Brine shrimps adrift: historical species turnover in Western Mediterranean Artemia (Anostraca)

Lucía Sainz-Escudero⋅
E. Karen López-Estrada⋅
Paula C. Rodríguez-Flores⋅ Mario García-París

Received: 3 November 2021 / Accepted: 9 March 2022 / Published online: 18 April 2022
© The Author(s) 2022

Abstract  Brine shrimps (Artemia) have undergone geographic range and demographic expansions as a result of their interaction with humans since the beginning of salt harvesting. This interaction has favoured the expansion of some species but compromising the survival of others. Mediterranean native populations of Artemia salina from coastal salterns and lagoons are facing the presence and expansion of the introduced and invasive American species Artemia monica (=A. franciscana). However, this species could not be the only threat. Parthenogenetic populations of the Asian species A. urmiana and A. sinica are widespread along the Mediterranean and other areas of the world. In this work, with the use of large cox1 and mitogenomic datasets, phylogenetic and phylogeographic inferences, and a time calibrated tree, we confirmed the Asian origin and recent arrival of the current Western Mediterranean parthenogenetic populations of Artemia. In addition, the replacement of Iberian populations of A. salina by Asiatic parthenogenetic populations lead us to recognize parthenogens as invasive. Current salterns development and commercial importance of Artemia make human-mediated introduction probable. These results demonstrate again the impact that changing human interests have on population expansion or decline of species adapted to anthropogenic habitats. Artemia salina decline makes urgent the implementation of conservation measures such as its use in fish farming and salt production or its inoculation in inland salterns.

Resumen  Las artemias (Artemia) han experimentado expansiones demográficas y cambios en sus áreas de distribución como resultado de su interacción con los humanos desde los inicios de la producción de sal. Esta interacción ha favorecido la expansión de algunas especies, pero comprometido la supervivencia de otras. Las poblaciones nativas mediterráneas de Artemia salina de las salinas y lagunas costeras se enfrentan a la presencia y expansión de la especie americana Artemia monica (=A. franciscana), introducida e invasora. Sin embargo, esta especie podría no ser la única amenaza. Las poblaciones asiáticas partenogenéticas...
de las especies *A. urmiana* y *A. sinica* están repartidas tanto en el Mediterráneo como en otras áreas del mundo. En este trabajo, confirmamos el origen asiático y la llegada reciente de las poblaciones partenogenéticas que se encuentran actualmente en el Mediterráneo occidental, a través del uso de inferencias filogenéticas y filogeográficas y un árbol calibrado, obtenidos a partir de un amplio conjunto de datos mitogenómicos y del marcador *cox1*. Además, el reemplazo de poblaciones ibéricas de *A. salina* por poblaciones partenogenéticas asiáticas, nos lleva a reconocerlas como invasoras. El desarrollo actual de las salinas y la importancia comercial de *Artemia* hacen probable su introducción antrópica. Estos resultados demuestran de nuevo el impacto que los intereses cambiantes de los seres humanos provocan en la expansión o declive de especies adaptadas a hábitats modificados por el hombre. El declive de Artemia salina hace urgente la implementación de medidas de conservación tales como favorecer su uso en las piscifactorias y durante la producción de sal, o su inoculación en las salinas de interior.

**Keywords**  Conservation - Dispersal - Species introduction - Invasive species - Salt harvesting - Phylogeny

**Palabras clave**  Conservación - Dispersión - Introducción de especies - Especies invasoras - Producción de sal - Filogenia

**Introduction**

Wild species able to colonize man-modified habitats are often capable to expand vastly their geographic ranges. The newly created habitats or transport provided by humans and lack of competitors may result in demographic bursts that significantly contribute to geographic expansions of their ranges (Senar et al. 2019; Tollenaere et al. 2010; Lewis et al. 2019). However, human needs and production systems change rapidly through time, and what was a favourable system for a certain species might change drastically, either favouring a different species, or limiting dramatically their available habitat (Bøhn et al. 2008; Dafni et al. 2010). As a consequence of these changes, populations of the once widespread, highly successful species, might suffer dramatic declines or become extirpated, and if the original natural habitat would be no longer available, the entire species might face extinction.

One of these taxa whose interactions with humans have produced local demographic bursts and vast geographic range expansions are brine shrimps, small crustaceans of the genus *Artemia* Leach, 1819 (Brachiopoda: Anostraca). The currently recognized five species of *Artemia* (Sainz-Escudero et al. 2021) are inhabitant of worldwide continental aquatic saline ecosystems (Van Stappen 2002). Their original habitat, inland saline lakes and coastal lagoons are limited, and their original distribution was probably restricted to certain geographic areas. This is still the case for the American Southern Cone species, *Artemia persimilis* Piccinelli and Prosdocimi, 1968 (Amat et al. 1994). However, Neolithic originated salt harvesting for human needs (Weller and Dumitroaia 2005; Figuls et al. 2007; Manrique 2011) was based for centuries in the creation of salterns, that accumulate salty water from wells or sources associated to subterranean diapirs or sea water, providing a completely new and suitable habitat for brine shrimps to be colonized (Martínez-Abraín and Jiménez 2015). The species of *Artemia* expanded their geographic ranges favoured by passive dispersal of their resistance eggs mediated by migratory birds (Green et al. 2005; Muñoz et al. 2013) or by anthropogenic movements motivated by salterns development or commercialization for fish farming (Vikas et al. 2012; Sorgeloos et al. 2001). However, human commercial interests have also come along with disturbances in brine shrimp species distribution, even compromising the survival of some. The large demand of *Artemia* for fish farming triggered the introduction and invasion of the North American cultivated species *Artemia monica* Verrill, 1869 (=*A. franciscana* Kellogg, 1906) into coastal salterns all over the world (Triantaphyllidis et al. 1994; Amat et al. 2007; Mura et al. 2006; Ruebhart et al. 2008; Scalone and Rabet 2013; Saji et al. 2019). This species is displacing the Mediterranean native species *Artemia salina* (Linnaeus, 1758) (Oscoz et al. 2010; Horváth et al. 2018) possibly due to its high adaptive potential and physiological plasticity that enhance its invasion range capacity (Dlugosch and Parker 2008).

However, *A. monica* is not the only species that occupy the native area of *A. salina*. The presence of parthenogenetic populations of *Artemia urmiana* Günther 1899 and *Artemia sinica* Cai 1989
Brine shrimps adrift: historical species turnover in Western Mediterranean Artemia…

(Sainz-Escudero et al. 2021; Rode et al. 2021) settled in inland and coastal Mediterranean salterns (Amat 1979, 1980; Abatzopoulos et al. 2009; Eimanifar et al. 2014; Asem et al. 2016; Triantaphyllidis et al. 1998), apart from other areas of the world (Geddes 1979; McMaster et al. 2007; Kaiser et al. 2006; Ben Naceur et al. 2012; Mlingi et al. 2019; Triantaphyllidis et al. 2011) was identified as the result of recent colonizations (Baxevanis et al. 2006; Muñoz et al. 2010; Maniatsi et al. 2011; Maccari et al. 2013b). But so far, these populations were not generally considered as invasive nor as a threat to the native A. salina (Céspedes et al. 2017; Green et al. 2005; Pinto et al. 2013; Sala et al. 2017; Amat et al. 2007).

Contrary to this idea, we hypothesize that the historically expanded populations of the Mediterranean species A. salina (Muñoz et al. 2014) are being affected not only by the human-induced introduction and invasion of A. monica but also by those of the Asian parthenogenetic populations of A. urmiana and A. sinica, which likely would have arrived recently. These invasions, probably associated to salt farming interests, seem to be displacing the native populations of A. salina, which might lead the species at the brink of extinction in some areas. However, there is very little previous evidence about the introduced and invasive character of parthenogens in the Mediterranean region. To test for these hypotheses, we need to check the historical and current presence of A. salina in Western Mediterranean salterns, confirm the recent arrival of the Asian parthenogenetic populations, and discuss the potential damage they are causing on A. salina. To do this, (1) we surveyed Iberian and some other Western Mediterranean inland and coastal salterns to document the current presence of Artemia and confirmed their identity by studying their morphology and sequencing a fragment of the mitochondrial cytochrome c oxidase I gene (cox1). Historical data on the presence of Artemia in Iberian salterns contained in previous bibliography was used to discuss the invasive character of parthenogens. (2) We identified the approximate geographical origin of the parthenogenetic A. urmiana and A. sinica populations in the Western Mediterranean region by using our cox1 data, together with all the previously available cox1 dataset of Artemia. Finally, (3) we used next generation sequencing (NGS) to generate the first complete mitogenomes for parthenogenic individuals of A. urmiana and A. sinica, and phylogenetically compare them with already published bisexual Artemia mitogenomes. We inferred divergence times estimation on our mitogenomic tree in order to find out the approximate time of appearance of parthenogenesis across the evolutionary history of the genus, and estimate their possible timing of expansion into the Western Mediterranean region.

In this work, we use Western Mediterranean brine shrimps (mainly from Iberian Peninsula) as a model to exemplify the human dependent fate to which human-favoured species are subjected following changes during the economic and productive procedures.

Materials and methods

Sampling and sequencing

We collected around five to ten adult individuals of Artemia from artificial inland and coastal salterns from a total of 30 localities, of which 25 correspond to Iberian Peninsula and 5 to other Western Mediterranean and North Africa locations. Information about sampling localities and their geographical coordinates is included in Table 1. Specimens were captured with an aquarium net, photographed (Fig. 1), georeferenced, and preserved in absolute ethanol and stored at –20 °C at the Museo Nacional de Ciencias Naturales (MNCN-CSIC) from Madrid (Spain).

Total genomic DNA (samples deposited at the MNCN DNA-tissues Collection) was isolated according to the protocols described by Hwang et al. (2019). A partial fragment of the cytochrome c oxidase subunit I (cox1) was amplified via the polymerase chain reaction (PCR) employing the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). PCR reactions were performed in a total volume of 25 µL that contained 2µL of extracted DNA, 1 µL of dNTP (10 mM), 2.5µL of reaction buffer 10x (Tris–HCl, pH 8.3, Biotools), 0.8 of µL MgCl2 (50 mM), 0.5 µL of each primer (10 µM), 17.3 µL of distilled water and 0.4 µL of Taq DNA polymerase (Biotools, 5 ud/ µL). PCR reactions consisted of 1 cycle of 5 min at 95 °C for initial denaturation, 40 cycles of 45 s at 42 °C and 1 min at 72 °C, and a final extension of 10 min at 72 °C. Amplified PCR products were visualized by electrophoresis in a 1.5% agarose gel. Samples
Table 1  Own mitogenomic and *cox1* data included in phylogenetic and phylogeographic analyses of parthenogenetic *A. urmiana* and *A. sinica* and bisexual *A. salina* individuals from Mediterranean localities

| MNCN DNA codes | Field number | GenBank accession numbers | Sample size | Species | Locality | Geographical coordinates |
|---|---|---|---|---|---|---|
| Mitochondrial genome | | | | | | |
| – | mtArt_1 | OM686871 | 1 | *Artemia urmiana* (parthenogenetic) | Spain: Murcia, San Pedro del Pinatar, Coterillo Salterns | 37°49′22.2″N, 0°45′37.4″W |
| – | mtArt_2 | OM686872 | 1 | *Artemia sinica* (parthenogenetic) | Portugal: Santarem, Rio Maior Salterns | 39°21′49.0″N, 8°56′35.6″W |
| – | mtArt_3 | OM686873 | 1 | *Artemia sinica* (parthenogenetic) | Spain: Guadalajara, Alcuneza Salterns | 41°05′38.6″N, 2°36′55.7″W |
| – | mtArt_4 | OM686874 | 1 | *Artemia sinica* (parthenogenetic) | Spain: Zaragoza, Bujalarolz, La Playa Salterns | 41°25′17.9″N, 0°11′49.3″W |
| Partial Mitochondrial marker Cytochrome oxidase subunit 1 | | | | | | |
| 119948, 119949 | art05, art06 | OM486980, OM486981 | 2 | *Artemia salina* | Chipre: Larnaca Salt Lake | 34°50′55.0″N, 33°37′1.0″E |
| 119960, 119961 | art07, art08 | OM486982, OM486983 | 2 | *Artemia salina* | Morocco: Casablanca-Settat, Sidi Bennour | 32°47′12.9″N, 8°57′58.5″W |
| 119962, 119963 | art09, art10 | OM486984, OM486985 | 2 | *Artemia salina* | Morocco: Casablanca-Settat, Sidi Bennour | 32°47′14.2″N, 8°57′57.4″W |
| 119964, 119965 | art11, art12 | OM486986, OM486987 | 2 | *Artemia salina* | Sicily: Trapani Salterns | 37°59′22.2″N, 12°30′59.6″E |
| 119967 | art13 | OM486988 | 1 | *Artemia salina* | Spain: Formentera, Savina Salterns | 38°43′47.6″N, 1°25′09.7″E |
| 119958, 119959 | art15, art16 | OM486989, OM486990 | 2 | *Artemia salina* | Spain: Granada, La Malahá I Salterns | 37°06′10.5″N, 3°43′17.7″W |
| 119954, 119955 | art17, art18 | OM486991, OM486992 | 2 | *Artemia salina* | Spain: Jaén, Brujuelo Salterns | 37°52′31.4″N, 3°40′21.5″W |
| 119956, 119957 | art19, art20 | OM486993, OM486994 | 2 | *Artemia salina* | Spain: Jaén, Lagartijo Salterns | 37°51′20.7″N, 3°39′15.1″W |
| 119952, 119953 | art21, art22 | OM486995, OM486996 | 2 | *Artemia salina* | Spain: Jaén, Salinas San Carlos Salterns | 37°53′13.0″N, 3°40′07.0″W |
| 119932, 119933 | art23, art24 | OM486997, OM486998 | 2 | *Artemia salina* | Spain: Mallorca, Salobral de Campos-Es Trenc | 39°20′54.2″N, 3°00′19.3″E |
| 119930, 119931 | art25, art26 | OM486999, OM487000 | 2 | *Artemia salina* | Spain: Mallorca, Snt Jordi, A’Avall Salterns | 39°19′24.0″N, 2°59′19.2″E |
Table 1 (continued)

| MNCN DNA codes | Field number | GenBank accession numbers | Sample size | Species | Locality | Geographical coordinates |
|----------------|--------------|---------------------------|-------------|---------|----------|-------------------------|
| 119922, 119923 | art27, art28 | OM487001, OM487002       | 2           | *Artemia sinica* (parthenogenetic) | Portugal: Santarem, RIo Maior Salterns | 39°21'49.0"N, 8°56'35.6"W |
| 119942, 119943 | art29, art30 | OM487003, OM487004       | 2           | *Artemia sinica* (parthenogenetic) | Spain: Àlava, Añana Salterns | 42°48'05.2"N, 2°59'09.8"W |
| 119944, 119945 | art31, art32 | OM487005, OM487006       | 2           | *Artemia sinica* (parthenogenetic) | Spain: Burgos, Poza de la Sal | 42°40'11.8"N, 3°30'19.1"W |
| 119936, 119937 | art33, art34 | OM487007, OM487008       | 2           | *Artemia sinica* (parthenogenetic) | Spain: Cuenca, Manzano Salterns | 40°05'20.6"N, 1°33'26.3"W |
| 119926, 119927 | art35, art36 | OM487009, OM487010       | 2           | *Artemia sinica* (parthenogenetic) | Spain: Guadalajara, Saelices de las Sal Salterns | 40°54'10.0"N, 2°19'39.9"W |
| 119938, 119939 | art37, art38 | OM487011, OM487012       | 2           | *Artemia sinica* (parthenogenetic) | Spain: Huesca, Naval, Rolda Salterns | 42°11'14.1"N, 0°09'09.2"E |
| 119918, 119916, 119917 | art39, art40, art41 | OM487013, OM487014, OM487015 | 3 | *Artemia sinica* (parthenogenetic) | Spain: Toledo, Quero, Laguna Grande Salterns | 39°30'15.8"N, 3°14'51.8"W |
| 119924, 119925 | art42, art43 | OM487016, OM487017       | 2           | *Artemia sinica* (parthenogenetic) | Spain: Toledo, Villacañas, Peña Hueca Lagoon | 39°31'10.8"N, 3°20'09.5"W |
| 119919 | art44 | OM487018                 | 1           | *Artemia sinica* (parthenogenetic) | Spain: Toledo, Villacañas, Tirez Lagoon | 39°32'36.0"N, 3°21'3.3"W |
| 119968 | art45 | OM487019                 | 1           | *Artemia sinica* (parthenogenetic) | Spain: Zaragoza, Bujaraloz, Playa Salterns | 41°25'17.9"N, 0°11'49.3"W |
| 119950, 119951 | art46, art47 | OM487020, OM487021       | 2           | *Artemia urmi-mana* (parthenogenetic) | Portugal: Aveiro, Aveiro Salterns | 40°37'43.7"N, 8°39'40.4"W |
| 119966 | art48 | OM487022                 | 1           | *Artemia urmi-mana* (parthenogenetic) | Sardegna: Cagliari, Stagno di Quarto | 39°13'15.3"N, 9°11'11.7"E |
| 119969, 119970 | art49, art50 | OM487023, OM487024       | 2           | *Artemia urmi-mana* (parthenogenetic) | Spain: Alicante, Calpe, Calpe Salterns | 38°38'48.6"N, 0°40'01.0"E |
| 119946, 119947 | art51, art52 | OM487025, OM487026       | 2           | *Artemia urmi-mana* (parthenogenetic) | Spain: Almería, Roquetas de Mar, La Romanilla Salterns | 36°46'49.6"N, 2°36'02.9"W |
| 119914, 119915 | art53, art54 | OM487027, OM487028       | 2           | *Artemia urmi-mana* (parthenogenetic) | Spain: Formen-terra, Savina Salterns | 38°43'47.6"N, 1°25'09.7"E |
containing visible and single bands were sequenced in forward direction at Macrogen Inc (Macrogen, Spain).

All sequences were edited using Geneious CS5.1 software (Kearse et al. 2012), aligned with MAFFT algorithm (Katoh and Toh 2008), and revised with Mesquite v.3.51 (Maddison and Maddison 2018).

Additionally, to our data, all the available *Artemia cox1* sequences available in GenBank (Valsala et al. 2005; Hou et al. 2006; Tizol-Correa et al. 2009; Muñoz et al. 2008, 2010, 2013; Maniatsi et al. 2009, 2011; Maccari et al. 2013b; Eimanifar and Wink 2013; Eimanifar et al. 2014, 2015, 2016; Asem et al. 2016, 2019, 2020; Naganawa and Mura 2017; Horváth et al. 2018) and one of *Branchinecta ferox* used as outgroup (LT821334 [Rodríguez-Flores et al. 2017]) were retrieved in order to build a dataset represented by 1505 sequences, that allowed us to depict

| MNCN DNA codes | Field number | GenBank accession numbers | Sample size | Species | Locality | Geographical coordinates |
|----------------|--------------|---------------------------|-------------|---------|----------|--------------------------|
| 119940, 119941 | art55, art56 | OM487029, OM487030        | 2           | *Artemia urmiana* (parthenogenetic) | Spain: Guadalajara, Imón Salterns | 41°09′32.1″N, 2°43′34.8″W |
| 119934, 119935 | art57, art58 | OM487031, OM487032        | 2           | *Artemia urmiana* (parthenogenetic) | Spain: Madrid, Villaconejos, La Seca Lagoon | 40°05′14.8″N, 3′32′05.8″W |
| 119920, 119921 | art59, art60 | OM487033,OM487034         | 2           | *Artemia urmiana* (parthenogenetic) | Spain: Murcia, San Pedro del Pinatar, Coterillo Salterns | 37°49′22.2″N, 0°45′37.4″W |
| 119928, 119929 | art61, art62 | OM487035, OM487036        | 2           | *Artemia urmiana* (parthenogenetic) | Spain: Navarra, Oro Salterns | 42°46′56.3″N, 1°52′36.5″W |

MNCN DNA Codes: DNA sample codes at the Museo Nacional de Ciencias Naturales DNA Collection

![Fig. 1](image)
Specimens of different lineages of *Artemia* found in Spain. A Mating specimens of the native *A. salina* from Es Trenc (Mallorca). B High density of different cohorts of *A. sinica* parthenogens in Quero salterns (Toledo). C Ventral view of a parthenogenetic specimen of *A. sinica* from the Medieval salterns of Alcuneza (Guadalajara). D Dorsal view of a parthenogenetic specimen of *A. urmiana* from the pre-industrial salterns of Roquetas de Mar (Almería). Photographs by PCR-F and MG-P.
the structuring of the genus through the Neighbour Joining analysis and to perform phylogeographic analyses. Some dissimilar sequences that featured stop codons when translated to amino Acids were removed from the analyses due to the existence of pseudogenes according to Rode et al. (2021).

In parallel, four parthenogenetic individuals (one corresponding to A. urmiana lineage and three to A. sinica) from four localities in the Iberian Peninsula were chosen to be high-throughput sequenced (at AllGenetics, A Coruña, Spain; Table 1). The DNA extraction protocol, library preparation, and sequencing process, were the same as those explained in Sainz-Escudero et al. (2021). Genome assembly of the parthenogenetic individuals of A. urmiana and A. sinica was carried out using the cox1 sequence as reference. Finally, annotation was performed using MITOS2 (Bernt et al. 2013), checking manually the start and stop codons of all coding genes. Mitogenomes of parthenogenetic Artemia urmiana and A. sinica were deposited in GenBank. GenBank accession numbers of the mitogenomes are indicated in Table 1.

Mitochondrial (cox1) analyses and phylogeography

In order to visualize the general structure inside the genus Artemia, the cox1 dataset and a sequence of Branchinecta ferox as outgroup (Rodríguez-Flores et al. 2017) were used to build the matrix. This matrix was aligned using MAFT algorithm (Katoh and Toh 2008), cleaned through Gblocks DNA information criterion (Castresana 2000) to exclude several contiguous non-conserved positions, and collapsed into haplotypes using ALTER (Glez-Peña et al. 2010). Then, this matrix was analysed with the Neighbour Joining (NJ) distance method using PAUP* 4.0 (Wilgenbusch

---

**Fig. 2** Mitochondrial phylograms for Artemia. A Own and GenBank cox1 data analysed with Neighbour Joining (not intended to show relationships, but general clade structure); B Bayesian phylogenetic tree based on mitogenomic data. Posterior probabilities > 0.90 are indicated by black dots (mtArt samples correspond to the first mitogenomes of parthenogens, all generated for this study). *Indicates that the clade includes parthenogenetic and bisexual specimens, **indicates that the clade is only composed by parthenogenetic specimens.

---
and Swofford 2003) (Fig. 2A). A bootstrap (BS) analysis (1000 replicates) was used to assess node support (implemented in PAUP*).

Part of this dataset was also used to perform phylogeographic analyses. We made two cox1 haplotype networks with sequences related to A. urmiana and A. sinica clades (Fig. 3) in order to represent the geographical distribution of the allele diversity and find the position of the diploid and tetraploid parthenogenetic populations from Iberian salterns inside these networks. We first used DNA Sequences Polymorphism 6.12.01 (Rozas et al. 2017) to generate the collapsed matrix of unique alleles, and then, networks were constructed using the TCS algorithm applied through Population Analysis with Reticulate Trees (PopART) 1.7 software (Leigh and Bryant 2015) to shape the relationships between the population individuals. Sequences coming from GenBank which had more than the 50% of missing data were removed from the collapsed matrix. Information about the sequence-haplotype correspondence and their bibliographic source is included in Tables 2 and 3 (A. urmiana and A. sinica, respectively).

Mitogenomic phylogeny and divergence time estimates

We reconstructed a new phylogenetic hypothesis based on complete mitochondrial genomes, to sort out the main Artemia lineages, and to identify a possible time of appearance of the parthenogenetic populations (Figs. 2, 4). The data matrix of Artemia mitochondrial genomes was composed by those available at the GenBank database (see Sainz-Escudero et al. 2021) and the new four genomes from the Asian parthenogenetic individuals of A. urmiana and A. sinica. The complete matrix includes 16 terminal taxa.

Phylogenetic reconstruction was performed using a Bayesian Inference approach implemented in MrBayes version 3.2.6 (Ronquist et al. 2012) and divergence time estimation was carried out in BEAST 1.7 (Drummond et al. 2012). These analyses were performed following the protocol and considerations concerning molecular clock calibration and priors are specified in Sainz-Escudero et al. (2021).

Results

Cytochrome c oxidase I allele diversity and phylogeography

Although the Neighbour Joining analysis was not aimed to clarify the phylogenetic relations between Artemia groups, it helped us to visualize sequence similarities. Accordingly, ten more or less differentiated mitochondrial groupings exist all over the world (Fig. 2A). This exploratory method supports the monophyly of all recognized species (BS values over 95%). The bisexual species A. monica shows a large genetic diversity with many geographical or ecologically isolated populations (Browne and Bowen 1991; Muñoz et al. 2013). Artemia urmiana is represented by three main clades constituted by bisexual populations from Western Asia, Tibet, Kazakhstan, and diploid/ triploid parthenogenetic populations from Western Asia, Africa, Australia, Europe, Madagascar and Russia. Artemia sinica is formed by two reciprocally
monophyletic clades, constituted by bisexual individuals from Eastern Asia and tetraploid/pentaploid parthenogens from China, Russia and the Mediterranean area. Two bisexual specimens identified as *A. sinica* “2” (Eimanifar et al. 2014) remain separated. *Artemia salina* is represented by two reciprocally monophyletic clades: A Mediterranean Lineage and a South African one (Muñoz et al. 2008). Finally, the Southern Cone Lineage is represented by *A. persimilis* from Argentina and Chile (Fig. 2A).

The phylogeographic analyses of the mitochondrial *cox1* for the Eastern Asian Clade (*A. sinica*) resulted in a total of 29 haplotypes (Fig. 3A, Table 2). Three main groups are detected. One central group is formed by haplotypes 1 to 24: 1–13 group contains only bisexual populations from Asia (China) and 14–24 corresponds to parthenogenetic populations from Asia (China), Russia and Mediterranean region (Egypt, Greece, Iberian Peninsula, Italy). Genetically distant from the previous core group, haplotypes 25–27 are three pentaploid sequences from Yinggehai Saltern, China (Asem et al. 2016) and haplotypes 28 and 29 differ in 27 mutations from the rest of bisexual haplotypes (Eimanifar et al. 2014).

The Western Asian Clade (*A. urmiana*) presents 161 haplotypes (Fig. 3B, Table 3). One group is

### Table 2

| No. Haplotype | Sample size | GenBank accession numbers | Bibliographic source |
|---------------|-------------|---------------------------|----------------------|
| 1             | 1           | DQ119648                  | Hou et al. (2006)    |
| 2             | 6           | KF707885,90–94            | Maccari et al. (2013b) |
| 3             | 4           | KF707886-89               | Maccari et al. (2013b) |
| 4             | 1           | HM998990                  | Maniatsi et al. (2011) |
| 5             | 1           | DQ119649                  | Hou et al. (2006)    |
| 6             | 1           | DQ119650                  | Hou et al. (2006)    |
| 7             | 3           | KF691272,75,99            | Eimanifar et al. (2014) |
| 8             | 1           | KF691273                  | Eimanifar et al. (2014) |
| 9             | 6           | KF691274,76,77,98, KF691300,02 | Eimanifar et al. (2014) |
| 10            | 1           | KF691301                  | Eimanifar et al. 2014 |
| 11            | 1           | KF691269                  | Eimanifar et al. (2014) |
| 12            | 1           | KF691270                  | Eimanifar et al. (2014) |
| 13            | 1           | KF691271                  | Eimanifar et al. (2014) |
| 14            | 1           | LC195586                  | Naganawa and Mura (2017) |
| 15            | 5           | OM487019; KU183954,59,60,57 | This work; Asem et al. (2016) |
| 16            | 2           | OM487003, OM487004        | This work            |
| 17            | 3           | KU183975-77               | Asem et al. (2016)   |
| 18            | 13          | HM998993,94               | Maniatsi et al. (2011) |
| 19            | 1           | KU183956                  | Asem et al. (2016)   |
| 20            | 14          | OM487001, OM487002, OM487007-18 | This work |
| 21            | 2           | OM487005, OM487006        | This work            |
| 22            | 3           | KU183972,73,74            | Asem et al. (2016)   |
| 23            | 1           | KU183971                  | Asem et al. (2016)   |
| 24            | 1           | KU183955                  | Asem et al. (2016)   |
| 25            | 1           | KU183968                  | Asem et al. (2016)   |
| 26            | 1           | KU183969                  | Asem et al. (2016)   |
| 27            | 1           | KU183970                  | Asem et al. (2016)   |
| 28            | 1           | KF691157                  | Eimanifar et al. (2014) |
| 29            | 1           | KF691158                  | Eimanifar et al. (2014) |
### Table 3 MtDNA (coxI) sequences of *A. urmiana* (bisexuals and parthenogens) used in this study; in the “GenBank accession number” column, a semi-colon separates sequences by groups according to bibliographic sources, as indicated in “Bibliographic source” column

| No. | Haplotype | Sample size | GenBank accession numbers | Bibliographic source |
|-----|-----------|-------------|---------------------------|----------------------|
| 1   | 274       |             | OM487022, OM487025, OM487026, OM487031, OM487032, OM487035, OM487036; KC193640-54, KC193666-72; DQ426485; KF707700-98, KF707800; 02–09, 20, 24, 27, 32, 33, 36–40, 42, 44–46, 52, 53, 67, 73; DQ119653; KF691148-53, 66–72, 87–89, KF691208-12, 14, 24-26, 33–35, 87–90, KF691338-42, 44, 46, 48, 73, 74, 75, 91–97, KF691400, 04–34, 42–45, 47, 48, 55, 56, 58–61, 63, 65, 67–75, 77, 78, 80, 85–91, 93, 95–97, KF691534, 48–53, 55; KU183949, 50, 52, 53, 88–92 | This work; Maccari et al. (2013a), Muñoz et al. (2010), Maccari et al. (2013b), Hou et al. (2006), Eimanifar et al. (2014), Asem et al. (2016) |
| 2   | 1         |             | DQ426826                   | Muñoz et al. (2010)  |
| 3   | 1         |             | GU591382                   | Muñoz et al. (2010)  |
| 4   | 1         |             | DQ426824                   | Muñoz et al. (2010)  |
| 5   | 1         |             | GU591380                   | Muñoz et al. (2010)  |
| 6   | 13        |             | OM487027, OM487028, OM487033, OM487034; KC193638,39; KF691236,38, KF691398,99, KF691401-03 | This work; Maccari et al. (2013a), Eimanifar et al. (2014) |
| 7   | 1         |             | KF691213                   | Eimanifar et al. (2014) |
| 8   | 4         |             | KF691457; KF707829, 30, 43 | Eimanifar et al. (2014), Maccari et al. (2013b) |
| 9   | 5         |             | KF691479, 82, 84, 92, 94   | Eimanifar et al. (2014) |
| 10  | 1         |             | KF691547                   | Eimanifar et al. (2014) |
| 11  | 1         |             | KF691554                   | Eimanifar et al. (2014) |
| 12  | 1         |             | KF707871                   | Maccari et al. (2013b) |
| 13  | 5         |             | KU183983-87                | Asem et al. (2016) |
| 14  | 9         |             | KF691464, 66, 76; KF707826, 28, 31, 34, 35, 41 | Eimanifar et al. (2014), Maccari et al. (2013b) |
| 15  | 1         |             | KF691483                   | Eimanifar et al. (2014) |
| 16  | 2         |             | KC193655, 56               | Maccari et al. (2013a) |
| 17  | 1         |             | KF707821                   | Maccari et al. (2013b) |
| 18  | 1         |             | KF691481                   | Eimanifar et al. (2014) |
| 19  | 7         |             | KF691360, 67–72            | Eimanifar et al. (2014) |
| 20  | 60        |             | OM487023, OM487024, OM487029, OM487030; GU591381; KF707765, 66, 70, 85, 88, 90–93, 95–97, 99, KF707850; KF691333–37, 43, 45, 57–59, 60, 61, KF691521, KF691530–32; KP090097-317, 319–324 | This work; Muñoz et al. (2010), Maccari et al. (2013b), Eimanifar et al. (2014, 2015) |
| 21  | 27        |             | KF707755-64, KF707823, 25; KU183961-67; KF691199-1204, KF691265, 68 | Maccari et al. (2013b), Asem et al. (2016), Eimanifar et al. (2014) |
| 22  | 1         |             | KF707822                   | Maccari et al. (2013b) |
| 23  | 7         |             | KP090318, 21; KU183978-82  | Eimanifar et al. (2015), Asem et al. (2016) |
| No. Haplotype | Sample size | GenBank accession numbers                                                                 | Bibliographic source                          |
|--------------|-------------|--------------------------------------------------------------------------------------------|-----------------------------------------------|
| 24           | 9           | KF707865, 66, 68–70, 72, 74; KF691183, KF691462                                            | Maccari et al. (2013b), Eimanifar et al. (2014) |
| 25           | 1           | KU183951                                                                                   | Asem et al. (2016)                            |
| 26           | 9           | HM998995                                                                                   | Maniatsi et al. (2011)                        |
| 27           | 1           | HM998996                                                                                   | Maniatsi et al. (2011)                        |
| 28           | 1           | HM999000                                                                                   | Maniatsi et al. (2011)                        |
| 29           | 1           | HM999002                                                                                   | Maniatsi et al. (2011)                        |
| 30           | 7           | HM998997                                                                                   | Maniatsi et al. (2011)                        |
| 31           | 1           | HM998998                                                                                   | Maniatsi et al. (2011)                        |
| 32           | 1           | HM998999                                                                                   | Maniatsi et al. (2011)                        |
| 33           | 3           | HM999001                                                                                   | Maniatsi et al. (2011)                        |
| 34           | 2           | KC193657, 58                                                                               | Maccari et al. (2013a)                        |
| 35           | 2           | KC193659, 60                                                                               | Maccari et al. (2013a)                        |
| 36           | 2           | OM487020, OM487021                                                                         | This work                                    |
| 37           | 1           | GU591388                                                                                   | Muñoz et al. (2010)                           |
| 38           | 22          | KF707855-64, 95–99, KF707919-22, 24–26                                                     | Maccari et al. (2013b)                        |
| 39           | 1           | KF707923                                                                                   | Maccari et al. (2013b)                        |
| 40           | 1           | KF707927                                                                                   | Maccari et al. (2013b)                        |
| 41           | 1           | GU591385                                                                                   | Muñoz et al. (2010)                           |
| 42           | 1           | GU591386                                                                                   | Muñoz et al. (2010)                           |
| 43           | 2           | KC193657, 78                                                                               | Maccari et al. (2013b)                        |
| 44           | 5           | GU591387; KF707673, 76, 77, 80                                                             | Muñoz et al. (2010), Maccari et al. (2013b)   |
| 45           | 5           | GU591389; KF707671, 72, 74, 79                                                             | Muñoz et al. (2010), Maccari et al. (2013b)   |
| 46           | 1           | KF707928                                                                                   | Maccari et al. (2013b)                        |
| 47           | 2           | KC193661, 62                                                                               | Maccari et al. (2013a)                        |
| 48           | 2           | KC193664, 65                                                                               | Maccari et al. (2013a)                        |
| 49           | 2           | KF707876, 83                                                                               | Maccari et al. (2013b)                        |
| 50           | 5           | JX512775, 91, JX512805; MK682353, 58                                                        | Eimanifar and Wink (2013), Asem et al. (2019) |
| 51           | 1           | JX512785                                                                                   | Eimanifar and Wink (2013)                     |
| 52           | 1           | JX512784                                                                                   | Eimanifar and Wink (2013)                     |
| 53           | 1           | JX512760                                                                                   | Eimanifar and Wink (2013)                     |
| 54           | 1           | JX512767                                                                                   | Eimanifar and Wink (2013)                     |
Table 3 (continued)

| No. | Haplotype | Sample size | GenBank accession numbers | Bibliographic source |
|-----|-----------|-------------|---------------------------|----------------------|
| 55  |           | 39          | DQ19651; KF707684,89, KF707875, 77, 79, 80, 81; JX512748, 55, 58, 62, 66, 69, 71, 76, 78, 80, 83, 88, 90, 95, 96, JX512803, 04, 08; MK682333, 38, 39, 46, 47, 51, 56, 66, 68, 70, 72, 79; HM998991 | Hou et al. (2006), Maccari et al. (2013b), Eimanifar and Wink (2013), Asem et al. (2019), Maniatsi et al. (2011) |
| 56  | MK682336  | 1           | MK682371                  | Asem et al. (2019)   |
| 57  | 1 MK682371 | 1           | MK682371                  | Asem et al. (2019)   |
| 58  | 7 7       |             | 7 KF707682; MK682359, 65, 69, 74; JX512756, 64 | Maccari et al. (2013b), Asem et al. (2010), Eimanifar and Wink (2013) |
| 59  | MK682335  | 1           | MK682335                  | Asem et al. (2019)   |
| 60  | 2 2       |             | JX512774; MK682367        | Eimanifar and Wink (2013), Asem et al. (2019) |
| 61  | MK682320  | 1           | MK682320                  | Asem et al. (2019)   |
| 62  | MK682323  | 1           | MK682323                  | Asem et al. (2019)   |
| 63  | 1 MK682324 | 1           | MK682324                  | Asem et al. (2019)   |
| 64  | 1 MK682327 | 1           | MK682327                  | Asem et al. (2019)   |
| 65  | 1 MK682331 | 1           | MK682331                  | Asem et al. (2019)   |
| 66  | 1 MK682332 | 1           | MK682332                  | Asem et al. (2019)   |
| 67  | 3 MK682330, 44, 78 |             | MK682330, 44, 78          | Asem et al. (2019)   |
| 68  | 1 MK682341 | 1           | MK682341                  | Asem et al. (2019)   |
| 69  | 1 MK682342 | 1           | MK682342                  | Asem et al. (2019)   |
| 70  | 1 MK682354 | 1           | MK682354                  | Asem et al. (2019)   |
| 71  | 1 KF707683 | 1           | KF707683                  | Maccari et al. (2013a, b) |
| 72  | 1 JX51277 | 1           | JX51277                   | Eimanifar and Wink (2013) |
| 73  | 2 JX512777, JX512801 | 2           | JX512777, JX512801        | Eimanifar and Wink (2013) |
| 74  | 1 JX512794 | 1           | JX512794                  | Eimanifar and Wink (2013) |
| 75  | 1 JX512797 | 1           | JX512797                  | Eimanifar and Wink (2013) |
| 76  | 1 JX512802 | 1           | JX512802                  | Eimanifar and Wink (2013) |
| 77  | MK682361  | 1           | MK682361                  | Asem et al. (2019)   |
| 78  | 1 MK682376 | 1           | MK682376                  | Asem et al. (2019)   |
| 79  | 1 JX512773 | 1           | JX512773                  | Eimanifar and Wink (2013) |
| 80  | 1 KF691533 | 1           | KF691533                  | Eimanifar et al. (2014) |
| 81  | 1 MK682373 | 1           | MK682373                  | Asem et al. (2019)   |
| 82  | 1 JX512779 | 1           | JX512779                  | Eimanifar and Wink (2013) |
| 83  | 1 KF707884 | 1           | KF707884                  | Maccari et al. (2013a, b) |
| 84  | 1 JX512781 | 1           | JX512781                  | Eimanifar and Wink (2013) |
| 85  | 2 JX512800; MK682377 | 2           | JX512800; MK682377        | Eimanifar and Wink (2013), Asem et al. (2019) |
Table 3 (continued)

| No. | Haplotype | Sample size | GenBank accession numbers | Bibliographic source |
|-----|-----------|-------------|---------------------------|----------------------|
| 86  | 1         | MK682321    |                           | Asem et al. (2019)   |
| 87  | 1         | JX512765    |                           | Asem et al. (2019)   |
| 88  | 1         | JX512786    |                           | Eimanifar and Wink (2013) |
| 89  | 1         | JX512752    |                           | Eimanifar and Wink (2013) |
| 90  | 1         | MK682360    |                           | Asem et al. (2019)   |
| 91  | 10        | GU591383; KC193663; KF707726; KF691520, 22–25; 28, 29 | Muñoz et al. (2010), Maccari et al. (2013a, b), Eimanifar et al. (2014) |
| 92  | 28        | GU591384; KF707810, 11, 13, 14, 16–18; KU053797-810, 812, 13, 15–18 | Muñoz et al. (2010), Maccari et al. (2013b), Eimanifar et al. (2016) |
| 93  | 2         | KU053811, 14 |                           | Eimanifar et al. (2016) |
| 94  | 1         | KU053819    |                           | Eimanifar et al. (2016) |
| 95  | 6         | KC193676; KF707847-49; KF707851, 54 | Maccari et al. (2013a, b) |
| 96  | 7         | KC193675; KF707749, 51, 52, KF707812, 15, 19 | Maccari et al. (2013a, b) |
| 97  | 5         | KF707691, 93, 95, 97, 98 | Maccari et al. (2013b) |
| 98  | 1         | KC193677    |                           | Maccari et al. (2013a) |
| 99  | 4         | KF707692, 94, 96, 99 | Maccari et al. (2013b) |
| 100 | 2         | KC193673; KF707746 | Maccari et al. (2013a, b) |
| 101 | 2         | KC193674; KF707801 | Maccari et al. (2013a, b) |
| 102 | 1         | KF691527    |                           | Eimanifar et al. (2014) |
| 103 | 1         | JX512757    |                           | Eimanifar and Wink (2013) |
| 104 | 4         | JX512754; MK682329, MK682348, MK682363 | Eimanifar and Wink (2013), Asem et al. (2019) |
| 105 | 1         | MK682345    |                           | Asem et al. (2019)   |
| 106 | 1         | JX512806    |                           | Eimanifar and Wink (2013) |
| 107 | 1         | MK682362    |                           | Asem et al. (2019)   |
| 108 | 1         | JX512787    |                           | Eimanifar and Wink (2013) |
| 109 | 1         | JX512761    |                           | Eimanifar and Wink (2013) |
| 110 | 1         | MK682349    |                           | Asem et al. (2019)   |
| 111 | 1         | MK682352    |                           | Asem et al. (2019)   |
| 112 | 1         | MK682334    |                           | Asem et al. (2019)   |
| 113 | 1         | MK682357    |                           | Asem et al. (2019)   |
| 114 | 1         | JX512798    |                           | Eimanifar and Wink (2013) |
| 115 | 1         | KF707686    |                           | Maccari et al. (2013b) |
| 116 | 1         | MK682322    |                           | Asem et al. (2019)   |
Table 3 (continued)

| No. Haplotype | Sample size | GenBank accession numbers | Bibliographic source |
|---------------|-------------|--------------------------|----------------------|
| 117           | 1           | KF707690                 | Maccari et al. (2013b) |
| 118           | 2           | KF707878, KF707882       | Maccari et al. (2013b) |
| 119           | 1           | KF707687                 | Maccari et al. (2013b) |
| 120           | 1           | JX512789                 | Eimanifar and Wink (2013) |
| 121           | 1           | MK682375                 | Asem et al. (2019)    |
| 122           | 1           | MK682326                 | Asem et al. (2019)    |
| 123           | 1           | JX512807                 | Eimanifar and Wink (2013) |
| 124           | 1           | JX512770                 | Eimanifar and Wink (2013) |
| 125           | 1           | JX512799                 | Eimanifar and Wink (2013) |
| 126           | 1           | JX512782                 | Eimanifar and Wink (2013) |
| 127           | 1           | JX512768                 | Eimanifar and Wink (2013) |
| 128           | 1           | MK682364                 | Asem et al. (2019)    |
| 129           | 1           | MK682343                 | Asem et al. (2019)    |
| 130           | 1           | JX512759                 | Eimanifar and Wink (2013) |
| 131           | 1           | JX512763                 | Eimanifar and Wink (2013) |
| 132           | 1           | KF707685                 | Maccari et al. (2013b) |
| 133           | 1           | JX512750                 | Eimanifar and Wink (2013) |
| 134           | 1           | KF707688                 | Maccari et al. (2013b) |
| 135           | 1           | MK682325                 | Asem et al. (2019)    |
| 136           | 1           | MK682350                 | Asem et al. (2019)    |
| 137           | 1           | MK682328                 | Asem et al. (2019)    |
| 138           | 1           | MK682337                 | Asem et al. (2019)    |
| 139           | 1           | MK682340                 | Asem et al. (2019)    |
| 140           | 1           | HM999003                 | Maniatsi et al. (2011) |
| 141           | 1           | HM999004                 | Maniatsi et al. (2011) |
| 142           | 1           | HM999005                 | Maniatsi et al. (2011) |
| 143           | 1           | MK682355                 | Asem et al. (2019)    |
| 144           | 1           | KF707681                 | Maccari et al. (2013b) |
| 145           | 1           | KF691526                 | Eimanifar et al. (2014) |
| 146           | 6           | KF707902, 05, 07, 08; KF691247, 49 | Maccari et al. (2013b), Eimanifar et al. (2014) |
| 147           | 1           | KF691216                 | Eimanifar et al. (2014) |
| 148           | 1           | KF691245                 | Eimanifar et al. (2014) |
Brine shrimps adrift: historical species turnover in Western Mediterranean Artemia…

constituted by haplotypes 1–36, which contains parthenogenetic populations from Asia (China, India, Iraq, Iran, Israel, Kazakhstan, Pakistan, Russia, Sri Lanka, Tibet Area, Turkey, Turkmenistan, Uzbekistan), Mediterranean region (Albania, Bulgaria, Egypt, France, Greece, Iberian Peninsula, Italy, Morocco, Sardinia, Ukraine) and other locations in the Old World (Madagascar, Namibia). The only bisexual specimen within this group is from Kazakhstan, and it is included in haplotype 1 (Hou et al. 2006). Haplotypes 37–46 correspond exclusively to bisexual populations from Tibet and Kazakhstan. Haplotypes 47 and 48 are parthenogenetic populations from the Iberian Peninsula (La Mata, Alicante) and Ukraine respectively. Haplotypes 49 to 144 correspond to bisexual and parthenogenetic populations from Asia (China, Iran, Tibet, Turkey) and the Mediterranean region (Bulgaria, Greece, Iberian Peninsula, Ukraine). Haplotype 145 is from a parthenogenetic population from Turkey. Genetically distant, haplotypes 146 to 161 are from bisexual populations from Tibet, and northern China.

Phylogeny of Artemia

The topology of the mitogenomic Bayesian analysis was totally congruent with the topology of the ultrametric tree obtained with BEAST (Fig. 2B). All nodes are supported with a posterior probability of 1 (PP = 1). Phylogenetic relationships within the main Artemia lineages concur with those previously proposed by Sainz-Escudero et al. (2021). Parthenogenetic samples were recovered in two non-sister clades. One parthenogenetic sample from Murcia (Spain) with unknown ploidy (mtArt_1), falls within the A. urmiana clade closely related with the sample of Urmia Lake, Iran (Fig. 2B). The samples of unknown ploidy from Santarem (Portugal) (mtArt_2), Guadalajara (Spain) (mtArt_3) and Zaragoza (Spain) (mtArt_4) conform a monophyletic group closely related with A. sinica (Fig. 2B).

The divergence time estimates for the main clades concur with that previously proposed by Sainz-Escudero et al. (2021) (Fig. 4). The time to the most recent common ancestor of the clades of bisexual A. urmiana and its parthenogenetic variants is placed during the Pleistocene (Mean 1.25 Ma, 95% HPD 0.90–0.47 Ma). Separation between the A. sinica lineage and its parthenogenetic variants occurred about
more than a half million years later (Mean 0.68 Ma, 95% HPD 1.59–0.95 Ma) (Fig. 4).

Discussion

Our study supports the multiple independent origin of parthenogenesis in Artemia (Muñoz et al. 2010; Maniatsi et al. 2011; Asem et al. 2016) and the geologically recent origin of parthenogenetic populations within A. urmiana and A. sinica lineages (Eimanifar et al. 2015; Sainz-Escudero et al. 2021; Rode et al. 2021). In the Iberian Peninsula, parthenogenetic populations were noted since the end of the twentieth century (Amat 1979, 1980; Triantaphyllidis et al. 1998). Later, a few populations from coastal and inland salterns were identified as part of the Asian lineages because of their phylogenetic relation with bisexual A. urmiana and A. sinica (Muñoz et al. 2010; Maniatsi et al. 2011; Maccari et al. 2013a). Our phylogeographic analyses corroborate the Asian origin of Iberian parthenogenetic samples. Cox1 haplotypes shown by parthenogenetic Iberian populations are shared with those of parthenogens from Asian localities, or are differentiated from them by no more than three point mutations, indicating shallow and recent divergences. Parthenogenetic populations of the A. urmiana lineage inhabit Iberian inland and coastal salterns, although they are more frequent in coastal ones (Amat 1980; Amat et al. 1995; Muñoz et al. 2010, 2014; Maccari et al. 2013a; this study). By contrast, parthenogenetic populations of the A. sinica lineage are mainly found in inland salterns (Amat 1980, this study) with only two coastal locations previously registered (Maniatsi et al. 2011; Maccari et al. 2013a).

Causes for the arrival of parthenogens to the Iberian Peninsula might be diverse. Bird-mediated dispersal seems likely for the colonization of large coastal salterns currently dominated by A. urmiana parthenogens (Persoone and Sorgeloos 1980; Green et al. 2005; Sánchez et al. 2012; Muñoz et al. 2013, 2014), but not for inland ones, predominantly occupied by A. sinica parthenogens, because of their smaller size, and generally less suitable conditions for bird feeding or nesting. The rise of salterns development makes anthropogenic transport also likely
Brine shrimps adrift: historical species turnover in Western Mediterranean Artemia

Léger et al. 1986; Sorgeloos et al. 2001; Dhont and Sorgeloos 2002; Van Stappen et al. 2007; Muñoz et al. 2008). The deliberate inoculation of Artemia by man in salterns have been a common practice because of the benefits that they generate for sea-salt production (Davis 1974; Persoone and Sorgeloos 1980; Dhont and Sorgeloos 2002), as it has been already reported in Australia (McMaster et al. 2007). In the Iberian case, the presence of A. urmiana in only a few inland salterns could be explained by current anthropogenic dispersal from coastal salterns to these precise inland salterns, most of them recently remodelled and conditioned for salt production (e.g. Oro and Imón salterns, in Navarra and Guadalajara respectively) or where the species has been possibly introduced for aquaristic purposes (La Seca, Madrid).

But, is the presence of parthenogenetic strains disturbing the stability of Mediterranean native A. salina populations? Historical records indicate that the native A. salina was present in many Iberian coastal and inland salterns (Amat 1980; Amat et al. 1995; Triantaphyllidis et al. 1998). Previous data also indicate that A. salina and Asian parthenogens were able to coexist in some places (Amat 1980; Amat et al. 1995; Barata et al. 1995; Van Stappen 2002). However, two salterns sampled in this study, Poza de la Sal (Burgos) (Fig. 5C) and San Pedro del Pinatar (Murcia) have apparently lost their populations of A. salina (Amat 1980; Amat et al. 1995), and are now occupied only by A. sinica and A. urmiana parthenogens respectively (Amat et al. 2007, this study). Some other salterns, like Bras del Port (Alicante), Savina (Formentera) or San Fernando ( Cádiz), have been recently colonized by parthenogens (Maccari et al. 2013a, b; this study) and probably will have a similar fate. The decline of A. salina likely reflects the higher colonization success of polyploid parthenogens (Browne and MacDonald 1982; Browne et al. 1988; Zhang and King 1993; but see Browne and Wanigasekera 2000). In fact, the colonization ability of parthenogens may even overcome that of the invasive American species in continental environments, because so far, only one population of this species has been found in Iberian inland salterns (Gerri de la Sal-Lleida, Amat et al. 2007; Muñoz et al. 2014 sub A. franciscana). These data provide an evidence of the displacement effect and competitive potential of parthenogens against A. salina populations and thus, confirming their invasive character.

However, the decline of A. salina in Iberia (Muñoz et al. 2008; this study) is not only a consequence of the parthenogens invasion, but also of a generalized abandonment and decay of inland salterns (Amat et al. 2007). Many inland salterns where the presence of Artemia was previously reported (Triantaphyllidis et al. 1998; Muñoz et al. 2008) are no longer in use and their tanks and ponds abandoned and dried-out (Armallá and Rienda in Guadalajara, Arcos de las
Salinas in Teruel, Peralta de la Sal in Huesca, and a long etc.) (Fig. 5A, B). New surveys are still needed in order to check for the continuity of the remaining populations in the near future (Fig. 5D). The evident decline of *A. salina*, together with the relative facility to reverse the situation in inland habitats, makes conservation actions urgent.

**Conclusions**

Our phylogenetic and phylogeographic analyses support a recent origin of parthenogenesis within the lineages of *A. sinica* and *A. urmiana* and also the recent colonization of the Western Mediterranean by Asian parthenogenetic populations. In Iberia, Asian parthenogens have replaced previously established populations of *A. salina*, which together with a dramatic loss of habitat due to the abandonment and decay of inland salterns, is driving *A. salina* to a critical situation, worsened by the invasion of coastal salt lakes by the American species, *A. monica* (Amat et al. 1995, 2007; Muñoz et al. 2008). In this situation, feasible conservation measures are necessary. Development of *A. salina* hatcheries would be desirable to avoid the import and spread of the American and Asian species. The restoration of Iberian historical inland salterns (Neolithic, Roman, Medieval, or pre-Industrial Revolution) and the inoculation of *A. salina* native cysts would be useful and effective steps to maintain an inland network of populations less susceptible to further replacement.

The case of *Artemia* invasions shows that the distribution range and population fate of the species of *Artemia* currently depends on human economical activities, not only because of the strong commercial displacement to which they are subjected, but because of their almost complete affiliation and success in artificial salterns (Carscadden et al. 2020). If human activities have been responsible to drive a species from centuries of historical population success to a current dramatic population decline, as it is the case of Mediterranean *A. salina*, we should be also responsible to revert its current pathway to extinction.

**Acknowledgements** We thank Alberto Sánchez, Cecilia Díaz, Ernesto Recuero, Jose Luis Ruiz, Leticia Puerta, Marta Calvo, Nerea Quesada, Nuria Cardo, Paloma Mas and Rubén González for their help during sampling. We greatly appreciate the kind help received in the salterns of Aveiro, the Añana Neolithic salterns and in the historical salterns of Peralta de la Sal. We also thank Begoña Sánchez, curator of the Arthropod Collection of the Museo Nacional de Ciencias Naturales and Beatriz Álvarez from the Tissue and DNA Collection for all the resources provided, and Luis M. Bautista, Juan E. Uribe and Natalía Rosas Ramos for their research advice and manuscript improvements. Finally, we thank the Comunidad Autónoma de Madrid (Spain), for the concession of the “Doctorado Industrial” grant (see funding acknowledgments), and the MNCN-CSIC for all facilities provided.

**Authors contribution** LS-E Conceptualization, methodology, formal analysis, resources, writing (original draft), supervision. EKL-E Conceptualization, methodology, formal analysis, supervision. PCR-F Conceptualization, resources, supervision. MG-P Conceptualization, methodology, resources, writing (original draft), supervision, project administration, funding acquisition.

**Funding** Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This research was funded by the project-grant IN2018/AMB9692 (“Doctorado Industrial” program of Comunidad Autónoma de Madrid) to MG-P. LS-E is supported by the “Doctorado Industrial” grant (IND2018/AMB9692), through the Fundación Global Nature, with support of MITECO (Ministerio para la Transición Ecológica y el Reto Demográfico, Spain). EKL-E is supported by a doctoral scholarship from CONACyT-México (330519/472100). PCR-F is supported by a E.O Wilson post-doctoral fellowship from the MCZ (Museum of Comparative Zoology) at Harvard University.

**Availability of data and material** The datasets generated and analysed during the current study are available for their visualization and download at the GenBank repository. GenBank accession numbers are included in the manuscript. DNA samples have been deposited at the Crustacean Collection of the MNCN (Museo Nacional de Ciencias Naturales) in Madrid, Spain. MNCN codes are included in the manuscript.

**Declarations**

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Consent for publication** All authors agreed to publish the manuscript.

**Consent to participate** All authors agreed to participate in the study and its publication.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included.
in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Abatzopoulos TJ, Amat F, Baxevanis AD, Belmonte G, Hontoria F, Maniatis S, Moscatoello S, Mura G, Shadrin NV (2009) Updating geographic distribution of Artemia urmi-ana Günther, 1890 (Branchiopoda: Anostraca) in Europe: an integrated and interdisciplinary approach. Int Rev Hydrobiol 94(5):560–579. https://doi.org/10.1002/irh.200911147

Amat F, Barata C, Hontoria F (1995) A Mediterranean origin for the Veldrif (South Africa) Artemia Leach population. J Biogeogr 22:49–59. https://doi.org/10.2307/2846072

Amat F, Hontoria F, Navarro JC, Vieira N, Mura G (2007) Biodiversity loss in the genus Artemia in the Western Mediterranean Region. Limn 26(2):387–404

Amat F, Hontoria F, Navarro JC, Cohen RG, Rodríguez-Gil SG (1994) Aproximación preliminar a la distribución del genero Artemia (especie A. persimilis) en Argentina. Provincias de Buenos Aires y La Pampa. VIII Congreso Latinoamericano de Acuicultura. Santa Fe de Bogotá, Colombia, pp 73–84

Amat F (1979). Diferenciación y distribución de las poblaciones de Artemia (Crustáceo branquiópodo) de España. Doctoral dissertation, Universidad de Barcelona, Barcelona, Cataluña.

Amat F (1980). Differentiation in Artemia strains from Spain. In: Persoone G, Sorgeloos P, Roels OA, Jaspers E (eds) The brine shrimp, Artemia, vol1. Morphology, genetics, radiobiology, toxicology. Universa Press, Belgium, pp 19–40

Asem A, Eimanifar A, Sun SC (2016) Genetic variation and evolutionary origins of parthenogenetic Artemia (Crustacea: Anostraca) with different ploidies. Zool Scr 45:421–436. https://doi.org/10.1111/zsc.12162

Asem A, Eimanifar A, Li W, Wang PZ, Brooks SA, Wink M (2020) Phylogeography and population genetic structure of an exotic invasive brine shrimp, Artemia Leach, 1819 (Crustacea: Anostraca), in Australia. Aus J Zool 66:307–316. https://doi.org/10.1071/ZO18077

Asem A, Eimanifar A, van Stappen G, Sun SC (2019) The impact of one-decade ecological disturbance on genetic changes: a study on the brine shrimp Artemia urmiana from Urmia Lake, Iran. PeerJ. https://doi.org/10.7717/peerj.7190

Asem A, Rastegar-Pouyani N, De Los Ríos-Escalante P (2010) The genus Artemia Leach, 1819 (Crustacea: Branchiopoda). I. True and false taxonomical descriptions. Lat Am J Aquat Res 38(3):501–506

Barata C, Hontoria F, Amat F (1995) Life history, resting egg formation, and hatching may explain the temporal-geographical distribution of Artemia strains in the Mediterranean basin. Hydrobiol 298:295–305. https://doi.org/10.1007/978-94-011-0291-9_28

Baxevanis AD, Kappas I, Abatzopoulos TJ (2006) Molecular phylogenetics and asexuality in the brine shrimp Artemia. Mol Phylogenet Evol 40:724–738. https://doi.org/10.1016/j.ympev.2006.04.010

Ben Naceur H, Ben-Rejeb JA, Romdhane MS (2012) Review of the biogeography of Artemia Leach, 1819 (Crustacea: Anostraca) in Tunisia. Int J Artemia Biol 2:24–39

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF (2013) MITOS: Improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol 69(2):313–319. https://doi.org/10.1016/j.ympev.2012.08.023

Böhn T, Amundsen PA, Sparrow A (2008) Competitive exclusion after invasion? Biol Invasions 10:359–368. https://doi.org/10.1007/s10530-007-9135-8

Browne RA, Bowen ST (1991) Taxonomy and populations genetics of Artemia. In: Browne R, Sorgeloos P, Trotman C (eds) Artemia biology. CRC Press, Boca Raton, Florida, pp 221–235

Browne RA, MacDonald GH (1982) Biogeography of the brine shrimp, Artemia: distribution of parthenogenetic and sexual populations. J Biogeogr 9(4):331–338. https://doi.org/10.1038/20744719

Browne RA, Wanigasekera G (2000) Combined effects of salinity and temperature on survival and reproduction of five species of Artemia. J Exp Mar Biol Ecol 244:29–44. https://doi.org/10.1016/S0022-0981(99)00125-2

Browne RA, Davis LE, Sallee SE (1988) Effects of temperature and relative fitness of sexual and asexual brine shrimp Artemia. J Exp Mar Biol Ecol 124(1):1–20. https://doi.org/10.1016/0022-0981(88)90201-8

Cai Y (1989) New Artemia sibling species from PR China. Artemia News 11(1998):40–41

Carscadden KA, Emery NC, Arnillas CA, Cadotte MW, Afkhami ME, Gravel D, Linvingstone SW, Wiens JJ (2020) Niche breadth: causes and consequences for ecology, evolution, and conservation. Q Rev Biol 95(3):179–214. https://doi.org/10.1086/710388

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17(4):540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Céspedes V, Sánchez MI, Green AJ (2017) Brine shrimp Artemia parthenogenetica and the alien boatman Trichocorixa verticalis: influence of salinity, predator sex, and size, abundance and parasitic status of prey. PeerJ 5:e3554. https://doi.org/10.7717/peerj.3554

Dafni A, Kevan P, Gross CL, Goka K (2010) Bombus terrestris, pollinator, invasive and pest: an assessment of problems associated with its widespread introductions for commercial purposes. Appl Entomol Zool 45(1):101–113. https://doi.org/10.1030/aez.2010.101

Davis JS (1974) Importance of microorganisms in solar salt production. In: Coogan AL (ed), 4th Symposium on salt, vol 1. Northern Ohio Geological Society, Cleveland

Dhont J, Sorgeloos P (2002) Applications of Artemia. In: Abatzopoulos TJ, Beardmore J, Clegg JS, Sorgeloos P (eds)
Artemia: basic appl biol. Springer Science, Dordrecht, pp 251–277

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol 29(8):1969–1973. https://doi.org/10.1093/molbev/mss075

Eimanifar A, Wink W (2013) Fine-scale population genetic structure in Artemia urmiana (Günther, 1890) based on mtDNA sequences and ISSR genomic fingerprinting. Org Divers Evol 13:531–543. https://doi.org/10.1007/s13127-013-0135-5

Eimanifar A, Van Stappen G, Marden B, Wink M (2014) Artemia biodiversity in Asia with the focus on the phylogeography of the introduced American species Artemia franciscana Kellogg, 1906. Mol Phylogenet Evol 79:392–403. https://doi.org/10.1016/j.ympev.2014.06.027

Eimanifar A, Van Stappen G, Wink M (2015) Geographical distribution and evolutionary divergence times of Asian populations of the brine shrimp Artemia (Crustacea, Anostraca). Zool J Linnean Soc 174(3):447–458. https://doi.org/10.1111/zool.12242

Eimanifar A, Asem A, Djamali M, Wink M (2016) A note on the biogeographical origin of the brine shrimp Artemia urmiana Günther, 1899 from Urmia Lake, Iran. Zootaxa 4097(2):294–300. https://doi.org/10.11646/zootaxa.4097.2.12

Figuls A, Weller O, Bonache J, González J (2007) El método Eimanifar A, Sánchez MI, Amat F, Figuerola J, Hontoria F, Ruiz Horváth Z, Lejeune C, Amat F, Sánchez-Fontenla J, Vad CF, Green AJ (2018) Eastern spread of the invasive Artemia franciscana in the Mediterranean Basin, with the first record from the Balkan Peninsula. Hydrobiol 822(1):229–235. https://doi.org/10.1007/s10750-018-3683-z

Hou L, Bi X, Zou X, He C, Yang L, Qu R, Liu Z (2006) Molecular systematics of bisexual Artemia populations. Aquac Res 37(7):671–680. https://doi.org/10.1111/j.1365-2109.2006.01480.x

Hwang JY, Haque N, Lee DH, Kim BM, Rhee JS (2019) Complete mitochondrial genome of the intertidal hermit crab, Pagurus similis (Crustacea, Anomura). Mitochondrial DNA Part B 4(1):1861–1862. https://doi.org/10.1080/23802359.2019.1613183

Kaiser H, Gordon AK, Paulet TG (2006) Review of the African distribution of the brine shrimp genus Artemia. Water Res 32(4):597–603. https://doi.org/10.4314/wsa.v32i4.5284

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 9(4):286–298. https://doi.org/10.1093/bib/bbn013

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinform 668(28):1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Leach WE (1819) Entomostracés. In: Dictionnaire des Sciences Naturelles, dans lequel on traité méthodiquement des différens êtres de la nature, considérés soit en eux-mêmes, d’après l’état actuel de nos connaissances, soit relativement a l’utilité qu’en peuvent retirer la médecine, l’agriculture, le commerce et les arts. Suivi d’une biographie des plus célèbres naturalistes. Tome quatorzième. XIVParis: Levraut

Léger P, Bengtson DA, Simpson KL, Sorgelosop P (1986) The use and nutritional value of Artemia as food source. Oceanogr Mar Biol Ann Rev 24:521–623

Leigh JW, Bryant D (2015) PopART: full-feature software for haplotype network construction. Methods Ecol Evol 6(9):1110–1116. https://doi.org/10.1111/2041-210X.12410

Lewis JS, Corn JL, Mayer JJ, Jordan TR, Farnsworth ML, Burdett CL, VerCauteren KC, Sweeney SJ, Miller RS (2019) Historical, current, and potential population size estimates of invasive wild pigs (Sus scrofa) in the United States. Biol Invasions 21:2373–2384. https://doi.org/10.1007/s10530-019-01983-1

Linnaeus C (1758) Systema Naturae per Regna tria Naturae, secundum Classes, Ordines, Genera, Species, cum characteribus, differentiis, synonymis, locis. Ed X vol 1. London: Natural History Museum, 824

Maccari M, Gómez A, Hontoria A, Amat F (2013a) Functional rare males in diploid parthenogenetic Artemia. J Evol Biol 26:1934–1948. https://doi.org/10.1111/jeb.12191

Maccari M, Amat F, Gómez A (2013b) Origin and Genetic Diversity of diploid parthenogenetic Artemia in Eurasia. PLoS ONE 8(12):e83348. https://doi.org/10.1371/journal.pone.0083348

Maddison WP, Maddison DR (2018) Mesquite: a modular system for evolutionary analysis. Version 3.51. http://www.mesquiteproject.org

Maniatis S, Kappas I, Baxevanis AD, Farmaki T, Abatzopoulos TJ (2009) Sharp phylogeographic breaks and patterns.
of genealogical concordance in the brine shrimp *Artemia franciscana*. Int J Mol Sci 10(12):5435–5470. https://doi.org/10.3390/ijms10125455

Maniatis S, Baxevanis AD, Kappas I, Deligiannidis P, Triantafyllidis A, Papakostas S, Bougioukis D, Abatzopoulos TJ (2011) Is polyplody a preserving accident or an adaptive evolutionary pattern? The case of the brine shrimp *Artemia*. Mol Phylogenet Evol 58:353–364. https://doi.org/10.1016/j.ympev.2010.11.029

Manrique JT (2011) La producción de sal n la Prehistoria de la Península Ibérica: estado de la cuestión. Arqueol Territ 8:71–84

Manzi V, Lugli S, Roveri M, Dela Pierre F, Gennari R, Lozar Maniatsi S, Baxevanis AD, Kappas I, Deligiannidis P, Trian-

Naganawa H, Mura G (2017) Two new cryptic species of *Arte-
mia* (Branchiopoda, Anostraca) from Mongolia and the possibility of invasion and disturbance by the aquaculture industry in East Asia. Crustaceana 90(14):1679–1698. https://doi.org/10.1163/15685403-00003744

Oscoz J, Tomás P, Durán C (2010) Review and new records of non-indigenous freshwater invertebrates in the Ebro River basin (Northeast Spain). Aquat Invasions 5(3):263–284. https://doi.org/10.3391/ai.2010.5.3.04

Persoone G, Sorgeloos P (1980) General aspects of the ecology and biogeography of Artemia. In: Persoone G, Sorgeloos P, Roels O, Jaspers E (eds) The brine shrimp *Artemia*, Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Belgium, pp 3–24

Pinto PM, Bio A, Hontoria F, Almeida V, Vieira N (2013) Portuguese native *Artemia parthenogenetica* and *Artemia franciscana* survival under different abiotic conditions. J Exp Mar Biol Ecol 440:81–89. https://doi.org/10.1016/j.jembe.2012.11.016

Rode NO, Jabbour-Zahab R, Boyer L, Flaven E, Hontoria F, Van Stappen G, Dufresne F, Haag C, Lenormand T (2021) The origin of asexual brine shrimps. Available as preprint at bioRxiv https://doi.org/10.1101/2021.06.11.440804

Rodríguez-Flores PC, Jiménez-Ruiz Y, Forró L, Vörös J, García-París M (2017) Non-congruent geographic patterns of genetic divergence across European *Branchinecta* (Anostraca: Branchinectidae). Hydrobiol 801(1):47–57. https://doi.org/10.1007/s10750-017-3266-4

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Lartet B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61(3):539–542

Rozas J, Ferrer-Mata A, Sánchez-Del Barrio JC, Guirao-Rico S, Llibre D, Ramos-Onsins SE, Sánchez-García A (2017) DnaSP 6: DNA sequence polymorphism analysis of large datasets. Mol Biol Evol 34:3299–3302. https://doi.org/10.1093/molbev/msx248

Ruebhart DR, Cock IE, Shaw GR (2008) Invasive character of the brine shrimp *Artemia franciscana*. Kellogg 1906 (Branchiopoda: Anostraca) and its potential impact on Australian inland hypersaline waters. Mar Freshw Res 59(7):587–595. https://doi.org/10.1071/MF07221

Sainz-Escudero L, López-Estrada EK, Rodríguez-Flores PC, García-París M (2021) Settling taxonomic and nomenclatural problems in brine shrimps, *Artemia* (Crustacea: Branchiopoda: Anostraca), by integrating mitogenomics, marker discordances and nomenclature rules. PeerJ 9:e10865. https://doi.org/10.7717/peerj.10865

Saji A, Eimanifar A, Soorae PS, Al Dhaheri SA, Li W, Wang PZ, Asem A (2019) Phylogenetic analysis of exotic invasive species of the brine shrimp *Artemia Leach*, 1819 (Branchiopoda, Anostraca) in Al Wathba Wetland Reserve (U.A.E.; Abu Dhabi). Crustaceana 92(4):495–503. https://doi.org/10.1163/15685403-00003884

Sala J, Gascón C, Cunillera-Montcusi D et al (2017) Defining the importance of landscape metrics for large branchiopod biodiversity and conservation: the case of the Iberian Peninsula and Balearic Islands. Hydrobiol 801(1):81–98. https://doi.org/10.1007/s10750-017-3293-1

Sánchez M, Horta F, Figueroa J, Green AJ (2012) Comparing the potential for dispersal via waterbirds of a native and an invasive brine shrimp. Freshw Biol 57:1869–1903. https://doi.org/10.1111/j.1365-2427.2012.02852.x

Scalone R, Rabet N (2013) Presence of *Artemia franciscana* (Branchiopoda, Anostraca) in France: morphological,
genetic, and biometric evidence. Aquat Invasions 8(1):67–76. https://doi.org/10.3391/ai.2013.8.1.08

Senar JC, Carrillo-Ortiz JG, Ortega-Segaleria A, Dawson-Pell FSE, Pascual J, Arroyo L, Mazzoni D, Montalvo T, Hatchwell BJ (2019) The reproductive capacity of Monk Parakeets Myiopsitta monachus is higher in their invasive range. Bird Study 66(1):136–140. https://doi.org/10.1080/00063657.2019.1585749

Sorgeloos P, Dhert P, Candreva P (2001) Use of the brine shrimp, Artemia spp., in marine fish larviculture. Aquac 2001:147–159. https://doi.org/10.1016/S0044-8486(01)00698-6

Tizol-Correa R, Maeda-Martínez AM, Weekers PH, Torrentera L, Murugan G (2009) Biodiversity of the brine shrimp Artemia from tropical saltlens in southern Mexico and Cuba. Curr Sci 96(1):81–87

Tollenaere C, Brouat C, Duplantier JM, Rahalison L, Rahelinirina S, Pascal M, Moné H, Mouahid G, Leirs H, Cosson JF (2010) Phylogeography of the introduced species Rattus rattus in the western Indian Ocean, with special emphasis on the colonization history of Madagascar. J Biogeogr 37:398–410. https://doi.org/10.1111/j.1365-2699.2009.02228.x

Triantaphyllidis GV, Pilla EJS, Thomas KM, Abatzopoulos TJ, Beardmore JA, Sorgeloos P (1994) International Study on Artemia. LII. Incubation of Artemia cyst samples at high temperature reveals mixed nature with Artemia franciscana cysts. J Exp Mar Biol Ecol 183:273–282. https://doi.org/10.1016/0022-0981(94)90092-2

Triantaphyllidis GV, Abatzopoulos TJ, Sorgeloos P (1998) Review of the biogeography of the genus Artemia (Crustacea, Anostraca). J Biogeogr 25:213–226. https://doi.org/10.1046/j.1365-2699.1998.252190.x

Triantaphyllidis GV, Abatzopoulos TJ, Miasa E, Sorgeloos P (1996) International study on Artemia. LVI. Characterization of two Artemia populations from Namibia and Madagascar: cytogenetics, biometry, hashing characteristics and fatty acid profiles. Hydrobiol 335(2):97–106. https://doi.org/10.1007/BF00015271

Valsala G, Sugathan S, Bharathan H (2015) Loss of indigenous brine shrimp Artemia parthenogenetica due to the invasion by American species Artemia franciscana at Thamaraikulam salt pan. Indian J Mar Sci 44(11):1712–1715

Van Stappen G (2002) Zoogeography. In: Abatzopoulos TJ, Beardmore J, Clegg JS, Sorgeloos P (eds) Artemia: basic and applied biology. Springer Science, Dordrecht, pp 171–224

Van Stappen G, Yu H, Wang X, Hoffman S, Cooreman K, Bossier P, Sorgeloos P (2007) Occurrence of allochthonous Artemia species in the Bohai Bay area, PR China, as confirmed by RFLP analysis and laboratory culture tests. Fundam Appl Limnol 170(1):21–28. https://doi.org/10.1127/1863-9135/2007/0170-0021

Vanhaecke P, Tackaert W, Sorgeloos P (1987) The biogeography of Artemia: an updated review. In: Sorgeloos P, Bengtson DA, Decleir W, Jaspers E (eds) Artemia research and its applications vol 1. Morphology, genetics, strain characterization, toxicology. Universa Press, Belgium, pp 129–155

Verrill AE (1869) Descriptions of some new American Phyllopod Crustacea. American Journal of Science and Arts 48(143), 244–254 [Contributions to Zoology from the Museum of Yale College, 3]. Published again as: Verrill AE (1869) Descriptions of some new American Phyllopod Crustacea. Annals Mag Nat Hist 4:331–341

Vikas PA, Sajeshkumar NK, Thomas PC, Chakraborty K, Vijayan KK (2012) Aquaculture related invasion of the exotic Artemia franciscana and displacement of the autochthonous Artemia populations from the hypersaline habitats of India. Hydrobiol 684(1):129–142. https://doi.org/10.1007/s10750-011-0976-x

Weller O, Dumitroaia G (2005) The earliest salt production in the world: an early Neolithic exploitation in Poiana Slatinei-Lunca, Romania. Antiquity 79(306). https://hal.archives-ouvertes.fr/hal-03036529

Wilgenbusch JC, Swoford D (2003) Inferring evolutionary trees with PAUP*. Curr Protoc Bioinform 6.4.1–6.4.28. https://doi.org/10.1002/0471250953.bi0604s00

Zhang L, King CE (1993) Life history divergence of sympatric diploid and polyploid populations of brine shrimp Artemia parthenogenetica. Oecologia 93:177–183. https://doi.org/10.1007/BF00317668

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.