



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

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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.

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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 piscifactorías 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 (Branchiopoda: 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 Prosdociami,

1968 (Amat et al. 1994). However, Neolithic originated salt harvesting for human needs (Weller and Dumitroaia 2005; Fíguls 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

(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. 1996; Vanhaecke et al. 1987; Maniatsi 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 parthenogenetic 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 $-20\text{ }^{\circ}\text{C}$ 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 2 μL of extracted DNA, 1 μL of dNTP (10 mM), 2.5 μL of reaction buffer 10x (Tris-HCl, pH 8.3, Biotools), 0.8 μL of MgCl_2 (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 $95\text{ }^{\circ}\text{C}$ for initial denaturation, 40 cycles of 45 s at $42\text{ }^{\circ}\text{C}$ and 1 min at $72\text{ }^{\circ}\text{C}$, and a final extension of 10 min at $72\text{ }^{\circ}\text{C}$. Amplified PCR products were visualized by electrophoresis in a 1.5% agarose gel. Samples

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

Table 1 (continued)

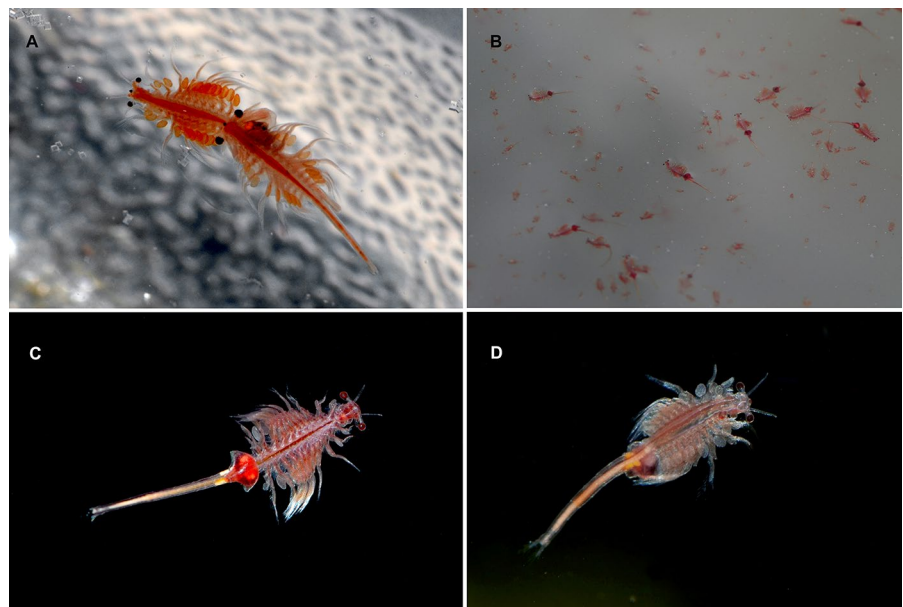
MNCN DNA codes	Field number	GenBank accession numbers	Sample size	Species	Locality	Geographical coordinates
119922, 119923	art27, art28	OM487001, OM487002	2	<i>Artemia sinica</i> (parthenogenetic)	Portugal: Santarem, Rio Maior Salterns	39°21'49.0"N, 8°56'35.6"W
119942, 119943	art29, art30	OM487003, OM487004	2	<i>Artemia sinica</i> (parthenogenetic)	Spain: Álava, Añana Salterns	42°48'05.2"N, 2°59'09.8"W
119944, 119945	art31, art32	OM487005, OM487006	2	<i>Artemia sinica</i> (parthenogenetic)	Spain: Burgos, Poza de la Sal	42°40'11.8"N, 3°30'19.1"W
119936, 119937	art33, art34	OM487007, OM487008	2	<i>Artemia sinica</i> (parthenogenetic)	Spain: Cuenca, Manzano Salterns	40°05'20.6"N, 1°33'26.3"W
119926, 119927	art35, art36	OM487009, OM487010	2	<i>Artemia sinica</i> (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	<i>Artemia sinica</i> (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	<i>Artemia sinica</i> (parthenogenetic)	Spain: Toledo, Quero, Laguna Grande Salterns	39°30'15.8"N, 3°14'51.8"W
119924, 119925	art42, art43	OM487016, OM487017	2	<i>Artemia sinica</i> (parthenogenetic)	Spain: Toledo, Villacañas, Peña Hueca Lagoon	39°31'10.8"N, 3°20'09.5"W
119919	art44	OM487018	1	<i>Artemia sinica</i> (parthenogenetic)	Spain: Toledo, Villacañas, Tirez Lagoon	39°32'36.0"N, 3°21'3.3"W
119968	art45	OM487019	1	<i>Artemia sinica</i> (parthenogenetic)	Spain: Zaragoza, Bujaraloz, Playa Salterns	41°25'17.9"N, 0°11'49.3"W
119950, 119951	art46, art47	OM487020, OM487021	2	<i>Artemia urmiana</i> (parthenogenetic)	Portugal: Aveiro, Aveiro Salterns	40°37'43.7"N, 8°39'40.4"W
119966	art48	OM487022	1	<i>Artemia urmiana</i> (parthenogenetic)	Sardegna: Cagliari, Stagno di Quartu	39°13'15.3"N, 9°11'11.7"E
119969, 119970	art49, art50	OM487023, OM487024	2	<i>Artemia urmiana</i> (parthenogenetic)	Spain: Alicante, Calpe, Calpe Salterns	38°38'48.6"N, 0°4'0.1"E
119946, 119947	art51, art52	OM487025, OM487026	2	<i>Artemia urmiana</i> (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	<i>Artemia urmiana</i> (parthenogenetic)	Spain: Formentera, Savina Salterns	38°43'47.6"N, 1°25'09.7"E

Table 1 (continued)

MNCN DNA codes	Field number	GenBank accession numbers	Sample size	Species	Locality	Geographical coordinates
119940, 119941	art55, art56	OM487029, OM487030	2	<i>Artemia urmiana</i> (parthenogenetic)	Spain: Guadajajara, Imón Salterns	41°09'32.1"N, 2°43'34.8"W
119934, 119935	art57, art58	OM487031, OM487032	2	<i>Artemia urmiana</i> (parthenogenetic)	Spain: Madrid, Villaconejos, La Seca Lagoon	40°05'14.8"N, 3°32'05.8"W
119920, 119921	art59, art60	OM487033, OM487034	2	<i>Artemia urmiana</i> (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	<i>Artemia urmiana</i> (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 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



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 (Kato and Toh 2008), and revised with Mesquite v.3.5.1 (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

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 aminoacids were removed from the analyses due to the existence of pseudogenes according to Rode et al. (2021).

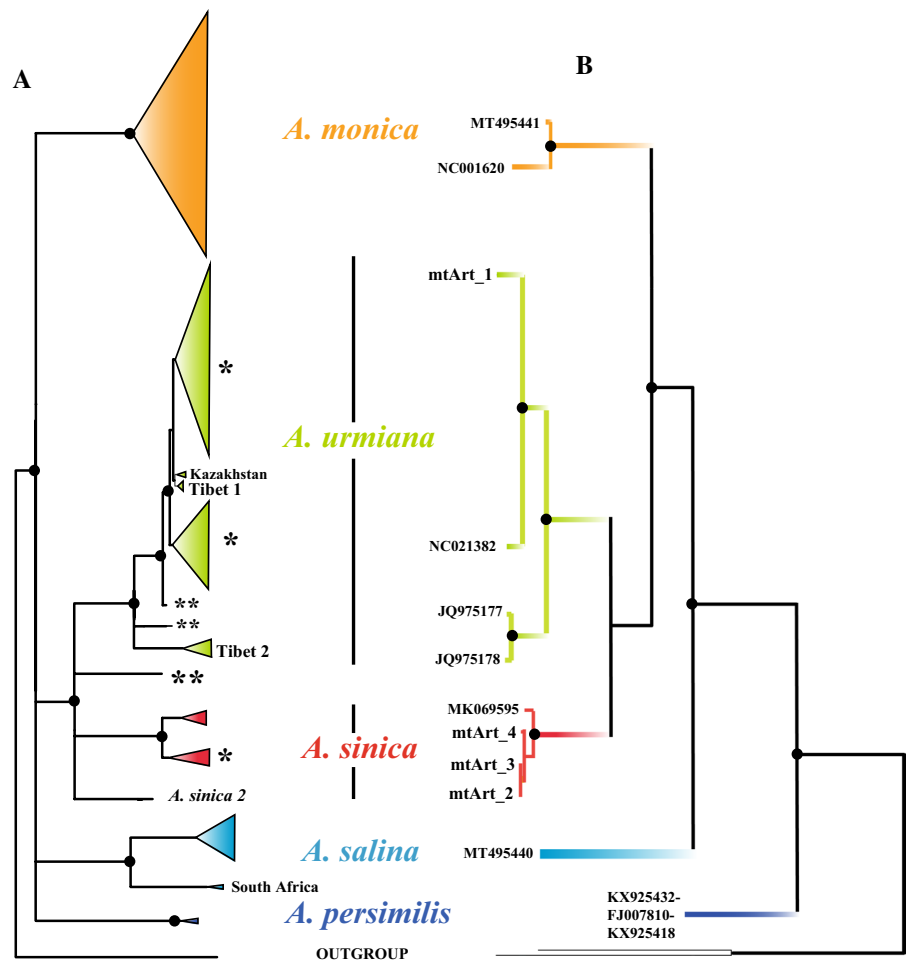
In parallel, four parthenogenetic individuals (one corresponding to *A. urmiana* lineage and three to *A. sinica*) from four localities in the Iberian Peninsula were chosen to be high-throughput sequenced (at AllGenetics, A Coruña, Spain; Table 1). The DNA extraction protocol, library preparation, and 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 *coxI* 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 (*coxI*) analyses and phylogeography

In order to visualize the general structure inside the genus *Artemia*, the *coxI* 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 *coxI* 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

Fig. 3 Haplotype networks based on *cox1* sequence data; **A** corresponds to parthenogenetic specimens of *A. sinica* and **B** to *A. urmiana*. Size of circles is proportional to the number of individuals sharing the haplotype. Numbers identify haplotypes. Black dots between haplotypes represent nucleotide substitutions or mutations. Information about sequence-haplotype correspondence is included in Tables 2 and 3

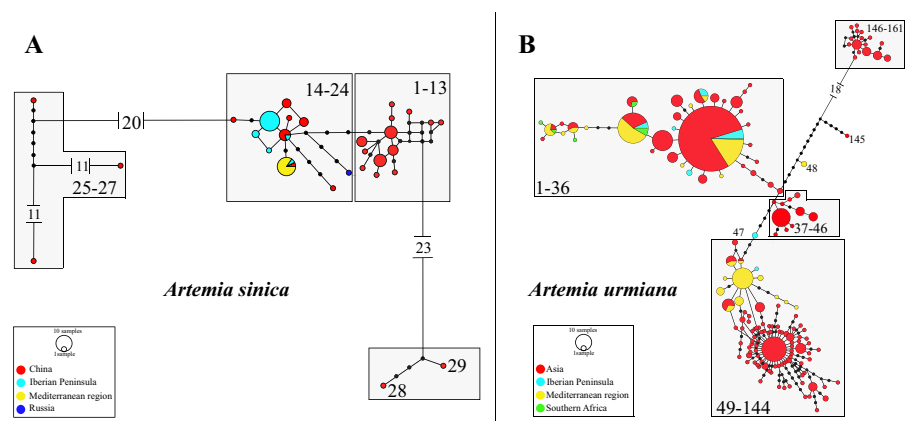


Table 2 MtDNA (*coxI*) sequences of *A. sinica* (bisexuals and parthenogens) used in this study; in the “GenBank accession number” column, a semi-colon separates sequences by groups according to bibliographic sources, as indicated in “Bibliographic source” column

No. Haplotype	Sample size	GenBank accession numbers	Bibliographic source
1	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)

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 *coxI* 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 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; DQ426825; 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; KP090297-317, 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)

Table 3 (continued)

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	KF707675, 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	DQ119651; 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	1	MK682336	Asem et al. (2019)
57	1	MK682371	Asem et al. (2019)
58	7	KF707682; MK682359, 65, 69, 74; JX512756, 64	Maccari et al. (2013b), Asem et al. (2010), Eimanifar and Wink (2013)
59	1	MK682335	Asem et al. (2019)
60	2	JX512774; MK682367	Eimanifar and Wink (2013), Asem et al. (2019)
61	1	MK682320	Asem et al. (2019)
62	1	MK682323	Asem et al. (2019)
63	1	MK682324	Asem et al. (2019)
64	1	MK682327	Asem et al. (2019)
65	1	MK682331	Asem et al. (2019)
66	1	MK682332	Asem et al. (2019)
67	3	MK682330, 44, 78	Asem et al. (2019)
68	1	MK682341	Asem et al. (2019)
69	1	MK682342	Asem et al. (2019)
70	1	MK682354	Asem et al. (2019)
71	1	KF707683	Asem et al. (2019)
72	1	JX51277	Maccari et al. (2013a, b)
73	2	JX512777, JX512801	Eimanifar and Wink (2013)
74	1	JX512794	Eimanifar and Wink (2013)
75	1	JX512797	Eimanifar and Wink (2013)
76	1	JX512802	Eimanifar and Wink (2013)
77	1	MK682361	Eimanifar and Wink (2013)
78	1	MK682376	Asem et al. (2019)
79	1	JX512773	Asem et al. (2019)
80	1	KF691533	Eimanifar and Wink (2013)
81	1	MK682373	Eimanifar et al. (2014)
82	1	JX512779	Asem et al. (2019)
83	1	KF707884	Eimanifar and Wink (2013)
84	1	JX512781	Maccari et al. (2013a, b)
85	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)

Table 3 (continued)

No. Haplotype	Sample size	GenBank accession numbers	Bibliographic source
149	1	KF691246	Eimanifar et al. (2014)
150	1	KF691318	Eimanifar et al. (2014)
151	1	KF691215	Eimanifar et al. (2014)
152	1	KF707904	Maccari et al. (2013b)
153	1	KF691248	Eimanifar et al. (2014)
154	1	KF691217	Eimanifar et al. (2014)
155	1	KF691218	Eimanifar et al. (2014)
156	1	KF691317	Eimanifar et al. (2014)
157	3	KF707900, 01, 03	Maccari et al. (2013b)
158	5	KF707912, 14–17	Maccari et al. (2013b)
159	5	KF707906, 09, 11, 13, 18	Maccari et al. (2013b)
160	1	KF707910	Maccari et al. (2013b)
161	1	KF691316	Eimanifar et al. (2014)

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

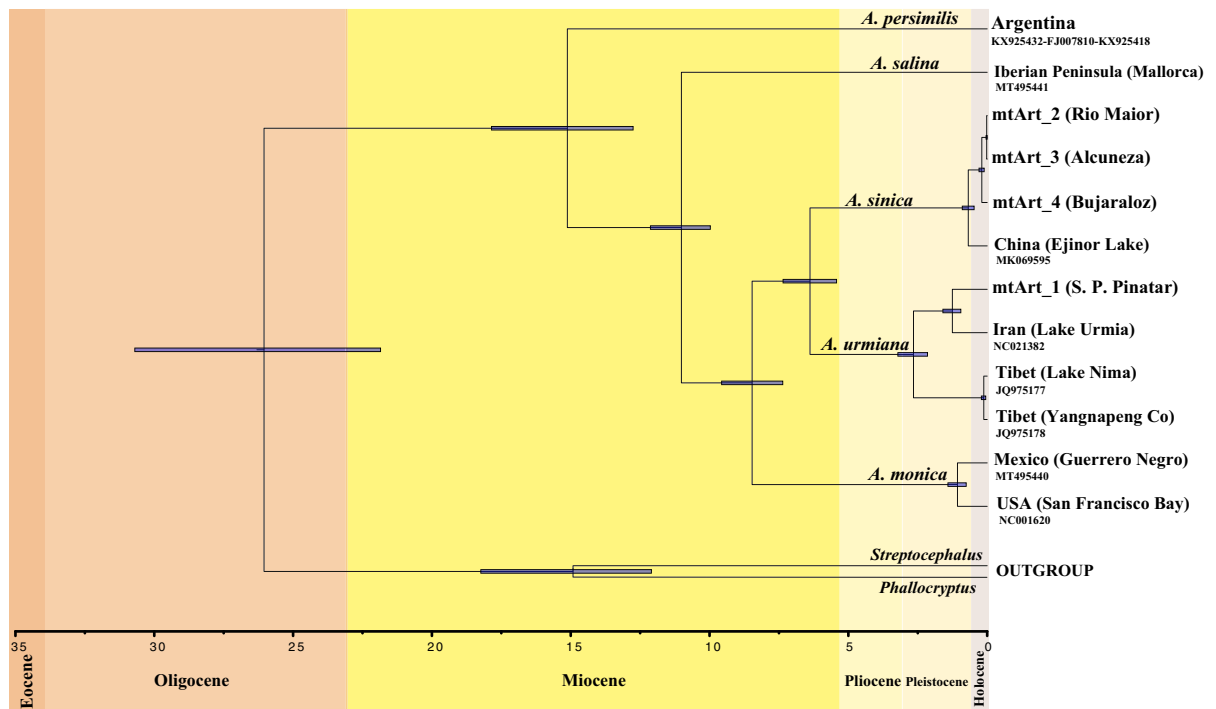


Fig. 4 Chronogram showing lineage divergence times in *Artemia* obtained using BEAST. Time is indicated in million years (Ma). Purple horizontal bars represent 95% HPD (High Posterior Density). A posterior probability value of 1 was obtained for all nodes

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. *CoxI* 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; 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

Fig. 5 Some examples of Iberian inland salterns where the presence of *Artemia* was documented. **A, B** Currently abandoned Medieval inland salterns not so long ago inhabited by *Artemia* populations **A**: Salterns of Armallá (Guadalajara); **B** Salterns of Rienda (Guadalajara). **C** Brine-well in the Medieval salterns of Poza de la Sal (Burgos). In these salterns *A. sinica* parthenogens have replaced native populations of *A. salina*. **D** Recently modified brine-pool in original Roman salterns, still inhabited by *A. salina* (La Malahá, Granada). Photographs by MG-P



(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 work). 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. franciascana*). 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.

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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.

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