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

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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

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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

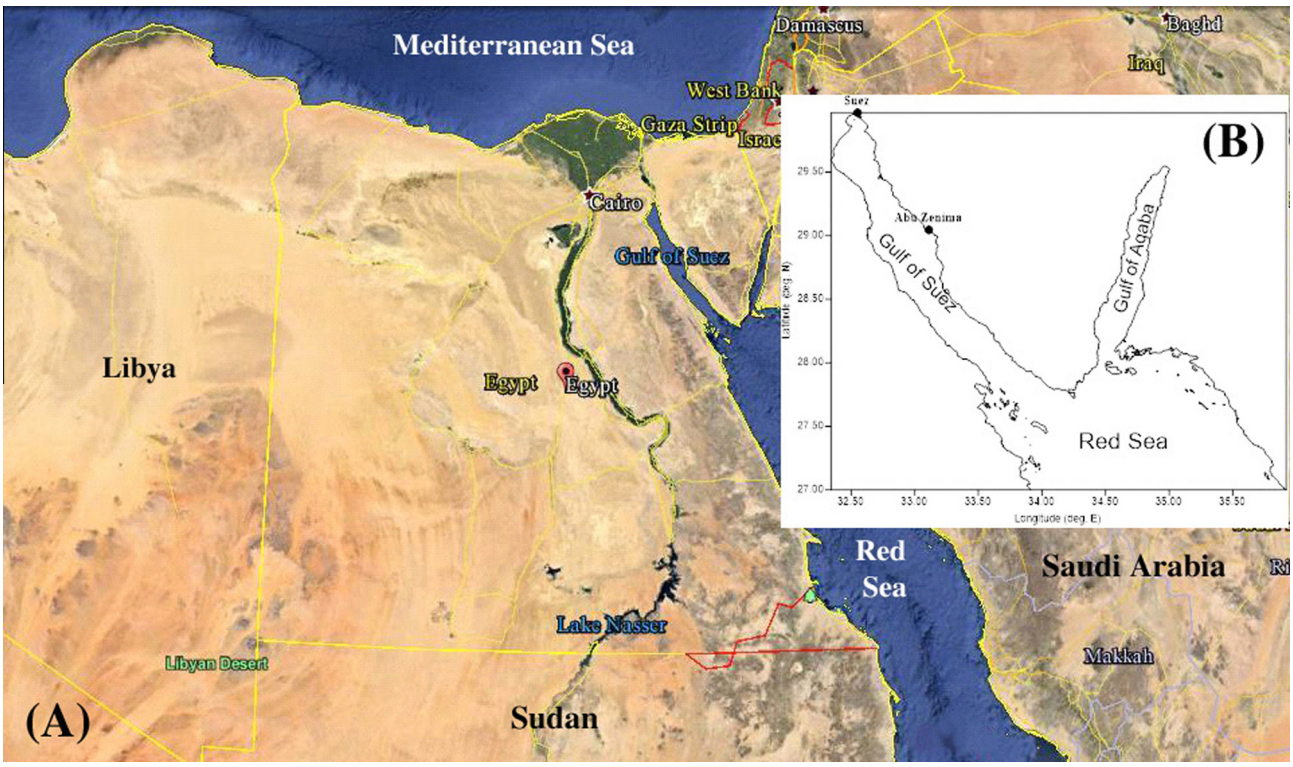

Figure 1 Location map. (A). Geographical location of Egypt. (B). Enlarged map showing the sampling area between Suez City and Abo Zenima region in the Suez Gulf (north of the Red Sea).

0.5 mg mL-1 proteinase K. The homoenates were incubated for one hour at 50 °C. The genomic DNA was then extracted from each species using conventional phenol–chloroform procedure and the resulting DNA was dissolved in TE buffer (100 mM Tris–HCl, 10 mM EDTA, pH 8). DNA was stored at 4 °C and its concentration was spectrophotometrically estimated. The partial coding regions of cytochrome oxidase subunit 1 (COI) gene were amplified by PCR using the set of primers described by Folmer et al. (1994), that are LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). The amplification reaction was set up as 20 ng of template DNA from each sample, 1X RBC SensiZyme® Hotstart Taq Premix (RBC Bioscience), 0.4 µM each of primer, in a total volume of 25 µL. The conditions of PCR included an initial denaturation for 2 min at 95 °C, followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 1 min at 42 °C and extension for 2 min at 72 °C. A final elongation step at 72 °C was appended to the PCR program and lasted for 7 min. The amplified products were visualized using 2 % agarose gel electrophoresis stained by 25 µg of ethidium bromide. The produced singular bands at the expected band sizes for each sample were purified using HiYield Gel/PCR DNA Fragments Extraction Kit (RBC Bioscience). The purified DNA fragments were subjected to sequencing. DNA sequencing was performed using the Big Dye Terminator version. 3.1 Cycle Sequencing kit (Applied Biosystems) and ABI3730 Sequencer (Applied Biosystems).The sequencing PCR reaction was performed at 96 °C for 2 min, followed by 25 cycles of 10 s at 96 °C, 5 s at 50 °C and 4 min at 60 °C.

DNA sequencing data analysis

The resulting raw sequences of COI were edited using the software Chromas Lite version 2.1 (Technelysium Pty Ltd, available from the URL http://technelysium.com.au/). The partial coding sequences for COI gene of the five collected crab species were compared in the GenBank database using BLAST algorithm. COI sequences for these five crab species and other 24 other COI sequences of crabs belonging to the species *Portunus trituberculatus*, *Atergatis floridus*, *Atergatis integerrimus*, *Liocarcinus holsatus*, *Tachypleus tridentatus*, *T. gigas* – were all retrieved from the GenBank database and aligned using Clustal W software (Thompson et al., 1994) that was included in the MEGA6 software package (Tamura et al., 2013). Pair wise genetic distances were calculated for COI gene in the five studied species also using the software MEGA6. The aligned sequences were saved as .fas (fasta) format. The alignment was saved as Fasta file and exported to JModelTest software V. 2.1.10 for detection of best nucleotide substitution model in order to construct a maximum likelihood phylogenetic tree between different species of freshwater tetraodontids. Then, the Fasta file was uploaded to Beauti software V. 1.8.3, with the substitution model determined using JModelTest, and 10,000,000 Markov chains were selected. The program was run once with this number of Markov chains, then two other runs were carried out, one with 50,000,000 chains and another with 100,000,000 chains. The resulting .xml files were opened using BEAST software V. 1.8.3 for estimating tree topologies. The resulting .log files from the three trials were uploaded to the program Tracer v1.6 to assess the quality of the results, accepting only theses with Effective Sample Size (ESS) above

200. The resulting trees were combined using LogCombiner software V. 1.8.3, then uploaded to TreeAnnotator software V. 1.8.3 for summarizing the information retrieved from tree samples produced through BEAST. The resulting consensus tree was then finally obtained using FigTree software V. 1.4.2. The horseshoe crabs from the family Limulidae, *T. tridentatus* and *T. gigas*, were used as outgroup as they are considered the oldest group of crab species with living representatives in the current geological era, i.e. living fossil. Later on, the sequences of the five crab species obtained from the Northern Red Sea in the current study were deposited in GenBank/EMBL/DDBJ International Databases with accession numbers KF793329, KF793328, KF793332, KF793331 and KF793330, respectively. That was done based on the morphological identification, BLAST comparison of COI barcode region results, and the phylogenetic tree's results.

Results

Morphological and morphometric description

Five species of crabs were identified in the present study, namely: *Charybdis (Charybdis) natator* (Herbst, 1794), *Charybdis (Charybdis) hellerii* (A. Milne-Edwards, 1867), *Portunus (Portunus) pelagicus* (Linnaeus, 1758), *Liocarcinus corrugatus* (Pennant, 1777), and *Atergatis roseus* (Rüppell, 1830).

Systematics accounts
Infraorder: Brachyura
Superfamily: Portunoidea

Family: Portunidae
Charybdis (Charybdis) natator (Herbst, 1794)
Fig. 2

Synonyms
Cancer natator Herbst, 1794: 156 pl. 40, Fig. 1.
Charybdis natator – Sakai, 1939: 407, Fig. 9b.
Charybdis (Charybdis) natator – Leene, 1938: 93, Figs. 50, 51.

Carapaces are densely covered with very short pubescence (hairs not easily scraped off) which are absent from several distinct transverse granulated ridges in the anterior half. The color is overall orange-red, with ridges on carapace while the legs are dark reddish brown. The width of the frontal-orbital border is distinctly narrower than the width of the carapace. Carapaces have distinct ridges or granular patches behind the level of the last pair of the anterolateral teeth. The fronto-orbital margin has 8 teeth, 6 teeth on the anterolateral margin with the first anterolateral tooth being truncated. The posterior border of the carapace forms a curve with the posterolateral border. Regarding the cheliped, the anterior border of merus has three spines and one distal spine on the posterior border; the carpus holds a strong spine on the inner angle and three spinules on the outer angle.

Charybdis (Charybdis) hellerii (A. Milne-Edwards, 1867)
Fig. 3

Synonyms
Goniosoma hellerii A. Milne Edwards, 1867: 282.
Charybdis hellerii – Edmondson, 1954: 247, Figs. 32a-f.
Charybdis (*Charybdis*) *hellerii* – Leene, 1938: 44, Figs. 15, 16a-d, 17a-c

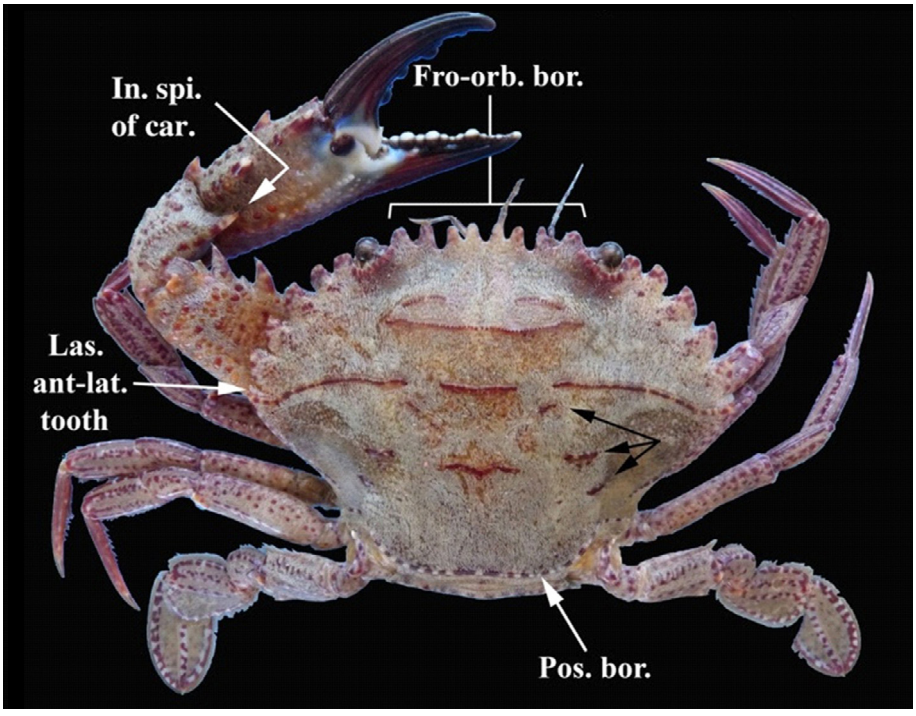

Figure 2 *Charybdis* (*Charybdis*) *natator* (Herbst, 1794). The dorsal view shows the fronto-orbital border (Fro-orb. bor.), posterior border (Pos. bor.), inner spine of carpus (In. spi. of car.) and last anterolateral tooth (Las. ant-lat. tooth) of the carapace. Black arrows show the 3 mesobranchial ridges.

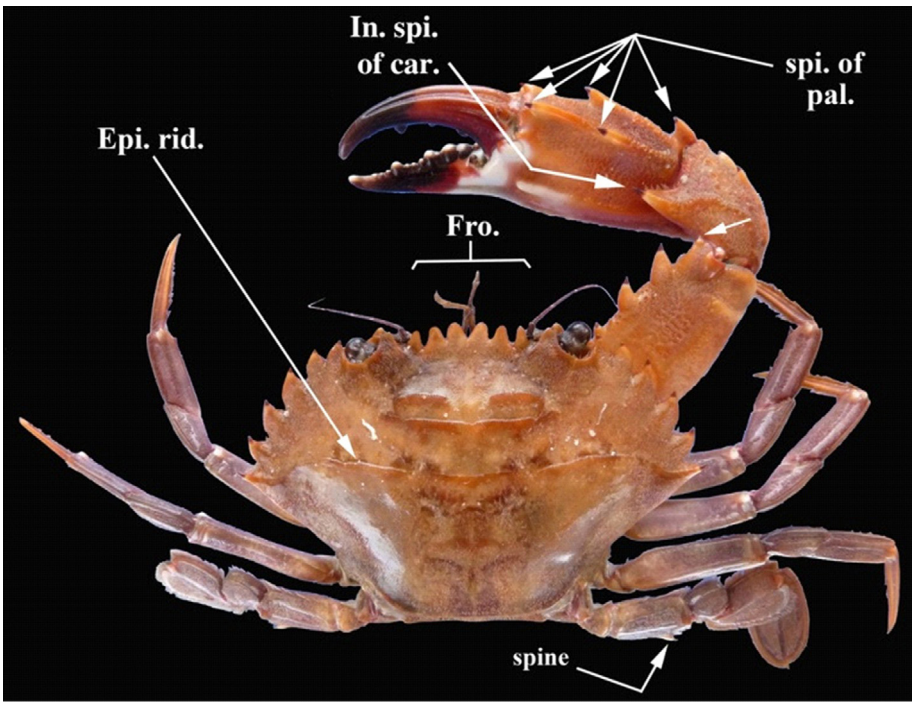

Figure 3 *Charybdis* (*Charybdis*) *hellerii* (A. Milne-Edwards, 1867). The dorsal view shows the front (Fro.) with six teeth, six teeth on each anterolateral margin of the carapace, epibranchial ridge (Epi. rid.), the strong inner spine of carpus (In. spi. of car.), the five spines of palm (spi. of pal.) and the spine of merus (spine) found on the natatory leg on the upper surface. White arrow shows the spinule of merus (cheliped) at the distal corner of the anterior border.

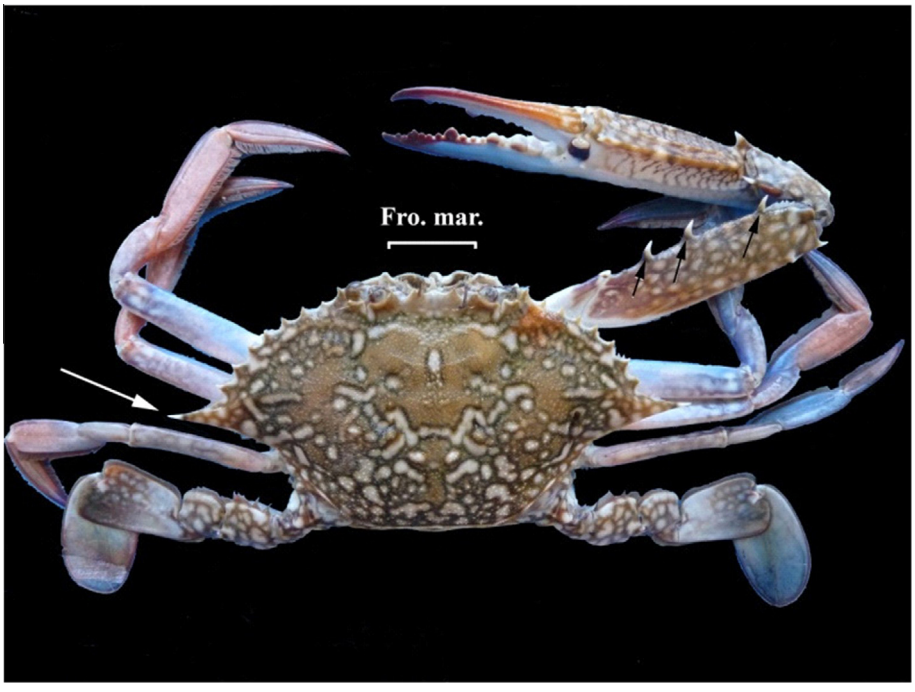

Figure 4 *Portunus* (*Portunus*) *pelagicus* (Linnaeus, 1758). The dorsal view shows the front margin (Fro. mar.) with 4 triangular teeth; white arrow shows the last anterolateral tooth; black arrows show the 3 spines on the inner margin of merus (cheliped).

Specimens showed an orange color on both sides of the anterior median line, a brown color on the entire posterior surface of the carapace and a purple color on the legs. Anterior carapace ridges are present and granular without any epibranchials at the back. Carapaces have six frontal teeth behind and six anterolateral teeth on each side, the last one is elongated and spiniform.

Chelipeds are stout; their anterior borders are of merus with three spines and a spinule at their distal corner; carpus have strong spines on the inner angle and three spinules at the outer angle, while the palm has five spines on the upper surface.

Portunus (Portunus) pelagicus (Linnaeus, 1758)
Fig. 4

Synonyms:
Cancer pelagicus Linnaeus, 1758: 626.
Portunus pelagicus – Fabricius, 1798: 367.
Portunus (*Portunus*) *pelagicus* – Utinomi, 1969: 87, Pl. 44

Carapaces are greenish-brown in color, with irregular pale mottling edged with dark brown; chelipeds are purplish, mottled and their fingers are blue. Carapace is wide in breadth with transverse granulate lines. The breadth-length ratio is 2.13. The front edge has 4 acutely triangular teeth; 9 teeth on each anterolateral margin (including acute lateral orbital angle tooth), the last tooth is 2–4 times larger than the preceding teeth. Chelipeds are long, massive, spinous and ridged. The inner margin of the merus (cheliped) has 3 spines.

Family: Polybiidae
Liocarcinus corrugatus (Pennant, 1777)
Fig. 5

Synonyms
Cancer corrugatus Pennant, 1777:5
Macropipus corrugatus – Pennant, 1777.
Portunus corrugatus – Pennant, 1777.
Liocarcinus corrugatus – Stimpson, 1870.

Carapaces are suboval and are broader than they are long. It is reddish-brown in color. The dorsal surface of carapace is moderately convex and has numerous distinctive transverse ridges that give a corrugated appearance. There are 5 prominent equally-developed antero-lateral teeth on each side of the carapace. The margin of the frontal region has a shallow median lobe and a pair of very broad concave sub-median lobes. Pereiopods are stout and setose, the carpus-dactylus of second to fourth is with longitudinal carinae. However, the dactylus of second to fourth pereiopods is styliform, while the fifth one is broadly lanceolate with a pronounced median carina.

Superfamily: Xanthoide
Family: Xanthidae
Atergatis roseus (Rüppell, 1830)
Fig. 6

Synonyms:
Carpilius roseus Rüppell, 1830: 13, Pl. 3, Fig. 3.
Cancer roseus – H. MilneEdwards, 1834: 374.
Atergatis roseus – De Haan, 1835: 17.

The specimens under study clearly belong to the group having neither traces of teeth nor ridge marking at the junction of the antero-lateral and postero-lateral margins of the carapace. They have uniform reddish brown carapaces. The carapace is transversely suboval, broader than it is long, slightly convex, with minute punctures distributed all over its surface. Punctuations occur also on chelipeds, walking legs, abdomen, sternum; minute setae are distributed on abdomen, sternum and coxae. The front is narrow and undetectably convex; it is

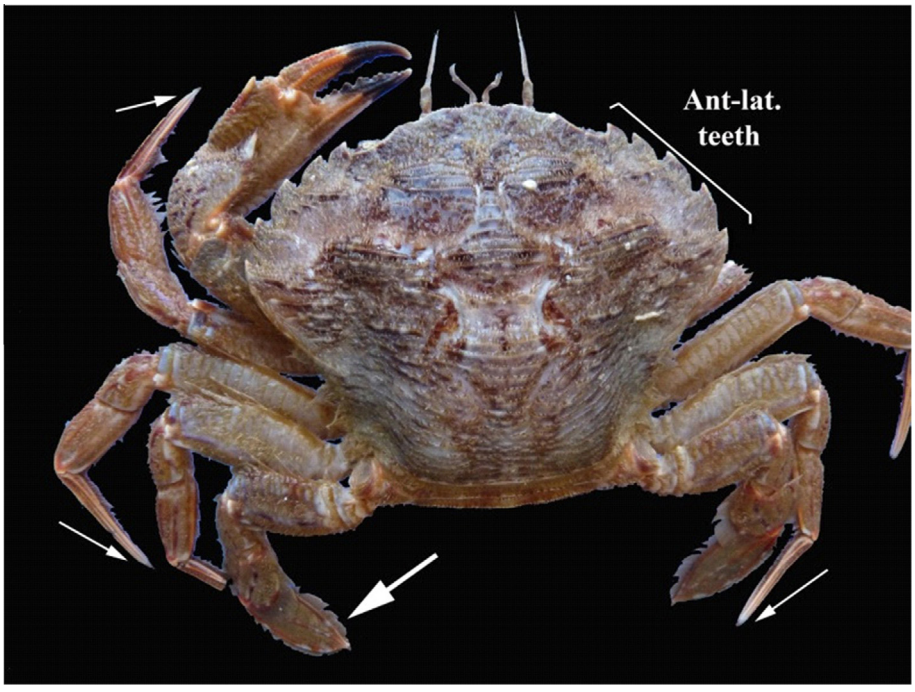

Figure 5 *Liocarcinus corrugatus* (Pennant, 1777). The dorsal view shows the Anterolateral teeth (Ant-lat. teeth). Small arrows show the styliform dactylus of second to fourth pereiopods. Large arrow demonstrates the broadly lanceolate dactylus of the fifth pereiopod with pronounced median carina.

Figure 6 *Atergatis roseus* (Rüppell, 1830). The dorsal view shows the punctuations on the carapace and cheliped. White arrows show the tufts of hairs at the inner angle of carpus (cheliped). Black arrow demonstrates the small notch of the front.

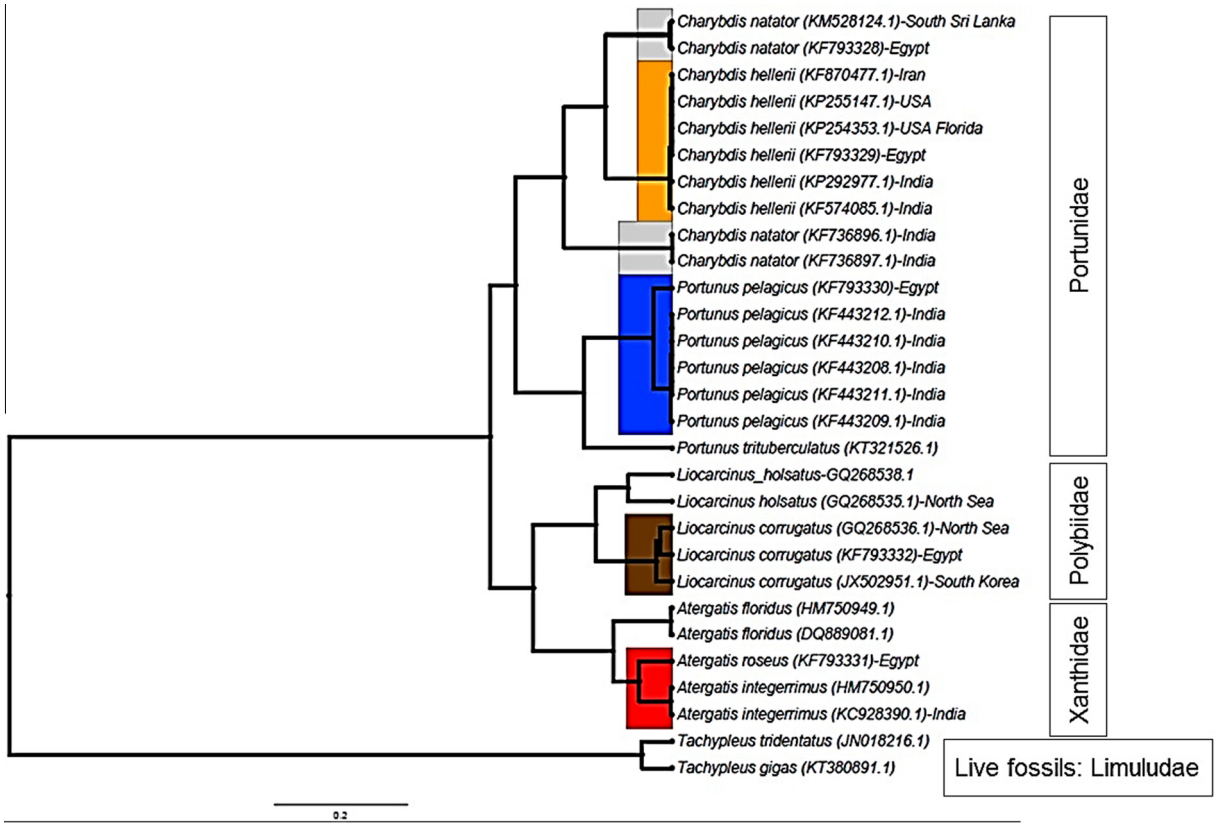

Figure 7 COI-based maximum likelihood (ML) phylogenetic tree for the five species of Egyptian crabs and their counterparts in other areas of the world, rooted to the living fossil crabs from the family Limuludae. Colors are shown according to external coloration of the crab species, as Grey: *C. natator*, Orange : *C. hellerii*, Blue : *P. pelagicus*, Brownish : *L. corrugatis*, Red: *A. roseus*.

Table 1 Pairwise genetic distance inferred from COI gene of the five crab species in Egypt included in this study. The Egyptian samples are marked by an asterisk. Representatives for genus *Atergatis* were included in the comparison to exhibit their distance to the Egyptian sample of *A. roseus* due to the complete absence of genetic barcodes for the latter species.

	Atergatis integerrimus – HM750950.1	*Atergatis floridus* – DQ889081.1	*Atergatis roseus** – KF793331	*Charybdis hellerii** – KF793329	*Charybdis natator** – KF793328	*Liocarcinus corrugatus** – KF793332
Atergatis integerrimus – HM750950.1	0.000	0.000	0.000	0.000	0.000	0.000
Atergatis floridus – DQ889081.1	0.222	0.000	0.000	0.000	0.000	0.000
*Atergatis roseus** – KF793331	0.156	0.177	0.000	0.000	0.000	0.000
*Charybdis hellerii** – KF793329	0.379	0.309	0.419	0.000	0.000	0.000
*Charybdis natator** – KF793328	0.396	0.279	0.362	0.148	0.000	0.000
*Liocarcinus corrugatus** – KF793332	0.325	0.345	0.313	0.301	0.303	0.000
*Portunus pelagicus** – KF793330	0.410	0.461	0.351	0.411	0.446	0.476

divided into two lobes by a small notch. The lower posterior margin of carapace is smaller than the frontorbital width. The carpus of cheliped has blunt teeth with tufts of hairs on the inner angle.

Molecular phylogenies

The final COI datasets were 652 bp-long for *C. natator* and 658 bp-long in *C. hellerii*, *L. corrugatus*, *P. pelagicus* and *A. roseus* (excluding primers). Best fitting models were applied for the COI datasets of nucleotide composition and divergence values depending on General Time Reversible (GTR) model and a tool from MEGA6 software. The maximum likelihood (ML) tree of the studied crabs and those retrieved from GenBank database, is shown in Fig. 7. The ML tree showed three major clades, each belonging to a single family. *C. hellerii; C. natator*, and *P. pelagicus* grouped together with the clade combining all representatives of the family Portunidae. *L. corrugatus* and other representatives of the family Polybiidae grouped also in a single clade. Finally, *A. roseus* fallen in the clade containing other representatives of the family Xanthidae. Pairwise genetic distances among the five crab species sampled in Egypt showed the highest value (0.476) between *L. corrugatus* and *P. pelagicus*, and the lowest (0.148) between *C. hellerii* and *C. natator* (Table 1). In both phylogenetic tree and pairwise genetic distances table, the absence of genetic barcodes for *A. roseus* in GenBank database obligated us to use a same-genus-level of comparison in order to assign the sample to a proper crab genus based on its studied morphology. Genetic assignment of *A. roseus* to the genus *Atergatis* was correct, since it clustered perfectly with other species of this genus in the constructed ML tree, and also showed the least pairwise distances with *A. integerrimus* and *A. floridus* (0.156 and 0.177, respectively) in comparison to all other tested crab species (Fig. 7, Table 1).

Discussion

About 12% of the brachyuran species colonizing the Mediterranean Sea now are Indo-West Pacific species that have reached there through the Suez canal (Almaça, 1985). Several species that have been highlighted by the current study are not autochthonous to the Egyptian waters. Moreover, before the present study no COI sequence data for these crabs collected from Egyptian Red Sea appeared in the GenBank/EMBL/DDBJ genetic databases; we have also added new data for these species. Some crab species assessed in this study came from distant geographical regions through several means of transport as highlighted before in the introduction section herein. These species are supported by special sturdiness and flexibility to both the transport route and the new-settling habitats. In this sense, *C. hellerii had* reached the Western Atlantic Ocean from its native range in the Indo-West pacific since the late 1980s. It has exhibited many life characteristics that would aid its transportation and rapid expansion in its new habitats (Dineen et al., 2001; Bolaños et al., 2011). These characteristics include its larval time, which is long enough - 44 days- to enable its survival in ballast waters. Also, its rapid growth and maturation, within one year only, causes a short generation time. Adult males exhibit a good capability of sperm storage for production of multiple successive broods,

together with a whole-year-long reproduction season, further rendering the population duplication time as very short. Moreover, *C. hellerii* could be obtained from a very wide variety of bottoms, including muddy, sandy and rocky ones. It was also found between corals, algae and mangrove prop roots. It is also capable of consuming a wide variety of preys, hence increasing the possibility of its opportunistic occupation to new habitats (Dineen et al., 2001; Bolaños et al., 2012). However, the other sympatric species of the same genus, *C. natator*, showed more limited capabilities of expansion than *C. hellerii*, such as two peaks of reproduction in the year only and a preference to rocky habitats (Sumpton, 1990). Very limited works pointed to the presence of *C. natator* in the Red Sea, an example of those is Vine (1986), and even fewer studies were concerned with its presence in the North of the Red Sea (Sallam and Gab-Alla, 2010). Therefore, and in contrast to *C. hellerii*, that could be identified in several points in the Eastern and Western Atlantic coasts where it could form self-sustaining populations (Mantelatto and Dias, 1999; Galil, 2000), *C. natator* seems to have less capabilities and slower rates of extension out of its main homelands in the Indo-West Pacific area.

P. pelagicus is naturally found throughout Indo-West Pacific (Ng, 1998), too. It migrated to the Mediterranean through Suez Canal very early after its construction, with the first sample being recorded in the Mediterranean at the Levantine Basin in 1898. Its Mediterranean migration mainly followed the Anatolian coasts and continued westwards (Corsini-Foka et al., 2004). It possesses a short larval development period (14–17 days) (Josileen and Menon, 2004) and exhibits a wide variety of diets, e.g. crustaceans, gastropods, bivalves, ophiuroids, that mainly depend upon the local availability of prey species (Williams, 1982). It also shows high fecundity and an all-year-long spawning time (Batoy et al., 1987; Zairion et al., 2015) all of which supports its great geographical expansion and its success in the recipient areas.

L. corrugatus has a wide range of distribution also. It has been reported in the Indo-Pacific coasts of Korea, China, Japan, South Australia, New Zealand and even in the Mediterranean Sea and the British Isles (Bennett, 1964; Zariquiey-Álvarez, 1968; Kim, 1973; Sakai, 1976; Ingle, 1980; Dai and Yang, 1991). However, it was never reported before in the Red Sea, so our study provides its first recording in the area of study. The species is mainly found in beaches with coarse sand and gravel. Its larval development completes in about 26–60 days (Kim and Hong, 1999). However, no data are available for its biology and ecology to suggest a special invasion route. For our knowledge, the species was not recorded in the zone between South East Asia to the Mediterranean. Hence, and coupling this to its relatively long larval period, the possibility of its transport through ballast waters cannot be ruled-out.

A. roseus was introduced to the Mediterranean from its native Indo-Pacific range, with the least records in the Eastern Mediterranean region (Corsini-Foka and Pancucci-Papadopoulou, 2010; Grosholz, 2011). No clear records were found for its presence in the Northern Red Sea. However, its presence in the Southern aperture of the Suez Canal further confirms the suggestion of Serène (1984) that the species entered the Mediterranean Sea as a Lessepsian migrant through the Suez Canal corridor from the Red Sea. Not enough data could be found for its biology and ecology, for example the feeding behavior and preferences, length of larval period, etc... Therefore, neither its real routes of introduction nor its post- introduction success can be clearly judged.

In conclusion, the current study successfully identified several crab species in the fundamental zone of the Northern Red Sea, just before the major corridor that permits the Lessepsian migrations to the Mediterranean, which is the Suez Canal. All species recorded in this study were naturally present in the Indopacific and migrated to the Mediterranean, with some migrating even farther, to the Western Atlantic. This study could reveal the necessity for more data about the biology, ecology and molecular genetics of the crab species assessed and other crab species in general. Finally, the area of study seems to be very promising and fundamental for proper surveillance of species-transfer among different aquatic environments. Combined analysis of morphology and DNA barcoding-based identification may provide a corner stone for the purpose of surveillance.

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