

The impact of the colonization of the invasive American *Artemia franciscana* (Crustacea: Anostraca) on genetic differentiation in the United Arab Emirates (Asia)

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Abstract

Artemia franciscana, native to America, has recently colonized non-indigenous populations in Eurasia, Mediterranean regions and Australia. In present we sought to evaluate the potential effects of colonization of *A. franciscana* on genetic differentiation in the new environments in UAE. We used the *COI* marker to determine population genetic structure and identify the origins of exotic populations in UAE. Our findings have confirmed the colonization of both localities by *A. franciscana*. Genetic variation of invasive *A. franciscana* were exclusively lower than native population in Great Salt Lake and San Francisco Bay. Results have showed the studied population could not possibly have colonized directly from natural American localities, perhaps resulting from secondary introduction events from other non-indigenous populations. Genetic analysis have yielded different demographic patterns for invasive studied populations. Al Wathba Wetland Reserve (AWWR) population have represented demographic expansion. In contrast, Godolphin Lakes (GL) population was at demographic equilibrium. Neutrality tests have documented the excess of both recent and historical mutations in the *COI* gene pool of invasive AWWR *Artemia* throughout establishment in the new environment.

Key words: *Artemia franciscana*, non-native population, mtDNA-*COI*, genetic variation, biodiversity, UAE

Introduction

The introduction of an exotic species to the natural environments can diminish biodiversity and generate a prominent change on biological community structure^{1,2}. Although only nearby 1% of introduced non-natives become invasive³, but establishment of invasive species in new habitats have caused widespread ecological effects and economic damage⁴.

Genetic structure of exotic species is one of the most important factors in their successful colonization and dispersion⁵. Regarding to the genetic contexts, the capacity of a non-native species to adapt to a new environmental conditions depends on the potential of genetic diversity of the species⁶. It is expected exotic species are experienced loss of genetic variation during colonization in non-native habitats due to the founder effect which resulted by genetic drift following small size of population⁷.

The brine shrimp genus *Artemia* is widely used in larviculture⁸. Annual *Artemia* cysts consumption by aquaculture and fishery industry raised rapidly from 60 tons in 1980 to nearly 2000 in 1994. At the beginning of the 21th century, the rapid development of aquaculture increased the harvest of *Artemia* cysts up to 9000 tons in 2000-2001 (wet weight) from Great Salt Lake, USA^{see9}. Although there is no official statistics, it was estimated that 1000 tons of *Artemia* cysts have been consumed alone in China in 2016¹⁰.

Since 1950, American *A. franciscana* from two major natural sources in the USA, the Great Salt Lake (GSL) and San Francisco Bay (SFB), were exported overseas for larviculture and fishery hatcheries¹¹⁻¹³. Because of *Artemia* cysts have been harvested in a limited number of natural hypersaline lakes, growing importance of *Artemia* in aquaculture needed to culture *Artemia* in other saltwater sources. Due to higher reproductive rate and adaptation ability of *A. franciscana*^{14,15}, this species has been selected to culture in non-indigenous natural and artificial

environments for industrial aquaculture and fishery activities, so currently it has a wide geographical distribution in the world containing inland salt lakes, coastal saltworks, salt ponds and lagoons. Now, *A. franciscana* has been colonized in numerous regions across Eurasia, especially in the Mediterranean^{9, 11-13, 16-20} and Australia²¹.

Two *Artemia* sites have been reported from the United Arab Emirates^{22,23}. Saji et al.²⁰ have documented invasive *A. franciscana* in Al Wathba Wetland Reserve. There is no evidence that *Artemia* had been introduced intentionally into these localities for commercial activity, but it has prepared a suitable habitat for the greater flamingos and other native shore birds²⁰. In 1998, before introduction of the greater flamingos in Godolphin Lakes, cysts of *Artemia* were distributed in the water body²³. Although previous studies have referred existence of *Artemia* in Godolphin Lakes to *A. franciscana*, there is a lack of phylogenetic proof to support this claim. The aim of the present study was to perform a phylogenetic analysis of populations from Godolphin Lakes to confirm the taxonomical status of *Artemia* in this locality. Here we sequenced the mitochondrial *COI* gene and calculated the genetic diversity and population genetic structure of *Artemia* populations in UAE to compare evolutionary progresses and genetic differentiation of invasive species in new environments.

Materials and Methods

Study area and sampling

In total, 82 cysts of *Artemia* were collected from two geographical localities in United Arab Emirates including Godolphin Lakes (GL) and Al Wathba Wetland Reserve (AWWR). (Fig. 1). The sampling sites with their abbreviations, geographical coordinates and number of specimens analyzed were summarized in Table 1.

Total DNA was extracted from each decapsulated *Artemia* cyst following the Chelex® 100

Resin method (Bio-Rad Laboratories, USA). The samples were crushed, incubated for 3 h at 56°C and finally 10 min at 80°C (tubes were vortexed every 30 min). The tubes were centrifuged at 10,000 rpm for 2 min. and the supernatant phase was used as a template in the PCR reaction^{13,24}. The extracted DNAs were stored at -80°C for further studies.

A fragment of mitochondrial marker *cytochrome c oxidase subunit I (COI)* was amplified using the universal primers LCO1490/HC02198²⁵. PCR was performed on a total volume of 20 µl containing 8 µl of ddH₂O, 10 µl Taq polymerase (2× Easy Taq® PCR SuperMix, Code#AS111 +dye, TransGen Biotech CO., Ltd. CHN), 0.4 µl of DNA solution and 0.8 µl of each primer. The PCR cycling program 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^{see13,24}.

Sequence alignment and phylogenetic analyses

Sequences were aligned using MEGA X with default setting²⁶. To identify taxonomical status of the collected samples, the *COI* reference sequences from bisexual species and parthenogenetic populations were downloaded from GenBank (Table 2) and utilized to draw phylogenetic trees. The phylogenetic tree was generated based on Bayesian inference (BI) as performed in MrBayes 3.2.2 on XSEDE²⁷. The best fitting nucleotide substitution model was estimated using MrModeltest 2.2²⁸ and HKY+G was selected as the best-fit model.

The sequences of *A. franciscana* were analyzed from two natural localities from U.S.A consisting of San Francisco Bay (SFB) (37 sequences: KF662960, KF662968, KF662970, KF662975) and Great Salt Lake (GSL) (29 sequences: KF662960, KF662968, KF662970, KF662971, KF662976, KF662977), respectively²⁹. To detect the genealogical relationships and origin of UAE samples with American *A. franciscana* (more information in Results), a median network was performed using the median-joining algorithm in the Network program ver.

5.0.0.3³⁰.

For each population (incl. UAE and American *A. franciscana*), the number of polymorphic sites (S), total number of mutations (Eta), number of haplotypes (h), haplotype diversity (Hd), haplotype ratio (Hr), nucleotide diversity (Pi), average number of nucleotide differences (k) and neutrality tests (i.e. Tajima *D*, Fu and Li's *D**, Fu's *F*s) were computed using DnaSP v.5.10 program³¹. Expected heterozygosity, *F*_{ST} (an overall population differentiation index), AMOVA and mismatch distribution were computed by Arlequin v.3.5³². Two parameters for population differentiation (*G*_{ST}, *N*_{ST}) were considered using the program Permut version 1.0³³.

Results

The Bayesian inference (BI) phylogenetic tree have utilized all studied *Artemia* samples from GL and AWWR were clustered in the clade of *A. franciscana* (Fig. 2). The results have documented both localities have been invaded by the exotic American species *A. franciscana*.

COI sequences of *A. franciscana* from UAE have displayed 16 variable sites where 8 sites were parsimony informative and 8 sites were singletons. The *COI* sequences of native American *A. franciscana* have represented 12 variable sites where 10 sites were parsimony informative and 2 sites were singletons, respectively.

The differentiation of haplotype frequencies was exclusively non-significant between both exotic populations in UAE (Table 3). Figure 3 showed the haplotype distribution network of the *A. franciscana* among native and invaded habitats. The 170 *COI* sequences of *A. franciscana* have represented 14 distinct haplotypes, that H1, H10 and H12 were the major types, grouped with 57.06% (97 ind.), 18.24% (31 ind.) and 12.94% (22 ind.), respectively (Table S1). In addition, the majority of sequences belonged to H1, composed of 47.42% AWWR, 46.39% GL, 4.12% SFB and 2.07% GSL sequences, respectively (Fig. 3 and Table 4).

The haplotypes distribution and frequency in each locality were determined in Table 5. The majority of haplotype frequency of the native American *A. franciscana* from SFB and GSL were located in H10 (83.87%) and H12 (95.45%), respectively. Two localities from UAE have possessed the greatest H1 haplotype frequency consisting of AWWR (88.46%: 46 individuals out of 52) and GL (86.54%: 45 individuals out of 52).

The estimated genetic indices for the studied localities were displayed in Table 6. The highest-ranking amounts of H_r (0.206), P_i (0.0038 ± 0.002) and K (2.334 ± 1.013) were documented in GSL, whereas, the SFB locality had the highest quantities of H_d (0.480 ± 0.087) and H_{exp} (0.277 ± 0.136). The lowest genetic variations has observed in the AWWR location, excepting H_r which the lowest value was noticed in GL (0.096). Neutrality tests have yielded negative values with significant and non-significant levels.

The mismatch distributions for invasive UAE and American populations of *A. franciscana* have showed that GSL, SFB and GL localities had a multimodal pattern, while AWWR location revealed a pattern likely to be unimodal (Fig. 4).

The lowest and non-significant values of the pairwise genetic differentiation index (F_{ST}) were observed between UAE populations (2.68%). The significant population differentiations were represented between UAE and American populations ($P < 0.01$). Additionally, the lowest and significant value of F_{ST} was determined between native American populations (SFB-GSL: 58.39%) (Table 7).

The permutation test has indicated that the difference of G_{ST} and N_{ST} was exclusively significant between UAE populations where N_{ST} (0.027) was higher than G_{ST} (0.0001) (Table 8). AMOVA analysis has documented that more than a third of genetic variation (37.20%) is attributed within populations (Table 9).

Discussion

The occurrence of *Artemia* in UAE had been recorded in two geographical sites including in Al Wathba Wetland Reserve²² and Godolphin Lakes²³. A molecular phylogenetic study has confirmed that population in Al Wathba Wetland Reserve belonged to an exotic American *A. franciscana*²⁰. Our results have also documented the colonization of same species in Godolphin Lakes locality.

The San Francisco Bay (SFB) and Great Salt Lake (GSL) are the two main sources of *Artemia* that have usually been used to culture in other saline ecosystems for industrial aquaculture and fishery activities to produce *Artemia* cysts and biomass^{13,20,21,34}, for this reason these populations were considered in this analysis to find out the genetic alterations of the colonized populations in new non-native environments.

Mitochondrial DNA represented some exceptional characteristics consisting rapid evolutionary rates, maternal origin, and lack of recombination^{35,36}. Then mitochondrial markers are important for apprehension the tracing and explanation the source of non-indigenous species in new habitats^{20,21,37-40}.

Phylogeographical analysis using mitochondrial *COI* markers have clearly demonstrated that GSL and SFB were origin of invasive *A. franciscana* in the Mediterranean area^{34,19}. Genetic pattern of Asian populations have shown *A. franciscana* were colonized by multiple origins, direct establishment from America and secondary introduction from Europe¹³. Saji et al. (20) studied on phylogeography of *A. franciscana* in Al Wathba Wetland Reserve (Abu Dhabi; UAE). Regarding to haplotype distribution, it was proposed the commercialized GSL source might be the origin of invasive *Artemia* in Abu Dhabi. A recent study on geographical origin of invasive American *Artemia* in Australia has documented different results. Genetic analyses evidenced that

populations from Port Hedland and St Kilda have genetically originated from SFB population, *Artemia* from Mulgundawa and Dampier resulting from secondary introduction events or introduced from admixture sources containing GSL and SFB²¹. In contrast Saji et al.²⁰ suggested that GSL as origin of Al Wathba Wetland Reserve (Abu Dhabi; UAE), our finding could not support any evidence that the origin of both localities in UAE (Al Wathba Wetland Reserve and Godolphin Lakes) came from native populations in America (GSL and/or SFB). Because the most sequences in major haplotype (H1) contained 47.42% of AWWR locality and 46.39% of GL localities, and both GSL and SFB could not share significant contribution in H1. Additionally, there is no meaningful relationship between American and invasive populations sequences in other haplotypes (Fig. 3 and Table 4). On the other hand the highest and significant F_{ST} values between American and exotic populations have strongly supported that GSL and SFB could not be an introduced source for UAE localities (Table 7). The paradox between our result and previous study on Al Wathba Wetland Reserve can be referred to technical error using short sequences of *COI* in the previous study (446 bp vs 604 bp). Regarding to results of the current study, the geographical origin of both UAE populations might be secondary introduction from other *Artemia* production sources, especially Eastern Asia including Mekong Delta (Vietnam) and Bohai Bay (China) where these are commercially available in aquaculture markets^{21, 34,41,42}.

Golani et al.⁴³ showed that the invasive populations generally possess lower genetic variation in non-indigenous new environments in comparison with the source populations. An introduced population of *A. franciscana* in Vinh Chau (Vietnam) has displayed low intraspecific genetic variation and reduced haplotype diversity as compared with its original population from SFB⁴⁴. In contrast, a comprehensive study on Asian *A. franciscana* has documented invasive Asian *A. franciscana* populations had higher genetic diversity than American GSL population

and native Asian species¹³. Identical findings also have been found in some Mediterranean invasive populations^{45,34}. Asem et al.²¹ find an equilibrium population of exotic *Artemia* from Dampier in Australia without genetic variation where population showed single haplotype. As well as other invasive populations in Australia represented different levels of genetic variations as compared with American native populations. Our results indicated the low level of genetic differentiation in both exotic UAE populations which Al Wathba Wetland Reserve revealed lower variation than Godolphin Lakes (Table 6).

Totally low genetic variation of exotic populations can be result of founder effect⁴⁴ or population bottleneck during the process of colonization²¹. In the other hand, higher genetic diversity can be attributed to adaptive ability and/or physiological plasticity in non-native populations^{7,13,34,46,47}. We suggest different ecological conditions in new environments can exert selective pressures during introduction exotic population which could affect genetic variation.

Our results have revealed a negative and significant Tajima's *D* value (-2.304) only for exotic population in AWWR location (Table 6), which demonstrated an excess of rare haplotypes following with population expansion or purifying selection⁴⁸⁻⁵². Given that high values of polymorphic sites (12 sites), number of mutations (12 mutations) and number of Haplotypes (12 haplotypes) in comparison with other localities, then Tajima's *D* result might be referred to the demographic expansion of *Artemia* population in AWWR. Additionally, the unimodal mismatch distribution has demonstrated the demographic expansion in this population (Fig. 4). The negative value of Fu's *F_s* test and Fu and Li's *D** test rare recent mutations⁵³⁻⁵⁶ and an excess of rare historical mutations⁵⁵⁻⁵⁸, respectively. The results of both neutrality tests were negative and significant for AWWR population that could document the excess of both novel and ancient mutations in the *COI* gene pool of invasive AWWR *Artemia* during colonization in

the new environment.

Regarding to non-significant value of neutrality tests and multimodal of mismatch distribution, Godolphin Lakes have shown a demographic equilibrium. American *Artemia* (GSL and SFB) has presented negative and non-significant value for neutrality tests and also mismatch distribution have revealed multimodal. These findings have evidenced that these populations were at demographic equilibrium. In contrast, Asem et al.²¹ found that GSL represented a unimodal mismatch distribution and have recorded this population was under demographic expansion. This difference can be attributed using sequences in different period from GSL. Asem et al.⁵⁶ have proved ecological variation could alter genetic structure of *Artemia* from Urmia Lake. It seems a comprehensive study needs to be performed on population genetic of both native American *Artemia* in GSL and SFB to estimate ability of genetic variation in two major sources in long-term.

The N_{ST} showed high and significant value (0.027) than G_{ST} (0.0001) between invasive populations in UAE. A significantly higher N_{ST} over G_{ST} generally point to the existence of phylogeographic structure (Pons and Petit, 1996). The results have indicated that there was a differentiation of geographical structure between exotic populations of Godolphin lakes and Al Wathba Wetland Reserve, while there was no phylogeographic differentiation between GSL and SFB populations and also among UAE and American populations.

Overall, the Godolphin Lakes and Al Wathba Wetland Reserve are close localities (approximately with 120 km distance) but represented strongly geographical structure. In addition, population genetic have showed these two populations had different demographic history. Consequently, these findings could be documented the effect of colonization on genetic variation in new environments. Although there is no enough information about ecological

conditions in these sites, but previous studies have recorded average salinity in 100 ppt and 180 ppt for Godolphin Lakes and Al Wathba Wetland Reserve localities, respectively^{23,59}. In hypersaline environments, salinity is the most important ecological parameter for biology of *Artemia* populations (incl. survival, growth and reproduction). The existing of geographical structure and differentiation in demographic histories in UAE populations can be referred to dissimilarity ecological conditions and different adaptation progresses in new environments.

Consequently, present study could provide evidences to demonstrate the impact of introducing exotic economical species in new environments on genetic and geographical differentiation. Our results have showed invasive species could present genetic pattern in different with native populations. Also, exotic populations should not necessarily have the similar genetic pattern in neighboring environments.

In conclusion, the impact of exotic species in aquatic ecosystems has considered less regard than terrestrial habitats⁶⁰. Additionally, there are a few information about colonization effects in biodiversity of hypersaline ecosystems which needs more considerations. They usually have a more limited number of species, compared to marine and freshwater habitats, and this makes their biodiversity more vulnerable. Therefore, introduction of exotic species could be caused a major threat on biodiversity of native species/populations. For example, colonization of American *A. franciscana* in Port Hedland and Dry Creek (Australia) has caused the extinction of native parthenogenetic populations^{see²¹}. There are several studies on biology of invasive *A. franciscana* in non-native environments and it can be suggested as suitable invasive modal organism to investigate on biological effect of exotic species particularly on patterns of genetic variation and impact on native communities.

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Acknowledgements

This project was funded by Hainan Province Science and Technology Department Key Research and Development Program (ZDYF2019154), and Local Government fund in SanYa City (2019YD06).

Author contributions

Conceived and designed experiments: A.A. and A.E. Sampling: R.S. Performed the experiments: H.L. M.L. and C.L. Performed the analysis and Interpretation of results: A.A. and A.E. Prepared manuscript: A.A and A.E. Edited manuscript: R.S., P-Zh. W. and W.L.

Competing interests

The authors declare no competing interests.

Table 1. Origin of *Artemia* samples from United Arab Emirates.

Site	Abbreviation	Geographic Coordinates	No. Ind.
Godolphin Lakes	GL	25°10'31.60"N, 55°15'46.81"E	52
Al Wathba Wetland Reserve	AWWR	24°15'15.59"N, 54°36'38.57"E	30

Table 2. Species information and GenBank accession numbers.

Species/ population	Abbreviation	Individual	Accession numbers	Ref.
<i>A. urmiana</i>	URM	4	JX512748-751	1
<i>A. sinica</i>	SIN	4	KF691298-301	2
<i>A. tibetiana</i>	TIB	4	KF691215-218	2
<i>A. salina</i>	SAL	4	KF691512-515	2
<i>A. persimilis</i>	PER	4	DQ119647	3
			HM998992	4
			EF615594	5
			EF615593	5
<i>A. franciscana</i>	FRA	4	KJ863440-443	2
Diploid Pop.	DI	4	KU183949-952	6
Triploid Pop.	TRE	3	HM998997-999	4
Tetraploid Pop.	TETR	4	KU183954-957	6
Pentaploid Pop.	PEN	4	KU183968-971	6
UAE pops.	GL	52	xxxxxxx-xx	7
	AWWR	30	xxxxxxx-xx	7
		22	MH221175-196	8

Ref: 1: Eimanifar and Wink 2013; 2: Eimanifar et al. 2014; 3: Hou et al. 2006; 4: Maniatsi et al. 2011; 5: Wang et al. 2008; 6: Asem et al. 2016; 7: Saji et al., 2019; 8: this study

Table 3. Differentiation of haplotype frequencies among localities (results represent as P value).

	GL	AWWR	GSL
AWWR	0.113		
GSL	0.0000	0.00001	
SFB	0.00001	0.00001	0.00001

Table 4. Distribution and frequency of observed localities in each haplotype.

Haplotype	Individuals	Locality	Percentage
H1	4	SFB	4.12
	2	GSL	2.07
	45	GL	46.39
	46	AWWR	47.42
H2	1	AWWR	100
H3	1	AWWR	100
H4	1	AWWR	100
H5	1	GL	50
	1	AWWR	50
H6	2	GSL	100
H7	1	AWWR	100
H8	1	GL	100
H9	1	GL	100
H10	26	SFB	83.87
	1	GSL	3.22
	4	GL	12.91
H11	1	SFB	100
H12	21	GSL	95.46
	1	AWWR	4.54
H13	6	SFB	75
	2	GSL	25
H14	1	GSL	100

Table 5. Distribution and frequency of observed haplotypes in each locality.

Population	Haplotype	Individual	Percentage
SFB	H1	4	10.81
	H10	26	70.27
	H11	1	2.70
	H13	6	16.22
GSL	H1	2	6.90
	H6	2	6.90
	H10	1	3.45
	H12	21	72.41
	H13	2	6.90
	H14	1	3.45
GL	H1	45	86.54
	H5	1	1.92
	H8	1	1.92
	H9	1	1.92
	H10	4	7.69
AWWR	H1	46	88.46
	H2	1	1.92
	H3	1	1.92
	H4	1	1.92
	H5	1	1.92
	H7	1	1.92
	H12	1	1.92

Table 6. Population genetic indices for invasive and native American *A. franciscana* based on *COI* loci.

Genetic indices	AWWR	GL	GSL	SFB
Number of sequences:	52	52	29	37
Number of polymorphic (segregating) sites (S)	12	7	11	7
Total number of mutations (Eta)	12	7	11	7
Number of Haplotypes (h)	7	5	6	4
HR	0.134	0.096	0.206	0.108
Haplotype (gene) diversity (Hd)	0.219 ± 0.077	0.249 ± 0.077	0.475 ± 0.111	0.480 ± 0.087
Nucleotide diversity Pi	0.0009 ± 0.001	0.0014 ± 0.001	0.0038 ± 0.002	0.0032 ± 0.002
Average number of nucleotide differences (k)	0.572 ± 0.471	0.865 ± 0.619	2.334 ± 1.313	1.945 ± 1.129
Ext Het	0.047 ± 0.016	0.123 ± 0.059	0.212 ± 0.145	0.277 ± 0.136
Tajima's D	-2.304**	-1.158 ^{ns}	-0.539 ^{ns}	0.451 ^{ns}
Fu and Li's D*	-3.433**	-0.329 ^{ns}	-0.545 ^{ns}	0.529 ^{ns}
Fu's Fs	-4.053*	-0.473 ^{ns}	0.826 ^{ns}	2.676 ^{ns}

ns: non-significant, * P < 0.05; ** P < 0.01

Fu's Fs should be regarded as significant if P < 0.02.

Table 7. Pairwise population matrix of F_{ST} values based on *COI* loci (results in percentage).

	GL	AWWR	GSL
AWWR	2.68 ^{ns}		
GSL	66.59**	70.58**	
SFB	66.00**	73.06**	58.39**

ns: non-significant, * P < 0.05; ** P < 0.01

Table 8: Pairwise population G_{st}/N_{st} values based on *COI* loci.

	GL	AWWR	GSL
AWWR	0.0001/0.027*		
GSL	0.614/0.632 ^{ns}	0.624/0.663 ^{ns}	
SFB	0.572/0.644 ^{ns}	0.613/0.710 ^{ns}	0.501/0.581 ^{ns}

ns: non-significant, * P < 0.05; ** P < 0.01

Table 9. Molecular variation (within and among populations) for among and between populations of invasive and native *A. franciscana* (by AMOVA).

Source of variation	d.f.	Sum of squares	Variance components	Variation (%)
Among Pop.s	3	134.804	1.06174	62.80
Within Pop.s	166	104.390	0.62885	37.20
Total	169	239.194	1.69060	100

Table S1. Haplotype information for the network of *Artemia* based on *COI* loci.

Haplotype	Individuals	Percentage
H1	97	57.06
H2	1	0.59
H3	1	0.59
H4	1	0.59
H5	2	1.18
H6	2	1.18
H7	1	0.59
H8	1	0.59
H9	1	0.59
H10	31	18.24
H11	1	0.59
H12	22	12.94
H13	8	4.71
H14	1	0.59
Total	170	100

Legends for figures

Figure 1. Map of the study areas: the Al Wathba Wetland Reserve and Godolphin Lakes, United Arab Emirates (U.A.E.), Map data ©2018 Google.

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

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

Figure 4. Observed mismatch distributions and their curve fit to simulated model of demographic expansion.



Figure 1

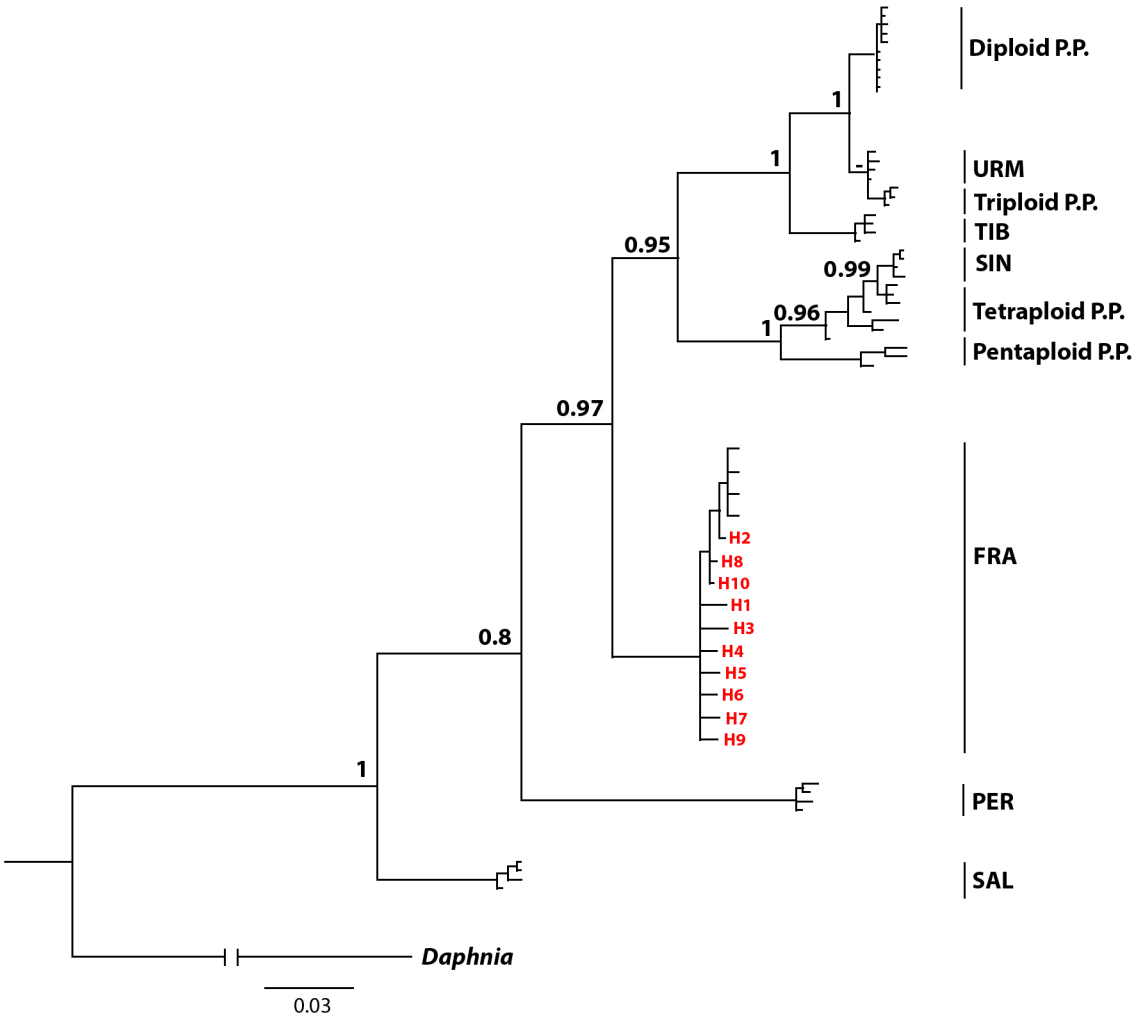


Figure 2

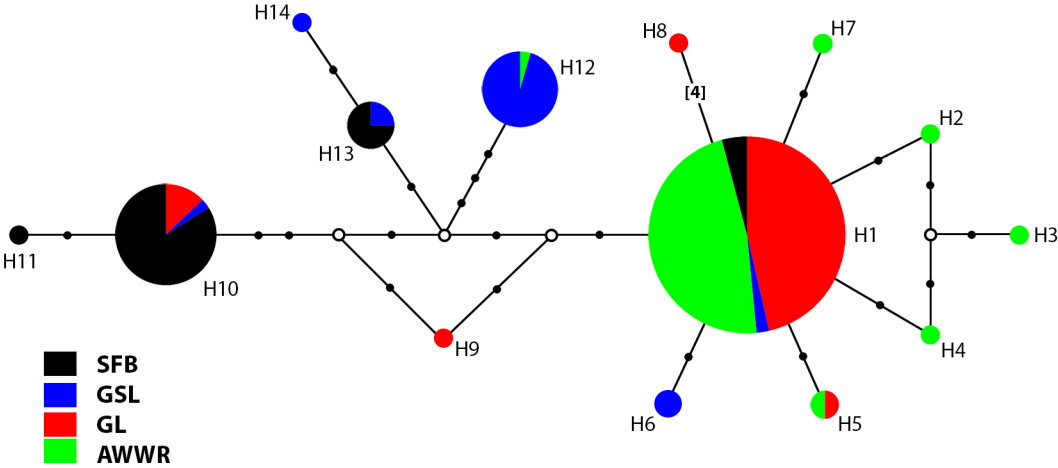


Figure 3

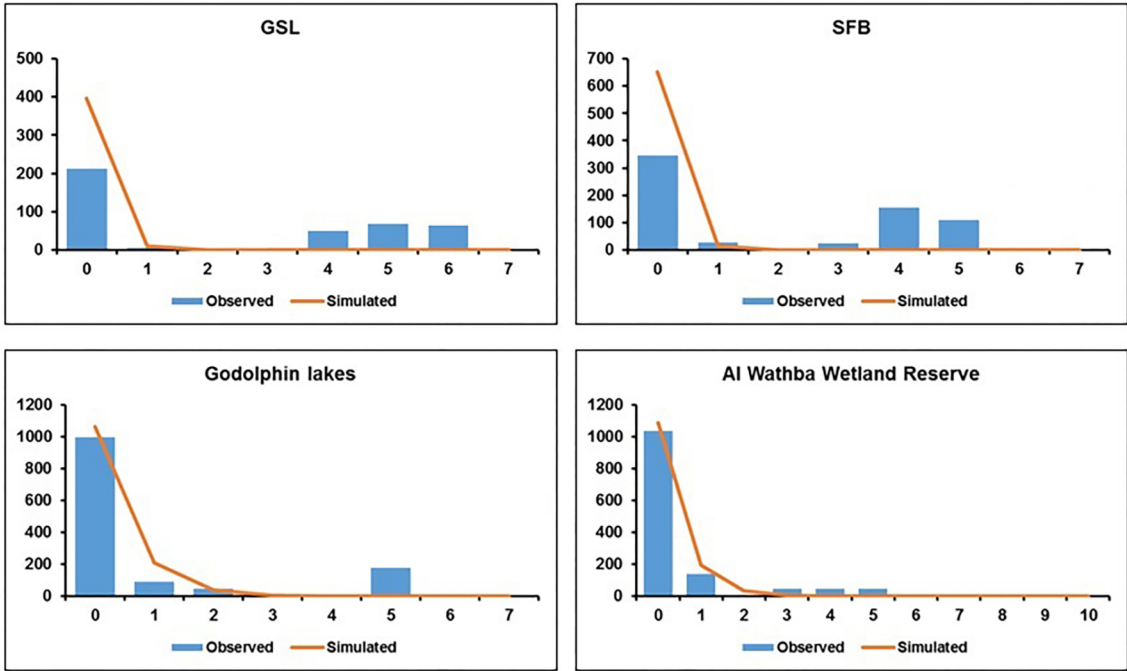


Figure 4