Phylogenetic relationship and systematic identification of different shrimp and prawn species in Egypt

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ARTICLE INFO

ABSTRACT

Among the Egyptian catch fisheries, marine shrimp species represent the most economically important invertebrate. Likewise, this species is distributed all over the world as a diverse one. The current study merged DNA barcoding as a valuable complementary tool with morphology-based species identification. Ten different maritime shrimp species were phenotypically delineated and identified, based on the structure of the carapace; the crest, groove, and spines, the rostrum with its teeth; dorsally and ventrally, sex determination; the shape of the petasma and thelycum, armed or unarmed telson, and the body colour. Phylogenetic relationships between ten Egyptian shrimp species were examined with the nucleotide sequence data stored in the GenBank database of partly region for the mitochondrial subunit I of cytochrome oxidase gene (COI). Most of the produced sequences had 99–100% similarity to conspecific sequences. Sequence assessment of the COI gene for the phylogenetic tree distinguished all ten shrimp species into three definite clades, which are genetically distinct from one another and demonstrated the same phylogenetic reservations to their respective genus. The percent GC content ranged between 41.4% in Metapenaeus monoceros and 36.6 % in Trachypenaeus curvirostris and Marsupenaeus japonicas. The highest genetic distance (0.3) was found between Palaemon serratus and Parapenaeus longirostris of Egypt, and Penaeus semisulcatus of India and Trachypenaeus curvirostris of Egypt. The lowest genetic distance (0.0) was observed between Parapenaeus longirostris of Egypt and Turkey, and Xiphopenaeus kroyeri of Egypt, Mexico, and the USA, overlapping each other. The current study clearly shows that DNA barcoding may be used for the differentiation between shrimp species, which will help researchers better understand the biology of evolution and conservation.

INTRODUCTION

Crustaceans thrive in a variety of environments including the ocean, brackish water, estuaries and freshwater; they exhibit a spectacular diversity of forms, habits and sizes. Amongst the variety of crustaceans, shrimps (Decapoda, Dall et al., 1990) are considered a source of global fisheries economy (Chanda, 2014). About 3000 shrimp and prawn species...
species were divided into four main categories (Penaeoidea, Caridea, Sergestoidea and Stenopodidea).

The superfamily Penaeoidea, with five families (Penaeidae, Solenoceridae, Aristeidae, Sergestidae, and Sicynoiiidae) with approximately 26 genera and 205 species, has the most commercial species (Sharawy et al., 2017). Identification of the crustacean species traditionally stands for morphological characteristics. Though, because of its wide variety and morphological similarities, shrimp can be difficult to identify through morphological characteristics alone in many cases (Hebert et al., 2003). Morphological features for crustacean species identification are sometimes ineffective and misleading for many reasons, such as the shrimp stages that can lead to incorrect species identification (Rajkumar et al., 2015), and the rough handling which causes damage to the shrimp species, giving ambiguous morphological identification (Rajkumar et al., 2015). In addition, shrimp species, like most of the other crustaceans, can change their colour during the molting process; thus, sometimes it is difficult to distinguish between the different species (CSIRO, 2013). However, molecular identification represented in DNA barcoding can overcome those problems. Yet, the molecular identification of the species correctness depends upon the reliability and whole reference database (Chang et al., 2017). Distinctive DNA markers have been utilized in various taxa. Bingpeng et al. (2018) evidenced the advantage of DNA barcoding technology compared to the traditional morphological classification where: (1) DNA barcoding can assist in correctly discriminating species that have highly similar morphological features. (2) Using DNA barcoding can differentiate the species at the level of various developmental stages. (3) The use of DNA barcoding technology might facilitate the discovery of cryptic species, with similar external morphologies. Additionally, molecular identification based on DNA technology can help identifying new invasive and translocated species (Wang & Qiao, 2009).

For species identification, the mitochondrial cytochrome c oxidase subunit I (COI) gene was used as a barcode sequence by Hebert et al. (2003). A set of primer DNA can amplify 648 base per (bp) of the COI gene (Folmer et al., 1994), which has a fast mutation rate and is considered perfect for differentiating between closely related species since its sequence is fixed among the conspecific species (Rajkumar et al., 2015). COI has been used as a mitochondrial genetic marker for the molecular identification of various crustacean species (Wilson & Sing, 2013; Rajkumar et al., 2015; Abbas et al., 2016; Karuppasamy et al., 2020). Some studies in Egypt were conducted using COI as a genetic marker for molecular identification, authentication and morphological characterization of the shrimp species (Sharawy et al., 2016; Sharawy et al., 2017; Abbas et al., 2018; Abbas et al., 2021). Hence, Egypt has coastlines on the two major seas, the Mediterranean and the Red Seas. Based on the importance of the Egyptian geographic location, it has been given much attention and interest for studying the species identification that can impact fisheries and biodiversity.

So far, still, morphological identification is used as a classic taxonomy in Egypt. The present study could be the first in Egypt, to the best of our knowledge, to give important information regarding the genetic identification (DNA barcoding) and morphological description conducted together on the shrimp and prawn species in Egypt. Moreover, the current study aimed to provide a better understanding of the origin and authentication of the species that exist in Egypt and determine the phylogenetic relationship between our species and the other related species. Consequently, this study would help researchers in monitoring and conserving shrimp diversity in Egypt.
Phylogenetic relationship and systematic identification of different shrimp species

MATERIALS AND METHODS

1. Samples collection and identification
   The ten shrimp species were collected freshly from the commercial catch of the west of the Mediterranean Sea and the Gulf of Suez, Red Sea in Egypt. Samples were directly preserved in iceboxes and transferred to the National Institute of Oceanography and Fisheries (Genetics Laboratory in Alexandria and Invertebrates Laboratory in Suez, Egypt). For molecular examination, a small part of muscle from each shrimp species was preserved in 99% ethanol until used for the next steps. The shrimp samples were examined morphologically and identified as follows: eight species from the family Penaeidae, *Penaeus kerathurus*, *Parapenaeus longirostris*, *Xiphopenaeus kroyeri*, *Metapenaeus Monoceros*, *Trachysalambria curvirostris*, *Penaeus semisulcatus*, *Penaeus japonicas*, *Melicertus latisulcatus*, one species of family Palaemonidae (*Palaemon serratus*), and one species of family Solenoceridae (*Solenocera crassicornis*). The morphological features of the ten shrimp and prawn species were characterized depending on some visible external characters i.e., the carapace shape with its crest, the spines and grooves, the rostrum with its dorsal and ventral margin teeth, the projections of thelycum of the female and petasma of the male and the distinct shape of telson and body colour. For accurate investigation and identification, the external morphological characterization was compared with standing publications (Pérez-Farfante & Kensley, 1997; Carpenter & Niem, 1998).

2. Molecular identification
   Precise muscle tissue from the collected samples (50-100 mg) was used for genomic DNA extraction according to Sambrook et al. (1989). TES buffer (10 mM Tris-HCl, 25 mM EDTA, 140 mM NaCl, pH 7.8) was added to homogenize the tissue, which includes 0.5 mg proteinase K and 1% SDS. Then, the homogenates were then incubated at 56°C for 2 hours. The genomic DNA was isolated and precipitated with 100% ethanol using a standard phenol-chloroform technique. TE buffer was used to elute the DNA pellets (100 mM Tris-HCl and 10 mM EDTA, pH 8). A nanodrop was used to assess the concentration of DNA samples using spectrophotometry (Biodrop, Cambridge, England). A 652 bp fragment of the mitochondrial gene cytochrome c oxidase subunit I (COI) was amplified via the PCR-Thermal Cycler for DNA barcoding of the shrimps (Applied Biosystems verity 96 wells, USA). The primer pair described in the study of Folmer et al. (1994), LCO1490 (5’-GGTCAACAAATCATAAAGATATTGG-3’) and HCO2198 (5’-TAAACTTCAGGGTGCCAAAAAATCA-3’), were used for the amplification of the COI gene. The amplification reaction was performed in a 25µl reaction volume containing 12.5µl of 1X MyTaq™ HS Red Mix (Bioline, London, UK), 20 ng of template DNA from each sample and 0.4µM of the primer. The PCR program consisted of 35 cycles of initial denaturation at 94°C for 5min, denaturation at 94°C for 30s, annealing at 45-50°C for 1.0min, extension at 72°C for 1.30min, and final steps of elongation at 72°C for 7min. The amplified product was electrophoresed at 130V using 1.4% agarose gel (100 mg/ml) stained with ethidium bromide. PCRs generated with targeted bands were purified using Isolate II PCR and Gel Kit (Bioline). Purified DNA fragments were sequenced using the BigDye Terminator cycle sequencer version 3.1 according to the method of Abbas et al. (2011) and applied to the ABI 3730 sequencing kit (both the kit and machine are applied biosystems). The condition of a sequencing PCR was performed as follows: 96°C for 2min, followed by 25 cycles at 96°C for 10s, at 50°C for 5s, and for 4 min at 60°C.
3. DNA sequencing data and analysis

COI gene sequences for the ten shrimp species were edited with Chromas Lite v2.1 software (Technelysium Pty Ltd., available at http://technelysium.com.au/) and read with the BioEdit 7.2.6.1 test tool. For nucleotides’ gaps and alignment, the Clustal W software was used. The sequence of the coding region of the COI gene portion of a shrimp species was compared to a reference sequence in the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for identity confirmation using BLAST search. Partial coding regions of the COI gene for the ten tested samples were submitted to the GenBank/EMBL/DDBJ database with the accession numbers (LC477199, LC477196, LC477193, LC477201, LC477205, LC175470, LC175472, LC155215, LC597232 and LC189473). COI sequences from other countries and related species were extracted from the GenBank database and sequenced with the Egyptian species. The percentage of total GC (% GC) and the base composition (%) of the COI gene among the ten shrimp species and retrieved sequences for the related species from different countries were also calculated using MEGA X software (Kumar et al., 2018). The FASTA format for the sequences was applied to MEGA X software to calculate the pairwise genetic distances for all the species. The Neighbour-Joining (NJ) tree was constructed based on the Kimura 2-parameter method (Kimura, 1980) with 1000 replicates of the bootstraps for the shrimp species.

RESULTS

1. Species identification

The morphological features of the studied shrimp and prawn species were represented in Table (1), based on the carapace’s shape; crest, groove and spines, and the rostrum's teeth; dorsally and ventrally, sex determination; the shape of petasma and thelycum, armed or unarmed telson and the body colour.

2. Molecular identification

COI sequences for the Egyptian shrimp species produced 652bp in length. All the sequences were simple and unambiguous. Eventually, there were no insertions, deletions, or stop codons in any of the sequences. Similarity search was analysed using BLAST to compare the obtained sequences with their counterparts and revealed high similarity (≤ 99%) with the compared sequences in the GenBank database, except for Trachypenaeus curvirostris (87%) and Melicertus latisulcatus (88%) sequence identity. Among the studied sequences for the ten shrimp species from Egypt and some related species from other countries, the GC percent fluctuated from 41.4% for Metapenaeus monoceros (Egypt) to 36.6% for Trachypenaeus curvirostris (India) and Marsupenaeus japonicas (China). The average nucleotide frequencies observed in the sequencing analysis were A = 27.01%, T = 34.23%, C = 20.66%, and G = 18.10 %. The COI gene's nucleotide substitution pattern is shown in Table (2).

The rate of substitution with the greatest rate was 18.58 (transitional substitution from G to A), while the rate with the lowest rate was 0.95 (transitional substitution from T to C). The “A + T” nucleotide frequencies were 61.24 %, whereas “C + G” nucleotide frequencies were 38.76 %. k1 = 2.07 (purines) and k2 = 0.14 (transversion rate ratios) are the transition/transversion rate ratios (pyrimidines).
Table 1. Identification of the studied penaeid shrimps and prawn species collected from the commercial catch of the Egyptian Mediterranean and Red Seas

<table>
<thead>
<tr>
<th>Species</th>
<th>Rostrum</th>
<th>Carapace</th>
<th>Sex determination</th>
<th>Telson</th>
<th>Body-color</th>
<th>Total length (max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. kerathurus</em></td>
<td>Dorsally: 8-13 teeth/ Ventrally: 1 tooth</td>
<td>Vast and chitinous carapace with 14 segments</td>
<td>Petasma: Ventrolateral lobule is strongly protruding distally into a conspicuous flap overreaching the ventromedial lobule. <strong>Thelycum:</strong> Closed; between 4&lt;sup&gt;th&lt;/sup&gt; and 5&lt;sup&gt;th&lt;/sup&gt; pairs of the thoracic legs.</td>
<td>Armed</td>
<td>Beige with dark transverse bands; blue uropod’s</td>
<td>F.: 23.5 cm M.: 18.0 cm</td>
</tr>
<tr>
<td><em>P. longirostris</em></td>
<td>Dorsally: 7 teeth+1 epigastric; shorter in male</td>
<td>Orbital, hepatic and branchiostegal spines; well-marked carina</td>
<td>Petasma: Symmetrical; semi-closed; <strong>Subovoid.</strong> <strong>Thelycum:</strong> Closed; a single plate of sternite XIV</td>
<td>Armed</td>
<td>Red-pale translucent; deep-red uropods.</td>
<td>F.: 23.5 cm M.: 18.0 cm</td>
</tr>
<tr>
<td><em>X. kroyeri</em></td>
<td>Dorsally: 5 teeth at base; elongated and curvy (upward) tip.</td>
<td>Smooth and glabrous crest; orbital and hepatic spines</td>
<td>Petasma: Subcircular; rounded projection; T-shaped; small spins ventrally; dorsomedial margin with cincinnuli. <strong>Thelycum:</strong> Closed; Single and smooth broad plate; between 13-14 sternites; round and curvy shape gonopores.</td>
<td>Unarmed</td>
<td>White to yellow</td>
<td>F.: 14.0 cm M.: 11.5 cm</td>
</tr>
<tr>
<td><em>M. monoceros</em></td>
<td>Dorsally: 8-10 teeth +1 epigastric.</td>
<td>Hepatic groove is descending vertically/ straight cervical groove</td>
<td>Petasma: Symmetrical; 2 rigid segments tightly folded longitudinally. <strong>Thelycum:</strong> Concaves and bounded laterally by a pair of ear-like lobes; Bounded anteriorly by the median projecting tongue; No exopodite on the fifth.</td>
<td>Armed</td>
<td>Pink with brown specks; Red uropod’s</td>
<td>F.: 18.6 cm M.: 16.0 cm</td>
</tr>
<tr>
<td><em>T. curvirostris</em></td>
<td>Dorsally: 6-9 teeth +1 epigastric; Slightly up curved at the tip in females almost straight in males.</td>
<td>Obscure grooves and crests; short longitudinal suture; Present hepatic sulcus</td>
<td>Petasma: T-shaped distolateral projections broadly aliform/Small distomedian projections. <strong>Thelycum:</strong> Posterior and middle components are fused and extend between the coxae of fifth pereiopods; V-shaped anterior edge with obtuse angle in-between its sides.</td>
<td>Armed</td>
<td>Pink to the red-brown body with white pereiopods</td>
<td>F.: 10.5 cm M.: 8.1 cm</td>
</tr>
<tr>
<td><em>P. semisulcatus</em></td>
<td>Dorsally: 6-7 teeth; Ventrally: 2-3 teeth</td>
<td>Smooth crest with a groove extended beyond epigastric tooth</td>
<td>Petasma: Ventral costa is unarmed or minutely serrate near apex; the outer surface of lateral lobes is minutely tuberculate. <strong>Thelycum:</strong> Closed type; lies ventrally between the coxae of fifth and fourth pereiopods.</td>
<td>Unarmed</td>
<td>Brown-grey and pale-yellow body with dorsal transverse bands.</td>
<td>F.: 23.0 cm M.: 19.0 cm</td>
</tr>
<tr>
<td><em>P. japonicus</em></td>
<td>Dorsally: 9-11 teeth/ Ventrally: 1 tooth</td>
<td>Smooth with wide and long groove; present of the gastro-frontal crest.</td>
<td>Petasma: Long distomedian projections; unarmed ventral costae; the outer surface of lateral lobes not tuberculate. <strong>Thelycum:</strong> No lateral plates; widely open anteriorly; the triangular shape of posterior and anterior processes (concaved plate).</td>
<td>Armed</td>
<td>Pale-yellow to pink; red/dark-brown transverse bands.</td>
<td>F.: 23.5 cm M.: 20.0 cm</td>
</tr>
<tr>
<td><em>M. latisulcatus</em></td>
<td>Dorsally: 9-12 teeth/ Ventrally: 1 tooth</td>
<td>Smooth and uniformly glabrous; bearing gastro-frontal carina and hepatic crest.</td>
<td>Petasma: United dorsomedial lobules along midline; the outer surface of lateral lobes not tuberculate. <strong>Thelycum:</strong> Two subtriangular shapes; curved lateral surface; rounded apex.</td>
<td>Armed</td>
<td>Pale-yellow to brown; a dark-brown abdominal crest.</td>
<td>F.: 20.2 cm M.: 17.0 cm</td>
</tr>
<tr>
<td><em>P. serratus</em></td>
<td>Dorsally: 6-9 teeth/ Ventrally: 4-5 teeth; elongated and nearly straight</td>
<td>Longitudinal ornamented with oblique dark lines.</td>
<td>Sexual dimorphism; the presence (♀) or absence (♂) of the appendix masculine.</td>
<td>Armed</td>
<td>Pale pink</td>
<td>12.0 cm</td>
</tr>
<tr>
<td><em>S. crassicornis</em></td>
<td>Dorsally: 8-12 teeth/ Ventrally: robustly convex</td>
<td>Smooth and glabrous; cervical groove deep</td>
<td>Petasma: Tubular and bearing a pair of spatulate projections on ventral free edges with many setae on distal borders. <strong>Thelycum:</strong> Open; simple basin shape</td>
<td>Unarmed</td>
<td>Reddish body and legs; dark-red uropods.</td>
<td>F.: 14.0 cm M.: 11.0 cm</td>
</tr>
</tbody>
</table>
The overall transition/transversion bias (R) is 0.45. The percent of GC content varies among species from 41.4% for both *Metapeneaeus monoceros* (Egypt), *Metapeneaeus Monoceros* (Mozambique), to 36.6% for *Trachypeneaeus curvirostris* (India), *Marsupeneaeus japonicas* (China) (Fig. 1) according to the sequence analysis of COI gene. The Neighbor-Joining phylogenetic tree revealed that three main clades were performed. The first clade included the species of *Penaeus japonicas*, *Melicetruus latisulcatus*, *Penaeus Kerathurus*, *Solenocera crassiconis* and *Penaeus semisulcatus* that clustered together. On the other hand, the second clade included the species of *Parapeneaeus longirostris*, *Metapeneaeus monoceros*, *Trachypeneaeus curvirostris* and *Xiphopenaeus kroyeri*. While, *Palaemon serratus* was separated in the third clade (Fig. 2). The Kimura 2-parameter (K2P) model was exploited to evaluate the genetic distance between the ten Egyptian shrimps and other closely related species from the GenBank database (Table 3). The pairwise distances, based on COI, were investigated where the highest genetic distance was (0.30) that verified between *Palaemon serratus* (Egypt, accession no. LC477193.1) and *Parapeneaeus longirostris* (Egypt, accession no. LC477196.1), and *Penaeus semisulcatus* (India, accession no. KY069066.1) and *Trachypeneaeus curvirostris* (Egypt, accession no. LC175472.1). However, the least genetic distance was (0.00) that verified for the following species’ sequences: between *Parapeneaeus longirostris* (Egypt, accession no. LC477196.1) concerning the GenBank database's reference *Parapeneaeus longirostris* (Turkey, accession no. KJ841702.1), and *Xiphopenaeus kroyeri* (Egypt, accession no. LC477201.1) with its GenBank references (Mexico, accession no. MH737706.1 and the USA, accession no. KY449154.1).

Table 2. Maximum composite likelihood estimates of the pattern of nucleotide substitution

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<th>A</th>
<th>T</th>
<th>C</th>
<th>G</th>
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<td>A</td>
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<td>11.37</td>
<td>6.86</td>
<td><strong>12.45</strong></td>
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<tr>
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<td><strong>G</strong></td>
<td><strong>18.58</strong></td>
<td>11.37</td>
<td>6.86</td>
<td>-</td>
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</tbody>
</table>
Phylogenetic relationship and systematic identification of different shrimp species

Fig. 1. The percent of the total GC content of COI sequences obtained in the current study and available in the GenBank from the other countries.

Fig. 2. Neighbor Joining (NJ) phylogenetic tree for Egyptian shrimp and prawn species and their related sequences obtained from the GenBank database, based on partial Cytochrome oxidase subunit I (COI) gene using K2P distance. The numbers above the branches are bootstrap values.
<table>
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**Table 3.** Pairwise genetic distances between the ten Egyptian shrimps evaluated in the current study and some related shrimp species available in the GenBank database based on the COI gene

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et al., 2022
**DISCUSSION**

Molecular taxonomy is commonly recognized as a valuable tool for accurate specimen designation, the exploration of new species, and in some cases, the recognition of cryptic species (Liu et al., 2014; Ferrari et al., 2021). Moreover, its significance extends to the capability of separating between complex species (Arlyza et al., 2008; Ward et al., 2008). DNA barcoding does not ignore morphological aspects evaluation, and its overall goal is to create a partnership between molecular and morphological taxonomists for rapid and unambiguous species recognition (Sharawy et al., 2017). Indeed, some studies on molecular identification and DNA barcoding have been conducted among shrimp species (Bilgin et al., 2015; Rajkumar et al., 2015; Sharawy et al., 2017; Abbas et al., 2018; Karuppasamy et al., 2020; Abbas et al., 2021). Sequence variation in a 650-bp region around the 5' end of the mtCOI gene has been shown in several studies to give a species-level resolution for a variety of animal taxa, including birds (Hebert et al., 2004; Aliabadian et al., 2013), springtails (Hogg & Hebert, 2004; Porco et al., 2012), spiders (Barrett & Hebert, 2005; Blagoev et al., 2013), moths (Janzen et al., 2005; Liu et al., 2014) and fishes (Ward et al., 2005; Cawthorn et al., 2012). The mtCOI gene has proved particularly efficient in evaluating crustacean phylogeny at the species level (Chu et al., 2003). The concordance of DNA and morphological-based classification in the current study confirms the COI sequence identification platform's expected capability for taxonomy evaluation (Rajkumar et al., 2015; Sharawy et al., 2017; Karuppasamy et al., 2020). In our sequencing analysis of the 652 bp COI gene, the lowest average percent of GC content was found in Xiphopenaeus kroyeri (36.9%), while the highest percentage (41.5%) was found in Metapenaeus monoceros. These results approximately agree with the findings from Iran (Samadi et al., 2016) for five Panaeidean shrimps and India (Karuppasamy et al., 2020), particularly with the increase in the GC content of the shrimp species. Higher GC content is critical for mtDNA stability (Johnston & Williams, 2016). This is due to the fact that genetic material with greater GC bonds might be less predisposed to spontaneous mutation and thus could be better protected against environmental mutagens (Samuels, 2005). Environmental factors such as temperature, salinity, light, oxygen, and pH are thought to cause GC-rich DNA to create a more heat-stable helix, enabling it selectively beneficial in the different species with strong metabolic regulation (Matzen et al., 2011; Apreshgi et al., 2016). The average nucleotide frequencies in this study were A = 27.01%, T = 34.23%, C = 20.66% and G = 18.10%, which were identical to those found in previous studies (Kundu et al., 2018; Ahmed et al., 2021). When the resulting COI sequences of the ten collected species were compared with available data in the GenBank database using BLAST search, most species collected showed high similarity (≤ 99%) compared to the reference in the database of GenBank, displaying a higher level of high marker recognition and molecular properties at the taxonomic level corresponded to the morphological identification of the species. However, Trachypenaeus curvirostris and Melicertus latisulcatus had less sequence similarity (87 % and 88 %, respectively) to the GenBank sequences' data. Due to the scarcity of publicly available sequences of these species in GenBank database, the similarity search results are not definite to identify Trachypenaeus curvirostris and Melicertus latisulcatus on the molecular levels. Whereas both species were identified at the morphological level, which is compatible with the previous studies (Sharawy et al., 2017; Abbas et al., 2018).
Nevertheless, in some cases, there was sectional sequence’s overlap between the reference and query species, and various sequences could not be accurately identified at the species level (Bariche et al., 2015). However, for certain species, a genus-level match could be achievable. The present study introduces an existing marine Egyptian shrimp species' first evolutionary relationship to the best of our knowledge. The generated phylogenetic tree allowed us to conclude the evolutionary relationships of Penaeidae, Palaemonidae, and Solenoceridae. The use of COI gene sequencing data provided accurate authentication and identification to understand the phylogeny of closely related species and supported morphological characterization. In the current study, the Kimura 2-parameter (K2P) for the COI gene was varied between (0%) and (3%) among the ten Egyptian shrimp species and the retrieved sequences related to the same shrimp spices. That variation in the genetic distance between species is consistent with the shrimp barcoding studies, which support the genetic divergence at the species level (Hubert et al., 2008; Samadi et al., 2016; Huzaid et al., 2020). The maximum genetic distance was (3%) between Penaeus semisulcatus (India) and Trachypenaeus curvirostris (Egypt) and between Palaemon serratus (Egypt) and Parapenaeus longirostris (Egypt). Therefore, these species are not closely related to each other which the genetic distance between them is 3% (Hebert et al., 2004). Unsurprisingly, such high genetic distance between these two shrimp species was due to that they belong to two different families “Palaemon serratus and Parapenaeus longirostris” belong to the family Penaeidae and Palaemonidae, respectively. The genetic distance between Penaeus semisulcatus (India) and Trachypenaeus curvirostris (Egypt) might be due to the origin of these two species, whereas the origin of Penaeus semisulcatus is the Indo-West Pacific while the origin of Palaemon serratus is the Eastern Atlantic (Holthuis, 1980). Phylogenetic relationships between Egyptian shrimps and the same species from some of the other countries proved the close relationship between individuals from Egypt and their peers from these countries, which clustered together in the same clades and branched in the sub-clades. These phylogenetic relationships clarified that the origin of these species under the study even from the Indo-West Pacific origin or Eastern-Atlantic (Holthuis, 1980, Dall et al., 1990) and provided the probability of their existing as a migratory species in the Red Sea which was translocated either from the Indian Ocean or from the Mediterranean Sea as a translocated shrimp from the Atlantic Ocean. So far, shrimps and prawns are well-known as active migratory species and can migrate through very long distances (Gokoglu and Kaya, 2008). 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 (Abbas et al., 2018). This may clarify the high similarity Regardless of the natural geographical barrier that separates these remote regions. Various molecular and morphological changes between species are actually due to responses to evolutionary variation (Chan et al., 2008; Ahmed et al., 2021). The species recognized morphologically were precise when using the molecular identification technique. The coupling between morphological and genetic recognition has resulted in a dependable platform for shrimp species accreditation. This combination technique can also resolve molecular identification confusion.

CONCLUSION

The present study is the first of its kind in Egypt to provide vital information on molecular identification and morphologically systematic for these number of the
Phylogenetic relationship and systematic identification of different shrimp species

important shrimp species. The present work used the mitochondrial gene (COI) for studying the phylogenetic relationship of the endemic marine Egyptian shrimp species. This study validates the COI genes’ effectiveness for shrimp species authentication and distinguishes the closely related species. It is also worth mentioning that the current study has contributed to the expansion of the GenBank database with Egyptian invertebrates like the marine shrimp species from the Mediterranean and the Red seas.

REFERENCES


