Marine Genomics 26 (2016) 29–39

Contents lists available at ScienceDirect

Marine Genomics

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

Genomics/technical resources

# Extracellular DNA amplicon sequencing reveals high levels of benthic eukaryotic diversity in the central Red Sea

John K. Pearman *, Xabier Irigoien, Susana Carvalho

*KAUST — King Abdullah University of Science and Technology, Red Sea Research Center, Thuwal 23955-6900, Saudi Arabia*

ARTICLE INFO

*Article history:*
Received 18 August 2015
Received in revised form 22 October 2015
Accepted 23 October 2015
Available online 11 November 2015

*Keywords:*
Marine sediment
Extracellular DNA
Biodiversity
Amplicon sequencing
Eukaryota
Red Sea

ABSTRACT

The present study aims to characterize the benthic eukaryotic biodiversity patterns at a coarse taxonomic level in three areas of the central Red Sea (a lagoon, an offshore area in Thuwal and a shallow coastal area near Jeddah) based on extracellular DNA. High-throughput amplicon sequencing targeting the V9 region of the 18S rRNA gene was undertaken for 32 sediment samples. High levels of alpha-diversity were detected with 16,089 operational taxonomic units (OTUs) being identified. The majority of the OTUs were assigned to Metazoa (29.2%), Alveolata (22.4%) and Stramenopiles (17.8%). Stramenopiles (Diatomea) and Alveolata (Ciliophora) were frequent in a lagoon and in shallower coastal stations, whereas metazoans (Arthropoda: Maxillopoda) were dominant in deeper offshore stations. Only 24.6% of total OTUs were shared among all areas. Beta-diversity was generally lower between the lagoon and Jeddah (nearshore) than between either of those and the offshore area, suggesting a nearshore–offshore biodiversity gradient. The current approach allowed for a broad-range of benthic eukaryotic biodiversity to be analysed with significantly less labour than would be required by other traditional taxonomic approaches. Our findings suggest that next generation sequencing techniques have the potential to provide a fast and standardised screening of benthic biodiversity at large spatial and temporal scales.



## 1. Introduction

The Red Sea has long been recognized as one of the world's biodiversity hotspots and an area of high endemism (Roberts et al., 2002; Allen, 2008). The unique characteristics of the Red Sea (high water temperature and salinity), as well as the restricted exchanges with the Mediterranean and the Indian Ocean (Tyler, 2003) probably account for its high levels of biodiversity and endemism. Despite the biogeographic interest, the habitats of the Red Sea remain understudied (see review by Berumen et al. (2013) for coral reefs), particularly in relation to other biodiversity hotspots such as the Great Barrier Reef, Australia. In particular, soft-bottoms, such as seagrass meadows and mangroves, have been largely neglected. Nevertheless, benthic fauna inhabiting soft-bottoms have a relevant role in nutrient recycling and water quality regulation (Carstensen et al., 2014), which are crucial for the maintenance of other habitats such as coral reef systems.

Traditional soft-bottom benthic biodiversity surveys require lengthy and time-consuming laboratory tasks such as sorting and identification based on morphological characteristics, which is currently complicated by a lack of taxonomists. These methodologies are, however, crucial starting points for creating an inventory of local taxonomic diversity and for species discovery and description (Plaisance et al., 2009). Phenotypic characteristics alone though have limited efficiency in identifying cryptic species, eggs, larvae and juvenile life stages, or for less well-studied taxonomic groups (Kochzius et al., 2008). Identification of benthic fauna can be particularly problematic in areas like the Red Sea, where specific identification keys are either scarce or not available. Also, currently most surveys focus on single biological components, such as macrofauna, i.e. organisms retained in 0.5 or 1 mm meshes (van Hoey et al., 2010; Seo et al., 2014; Stark et al., 2014), meiofauna (organisms <0.5 mm) (Frenzel et al., 2009; Riera et al., 2011; Mirto et al., 2012), or prokaryotes (Ramaiah and Chandramohan, 1993; Caruso et al., 2003; La Rosa et al., 2004), hampering a comprehensive understanding of the relative contribution of different phyla as well as biodiversity patterns of the whole system.

Environmental sampling relies on the extraction of bulk DNA and the amplification of suitable genes, such as the nuclear ribosomal RNA (rRNA) gene. High-throughput sequencing has been utilized in the marine community mainly to study the diversity of bacteria and archaea (Sogin et al., 2006; Brazelton et al., 2010; Bougouffa et al., 2013), although marine eukaryotes have also been studied including microbial eukaryotes (Cheung et al., 2010; Logares et al., 2012; Orsi et al., 2013; Pawlowski et al., 2011), zooplankton (Lindeque et al., 2013; Pearman et al., 2014) and fish (Thomsen et al., 2012). In the marine benthic environment it has been validated and used to study a variety of habitats including estuaries (Chariton et al., 2010), deep sea (Bik et al. 2012a,b) and coastal areas (Fonseca et al., 2010). It is a technique with the potential to greatly simplify comprehensive studies in areas of complex diversity and taxonomy, due to the fact that experts in morphological

* Corresponding author.
*E-mail address:* john.pearman@kaust.edu.sa (J.K. Pearman).

http://dx.doi.org/10.1016/j.margen.2015.10.008
1874-7787/

identification for a wide range of taxa are not required for the analysis of samples.

The present study aims to investigate the broad-scale taxonomic diversity of the eukaryotic benthos in shallow coastal areas of the central Red Sea, using extracellular DNA. Extracellular DNA constitutes a significant fraction of the total DNA (Nielsen et al., 2006) (up to 90% of total DNA in deep-sea sediments; Dell'Anno and Danovaro, 2005). Taberlet et al. (2012) have undertaken pilot experiments targeting ice worms (Enchytraeidae), earthworms (Lumbticina) and fungi using specific primers and found that the expected sequences were retrieved from the extracellular DNA. It has also been used as a census for marine fishes in a large mesocosm experiment (Kelly et al., 2014). Furthermore using extracellular DNA also offers the advantage of simplified extraction procedures, as protocols for cell lysis are not required making protocols quicker and cheaper. Our intention, instead of providing a detailed inventory of biodiversity, is to compare the eukaryotic benthic diversity across different coastal habitats, encompassing as many phyla as possible, using the cheap and previously tested protocol developed by Taberlet et al. (2012). Our main questions were: i) how benthic eukaryotic patterns (alpha- and beta-diversity, assemblage composition and structure) change across shallow coastal habitats; ii) are the patterns of variability consistent at different taxonomic levels; and iii) are the general biodiversity patterns observed in the Red Sea consistent with those observed on a global scale.

## 2. Methods

### 2.1. Sample collection.

A total of 32 sediment samples (16 sites, two replicates per site) were collected from intertidal (mangrove) to subtidal areas (up to 60 m depth) in the central Red Sea between January and March 2014 (Fig. 1). Samples were mainly collected using a Van Veen grab (0.1 $m^2$); intertidal samples in the mangrove area were collected

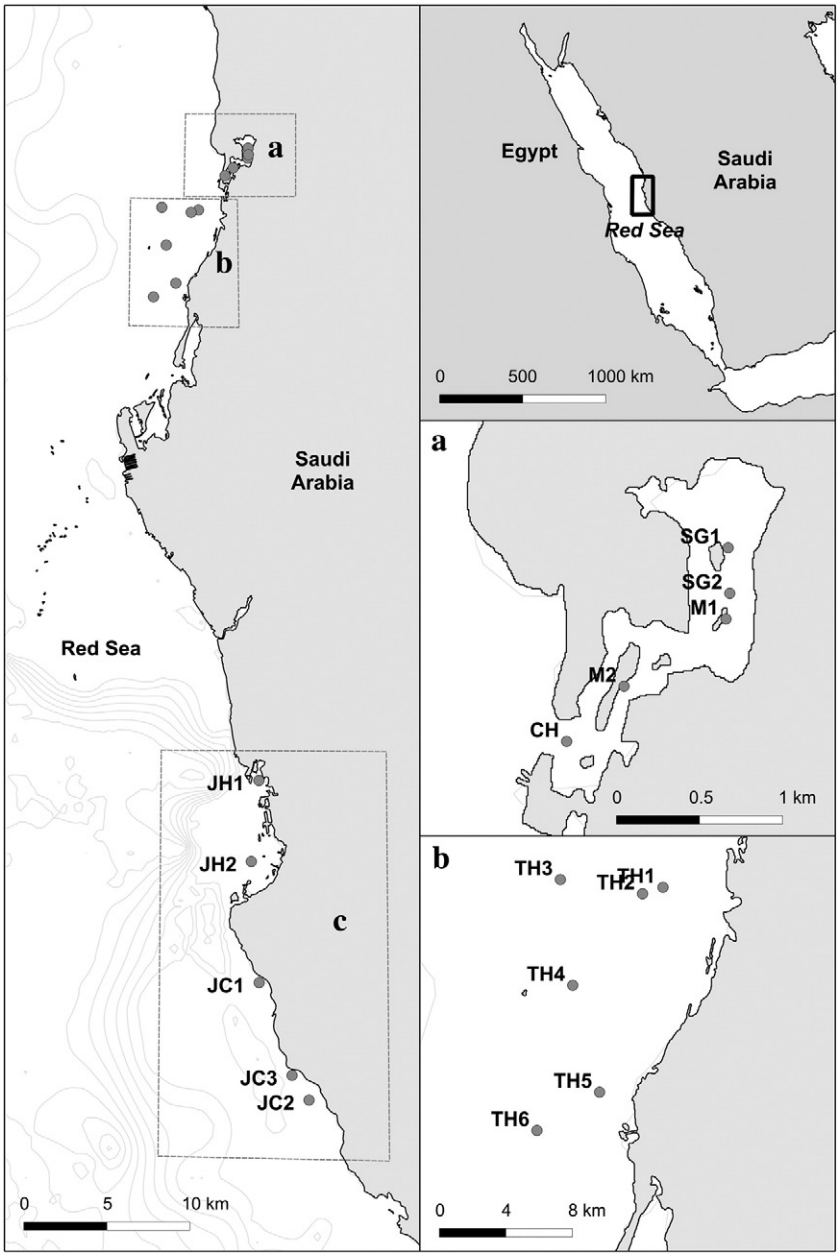


**Fig. 1.** Map illustrating the sampling stations in the 3 different areas in the central Red Sea, specifically, coastal lagoon (mangrove, M1, M2; seagrass, SG1, SG2; lagoon channel, CH), Thuwal (TH1, TH2, TH3, TH4, TH5, TH6) and Jeddah (harbour, JH1, JH2; coastal, JC1, JC2, JC3). Map was created using the software QGIS v2.6 (http://www.qgis.org/en/site/).