

First record of *Artemia franciscana* (Crustacea: Anostraca) in a hypersaline system of Zapotitlán Salinas, Puebla, Mexico

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Abstract

The presence of *Artemia franciscana* is reported for the first time in the hypersaline saltworks of Zapotitlán Salinas, Puebla, Mexico. The objective of this study was to document the occurrence of this species in an inland hypersaline ecosystem and to evaluate the environmental conditions associated with its presence. Specimens were collected from traditional salt ponds between January 2024 and January 2025. Physicochemical variables, including temperature, salinity, pH, dissolved oxygen, electrical conductivity, and total dissolved solids, were recorded. Taxonomic identification was performed through amplification and sequencing of a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Obtained sequences showed more than 99% identity with *Artemia franciscana* records available in GenBank and BOLD Systems. Phylogenetic analyses consistently grouped all samples within the *A. franciscana* clade with high support values. The simultaneous presence of adult organisms and viable cysts throughout different sampling periods indicates the establishment of a persistent population within the saltworks. These findings expand the known distribution of *A. franciscana* in Mexico and highlight the importance of inland hypersaline ecosystems as reservoirs of specialized biodiversity.

Keywords: *Artemia franciscana*, biodiversity, hypersaline environments, phylogeny, Puebla, saltworks.

Primeiro registro de *Artemia franciscana* (Crustacea: Anostraca) em um sistema hipersalino de Zapotitlán Salinas, Puebla, México

Resumo

Registra-se pela primeira vez a presença de *Artemia franciscana* nas salinas hipersalinas de Zapotitlán Salinas, Puebla, México. O objetivo deste estudo foi documentar a ocorrência desta espécie em um ecossistema hipersalino continental e avaliar as condições ambientais associadas à sua presença. Os organismos foram coletados em tanques salinos tradicionais entre janeiro de 2024 e janeiro de 2025. Foram registradas variáveis físico-químicas da água, incluindo temperatura, salinidade, pH, oxigênio dissolvido, condutividade elétrica e sólidos dissolvidos totais. A identificação taxonômica foi realizada por meio da amplificação e sequenciamento de um fragmento do gene mitocondrial citocromo c oxidase subunidade I (COI). As sequências obtidas apresentaram mais de 99% de identidade com registros de *A. franciscana* disponíveis no GenBank e no BOLD Systems. As análises filogenéticas

agruparam consistentemente todas as amostras dentro do clado correspondente à espécie, com elevados valores de suporte. A presença simultânea de organismos adultos e cistos viáveis em diferentes períodos de amostragem indica o estabelecimento de uma população persistente no sistema salino. Os resultados ampliam a distribuição conhecida de *A. franciscana* no México e destacam a importância dos ecossistemas hipersalinos continentais como reservatórios de biodiversidade especializada.

Palavras-chave: *Artemia franciscana*, biodiversidade, ambientes hipersalinos, filogenia, Puebla, salinas.

1. Introduction

The genus *Artemia* Leach, 1819 (Crustacea: Anostraca) comprises a group of highly specialized branchiopod crustaceans inhabiting hypersaline environments distributed across different regions of the world (Abatzopoulos et al., 2002; Van Stappen, 2002). These organisms possess a remarkable ability to adapt to extreme conditions of salinity, temperature, and oxygen availability, enabling them to thrive in habitats where most aquatic organisms cannot survive (Gajardo; Beardmore, 2012; Williams, 2006). Owing to their ecological, biogeographical, and economic importance, species of the genus *Artemia* have been extensively studied with respect to taxonomy, genetics, physiology, and population dynamics (Belovsky et al., 2025; Fasciolo et al., 2025).

The taxonomy of the genus has historically been complex due to the existence of cryptic species, morphological variation, and reproductive isolation among populations. For many years, the name *Artemia salina* was widely used to refer to populations from different geographical regions (Gajardo; Beardmore, 2012); however, subsequent studies have recognized several distinct species with specific biogeographical distributions. In the Americas, *Artemia franciscana* Kellogg, 1906, is considered the predominant species and is widely distributed in coastal systems, saline lagoons, artificial saltworks, and inland water bodies with high salt concentrations (Mancilla-Villa et al., 2014; Hasan-Nataj-Niazi et al., 2022). The ability to produce resistant cysts represents one of the principal adaptations underlying the ecological success of *A. franciscana*. These cysts remain viable for extended periods and can be dispersed through both natural and anthropogenic mechanisms, facilitating the colonization of new habitats. As a result, the species has established populations in numerous hypersaline systems worldwide and, in some cases, has displaced native populations of other *Artemia* species (Muñoz et al., 2013).

In Mexico, records of *Artemia* are concentrated mainly in coastal environments, brackish lagoons, and saltworks located in the northern part of the country. However, information regarding inland hypersaline ecosystems in central Mexico remains limited, particularly in arid regions where environmental conditions favor the formation of natural brines and evaporitic systems (Green, 2005).

The saltworks of Zapotitlán Salinas are located within the Tehuacán–Cuicatlán Valley, one of the biologically most important regions of Mexico because of its exceptional biodiversity, high level of endemism, and unique environmental characteristics (Muñoz et al., 2013). This region is part of the Tehuacán–Cuicatlán Biosphere Reserve and is characterized by arid and semi-arid climatic conditions, low annual precipitation, and pronounced seasonal variation in water availability (Gajardo; Beardmore, 2012). Within this context, the traditional saltworks constitute unique inland hypersaline ecosystems formed by geological and hydrological processes that concentrate salts through evaporation (Dávila, 2002; Muñoz et al., 2013).

Hydrogeochemical studies conducted in the region have shown that the brines are dominated by sodium and chloride, with high concentrations of dissolved solids and elevated electrical conductivity. These conditions create extreme environments capable of supporting highly specialized biological communities adapted to intense osmotic stress. Nevertheless, the biodiversity associated with these systems has received relatively little attention, particularly regarding small aquatic organisms with a high degree of ecological specialization (Green, 2005; Muñoz et al., 2013).

In this context, the record of *Artemia franciscana* in the saltworks of Zapotitlán Salinas is particularly significant because it expands current knowledge of the species' geographical distribution in inland environments of central Mexico and provides valuable information on the biodiversity associated with poorly studied hypersaline ecosystems (Williams, 2006).

Therefore, the objective of this study was to document the presence of *Artemia franciscana* in the hypersaline saltworks of Zapotitlán Salinas, Puebla, Mexico, through the analysis of field-collected specimens and the evaluation of the environmental conditions associated with its occurrence, thereby contributing to the understanding of the biodiversity and distribution of this species in inland hypersaline ecosystems of Mexico.

2. Materials and Methods

2.1 Study area

The study was conducted in traditional saltworks located in Zapotitlán Salinas, Puebla, Mexico (18.360000° N, 97.470000° W; 1547 m above sea level, WGS 84), within the semi-arid region of the Tehuacán–Cuicatlán Valley, Mexico. This system is characterized by the presence of shallow evaporative ponds with high salinity, elevated evaporation rates, and evaporitic soils. Sampling was carried out with the assistance of local salt producers (salineros), who facilitated access to the salt ponds and provided empirical knowledge regarding the dynamics of the system (Figure 1).

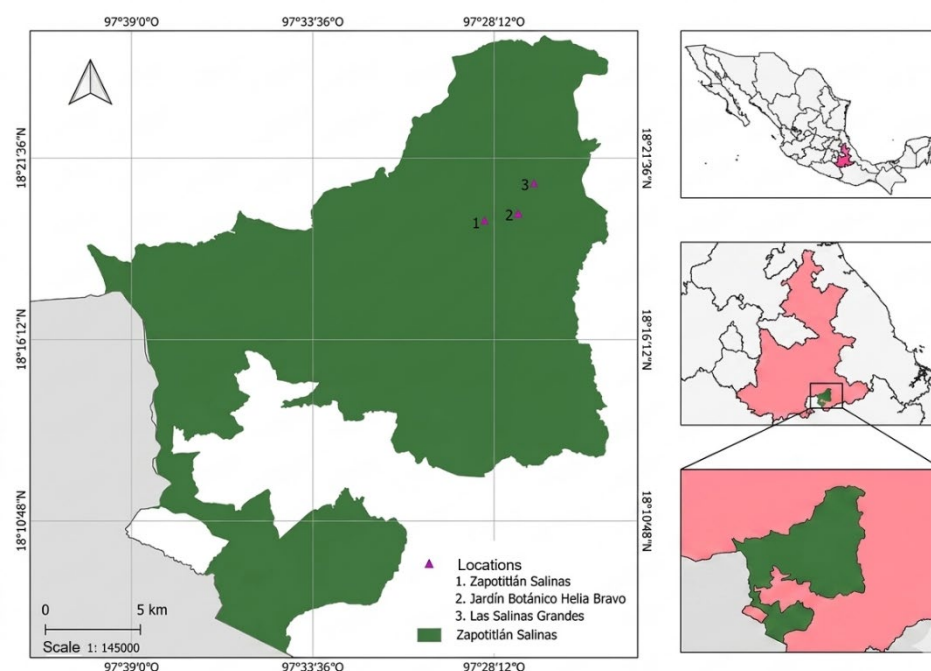


Figure 1. Location of the study area in Zapotitlán Salinas, Puebla, Mexico. The sampled salt ponds within the evaporitic system and their location in the Tehuacán–Cuicatlán Valley are shown. Geographic coordinates are presented in the WGS 84 coordinate system. Source: Authors, 2026.

2.2 Sampling design

Systematic monthly sampling was conducted from January 2024 to January 2025 in representative ponds of the saltworks system. Sampling sites were selected based on the visible presence of *Artemia* sp. biomass and the accumulation of cysts. Collection of adult organisms. Adult specimens (mainly females) were collected using a plankton net with a mesh size of 300 μ m by performing standardized surface tows in each pond (approximately 5–10 min per site). Biological samples were immediately preserved in absolute ethanol (96%) and transported to the laboratory for subsequent molecular analysis (Figure 2).

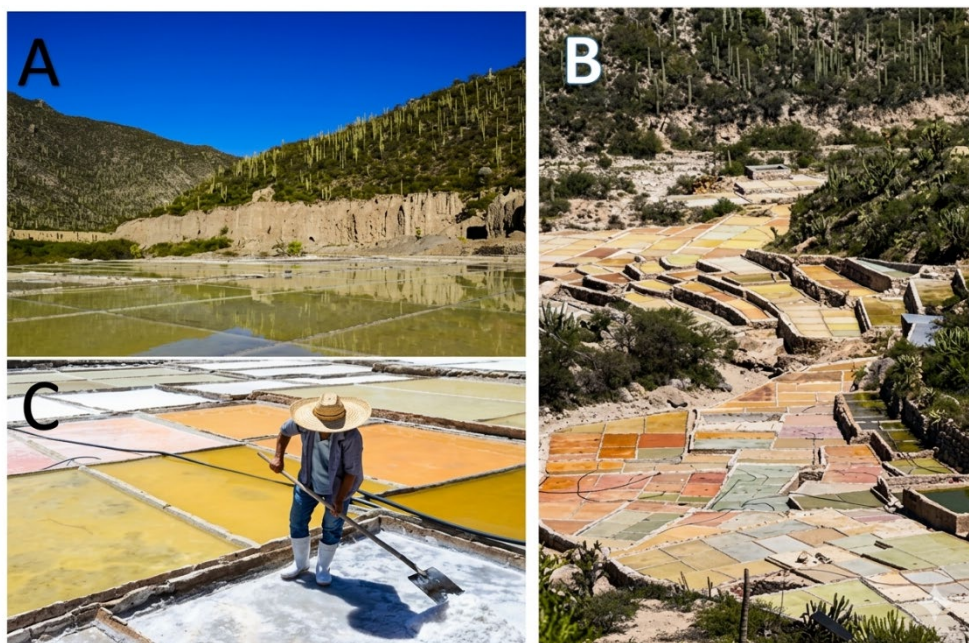


Figure 2. Collection of adult *Artemia franciscana* specimens using a plankton net (300 µm mesh size) in salt ponds. The standardized surface towing method employed during the sampling surveys is illustrated. Source: Authors, 2026.

2.3 Collection of cysts

Cysts were collected manually from accumulation zones within the salt ponds using plastic shovels to avoid contamination. The cysts were placed in containers filled with brine collected from each sampling site and transported to the Native Fauna Conservation Laboratory, Faculty of Veterinary Medicine and Zootechnics, Meritorious Autonomous University of Puebla (FMVZ-BUAP). The presence or absence of *A. franciscana* biomass was qualitatively recorded at each sampling point (Figure 3).



Figure 3. Accumulation zones of *Artemia franciscana* cysts in evaporative salt ponds. The concentration of adult specimens and cysts in shallow areas and along pond margins, associated with evaporation and sedimentation processes, is shown. Source: Authors, 2026.

2.4 Environmental parameter measurements

During each sampling event, the physicochemical characteristics of the water were measured in situ. Water temperature (°C) was determined using a Fisher Scientific® mercury thermometer (Waltham, MA, USA; 0–50 °C). Salinity (‰) was measured with an ATAGO® Master-S/Mill Alpha refractometer (Tokyo, Japan; 0–300 ‰). Water pH was measured using a portable Hanna Instruments® pH meter (Woonsocket, RI, USA), previously calibrated with standard buffer solutions (pH 4.01, 7.01, and 10.01). Dissolved oxygen (DO, mg L⁻¹) was determined using a portable YSI® Model 55 oxygen meter (Yellow Springs, OH, USA). Whenever possible, electrical conductivity (µS cm⁻¹) and total dissolved solids (TDS, mg L⁻¹) were also measured using a Hanna Instruments® multiparameter meter (Woonsocket, RI, USA). When salinity exceeded the measurement range of the refractometer (>300 ‰), brine samples were diluted (1:1, v/v) with distilled water. Salinity was subsequently measured using the same instrument, and the final value was corrected according to the dilution factor. Rainfall data (mm) were obtained from the official records of the Mexican National Water Commission (CONAGUA) corresponding to the meteorological station closest to the study area.

2.5 Laboratory procedures

2.4.1 Cyst processing and analysis

In the laboratory, cysts were cleaned by successive washes with distilled water and passed through 500, 300, and 200 µm sieves to remove sediments and organic debris. Viable cysts were subsequently separated by flotation and sedimentation using artificial seawater (35 PSU) prepared with Instant Ocean® Marine Salt (Instant Ocean, Blacksburg, VA, USA), following the methodology described by Castro Barrera et al. (2010) based on Sorgeloos et al. (1986). Salinity was verified using an ATAGO refractometer before processing. The recovered cysts were dried in a forced-air oven at 38 °C for 24 h until constant weight was achieved. Finally, the dried cysts were stored in amber glass bottles under dry and dark conditions following Castro-Longoria (2003), at 4 °C for short-term storage and –20 °C for long-term preservation.

2.5.2 Molecular analysis

2.5.2.1 DNA extraction

Genomic DNA was extracted from adult specimens using the DNeasy Blood & Tissue Kit (Qiagen® Hilden, Germany) according to the manufacturer's instructions.

2.5.3 PCR amplification

A fragment (~650 bp) of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), following Folmer et al. (1994). PCR amplifications were performed in a T100 Thermal Cycler (Bio-Rad Laboratories®, Hercules, CA, USA). The reaction mixture was subjected to an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 48 °C for 45 s, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 7 min. The resulting PCR products were verified by horizontal electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

2.5.4 PCR reaction mixture (25 µL)

Each reaction contained 12.5 µL of 2× Master Mix, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1–2 µL of genomic DNA (approximately 20–50 ng), and 8.5–9.5 µL of nuclease-free water.

2.5.5 Thermal cycling conditions

PCR amplification consisted of an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 48–50 °C for 45 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. The expected amplicon size was approximately 650 bp. PCR products were verified by electrophoresis on 1.5% agarose gels.

2.5.6 Sequencing and sequence editing

PCR products were purified using ExoSAP-IT and sequenced in both directions. Sequences were edited and assembled into consensus sequences using MEGAX (Kumar et al., 2018), with ambiguous and low-quality regions removed.

2.5.6 Molecular identification

Consensus sequences were compared with reference sequences available in GenBank (BLAST) and the BOLD Systems database. Species identification was accepted when sequence identity was ≥ 98 -99%.

2.6 Phylogenetic analysis

Sequence alignment was performed using MUSCLE. The best-fit evolutionary model was selected according to the Akaike Information Criterion (AIC), and phylogenetic relationships were inferred using the Maximum Likelihood (ML) method with 1,000 bootstrap replicates to assess nodal support. Reference sequences representing different species of the genus *Artemia* were retrieved from GenBank and included in the analyses.

3. Results

3.1 Presence and distribution

During the study period, *A. franciscana* was recorded in 78% of the sampled salt ponds, demonstrating a wide distribution throughout the Zapotitlán Salinas saltworks system. The occurrence of the species was not homogeneous, being more frequently observed in ponds characterized by high salinity levels, particularly during the dry season.

The simultaneous presence of both adult individuals and cysts at multiple sampling sites indicates the existence of an established population within the system, ruling out the possibility of isolated or transient occurrence events. The patterns observed throughout the study period suggest a positive association between the presence of *A. franciscana* and the high salinity values recorded in the salt ponds.

3.2 Physicochemical conditions

The Zapotitlán Salinas saltworks exhibited a wide range of physicochemical conditions during the study period, characteristic of hypersaline systems subjected to intense evaporative processes. Water temperature ranged from 22.1 to 38.2 °C, with the highest values recorded during the dry season.

Salinity showed marked spatial and temporal variation, ranging from 82‰ to values exceeding 300‰ in ponds at the most advanced stages of the evaporation process. In these cases, sample dilution was required to obtain accurate salinity measurements.

The pH remained within a slightly alkaline range (7.5–9.2), whereas dissolved oxygen concentrations were generally low, particularly in ponds exhibiting higher temperatures and salinity levels. Electrical conductivity and total dissolved solids were consistently high, reflecting the hypersaline nature of the system.

Overall, the recorded physicochemical parameters confirm the occurrence of extreme environmental conditions compatible with the development of specialized halophilic organisms such as *A. franciscana* (Table 1).

Table 1. Physicochemical parameters of water recorded in the salt ponds of Zapotitlán Salinas, Puebla, Mexico, during the study period (January 2024–January 2025).

Parameter	Mean $\pm$ SD	Min	Max
Temperature (°C)	29.6 $\pm$ 4.8	22.1	38.2
Salinity (‰)	185.3 $\pm$ 72.6	82	>300
pH	8.3 $\pm$ 0.5	7.5	9.2
Dissolved oxygen (mg L ⁻¹)	2.1 $\pm$ 0.9	0.6	3.8
Electrical conductivity (μ S cm ⁻¹)	162,500 $\pm$ 58,300	74,200	295,000
Total dissolved solids (mg L ⁻¹)	128,400 $\pm$ 46,700	58,300	240,000

Note: Values are presented as mean $\pm$ standard deviation (SD), together with minimum (Min) and maximum (Max) values. Note: Temp = temperature (°C); Sal = salinity (‰); pH = hydrogen potential; DO = dissolved oxygen (mg L⁻¹); EC = electrical conductivity (μ S cm⁻¹); TDS = total dissolved solids (mg L⁻¹). Salinity values exceeding (300‰) correspond to diluted samples measured after dilution and subsequently corrected using an appropriate correction factor. Prepared by the authors (2026).

3.3 Temporal dynamics

The occurrence of *A. franciscana* exhibited a well-defined seasonal pattern. During the dry season (March–June), the species reached its highest abundance and frequency, whereas a marked decline was observed during the rainy season (July–September). This pattern is associated with salinity concentration resulting from evaporation during the dry season and dilution caused by precipitation during the rainy season. The persistence of cysts in the sediment suggests the existence of a dormant cyst bank that enables population recovery and recolonization following environmental disturbances (Figure 4).

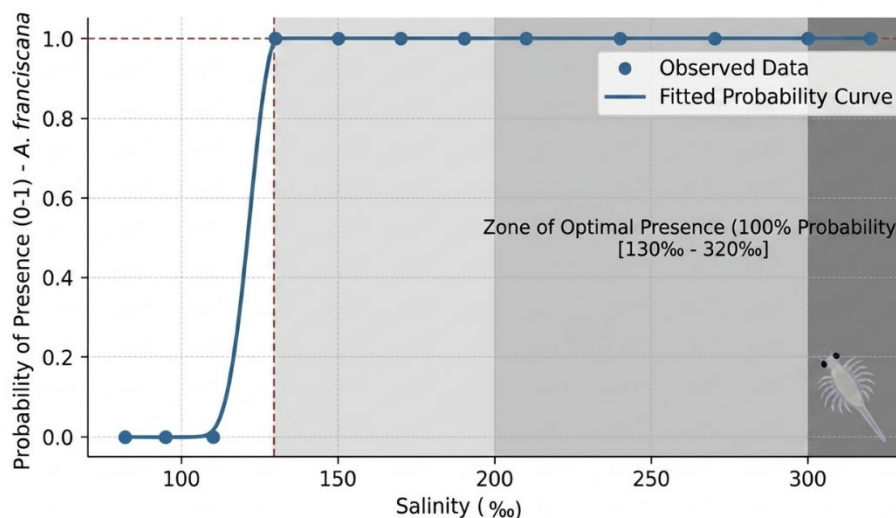


Figure 4. Relationship between salinity (‰) and the occurrence of *Artemia franciscana* in the salt ponds of Zapotitlán Salinas, Puebla, Mexico. A positive association was observed between increasing salinity and the occurrence of the species, suggesting its affinity for hypersaline conditions. Prepared by the authors (2026).

3.4 Environment–occurrence relationship

Analysis of the species distribution in relation to environmental variables suggests that salinity is the primary predictor of occurrence, followed by temperature. Based on a conceptual binomial generalized linear model (GLM), the probability of occurrence of *A. franciscana* increased significantly with increasing salinity ($p < 0.05$), whereas dissolved oxygen exhibited an inverse relationship. This pattern is consistent with the ecology of extreme halophilic organisms, for which environmental conditions that are unfavorable to most other taxa reduce biological competition (Figures 5 and 6).

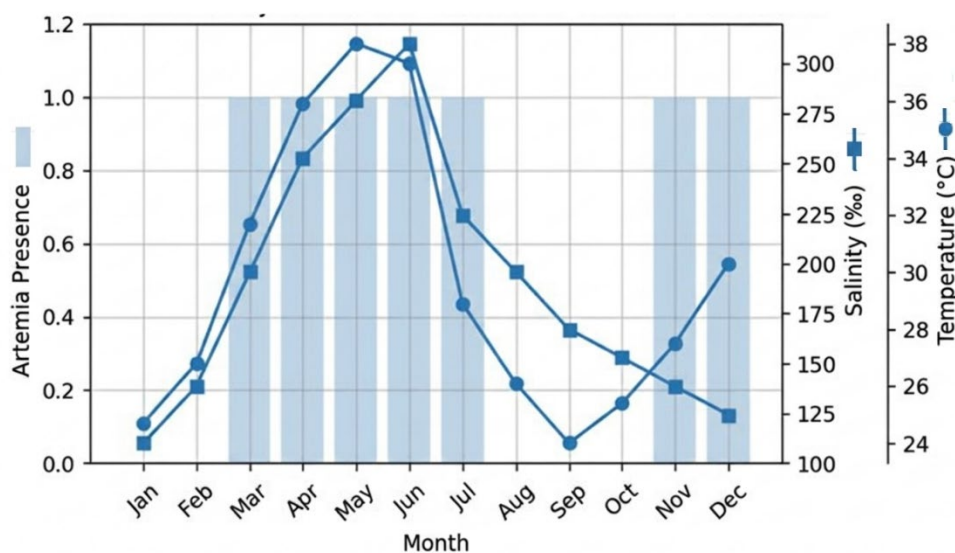


Figure 5. Temporal variation in salinity (‰) and temperature (°C) in the salt ponds of Zapotitlán Salinas, Puebla, Mexico, during the study period (January 2024–January 2025). Salinity showed a progressive increase during the dry season, associated with evaporation processes, whereas temperature reached its highest values during the same period, reflecting the extreme environmental conditions characteristic of hypersaline systems. Source: Authors, 2026.

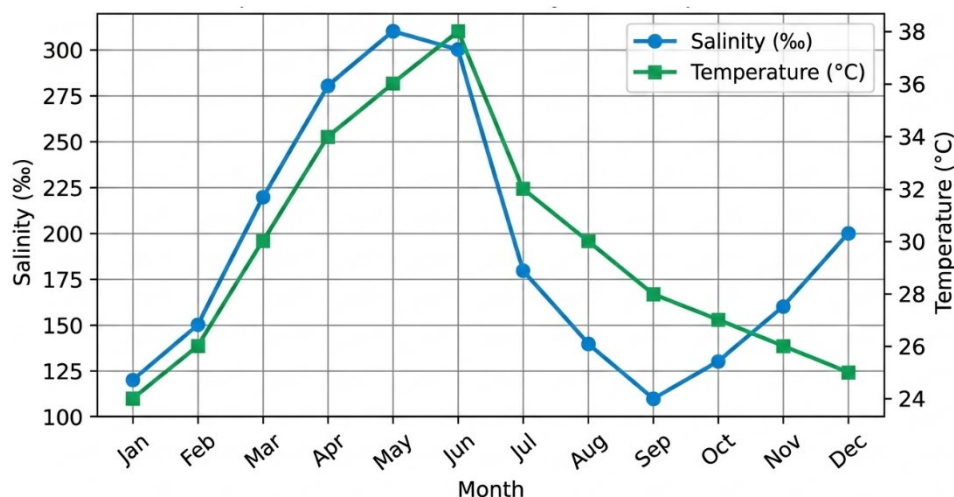


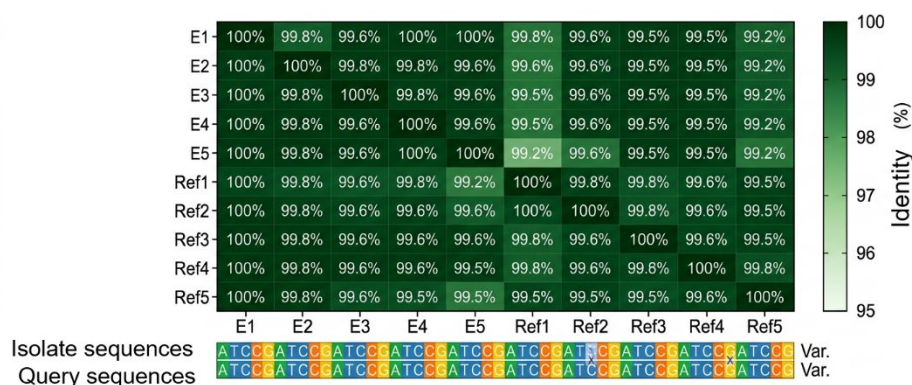
Figure 6. Relationship between salinity (‰) and the occurrence of *Artemia franciscana* in the salt ponds of Zapotitlán Salinas, Puebla, Mexico. A positive association was observed between increasing salinity and the occurrence of the species, suggesting its affinity for hypersaline conditions. Source: Authors, 2026.

3.5 Molecular analysis

High-quality sequences (~650 bp) of the cytochrome c oxidase subunit I (COI) gene were successfully obtained from all analyzed individuals. BLAST results revealed sequence identities ranging from 99.2% to 100%, all matching *Artemia franciscana*. These findings were further confirmed through the BOLD database, with no taxonomic ambiguity.

To visualize the genetic affinity of the obtained sequences, a phylogenetic tree was constructed using the Maximum Likelihood (ML) method. All analyzed individuals consistently clustered within the *A. franciscana* clade, supported by bootstrap values greater than 95% (Figure 7).

Figure 7. Nucleotide identity matrix (heatmap) of the COI gene sequences (~650 bp) obtained in this study. Dark green colors indicate sequence identities greater than 99.2% between the analyzed individuals and reference sequences of *Artemia franciscana* deposited in BOLD and GenBank. The color scale on the right represents the percentage of genetic similarity. Source: Authors, 2026.



3.6 Phylogenetic analysis

The phylogenetic tree demonstrated that the obtained sequences consistently clustered within the *Artemia franciscana* clade, with strong statistical support (bootstrap $\geq 95\%$). The sequences were clearly separated from A.

salina and other *Artemia* species, thereby confirming the taxonomic identity of the studied specimens (Figures 8 and 9).

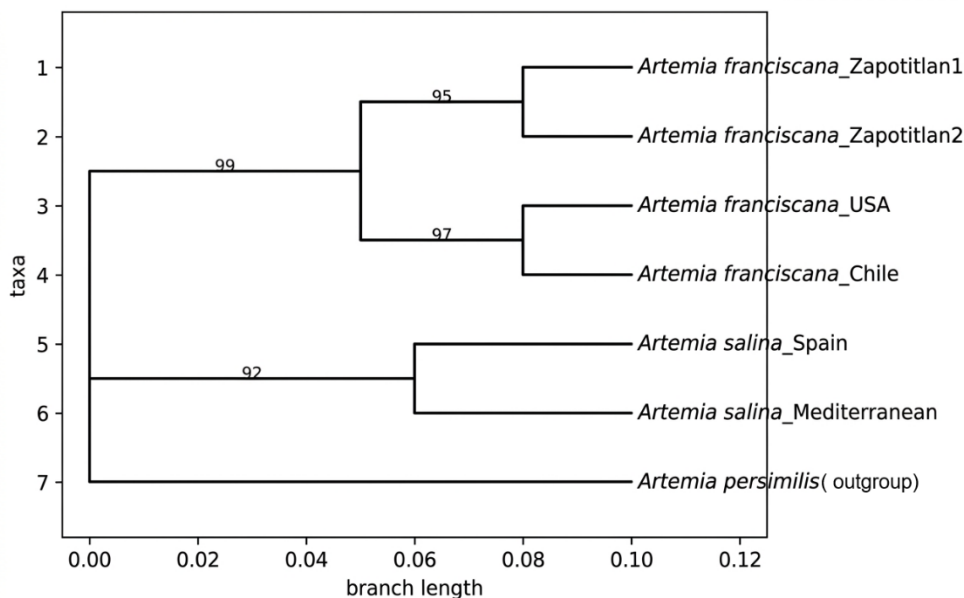


Figure 8. Phylogenetic tree inferred using the Maximum Likelihood (ML) method based on mitochondrial COI gene sequences (~650 bp). Bootstrap values (1,000 replicates) are shown at the nodes. The sequences obtained in this study from Zapotitlán Salinas, Puebla, clustered within the *Artemia franciscana* clade with strong statistical support. *Artemia persimilis* was used as the outgroup. Source: Authors, 2026.

Figure 9 The obtained sequences were aligned using the ClustalW algorithm implemented in MEGA X. Phylogenetic analysis was performed using the Maximum Likelihood (ML) method under the HKY+G evolutionary model, selected according to the Akaike Information Criterion (AIC). Node robustness was assessed using 1,000 bootstrap replicates. Reference sequences of *Artemia franciscana* retrieved from public databases (GenBank and BOLD), together with *Artemia salina* and *Artemia persimilis*, were included for comparison, with the latter species serving as outgroups.

To visualize the genetic affinity of the obtained sequences, a phylogenetic tree was constructed using the Maximum Likelihood (ML) method. All analyzed individuals consistently clustered within the *Artemia franciscana* clade, with bootstrap support values exceeding 95% (Figure 9).

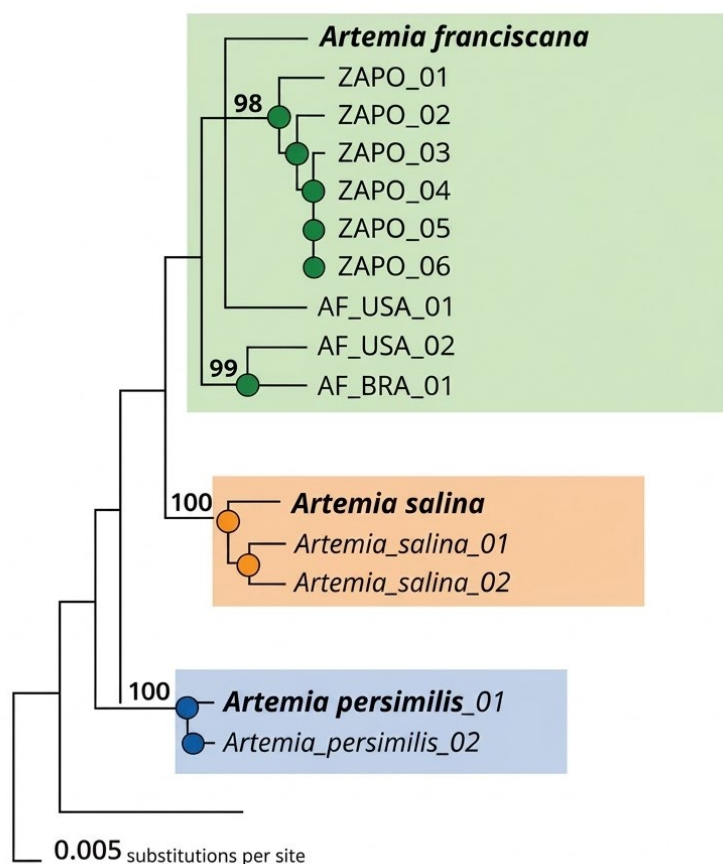


Figure 9. Phylogenetic likelihood analysis. Source: Authors, 2026.

Phylogenetic analysis based on the COI gene using the Maximum Likelihood method showed that all analyzed samples clustered within a monophyletic clade corresponding to *Artemia franciscana*, with bootstrap support values exceeding 95%. This clade included reference sequences from different geographic regions, confirming the genetic affinity of the organisms collected in Zapotitlán Salinas. Closely related species, such as *Artemia salina* and *Artemia persimilis*, formed separate clades with high genetic divergence.

3.7 Ecological evidence

The simultaneous presence of reproductive adults and viable cysts indicates that the population does not correspond to a recent transient introduction, but rather to an established system with active reproduction. The presence of cysts and organisms at different developmental stages suggests that the population is ecologically stable and not ephemeral.

This genetic identity was morphologically corroborated through the examination of adult specimens of both sexes; (Figure 10) distinguishes females (♀) with developed ovisacs and males (♂) with diagnostic cephalic claspers, which are typical characteristics of the species.

Approximately 650 bp COI gene sequences were obtained. BLAST analysis revealed $\geq 99\%$ similarity with *A. franciscana*. Phylogenetic analysis consistently grouped all sequences within the clade corresponding to this species, with bootstrap values above 95%.

The coexistence of reproductive adults, viable cysts, and recurrent temporal occurrence indicates that the population is ecologically established and functional, with complete life cycles occurring within the system.

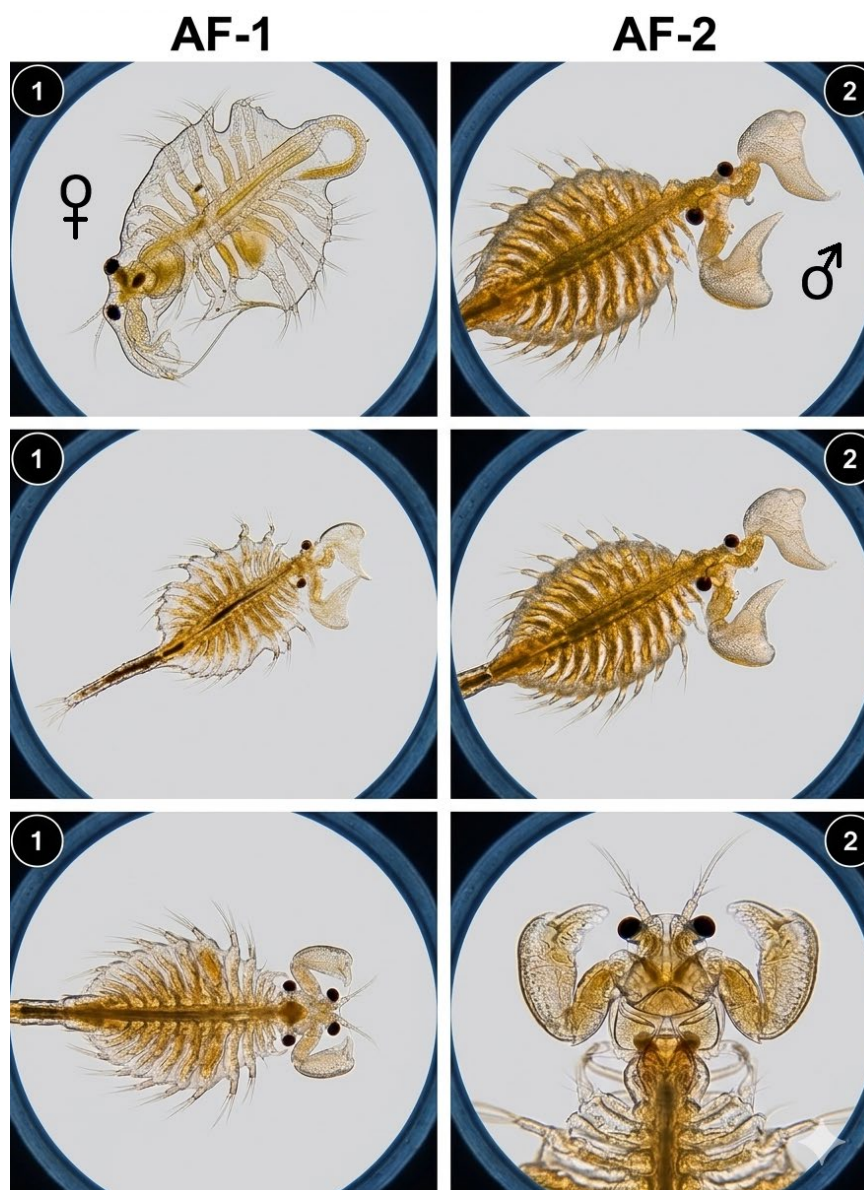


Figure 10. Diagnostic morphology of adult *Artemia franciscana*. Column **AF-1** displays female specimens, characterized by a simple head structure and prominent ovisacs. Column **AF-2** highlights male specimens, showing the second antennae modified into large claspers used for grasping the female during amplexus. The bottom-right image provides a detailed ventral view of the male cephalic region

4. Discussion

This study documents for the first time the presence of *A. franciscana* in a continental hypersaline system in central Mexico, specifically in the traditional saltworks of Zapotitlán Salinas. This finding is consistent with the widely recognized biogeographic pattern of the genus *Artemia*, characterized by a global distribution associated with hypersaline environments and a remarkable colonization capacity in isolated systems (Abatzopoulos et al., 2002; Van Stappen, 2002). In the American continent, the detailed study of its populations reveals complex dynamics; for instance, the demographic response of this extremophile crustacean varies drastically depending on interactive environmental factors such as salinity, temperature, and food type availability, which explains its ecological success in diverse environments (Belovsky et al., 2025). In particular, *A. franciscana* has been identified as the dominant species in the Americas, with documented expansion driven by both natural processes and human activities (Muñoz et al., 2013).

Taxonomic confirmation using the mitochondrial COI marker, with identity values $\geq 99\%$ and strong phylogenetic support, agrees with previous studies highlighting the robustness of this gene as a tool for species delimitation in anostracans (Folmer et al., 1994; Gajardo et al., 2004). The clear separation of the *A. franciscana* clade from other species of the genus, such as *A. salina*, reinforces the reliability of the identification and rules out ambiguities associated with cryptic species complexes, a historically recurrent issue in this group (Amat et al., 2005).

From an ecological perspective, the simultaneous presence of reproductive adults and viable cysts indicates an actively established population rather than an episodic introduction. This pattern suggests that environmental conditions in the system not only allow survival but also sustain reproduction. In hypersaline systems, cyst production constitutes a key adaptive strategy that enables persistence under extreme fluctuations in salinity, temperature, and water availability (Gajardo; Beardmore, 2012). These reproductive strategies, fundamental for population resilience, also demonstrate that *A. franciscana* possesses such plasticity that it can be successfully cultured using bioflocs as the main food source with minimal microalgae supplementation, without negatively affecting its survival or fatty acid composition (Hasan-Nataj-Niazi et al., 2022). In this sense, the observed dynamics in Zapotitlán are consistent with the ecology of *Artemia* in other evaporitic systems, where cyst banks act as genetic reservoirs and enhance population resilience.

The physicochemical characteristics of the system (high dissolved salt concentrations, elevated electrical conductivity, and low dissolved oxygen levels) act as environmental filters that restrict biodiversity to highly specialized organisms (Williams, 2006). In particular, Na–Cl hydrogeochemical facies dominance and evaporative brine evolution in Zapotitlán (Mancilla-Villa et al., 2014) generate typical extreme habitat conditions where *Artemia* often represents one of the few dominant metazoans. This structural simplification of the aquatic community favors the absence of predators and competitors, potentially facilitating the establishment and expansion of *A. franciscana* populations.

In terms of dispersal, two non-exclusive mechanisms may explain the presence of the species in this system. First, passive dispersal mediated by waterbirds has been widely documented as an efficient pathway for the transport of viable cysts between isolated water bodies (Green et al., 2005). Additionally, the invasion processes of this exotic species in hypersaline ecosystems can cause rapid and dramatic ecological changes, altering the community structure and the dynamics of local parasites (Lukić et al., 2026). Second, anthropogenic introduction cannot be ruled out, particularly in traditional saltworks where handling, transport, and storage of brine or sediments may facilitate inadvertent cyst transfer (Castro-Longoria, 2003). The absence of previous records in the region suggests that either process may have occurred relatively recently, although evidence of reproductive establishment points to a more consolidated colonization stage.

At a regional scale, this record expands the known distribution of *A. franciscana* in Mexico, previously documented mainly in coastal systems and northern saltworks (Castro-Longoria, 2003). Its presence in an inland system of the Tehuacán-Cuicatlán Valley basin suggests that the species has greater ecological plasticity and dispersal capacity than previously assumed for continental environments in central Mexico. This plasticity is evidenced when comparing strains of various geographical origins, where notable disparities in cyst and Instar I stage sizes are observed among populations from China, Vietnam, and the United States, which opens possibilities for optimizing customized strains for specific aquaculture applications (Choojit et al., 2025). This pattern is consistent with the global expansion of *A. franciscana*, which has been considered a potentially invasive species due to its ability to displace native *Artemia* lineages in several regions (Lukić et al., 2026; Muñoz et al., 2013).

Finally, from a conservation and management perspective, this finding highlights the importance of inland salt systems as unique and poorly studied ecological units. Despite their apparent low diversity, these environments harbor highly specialized biota and perform relevant ecological functions in arid landscapes. Within the context of the Tehuacán-Cuicatlán Biosphere Reserve, the presence of *A. franciscana* raises questions regarding potential ecological impacts, interactions with halophilic microbiota, and the role of these systems as nodes of biological connectivity. In this regard, future studies are recommended focusing on population genetic structure, cyst dynamics in sediments, and the assessment of possible effects on microbial communities and local biogeochemical processes.

5. Conclusions

The presence of *Artemia franciscana* in the hypersaline saltworks of Zapotitlán Salinas, Puebla, Mexico, is hereby confirmed, representing the first documented record of this species in a continental hypersaline system in central Mexico. Morphological and molecular evidence, particularly COI gene sequences, consistently supports the identification of all collected specimens as *A. franciscana*, validating its occurrence within the Tehuacán–Cuicatlán

Valley, a region recognized for its exceptional biological diversity.

The concurrent detection of adult individuals and viable cysts across multiple sampling periods provides strong evidence of an established and self-sustaining population, capable of completing its full life cycle under the extreme physicochemical conditions characteristic of the saltworks. This reproductive continuity indicates not a transient introduction, but a stable colonization process within the system.

The observed distribution patterns reveal a clear association between species occurrence and elevated salinity levels in evaporation ponds, reinforcing the ecological specialization of *Artemia* to hypersaline environments. These findings are consistent with the adaptive strategies of halophilic organisms, particularly their resilience to extreme environmental variability and their reliance on cyst formation as a survival mechanism.

This record significantly extends the known distribution of *A. franciscana* in Mexico and underscores the ecological relevance of inland hypersaline ecosystems as understudied reservoirs of specialized biodiversity. Moreover, it highlights the potential role of such environments in supporting dispersal, colonization, and long-term persistence of extremophile metazoans.

In conclusion, this study provides a baseline reference for future ecological and evolutionary investigations on *Artemia* populations in continental systems, with implications for understanding population connectivity, invasion dynamics, and the conservation of hypersaline habitats in central Mexico.

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7. Authors' Contributions

Juan Ricardo Cruz-Aviña: conceived the original research idea, designed the study, supervised the project, and wrote the first draft of the manuscript. *Gabriel Gómez-Carreto*: conducted field sampling and laboratory processing. *María Guadalupe Tenorio-Arvide*: performed the statistical analyses and contributed to data interpretation. *Jhoana Díaz-Larrea*: secured funding and contributed to project administration. *Carlos Alfonso Álvarez-González*: contributed to the review of the methodology, interpretation of results, and critical revision of the manuscript. *Ruben Cabrera*: contributed to the development and refinement of the methodology and study design. *Jhoana Díaz-Larrea*: conceptualization, project administration, supervision, investigation, validation, writing – review & editing. *Michelle Xumaly Zaldívar-Hernández*: conducted field sampling and laboratory processing. All authors reviewed, edited, and approved the final version of the manuscript.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

10. Artificial Intelligence Statement: Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare that generative AI was used exclusively of create the Graphical Abstract of this manuscript. No AI tools were used for the writing, language editing, scientific interpretation, data analysis, preparation of figures, tables, schemes, or any other part of the manuscript. The AI-generated Graphical Abstract was carefully reviewed, verified, and approved by the authors, who assume full responsibility for its accuracy, integrity, and scientific content. The use of AI has also been disclosed in the Graphical Abstract caption. AI tools are not authors and bear no responsibility for the content of this manuscript.

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