

Despite the abundant and wide distribution of these hypersaline areas, very little was known about the biodiversity of Artemia populations in them. In their first biogeographical work on world Artemia spots, Vanhaecke et al. (1987) listed no more than four Algerian Artemia sites. A first annotated check-list of large branchiopods of Algeria and an approach to the findings of A. salina can be found in Samraoui et al. (2006) with personal references to its presence in the Bethioua saltworks, in the Ezzemoul sebkha and in the chotts Merouane and Melghir in the Southern High Plains (Sahara). These authors also reference previous records due to Blanchard & Richard (1890), Gauthier (1928), Haddag (1991), Zemmouri (1991), and Beladjal et al. (1995). A limnological report worked out by McCarraher (1972) also quotes the presence of A. salina in two inland hypersaline Algerian sites. More recently, Artemia was cited by Kara et al. (2004) and by Amarouayache et al. (2009a, b) in the chott of Merouane. All these reports are referring the exclusive presence of A. salina, probably due to the lack of information related with the actual species biodiversity recently unveiled for the genus.

Morphometry has been chosen in this study as a tool to identify the different species and strains that are present in the sampled sites. It could be the easiest available methodology to be applied by the Algerian researchers in order to improve the knowledge of Artemia biodiversity

through an exhaustive prospection of hypersaline ecosystems in Algeria. Several authors working on this topic in North African countries (Tunisia, Algeria, Morocco, Egypt, etc.) have already used similar morphological and morphometric methods. In our case, the results obtained with these methods have been confirmed by using reference Mediterranean species and populations that were taxonomically and systematically described and employed in multidisciplinary approaches, including molecular biology studies (Mura et al., 2005, 2006; Muñoz et al. 2008, 2010b). In addition, morphometry is a valid first approach to survey the Artemia biogeography of an area; however there is no doubt that other complementary methods should be applied to obtain a complete picture of the species and population distribution.

The objectives of this study focus on the biogeographical localisation of hypersaline biotopes fit for the development of Artemia populations in the Northern Algeria. The study also aims to updating the actual biodiversity of Artemia populations found. Thus, it tries to discern on the real abundance of bisexual A. salina and/or to unveil the previous misled presence of parthenogenetic populations. The methods employed comprise the species differentiation allowed by morphologic (Amat 1980; Mura and Brecciaroli 2004) and morphometric studies, supported by a large morphometric data base on Mediterranean Artemia populations available at the Instituto de Acuicultura de Torre de la Sal (Hontoria & Amat, 1992).

MATERIAL AND METHODS

Samples of mixed Artemia cysts and debris floating on brines, or accumulated along the banks, were obtained during several surveys run between 2005 and 2009 on the hypersaline ecosystems cited in table I. Different samples of living Artemia specimens preserved in 70° ethanol were also collected when present. Debris and cysts were floated in brine in the laboratory, sieved through different mesh sizes (1 mm, 0.5 mm, 0.3 mm and 160 µm) and finally cysts were cleaned with standardized methods (Vanhaecke & Sorgeloos, 1980), oven dried at

39° C and vacuum stored at 4° C.

Culture conditions

Cysts were hatched under standard conditions in 1 L glass tubes, filled with filtered 35 gL⁻¹ seawater (pH 8) , at 28°C with continuous illumination and aeration. Nauplii obtained were grown up in transparent plastic cylindrical containers filled with 70–80 gL⁻¹ filtered brine (seawater plus crude salt), on a mixed microalgal diet of Dunaliella salina (Teodoresco, 1905) and Tetraselmis suecica (Butcher, 1959) balanced to the same salinity. The temperature was maintained at 24 ± 1°C under a mild aeration from the bottom and a light 12D : 12L regime. The culture was monitored every 3-4 days to assess the size and developmental stage of shrimps. When young and adult individuals became apparent, some of them were sampled randomly, and anaesthetized in a Petri dish with seawater and the addition of 5 mL chloroformed fresh water. They were observed under the microscope in order to verify the presence of males and females, and the presence of females belonging to one or more different species or strains (bisexual as well as parthenogenetic) according to the morphological traits defined by Amat (1980, 1985), and Mura & Brecciaroli (2004). This previous selection allowed a first approach to the specific composition of the Artemia populations obtained from the hatched original cysts. On the basis of the abundance of enough adult specimens (30) to develop a morphometrical study, they were maintained in the same culture conditions until more than 50% of the females had attained full ovisac development and produced the first offspring batch (living nauplii or cysts).

Multivariate morphometry of large samples

The hatching percentages for these samples ranged between 70 and 80%, and the survival to adulthood was over 50%.

Once attained adulthood, random samples of 30 females were removed from each culture,

anesthetized and measured under a dissecting microscope provided with a micrometer eyepiece (Hontoria & Amat, 1992). The following 13 morphometric parameters were examined: total body length (TL), abdomen length (AL), width of the third abdominal somite (AW), head width (HW), maximal distance between the compound eyes (ED), eye diameter (ES), length of the first antenna (ANL), length of the furca (FL), number of setae on the right and on the left branch of the furca (LS, RS), abdomen length / total length ratio (ATL), and ovisac width (OW).

The discriminant analysis is a multivariate procedure that provides a series of variables, which are linear functions of the morphometric variables studied. These functions maximise the separation among different groups of observations defined a priori. In the analysis, the separation criterion is the origin of each population, and the presence of diverse populations for the same species or strains as references reinforces the discriminating capacity of the analysis. For this reason, a number of other A. salina (4), diploid parthenogenetic (6) and tetraploid parthenogenetic (5) Artemia strains native from several countries around the Mediterranean basin and properly referenced, together with two A. franciscana populations (table II), were retrieved from the morphometric database of the Instituto de Acuicultura de Torre de la Sal (IATS-CSIC) and also included in the analysis. The morphometric data present in this database have been obtained from individuals chosen and cultured in the same way described above in this study. The full data matrix was subjected to multivariate discriminant analysis using the statistical package SPSS for Windows version 17.0 (SPSS Inc. Chicago, Ill, USA) (Hontoria & Amat 1992) in order to characterise the Algerian Artemia populations (bisexual and parthenogenetic), and to establish relationships with the other Mediterranean parthenogenetic and bisexual strains or species.

Morphology of small samples

Some very short cyst samples (less than 100 mg dry cysts after cleaning) from Bethioua,

Oran and Setif sebkhas, most probably produced in late winter, showed hatching efficiencies about 5 % and the few nauplii obtained scarcely attained adulthood (30 % survival approximately) under the standard culture conditions. Not enough adult specimens were obtained to apply the multivariate analysis. The few ones attaining adulthood, together with those sampled mainly in winter in these biotopes and preserved in ethanol, were compared with the morphological characters displayed by cultured adult specimens obtained from the Relizane, Ezzamoule and Garaet et Tarf sebkhas, according to the morphological characterisation described by Amat (1980), Mura (1990), Triantaphyllidis et al. (1997) and Mura & Brecciaroli (2004). Therefore, instead of the utilisation of the discriminant analysis method, the approach based on a visual identification (optical microscopy) following the morphological characters described by Amat (1980) and Mura & Brecciaroli (2004) was used to identify the presence of A. salina winter populations in those biotopes.

RESULTS AND DISCUSSION

Original samples monitored in the wild, as well as populations cultured in the laboratory after hatching original cyst samples, showed the presence of different Artemia populations which, according to morphological traits cited above (Amat 1980, 1985; Mura and Brecciaroli 2004), agreed with the occurrence of A. salina and both, diploid and tetraploid, parthenogenetic Artemia strains.

Other zooplankters were present, especially during the periods characterised by low salinities, in winter after the rainy season and in the early spring, among them the rotifer Brachionus plicatilis (Müller, 1786), the harpacticoid copepod Cletocampus retrogressus (Schmankevitch, 1875), or several cladocerans like Chirocephalus salinus (Daday, 1910) and Ch. diaphanus (Desmarest, 1823) (Alonso, 1996; Samraoui et al., 2006). Sebkha samples showed a certain diversity of aquatic invertebrate propagules together with Artemia cysts. It was possible

to distinguish cladoceran ephyra, those from Ch. salinus and Ch. diaphanus, as well as other large branchiopod cysts, likely Branchinecta spinosa (Milne Edwards, 1840) (Alonso, 1996; Ben Naceur et al., 2009). All the populations studied, either cultured under laboratory conditions after hatching original cysts or observed from preserved samples collected in the wild, showed more than one species or strain. The only exceptions were the samples obtained from the inland Goléa Mnea salt lake, and that from the Garaet et Tarf sebkha, which always produced a tetraploid parthenogenetic Artemia and the bisexual A. salina, respectively, as supported by the results of the discriminant analysis and the visual observations in the samples that could not be included in the multivariate procedure.

The results obtained from the multivariate analysis applied to the female morphology of the Algerian Artemia populations studied, together with those used as references, when the origin of the different populations was used as a separation factor, are displayed in fig. 1. The multivariate analysis produced twelve discriminant functions. The first four of these functions give a cumulative variance explanation percentage of 85.0 %. The first 11 functions calculated were highly significant ($P < 0.001$). The analysis allows to correctly classifying the 86.9% of the observations in their original population groups. Moreover, when the species/parthenogenetic ploidy is used as separation criterion in the discriminant analysis the observations are classified correctly in the 98.7% of the cases. The females pertaining to A. salina were grouped properly in the 98.8% of the cases studied, those diploid parthenogenetic in the 98.9%, the tetraploid parthenogenetic in the 98.1%, and the reference observations from A. franciscana are all them classified correctly.

The morphometric characters that were highly correlated with the first discriminant function were the length of the first antenna, the distance between compound eyes and the total length, while those related to the second function were the length of the furca and the number of setae on furca. Fig. 1 shows the centroids that can be considered as representative of each

population according to the significance levels of the discriminant functions that are used to calculate them. The groupings unveiled correspond to the parthenogenetic diploid and tetraploid strains, to A. salina and to A. franciscana for the female individuals studied.

The females of the Algerian populations studied in this work are placed by the discriminant analysis among the centroids pertaining to the native bisexual A. salina or any of the parthenogenetic groups of different ploidy level. Then it is possible to accept that the biodiversity of the Algerian Artemia populations is congruent with the biodiversity found for the rest of the western Mediterranean countries (Amat et al., 1995a). In the Iberian Peninsula, there is a frequent co-occurrence of the bisexual A. salina and the diploid parthenogenetic strain populations in the seashore saltmarshes and saltworks, with a marked seasonality. While the bisexual species shows a thermal preference for the low temperatures prevailing during the late winter and early spring period, the diploid parthenogenetic strains develop their populations during the spring and summer period (Amat, 1981). This is the same situation observed for the Algerian populations, at least in the northern area where sebkhas are found and where the mean thermal range varies between 10°-12° C in winter and between 21°-24° C in summer (ONM, Algerian National Office of Meteorology).

The morphologic comparison of the small samples obtained from Oran, Bethioua and Setif sebkhas, not included in the morphometric study, verified the presence of A. salina in these sites during late winter and spring. Therefore, instead of the utilisation of the discriminant analysis method, the approach based on a visual identification (optical microscopy) following the morphological characters described by Amat (1980) and Mura & Brecciaroli (2004) was enough to identify the presence of winter populations of A. salina in those biotopes. In summary, A. salina and the diploid parthenogenetic populations are present in all sebkhas and in the Chott Melghir, with the exception of Garaet et Tarf where only A.salina appeared. The tetraploid parthenogenetic population is absent in the Ezzamoule, Melghir and Oran sebkhas, but is

exclusive for the Goléa salt lake.

Until recently, and even today, biogeographical and biodiversity studies dealing with the distribution of Artemia species in the North of Africa have been limited to the identification of populations belonging to the bisexual A. salina, while the presence of parthenogenetic strains has been scarcely known (Amat 1983; El Bermawi et al., 2004). Our results confirm that the biodiversity pattern of the populations of Artemia in Algeria is similar to that found in the rest of the western Mediterranean.

The distribution of Algerian tetraploid parthenogenetic populations is quite astonishing. In the Iberian Peninsula, they are spread mainly in inland biotopes, while they are hardly present in the seashore saltworks. The inland hypersaline ecosystems are characterised by a thermal regime quite extreme, with cold winters and warm summers, but showing plentiful populations in summer, during the short period of salt production, at least in the case of inland solar saltworks (Amat et al., 1995a). The results for the Algerian populations do not match likely with this schema, with the exception of the population found in the inland Goléa Mnea salt lake. The mean thermal range for this area, in the northern Sahara, varies between 15°-18° C in winter and 40°-45° C in summer (ONM). The abundance and co-occurrence of the tetraploid parthenogenetic strain in the sebkhas cyst samples could be explained by the high salinities and temperatures attained in these saltmarshes during the short period of brine availability in the early summer, in the sebkha itself as well as in the exploited saltworks, especially in Bethioua and Relizane.

Crossbreeding experiments performed by Baratelli et al. (1990) suggested the conspecificity of the Mediterranean populations, from Italy as well as from the north of Africa, and SEM studies from Mura (1990) reinforced this conspecificity. Just recently it has been demonstrated that A. salina populations show substantial genetic diversity and a strong phylogeographical structure, with high regional endemisms in the Mediterranean Basin, the

Iberian Peninsula, the north of Africa and Italian islands (Sardinia and Sicily) (Muñoz et al., 2008). An accurate knowledge on the biodiversity of bisexual A. salina populations in Africa is urgent, especially due to the lack of information known from this continent. A. salina presence has been reported only in the Mediterranean area and in South Africa (Amat et al., 1995b), but the substantial genetic diversity and the presence of marked regional endemisms showed by Muñoz et al. (2008) for this species in the African continent allowed these authors to claim for a specific status for the South African bisexual populations.

This lack of biodiversity information is relying on the supposed exclusivity of A. salina presence in the north of Africa. In fact, all the publications reporting the presence of Artemia populations in Algeria, cited in the introduction to this study, are being referred exclusively to A. salina. Even today, the efforts developed by several authors in the north of Africa are likely focused to unveil the presence and distribution of the bisexual species A. salina, without any mention to the finding of other strains. This is the case for Morocco (Sadkaoui et al., 2000) for Libya (El Magsodi et al., 2005) as well as for Tunisia (Ben Naceur et al., 2008). However, there are a few previous studies, rather apart in time, confirming the presence of some clonal or parthenogenetic (diploid) Artemia populations in this area. This is the case of Larache salterns in Morocco (Amat, 1983) and also that of the Egyptian salterns from El-Max and Borg El Arab as well as the Qarun lake (El Bermawi et al., 2004) located at both longitudinal extreme ends of the North African Mediterranean shore. The great abundance of parthenogenetic Artemia populations in the Mediterranean countries (Amat et al., 1995a; Mura et al., 2005; Baxevanis et al., 2006) must have its congruent correspondence in the southern countries of the same geographical area or basin.

In the literature it is reported that parthenogenetic species or strains use to show widespread haplotypes (Law & Crespi, 2002), what suggests faster dispersal rates than their relatives or co-occurring bisexuals. For example, clonal lineages of parthenogenetic Daphnia are

widely distributed (Weider et al., 1999). In addition, several studies suggest that asexuals are better and faster colonizers, and that they are usually spread on a greater niche breadth than co-occurring bisexuals (Browne & MacDonald 1982; Browne et al., 1988; Triantaphyllidis et al., 1995; Muñoz et al., 2010). In the case of parthenogenetic Artemia, this colonising capability is supported by their physiological and ecological tolerances, as well as their genetic structure (ploidy level: automixis or apomixis, types of reproduction: ovoviviparity or oviparity) (Zhang & Lefcort, 1991; Baxevanis et al., 2006). These arguments are sufficiently confirmed today, together with the demonstrated presence of parthenogenetic Artemia populations in the whole Old World, to explain the presence of these strains in northern Africa.

It is worth to mention also that there is a generalized invasion of the western Mediterranean by the exotic species A. franciscana (Amat et al., 2005, 2007; Mura et al., 2006). Its presence has also been reported in diverse African countries, in the Bou Areg sebkha (Morocco) (Amat et al., 2005), in Fundisha salterns (Kenya) (Muñoz & Pacios, 2010), in Ifaty salterns (Madagascar) (Kaiser et al., 2006; Muñoz & Pacios, 2010), and in the Cape Verde archipelago (Ballell, 2006; Muñoz & Pacios, 2010). Consistently with a man-mediated introduction or dispersion via shorebirds (Green et al., 2005) it is possible to expect that viable A. franciscana cysts could eventually reach the coastal sebkhas or saltworks in the North African Mediterranean region. For this reason, it is worthy to have developed a previous task of identification and geographical distribution of the native Artemia populations. This would provide a basic knowledge useful to discern the presence of different species or strains, primarily based on a morphological differentiation. This knowledge would allow assessing the presence of original populations, or the unexpected arrival of the invasive species, preventing in this case its expansion, or facilitating the most effective measures for the conservation of the autochthonous species and strains.

ACKNOWLEDGEMENTS

This study was partially financed by the Spanish Ministry of Research and Innovation projects CGL2005-02306, CGL2008-03277, and by a Spanish Agency for International Cooperation and Development (AECID) project (A/017377/08). M. Ghomari was supported by a fellowship of the University of Mostaganem (Algeria) and by the project A/017377/08.

REFERENCES

- ABATZOPOULOS, T.J., J.A. BEARDMORE, J.S. CLEGG & P.SORGELOOS, 2002a. Artemia: Basic and Applied Biology: 1-286. (Kluwer Academic Publishers. Dordrecht, The Netherlands)
- ABATZOPOULOS, T.J., I. KAPPAS, P. BOSSIER, P. SORGELOOS & J.A. BEARDMORE 2002b. Genetic characterization of Artemia tibetiana (Crustacea, Anostraca). Biological Journal of the Linnean Society, **4**: 139-150.
- ABATZOPOULOS, T.J, F. AMAT, A.D. BAXEVANIS, G. BELMONTE, F. HONTORIA, S. MANIATSI, S. MOSCATELLO, G. MURA & N.V. SHADRIN. 2009. Updating geographic distribution of Artemia urmiana Günther, 1890 (Branchiopoda, Anostraca) in Europe: An integrated and interdisciplinary approach. International Review of Hydrobiology, **94** (5): 560-579.
- ALONSO, M., 1996. Crustacea, Branchiopoda. In : M.A. Ramos et al (eds.): Fauna Ibérica, **7**: 1-486. Museo Nacional de Ciencias Naturales (Consejo Superior de Investigaciones Científicas, Madrid, España).
- AMAROUAYACHE, M., F. DERBAL & M.H. KARA. 2009a. Biological data on Artemia salina (Branchiopoda, Anostraca) from Chott Marouane (northeast Algeria). Crustaceana, **82** (8): 997-1005.

- , 2009b. The parasitism of Flamingolepis liguloides (Gervais, 1847) (Cestoda, Hymenolepididae) in Artemia salina (Crustacea, Branchiopoda) in two saline lakes in Algeria. *Acta Parasitologica*, **54** (4): 330-334.
- AMAT, F., 1980. Differentiation in Artemia strains from Spain. In: G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers (eds.), *The brine shrimp Artemia*. **1**: Morphology, Genetics, Radiobiology, Toxicology: 19-39 (Universa Press .Wetteren Belgium).
- , 1981. Zygogenetical and parthenogenetical Artemia in the Cadiz sea side salterns. *Marine Ecology. Progress. Series*, **13**: 291-293.
- , 1983. Diferenciación y distribución de las poblaciones de Artemia de España. VI. Biogeografía. *Investigación Pesquera*, **47** (2): 231-240.
- , 1985. Biología de Artemia. *Informes Técnicos del Instituto de Investigaciones Pesqueras*, **126-127**.
- AMAT, F., C. BARATA, F. HONTORIA, J.C. NAVARRO & I.VARO, 1995a. Biogeography of the genus Artemia (Crustacea, Branchiopoda, Anostraca) in Spain. *International Journal of Salt Lake Research*, **3**: 175-190.
- AMAT, F., C. BARATA & F.HONTORIA, 1995b. A Mediterranean origin for the Veldrif (South Africa) Artemia Leach population. *Journal of Biogeography*, **2**: 49-59.
- AMAT, F., F. HONTORIA, O. RUIZ, A. GREEN, M. SANCHEZ, J. FIGUEROLA & F. HORTAS, 2005. The American brine shrimp as an exotic invasive species in the Western Mediterranean. *Biological Invasions*, **7**: 37-47.
- AMAT, F., F. HONTORIA, J.C. NAVARRO, N. VIEIRA & G. MURA, 2007. Biodiversity loss in the Genus Artemia in the Western Mediterranean Region. *Limnetica*, **26**: 177-194.
- BARATELLI, L., V. VAROTTO, G. BADARACCO, G. MURA, B. BATTAGLIA & C. BARIGOZZI, 1990. Biological data on the brine shrimp Artemia living in the Italian saltworks. *Atti Accademia Nazionale dei Lincei. Memorie*, **1**: 45-53.

- BALLELL, L, 2006. Caracterización de las poblaciones de Artemia (Crustacea, Branchiopoda) presentes en la República de Cabo Verde. Tesis del Master Internacional en Acuicultura: 1-50. (Universidad de Las Palmas de Gran Canaria, España).
- BAXEVANIS, A. D., I. KAPPAS & T. J. ABATZOPOULOS, 2006. Molecular phylogenetics and asexuality in the brine shrimp Artemia. *Molecular Phylogenetics and Evolution*, **40**: 724-738.
- BELADJAL, L., J. MERTENS & H.J. DUMONT, 1995. An analysis of the setation pattern of the limbs in Anostraca (Crustacea); using the Algerian species as an example. *Hydrobiologia*, **298**: 263-280.
- BEN NACEUR, H., A. BEN REHEB JENHANI, M. EL CAFSI & M. ROMDHANE, 2008. Determination of biological characteristics of Artemia salina (Crustacea, Anostraca) population from Sabkhet Sijoumi (NE Tunisia). *Transitional Waters Bulletin*, **3**: 65-74.
- BEN NACEUR, H., A. BEN REHEB JENHANI & M. ROMDHANE, 2009. Note on the distribution of Branchinella spinosa (Milne Edwards, 1840) (Branchiopoda, Anostraca) in Tunisia. *Notes and News. Crustaceana*, **82** (5): 635-641.
- BLANCHARD, R & J. RICHARD, 1890. Sur les crustacés des Sebkhas et des Chotts d'Algérie. *Bulletin de la Société Zoologique de France*, **45**: 136-138.
- BOWEN, S.T & G. STERLING, 1978. Esterase and malate dehydrogenase isozyme polymorphisms in 15 Artemia populations. *Comparative Biochemistry and Physiology*, **61B**: 593-595.
- BROWNE, R.A & G.H. MACDONALD, 1982. Biogeography of the brine shrimp, Artemia: distribution of parthenogenetic and sexual populations. *Journal of Biogeography*. **9**: 331-338.
- BROWNE, R.A, L.E. DAVIS & S.E. SALLEE , 1988. Effects of temperature and relative fitness of sexual and asexual brine shrimp Artemia. *Journal of Experimental Marine Biology and*

Ecology. **124**: 1-20.

- COHEN, R.G., F. AMAT, F. HONTORIA & J.C. NAVARRO, 1999. Preliminary characterization of some Argentinean Artemia populations from La Pampa and Buenos Aires provinces. International Journal of Salt Lake Research, **8** (4): 329-340.
- EL BERMAWI, N., A.D. BAXEVANIS, T.J. ABATZOPOULOS, G. VANSTAPPEN & P. SORGELOOS, 2004. Salinity effects on survival, growth and morphometry of four Egyptian Artemia populations (International Study on Artemia LXVII). Hydrobiologia, **523**: 175-188.
- EL MAGSODI, M.O., H.M. EL GHEBLI, M. ENBAYA, M. HAMZA, U. DREBIKA & P. SORGELOOS, 2005. Reproductive and lifespan characteristics of Artemia from Lybian Abu Kammash Sabkh. Lybian Journal of Marine Science, **10**: 1-8.
- GAUTHIER, H., 1928. Nouvelles recherches sur la faune des eaux continentales de l'Algérie et de la Tunisie. (Minerva. Alger).
- GAJARDO, G., J. CRESPO & C.D. TRIANTAPHYLIDIS, 2004. Species identification of Chilean Artemia populations based on mitochondrial DNA RFLP analysis. Journal of Biogeography, **31**: 547-555.
- GREEN, A.J., M.I. SANCHEZ, F. AMAT, J. FIGUEROLA, F. HONTORIA, O. RUIZ & F. HORTAS, 2005. Dispersal of invasive and native brine shrimps Artemia (Anostraca) via waterbirds. Limnology and Oceanography, **50**(2): 737-742.
- HADDAG, M., 1991. Contribution a l'étude d'une souche d'Artemia (Artemia tunisiana) endémique aux eaux de la saline d'Arzew, Algérie : 1-72 (Thèse de Magister. Institut des Sciences de la Mer et de l'Aménagement du Littoral. Alger).
- HONTORIA, F & F. AMAT, 1992. Morphological characterization of adult Artemia (Crustacea, Branchiopoda) from different geographical origin. Mediterranean populations. Journal of Plankton Research, **14** (7): 949-959.

- KAISER, H., A.K. GORDON & T.G. PAULET, 2006. Review of the African distribution of the brine shrimp genus Artemia. Water. S.A., **32**(4): 597-604.
- KARA, M.H., K.A. BENGRINE, F. DERBAL, L. CHAOUI & M. AMAROUAYACHE, 2004. Quality evaluation of a new strain of Artemia from Chott Marouane (Northeast Algeria). Aquaculture, **235**: 361-369.
- LAW, J.H. & B.J. CRESPI, 2002. The evolution of geographic parthenogenesis in Timema walking-sticks. Molecular Ecology, **11**: 1417-1489.
- McCARRAHER. D.B., 1972. A preliminary bibliography and lake index of the inland mineral waters of the world: 1-38. Nebraska Game and Parks Commission. Research Publications. (University of Nebraska. Lincoln)
- MUÑOZ, J., A. GOMEZ, A.J. GREEN, J. FIGUEROLA, F. AMAT & C. RICO, 2008. Phylogeography and local endemism of the native Mediterranean brine shrimp Artemia salina (Branchiopoda: Anostraca). Molecular Ecology, **17**: 3160-3177.
- MUÑOZ, J. & F. PACIOS, 2010. Global biodiversity and geographical distribution of diapausing aquatic invertebrates: the case of the cosmopolitan brine shrimp Artemia (Branchiopoda, Anostraca). Crustaceana **83** (4): 465-480.
- MUÑOZ, J., A. GÓMEZ, A.J. GREEN, F. FIGUEROLA, F. AMAT & C. RICO, 2010b. Evolutionary origin and phylogeography of the diploid obligate parthenogen Artemia parthenogenetica (Branchiopoda : Anostraca). Plos One, **5** (8): e11932. doi:10.1371/journal.pone.0011932.
- MURA, G., 1990. Artemia salina (Linnaeus, 1758) from Lymington, England: frontal knob morphology by scanning electron microscopy. Journal of Crustacean Biology, **10**: 364-368.
- MURA, G. & B. BRECCIAROLI, 2004. Use of morphological characters for species separation within the genus Artemia (Crustacea, Branchiopoda). Hydrobiologia, **520**: 179-188.

- MURA, G., A.D. BAXEVANIS, G. MEDINA, F. HONTORIA, I. KAPPAS, S. MOSCATELLO, G. FANCELLO, F. AMAT & T.J. ABATZOPOULOS, 2005. The use of a multidisciplinary approach for the characterization of a diploid parthenogenetic Artemia population from Torre Colimena (Apulia, Italy). *Journal of Plankton Research*, **27**(9): 895-907.
- MURA, G., I. KAPPAS, A. BAXEVANIS, S. MOSCATELLO, Q. D'AMICO, G. MEDINA, F. HONTORIA, F. AMAT & T.J. ABATZOPOULOS, 2006. Morphological and Molecular data reveal the presence of the invasive Artemia franciscana in Margherita di Savoia salterns (Italy). *International Review of Hydrobiology*, **91**: 539-554.
- SADKAOUI, F., M. MONCEF, J. SIF & R. GAUDY, 2000. Preliminary report on the brine shrimp (Artemia salina) from El Jadida saltworks, Morocco. *Marine Life*, **10** (1-2): 33-42.
- SAMRAOUI, B., K. CHAKRI & F. SAMRAOUI, 2006. Large branchiopods (Branchiopoda: Anostraca, Notostraca and Spinicaudata) from the salt lakes of Algeria. *Journal of Limnology*, **65**(2): 83-88.
- TRANTAPHYLLOIDIS. G.V., K. POULOPOULOU, T.J. ABATZOPOULOS, C.A. PEREZ & P. SORGELOOS, 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.
- TRANTAPHYLLOIDIS, G.V., G.R.J. CRIEL, T.J. ABATZOPOULOS, K.M. THOMAS, J. PELEMAN, J.A. BEARDMORE & P. SORGELOOS, 1997. International Study on Artemia. LVII. Morphological and molecular characters suggest conspecificity of all bisexual European and North African Artemia populations. *Marine Biology*, **129**: 477-487.
- TRANTAPHYLLOIDIS, G.V., T.J. ABATZOPOULOS & P. SORGELOOS, 1998. Review of

- the Biogeography of the Genus Artemia (Crustacea, Anostroca). Journal of Biogeography, **25**: 213-226.
- VANHAECKE, P., W. TACKAERT & P. SORGELOOS, 1987. The biogeography of Artemia: an updated review. In: P. Sorgeloos, D.A. Bengtson, W. Decleir & E. Jaspers (eds.), Artemia Research and its Applications. Vol. **1**: 129-155. (Universa. Press. Wetteren Belgium).
- VANHAECKE, P & P. SORGELOOS, 1980. International Study on Artemia IV. The biometrics of Artemia strains from different geographical origin. In: G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers (eds.), The Brine Shrimp Artemia, **3**, Ecology, Culturing, Use in Aquaculture : 393-405. (Universa. Press. Wetteren. Belgium).
- VANSTAPPEN, G., 2002. Zoogeography. In: T.J. Abatzopoulos, J.A. Beardmore, J.S. Clegg & P. Sorgeloos (eds.), Biology of Aquatic Organisms. Artemia: Basic and Applied Biology: 171-224. (Kluwer Academic Publishers. Dordrecht. The Netherlands).
- WEIDER, L.J., A. HOBÆK, J.K. COLBOURNE, T.J. CREASE, F. DUFRESNE & P.D.N. HEBERT, 1999. Holarctic phylogeography of an asexual species complex I. Mitochondrial DNA variation in arctic Daphnia. Evolution, **53**: 777–792.
- ZEMMOURI, A., 1991. A note on the genus Artemia in Algeria. Hydrobiologia, **212**: 231-233.
- ZHANG, L. & H. LEFCORT, 1991. The effects of ploidy level on the thermal distributions of brine shrimp Artemia parthenogenetica and its ecological implications. Heredity, **66**: 445-452.

FIGURE CAPTIONS

Figure 1. Population centroids for the two first discriminant functions of the discriminant analysis on the morphology of females from different Algerian Artemia populations compared to several related populations from the Mediterranean area.

Table I. Algerian hypersaline ecosystems surveyed, localisation and geographical coordinates.

Bethioua sebkha and saltworks	Oran	35°42'31" N 0°16'53" W
Oran Grande sebkha	Oran	35°44'28" N 0°23'13" W
Relizane sebkha and saltworks	Sidi Bouziane	35°50'31" N 0°39'10" E
Setif sebkha	Ain Arnat	36°09'45" N 5°17'28" E
Ezzamoule sebkha and saltworks	Oum Bouaghi	35°52'54" N 6°30'14" E
Goléa Mnea salt lake	Ghardaia	30°28'39" N 2°55'29" E
Garaet et Tarf sebkha	Oum Bouaghi	35°40'33" N 7°04'47" E
Melghir chott	Biskra	34°10'46" N 6°07'05" E

Table II. Species/strain designation, origin and location of the populations retrieved from the IATS morphometrical database and used in the discriminant analysis.

Bisexual <u>A. salina</u>		
Souzama (Oualidia, Morocco)	32°44'N 9°01'W	Muñoz et al. 2008
Rocío (Cádiz, Spain)	36°52'N 6°19'W	Muñoz et al. 2008
Bonmatí (Alicante, Spain)	38°10'N 0°37'W	Muñoz et al. 2008
Trapani (Sicilia, Italy)	37°59'N 12°32'E	Muñoz et al. 2008
Diploid parthenogenetic		
Wadi Natron (El Cairo, Egypt)	30°23'N 30°19'E	Muñoz et al. 2010b
Odiel (Huelva, Spain)	37°15'N 6°59'W	Muñoz et al. 2010b
La Mata lagoon (Alicante, Spain)	38°02'N 0°42'W	Mura et al. 2005
Aigües Mortes (Languedoc, France)	43°32'N 4°10'E	Mura et al. 2005
Margherita di Savoia (Foggia, Italy)	41°22'N 16°05'E	Muñoz et al. 2010b
Torrecolimena (Apulia, Italy)	40°18'N 17°43'E	Mura et al. 2005
Tetraploid parthenogenetic		
Odiel (Huelva, Spain)	37°15'N 6°59'W	Mura et al. 2006
Pinilla (Albacete, Spain)	38°45'N 1°40'W	Amat et al. 1995a
Delta del Ebro (Tarragona, Spain)	40°34'N 0°40'E	Mura et al. 2006
Larache (Tanger, Morocco)	35°11'N 6°06'W	Hontoria & Amat 1992
Margherita di Savoia (Foggia, Italy)	41°22'N 16°05'E	Mura et al. 2006
Bisexual <u>A. franciscana</u>		

San Francisco Bay (California, USA)	37°30′N 122°12′W	Mura et al. 2006
Great Salt Lake (Utah, USA)	41°00′N 112°30′W	Mura et al. 2006