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A Population-Specific Marker within the Superspecies *Artemia franciscana*

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Artemia franciscana is one of the two *Artemia* species that are found in the New World with a wide distribution in the American continent. Commercially available cysts mainly originate from Great Salt Lake, Utah and from San Francisco Bay, California. Cysts from the latter have been used in many (deliberate or not) inoculations around the world. These two populations show a number of differences (i.e., in diapause deactivation treatment or in temperature tolerance) supporting the notion that *A. franciscana* is a "superspecies". In addition, evidence from morphological, karyological, electrophoretic, and DNA sequencing data has shown significant sub-structuring and differentiation within *A. franciscana*. In this work, the second p26 intron was studied in order to trace the "hidden diversity" within the *A. franciscana* complex and to evaluate its utility as a population-specific marker for various applications. Ten *A. franciscana* populations from USA, Chile, Brazil, and Vietnam were screened and the sizes of the amplicons revealed six different genotypes (Table 1). Primer design was based on the conserved regions of the second and third exons of p26 gene sequences of *A. franciscana* (GenBank accession numbers DQ310577, DQ310575 and AF031367). BLAST searches confirmed the identity of the PCR product on the basis of detected similarities with second and third exons of p26 sequences from other *Artemia* representatives. San Francisco Bay yielded a single fragment of ~1500 bp (using 1.8% agarose gels and visualized under UV light) whereas Great Salt Lake showed two patterns, namely ~2000 bp and 2000/1500 bp. Populations that are known to originate from San Francisco Bay inoculated material, such as MAC (Macau, Brazil) and VC (Vinh Chau, Vietnam), showed the same genotype as the source population. Among the six Chilean populations studied, substantial heterogeneity in genotypic profiles was revealed (Table 1). Populations CHA, LLA, LVI, and IQU were monomorphic while observed heterozygosity values for CON and CEJ were 0.45 and 0.49, respectively. The same estimate for the GSL population was 0.55. For the whole data set, 30 of 45 comparisons for population differentiation showed significant ($p < 10^{-5}$) heterogeneity in allele frequencies. Twenty single-pair crosses between Great Salt Lake (~2000 bp) and San Francisco Bay (~1500 bp) were performed and screened with the p26 marker in order to confirm the pattern of inheritance. Although preliminary, our results indicate that both fixed differences and polymorphic patterns exist in p26 genotypes within the *A. franciscana* complex. The developed marker seems to be of diagnostic value in stock identification as shown by the obtained genotypes in feral populations (MAC, VC). The p26 gene could be informative in population genetic surveys investigating levels of variability, population structure and differentiation, and patterns of gene flow. The obtained genetic diversity estimates reflect a common fact in *A. franciscana* populations which is a trend towards the maintenance of high levels of genetic variability in their gene pools and great interpopulation genetic heterogeneity.

Table 1–Numbers of individuals (*N*) with different p26 genotypes in the examined *Artemia franciscana* populations (SFB: San Francisco Bay, California, USA; GSL: Great Salt Lake, Utah, USA; CHA: Chaxas, Chile; CON: El Convento, Chile; LLA: Llamara, Chile; LVI: Los Vilos, Chile; IQU: Iquique, Chile; CEJ: Cejas, Chile; VC: Vinh Chau, Vietnam; MAC: Macau, Brazil).

	SFB	GSL		CHA	CON					LLA	LVI	IQU	CEJ			VC	MAC
N	48	23	28	38	33	5	3	10	18	40	40	30	8	13	20	65	25
2000 bp		-	-			-	-		-				-		-		
1500 bp	-		-	-			-	-			-	-		-	-	-	-
1400 bp					-			-	-	-							