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FULL LENGTH ARTICLE

Phylogenetic characterization of two echinoid species of the southeastern Mediterranean, off Egypt

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Abstract In this study we investigated the phylogenetics of two sea urchin species, *Arbacia lixula* and *Paracentrotus lividus* from the Mediterranean Sea. Specimens were 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 (COI) and 16S ribosomal RNA (16S) 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 (COI; $H = 0.9500$ and 16S; $H = 0.8580$) and low values of nucleotide diversity (COI; $\pi = 0.0075$ and 16S; $\pi = 0.0049$), indicating a high degree of polymorphism within this species. This study represents the first attempt at DNA barcoding of echinoid species in the southeast Mediterranean off the Egyptian coast, and will provide a base for future phylogenetic analyses.

Introduction

Measuring marine biodiversity is one of the main tools for effective management of the resources in the Mediterranean basin (Penant et al., 2013). Within marine resources, the sea urchins (Echinodermata: Echinoidea) are keystone animals in coastal areas due to their ability to alter the composition and

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the dynamics of algal resources by their grazing (Elmasry et al., 2013). Studies on the status of echinoid populations of the southeastern Mediterranean basin off Egypt are scarce including such basic data as their phylogenetics and population structure, distribution and dynamics, and fisheries status (Elmasry et al., 2013).

Echinodermata are characterized by polychromatism. Such color polymorphism is displayed in the sea cucumber *Apostichopus japonicus* (Kanno et al., 2006), the sea star (Asteroidae) *Pisaster ochraceus* (Calderon et al., 2010), the brittle star (Ophiuroidea) *Ophiothrix spiculata* (Ebert, 1996), and in sea urchins (Echinoidea) such as *Echinometra* sp., *Paracentrotus* sp. (Calderon et al., 2010), and *Heterocentrotus mammillatus* (Ebert, 1996). Calderon et al. (2010) stated that the evolution of color morphotypes within sea urchin species may be recent, and that species with polychromatism may be on their way toward becoming new species through sympatric speciation.

In this study, we investigated two species, *Paracentrotus lividus* (Lamarck, 1816) and *Arbacia lixula* (Linnaeus, 1758). The purple sea urchin *P. lividus* inhabits the entire Mediterranean basin and the eastern Atlantic from the British Isles to Morocco (west coast of Africa), including the Macaronesian Islands (Azores, Canary, Madeira and Cape Verde Islands) (Calderon et al., 2010). Similarly, the black sea urchin *A. lixula* is also widespread, and has been reported from the Mediterranean Sea and Macaronesian Islands, as well as from the Atlantic coasts of western Africa and Brazil (Wangensteen et al., 2012).

There is a high demand for commercially harvested sea urchins in Egypt, particularly during the summer season from the part of the Egyptian local consumers, tourists, recreational divers and restaurants serving seafood and sushi. *P. lividus* is the most desirable sea urchin in Egypt, and fishing workers consider it female in gender, calling it "netaya", while the black urchin *A. lixula* (Linnaeus, 1758) is considered as non-edible male "dakkar" due to the bitterness of their gonads, which are quite inferior in their taste compared to those of *P. lividus*. Unfortunately, there are no fishery records or statistics available for these two species, or for any other Egyptian commercial echinoid species. However, the collapse of *P. lividus* populations from the eastern Mediterranean has been recently reported, and this may have occurred within the last 15 years (Yeruham et al., 2015). Therefore, there is a critical need for genetic information of Egyptian populations of echinoids to help more effectively manage and conserve populations.

Thus, the aims of this study were to obtain information on the phylogenetic variation of *P. lividus* and *A. lixula* in the southeast Mediterranean off Egypt. Additionally, we examined if phenotypic coloration of different sympatric color morphotypes of *P. lividus* could be correlated with any underlying genetic structure.

Materials and methods

Sampling of sea urchins

Sampling of *P. lividus* and *A. lixula* sea urchins from different locations off the coast of Alexandria City, Egypt, was performed between January 2013 and October 2014. Specimens were collected from eastward coastline of Alexandria City (Sidi Bishr) (Fig. 1). Specimens were collected by SCUBA diving at depths ranging between 3 and 17 m. Specimens were transported alive to the laboratory for further analyses.

DNA extraction, PCR amplification, phylogenetic analyses

Twenty individuals were selected from Sidi Bishr (16 specimens of *P. lividus* and 4 specimens of *A. lixula*), dissected and the gut and gonads from samples were preserved in absolute ethanol (99.5%). Genomic DNA was extracted from 20 individual sea urchins from ~0.1 g of gonads using a DNeasy Tissue Kit (Qiagen), following the manufacturer's protocol. Two DNA markers were used in this study. 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′ (Arndt et al., 1996). 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′ (Palumbi et al., 1991). 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 (http://www.fasmac.co.jp/index.html), 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 (http://www.geneious.com; Kearse et al., 2012). 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 Calderon et al., 2009; COI sequences from Duran et al., 2004), 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 Wangensteen et al. (2012)). Maximum likelihood (ML) and Neighbor-Joining (NJ) phylogenetic trees were constructed in MEGA 6 (Tamura et al., 2013) with 1000 bootstraps using a Tamura 3-parameter model (Tamura et al., 2013) as the best-calculated model for both markers without outgroups.