



Molecular and biometric characterizations of the Western king prawn *Melicertus latisulcatus* (Kishinouye, 1896) in the Egyptian Red Sea.

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ABSTRACT

Although the western king prawns *Melicertus latisulcatus* (Kishinouye, 1896) are among the highly-priced shrimp species in the world, there is a great shortage in research dealing with both morphological and molecular clues for the identification of these species. Therefore, the morphological study was designed to provide a full morphological description of this important species. The morphometric analysis proved the variability of the relative growth between sexes suggesting that length-weight relationships could be used as important criteria for selective breeding programs. For molecular identification DNA was extracted from the samples to provide genetic barcodes of this species using polymerase chain reaction (PCR)-amplification of 16SrRNA and cytochrome oxidase subunit 1 (COI) genes. The results clearly showed that, the Egyptian *M. latisulcatus* species is genetically closely related to all *Melicertus* species, while both barcoding and phylogeny of this species resulted in clear species discrimination. Furthermore, the samples identified in the current study seem to belong to a single origin, differing from those from other geographical locations where the species was found. Overall, this study suggested a valuable tool for assessment of prawn populations at both fisheries and genetic levels, providing a tool for management as well as for the optimization of its aquaculture.

INTRODUCTION

Penaeid shrimps and prawns have a worldwide distribution in both the tropics and the subtropics regions with a global catch of 3.4 million tonnes/year, with more than half of it is originated from both Western and Northwest Central Pacific and other essential fisheries are in the Indian Ocean and in the Western Atlantic Ocean (FAO, 2014). However, the greatest differences of the Penaeidae's family are the preferred habitats of their different stages; either they are predominantly estuarine in/offshore, demersal or pelagic (Dall *et al.*, 1990). Such benthic prawn prefers a sandy to seagrass or vegetated habitats (Tanner and Deakin, 2001).

Prawn juveniles and adults show a strong diel behavioral pattern of daytime burial and nocturnal activity (Primavera and Lebata, 2000), where higher catchability is greater during the lunar cycle as well as warmer months.

Moreover, the abundance of it within the waterbody is influenced by salinity, where more abundance is associated with salinities above 30 psu (Dixon *et al.*, 2011).

The western king prawn was previously classified as *Penaeus latisulcatus*, kishinouye (1896). In 1997, Pérez Farfante and Kensley suggested a taxonomic reduction using the former subgenera in the genus *Penaeus* to a generic rank; replacing *Penaeus* with *Melicertus*. In 2007, Flegel refined the taxonomic name, claiming confusion raised using Pérez Farfante and Kensley's taxonomic revisions, and proposed to include a statement in brackets after the new binomial the first time it is mentioned as *Penaeus (Melicertus) latisulcatus*.

The Egyptian Red Sea includes two Gulfs (Suez and Aqaba) and the intervening Sinai Peninsula with a total length of about 1500 km. According to the General Authority of Fish Resource Development (GAFRD, 2014), the annual Egyptian commercial fisheries and aquaculture production of shrimp and prawn are 21804 tonnes distributed as 7235, 4562, 8061 and 1946 tonnes from aquaculture, Lakes, Mediterranean and Red Seas, respectively.

Basically, adult prawns exploit the offshore during the breeding phase where drift and growth of larvae occur, while the settlement of postlarvae and their development to juvenile stage occur in the estuaries (inshore phase). Such deviation can be a cause of the conflict between postlarvae, juvenile. In addition, fishermen were claiming that their right in fishing areas is being breached. To make the matter worse, more pressure has been developed, that the immigrating postlarvae and adults are collected for sale to commercial prawn farms as a seed stock or even as a source of seed. The deviation is not recorded in the Red Sea species.

The Suez Gulf is one of the most productive wild fishing areas in the Egyptian Red Sea. It contains a huge variety of fish, molluscs and crustaceans of its commercial catch, prawn and shrimp are the most important commercial species representing about 7% of the total trawl landings (El-Ganainy and Yassien, 2012). Based on shrimp and prawn commercial sizes, they are categorized by fishers to small (*Trachysalambria curvirostris* and *Metapenaeopsis stridulans*) and large categories (*Melicertus latisulcatus*, *Penaeus semisulcatus*, and *Marsupenaeus japonicas*).

Melicertus latisulcatus, kishinouye, 1896 (formerly *Penaeus (Melicertus) latisulcatus*) is the most economically important prawns in both wild fisheries and aquaculture; due to their larger size compared with other shrimp and prawn species in the Gulf of Suez. Furthermore, it is a dominant and important species in the Egyptian Red Sea production. Despite their economic importance in Egypt, there are no studies about its molecular identification as well as the morphological characters. Furthermore, a survey of the literature shows that only a few studies have been conducted concerning its morphometry in Egypt (Yasin, 1992; Zaglol, 1995; Abdel-Razek *et al.*, 1996; El-Ganainy and Yassien, 2012) as well as in different regions around the world (Penn and Hall, 1974; Ivanov and Krylov, 1980; Farmer, 1986; Potter *et al.*, 1991; Kangas, 1999; Carrick, 2003; Sundaramoorthy *et al.*, 2013).

DNA barcoding is a technique that was originally set to assign unknown samples to certain species and to enhance the discovery of new species. It mainly relies on the retrieval of a standard short inter-specifically hypervariable sequence of the genome, the barcode, and comparing this to a database of known reference sequences of known identity (Hajibabaei *et al.*, 2007). Until now, the 5' region of the

mitochondrial cytochrome oxidase subunit 1 (CO1) gene is the universal standard barcode for animals (Hajibabaei *et al.*, 2007). Therefore, DNA barcoding was successfully used in resolving several issues related to aquatic and terrestrial animals discrimination. The concept of DNA barcoding resulted in significant advances in complicated issues related to the taxonomy of the species, mainly biodiversity description in the given regions; characterization of cryptic species; elucidation a similar phenotypic species and life history polymorphism (Tavares *et al.*, 2017). Genetic identification of decapods, especially when coupled to the morphological description, is gaining wide popularity; due to the morphological similarity in this group, besides its economic importance (Sharawy *et al.*, 2017). Our team members are directly involved in both morphological and genetic description, and authentication of the Egyptian decapod species that exist within the Egyptian territories, being either autochthonous or allochthonous species (e.g. Abbas *et al.*, 2016; Sharawy *et al.*, 2017). Again, as *M. latisulcatus* is the most economically important species for the Egyptian capture fisheries in the Suez Gulf, it has been of a prime importance to develop both molecular and morphological tools for its exploration and authentication. In the current study, *M. latisulcatus* was subjected to DNA barcoding through isolation, polymerase chain reaction (PCR) amplification and sequencing of the 5 hypervariable barcode region of CO1 gene, alongside with conducting various morphological measurements over it, in order to verify and authenticate it as a major member of the Egyptian economic shrimps and prawn species.

MATERIALS AND METHODS

Sampling and biometric analyses:

From November 2014 to October 2015, a total of 2865 individuals (987 males and 1878 females) of *M. latisulcatus* were freshly monthly collected from the commercial catch of the Red Sea. Samples were immediately transferred to the National Institute of Oceanography and Fisheries laboratories, Suez, Egypt on ice boxes where the measurements were carried out.

To avoid bias due to different measuring tools, or due to human errors, and seeking more accuracy (Sharawy *et al.*, 2017), individual lengths were measured to the nearest 0.1 cm applying a Matlab routine (The Math works, Inc., Natick, MA) on the individual prawn snapshot that has been taken using a USB camera (Leica MEGA 0.1S) equipped with a DC VARIO-ELMARIT 1:2.8-4916.3-25.2 ASPH lens. The snapshots were stored for further measurements or for double check whenever needed. However, only prawns with complete rostrum have been manually straightened on millimeter paper to avoid the natural curvature of prawn body. The measurements were defined as: a) total length (TL, cm); from tip of the rostrum to the end of the telson, b) carapace length (CL, cm); distance from the posterior margin of orbit to the posterior edge of the carapace, c) carapace width (CW, cm), greatest width of the point of last dorsal rostral tooth and d) the wet weight (Wt, g) that was weighed by electronic digital balance (Sartorius \pm 0.01 g).

The external morphological characteristics of prawns; the carapace and its spines, the rostrum together with its dorsal and ventral teeth, the ridges, the grooves, telson, appendages and their segments, and the different sexual characteristics, were identified. Thereafter, they were compared to those in the literature; in order to confirm the identification (e.g. Pérez Farfante and Kensley, 1997; Nizinski, 2003).

The Following equation was used to establish the length-weight relationships: $Wt = a TL^b$, where TL is the total length in cm and Wt is the total body weight (wet

weight) in g, while *a* and *b* are constants. Meanwhile, the length-weight parameters were estimated by fitting a nonlinear model while the length-length parameters were estimated by fitting a linear model in PASW statistics 18 software package.

DNA extraction and PCR amplification:

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 0.5 mg mL⁻¹ proteinase K. The homogenates were incubated for one hour at 50 °C. The genomic DNA was then extracted from each sample as described in (Sambrook *et al.*, 1989) using the conventional phenol-chloroform procedure. The resulting DNA was dissolved in TE buffer (100 mM Tris-HCl, 10 mM EDTA, pH 8). DNA was then stored at 4°C and its concentration was estimated by NanoDrop (Biodrop, England).

The partial coding regions of cytochrome oxidase subunit 1 (COI) gene were amplified by PCR (Veriti Thermal cycler, Applied Biosystems, USA) using the set of primers, described by Folmer *et al.* (1994), which 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 MyTaqTM HS Red Mix (Bioline, London, UK), 0.4 µM each of primer, in a total volume of 25 µL. However, the PCR was initially denatured for 5 min at 94°C, and then by 15 cycles of denaturation for 1 min at 94°C annealing for 1 min at 40°C, extension for 1 min at 72°C, and 25 cycles of denaturation for 1 min at 94°C annealing for 1 min at 45°C, extension for 1 min at 72°C, and a final elongation at 72°C for 7 min in a GeneAmp PCR System 9700 (Applied Biosystems, California, USA).

The 16S rRNA gene partial fragments were amplified using the 16Sbr universal primer (5'-CCGGTCTGAACTCAGATCACGT-3'), and 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') set. The amplification (25 µl) contained 1 µl DNA and 12.5 µl PCR-Master Mix, 0.4 µM each of primer. The PCR amplification consisted of an initial denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min, extension at 72°C for 1.30 min, and a final extension at 72°C for 7 min.

The amplified products were visualized using 1.4 % 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 Isolate II PCR and Gel Extraction Kit (Bioline, London, UK). The purified DNA fragments were subjected to sequencing, which was performed using the Big Dye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems) and ABI3730 Sequencer (Applied Biosystems, California, USA). The sequencing PCR reaction was performed at 96°C for 2 min, followed by 25 cycles of 10 sec at 96°C, 5 sec at 50°C and 4 minutes at 60°C.

DNA sequencing and phylogenetic analysis:

The resulting raw sequences of COI and 16S rRNA genes were edited by the Chromas Lite software version 2.1 (Technelysium- Pty Ltd, available from the URL <http://technelysium.com.au/>). The partial coding sequences for COI gene and/or the 16SrRNA sequences of the Egyptian samples of *M. latisulctus* were compared to the GenBank database using BLAST algorithm. COI sequences for *M. latisulctus* and other species of the genus *Melicertus* that are available in the GenBank database were retrieved from GenBank database and aligned using Clustal W software (Thompson *et al.*, 1994) included in the MEGA6 software package (Tamura *et al.*, 2013). These sequences came from *Melicertus latisulcatus*, *M. canaliculatus*, *M. kerthurus*, *M. plebejus*, *M. hathor*, *M. margiatus*, *Farfantepenaeus aztecus*, *F. brasiliensis*, *F.*

paulensis, *Fenneropenaeus indicus*, *F. merguensis*, *F. penicillatus*, *Hymenopenaeus debilis*, *Litopenaeus vannamei*, and *Marsupenaeus japonicus*. The aligned sequences were either used for calculation of pairwise distances or saved as fasta format. Then, the fasta file was exported to JModel-Test software V. 2.1.10 for finding the fit nucleotide substitution model to construct the maximum likelihood phylogenetic tree. Then, the fasta file was uploaded to Beauti software V. 1.8.3. To determine the substitution model, the JModel-Test was used and Markov chains (10.000.000) were selected. This number of Markov chains was used once and the program ran again twice with 50.000.000, and 100.000.000 chains. To estimate the tree topologies, BEAST software V. 1.8.3 was used with the XMLfiles. The log files resulted from the three trials was uploaded to the program Tracer v1.6; to evaluate the quality of the results and the only accepted results were that above 200 Effective Sample Size (ESS). LogCombiner software V. 1.8.3 was used to combine the results from the trees, and then it was uploaded, to Tree Annotator software V. 1.8.3; in order to summarize the information that was retrieved from tree samples and created by BEAST. The FigTree software V. 1.4.2 was applied to obtain the final consensus tree.

RESULTS

Morphology of *M. latisulcatus*:

The body of *M. latisulcatus* has pink to reddish color in adults, while the abdominal segments have a short vertical dark bar on pleuron with 2 dark stripes on the right angle of the carapace too. The carapace is uniformly glabrous and has no longitudinal sutures with very distinct grooves and crests that bearing both gastro-frontal carina and hepatic crests. The rostrum is well developed, straight and slightly curved at the tip, and armed with 9-12 dorsal teeth and 1 ventral tooth. Its postrostral crest is well developed with a deep median groove through it, while the adrostral groove is extended to the posterior margin of the carapace, which is wider than the postrostral crest, while the posterior end of the gastro-frontal groove is visibly divided into two parts (Fig. 1).

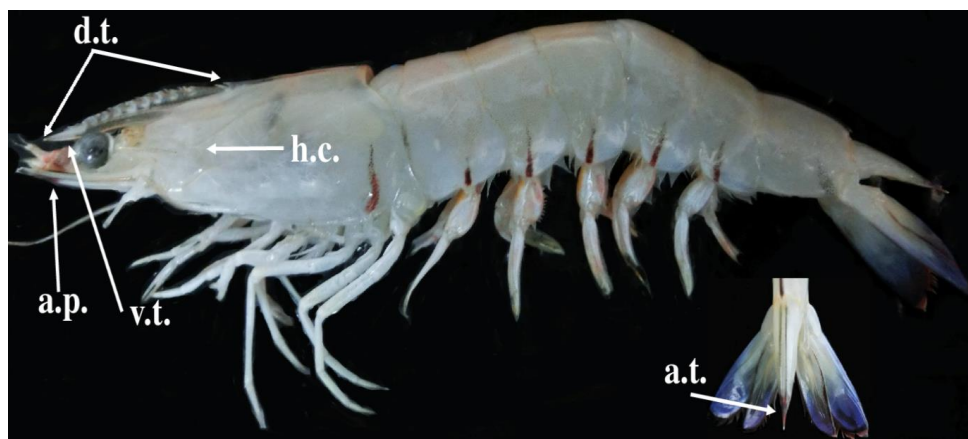


Fig. 1: Lateral view of western king prawn shows the antennal peduncle (a.p.), dorsal teeth (d.t.), ventral tooth (v.t.), and hepatic crest (h.c.) together with its dorsal view of armed telson (a.t.) and tail fan.

Prawns petasma has an involute pod-like structure, in which the dorsomedial lobules are united along midline, while the dorsolateral lobules are reaching, distally, the same level as ventromedial lobules below the tip of dorsomedial lobules. Prawn

males have subtriangular appendix masculine with a curved lateral surface and rounded apex.

The anterior portion of thelycum is consisting of well-calcified swelling with strongly branched apical projections into a pair of flattened round-tipped horns between the fourth pair of pereopods. The posterior portion contains two almost concave lateral plates that meeting along the midline, which forming the seminal receptacles. Overall, the abdomen is dorsally carinated from the posterior half of the fourth segment to the tip of sixth while the telson has 3 pairs of movable lateral spines (Fig. 2).



Fig. 2: Dorsal view of thelycum (left) shows the anterior process (a.p.), the posterior process and the lateral plates (l.p.), and petasma photographs (right) shows the distomedian projections (d.p.), the ventral costae (v.c.) and the lateral lobules (l.l.) of the western king prawn collected from the Gulf of Suez, Red Sea from November 2014 to October 2015.

Morphometric characteristics and Size distribution:

Specimens from all sampling dates were pooled by sex to determine relationships between lengths as well as length-weight relationships. The morphometric relationships equations (length-length relationships) computed for 987 males and 1878 females as:

Total length-carapace length (TL-CL) relationships

For males: $CL = 0.3477TL + 0.072 (\pm 0.003) (r^2 = 0.938)$

For females: $CL = 0.3643TL - 0.098 (\pm 0.007) (r^2 = 0.950)$

Carapace length-carapace width (CL-CW) relationships

For males: $CW = 0.2994CL + 0.025 (\pm 0.002) (r^2 = 0.793)$

For females: $CW = 0.3356CL - 0.112 (\pm 0.009) (r^2 = 0.829)$

While the relationships between the total weight (Wt) and both total length (TL) as well as carapace length (CL) are presented in Table (1). The TL-Wt nonlinear regression analysis for males and females showed that the slopes were significantly differed between sexes (Kolmogorov-Smirnov test, $p < 0.001$). Females were increasingly heavier than males with growing TL. Meanwhile the linear regressions of TL on CL were also significant for both sexes, total length scaled isometrically to CL in males and negatively in females. It shows that males body proportion change constantly but increased in females, i.e., in large females the CL (abdomen) were relatively shorter in comparison with the small ones.

Table 1: Published studies estimates of length-weight relationship equations of western king prawn *Melicertuslatisulcatus* (in chronology order).

Author	Equation	Region	Year
Penn and Hall, 1974	Male: $Wt = 0.000847 CL^{2.899}$ Females: $Wt = 0.000156 CL^{2.701}$	Shark Bay, Western Australia	-
Ivanov and Krylov 1980	Male: $Wt = 0.0000101 L^{2.96}$ ($r = 0.99, n = 11$) Females: $Wt = 0.000103 L^{2.53}$ ($r = 0.95, n = 37$) Sex combined: $Wt = 0.00189 L^{2.85}$ ($r = 0.96, n = 48$) Male: $Wt = 0.001612 L^{2.70}$ ($r = 0.972, n = 23$) Females: $Wt = 0.000998 L^{2.20}$ ($r = 0.95, n = 57$) Sex combined: $Wt = 0.00753 L^{2.28}$ ($r = 0.97, n = 80$)	Western Indian Ocean	Dec. 1975/ April 1976
Farmer 1986	Male: $Wt = \exp(0.00195 + 2.746 \log CL + e)$ Females: $Wt = \exp(0.00265 + 2.648 \log CL + e)$	Arabian Gulf	-
Potter et al 1991	Male: $Wt = 2.617 \log CL^{2.633}$ ($r^2 = 0.97, n = 1580$) Females: $Wt = 2.532 \log CL^{2.508}$ ($r^2 = 0.95, n = 1140$)	Pee-Harvey Estuary, south-western Australia	May 1985/ April 1988
Yassein 1992	Male: $Wt = 0.00448 TL^{3.193}$ ($r = 0.999, n = 1083$) Females: $Wt = 0.00375 TL^{3.275}$ ($r = 0.999, n = 1183$) Sex combined: $Wt = 0.00345 TL^{3.299}$ ($r = 0.96, n = 2266$)	Suez Gulf, Red Sea	Feb. 1984/Dec. 1986
Zaglol 1995	Male: $Wt = 0.167 CL^{2.782}$ ($r^2 = 0.999, n = 350$) Females: $Wt = 0.152 CL^{2.865}$ ($r^2 = 0.998, n = 378$)	Suez Gulf, Red Sea	1990/1991
Kangas (1999)	Juvenile: $Wt = 0.000662 CL^{2.908}$ ($n = 325$)	Port Wakefield, Gulf St Vincent	April 1997
Carrick (2003)	Male: $Wt = 0.000124 CL^{2.76}$ Females: $Wt = 0.000175 CL^{2.66}$	Spencer Gulf	-
El-Ganainy and Yassien (2012)	Sex combined: $Wt = 0.2615 CL^{2.6875}$	Suez Gulf, Red Sea	-
Sundaramoorthy et al 2013	Sex combined: $Wt = 0.00418 TL^{3.003}$	Thoothukudi coast, Southeast India	April 2006/March 2008
Recent study	Male: $Wt = 0.0044 TL^{3.14}$ ($r^2 = 0.956, n = 987$) Females: $Wt = 0.0038 TL^{3.21}$ ($r^2 = 0.975, n = 1878$) Sex combined: $Wt = 0.0039 TL^{3.20}$ ($r^2 = 0.969, n = 2865$) Male: $Wt = 0.134 CL^{3.06}$ ($r^2 = 0.952$) Females: $Wt = 0.135 CL^{3.05}$ ($r^2 = 0.941$) Sex combined: $Wt = 0.132 CL^{3.063}$ ($r^2 = 0.969$)	Suez Gulf, Red Sea	Nov. 2014/Oct. 2015

Furthermore, the ratio between CW:TL was plotted on TL to investigate the deference's in body proportion between males and females. Males and females grew constantly (same trends) up to 10.3 cm TL (= 3.9 cm CL) (Fig.3). In contrast, the ratio has been decreased in males while it increased constantly in females where the abdomen suddenly broadened, indicating amorphological shift between immature and mature females.

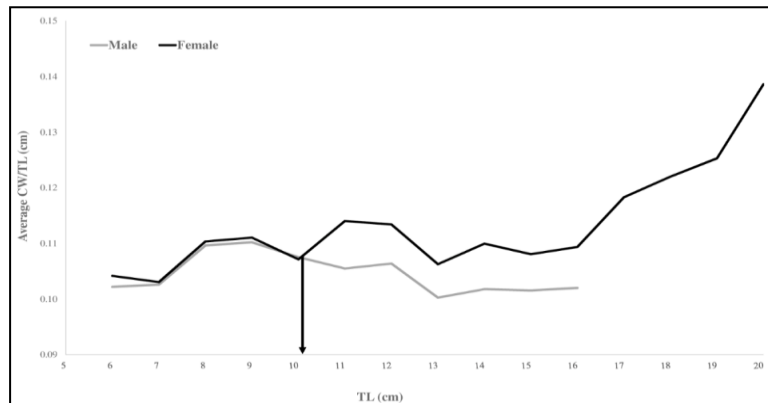


Fig. 3: Average Carapace width divided by total length (CW/TL) on total length (TL) for males and females western king prawn collected from the Seuz Gulf, Red Sea from November 2014 to October 2015.

Overall, a total number of individuals analyzed for this study was 2865 individuals, females dominated the catch with 65.6% (1878 individuals) while 34.4% were males with 987 individuals (1.9:1 ratio). Males were on average smaller (TL ranged from 6.3cm to 17.0cm) than females (TL ranged from 7.3cm to 20.2cm), the mean TL was 13.4 ± 1.7 cm (\pm sd) and 14.7 ± 2.8 cm (\pm sd) for males and females, respectively. The mean TL of females was 3.2cm longer than that of males. Males and females of the smallest size class (6.0-11 cm) have been observed only during the cold seasons, especially during the winter.

The overall mean weight (Wt) of females were 14 – 20% heavier than that of male prawns; the mean Wt was 16.6 ± 2.3 g and 23.4 ± 3.1 g for male and female prawns, respectively. Minimum and maximum Wt were 1.2g, 40.7 g, 2.3 g and 68.3 g for males and female prawns, respectively.

Molecular identification:

The COI sequences of the Egyptian Red Sea *M. latisulcatus* were deposited in GenBank/EMBL/DDBJ databases with accession numbers LC189472.1, LC189473.1, and LC189476.1-LC189481.1. This was carried out according to the morphological identification, BLAST comparisons for the obtained sequences with their counterparts in the GenBank database, and the phylogenetic tree's results, as well be mentioned. No COI sequences for COI barcode region were found in the GenBank database. The present sequences showed 99% similarity with *M. hathor* (acc. no. KU950454.1). Much lower similarities were found with other species of family Penaeidae, including, for example, 86 %-88% with *Marsupenaeus japonicas* (acc. no. KC409392.1, KP297916.1); 85 % with *Fenneropenaeus merguensis* (acc. no.KJ879290.1), and 85 % with *Penaeus indicus* (acc. no.AF284431.1).

The 16SrRNA sequences were deposited in the same databases under the accession numbers LC198474.1, LC198475.1, and LC202982.1-LC202986.1. Egyptian *M. latisulcatus* samples showed 100 % similarity with *M. Hathor* (acc. no. KU950453.1, JF899806.1), 99 % with *M. latisulcatus* (acc. no.LC189475.1), and lower similarities with other penaeid species, like 94 % with *M. plebejus* (acc. no.AF279822.1) and 93 % with *Penaeusmonodon* (acc. no.HQ127457.1).

Phylogenetic analysis for both 16SrRNA and COI genes exhibited a close proximity among all *Melicertus* species, especially for Egyptian *M. latisulcatus* presented in the current study and *M. hathor*, being the two species located in the same subclade in the phylogenetic trees of both analyzed genes. Interestingly, 16SrRNA gene sequences of some *M. latisulcatus* collected from Egypt and Australia in other studies than the current one (acc. no. KU324868.1 and KR153528.1) were located in a single subclade with another Indo-West Pacific species, *M. plebejus*. In addition, a sample of *M. latisulcatus* collected from Hong Kong (acc. no. AF279821.1) and Egypt (acc. no. KU324867.1) in other studies than the current one were grouped together in a single subclade. Both of these subclades differed from the subclade, which *M. latisulcatus* morphologically verified in the current study (Figs. 4 and 5).

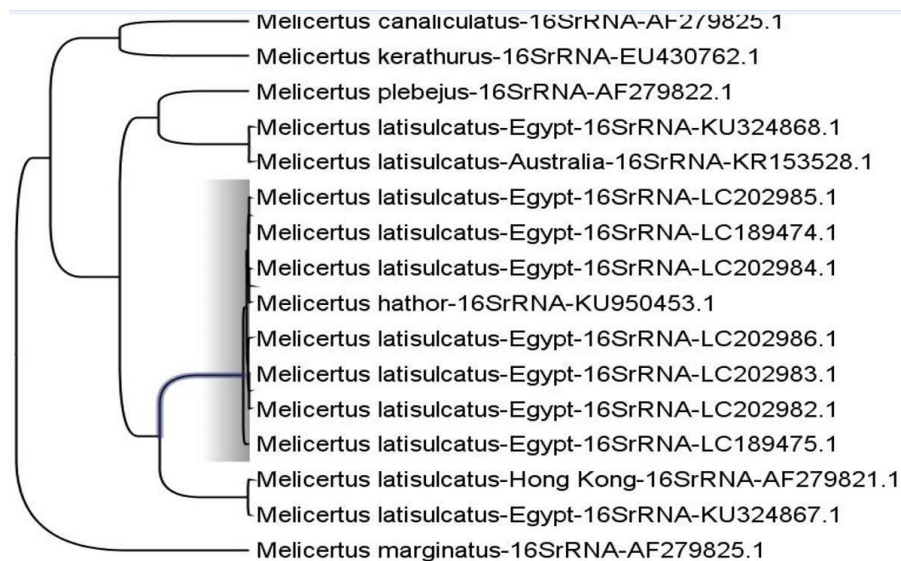


Fig. 4: Maximum likelihood Phylogenetic tree for 16SrRNA gene sequences of *M. latisulcatus* collected from the Red Sea in the current study and others retrieved from the GenBank database for the same species and others from the same genus but different species.

Table 3: 16SrRNA gene-based pairwise distances among Egyptian and international samples of *M. latisulcatus* and other shrimp species.

	<i>T. curvirostris</i> -Egypt-LC150891.1	<i>M. kerathurus</i> -EU430762.1	<i>M. latisulcatus</i> -Egypt-KU324868.1	<i>M. latisulcatus</i> -Egypt-KU324867.1	<i>M. hathor</i> -KU950453.1	<i>M. latisulcatus</i> -Egypt-LC202986.1	<i>M. latisulcatus</i> -Egypt-LC202985.1	<i>M. latisulcatus</i> -Egypt-LC202984.1	<i>M. latisulcatus</i> -Egypt-LC202983.1	<i>M. latisulcatus</i> -Egypt-LC202982.1	<i>M. latisulcatus</i> -Egypt-LC189474.1	<i>M. latisulcatus</i> -Egypt-LC189475.1	<i>M. canaliculatus</i> -AF279825.1	<i>M. marginatus</i> -AF279825.1	<i>M. plebejus</i> -AF279822.1	<i>M. latisulcatus</i> -Australia-KR153528.1	<i>M. latisulcatus</i> -Hong Kong-AF279821.1
<i>T. curvirostris</i> -Egypt-LC150891.1																	
<i>M. kerathurus</i> -EU430762.1	0.158																
<i>M. latisulcatus</i> -Egypt-KU324868.1	0.187	0.106															
<i>M. latisulcatus</i> -Egypt-KU324867.1	0.165	0.092	0.070														
<i>M. hathor</i> -KU950453.1	0.158	0.088	0.081	0.066													
<i>M. latisulcatus</i> -Egypt-LC202986.1	0.158	0.088	0.081	0.066	0.000												
<i>M. latisulcatus</i> -Egypt-LC202985.1	0.158	0.088	0.081	0.066	0.000	0.000											
<i>M. latisulcatus</i> -Egypt-LC202984.1	0.158	0.088	0.081	0.066	0.000	0.000	0.000										
<i>M. latisulcatus</i> -Egypt-LC202983.1	0.158	0.088	0.081	0.066	0.000	0.000	0.000	0.000									
<i>M. latisulcatus</i> -Egypt-LC202982.1	0.158	0.088	0.081	0.066	0.000	0.000	0.000	0.000	0.000								
<i>M. latisulcatus</i> -Egypt-LC189474.1	0.158	0.084	0.084	0.062	0.004	0.004	0.004	0.004	0.004	0.004							
<i>M. latisulcatus</i> -Egypt-LC189475.1	0.158	0.088	0.081	0.066	0.000	0.000	0.000	0.000	0.000	0.000	0.004						
<i>M. canaliculatus</i> -AF279825.1	0.168	0.062	0.088	0.077	0.088	0.088	0.088	0.088	0.088	0.088	0.084	0.088					
<i>M. marginatus</i> -AF279825.1	0.183	0.106	0.103	0.106	0.106	0.106	0.106	0.106	0.106	0.106	0.103	0.106	0.092				
<i>M. plebejus</i> -AF279822.1	0.165	0.077	0.055	0.044	0.059	0.059	0.059	0.059	0.059	0.059	0.055	0.059	0.070	0.095			
<i>M. latisulcatus</i> -Australia-KR153528.1	0.187	0.106	0.000	0.070	0.081	0.081	0.081	0.081	0.081	0.081	0.084	0.081	0.088	0.103	0.055		
<i>M. latisulcatus</i> -Hong Kong-AF279821.1	0.165	0.092	0.070	0.000	0.066	0.066	0.066	0.066	0.066	0.066	0.062	0.066	0.077	0.106	0.044	0.070	

For the first time in Egypt and the world, we provided a full morphological description and DNA barcodes for the western king prawn *M. latisulcatus*. Females *M. latisulcatus* collected from the Suez Gulf were larger and heavier, with the wider abdomen, than males sampled during 2014/2015. In the present study, the recurrent allometric growth for prawns (*M. latisulcatus*) were in agreement with previous studies in the Gulf of Suez (Yassein, 1992; Zaglol, 1995) where females are heavier (higher b values) than males with increasing total length. In contrast, smaller b values in males were reported for *M. latisulcatus* in Australia (Penn and Hall, 1974; Potter *et al.*, 1991; Kangas, 1999; Carrick, 2003), Western Indian Ocean (IvanovandKryloy, 1980), Arabian Gulf (Farmer, 1986). Such different morphometric equations were reported for a number of shrimps and prawns species (Sharawy *et al.*, 2016 and 2017), which clearly indicate the occurrence of ontogenetic sexual dimorphism of *M. latisulcatus* in the present study. Furthermore, it has been suggested that the morphometric differences are due to different growth (in size) and metabolism between males and females, especially during the sexual maturation (Accioly *et al.*, 2013). Moreover, Accioly *et al.* (2013) stated that such allometry of the sexual characteristics is an example of the interspecific heterochrony identification.

The present results of length-weight relationships (TL-WT and CL-Wt) of males and females revealed that females started to be heavier than males from 10.3 cm and more. At the same total length, females start to develop a wider carapace, while the males show the opposite pattern. Previous morphometric relationships showed only a general increase in the size of females when changing from immature to sexual maturity stages. Such obvious size increase in the carapace width/total length proportions (Fig. 3) is actually related to sexual maturity, unfortunately, can only be verified by gonad histology. However, these lengths (10.3 cm TL = 3.8 cm CL) are almost identical to the length of *M. latisulcatus*, in which 50% of all females were mature, that estimated in the Spencer Gulf (CL₅₀ = 3.65 cm, Carrick, 2003), the Queensland's East Coast (CL₅₀ = 3.4 cm, Courtney and Dredge, 1988) and

the Suez Gulf ($TL_{50} = 10.8$ cm, Yassien, 1992). While small ripe females were recorded by Penn (1980) in the Western Australian population ($CL_{50} = 2.9$ cm). In fact, a similar pattern occurs for a number of commercially important penaeid species (Accioly *et al.*, 2013; Sharawy *et al.*, 2016 and 2017).

In the present work, the recorded ratio deviation toward females (1.9:1) is common in penaeid species (see Sharawy *et al.*, 2016 and 2017), which may be attributed to the differences in life cycle, reproductive patterns, migration, mortality, nets selectivity and the aforementioned growth rates.

Barcoding of species belonging to the crustacean genus *Melicertus* is still very poor (e.g. Keskin and Atar, 2013). Genus *Melicertus* encompasses nine species, namely: *M. canaliculatus*, *M. hathor*, *M. kerathurus*, *M. latisulcatus*, *M. longistylus*, *M. marginatus*, *M. plebejus*, *M. similis* and *M. tigrinus*. Recently, barcoding coupled to morphological clues for authentication of species belonging to this genus is completely lacking. Therefore, this study, to the best of our knowledge, is the first to provide both morphological and genetic markers for identification of *M. latisulcatus*, and also for identification of the genus *Melicertus* in the world. It is worth to mention that, not only COI was proven as a good marker for discrimination of crustacean species (Sharawy *et al.*, 2017) but also 16SrRNA gene has been suggested as a better marker especially for higher crustacean species. Therefore, we used both markers; in order to provide adequate molecular clues for *M. latisulcatus* identification.

Yet, the present phylogenetic trees showed two very interesting points: a) both 16 Sr RNA gene and COI-based phylogenetic trees placed *M. hathor* and *M. latisulcatus* in the same subclade. BLAST comparisons were completely in agreement with our phylogenetic data, showing about 99 % sequence identity among *M. latisulcatus* samples analyzed in the current study and *M. hathor* present in the GenBank database, and b) some Egyptian *M. latisulcatus* 16SrRNA gene sequences that we retrieved from the GenBank database were divergent from the samples we morphologically and genetically authenticated as *M. latisulcatus*. Both points may suggest some phenotypic plasticity of this species, and confirm the need for including both morphological and genetic descriptions for future identification and authentication of both species *M. latisulcatus* and *M. hathor*.

Penaeid shrimps and prawns are well-known as an active migratory species. As previously mentioned, they perform an inshore reproductive migration towards estuaries to reproduce, and their juveniles carry out an offshore migration towards the deep waters of the open sea. Moreover, some penaeids can migrate through very long distances. For example, an individual sample of *P. plebejus* was recaptured about 900 km away from the first site where they were initially recorded (Ruello, 1975). Likewise, *M. hathor* crossed the Suez Canal from its original habitats in the Red Sea, and even the nearest Mediterranean shores, and reached the Northern Mediterranean, where it was recorded in Turkey (Gokoglu and Kaya, 2008). Therefore, the widespread of the *M. latisulcatus* in the entire area of the Western Indo-Pacific and the Indian Ocean to the Red Sea and the Mediterranean Sea may be attributed to the active migratory capability of such species. Until now, there are not enough studies that cover the morphological/genetic variations in this species in a wide geographical range to give clear vision about its origin and expansion; possibly owing to the scarcity of genetic data. So far, this study could provide a step forward for the identification of spatial distribution patterns of this species, in addition to identifying its genetic diversity as tools for its management, economic uses, and conservation.

In Conclusion and for the first time, the present work verified the variability of the relative growth between *M. latisulcatus* sexes and estimated the size of sexual

maturity based on a morphometric analysis, which represents an important tool for the management of such commercial species. Moreover, genetic barcodes based on both 16SrRNA and COI genes were provided for the first time in the Red Sea area in Egypt. The phylogeny based on mitochondrial DNA sequences provides a plurality of phylogenetically instructive characters, even with the species under the study or within the genus *Melicertus*. On the other hand, one of the outcome results from the present study is adding more sequences to the GenBank database regarding the Egyptian marine invertebrates especially from the Gulf of Suez, Red Sea. Our results also suggested that length-weight relationships could be used as an important criterion for selective breeding programs. Furthermore, based on these results, subsequent studies will be able to estimate the sexual differentiation through early ontogenetic stage; in order to 1) separate females from males during culture, ensuring similar prawn size in tanks/ponds, and 2) boost a protocol to secure monosex (all females) stocks which have a significant impact on shrimp and prawn culture sector so far.

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ARABIC SUMMARY

الخصائص الجزيئية والبيومترية المميزه للجمبرى الملكى الغربى *Melicertus latisulcatus* فى مياه البحر الأحمر المصرى

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على الرغم من ان الجمبرى اللاتى او الجمبرى الملكى الغربى يعتبر من بين الأنواع غالية الثمن فى العالم، الا ان هناك نقص شديد فى الدراسات التى تتناول كل من الخصائص المورفولوجية والدلائل الجزيئية الجينية لتحديد هذا النوع. ولهذا فان الهدف الأساسى لهذه الدراسة هو الجمع بين الوصف المورفولوجى الكامل والتأكد والتعريف الجينى لهذا النوع الهام من الجمبرى الذى يفتقر لمثل هذا النوع من الدراسات. وقد اثبتت التحاليل المورفومترية تباين النمو النسبى بين الجنسين مما يشير الى أن علاقة الطول بالوزن يمكن ان تستخدم كمعايير هامة لبرامج التربية الانتخابية. فى هذه الدراسة تم عزل الحمض النووى من العينات لمعرفة الباركودينج الوراثى لهذا النوع عن طريق استخدام تفاعل البوليمر المتسلسل للجينين الميتوكوندريال 16S rRNA والسيتوكروم اوكسيداز واحد. واطهرت النتائج بوضوح ان النوع المصرى للجمبرى اللاتى ترتبط ارتباطا وثيقا وراثيا بجميع الأنواع من الجنس الميليسترس، فى حين أن كلا من الباركودينج والفيلوجينى ساهموا فى تمييز واضح لهذا النوع وتحديد وتأكيد جينيا. وبوجه عام فان هذه الدراسة تعتبر أداة قيمة لتقييم عشائر الجمبرى على مستوى مصايد الأسماك وعلاقتها بالوراثة، وكذلك توفير أداة للتحكم والإدارة وذلك للاستغلال الأمثل للأستزراع المائى.