APPLICATION OF RFLP ANALYSIS TO IDENTIFY
CYST POPULATIONS OF ARTEMIA URMIANA
GÜNTHER, 1899 (BRANCHIOPODA, ANOSTRACA)
FROM URMIA LAKE, IRAN

BY

AMIN EIMANIFAR1,4, SOHRAB REZVANI2) and JIRAIR CARAPETIAN3)

1) Artemia Reference Center in Middle and Western Asia, P.O. Box 57135-1367, Urmia, Iran
2) Iranian Fisheries Research Organization (IFRO), P.O. Box 14155-6116, Teheran, Iran
3) Department of Biology, Faculty of Science, Urmia University, Urmia, Iran

ABSTRACT

A rapid and reliable PCR-RFLP method was optimized to identify cyst batches of Artemia urmiana collected from different regions of Urmia Lake. Following DNA extraction, a 1564 bp region of a mitochondrial gene encoding the ribosomal RNA was successfully amplified by the PCR technique. Eleven restriction endonucleases were subsequently employed in order to digest the PCR product. These enzymes gave specific restriction patterns for discriminating cyst batches collected from top and bottom layers of three main geographical areas of Urmia Lake. Detailed analysis revealed that the RFLP patterns of four restriction enzymes (HinfI, TaqI, MspI, and MboI) were sufficient to differentiate between the samples studied. The method described is sensitive, rapid, and reliable, and can be a useful tool for the identification of various populations of A. urmiana existing in the ecosystem of Urmia Lake.

RÉSUMÉ

Une méthode rapide et fiable de PCR-RFLP a été optimisée pour identifier les œufs de durée d’Artemia urmiana récoltés dans différentes régions du lac Urmia. Après extraction du DNA, une région de 1564 bp du gène mitochondrial codant pour le RNA mitochondrial a été amplifiée avec succès par la technique de PCR. Onze endonucléases de restriction ont été ensuite utilisées afin de digérer le produit de la PCR. Ces enzymes ont donné des patrons de restriction spécifiques permettant de discriminer les œufs de durée collectés dans les couches supérieure et inférieure de trois principales régions géographiques du lac Urmia. L’analyse détaillée a révélé que les patrons RFLP de quatre enzymes de restriction (HinfI, TaqI, MspI et MboI) suffisaient pour distinguer les échantillons étudiés. La méthode décrite est sensitive, rapide et fiable, et peut être un outil utile pour l’identification des diverses populations de A. urmiana présentes dans l’écosystème du lac Urmia.

4) To whom correspondence should be addressed; Fax: +98.4414362728; e-mail: amineimanifar@yahoo.com

© Koninklijke Brill NV, Leiden, 2006
Crustaceana 78 (11): 1311-1323
Also available online: www.brill.nl
INTRODUCTION

The genus *Artemia* (Branchiopoda, Anostraca), distributed in hypersaline environments in all continents except the Antarctic (Triantaphyllidis et al., 1998; Van Stappen, 2002), is a complex of eight biparental sexual species and many parthenogenetic populations (Browne & Bowen, 1991). Several recent publications have shown that *Artemia* is an exceptionally interesting and useful biological subject for a wide variety of experimental studies (Gajardo et al., 2002; Bagshaw, 1989). Additionally, the ease of cyst transportation and the culture under laboratory conditions have enabled extended interpopulation comparisons (Gajardo et al., 2001). In addition, comparisons using allozyme analysis (Abreu-Grobois & Beardmore, 1982; Gajardo & Beardmore, 1989, 1993; Pilla & Beardmore, 1994; Gajardo et al., 1995, 1999), cytogenetics (Barigozzi, 1974, 1980; Barigozzi et al., 1984; Abatzopoulos et al., 1986, 1987; Colihueque & Gajardo, 1996), mitochondrial (Perez et al., 1994; Gajardo et al., 2004), and nuclear DNA traits (Badaracco et al., 1987, 1995; Triantaphyllidis et al., 1997; Sun et al., 1999), enable one to do molecular systematics based on DNA polymorphism and molecular variation. So, it is important to choose an appropriate genetic marker for targeted use. Thus, in making such a choice, we point out the following: (a) the development of universal primers for PCR amplification of specific mtDNA regions has facilitated analysis of mtDNA (Cronin et al., 1993; Meyer, 1994; Palumbi et al., 1991); (b) the high rate of mtDNA evolution, the maternal mode of inheritance, and the lack of recombination have made mtDNA the most useful molecule in this kind of analysis (Avise, 1986; Mortiz et al., 1987; Wilson et al., 1987); (c) mitochondrial DNA has proven to be an excellent tool for examining biogeographical distributions, above and below the species level (Avise, 1994); and (d) mtDNA has emerged as a genetic marker able to discriminate stocks of organisms (Billington & Hebert, 1991). Hence, we have chosen to employ mtDNA to discriminate between cyst batches for the purpose of characterizing distinct populations of *Artemia urmiana* Günther, 1899 collected from various ecological zones of the huge salt lake (Lake Urmia), which is located in the northwest of Iran. It is expected that this rapid procedure can be employed for other *Artemia* populations found in the neighbouring west Asian countries and the observed variation can be used for ecological research with the aim of detecting a meaningful phylogeographical variation.

MATERIALS AND METHODS

*Artemia* samples

The geographic origins of the *Artemia* populations investigated in this study were all from Urmia Lake, located in the northwest of Iran (fig. 1). Three