Egyptian Journal of Aquatic Research (2016) 42, 319–329

HOSTED BY

ELSEVIER

National Institute of Oceanography and Fisheries

Egyptian Journal of Aquatic Research

http://ees.elsevier.com/ejar
www.sciencedirect.com

Genetic and morphological identification of some crabs from the Gulf of Suez, Northern Red Sea, Egypt

Eman M. Abbas [a,*], Khaled M. Abdelsalam [b], Khaled Mohammed-Geba [c,*], Hamdy O. Ahmed [a], Mikio Kato [d]

[a] *Aquaculture Division, National Institute of Oceanography and Fisheries, Egypt*
[b] *Marine Environment Division, National Institute of Oceanography and Fisheries, Egypt*
[c] *Genetic Engineering and Molecular Biology Division, Faculty of Science, Menoufia University, Egypt*
[d] *Department of Biological Science, Graduate School of Science and Biology Laboratory of Faculty of Liberal Arts and Science, Osaka Prefecture University, Japan*

Received 14 April 2016; revised 1 August 2016; accepted 2 August 2016
Available online 27 August 2016

KEYWORDS

Crabs;
DNA barcoding;
Morophological identification;
Cytochrome oxidase subunit I (COI) gene

Abstract Most crab species inhabiting the Red Sea have not been characterized morphologically and genetically. In the current work, five different crab species were collected from the northern part of the Egyptian Red Sea. They were morphologically identified through description of colors, dentations of the carapace and shapes of chelipeds and pereiopods. They were also genetically characterized by the partial sequencing of the barcode region in the mitochondrial cytochrome oxidase subunit I (COI) gene, which is known to be hypervariable among different crab species. Morphological and genetic characterization identified the crab species as: *Charybdis* (*Charybdis*) *hellerii* (A. Milne-Edwards, 1867), *Charybdis* (*Charybdis*) *natator* (Herbst, 1794), *Portunus* (*Portunus*) *pelagicus* (Linnaeus, 1758), *Liocarcinus corrugatus* (Pennant, 1777), and *Atergatis roseus* (Rüppell, 1830). This is the first record of *L. corrugatus* in the Egyptian Red Sea, despite being previously recorded in the Indian and Atlantic Ocean as well as in the Mediterranean Sea. DNA barcoding with precise morphological identification was effective in characterizing the crab species collected from the Egyptian Red Sea water.

Introduction

The flow of organisms, which is considered a biological invasion, can occur naturally through changing climates and currents or by human activities. The second type is sometimes unpredictable because it is capable of crossing many borders and because it exhibits many vectors (Carlton, 1996). As

* Corresponding authors at: Lab of Genetics, Aquaculture Division, National Institute of Oceanography and Fisheries, Alexandria Branch, Egypt (E.M. Abbas). Genetic Engineering and Molecular Biology Division, Department of Zoology, Faculty of Science, Menoufia University, Egypt (K. Mohammed-Geba).
E-mail addresses: emanabbas03@yahoo.com (E.M. Abbas), khaled-spain@yahoo.com (K. Mohammed-Geba).

Peer review under responsibility of National Institute of Oceanography and Fisheries.

http://dx.doi.org/10.1016/j.ejar.2016.08.003
1687-4285

reviewed by Hulme et al. (2008), biological invasions occur mainly through one of three routes, transfer through goods importation, transfer through transportation vehicles and dispersal through man-made channels and pathways between distant areas. The importation of goods causes an unintentional introduction to the contaminant species, as in the common cases of crustaceans and jellyfish that have been introduced to some Egyptian and international water bodies with animals brought for aquaculture from distant zones (for example, see Ishak, 1980; Minchin, 1997; El-Serafy et al., 2014; El-Shabrawy and Dumont, 2016). Regarding the second category, introduction through human transportation vehicles, alien species can be transported through ballast waters, as in the case of the introduction of the North Atlantic spider crab *Hyas araneus* to Antarctica and the Chinese mitten crab *Eriocheir sinensis* to Northern Europe (Herborg et al., 2003; Tavares and De Melo, 2004). Finally, there comes the third introduction pathway for non-native species, most importantly in Egypt and the Mediterranean Sea in general, named as the "corridor" pathway. This one aids the transfer of species through geographical areas that were linked by man-made canals, like these of Suez (Asia/Africa), Panama (the Americas) and Rhine-Main-Danube (Europe) (Por, 1978, 1990; Tavares & De Melo, 2004; Hulme, 2015).

Since its completion in the 19th century in Egypt, the Suez Canal has been considered the international corridor for Lesspsian migrants, including many finfish and shell fish species that move from the Red Sea to the Mediterranean Sea and vice versa. Some of these species could establish successful populations in their new habitats, including Rabbitfishes *Siganus rivulatus* and *Siganus luridus* (Hassan et al., 2003), the dusky sweeper *Pempheris rhomboidea* (Azzurro et al., 2015), the decapod crabs *Portunus pelagicus* (Corsini-Foka et al., 2004), *Atergatis roseus* (Galil, 2011), *Actaea savignii* (Karhan et al., 2013) and others.

Crabs are decapod crustaceans; their eyes are on short stalks and they have short, broad and more or less flattened bodies (carapace) with small abdomens that are folded under the thorax. Brachyuran crabs (the subject of the present study) are one of the most diverse animal groups at the infra-order level (Števčić, 2005), comprising about 1,271 genera and 6793 species worldwide (Ng et al., 2008; De Grave et al., 2009). According to Boudreau and Worm (2012), Brachyuran crabs play an important role in marine benthic communities, ranging from intertidal to deep waters. They are preys for a wide range of invertebrates and vertebrates that are successful and versatile predators, preying at more than one trophic level. Crabs interact with the habitat and its inhabitants in a variety of ways, including providing habitat for smaller invertebrates and competing for food and shelter.

The systematics of the brachyuran crabs are usually based on the morphological diagnostic characters. However, there are many new approaches using various methods and novel data from sperm or molecular studies (Števčić, 2005).

DNA barcoding technology, using short sequences that belong to the mitochondrial gene cytochrome oxidase subunit 1 (COI), has been proposed as a method for enabling rapid and accurate detection and identification of species (Hebert et al., 2003; Marshall, 2005; Hajibabaei et al., 2007). Through studying the divergence of the barcode region of COI gene in 150 crustacean families, Costa et al. (2007) confirmed its effectiveness in placing different decapod species in their proper taxonomic order. Crustacean COI barcode region exhibited the highest species-level divergence rate among all animal groups. Therefore, the COI gene barcode region provides one of the best known systems for crab identification. Since then, this tool has been extensively applied for new crab species identification, zoogeographical description of crab species in certain zones and even mislabeling detection of crab species in fish markets (for example, see Knowlton and Leray, 2015; Raupach et al., 2015; Van der Meij et al., 2015). Congruent DNA barcoding and morphological description of animal species enable the accurate discrimination of different species, including cryptic ones (Keenan et al., 1998; Lai et al., 2010). There is still so much work needed for the identification and barcoding of crab species. In the present study, we have applied different DNA analyses, mainly barcoding and phylogeny, along with morphological parameter analysis to identify various crab species inhabiting the Egyptian territories of the Red Sea (Suez City and Suez Gulf), where the composition of crab species is still unknown in detail.

Materials and methods

Sample collection

Crab samples were collected from Suez City (29°10′N, 32°54′E) and the Abo Zenima area (29°05′N, 33°10′E) in the Gulf of Suez, north of the Red Sea in Egypt (Fig. 1). For each species, two or three specimens were sampled and preserved in an ice box until transferred to the laboratories. In the Laboratories of Genetics and Marine Biota Taxonomy of the National Institute of Oceanography and Fisheries (Alexandria, Egypt), the second pereopod (the first walking leg) was removed, sliced and placed immediately in 99% ethanol for preservation until DNA analysis. The species were identified by examining the external morphology. Then, they were subjected to DNA extraction for analyzing the COI gene barcode region in all of them as described below.

Morphological and morphometric characteristics

The external morphology of crabs was characterized in regards to the following parameters: color and measurement of the carapace. The carapace length was measured from the tip of the median frontal teeth, along the median axis to the posterior border of the carapace. The breadth was measured across the widest points, usually found between the last pair of anterolateral spines. Teeth on the anterolateral margin of carapace, as well as teeth or ridges of carapace were indicated. The shapes of cheliped and/or pereiopods were also used as useful taxonomic features. The obtained parameters were compared to those in the literatures to confirm the identification (e.g. Stephenson, 1972; Serène, 1984; Moyse and Smaldon, 1990; Wee and Ng, 1995; Ng, 1998).

Molecular identification

DNA extraction, PCR amplifications and sequencing

Briefly, 100–250 mg of muscle tissue samples were excised and homogenized in TES buffer (10 mM Tris–HCl, 140 mM NaCl, 25 mM EDTA, pH 7.8) that contained 1% SDS and