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Descriptive analysis and molecular identification of the green tiger shrimp *Penaeus semisulcatus* (De Haan, 1844) in Suez Gulf, Red Sea

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Abstract
Extensive sampling was conducted to investigate both population dynamics and morphometric characteristics of *Penaeus semisulcatus* from Suez Gulf, Red Sea. Growth parameters, mortalities, exploitation rate and yield per recruit were estimated for males and females separately. The equilibrium constants of carapace length-weight relationships revealed an isometric growth for both sexes. Furthermore, the exploitation rate of females was higher than that estimated for males, indicating sex- and size-related over-fishing. In the present study, two genetic markers were used to molecularly identify *P. semisulcatus* from Suez Gulf; mitochondrial gene Cytochrome Oxidase subunit 1 (COI), and nuclear gene 18S rRNA. Our results showed that Egyptian *P. semisulcatus* appeared to cluster with the Arabian Gulf's species, while the genetic distance between them was short. This present study can be considered as the first investigation covering both morphometric and molecular tools together in the Gulf of Suez, Red Sea or even in the other Egyptian coasts.

Keywords: Molecular identification, morphometric characteristics, population dynamics, *Penaeus semisulcatus*, Suez Gulf, Red Sea

1. Introduction
Marine crustaceans, such as penaeidae shrimps, are very important world fisheries and aquaculture due to their high demand in the world's markets [1]. The annual global catch of shrimp species has been about 3.4 million tonnes and more than half of it is from the Western and Northwest Central Pacific [2]. Shrimp remains to be the largest single product in value terms, which accounts for about 15% of the internationally traded fishery products' total value in 2012. Moreover, shrimps are primarily produced in developing countries, and much of this production progressively expands in the international trade. However, as economic conditions develop in these countries, growing demand leads to high domestic consumption and therefore fewer exports. This high demand and subsequent overexploitation negatively impact the standing stocks of shrimp, which falls below the healthy standard levels. This requires regular monitoring, assessment and then management of each species within the population to keep track the effects of such exploitation on fisheries stock. Therefore, fundamental knowledge of stock composition is vital for effective management of fisheries in order to overcome or reduce such overutilization. In Egypt, very little is published to investigate how penaeidae shrimp stocks differ morphologically and genetically based on its spatial distribution.
Morphological identifications of crustaceans are very critical since it has different larval stages, gender, phenotypic plasticity [3]. It is also worth to mention that most researchers still rely on classical taxonomy for validating shrimp and prawn species, e.g. shrimp and most other crustaceans are able to change colour depending upon the sex, growth, background coloration and time of day due to chromatophores. Accordingly, the classification of most shrimps is sometimes unsuccessful and ambiguous, and therefore cannot be assigned to the correct species [3, 4]. Such problem can recently be avoided by using molecular identifications. Recently, molecular methods are expected to provide new and more precise means for generating phylogenetic distances among species. Molecular biomarkers are strong tools to identify the species' genetic structure and the evolutionary history of populations [5]. Recently, the application of mtDNA in fisheries assessment programmes has given satisfactory results [6]. The evolutionary history of different penaeidae species was resolved in prior studies.

Penaeidae species have molecularly identified to examine penaeidae phylogenetic by mitochondrial marker cytochrome oxidase subunit 1 gene (CO1), mitochondrial 16S ribosomal RNA (16S rRNA) and randomly amplified polymorphic DNA (RAPD) analysis [7-9, 4, 10]. In Egypt, the annual commercial fisheries production of shrimp is 14,569 tonnes [11]. The Red Sea participates by about 1,948 tonnes which stand for 4% of the total production [11]. In the Egyptian Red Sea fisheries, the shrimp species include *Penaeus semisulcatus*, *Melicertus latisulcatus*, *Marsupenaeus japonicus*, *Metapenaeopsis stridulans* and *Trachysalambria curvirostris* [12]. Fortunately, the habitat and description of penaeidae shrimp are well known in the Red Sea that includes a vital part of the fishing area illustrated [13]. It is well documented that almost all shrimp species have the same complex life cycles. They involve the demersal eggs that hatch into pelagic larvae which pass through a succession of larval stages before reaching their nursery ground. This leads to the development of two diverse types of shrimp fisheries; artisanal fisheries that object shrimps in deeply constricted appearance during the migration process from the near shore into the open sea, and the trawling fisheries in deep water.

Gulf of Suez is one of the most productive wild fishing areas in the Egyptian Red Sea. It contains a huge variety of fish, molluscs and crustaceans. Off its commercial catch, penaeidae shrimps are the most important commercial species which represent about 7% of the total trawl landings [12]. Based on their size, they can be categorized by fishermen into small (*T. curvirostris* and *M. stridulans*) and large categories (*M. latisulcatus, P. semisulcatus* and *M. japonicas*).

P. semisulcatus shrimp is the most economically important penaeidae shrimps in both wild fisheries and aquaculture due to their larger size compared with other shrimps. Despite their wide distribution and economic importance of the Red Sea, little is known about their molecular identifications (only one in GenBank) especially when combined with the morphological characters. However, several studies have been conducted concerning the biology and population structures of the green tiger shrimp *P. semisulcatus* in different regions around the world [14, 15, 12, 16, 17]. In the present investigation, morphological characters and population structure, including basic identification procedure using COI and nuclear 18S rRNA gene were used to validate shrimp species to give a descriptive characterization of *P. semisulcatus* shrimps. Therefore, and based on shrimp validation, population dynamics study was conducted to evaluate the stock status of *P. semisulcatus* shrimps in the Suez Gulf, Red sea over 12 months. This study is the first trial, at the best of our knowledge, to get a fundamental knowledge about the diversity of one of the most commercial penaeidae shrimp species in Egypt. Furthermore, size and growth parameters are significant advantages for economically required species, as well as for stock management of wild *P. semisulcatus.* They both can also help to select the appropriate stock for successful hatcheries and grow out productions for using in the field of aquaculture.

2. Material and Methods

2.1 Data collection

Over 12 months, a total of 1,166 individuals (about 100 individuals/month) of *P. semisulcatus* shrimp were freshly collected from the commercial catch centre (Al-Attaka) of Suez Gulf, the Red Sea from November, 2014 to October, 2015. The Gulf of Suez extends about 314 km between 28°45′N and 33°00′E coordinates with a maximum depth of 32 km and an average depth of 40 km. Samples were immediately transferred to the National Institute of Oceanography and Fisheries laboratories in Suez, Egypt on the ice where the measurements were carried out. For *DNA* samples, pieces of tail muscle were sliced and placed immediately in 99% ethanol for preservation until *DNA* further analysis.

2.2 Analysis of data

2.2.1 Population structure

To avoid bias due to different measuring tools or scientists and for more accurate procedures, individual lengths were measured to the nearest 0.1 cm applying a Matlab routine (The Math works, Inc., Natick, MA) on the individual shrimp snapshot that has been taken using a USB camera (Leica MEGA 0.I.S) equipped with a DC VARIO-ELMARIT 1:2.8-4916.3-25.2 ASPH lens. Furthermore, the snapshots can be easily stored for further measurements or double checked if needed. Individual shrimp with complete rostrum have been manually straightened on millimetre paper (to avoid the natural curvature of shrimp 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 and c) wet weight (Wt, g) was weighed by electronic digital balance (Sartorius ± 0.01 g).

2.2.2 Growth parameters, mortalities and exploitation rates

By applying ELEFAN1 program [18] with length frequencies inputs, asymptotic length (L_∞) and the values of growth co-efficient (*K*) were estimated for each sex separated using VBGF growth function [19]: $L_t = L_\infty [1\text{-}e^{-K(t-t_0)}]$ where L_t is *CL* at age (t), L_∞ is the asymptotic CL, K is the curvature value and t_0 is the age at theoretical CL is zero. Instantaneous rate of natural mortalities (M) was also estimated by Pauly's [20] empirical equation as: Log (M) = - 0.0066 - 0.279 Log (L_∞) + 0.6543 Log (K) + 0.4634 Log (T) where T is the water temperature. The instantaneous rate of total mortality (Z) was estimated using FiSAT II program. Hence, fishing mortalities (F) for both sexes can be calculated as: F=Z–M and then exploitation rates (E) were estimated by Gulland's [21] formula as: E=F/ Z. According to Pauly [18], the ascending left arm of the CL converted catch curve was used to examine the probability of capture of each length class. The modified Beverton and Holt [22] relative yield per recruit (Y/R) model by Pauly and Soriano [23] was used and then integrated into FiSAT II software to estimate the levels of exploitation that would give optimum yields. From the analysis, E_{max} (the exploitation rate giving maximum relative yield per recruit), $E_{0.1}$ (exploitation rate at which the marginal increase in Y/R is 10% of its value at E=0), and $E_{0.5}$ (the exploitation corresponding to 50% of the unexploited relative biomass per recruit), were also estimated (B/R). To establish CL-Wt relationships, Le Cren's [24] equation was used as: $Wt=aCL^b$ where CL is carapace length in cm, Wt is total body wet weight in g while a and b are constants.

2.2.3 DNA samples and analysis

The molecular biology work was done in the laboratory of Evolutionary Genomics, Graduate School of Science and Biology, Osaka Prefecture University. Samples of genomic DNA was extracted from shrimp muscles (two samples for

mitochondrial gene COI analysis and one sample for nuclear gene 18S ribosomal RNA) as described in Sambrook *et al.,* [25] and homogenized in the DNA isolation buffer TES [10 mM Tris-HCl (Wako, Japan), 140 mM NaCl (Wako, Japan), 25 mM EDTA (Bio-Rad), pH 7.8] containing 1% SDS-Wako, Japan and 0.5 mg mL^{-1} proteinase K (Biolabs, New England). The reaction mixtures were incubated for one hour at 50 °C. Total genomic DNA was then extracted using a conventional phenol-chloroform procedure, and then was recovered by standard precipitation with ethanol. The resulting DNA was dissolved in TE buffer (100mM Tris-HCl, 10mM EDTA, pH 8). The concentration of the extracted *DNA* was spectrophotometrically assessed (Eppendorf, Hamburg, Germany) and stored at 4 °C. The partial coding regions of mitochondrial gene (COI) were then amplified by PCR using a set of primers as: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 5'- TAAACTTCAGGGTGACCAAAAAATCA-3') [26]. The amplification reaction was set up with 20 ng of template DNA from each sample and was performed using the following mixture: 1X RBC SensiZyme®HotstartTaq Premix (RBC Bioscience, Taipei, Taiwan) and 0.4 µM each of primer (the total volume is 25 µl). This mixture 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). 18S rRNA partial fragment was amplified using pairs of primers, Ebi18S-F (CGCCTACAATGGCTATAACGGGTAAC), and Ebi18S-R (GGTTAGAACTAGGGCGGTATCTGATC), which were newly designed to target conserved regions at the 5′ and 3′ boundaries of the 18S rRNA gene. These conserved regions, separated by ~700 bp, were based on the aligned sequences for the family penaeidae available in GenBank database. The amplification (25 µl) contained 1 µl DNA and 12.5 µl PCR-Master Mix, 0.4 µM each of primer and used a cycling profile of 30 sec at 95 °C, 0.30 sec at 48 °C and 2 min at 74 °C for 35 cycles with an initial denaturing step at 95 °C for 5 min and a final extension step at 74 °C for 7 min. The products were then purified using HiYield Gel/PCR *DNA* Fragments Extraction Kit (RBC Bioscience, Taipei, Taiwan). The purified DNA fragments were subjected to sequencing. DNA sequencing was achieved using the Big Dye Terminator ver. 3.1 cycle Sequencing kit (Applied Biosystems, California, USA) 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 min at 60 °C.

The raw sequence data of COI (two sequences) and 18S rRNA (one sequence) were edited by a free software Chromas Lite version 2.1 (Technelysium Pty Ltd, available from the URL http://technelysium.com.au/). The partial coding sequences for two specimens of COI (658 bp) and one 18S rRNA (663 bp) of *P. semisulcatus* were deposited in GenBank/EMBL/DDBJ International databases with accession numbers LC155214, LC155215 and LC155213, respectively. The COI and 18S rRNA sequences for the *P. semisulcatus* were retrieved from the databases (KU324647, KM528140, KF613002, KF604893, KF604894, KR261587 and KR261588) and were aligned by Clustal W tool in MEGA7 software [27]. By running MEGA7, best fitting models were applied to the COI datasets of nucleotide composition and divergence values depending on General Time Reversible (GTR) model [28]. Phylogenetic trees were also reconstructed by MEGA7 using Maximum Parsimony (MP) method with 1000 replicates of bootstrapping.

3. Results

3.1 Population structure

The total number of individuals that have been analysed for this study was 1,166 individuals. Males were dominated with 55.6%, while females were 44.4%. Males were on average shorter than females CL by 1.8 cm. The mean CL was 5.3±0.6 cm (±SE) and 6.0±0.9 cm for male and female shrimps, respectively. The results are indicating significant difference ($p<0.001$) between both sexes. Overall mean Wt of female shrimps was about 50% heavier than that of male shrimps; the mean Wt was 21.3±6.4 g and 31.0±16.3 g for male and female shrimps, respectively. The minimum Wt for males and females were 2.5 g and 2.6 g respectively, while the maximum Wt for males was 44.8 g and was 90.1 g for females.

CL–Wt regression showed that females were increasingly heavier than males with growing CL (Fig. 1).

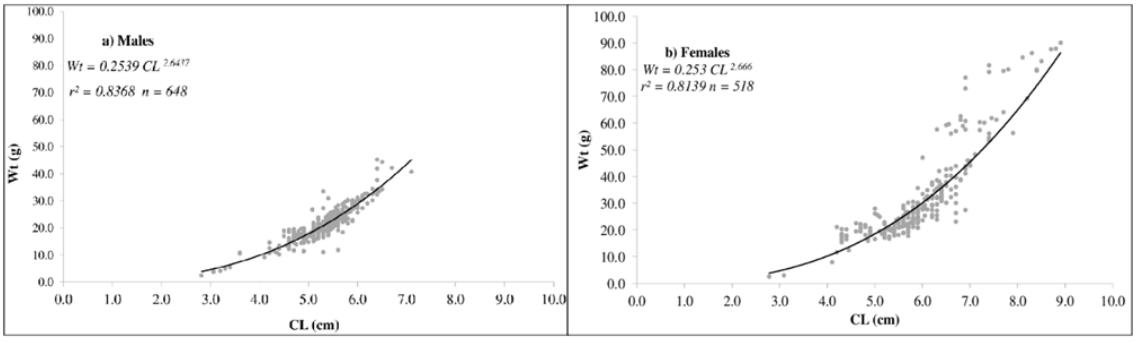

Fig 1: CL-Wt relationships for males (a) and females (b) of *P. semisulcatus*. Linear equation was estimated to describe TL–CL relationship for males and females separated and was TL = 0.1542+ 0.3467 (CL), $r^2 = 0.812$ for males while was TL = 0.231 + 0.347 (CL), $r^2 = 0.877$ for females.

3.2 Growth parameters, mortalities and exploitation rates

The growth parameter estimated using ELEFAN 1 were CL_∞ = 7.35 cm, K = 1.410 yr^{-1} and t_0 = - 0.887 for males and for females CL_∞ = 8.92 cm, K = 1.20 yr^{-1} and t_0 = - 0.685. VBGF in the present study were $Lt = 73.5 [1 - e^{-1.410 (t + 0.887)}]$ and $Lt = 89.2 [1 - e^{-1.20 (t + 0.685)}]$ for males and females respectively. The total mortality (Z) estimated was 7.755 yr^{-1} for male (Fig. 2a) and 8.840yr^{-1} for female (Fig. 2b), while the natural mortality (M) was 3.137 yr^{-1} and 2.678 yr^{-1} for males and females respectively.

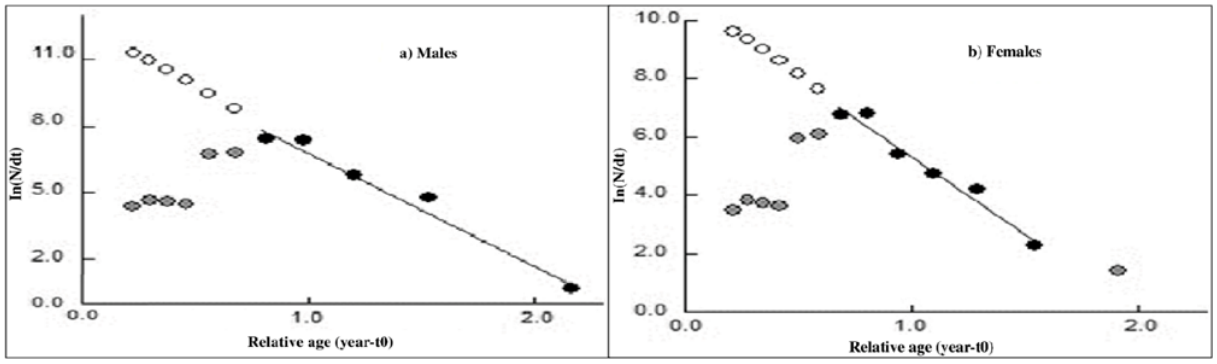

Fig 2: Probability of capture for males (a) and females (b) of *P. semisulcatus*. The darkened full dots represent the points used in calculating through least square liner regression while the open ones represent not fully recruited and or nearing to L

Fishing mortalities (F) also were estimated as 4.569 yr^{-1} and 6.1614 yr^{-1} for males and females respectively. Meanwhile, exploitation rates (E) were also estimated and recorded as 0.590 for males and 0.696 for Females. Length at first capture (L_C) at which 50% of shrimp are vulnerable to capture ($L_{50\%}$), was estimated as 3.47 cm and 4.38 cm for males and females respectively (Fig. 3).

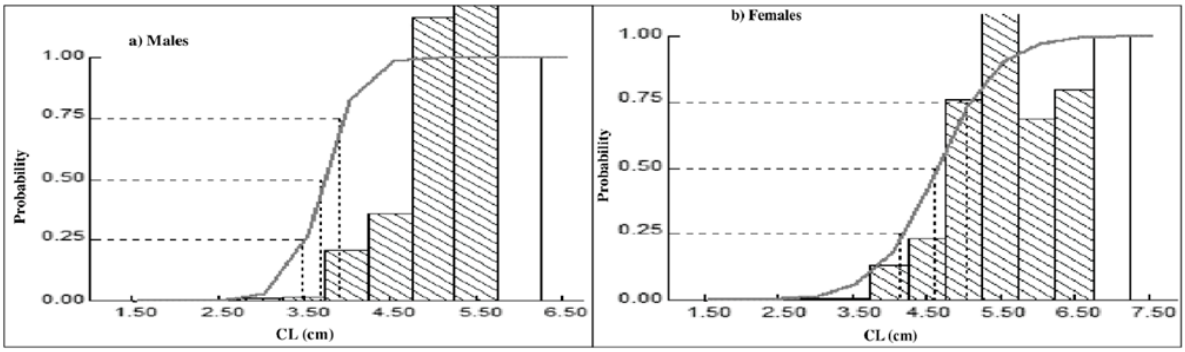

Fig 3: Probability of capture ($L_{50\%}$ or L_C) for males (a) and females (b) of *P. semisulcatus*.

Figure (4) expresses the relationships between the relative Y/R and the relative B/R of both males and females. It shows that the maximum allowable limit of exploitation level (E_{max}) was estimated at 0.878 for males and 0.894 for females. Meanwhile, the level of exploitation at which marginal increase in relative Y/R in 10% ($E_{0.1}$) of marginal increase computed at a very low value at E was 0.751 and 0.807 for males and females respectively. $E_{0.5}$, the exploitation level which corresponds to 50% of relative B/R of the unexploited stock was 0.414 and 0.417 for males and females green tiger shrimp respectively.

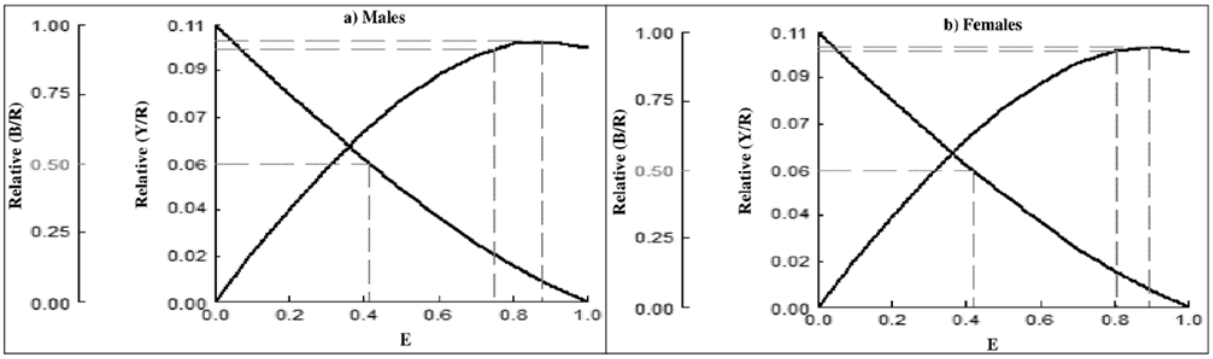

Fig 4: Relationship between the relative Y/R and relative B/R using knife-edge procedure for males (a) and females (b) of *P. semisulcatus*.

3.3 Molecular phylogenies

The final COI datasets were 585 bp-long in *P. semisulcatus* (excluding primers). Maximum Parsimony (MP) tree of the species we studied and those retrieved from databases are shown in Fig 5. The parsimony analysis of molecular data resulted in a single optimal tree with length 352, consistency index 0.93, composite index 0.94 and retention index 0.97. For the 585-nucleotide positions, parsimony-informative sites were 0.89. The top appropriate model of nucleotide substitution for COI was Hasegawa-Kishino-Yano model with gamma distribution with invariant sites (HKY+G+I). The MP tree showed two distinct clades, clade one for our individuals (LC155214 and LC155215) which are clustered with the individuals of *P. semisulcatus* from the Persian Gulf (KR261587 and KR261588). The other sequences from the same species are clustered to one main clade (Fig 5). Pairwise

genetic distances among the species in this study showed highest values (0.138 and 0.112) between the *P. semisulcatus* from the Egyptian Red sea, Philippines and Sri Lanka while the lowest genetic value was 0.002 from the Persian Gulf, Iran. The 18S rRNA partial gene for the species under the study was 663 bp in length and it confirmed the molecular identification of *P. semisulcatus*. The identity of the same species from the GenBank database was 99% within the same cover region. Although, the 18S rRNA sequences data of this species was very few (only four sequences including our sequence LC155213), it is the only one recorded in Egypt until now. Off the 663 characterises recorded, 53.75% was GC content, 46.2% was AT content and GC/AT was 1.16.

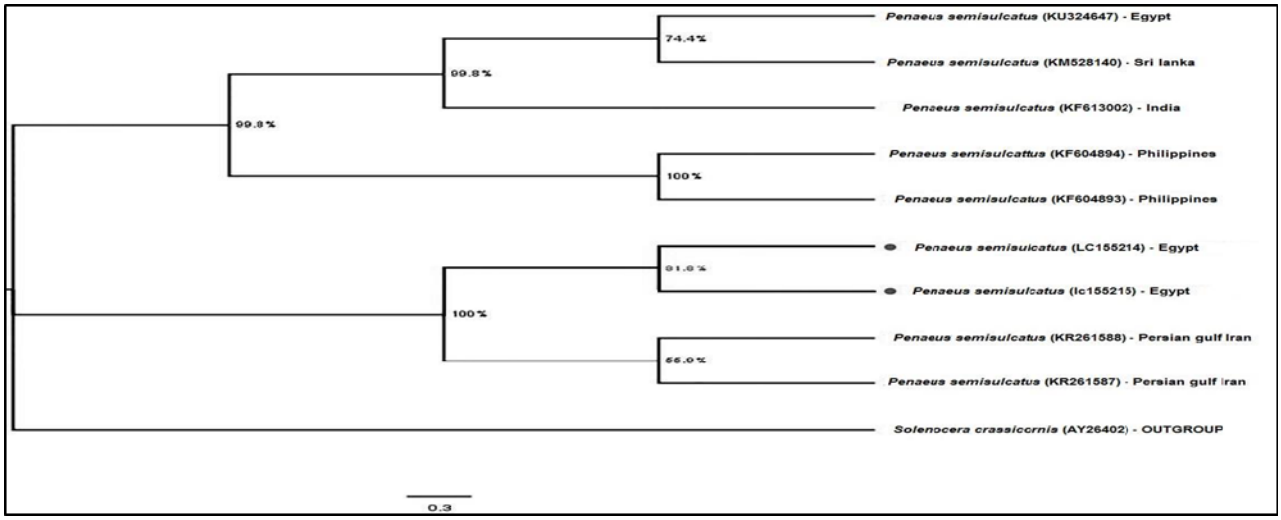

Fig 5: Maximum Parsimony (MP) phylogenetic tree of mtDNA-COI gene sequences of *P. semisulcatus* (bootstrap values of 1000 replicates are indicated next to the branches). LC155214 and LC155215 are accession numbers of sequences from present study. The other sequences were retrieved from GenBank with their accession numbers. *Solenocera crassicornis* -AY264902 was utilized as an out group. Asterisks represent the Egyptian green tiger shrimp.

4. Discussion

In the present work, monthly variations of length frequency were used to determine *P. semisulcatus* growth parameters, and then to estimate other population dynamic parameters. The results of morphometric measurements in the present study clearly showed that female shrimps reached both larger size and higher growth rate than males indicating sex-specific growth. This observed growth difference is common for many shrimps [29-33, 17]. The estimated values of growth parameters (L_∞ and K) of *P. semisulcatus* differ from the estimates of the species reported in other regions. As Abdul-Wahab [17] recorded both growth parameters (CL_∞ and K) and they were 44.65 mm and 1.2 y^{-1} for males, while were 58.8 mm and 1.4 y^{-1} for females respectively. Meanwhile, Mehanna *et al.,* [16] estimated higher values than pervious study. Such wide variations in the growth parameters estimated by different authors are due to different observed sizes, different age, sample size, and different environmental conditions. Based on the estimated values of L_∞ and K, Pauly's equation [20] commonly allows the estimation of natural mortality in marine organisms which can also be used in shrimps and other invertebrates [34]. However, the values of (M) in this study were 2.11 yr^{-1} for males and 2.39 yr^{-1} for females, which lie within the limits reported by Garcia and Le Reste [35]. Such high values estimated in the present work or even that recorded in previous studies most likely indicate that all World fisheries suffer from high fishing mortalities just like the case of Suez gulf. In addition, many natural factors interact to increase the natural mortalities, e.g., bad food condition due to competition with other predators [15]. On the other hand, the total mortality (Z) for females was slightly higher (Z) than that of males, while fishing mortalities (F) were higher than natural mortality (M) for both males and females. These high values of Z are comparable with other findings, e.g., values were 9.2 for males and 8.8 for females and were 6.7 for combined sexes in Kuwait [36], while they were lower in the Red Sea (8.18 for males and 6.77 for females) [31], and reached 3.61 and 5.65 for males and females in the Philippines [37]. In the present investigation, exploitation rate of males was better than that estimated for females. Such high exploitation rate of female shrimp suggests sex- and size-related overfishing for *P. semisulcatus* in the Gulf of Suez. Accordingly, females might have a higher susceptibility to fishing gear than males due to behavioural variances related to egg bearing. They may also occur in greater densities and/or have slower escape reactions when being fished. However, the values of *E* were slightly higher than the level suggested by Gulland [38]. In addition, the results of *Y/R* analysis support a previous finding that showed high levels of exploitation in the Gulf of Suez. Furthermore, our results were in agreement with other studies in different coastal areas i.e., in Bushehr at the Persian Gulf of Iran [15], in the Arabian Sea of Oman [16] and in Yemen [39]. Such over utilization may be due to using illegal nets, or illegal fishing of shrimp post-larvae or broodstock for aquaculture practice. Therefore, it is strongly recommended that such over-exploitation should be declined in order to maintain a sufficient biomass. Recently, the use of molecular tools gives an important highlights to improve our capability to recognize the genetic diversity of populations with different geographic distribution. Only a few studies have been published on the genetic difference of *P. semisulcatus* and they are limited in comparison to those done on other commercially essential species [40, 41, 9, 4]. In the present study, Egyptian individuals of *P. semisulcatus* were molecularly identified by using short sequences belonging to the mitochondrial gene COI and nuclear gene 18S rRNA. The COI partial coding gene appears to confirm the phylogenetic relationship of this species from Egypt and other locations.

We identified that the genetic distance of 0.2% was found between *P. semisulcatus* collected from Suez Gulf, Red Sea and the same species from the Persian Gulf. On the other hand, the genetic distances were 13.8% and 11.2% between the species collected from Egypt, Philippines, and Sri Lanka respectively. It is well known that *P. semisulcatus* is originally distributed from the Indo-Pacific region of the Red Sea as well as the Arabian Gulf [14], which might explain the higher range of similarity in spite of the natural geographical barrier between these two areas. These findings were supported by another sequence of this species from Egypt (accession number: KU324647) that was clustered with those recorded at the Indo-pacific Ocean (accession numbers: KM528140, KF613002). Furthermore, and based on 18S rRNA in the GenBank database for *P. semisulcatus* (accession numbers: KM486610, DQ079766 and AY315659), our data (accession number LC155213, the first one in the GenBank database for Egypt) suggested that the 18S rRNA sequences have discriminatory value mostly on higher taxonomic levels and confirmed the molecular taxonomy of the species.

5. Conclusion

In conclusion, the present study indicated that the molecular tool findings are consistent with the morphometric results. Hence, it could be demonstrated that morphometric characters together with phylogeny analysis can act as cooperative tools to confirm the phylogenetic relationship of these economically important species. On the other hand, the outcome of the present study has provided the fundamental information on morphometric relationships which is important for fisheries management of *P. semisulcatus* from the Gulf of Suez. It is also worth to mention that the present study has added more data to the GenBank database concerning the Egyptian marine invertebrate organisms. In undertaking this study, we had the advantages over other studies by: a) samples were collected over 12 months continuously (normally commercial fishing in Egypt is restricted during the spawning season of the most commercial fishes), b) aspect of accurate measurement of length has been standardized using digital images via a camera system which was specially established for the present work. Furthermore, it is considered as the first study addressing fisheries, morphometric and molecular tools together in the Gulf of Suez, Red Sea or even in the other Egyptian coasts for penaeidae species. Therefore, more investigations are needed to describe the population genetics and to examine the diversity of the genetic and the conservation situation of *P. semisulcatus* in the Egyptian coastal areas.

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