PHYLOGENETIC CHARACTERIZATION OF TWO ECHINOID SPECIES OF THE SOUTHEASTERN MEDITERRANEAN, OFF EGYPT

A. O. Hamdy [1]*, T. Soliman [1], F. Abdel Razek [1], A. El-Sayed [2], E. Elmasry [2] and J. Davis Reimer [3]
[1] National Institute of Oceanography and Fisheries - hamdy_nfra@yahoo.com
[2] Oceanography Department, Faculty of Science, Alexandria University
[3] Tropical Biosphere Research Center, University of the Ryukyus

Abstract
In this study we investigated the phylogenetics of two sea urchin species, *Arbacia lixula* and *Paracentrotus lividus* collected from the east coast of Alexandria City, Egypt. Pigmentation examination showed four sympatric color morphotypes (black, purple, reddish brown, and olive green). Mitochondrial DNA was extracted from specimens and mitochondrial cytochrome oxidase subunit I and 16S ribosomal RNA were sequenced. The results showed that all black specimens constituted the species *A. lixula*. All other colors belonged to *P. lividus*, with no apparent differentiation between color morphotypes. Moreover, *P. lividus* showed high haplotype diversity and low values of nucleotide diversity, indicating a high degree of polymorphism within this species.

Keywords: Echinodermata, Levantine Basin, Genetics

Materials and Methods. Sampling, DNA extraction & PCR amplification. Twenty individuals were selected from station Sidi Bishr, Alexandria, Egypt (Fig. 1)

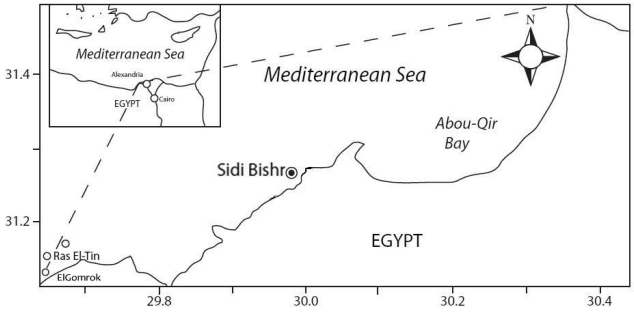

Fig. 1. Sampling site, Sidi Bishr, Alexandria, Egypt.

(16 specimens of *P. lividus* and 4 specimens of *A. lixula*), dissected and their gut and gonads were preserved in absolute ethanol (99.5%). Genomic DNA was extracted from 20 sea urchins from ~0.1 g of gonads using a DNeasy Tissue Kit (Qiagen), following the manufacturer's protocol. Mitochondrial cytochrome oxidase subunit I (COI) was amplified using the forward primer COIe-F 5′-ATA ATG ATA GGA GGR TTT GG-3′ and the reverse primer COIe-R 5'-GCT CGT GTR TCT ACR TCC AT-3′. 16S ribosomal RNA (16S) was amplified using the forward primer 16SA-R 5′-CGC CTG TTT ATC AAA AAC AT-3′ and the reverse primer 16SB-R 5′-GCC GGT CTG AAC TCA GAT CAC GT-3′. PCR reactions were carried out in a 20 µl total volume containing 5–20 ng of template DNA, 0.5 µM of each primer, and 10 µl of HotStarTaq™ Master Mix (Qiagen, Tokyo, Japan), in RNase-free distilled water. The PCR conditions for both markers consisted of an initial denaturing step at 95 °C for 15 min, 35 cycles (94 °C for 1 min, 46 °C for 1 min and 72 °C for 1 min) and a final step at 72 °C for 10 min for both DNA markers. PCR product sizes were checked by gel electrophoresis on 1.5% agarose gel. The amplified products were purified with Exonuclease I and Alkaline Phosphatase Shrimp (Takara) by being incubated at 37 °C for 20 min, followed by deactivation at 83 °C for 30 min. Purified PCR products were sequenced using an ABI Prism automated sequencer at Fasmac Co., Kanagawa, Japan, in both in forward and reverse directions.

Phylogenetic analyses
The sequences for both sea urchin species were edited and aligned using the software Geneious version 8.1. Novel sequences obtained in this study were deposited in GenBank (Accession Numbers KU172482–KU172520). New sequences obtained in this study for *P. lividus* were aligned with previously reported sequences from the eastern Atlantic and western Mediterranean (16S sequences from [1]; COI sequences from[1]), as well as outgroup sequences from *Psammechinus miliaris*. As *P. lividus* sequences were shown to form a well-supported monophyly to the exclusion of the outgroup, we subsequently generated and used unrooted trees in our analyses (without outgroup sequences) to improve their resolution. Sequence data of *A. lixula* were aligned with previous reported sequences in GenBank from the Atlantic Ocean and Mediterranean Sea (16S sequences from Chenuil et al., unpulished; COI sequences from [3]). Maximum likelihood (ML) and Neighbor-Joining (NJ) phylogenetic trees were constructed in MEGA 6 with 1000 bootstraps using a Tamura 3-parameter model as the best-calculated model for both markers without outgroups. There are no data available for these markers in congeners of either species in GenBank. Phylogenetic trees were constructed using Bayesian inference (MrBayes 3.2.2), with 1,000,000 cycles of Markov Chain Monte Carlo (MCMC), 4 heated chains and a burn-in of 100,000. For *P.lividus* (n = 16), the number of haplotypes (H), haplotype diversity (Hd), and nucleated diversity (π) was estimated using DNaSP version 5.1.

Results and Discussion
For the COI and 16S markers, fragments of 602 bp and 515 bp, respectively, were sequenced from specimens of *P. lividus* and *A. lixula*, respectively. Alignments showed clear patterns of differences between each species. A phylogenetic tree constructed with outgroups using the maximum likelihood method showed each species as a monophyly, one clade per species (data not shown). From 16 individuals of *P. lividus* a total of 12 and 9 haplotypes were found in COI and 16S, respectively, indicating a high degree of polymorphism within this species. *P. lividus* showed high values of haplotype diversity (COI; H = 0.9500 and 16S; H = 0.8580) and low values of nucleotide diversity (COI; π = 0.0075 and 16S; π = 0.0049). Genotyping results using 16S and COI sequences showed no relation between genetic differentiation and color morphotypes for *P. lividus*. Sequences of both mtDNA markers of *P. lividus* matched closely or were identical to previously reported sequences. For *A. lixula*, due to limited number of sequences of *A. lixula*, we could not perform meaningful comparisons with previous reports.The current study molecularly confirms the identity of *A. lixula* and *P. lividus* in Egyptian waters, and their close connectivity to previously reported populations in other Mediterranean regions. No significant phylogenetic patterns corresponded to the different observed color morphotypes of *P. lividus*.

References
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