MOLECULAR ECOLOGY
RESOURCES
Molecular Ecology Resources (2010) doi: 10.1111/j.1755-0998.2010.02908.x

MOLECULAR DIAGNOSTICS AND DNA TAXONOMY

SNP detection in Na/K ATP-ase gene α_1 subunit of bisexual and parthenogenetic *Artemia* strains by RFLP screening

R. MANAFFAR,* S. ZARE,† N. AGH,* N. ABDOLAHZADEH,† S. SOLTANIAN,‡ P. SORGELOOS,§ P. BOSSIER§ and G. VAN STAPPEN§

**Artemia and Aquatic Animals Research Institute, Urmia University, PO Box 165, Urmia, Iran, †Department of Biology, Faculty of Science, Urmia University, PO Box 165, Urmia, Iran, ‡Aquatic Animals Health & Disease Department, School of Veterinary Medicine, Shiraz University, Shiraz, 71345-1731, Iran, §Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Rozier 44, B-9000 Gent, Belgium*

Abstract

In order to find a marker for differentiating between a bisexual and a parthenogenetic *Artemia* strain, Exon-7 of the Na/K ATPase α_1 subunit gene was screened by RFLP technique. The results revealed a constant synonymous SNP (single nucleotide polymorphism) in digestion by the *Tru*1I enzyme that was consistent with these two types of *Artemia*. This SNP was identified as an accurate molecular marker for discrimination between bisexual and parthenogenetic *Artemia*. According to the Nei's genetic distance (1973), the lowest genetic distance was found between individuals from *Artemia urmiana* Günther 1890 and parthenogenetic populations, making the described marker the first marker to easily distinguish between these two cooccurring species.

Keywords: *Artemia*, Na/K ATPase, polymorphism, RFLP

Received 17 June 2010; revision received 7 July 2010; accepted 18 July 2010

Artemia is able to survive under extreme osmotic salt concentration because of specialized organs that extrude salt from the isosmotic internal haemolymph to the external medium (Browne & MacDonald 1982). The Na/K ATPase is a heterodimeric protein composed of two subunits; an α subunit that is homologous to p-type ATPases containing most of the active centres and a smaller β subunit. This protein involved in Na^+ and K^+ transport through the plasma membrane (Vasilets & Schwartz 1993) has been proposed to play an essential role in the extraordinary osmotic resistance of *Artemia* (Conte 1984; Cortas *et al.* 1989; Holliday *et al.* 1990). Considering the importance of Na/K ATPase activity, high levels of polymorphism are to be expected (García-Sáez *et al.* 1997).

The current study intended to search for a molecular marker suitable to distinguish between bisexual and parthenogenetic *Artemia*. Such a marker could be an effective tool for the ecological research on the natural and non-natural cooccurrence of parthenogenetic species and bisexual species in salt lakes and salt works.

To achieve this objective, overall 23 *Artemia* populations were included (Table 1). Genomic DNA was extracted from single individuals (cysts and adults, making up in total 730 individuals). A Chelex method was used to extract DNA from single cysts (Estoup *et al.* 1996). Extraction of DNA from adult individuals was carried out using the SDS–chloroform method (Sambrook *et al.* 1989). A couple of primers, allowing the amplification of a 280- bp fragment of exon-7 of Na/K ATPase, were designed according to the available sequences in the Genbank (National Center for Biotechnology Information, NCBI) and the found polymorphism in this genus by García-Sáez *et al.* (2000). The primers were as follows: forward: 5′-cag-cca-aac-gta-tgg-ctt-c-3′ and reverse primer: 5′-gaa-ttc-agc-acg-act-gca-aag-3′. The

Correspondence: Ramin Manaffar, Fax: 04413440295;
E-mail: Raminmanaffar@gmail.com