Biodiversity of the brine shrimp *Artemia* from tropical salterns in southern México and Cuba

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Using a fragment of the mitochondrial 16S ribosomal RNA (16S) and cytochrome oxidase I (COI) gene sequences, the biodiversity and phylogenetic relationships of the brine shrimp *Artemia* from tropical salterns of southern México (four sites in the Yucatán Peninsula, one site each in the states of Oaxaca and Campeche), and two sites in Cuba were examined. The Mexican populations showed genetic distances of 0–0.43 among themselves and 0.0298–0.0324 with the Cuban populations. Eight haplotypes (16S + COI) were identified from the studied populations, one for the Cuban populations and seven for the Mexican populations. Phylogenetic analyses of these haplotypes indicate that *Artemia* from these salterns are separated into two well-differentiated clades, one constituted by the Mexican populations and another by the Cuban populations of *A. franciscana*, suggesting that *Artemia* populations of Mexico are native and the Cuban are introduced.

Keywords: *Artemia*, cytochrome oxidase 1, genetic distance, 16S rRNA.

Introduction

THE branchiopod crustacean *Artemia* (Anostraca) has been reported in more than 600 coastal locations and inland waters around the world[1]. In America, it inhabits hypersaline water bodies from Canada to Chile, including the Caribbean. According to Gajardo *et al.*[2], there are three zygogenetic species in America: *Artemia franciscana* Kellogg, 1906; *A. monica* Verrill, 1869 and *A. persimilis* Piccinelli and Prosdocimi, 1968. *A. franciscana* has a wide distribution range and is found from Canada to Chile, whereas *A. persimilis* and *A. monica* have narrow distribution ranges, with the former found in Argentina and Chile, and the latter exclusively in Mono Lake, California, USA[2]. *A. franciscana* and *A. persimilis* show a convergence zone in Los Vilos, Yape and the Atacama Desert, Chile[3,4]. In Central and South America, *A. franciscana* was inoculated, with or without the presence of native *Artemia*, to increase the availability of 'new' stocks of *A. franciscana*[5], to meet the growing demand of aquaculture and to minimize dependence on *Artemia* from the Great Salt Lake (GSL), Utah, USA. However, the GSL is still the major world supplier of commercial cysts, despite the ecological degradation and poor harvests in some years[5].

In the 1970s, *Artemia* was introduced into five salt works in Cuba where no natural *Artemia* populations had been reported[6]. At present, stable populations of *Artemia* are found in only two salt works, Frank País (Guantánamo province) and Santa Lucía (Camagüey province), and with a temporary population in the Bidos salt work (Matanzas province)[7]. These populations are considered to be *A. franciscana*, though a conclusive characterization does not exist.

In México, natural populations of *Artemia* have been recorded in at least 29 locations in 11 states[8]. In spite of cytogenetic[9,10], allozymatic[11,12] and morphological[13–15] studies done on Mexican *Artemia*, 23 populations have not yet been determined at the species level[8].

Globally, despite the use of morphology, kariology and molecular analysis, almost half of the brine shrimps reported from 600 locations remain unidentified[1]. Several techniques using DNA have been used to investigate the diversity of the genus *Artemia*: random amplified polymorphic DNA (RAPD)[16–19], amplified fragment length polymorphism (AFLP)[20], restriction fragment length polymorphism (RFLP)[2,21], and analysis of nuclear DNA internal transcribed spacer 1 sequence[21,22]. Also, mitochondrial gene sequences were successfully used at species levels in *Artemia*[22–24].

In our study the mitochondrial 16S rRNA (16S) and cytochrome oxidase I (COI) gene sequences were used to investigate geographically separated *Artemia* populations from tropical salterns of Central America and the Caribbean. The genetic variation within and between these populations and their phylogenetic relationships were determined.

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Materials and methods

Samples of *Artemia* cysts were taken from eight locations: two in Cuba and six in México (four from Yucatán, one each from Campeche and Oaxaca). The geographic origin of the samples is shown in Figure 1.

DNA extraction, PCR amplification and sequencing

Cysts were decapsulated using sodium hypochloride and then rinsed with distilled water. DNA was isolated from single cysts crushed in a 1.7 ml Eppendorf tube with 65 µl of 5% Chelex. Samples were incubated at boiling temperature for 8 min. Next, 0.8 µl proteinase K (20 mg/ml) was added to each sample and they were incubated for 30 min at 55°C. They were once again incubated at boiling temperature for 4 min. Finally, the samples were centrifuged for 3 min at 9300 *g*. Using an aliquot of the supernatant as template, a fragment of the mitochondrial 16S gene was amplified using primers 16Sar and 16Sbr[25], and the COI gene was amplified using primers 22F (5′-GGTCAACAAATCATAAAGATATTGG-3′) (designed by GM) and HCO2198 (5′-TGATTTTTTGGTCACCCT-GAAGTTTA-3′)[26]. Cycling conditions for amplification of the 16S were from Murugan *et al.*[27]. The COI gene was amplified using the following cycling conditions: a preliminary denaturation at 95°C for 5 min, five cycles of denaturation at 94°C for 1 min, annealing at 45°C for 90 s, extension at 72°C for 90 s, 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s, extension at 72°C for 85 s, and a final extension at 72°C for 10 min. PCR products were verified by electrophoresis and purified using Amersham Pharmacia GFX columns. The purified PCR products were sequenced in an ABI 310 Genetic Analyzer and an ABI 377 DNA Sequencer.

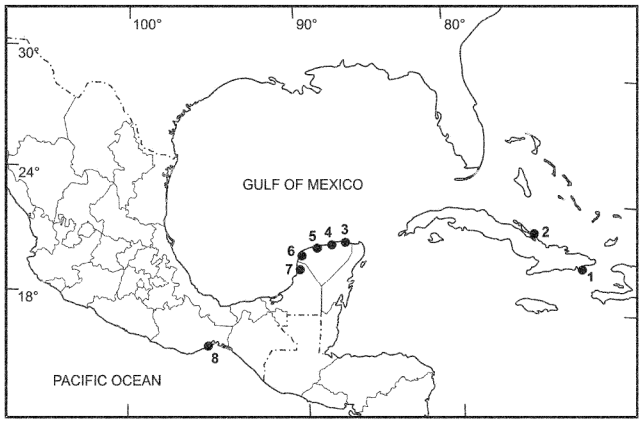

Figure 1. Approximate locations in Cuba and southern Mexico of *Artemia* populations used in this study. Cuba – 1, Salt work Frank País, Guantánamo (CGA); 2, Salt work El Real, Camagüey (CGA). Mexico – 3, Xtampú (YX); 4, San Crisanto (YSC); 5, Chuburna (YC); 6, Celestún (Yucatán); 7, Campeche (CP), and 8, Oaxaca (OX).

For each population a minimum of five replicates (cysts) were sequenced.

Sequence alignment and phylogenetic analyses

Sequences of the 16S and COI genes were aligned with ClustalX version 1.8 (ref. 28) using default settings and checked manually in GenDoc[29]. They were analysed as an independent (16S, COI) and a combined 16S–COI dataset. The GenBank 16S and COI sequences of *A. franciscana* (16S and COI, accession no. X69067), *A. persimilis* (16S, AF202766; COI, DQ119647), and *A. sinica* Cai, 1989 (16S, AF202754; COI, DQ119648) were included in the analyses.

The Akaike Information Criterion (AIC) in Modeltest 3.7 (refs 30, 31) was used to estimate the appropriate substitution model of DNA evolution from the data (16S, COI, and combined 16S–COI). With this model the sequence divergence was calculated using a pairwise sequence comparison on a Maximum-Likelihood-distance matrix for the combined dataset. Phylogenetic relationships were analysed using the Neighbour Joining (NJ), Maximum Parsimony (MP), and the Maximum Likelihood (ML) algorithms in PAUP* 4.0b10 (ref. 32) and Bayesian (MB) algorithm in MrBayes 2.01 (ref. 33). Topology support of the branches was assessed through nonparametric bootstrapping using pseudoreplicates[34], 10,000 for NJ, 1000 for MP and 100 for ML.

Bayesian analysis was done initiating the MCMC (Markov-chain Monte Carlo) process from a random starting point. Four chains were run simultaneously for 1,000,000 generations, with trees sampled every 100 generations for a total of 10,000 trees in the initial sample. Variations in the ML scores in the samples were examined by inspecting the MrBayes-logfile, and the position where the ML scores stopped improving was determined. The portion of the trees before the position (tree number) where the ML score stopped improving dramatically and only fluctuated around a plateau was discarded. The subsequent probability of the phylogeny and its branches was determined for all those trees in the plateau phase with nearly the best ML scores. The Bayesian estimates of subsequent probability and bootstrap analyses were included to assess support. Trees were displayed with TREEVIEW 1.6.6 (ref. 35).

Results

Characteristics of the 16S and COI gene fragments

Unambiguous sequences for the mitochondrial 16S and COI genes of eight *Artemia* populations from different geographic origins were obtained and deposited in GenBank (accession nos DQ401259–DQ401278). The length of the 16S and COI fragments of the eight *Artemia* popu-

lations used in the analysis was 483 and 656 bp respectively. The G + C base content in the 16S fragment of the Mexican and Cuban *Artemia* populations varied between 40.4 (YCE population) and 41% (CGA and CM populations), and for COI it was between 41 (OX, YC and YSC2 populations) and 41.8% (CGA and CM populations). Base frequencies were biased towards A and T for both genes. The frequency of A (30.4–30.6%) was higher than T (28.4–29.2%) in the 16S, whereas the reverse was found in the COI with 35.7–36.1% for T and 22.6–22.9% for A. The A + T base content was between 59 and 59.6%, and was higher than the G + C content in both genes. The *A. franciscana*, *A. persimilis*, and *A. sinica* sequences taken from the GenBank database also showed higher values for A than T in the 16S, whereas COI showed the reverse. Also, the A + T content in these species was greater than the G + C content for both genes and was 58.8–60.6% for 16S and between 59 and 60.1% for COI (Table 1).

The 16S fragment of the Cuban and Mexican *Artemia* populations plus *A. franciscana* showed 101 variable sites (20.7%) with *A. sinica* and *A. persimilis*, of which 31 were informative (30.7%). The Cuban and Mexican populations contained nine variable sites (1.9%), of which seven were informative (77.8%). The COI fragment of the Cuban and Mexican *Artemia* populations plus *A. franciscana* with *A. sinica* and *A. persimilis* showed 160 variable sites (24.4%), of which 68 were informative (42.5%). The Cuban and Mexican *Artemia* populations contained 25 variable sites (3.8%), of which 23 were informative (92%). Transitions (*Ts*) have predominance over transversions (*Tv*) in the 16S fragment (*Ts*/*Tv* ratio = 2), whereas no transversions were observed in the COI fragment. The 16S fragment showed only T–C transition, whereas the A–G transition was higher (56%) than the T–C transition (44%) in the COI fragment (Tables 2 and 3).

Genetic distance

The nucleotide variation and pairwise sequence comparison using distance measurements by ML with settings corresponding to the Hasegawa, Kishino, Yano model (HKY) with a shape parameter of the gamma distribution correction (HKY + G) are given in Table 4. Genetic distances indicated two well-defined groups, one by all Mexican populations and another by the Cuban populations, including *A. franciscana*. No difference was found between the two Cuban populations, but they differed from *A. franciscana* in 9 bp (ML distance 0.0083). The Mexican populations showed 0–5 bp (0.0000 to 0.0043) differences among themselves, and between 28 and 30 bp (0.0298 and 0.0324) when they were compared with the

Table 1. Fragment length (number of bases), base composition (%), G + C and T + A content, and the ratio T + A/G + C of the mitochondrial 16S and COI genes of *Artemia* populations

Gene	Origin	Population	No. of bases	T	C	A	G	G + C	T + A	T + A/G + C
16S	China	Asin	429	28.2	17.5	32.4	21.9	39.4	60.6	1.54
	Argentina	Aper	427	28.3	16.6	32.1	23.0	39.6	60.4	1.53
	USA	Afran	485	28.5	17.5	30.3	23.7	41.2	58.8	1.43
	Cuba	CGA	483	28.4	17.4	30.6	23.6	41	59	1.44
		CM	483	28.4	17.4	30.6	23.6	41	59	1.44
	México	CP	483	29.0	16.8	30.4	23.8	40.6	59.4	1.46
		OX	483	28.8	17.0	30.4	23.8	40.8	59.2	1.45
		YC	483	28.8	17.0	30.4	23.8	40.8	59.2	1.45
		YCE	483	29.2	16.6	30.4	23.8	40.4	59.6	1.48
		YSC1	483	29.0	16.8	30.4	23.8	40.6	59.4	1.46
		YSC2	483	29.0	17.0	30.4	23.6	40.6	59.4	1.46
		YX1	483	28.8	17.0	30.4	23.8	40.8	59.2	1.45
		YX2	483	28.8	17.0	30.4	23.8	40.8	59.2	1.45
COI	China	Asin	656	36.0	22.1	24.1	17.8	39.9	60.1	1.51
	Argentina	Aper	656	36.6	21.8	21.8	19.8	41.6	58.4	1.40
	USA	Afran	656	35.8	21.8	23.2	19.2	41	59	1.44
	Cuba	CGA	656	35.7	22.0	22.6	19.8	41.8	58.3	1.39
		CM	656	35.7	22.0	22.6	19.8	41.8	58.3	1.39
	México	CP	656	36.0	21.6	22.9	19.5	41.1	58.9	1.43
		OX	656	36.1	21.5	22.9	19.5	41	59	1.44
		YC	656	36.1	21.5	22.9	19.5	41	59	1.44
		YCE	656	36.0	21.6	22.9	19.5	41.1	58.9	1.43
		YSC1	656	36.1	21.5	22.7	19.7	41.2	58.8	1.43
		YSC2	656	36.1	21.5	22.9	19.5	41	59	1.44
		YX1	656	36.0	21.6	22.7	19.7	41.3	58.7	1.42
		YX2	656	36.0	21.6	22.9	19.5	41.1	58.9	1.43

Asin, *Artemia sinica*; Aper, *A. persimilis*; Afran, *A. franciscana*; CGA, El Real, Camagüey; CM, Frank País, Guantánamo; CP, Campeche; OX, Oaxaca; YC, Chuburna, Yucatán; YCE, Celestún, Yucatán; YSC1 and YSC2, San Crisanto, Yucatán; YX1 and YX2, Xtampú, Yucatán.

Cuban populations and *A. franciscana*. The Cuban and Mexican *Artemia* populations showed 154–162 bp (0.5363 and 0.5958) differences with *A. sinica*, and 205–206 bp (1.2950 and 1.3214) with *A. persimilis* (Table 4).

Phylogenetic analyses

The alignment of the *Artemia* taxa contained 1143 aligned positions (16S + COI). From the Cuban and Mexican *Artemia* taxa, eight haplotypes were found for the combined 16S and COI fragments, and eight and five haplotypes were found for the genes when they were analysed separately.

Two non-*Artemia* taxa, *Parartemia contracta* Linder, 1941 (16S, AF209048; COI, AF209059) and *P. cylindifera* Linder, 1941 (16S, AF209050; COI, AF308954), were included in the dataset to root phylogenetic trees. Evaluation of the AIC obtained from the Modeltest analysis showed that the Transition Model (TIM) using gamma correction for among-site rate variation and a correction for significant invariable sites (TIM + G + I) was the best model to fit our data. The model parameters have the values R = 1.0000, 17.8975, 3.9081, 3.9081, 34.9887, with the proportion of invariable sites P_{inv} = 0.2585. Considering the similarities observed among major clades in NJ, MP, ML, and MB trees, a single phylogram was presented to represent results of the different analysis (Figure 2).

The NJ analysis with distance measurement set to maximum likelihood using TIM + G + I showed that *Artemia* populations are monophyletic (bootstrap support (BS) = 100%), and that the Cuban and Mexican populations form two different clades (BS = 99%). The ML analysis, also using the TIM + G + I model, showed a high ML bootstrap support for the *Artemia* clade (BS = 100%, heuristic search). Again, the Cuban and Mexican populations were separated into different clades (BS = 78%). The MP analysis with heuristic search resulted in three most parsimonious trees of 614 steps (CI = 0.8567, RI = 0.7549, RC = 0.6467). The bootstrap 50% majority-rule consensus tree, with an almost identical topology as in the NJ and ML trees, showed the studied *Artemia* as a monophyletic group (BS = 100%, heuristic search), and the Cuban and Mexican populations as two different clades. The *Artemia* from Cuba together with *A. franciscana* formed a sister group to Mexican *Artemia* (BS = 100%; Figure 2). Bayesian analysis also produced high posterior probability support for the monophyly of *Artemia* (100%) and the separation of Cuban and Mexican populations (99%; Figure 2). The monophyly of *Artemia* was also observed in NJ, ML, MP, and MB phylogenetic analyses with six other anostracan taxa, *Artemiopsis stefanssoni* Johansen, 1921; *Branchinecta paludosa* (Müller, 1788), *Eubranchipus* sp., *Polyartemiella hazeni* (Murdock, 1884), *Streptocephalus dorothae* Mackin, 1942 and *Thamnocephalus platyurus* Packard, 1877 (not shown here).

Table 2. Variable sites in the mitochondrial 16S rRNA gene fragments of *Artemia* populations from southern México and Cuba

	Base position								
Population	152	227	252	254	275	297	306	324	432
CP	T	C	T	T	G	C	G	T	T
YCE	.	.	.	.	.	.	.	.	.
YSC1	C	.	.	.	.	T	.	.	.
YSC2	C	.	.	.	T	T	.	.	.
OX	C	.	.	.	.	.	.	.	.
YX1	C	.	.	.	.	.	.	.	.
YX2	.	.	.	.	.	.	.	C	.
YC	C	.	.	.	.	.	.	.	.
CGA	C	T	A	C	.	.	C	.	C
CM	C	T	A	C	.	.	C	.	C

Table 3. Variable sites in the mitochondrial COI gene fragments of *Artemia* populations from southern México and Cuba

	Base position																								
Population	29	38	68	167	182	195	212	236	239	266	275	317	338	362	437	470	476	492	515	528	540	542	572	620	638
CP	G	T	A	G	G	T	A	T	A	C	C	A	C	T	G	T	A	A	G	A	C	A	C	A	T
YCE	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
YSC1	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	G	.	.	.	.	.	.	.
YSC2	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OX	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
YX1	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
YX2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
YC	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CGA	A	C	G	A	A	C	G	C	G	T	.	.	T	C	A	C	G	.	A	G	T	G	T	G	C
CM	A	C	G	A	A	C	G	C	G	T	.	.	T	C	A	C	G	.	A	G	T	G	T	G	C

Table 4. Matrix of the total nucleotide differences (above diagonal), and genetic distances (below diagonal) based on maximum likelihood with settings corresponding to the HKY + G model, as determined by Modeltest of 16S + COI gene fragments of *Artemia* populations

	OX	YC	YSC2	YSC1	CP	YX2	YCE	YX1	CGA	CM	Afran	Asin	Aper
OX		0	1	2	2	3	3	2	28	28	27	159	205
YC	0.0000		1	2	2	3	3	2	28	28	27	159	205
YSC2	0.0009	0.0009		3	3	4	4	3	29	29	28	159	206
YSC1	0.0018	0.0018	0.0027		4	5	3	4	30	30	29	160	206
CP	0.0018	0.0018	0.0027	0.0036		1	1	2	28	28	27	161	205
YX2	0.0027	0.0027	0.0036	0.0045	0.0009		2	3	29	29	28	162	205
YCE	0.0027	0.0027	0.0036	0.0027	0.0009	0.0018		3	29	29	28	161	205
YX1	0.0018	0.0018	0.0027	0.0036	0.0018	0.0027	0.0027		28	28	27	160	206
CGA	0.0298	0.0298	0.0314	0.0324	0.0298	0.0311	0.0311	0.0298		0	9	154	206
CM	0.0298	0.0298	0.0314	0.0324	0.0298	0.0311	0.0311	0.0298	0.0000		9	154	206
Afran	0.0285	0.0285	0.0301	0.0310	0.0285	0.0298	0.0298	0.0285	0.0083	0.0083		154	206
Asin	0.5787	0.5787	0.5859	0.5844	0.5902	0.5958	0.5902	0.5843	0.5363	0.5363	0.5364		177
Aper	1.2950	1.2950	1.3214	1.3055	1.2950	1.2950	1.2950	1.3054	1.3340	1.3340	1.3345	0.883	

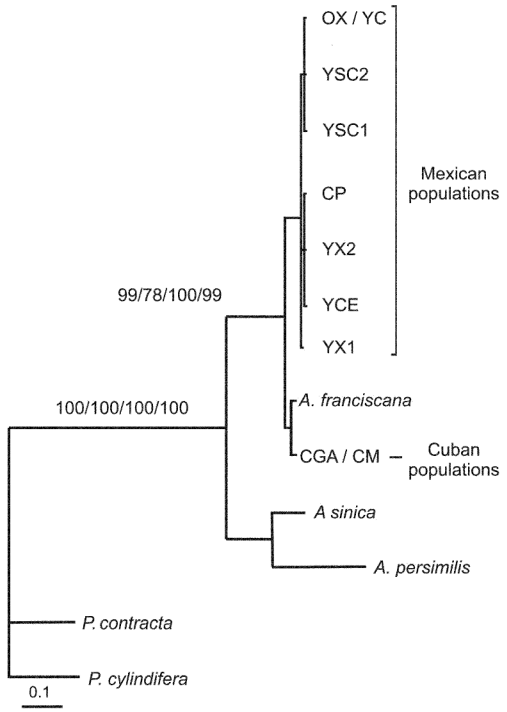

Figure 2. Phylogram (MrBayes) consensus tree for Neighbour Joining (NJ), Maximum Likelihood (ML), Maximum Parsimony (MP), and MrBayes (MB) analysis (numbers in that order) inferred from mitochondrial DNA (combined 16S and COI gene fragments). Mexican populations – OX, Oaxaca; YC, Chuburna, Yucatán; YSC1 and YSC2, San Crisanto, Yucatán; CP, Campeche; YX1 and YX2, Xtampú, Yucatán; YCE, Celestún, Yucatán. Cuban populations – CM, Frank País, Guantánamo; and CGA, El Real, Camagüey. Numbers at nodes represent bootstrap values of NJ, ML, MP and posterior probability value of MB.

Among the *Artemia* populations examined, two groups with strong support were observed: the Cuban populations with *A. franciscana* and the Mexican populations (99/78/100/99). Within the Mexican populations two clades appeared; one with OX, YC, YSC2 and YSC1 populations and the other with CP, YX2, YCE and YX1 populations. Separation of these clades was supported by bootstrap values and posterior probability value (MB) of 67/64/100/90 (NJ/ML/MP/MB; Figure 3).

Discussion

A greater proportion of T + A content than G + C content was found for both 16S and COI fragments with values of 52–65% in the *Artemia* populations studied. This is a characteristic property of arthropod mitochondrial genes[36,37]. The mitochondrial DNA of *Artemia* is relatively conserved in contrast to some other invertebrate species[23]. Despite independent rates of evolution, the 16S and COI genes proved to be useful for phylogenetic studies in *Artemia* and suited to evaluate taxa that diverged within the last 145–150 million years[38,39], as is the case for anostracans that are considered to have originated in the Lower Cretaceous (~145 mya)[40]. This is one of the reasons that both genes are often used in crustacean phylogeny studies[41]. Our results of 16S and COI sequences showed little differences between *A. franciscana* and the two Cuban populations. Based on the number of haplotypes obtained separately for the 16S (eight haplotypes) and COI (five haplotypes) genes, the former could be an appropriate genetic marker to evaluate genetic diversity among *Artemia* Mexican populations. Murugan *et al.*[27] noted that the 16S fragment alone was not informative enough for molecular comparison of American *Triops* forms, suggesting a different rate of evolutionary change in different regions of mtDNA. For halophylic crustaceans, and particularly *Artemia*, living under the influence of extreme environmental conditions, especially hypersalinity, clear evidence has been provided for a general acceleration of rates of molecular evolution (mitochondrial 16S, and nuclear 18S and 28S)[42].

The phylogenetic analyses in this study reveal two well-differentiated clades; one composed of the Mexican populations and another of the Cuban populations with *A.*

franciscana (Figure 2). This is also supported by the Maximum-Likelihood-distance analysis and the total nucleotide difference of the gene fragments (Table 4). Torrentera and Dodson[15], using canonical discriminant analyses, reported a morphological differentiation in the *Artemia* populations from Yucatán, San Francisco Bay, and the Great Salt Lake, USA. They suggested that the Yucatán populations might represent one or more distinct species. According to them, Yucatán populations are genetically distinct and ecologically separated from the North American species and from each other by habitat differences. In 2002, Torrentera and Abreu-Grobois[10] reported that the Yucatán *Artemia* populations had important cytogenetic differences when compared to the typical $2n = 42$ chromosomes and the 15.1 to 15.9 chromocenters of *A. franciscana* (Great Salt Lake and San Francisco Bay). Populations of Celestún, Chuburna, and Xtampú had a diploid chromosomal number of 40, 44 and 48, and a chromocentre number of 1–3, 1–8 and 1–4. The molecular and phylogenetic differences found between the Mexican *Artemia* strains and *A. franciscana* seem to favour the concept advanced by Torrentera and Dodson[15], that the southern Mexican populations may represent different species.

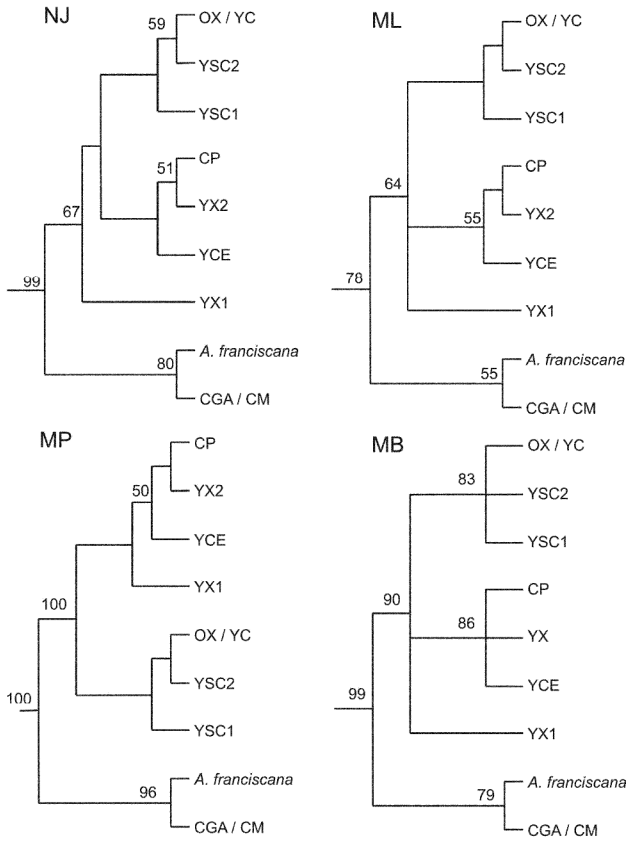

Figure 3. Cladogram tree sections for Cuban and Mexican populations for NJ, ML, MP, and MB analysis inferred from mitochondrial DNA (combined 16S and COI gene fragments). Numbers at nodes represent bootstrap values of NJ, ML, MP and posterior probability value of MB (bootstrap values less than 50% are not given).

The molecular similarity of the Cuban populations with *A. franciscana* indicates that this is a species introduced into Cuba[6,7]. The Cuban populations, despite their geographic distance (ca. 600 km) and different habitat conditions, had zero nucleotide difference (Table 4). El Real (CGA) is a seasonal salt works affected by periods of intensive rain, where the reproductive population almost disappears as a result of freshwater overflow. The *Artemia* population in the salt works Frank País is permanent, though it is mostly confined to the crystallization ponds where salinities are 160 g/l or higher, thus excluding the predator fish *Ciprinodon variegates*, which lives in the less saline ponds. However, the evolutionary potential of *Artemia* is well-documented in *A. franciscana* from Vinh Chau (Vietnam), where genetic differences have been found with the original inoculation source (San Francisco Bay) after nine years[43].

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