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A REPORT ON OCCURRENCE OF *Artemia franciscana* Kellogg 1906 IN NOUGH CATCHMENT (IRAN)

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ABSTRACT

Brine shrimp, *Artemia*, (Crustacean, Anostraca) is a genus of sexual and parthenogenetic forms with a global distribution in inland salt lakes, costal lagoons and solar saltworks except Antarctica. It is well suited for the study of evolutionary processes such as different adaptations, speciation and genetic differentiation. Due to occurrence of an unknown bisexual *Artemia* in Nough catchment, the main objective of the present study was to investigate the species of this un-endemic *Artemia* that its morphologic characters were similar to *A.franciscana*. Samples were obtained from Nough catchment as adult and form the Iranian Fisheries Research organization (Tehran, Iran) as dried cycts of *A.franciscana* GSL as the reference population. The study was assayed by RFLP analysis of a region of the mtDNA. A total of 4 mtDNA composite genotypes were identified, one composite genotype (H_1) occurred in two samples. The neighbor-joining distance tree shows all haplotypes appears well differentiated from each other and identifies the strong relationships between H_1 haplotype of Nough and *A.franciscana* (GSL). The results indicated on occurrence of *A.franciscana* in Nough catchment and the genetic differentiation of this species in new environment. They shows that Nough population that starting from the GSL source population is the first step of microevolutionary changes leading eventually to geographic differentiation and progressive adaptation to new environment.

Keywords: Investigate, RFLP, mtDNA, *Artemia*, Nough, Iran

INTRODUCTION

Brine shrimp, *Artemia*, (Crustacean, Anostraca) is a genus of sexual and parthenogenetic forms with a global distribution in inland salt lakes, costal

lagoons and solar saltworks except Antarctica [1, 2, 3]. The prime abiotic factor determining its presence is high salinity [4]. Although *Artemia* is restricted to hypersaline biotopes, other factors such as temperature, ionic composition and biotic interactionsalso play an important role in the patterns of its distribution [3].

Artemia has featured in the literature by virtue of its importance in aquaculture and as a model system for varied research [5]. Currently, eight bisexual species [6] and a heterogeneous group of obligate parthenogens are recognized. A sharp geographic boundary separates the New World bisexual (*A. franciscana*, *A. persimilis*, *A. monica*) from their Old World relative (*A. salina*, *A. urmiana*, *A. sinica*, *A. tibetiana* and *Artemia* sp. from Kazakhstan). Similarly, parthenogenetic populations are restricted to the Old World, where they comprise the majority. Bisexuals are diploid while parthenogenes rang in ploidy from 2n to 5n [7].

The economic importance of *Artemia* for shellfish and marine larviculture is substantial [8, 9, 10]. On the other hand, saltworks operations need to have viable *Artemia* population in order to control the algae blooms which results to the improvement of the quality of salt produced [10, 11].

Artemia is exceptionally well suited for the study of evolutionary processes such as different adaptations, marked biogeographic patterns, speciation and genetic differentiation [6, 12]. Many of the factors though to be responsible for genetic differentiation and speciation in other organism are observable in *Artemia* including: ecological isolation, formation of cline (in the content of heterochromatin), poly-, hetero- and aneuploidy [13, 14] and pre- or post- mating reproductive isolation [6]. Within their biological communities there is considerable diversification with respect to permanence, seasonality and predictability of the environment [4, 15].

Until almost the mid-1990s most genetic investigations utilized allozymic analyses to address various issues such as level of polymorphism, population structure, patterns of geographic divergence and others [6, 13, 16, 17, 18, 19, 20, 21] over the two last decade, interpretations of patterns of genetic differentiation and the distribution of diversity in several organisms have been considerably refined by the introduction of new molecular tools [22], the use of molecular markers [4, 12, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37] have provided additional data, albeit mainly on the evolutionary relationships and level of divergence between species of the genus.

Among the bisexual species of the *Artemia*

genus, *A.franciscana* has been the most extensively studied. *A.franciscana* is endemic to the New World, however both permanent and temporal populations also exist worldwide, due to introductions of different strains [4, 38]. Since introduction or dispersal, rapid evolutionary changes seem to have occurred in natural populations of *A.franciscana* as inferred from the accumulation of novel alleles apparently as a response to environmental differences [20, 39]. *A.franciscana*, which as a whole has a highly variable gene pool, displays considerable inter-populational genetic and life history heterogeneity [13]. It would not be an overstatement to say that the majority of distinctive features found in the genus [29] are harbored within *A.franciscana* alone. This fact, combined with the presence of many natural or introduced populations around the world, makes *A.franciscana* an invaluable genetic system for fine scale studies of microevolutionary divergence [4].

Nough catchment (30^o 60' E, 56^o 50' N; Kerman province) is a lake of about 0.4 km^2 has formed following the construction of a dam on salty river 40 km away from the town of Nough near the city of Rafsanjan. The maximum depth of this catchment is about 4.0 m while its average depth is 2.0 m. its water salinity fluctuates seasonally between 80 and 150 g/L.

Due to occurrence of an unknown bisexual *Artemia* in Nough catchment, the main objective of the present study was to investigate the species of this un-endemic *Artemia* that its morphologic characters were similar to *A.franciscana.*

MATERIALS AND METHODES

All samples used in this study were obtained form Nough catchment as adult and form the Iranian Fisheries Research organization (Tehran, Iran) as dried cycts of *A.franciscana* GSL as the reference population. Live adults were sampled with plankton net (250 μm) and preserved in ethanol % 100 (Merk) prior to DNA extractions. Cysts of *A.franciscana* were incubated in artificial 0.45 μm – filtered 35 ppt Dietrich and kale (D and K) medium which was prepared following the modification of Vanhaecke *et al.* [40]. Hatching conditions were according to Sorgeloos *et al.* [41]. After 30 hours of incubation nauplii were transferred to one litter cylindorconical glass tubes containing D and K medium of 50 ppt salinity. The animals were kept under mild aeration, at 25 ± 1^{oc} with 12 hr cool white fluorescent lighting daily. They were fed on a mixed diet of alga (Dunaliella) and the yeast – based formulated feed LANSY 1-PZ (INVE aquaculture SA, Belgium) following the feeding schedule of Triantaphyllidis *et al.* [11] until they reached the adult stage.

Adult samples were assayed by way of restriction fragment length polymorphism (RFLP) analysis of a region of the mtDNA and screened for variation within and differentiation between *Artemia* samples.

Following DNA extraction (phenol – chloroform method, [42]) samples were loaded on an automatic thermocycler (Corbett Research) for amplification of a 1566 bp long mtDNA target sequence. The primers were supplied from MWG – Biotech, for each amplification the total reaction volume of 25 ml consisted of 5 µl 10X reaction buffer (500 mM Kcl, 200 mM tris – Hcl, PH = 8.4, Cinnagen), 0.5 µL dNTPs (10 mM of each base, PH = 7.5, MBI – Fermentase) , 2 µL of each primers (final concentration of 20 pM), 0.5 µL Taq DNA polymerase (5 unit per µL, Cinnagen), 50 – 100 ng of template mtDNA , 2µl $Mgcl_2$ (50 ml M, Cinnagen) and dH_2o. Each of the 30 amplification cycles consisted of 1 min and 15 sec denaturation at 94 ^{oc}, 50 sec annealing at 54 ^{oc}, 1 min extension at 72 ^{oc} and a final extension of 4 min at 72 ^{oc}.

Ten restriction endonuclease (*AluI, EcoRI, Eco47I, HaeIII, HindIII, HinfI, MboI, MspI, RsaI* and *TagI*) were employed to assess variation in the amplified region. For each sample 4 – 6 ml of amplified DNA was digested and products were electrophoresed on 6 % vertical polyacrylamide and visualised by silver staining. Data (digested fragment approach) were analysed by REAP, version 4.0 [43]. Haplotype diversity [44] and nucleotide diversity [45] values with in population were computed. Genetic distance between haplotypes was computed based on Nei's [46], and cluster analysis performed to create a dendrogram using Kimura's [47] two – parameter method with PAUP 4.0b1b [48].

RESULTS

In total 60 individual animals were electrophoresed and enzymes *MspI*, *HaeIII* and *RsaI* detected polymorphism across samples. A total of 35 fragments were surveyed in the mtDNA target sequence as the average number of bases examined was 148.84 and the *A.franciscana* mitochondrial genome is estimated to be 15822 nucleotide long [24], 0.94 % of the *Artemia* mtDNA was screened.

Homology of fragments was established through side by side gel comparisons and also through comparisons of restriction patterns to those generated by gene runner computer package (version 3.0, Hastings soft ware). A total of 4 mtDNA composite genotypes were identified, one composite genotype (H_1) occurred in two samples. Haplotype frequency estimates together with haplotype and nucleotide diversity values are given in Table 1. The distance

matrix between haplotype (Table 2) was used for clustering (Figure 1). The dendrogram shows all haplotypes appears well differentiated from each other and identifies the strong relation ships between H_1 haplotype of nough and *A.franciscana* (GSL).

Obtained results indicated on occurrence of *A.franciscana* in Nough catchment and the genetic differentiation of this species in new environment.

Table 1: Mitochondrial DNA variability. Composite genotype (capital letter refer to a restriction endonuclease pattern in the order *MspI, HinfI, HindIII, Eco47I, AluI, MboI, HaeIII, RsaI, TaqI, EcoRI*), haplotype distribution and relative frequency, haplotype diversity percentage with SE and nucleotide diversity percentage.

Haplotype	composite genotype	Nough	*A.franciscana* GSL
H_1	AAAAAAAAAA	2 (7.4%)	0
H_2	BAAAAAAAAA	7 (25.9%)	0
H_3	BAAAAABAAA	1 (3.7%)	0
H_4	BAAAAABBAA	20 (62.9%)	30 (100%)
Haplotype diversity %		57.26	0.00
±SE		8.74	0.00
Nucleotide diversity %		0.87	0.00

Table 2: Nei's genetic distance based on FRLP data of haplotypes in the population studied.

	H_1	H_2	H_3	H_4
H_1	-			
H_2	0.0067	-		
H_3	0.0111	0.0038	-	
H_4	0.0251	0.0156	0.1075	-

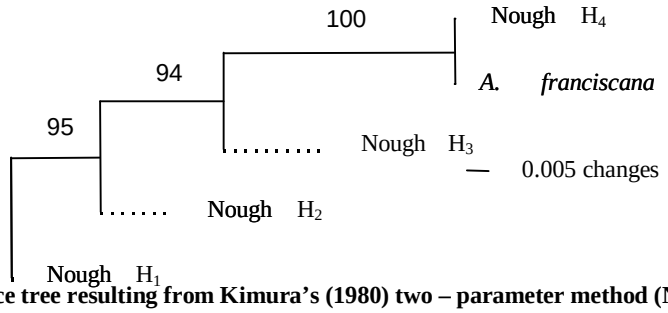

Figure 1: neighbor – joining distance tree resulting from Kimura's (1980) two – parameter method (Note scale bar).

DISCUSSION

The brine shrimp can be considered a model organism, offering numerous advantages for comprehensive, multi disciplinary studies. It is highly favourable for evolutionary studies as it has a range of key factor dividing speciation not very often seen in other organism [30].

Considering the importance of *Artemia* in aquaculture, monitoring of strains introduced to different areas world wide has acquired particular weight, not for the future success and further development of inoculation programs, but also for the advancement of certain issues related to tolerance life strategies in and response to different environments and others [4].

The *A.franciscana* super species complex certainly warrants in depth study. This is due to a variety of reasons including: the high genetic diversity of its natural populations, which are spread widely over a vast area with a variety of biotopes [3], its widespread use in aquaculture and the

occurrence of newly established populations, as a result of deliberate inoculation, which are developing a genetic adaptation to their new environment [49, 50].

Nough catchment is known to have been a suitable habitat for brine shrimp. Results showed the unknown bisexual *Artemia* that occurred in this catchment was *A.franciscana*. The genetic differentiation on Nough population that starting from the GSL source population is not of great magnitude, however, this is the first step of microevolutionary changes leading eventually to geographic differentiation and progressive adaptation to new environment. This explains the intermediate characteristics of Nough population and the low genotypic diversities in this population. The impact of the environment is relevant to understand the extent of differentiation in *Artemia* populations, often affected by geographical and ecological separation along with climatic and hydrobiological changes which as a whole, offer a mosaic of environmental conditions that tend to select local forms [4].

The mtDNA sequence analysed in this study has provided some diagnostic power in comparing strains GSL and Nough of *Artemia*. Estimates of gene diversity obtained from mtDNA are expected to exhibit a larger variance than similar estimates based on a large number of nuclear loci [44]. Despite the current debate over mtDNA transmission genetics [51], the occurrence of maternal and clonal inheritance reduce its effective population size to only about one – fourth that of nuclear genes. Therefore, population subdivision is not expected to have the some effect on the geographic distribution of variability in nuclear and mitochondrial genomes [52].

As a consequence, a population can be effectively more subdivided for organelle genes, while the nuclear genes are indicative for panmixia [4, 53].

The identification of strains with different genetic compositions is expected to contribute considerably to the understanding of molecular ecology and aquaculture practices involving *Artemia*.

CONCLUSION

This study indicated on occurrence of *A.franciscana* in Nough catchment and the genetic differentiation of this species in new environment. The genetic differentiation on Nough population that starting from the GSL source population is not of great magnitude, however, this is the first step of microevolutionary changes leading eventually to geographic differentiation and progressive adaptation to new environment.

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