PRELIMINARY STUDIES ON GENETIC DIVERGENCE OF SOME SEA URCHINS ON THE SOUTHERN LEVANTINE BASIN OF EGYPT

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Abstract
There's no assessment for the sea urchin diversity in the Mediterranean despite the critical role they have as keystone species. Recent surveys in the Mediterranean show new data about sea urchin diversity and newly introduced species. Data from western Mediterranean countries to Egypt show records of 4 species *Paracentrotus lividus, Arbacia lixula, Sphaerechinus granularis and Psammechinus microtuberculatus.* While the Eastern Mediterranean countries to Egypt show the presence of Diadema setosum which is a recent invader from the Red Sea. Still the southern Levantine, of Egypt coast, lack complementing data for such findings.

Keywords: Biodiversity, Levantine Basin, Echinodermata, Genetics

Introduction
The morphological similarity between the species most common in the Mediterranean Levantine basin along with the lack of studies in Egypt that tackles the biology and ecology of these organisms have resulted in confused nomenclature. Furthermore, the uncertain geographical extent due to continuous introduction of new species has resulted in inconclusive reliability on biogeography [1]. The purpose of this study is to use genetic analysis as a reliable way for classification. Molecular methods depend on isolation and identification of 16S mitochondrial DNA gene was successfully used as a most preferred model for molecular genetics ecology as it support the relationship between morphology and genetics [2]. Therefore, we aimed to isolate and identify 16S mitochondrial DNA gene from selected sea urchins from several locations in Egypt to be used further for sequencing and subsequently in taxonomic and phylogenetic analyses.

Materials and Methods
The samples are collected by SCUBA diving from 2 rocky shore stations of Alexandria coast Abu Qir bay and Miami area Fig (1).

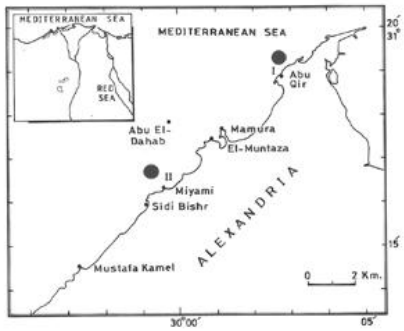

Fig. 1. Map showing locations of collection sites.

Four distinct morphological groups were selected. Gonad and gut specimens were collected from the 4 groups in order to examine their genetic variability. Specimens were quick frozen in liquid N2 and then ground in a clean, sterilized mortar. The genomic DNA was then extracted using Gene JET genomic DNA extraction kit following the manufacturer protocol (Fermentas, #K0721). To amplify partial 16S mitochondrial DNA region (approximately 450-500 bp), two universal primers (F:5'GACGAGAAGACCCTGTGGAGC3' and R:5'ACTTAGATAGAAACTGACCTG3') were designed using Primer 3.0 software based on conserved regions in published sea urchin and sea star sequence data [3]. The PCR was carried out following the manufacturer protocol (Fermentas, #K1071) in Techne TC-plus thermal cycler. PCR products were electrophoresed on 1% agarose gels using 1X TAE buffer containing 200 ng/ml ethidium bromide and then photographed.

Results and Discussion
The morphological examination resulted in 4 inconclusive regular sea urchin groups and they were expected to be *Arbacia lixula, Paracentrotus lividus, Psammechinus microtuberculatus and Sphaerechinus granularis*. However it is known that the juvenile individuals of *A. lixula* and *S. granularis* are sometimes confused with *P. lividus*. The *P. microtuberculatus* is also confused with *P. lividus* when the later has a lighter green color.
The amplified 16S mitochondrial DNA products from all four selected sea urchins showed four bands with different sizes (ranged from 450bp to 500bp) (Fig. 2).

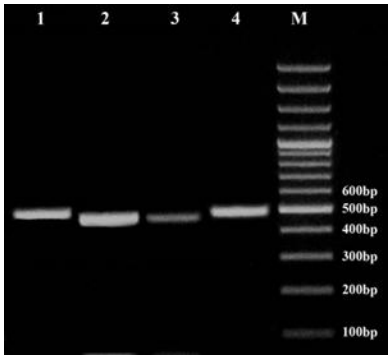

Fig. 2. PCR of 16S mitochondrial DNA fragment of 4 sea urchins (lanes 1-4), M= Marker.

The resulted PCR products are of different size, it is possible that these four sea urchins may be of different species or subspecies. Further confirmation using sequencing will be helpful for taxonomy and phylogenetic classification which will be done shortly after. We conclude that the molecular identification using 16S mitochondrial DNA is proved to be a more reliable way than the morphological approach to solve persisting problems in classification among individuals of sea urchins.

References
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