Journal of Sea Research 76 (2013) 146–153

Contents lists available at SciVerse ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

Deep genetic divergence in giant red shrimp *Aristaeomorpha foliacea* (Risso, 1827) across a wide distributional range

M.V. Fernández [a], S. Heras [a], F. Maltagliati [b], M.I. Roldán [a,*]

[a] Laboratori Ictiologia Genètica, Universitat de Girona, Campus de Montilivi, 17071 Girona, Spain
[b] Dipartimento di Biologia, Università di Pisa, Via Derna 1, 56126 Pisa, Italy

ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form 5 July 2012
Accepted 3 August 2012
Available online 14 August 2012

Keywords:
Mitochondrial Genetic Diversity
Allopatric Divergence
Fisheries
Mediterranean Sea
Indian Ocean

ABSTRACT

The giant red shrimp, *Aristaeomorpha foliacea*, is a commercially important species in the Mediterranean Sea (MED), Mozambique Channel (MOZ), and north western Australia (AUS). 685 bp of the mitochondrial COI gene was sequenced in 317 individuals from six Mediterranean and two Indian Ocean localities. Genetic diversity estimates of Indian Ocean samples were higher than those of MED counterparts. AMOVA, phylogenetic tree, haplotype network and Bayesian assignment analyses detected three haplogroups, corresponding to MED, MOZ and AUS, separated by three and 38 mutational steps, respectively. Within MED shallow genetic divergence between populations was dependent on local oceanographical characteristics. Mismatch distribution analysis and neutrality tests provided a consistent indication of past population expansion in each region considered. Our results provide the first evidence of genetic structure in *A. foliacea* and suggest a scenario of allopatric speciation within the Indian Ocean that, however needs deeper examination.

1. Introduction

The giant red shrimp, *Aristaeomorpha foliacea* (Risso 1827), is a demersal decapod that inhabits muddy bottoms of the continental slope with the highest densities generally in the upper 600 m and in zones where canyons cross the bottoms (D'Onghia et al., 1998); however, its bathymetric distribution is reported to range from123 m (Politou et al., 2004) to 1145 m depth (Capezzuto et al., 2010). The species is an economically important target for fisheries, being exploited in the Western Mediterranean Sea since 1959 (Campillo, 1994). In recent years, more fisheries were developed in north-western Australia (Wadley, 1994) and Mozambique Channel (Sobrino et al., 2009). Before the 1980s, the giant red shrimp was extensively captured in the Gulf of Lion but the pressure exerted by fisheries was considered as one of the causes for the decline of *A. foliacea* stock from those grounds (Campillo, 1994) and its lower abundance in the Western Mediterranean (Cau et al., 2002).

In the Mediterranean, the current geographical distribution of *A. foliacea* is patchy, with a longitudinal abundance gradient increasing from western to eastern regions (Cau et al., 2002; Company et al., 2004). In fact, the presence of *A. foliacea* in some areas of the Western Mediterranean is so scarce that no directed fisheries exist (Sardà et al., 2004); hence, major fishing grounds are located in the Tyrrhenian Sea, Sardinia Channel, Strait of Sicily and Western Ionian Sea (Cau et al., 2002; Politou et al., 2004; Sardà et al., 2004). Recently, the establishment of a new viable fishery in the Greek Ionian Sea grounds was examined (Mytilineou et al., 2006 and references therein).

Furthermore, Ghidalia and Bourgois (1961) and Politou et al. (2004) noted that *A. foliacea* seems to be associated with the more saline and warmer waters of the eastern basin of the Mediterranean than in the western counterpart. Capezzuto et al. (2010) detected an increase in *A. foliacea* biomass in the Western Ionian Sea that correlated significantly with increasing temperature and salinity. The particular hydrographical characteristics of Mediterranean basins are a consequence of a complex oceanic circulation system that determines the presence of oceanographical barriers (Sarà, 1985). Different environmental conditions and oceanographical barriers can make difficult the migration of individuals and eventually lead to genetic divergence (Kritzer and Sale, 2006).

Molecular markers are robust tools for detecting species' genetic structure, as well as evolutionary history of populations (Avise, 2004). In particular, implementation of mitochondrial DNA (mtDNA) in fishery management programmes has given satisfactory results (Waples et al., 2008), being extensively used to investigate patterns of intra-specific partitions of genetic polymorphisms (Avise, 2004). In addition, its utility for species identification has been demonstrated for a large array of crustacean species (Costa et al., 2007). In fishery management the identification of stock boundaries and the levels of connectivity among them is important to avoid mismanagement-related problems, such as overexploitation of the resource (Everhart and Youngs, 1981).

Results of a previous molecular work on *A. foliacea*, carried out by means of ISSRs (inter simple sequence repeats), were consistent with shallow genetic divergence between Mediterranean and Mozambique

* Corresponding author. Tel.: +34 972 418961; fax: +34 972 418277.
E-mail address: marina.roldan@udg.edu (M.I. Roldán).

1385-1101/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.seares.2012.08.004

Channel populations (Fernández et al., 2011a). In the present investigation, the mitochondrial gene coding for the subunit I of the Cytochrome C Oxidase (COI) was used to survey mtDNA variability and assess spatial population structuring in *A. foliacea*, to gather further information on species' genetic structure across harvesting areas. The COI gene exhibited medium to high levels of genetic differentiation in other Penaeoidea species across similar distributional ranges (Fernández et al., 2011b; Reuschel et al., 2010).

2. Materials and methods

2.1. Sampling

A total of 317 adults of *A. foliacea* were collected from six Mediterranean localities (Ibiza Channel, Tyrrhenian Sea, Mazara del Vallo, Portopalo, Ionian Sea and Aegean Sea), and two Indian Ocean localities (Mozambique Channel and north-western Australia) (Fig. 1). The *Instituto Español de Oceanografía* provided the samples from the Ibiza Channel (39°02′N, 02°39′E), and Mozambique Channel (25°57′S, 34°38′E, Mozambique 0308 survey); the Hellenic Centre for Marine Research collected the samples from the Aegean Sea (37°17′N, 22°53′E) and the Ionian Sea (37°31′N, 21°22′E; MEDITS08 survey), and the sample from the Tyrrhenian Sea (42°28′N, 9°43′E; MEDITS08 survey) was provided by the *Institut Francais de Recherche pour l'Exploitation de la Mer*. The sample from north-western Australia (14°51′S, 121°26′E, Voyage of Discovery survey SS05/2007) belongs to the collections of the Museum Victoria of Melbourne. The sample from Mazara del Vallo was purchased at a local market and the sample of Portopalo was obtained from local fishermen.

2.2. DNA extraction, amplification and sequencing

Genomic DNA was extracted from a portion of about 10 mg of abdominal muscle preserved in 95% ethanol, following the protocol described in Sambrook et al. (1989) with slight modifications: tissue was digested overnight at 37 °C in a 1.5 ml centrifuge tube containing 600 μl of TENS buffer (0.05 M Tris–HCl pH 8, 0.1 M EDTA, 5 M NaCl and 5 M SDS) and 40 μl of Proteinase K (20 mg/ml); total DNA was extracted with phenol followed by phenol:chloroform:isoamyl alcohol (25:24:1); genomic DNA was recovered by standard precipitation with ethanol. Finally DNA was resuspended with 75 ml of deionised water. Initially, primers and PCR conditions implemented in *Aristeus antennatus* (Roldán et al., 2009) were tested on *A. foliacea*, but amplifications did not provided satisfactory results. Universal primers from Palumbi et al. (1991) (COIaH, COIfL) were also tested with similar unsatisfactory results. Subsequently, different sequences of Dendrobranchiata species available in GenBank (Sergestidae, Luciferidae, Solenoceridae, Benthesicymidae, Sicyoniidae, Penaeidae and Aristeidae) were aligned and screened for conserved regions on the COI gene with the final design of new specific primers for *A. foliacea* (COI_H1: 5′-AATCTTCCTGGTAGGAGCTTAAA-3′, and COI_L4: 5′-TTACCAGTCTTAGCAGGAGCTATT-3′). Polymerase Chain Reactions (PCR) used 1× PCR Buffer (Ecogen), 2.5 mM of $MgCl_2$, 0.2 mM of each primer, 8 mM of dNTPs, 0.025 U of DNA polymerase (Ecotaq by Ecogen) and 25 ng of template DNA in a 30 μl final volume. PCR amplifications were performed on an MJ thermocycler programmed for one initial denaturation at 94 °C for 5 min, followed by 33 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 45 s and elongation at 72 °C for 1 min, and a final elongation at 72 °C for 5 min. PCRs were carried out with standard precautions to detect contamination and related problems. PCR products were verified on 1% agarose gel with ethidium bromide (0.5 mg/ml). Sequences were cleaned for sequencing by treating with exonuclease I and shrimp alkaline phosphatase (Werle et al., 1994). DNA sequencing reactions were carried out with COI_H1 primer and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. Labelled fragments were loaded onto an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) at the *Laboratori d'Ictiologia Genètica*, *Universitat de Girona*, Spain.

2.3. Sequence data analysis

Sequences were aligned and edited in SeqScape v2.5 (Applied Biosystems) and final editing was performed with BioEdit v7.0.4.1 (Hall, 1999) employing as reference the partial region of *A. antennatus* COI (Roldán et al., 2009; GenBank accession number EU908573). Each new distinct haplotype was submitted to GenBank (accession number JN676306–JN676362, Supplementary material, Table S1). A hierarchical series of tests based on the Akaike Information Criterion (AIC) was applied to identify the most appropriate nucleotide substitution model among 88 models tested, as implemented in jModelTest v3.7 (Posada, 2008). Haplotype diversity (h) and nucleotide diversity (π) were calculated using DnaSP v5 (Librado and Rozas, 2009). Pairwise F_{ST} among all localities and hierarchical analysis of molecular variance (AMOVA, Excoffier et al., 1992) were calculated with Arlequin v3.5 (Excoffier and Lischer, 2010). The latter analysis was used to examine the partition of genetic variance into the within-sample, among-sample and among-region components. Four analyses were carried out with the following groups: *i*) the total dataset without any structuring, *ii*) the total dataset considering three regions (Mediterranean, Mozambique Channel and Australia), *iii*) the Mediterranean dataset without any structuring,

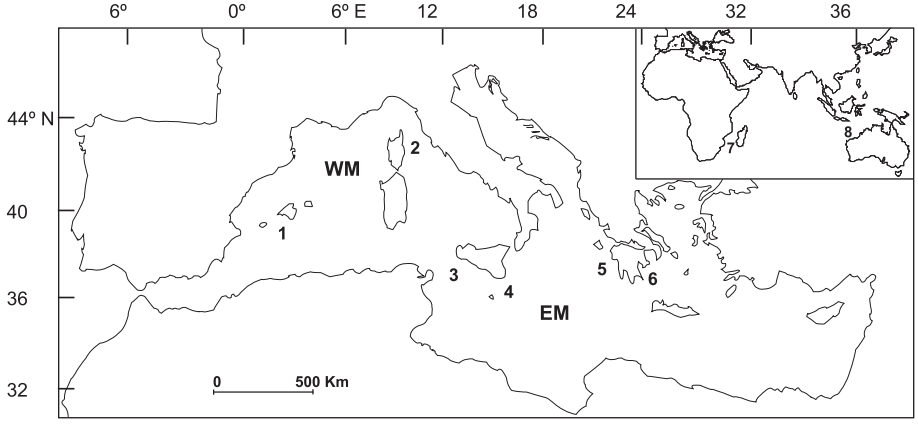

Fig. 1. *Aristaeomorpha foliacea*. Location of sampling sites: 1—Ibiza Channel (IBI), 2—Tyrrhenian Sea (TYR), 3—Mazara del Vallo (MAZ), 4—Portopalo (PPA), 5—Ionian Sea (ION), 6—Aegean Sea (AEG), 7—Mozambique Channel (MOZ), and 8—north-western Australia (AUS).