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The Occurrence of Parthenogenetic *Artemia* Leach, 1819 (Crustacea: Anostraca) in Cancun Saltern, Mexico

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ABSTRACT

Artemia, commonly known as brine shrimp, constitutes a globally distributed halophilic zooplankton organism, occupying hypersaline environments including inland lakes, salterns, and coastal salt lagoons. The genus *Artemia* encompasses regional endemic species and a diverse array of parthenogenetic lineages, which are characterized by various ploidy levels and distributed across Asia, Europe, Africa, and Australia. The aim of the study is to investigate and determine the taxonomic status of an unusual mitochondrial cytochrome oxidase subunit I (mt*COI*) sequence among 14 different populations of *Artemia franciscana* Kellogg, 1906, collected from Cancun saltern in Mexico. DNA extraction and mt*COI* gene amplification were conducted and taxonomic classification was achieved via BLAST analysis. A phylogenetic tree, constructed using Maximum Likelihood methodology, revealed the phylogenetic relationship between Mexican parthenogenetic *Artemia* and other parthenogenetic lineages of varying ploidy levels. The results unequivocally confirm the presence of parthenogenetic *Artemia* in Cancun saltern, aligning with sequences from Asia and Europe. Phylogenetic analysis positions revealed the Mexican specimens within the clade of diploid parthenogenetic lineages. The competitive vigor and reproductive capabilities of *A. franciscana* appear to have limited the expansion of parthenogenetic *Artemia* in North America. Further research endeavors are essential to unravel the enigmatic biogeography of parthenogenetic *Artemia* in North America and shed light on its potential native or introduced status.

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Introduction

The brine shrimp *Artemia* Leach, 1819, (Crustacea: Anostraca), a cosmopolitan halophilic zooplankton, is an inhabitant of hypersaline inland lakes, saltern, and coastal salt lagoons. The genus *Artemia* comprises several regional endemic species and a large number of parthenogenetic lineages (Asem *et al*., 2010, 2023; Asem and Sun, 2014, 2016; Eimanifar *et al*., 2020). Parthenogenesis in *Artemia* is obligatory and parthenogenetic lineages are characterized by different ploidy levels (di-, tri-, tetra-, and pentaploidy) which are widely distributed in Asia, Europe, Africa, and Australia (Maniatsi *et al*., 2011; Asem *et al*., 2016; Rode *et al*., 2022).

Nevertheless, the existence of parthenogenetic *Artemia* in North America has been assumed since the end of the 19th century. There are several reports on the occurrence of parthenogenetic *Artemia* from Great Salt Lake (UT, USA) (Packard, 1883; Cuellar, 1990).

Campos-Ramos *et al*., (2003) studied commercial eggs from the Great Salt Lake and San Francisco Bay to explore parthenogenetic *Artemia*. Regarding the parthenogenesis reproductive model and using 16S nucleotide sequences, they confirmed the presence of parthenogenetic *Artemia* in the Great Salt Lake. Later, Endebu *et al*., (2013) aimed to prove or invalidate the existence of parthenogenetic *Artemia* in the Great Salt Lake using field samples which had been collected during 1997-2005. They studied two molecular sequence markers (exon-7 of the Na/K-ATPaseα-1 subunit gene and exon-2 of the heat shock protein HSP26 gene) and confirmed the existence of parthenogenetic specimens in the field samples from the period 2000 to 2002.

During a study on genetic variation of 14 Mexican populations of *Artemia franciscana* Kellogg, 1906 (unpublished data), the present research observed an abnormal mt*COI* sequence from a field sample collected at Cancun saltern in Mexico. This report aims to identify its taxonomic status.

Materials and methods

Ten adult *Artemia* samples were obtained from Cancun saltern (21.171N, 86.807W) in Quintana Roo State (Yucatan Peninsula, Mexico) (Fig. 1).

Total DNA was extracted from a small piece of antennal tissue using the Chelex® 100 Resin method (Bio-Rad Laboratories, USA) (see Asem *et al*., 2016). A partial fragment of the mitochondrial cytochrome oxidase subunit I (mt*COI*) was amplified utilizing the invertebrate universal primers LCOI490/HC02198 (Folmer *et al*., 1994). The standard nucleotide BLAST online software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was utilized to distinguish taxonomic status.

Fig. 1. Geographical position of Cancun saltern (21.171N, 86.807W) from Quintana Roo State (Yucatan Peninsula, Mexico). Map data ©2022 Google.

To compare the phylogenetic relationship between reported parthenogenetic *Artemia* from Mexico and other parthenogenetic lineages with different ploidy levels, a phylogenetic tree was generated based on Maximum Likelihood (ML) using MEGA X (Kumar *et al*., 2018). Sequences of *Artemia urmiana* Günther, 1899 and *Artemia sinica* Cai, 1989 were used as ancestral reference species for parthenogenetic lineages following Asem *et al*., (2022).

Results and discussion

The result confirms that one of the studied individuals from Cancun saltern (GenBank accession no.: ON088931; 610 bp) is a parthenogenetic specimen with 100 percent identity overlay to 43 diploid sequences of eight localities from Asia (Iran and China) and Europe (Russia and France) (Table 1). Additionally, the result of the phylogenetic tree shows that Mexican parthenogenetic *Artemia* is located in the clade of diploid parthenogenetic lineage (Fig. 2).

The parthenogenetic specimens in Mexico were previously reported by Gallardo and Castro (1987) and Torrentera and Dodson (1995) from San Crisanto (Yucatan Peninsula) and Las Colorada (Pacific coast), respectively. The present report is the third confirmed record of the occurrence of parthenogenetic *Artemia* from field samples in Mexico.

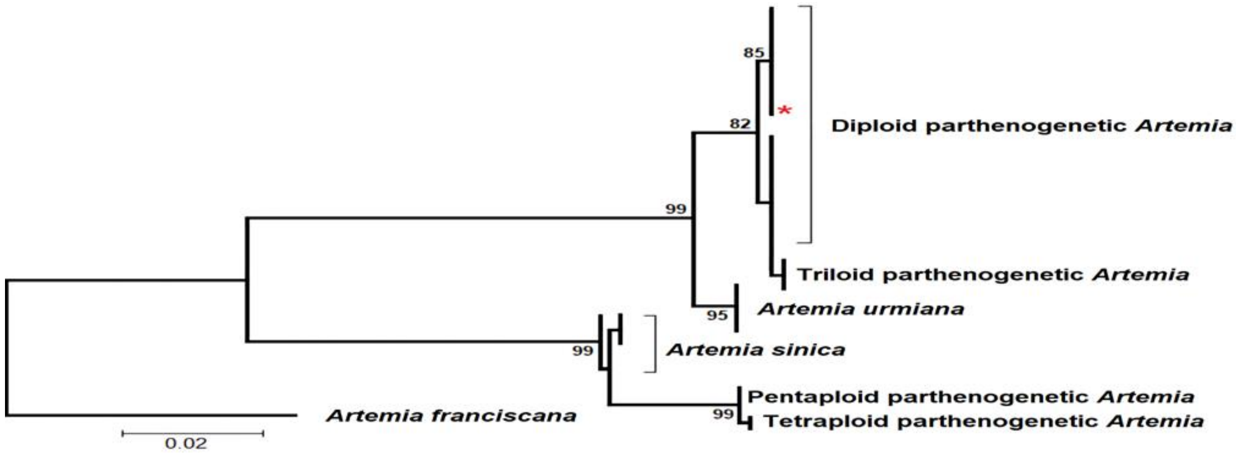

Fig. 2. A Maximum-likelihood (ML) phylogenetic tree based on mt*COI* marker: The maximum-likelihood bootstrap values are shown for each major node. GenBank accession numbers are available in Table 1 and Asem *et al*., (2022). *Artemia franciscana* (GenBank accession no. KJ863440) was used as an outgroup. The star symbol (*) shows the position of Mexican parthenogenetic *Artemia* from Cancun saltern on phylogenetic tree

Table 1. Habitats and sequence(s) information of Cancun saltern and eight diploid parthenogenetic lineages from Asia and Europe.

Locality	Geographic coordinates	GenBank accession no.	Sampling Year	Ref.
Cancun saltern (Mexico)	21.171N, 86.807W	ON088931	2005	This study
Maharlu Lake (Iran)	29.47N, 52.77E	MT791756-58 MT791760-64 MT791768	1997	Rode et al., 2022
Dongjiagou (China)	39.12N, 122.04E	MT791710-18 MT791720 MT791722-23 MT791725-26	1991	Rode et al., 2022
Yingkou (China)	40.55N, 122.32E	MT791795-96	1989	Rode et al., 2022
Bameng area (China)	23.29N, 106.37E	MT791704	1995	Rode et al., 2022
Kulundinskoye (Russia)	53.01N, 79.51E	MT791727	2007	Rode et al., 2022
Aigues-Mortes (France)	43.52N, 4.18E	MT791647 MT791649-52 MT791654-57 MT791659-63	2011	Rode et al., 2022
Salin De Giraud (France)	43.42N, 4.63E	MT791783	2011	Rode et al., 2022
Sete-Villeroy (France)	43.38N, 3.62E	MT791789	2012	Rode et al., 2022

Generally, *A. franciscana* has a high adaptation ability and reproductive rate (Amat *et al*., 2007; Sanchez *et al*., 2016); thus, it can be concluded that the competitive potential of *A. franciscana* has likely prevented the expansion of parthenogenetic *Artemia* in North America. Previous studies and current results could not provide scientific evidence to explain the origin of parthenogenetic *Artemia* in North America; it is not clear if they are native or anthropogenic. Further comprehensive studies are necessary to investigate North America's biogeography of parthenogenetic *Artemia*.

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Conflicts of Interest

The authors declare no conflict of interest.

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