



PHYLOGENETIC ANALYSIS OF EXOTIC INVASIVE SPECIES OF THE
BRINE SHRIMP *ARTEMIA* LEACH, 1819 (BRANCHIOPODA, ANOSTRACA)
IN AL WATHBA WETLAND RESERVE (U.A.E.; ABU DHABI)

BY

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ABSTRACT

The taxonomic identity of an unknown *Artemia* population inhabiting the Al Wathba Wetland Reserve in Abu Dhabi, U.A.E., was determined using phylogenetic analysis of the mitochondrial marker *Cytochrome Oxidase Subunit 1 (COI)*. The results showed that the examined population belongs to an exotic invasive species, *Artemia franciscana*. Based on the distribution pattern of haplotypes, the studied population could possibly have originated from that inhabiting the Great Salt Lake (Utah, U.S.A.).

Key words. — *Artemia*, invasive species, *COI*, Abu Dhabi, U.A.E., Great Salt Lake

RÉSUMÉ

L'identité taxonomique d'une population inconnue d'*Artemia*, habitant la réserve d'Al Wathba Wetland à Abu Dhabi, U.A.E., a été déterminée en utilisant l'analyse phylogénétique du marqueur mitochondrial *Cytochrome Oxidase Subunit 1 (COI)*. Les résultats montrent que la population examinée appartient à une espèce invasive exotique, *Artemia franciscana*. Sur la base du modèle de distribution des haplotypes, la population étudiée pourrait être originaire du Grand Lac Salé (Utah, U.S.A.).

Mots clés. — *Artemia*, espèce invasive, *COI*, Abu Dhabi, U.A.E., Grand Lac Salé

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INTRODUCTION

The brine shrimp *Artemia* Leach, 1819 is widely distributed in more than 600 geographical sites on all continents except Antarctica (Van Stappen, 2002), and includes seven described bisexual species and in addition a number of parthenogenetic populations (Asem et al., 2010). Four bisexual species are native to the Old World, namely *Artemia salina* (Linnaeus, 1758) (Mediterranean basin), *Artemia urmiana* Gunther, 1899 (Lake Urmia, Iran and the Crimean salt lakes, Russia), *Artemia sinica* Cai, 1989 (China and Mongolia) and *Artemia tibetiana* Abatzopoulos, Zhang & Sorgeloos, 1998 (Qinghai — Tibetan Plateau, China). The other three bisexual species live in the New World, *Artemia monica* Verrill, 1869 (Mono Lake, U.S.A.), *Artemia franciscana* Kellogg, 1906 (North America, Central America and South America) and *Artemia persimilis* Piccinelli & Prosdocimi, 1968 (Argentina and Chile) (Asem et al., 2010; Rogers, 2013). Parthenogenetic populations are found in the Old World and in Oceania (Van Stappen, 2004). Furthermore, there is a large number of *Artemia* populations reported with unknown taxonomic status across the world (Van Stappen, 2004), which should be conserved for their contribution to natural biodiversity.

Just four *Artemia* sites have been reported from the Arabian Peninsula, including Saudi Arabia (2 sites), Kuwait (1 site) and the United Arab Emirates (1 site), which were all reported as “unknown populations” (Vanhaecke et al., 1987; Aspinall & Hellyer, 1999; Van Stappen, 2002). The first report of *Artemia* in the Al Wathba Wetland Reserve dates back to that of Aspinall & Hellyer in 1999 (Van Stappen, 2002). The primary studies done so far documented the population as belonging to a bisexual species (Al Dhaheri & Saji, 2013; Saji et al., 2016; Saji et al., unpublished data). The aim of the present study thus was to determine the taxonomic status of an existing *Artemia* population found in the Al Wathba Wetland Reserve.

MATERIAL AND METHODS

Study area and sampling

The Al Wathba Wetland Reserve (AWWR) (24°15'15.59"N 54°36'38.57"E) is located at 40 km east of Abu Dhabi, has a 4.9 km² average surface area, with approximately 1.5 km in length and 0.5 km in width, and has a maximum depth of 2 m (Brook et al., 2004; Al Dhaheri & Saji, 2013) (fig. 1).

Adult specimens of *Artemia* were collected from 6 stations using a Sampling Two Conical Nets, (40 cm long by 30 cm diameter with 100 µm diameter mesh), by pulling for ~10 meters at the surface in November and December 2010.



Fig. 1. Map of the study area: the Al Wathba Wetland Reserve in Abu Dhabi, United Arab Emirates (U.A.E.).

DNA extraction, PCR amplification and sequencing

Total DNA was separately extracted from part of the antenna of 22 adult males and females (1:1) following the Chelex[®] 100 Resin method (Bio-Rad Laboratories, Hercules, CA, U.S.A.). A fragment of mitochondrial *Cytochrome C Oxidase Subunit I (COI)* was amplified using the invertebrate universal primers LCO1490/HC02198 (Folmer et al., 1994). The thermal cycler PCR conditions were as follows: a cycle of 3 min at 94°C, followed by 35 cycles of 45 s at 94°C, 60 s at 45°C, and 60 s at 72°C, with a final step of 5 min at 72°C (Asem et al., 2016).

Sequence alignment and phylogenetic analyses

Sequences were aligned using MEGA ver. 6.00 with the default parameters (Tamura et al., 2013). Our own DNA dataset was composed of 22 sequences with 740 bp, after the alignment. The absence of pseudogenes was confirmed using the sequence of protein coding for each one and the fact that there were no multiple mutations or deletion(s) and duplication(s) in the analysed sequences. To find the phylogenetic relationship among the collected samples from the AWWR and with other bisexual species, we included five *COI* sequences (four sequences for *A. persimilis*) for each species from GenBank (table I). The Online NCBI

TABLE I
Information on and *COI* GenBank accession numbers of *Artemia* species used in this study

Species	Abbreviation	Number of individuals	Accession numbers	References
<i>A. urmiana</i> Gunther, 1899	URM	4	JX512748-751	Eimanifar & Wink (2013)
<i>A. sinica</i> Cai, 1989	SIN	4	KF691298-301	Eimanifar et al. (2014)
<i>A. tibetiana</i> Abatzopoulos, Zhang & Sorgeloos, 1998	TIB	4	KF691215-218	Eimanifar et al. (2014)
<i>A. salina</i> (Linnaeus, 1758)	SAL	4	KF691512-515	Eimanifar et al. (2014)
<i>A. persimilis</i> Piccinelli & Prodocimi, 1968	PER	4	DQ119647 HM998992 EF615594 EF615593	Hou et al. (2006) Maniatsi et al. (2011) Wang et al. (2008) Wang et al. (2008)
<i>A. franciscana</i> Kellogg, 1906	FRA	4	KJ863440-443	Eimanifar et al. (2014)
Unidentified	AWWR	22	MH221175-196	This study

Genomic BLAST software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was utilized to confirm the taxonomic status of the different species sequences that were used for the phylogenetic analyses. The phylogenetic tree was reconstructed using Bayesian inference (BI) as implemented in MrBayes 3.2.2 on XSEDE (Miller et al., 2010). To estimate the genealogical relationships among haplotypes of the examined individuals and *A. franciscana*, a median network was generated using the median-joining algorithm as implemented in the Network program ver. 5.0.0.3 (Bandelt et al., 1999). The sequences of *A. franciscana* were chosen from two natural habitats in the U.S.A., including the Great Salt Lake (GSL), Utah (29 individuals: KF662960, KF662968, KF662970, KF662971, KF662976, KF662977) and San Francisco Bay (SFB), California (37 individuals: KF662960, KF662968, KF662970, KF662975) (Muñoz et al., 2013).

RESULTS AND DISCUSSION

During sampling, the average salinity, temperature and pH were recorded as 81 ppt, 25°C and 7.8, respectively.

According to the BI phylogenetic tree based on the *COI* dataset, all examined *Artemia* individuals from the AWWR were grouped in the clade of *A. franciscana*

(fig. 2). The results thus confirmed that the AWWR has been invaded by the American native species *A. franciscana*, and no evidence was found that could possibly document the existence of an endemic population of *Artemia* in the AWWR.

The 88 *COI* sequences of *A. franciscana* revealed six distinct haplotypes, of which H1 and H2 were the major types, grouped with 48 and 27 sequences, respectively (fig. 3 and table II). H1 appeared composed of 47.92% GSL, 43.75% AWWR and 8.33% SFB sequences, while H2 contained 96.29% and 3.71% of the sequences from SFB and GSL, respectively. There was one more sequence of the AWWR material that was shared with two others from GSL, i.e., in H4. With regard to the distribution of sequences in the haplotype network, the genetic structure of the *COI* marker in AWWR presented a high sequence similarity with the GSL source.

Artemia is a crustacean with commercial importance in the aquaculture industry (Sorgeloos et al., 2001). Various products of *Artemia*, especially enriched nauplii, are widely used as live food in fishery and aquaculture (Lavens & Sorgeloos, 1996; Touraki et al., 2012; Yin et al., 2012). Since the 1970s, *A. franciscana* has been introduced intentionally into many salt habitats in Asia, Australia and the Western Mediterranean (Amat et al., 2005, 2007; Ben Naceur et al., 2010; Muñoz & Pacios, 2010; Scalone & Rabet, 2013; Muñoz et al., 2014; Eimanifar et al., 2014).

A. franciscana, the inhabitant of the Great Salt Lake (GSL), has been generally used in the aquaculture industry for cysts and biomass production (Sorgeloos et al., 2001). According to our results, the distribution of sequences in the haplotype network verified a hypothesis that the Great Salt Lake can be the origin of an exotic, invasive *Artemia* in the AWWR. Although there is no evidence to confirm that *A. franciscana* had been introduced intentionally into Al Wathba Wetland Reserve for commercial purposes, it supports a suitable habitat for migratory birds, especially the greater flamingo (*Phoenicopterus roseus* Pallas, 1811). The first population of greater flamingos resided in the AWWR in 1993 (Al Dhaheri, 2004). Green et al. (2005) suggested migrating of shorebirds is one of the major tools for dispersal of invasive and native brine shrimps of the genus *Artemia*. It seems that migratory birds, particularly the greater flamingo, may play a key role in the dispersal of non-native *A. franciscana* into Al Wathba Wetland Reserve from neighbouring sites in the Middle East. There are a few confirmed sites in the Middle East that are invaded by exotic invasive *A. franciscana*, including Iran (Nough, Maharlu Lake, Mahshahr port, Nough catchment) and Iraq (Garmat Ali) (Eimanifar et al., 2014).

Therefore, in conclusion we can state, that comprehensive studies need to be carefully performed considering the biodiversity of American *A. franciscana* in Middle East, in order to get a clear picture of the dispersal and distribution of the species in this part of the world.

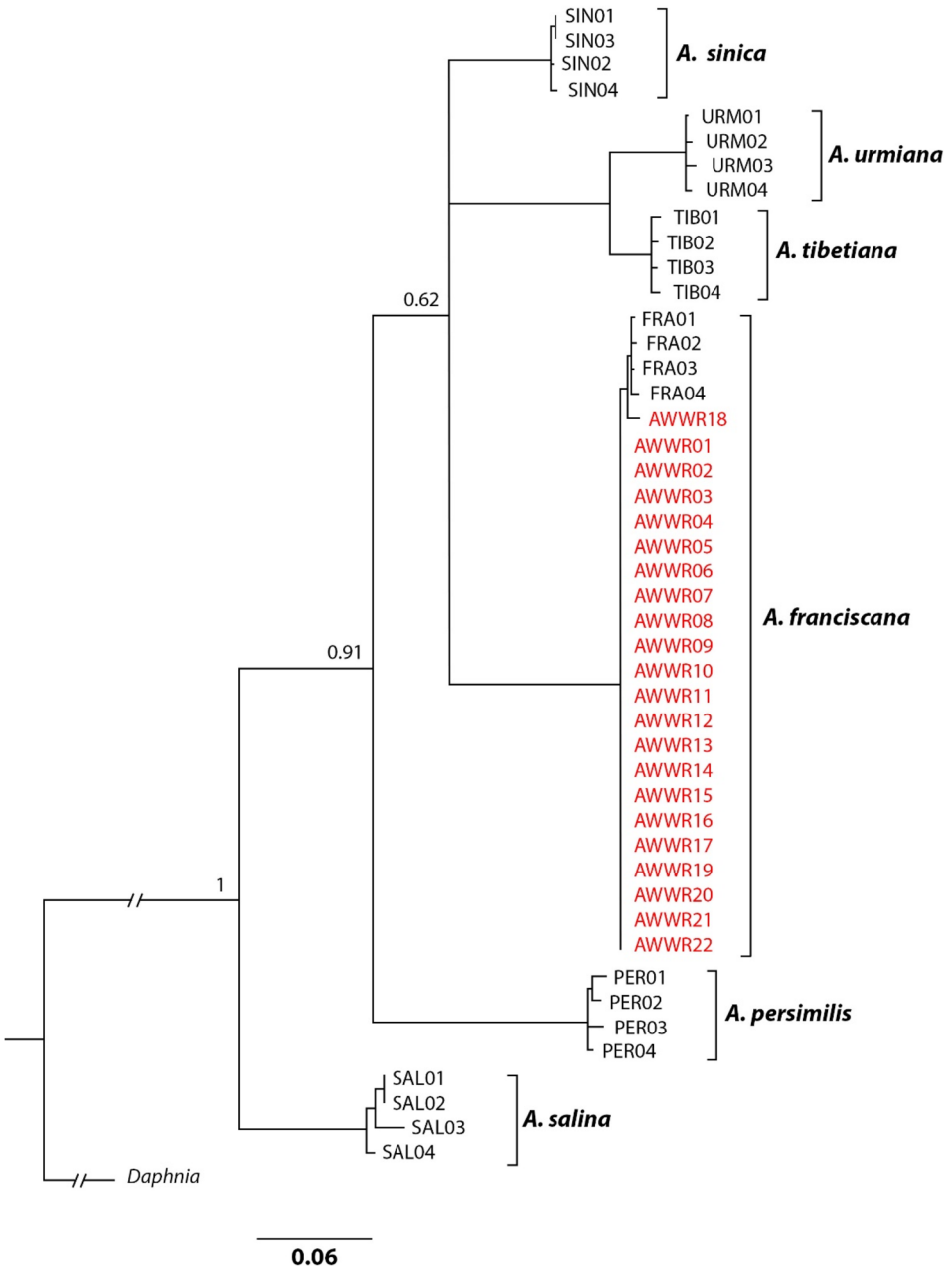


Fig. 2. The *COI* phylogeny of the *Artemia* specimens analysed, based on the Bayesian Inference approach. The numbers behind major nodes denote posterior probabilities. *Daphnia tenebrosa* G. O. Sars, 1898 (GenBank accession no. HQ972028) was used as an outgroup.

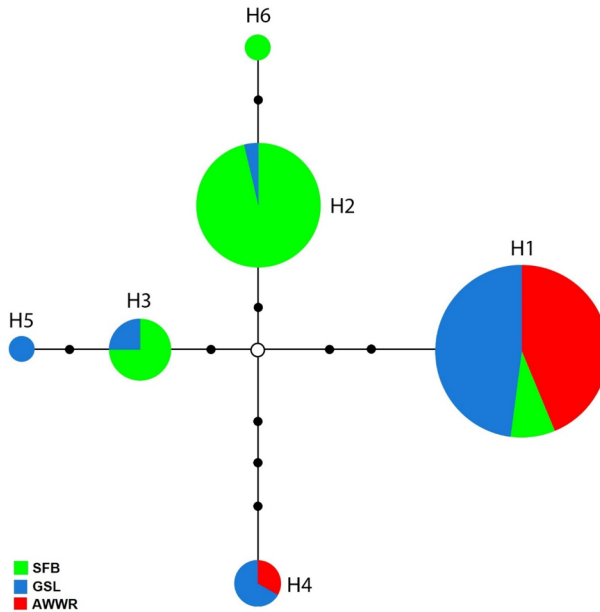


Fig. 3. The relationship of *COI* haplotypes distribution among *Artemia* individuals from the Great Salt Lake (GSL), San Francisco Bay (SFB) and the Al Wathba Wetland Reserve.

TABLE II
Haplotype information for the network of *Artemia* haplotypes designed in this study (see fig. 3)

Haplotype No.	Total number of individuals	GSL (No. — Percentage)	SFB (No. — Percentage)	AWWR (No. — Percentage)
H1	48	23 — 47.99%	4 — 8.33%	21 — 43.75%
H2	27	1 — 3.71%	26 — 96.29%	0 — 0%
H3	8	2 — 25%	6 — 75%	0 — 0%
H4	3	2 — 66.66%	0 — 0%	1 — 3.34%
H5	1	1 — 100%	0 — 0%	0 — 0%
H6	1	0 — 0%	1 — 100%	0 — 0%

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