Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(3): 565 – 578 (2022)
www.ejabf.journals.ekb.eg


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

**Eman M. Abbas[1], Mikio Kato[2], Zaki Z. Sharawy[1]**

1.National Institute of Oceanography and Fisheries (NIOF), Cairo 11516, Egypt
2.Biology Laboratory, Faculty of Liberal Arts and Sciences, Osaka Prefecture University, 1-1 Gakuencho, Naka-ku, Sakai, 599-8531, Japan

*Corresponding Author: emanabbas03@yahoo.com

## ARTICLE INFO

**Article History:**
Received: May 2, 2022
Accepted: June 1, 2022
Online: June 11, 2022

**Keywords**:
DNA barcoding,
*COI* gene,
Morphology,
Phylogeny,
Shrimp species

## 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 were divided into four main categories (Penaeoidea, Caridea, Sergestoidea and Stenopodidea).

The superfamily Penaeoidea, with five families (Penaeidae, Solenoceridae, Aristeidae, Sergestidae, and Sicynoiidae) 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.

## 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′ TAAACTTCAGGGTGACCAAAAAATCA-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$^{TM}$ 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 sequencing data 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

| Species | Rostrum | Carapace | Sex determination | Telson | Body-color | Total length (max.) |
|---|---|---|---|---|---|---|
| ***P. kerathurus*** | **Dorsally:** 8-13 teeth/ **Ventrally:** 1 tooth | Vast and chitinous carapace with 14 segments | **Petasma:** Ventrolateral lobule is strongly protruding distally into a conspicuous flap overreaching the ventromedial lobule. **Thelycum:** Closed; between 4$^{th}$ and 5$^{th}$ pairs of the thoracic legs | Armed | Beige with dark transverse bands; blue uropod's | F.: 23.5 cm M.: 18.0 cm |
| ***P. longirostris*** | **Dorsally:** 7 teeh+1 epigastric; shorter in male | Orbital, hepatic and branchiostegal spines; well-marked carina | **Petasma:** Symmetrical; semi-closed; Subovoid. **Thelycum:** Closed; a single plate of sternite XIV | Armed | Red-pale translucent; deep-red uropods. | F.: 23.5 cm M.: 18.0 cm |
| ***X. kroyeri*** | **Dorsally:** 5 teeth at base; elongated and curvy (upward) tip. | Smooth and glabrous crest; orbital and hepatic spines | **Petasma:** Subcircular; rounded projection; T-shaped; small spins ventrally; dorsomedial margin with cincinnuli. **Thelycum:** Closed; Single and smooth broad plate; between 13-14 sternites; round and curvy shape gonopores. | Unarmed | White to yellow | F.: 14.0 cm M.: 11.5 cm |
| ***M. monoceros*** | **Dorsally:** 8-10 teeth +1 epigastric. | Hepatic groove is descending vertically/ straight cervical groove | **Petasma:** Symmetrical; 2 rigid segments tightly folded longitudinally. **Thelycum:** Concaves and bounded laterally by a pair of ear-like lobes; Bounded anteriorly by the median projecting tongue; No exopodite on the fifth. | Armed | Pink with brown specks; Red uropod's | F.: 18.6 cm M.: 16.0 cm |
| ***T. curvirostris*** | **Dorsally:** 6-9 teeth +1 epigastric; Slightly up curved at the tip in females almost straight in males. | Obscure grooves and crests; short longitudinal suture; Present hepatic sulcus | **Petasma:** T-shaped distolateral projections broadly aliform/Small distomedian projections. **Thelycum:** 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. | Armed | Pink to the red-brown body with white pereiopods | F.: 10.5 cm M.: 8.1 cm |
| ***P. semisulcatus*** | **Dorsally:** 6-7 teeth; **Ventrally:** 2-3 teeth | Smooth crest with a groove extended beyond epigastric tooth | **Petasma:** Ventral costa is unarmed or minutely serrate near apex; the outer surface of lateral lobes is minutely tuberculate. **Thelycum:** Closed type; lies ventrally between the coxae of fifth and fourth pereiopods | Unarmed | Brown-grey and pale-yellow body with dorsal transverse bands. | F.: 23.0 cm M.: 19.0 cm |
| ***P. japonicus*** | **Dorsally:** 9-11 teeth/ **Ventrally:** 1 tooth | Smooth with wide and long groove; present of the gastro-frontal crest. | **Petasma:** Long distomedian projections; unarmed ventral costae; the outer surface of lateral lobes not tuberculate. **Thelycum:** No lateral plates; widely open anteriorly; the triangular shape of posterior and anterior processes (concaved plate). | Armed | Pale-yellow to pink; red-/dark-brown transverse bands. | F.: 23.5 cm M.: 20.0 cm |
| ***M. latisulcatus*** | **Dorsally:** 9-12 teeth/ **Ventrally:** 1 tooth | Smooth and uniformly glabrous; bearing gastro-frontal carina and hepatic crest. | **Petasma:** United dorsomedial lobules along midline; the outer surface of lateral lobes is not tuberculate. **Thelycum:** Two subtriangular shapes; curved lateral surface; rounded apex. | Armed | Pale-yellow to brown; a dark-brown abdominal crest. | F.: 20.2 cm M.: 17.0 cm |
| ***P. serratus*** | **Dorsally:** 6-9 teeth/ **Ventrally:** 4-5 teeth; elongated and nearly straight | Longitudinal ornamented with oblique dark lines. | Sexual dimorphism; the presence (♂) or absence (♀) of the appendix masculine. | Armed | Pale pink | 12.0 cm |
| ***S. crassicornis*** | **Dorsally:** 8-12 teeth/ **Ventrally:** robustly convex | Smooth and glabrous; cervical groove deep | **Petasma:** Tubular and bearing a pair of spatulate projections on ventral free edges with many setae on distal borders. **Thelycum:** Open; simple basin shape | Unarmed | Reddish body and legs; dark-red uropods. | F.: 14.0 cm M.: 11.0 cm |

The overall transition/transversion bias (R) is 0.45. The percent of GC content varies among species from 41.4% for both *Metapenaeus monoceros* (Egypt), *Metapenaeus Monoceros* (Mozambique), to 36.6% for *Trachypenaeus curvirostris* (India), *Marsupenaeus 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*, *Melicetrus latisulcatus*, *Penaeus Kerathurus*, *Solenocera crassiconis* and *Penaeus semisulcatus* that clustered together. On the other hand, the second clade included the species of *Parapenaeus longirostris*, *Metapenaeus monoceros*, *Trachypenaeus 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 *Parapenaeus longirostris* (Egypt, accession no. LC477196.1), and *Penaeus semisulcatus* (India, accession no. KY069066.1) and *Trachypenaeus curvirostris* (Egypt, accession no. LC175472.1). However, the least genetic distance was (0.00) that verified for the following species' sequences: between *Parapenaeus longirostris* (Egypt, accession no. LC477196.1) concerning the GenBank database's reference *Parapenaeus 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

| | **A** | **T** | **C** | **G** |
|---|---|---|---|---|
| **A** | - | 11.37 | 6.86 | **12.45** |
| **T** | 8.97 | - | **0.95** | 6.01 |
| **C** | 8.97 | **1.58** | - | 6.01 |
| **G** | **18.58** | 11.37 | 6.86 | - |

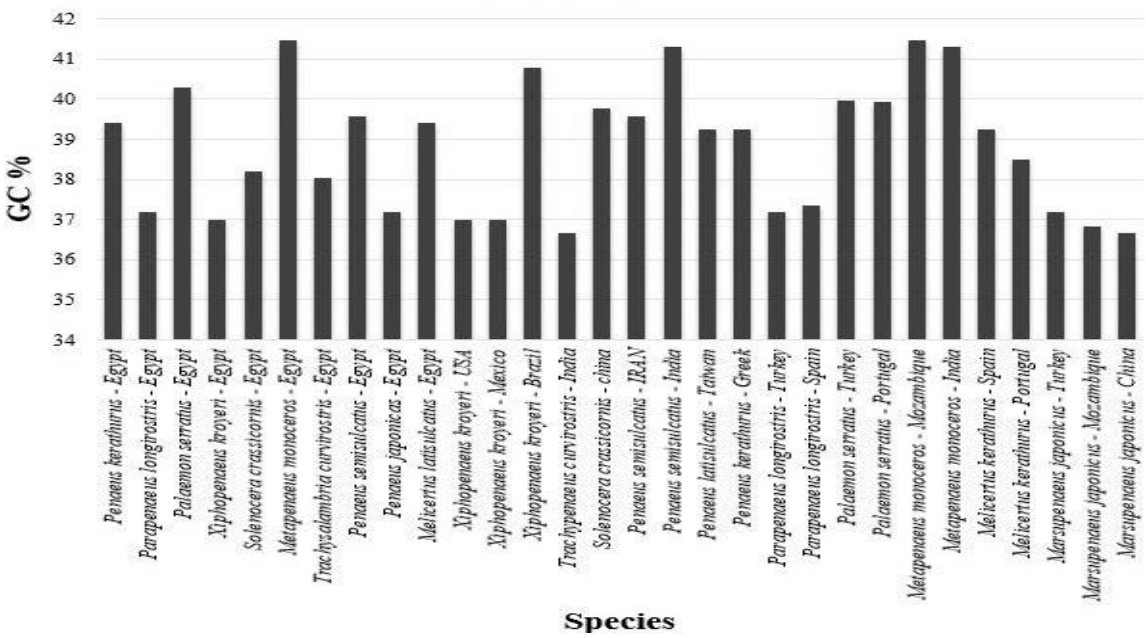


**Fig. 1**. The percent of the total GC content of *COI* sequences obtained in the current study and available in the GenBank from the other countries.

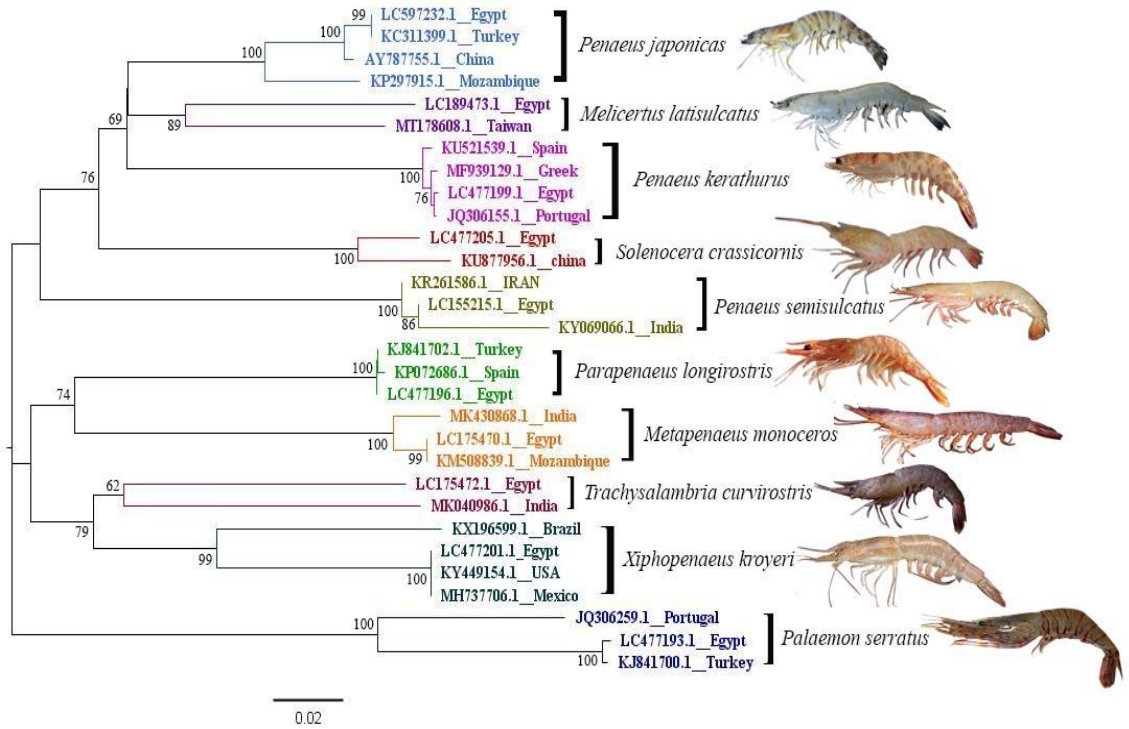


**Fig. 2**. Neighbor Joining (NJ) phylogenetic tree for Egyptian shrimp and prawn species and their related sequences obtained from the GenBank database, based on partial Cytochrome oxidase subunit I (COI*)* gene using K2P distance. The numbers above the branches are bootstrap values.

**Table 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

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Penaeus kerathurus* Egypt_LC477199.1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Parapenaeus longirostris* Egypt_LC477196.1 | 0.22 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Palaemon serratus* Egypt_LC477193.1 | 0.29 | **0.30** | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Xiphopenaeus kroyeri* Egypt_LC477201.1 | 0.23 | 0.23 | 0.27 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Solenocera crassicornis* Egypt_LC477205.1 | 0.19 | 0.19 | 0.29 | 0.23 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Metapenaeus monoceros* Egypt_LC175470.1 | 0.23 | 0.18 | 0.28 | 0.26 | 0.21 | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Trachysalambria curvirostris* Egypt_LC175472.1 | 0.22 | 0.206 | 0.28 | 0.18 | 0.21 | 0.21 | | | | | | | | | | | | | | | | | | | | | | | | |
| *Penaeus semisulcatus* Egypt_LC155215.1 | 0.21 | 0.22 | 0.27 | 0.22 | 0.22 | 0.23 | 0.25 | | | | | | | | | | | | | | | | | | | | | | | |
| *Penaeus japonicas* Egypt_LC597232.1 | 0.16 | 0.21 | 0.28 | 0.22 | 0.17 | 0.24 | 0.21 | 0.18 | | | | | | | | | | | | | | | | | | | | | | |
| *Melicertus latisulcatus* Egypt_LC189473.1 | 0.17 | 0.21 | 0.26 | 0.22 | 0.18 | 0.26 | 0.23 | 0.23 | 0.15 | | | | | | | | | | | | | | | | | | | | | |
| *Xiphopenaeus kroyeri* USA_KY449154.1 | 0.23 | 0.22 | 0.27 | **0.00** | 0.23 | 0.26 | 0.18 | 0.23 | 0.22 | 0.22 | | | | | | | | | | | | | | | | | | | | |
| *Xiphopenaeus kroyeri* Mexico_MH737706.1 | 0.23 | 0.22 | 0.27 | 0.00 | 0.23 | 0.26 | 0.18 | 0.22 | 0.22 | 0.22 | **0.00** | | | | | | | | | | | | | | | | | | | |
| *Xiphopenaeus kroyeri* Brazil_KX196599.1 | 0.23 | 0.21 | 0.28 | 0.12 | 0.24 | 0.23 | 0.21 | 0.22 | 0.24 | 0.25 | 0.12 | 0.12 | | | | | | | | | | | | | | | | | | |
| *Trachypenaeus curvirostris* India_MK040986.1 | 0.24 | 0.18 | 0.26 | 0.18 | 0.22 | 0.22 | 0.16 | 0.24 | 0.23 | 0.25 | 0.18 | 0.18 | 0.18 | | | | | | | | | | | | | | | | | |
| *Solenocera crassicornis* china_KU877956.1 | 0.21 | 0.22 | 0.29 | 0.24 | 0.04 | 0.21 | 0.23 | 0.22 | 0.18 | 0.18 | 0.24 | 0.24 | 0.24 | 0.22 | | | | | | | | | | | | | | | | |
| *Penaeus semisulcatus* IRAN_KR261586.1 | 0.20 | 0.22 | 0.27 | 0.22 | 0.21 | 0.22 | 0.25 | 0.003 | 0.18 | 0.22 | 0.22 | 0.22 | 0.22 | 0.24 | 0.22 | | | | | | | | | | | | | | | |
| *Penaeus semisulcatus* India_KY069066.1 | 0.25 | 0.26 | 0.32 | 0.27 | 0.26 | 0.27 | **0.30** | 0.03 | 0.22 | 0.26 | 0.27 | 0.27 | 0.26 | 0.29 | 0.27 | 0.03 | | | | | | | | | | | | | | |
| *Penaeus latisulcatus* Taiwan_MT178608.1 | 0.15 | 0.21 | 0.29 | 0.21 | 0.17 | 0.22 | 0.21 | 0.20 | 0.15 | 0.12 | 0.21 | 0.21 | 0.22 | 0.24 | 0.17 | 0.20 | 0.25 | | | | | | | | | | | | | |
| *Penaeus kerathurus* Greek_MF939129.1 | 0.01 | 0.22 | 0.29 | 0.23 | 0.19 | 0.23 | 0.23 | 0.21 | 0.16 | 0.17 | 0.23 | 0.23 | 0.22 | 0.24 | 0.21 | 0.20 | 0.25 | 0.16 | | | | | | | | | | | | |
| *Parapenaeus longirostris* Turkey_KJ841702.1 | 0.22 | **0.00** | 0.31 | 0.22 | 0.19 | 0.18 | 0.21 | 0.22 | 0.21 | 0.20 | 0.22 | 0.22 | 0.21 | 0.18 | 0.22 | 0.22 | 0.26 | 0.21 | 0.22 | | | | | | | | | | | |
| *Parapenaeus longirostris* Spain_KP072686.1 | 0.22 | 0.001 | 0.31 | 0.23 | 0.20 | 0.18 | 0.21 | 0.23 | 0.21 | 0.21 | 0.23 | 0.23 | 0.21 | 0.19 | 0.22 | 0.23 | 0.26 | 0.23 | 0.22 | 0.001 | | | | | | | | | | |
| *Palaemon serratus* Turkey_KJ841700.1 | 0.29 | 0.29 | 0.003 | 0.28 | 0.29 | 0.28 | 0.27 | 0.27 | 0.28 | 0.27 | 0.28 | 0.28 | 0.28 | 0.27 | 0.29 | 0.27 | 0.32 | 0.29 | 0.29 | 0.29 | 0.29 | | | | | | | | | |
| *Palaemon serratus* Portugal_JQ306259.1 | 0.27 | 0.27 | 0.12 | 0.28 | 0.29 | 0.28 | 0.25 | 0.26 | 0.26 | 0.26 | 0.28 | 0.28 | 0.28 | 0.27 | 0.28 | 0.26 | 0.30 | 0.27 | 0.28 | 0.27 | 0.27 | 0.12 | | | | | | | | |
| *Metapenaeus monoceros* Mozambique_KM508839.1 | 0.23 | 0.18 | 0.28 | 0.26 | 0.21 | 0.00 | 0.21 | 0.23 | 0.24 | 0.26 | 0.26 | 0.26 | 0.23 | 0.22 | 0.21 | 0.22 | 0.27 | 0.22 | 0.23 | 0.18 | 0.18 | 0.28 | 0.28 | | | | | | | |
| *Metapenaeus monoceros* India_MK430868.1 | 0.24 | 0.19 | 0.28 | 0.25 | 0.21 | 0.022 | 0.22 | 0.24 | 0.23 | 0.25 | 0.25 | 0.25 | 0.23 | 0.22 | 0.22 | 0.23 | 0.28 | 0.23 | 0.24 | 0.19 | 0.19 | 0.28 | 0.29 | 0.02 | | | | | | |
| *Melicertus kerathurus* Spain_KU521539.1 | 0.01 | 0.22 | 0.28 | 0.23 | 0.18 | 0.22 | 0.22 | 0.21 | 0.16 | 0.17 | 0.23 | 0.23 | 0.23 | 0.25 | 0.21 | 0.20 | 0.25 | 0.15 | 0.01 | 0.22 | 0.22 | 0.28 | 0.28 | 0.23 | 0.23 | | | | | |
| *Melicertus kerathurus* Portugal_JQ306155.1 | 0.001 | 0.22 | 0.29 | 0.23 | 0.19 | 0.23 | 0.23 | 0.21 | 0.16 | 0.17 | 0.23 | 0.23 | 0.23 | 0.24 | 0.21 | 0.20 | 0.25 | 0.15 | 0.002 | 0.22 | 0.22 | 0.29 | 0.28 | 0.23 | 0.24 | 0.01 | | | | |
| *Marsupenaeus japonicus* Turkey_KC311399.1 | 0.16 | 0.21 | 0.28 | 0.22 | 0.17 | 0.24 | 0.21 | 0.18 | 0.00 | 0.15 | 0.22 | 0.22 | 0.24 | 0.23 | 0.18 | 0.18 | 0.22 | 0.15 | 0.16 | 0.21 | 0.21 | 0.28 | 0.26 | 0.24 | 0.23 | 0.16 | 0.16 | | | |
| *Marsupenaeus japonicus* Mozambique_KP297915.1 | 0.15 | 0.202 | 0.29 | 0.24 | 0.16 | 0.24 | 0.22 | 0.20 | 0.05 | 0.13 | 0.24 | 0.24 | 0.22 | 0.23 | 0.16 | 0.19 | 0.24 | 0.15 | 0.15 | 0.20 | 0.20 | 0.29 | 0.26 | 0.24 | 0.24 | 0.15 | 0.15 | 0.05 | | |
| *Marsupenaeus japonicus* China_AY787755.1 | 0.15 | 0.20 | 0.28 | 0.22 | 0.16 | 0.23 | 0.21 | 0.18 | 0.01 | 0.14 | 0.22 | 0.22 | 0.24 | 0.23 | 0.17 | 0.17 | 0.22 | 0.14 | 0.15 | 0.20 | 0.20 | 0.28 | 0.26 | 0.23 | 0.24 | 0.15 | 0.15 | 0.01 | 0.05 | |

## 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 mt*COI* 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 mt*COI* 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

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

**Abbas, E. M.; Abdelsalam, K. M.; Mohammed-Geba, K.; Ahmed, H. O. and Kato, M.** (2016). Genetic and morphological identification of some crabs from the Gulf of Suez, Northern Red Sea, Egypt. E*gypt. J. Aquat. Res, 42*(3)**:** 319-329. doi:10.1016/j.ejar.2016.08.003

**Abbas, E. M.; Ali, F. S.; Desouky, M. G.; Ashour, M.; El-Shafei, A.; Maaty, M. M. and Sharawy, Z. Z.** (2020). Novel comprehensive molecular and ecological study introducing coastal mud shrimp (*Solenocera crassicornis*) recorded at the Gulf of Suez, Egypt. *J. Mar. Sci. Eng., 9*(1): 9. doi:10.3390/jmse9010009

**Abbas, E. M.; Geba, K. M. and Sharawy, Z. Z.** (2018). Molecular and biometric characterizations of the Western king prawn *Melicertus latisulcatus* (Kishinouye, 1896) in the Egyptian Red Sea. *Egypt. J. Aquat. Biol. Fish., 22*(1): 1-14. doi:10.21608/EJABF.2018.7181

**Abbas, E. M.; Takayanagi, A.; Shimizu, N. and Kato, M.** (2011). Methylation status and chromatin structure of the myostatin gene promoter region in the sea perch *Lateolabrax japonicus* (Perciformes). *Genetics Mol. Res., 10*(4): 3306-3315. doi:10.4238/2011.December.8.7

**Ahmed, M. S.; Salam, S.; Rumana, S. S. S. and Barua, A.** (2021). DNA barcoding and phylogenetic relationship of shrimps (Crustacea: Decapoda) of Bangladesh. *Bioresearch Communications (BRC), 7*(1): 941-946. doi:10.3329/ brc. v7i1.54248

**Aliabadian, M.; Beentjes, K. K.; Roselaar, H. V. B.; Nijman, V. and Vonk, R.** (2013). DNA barcoding of Dutch birds. *Zookeys,* (365): 25. doi.org/10.3897/ zookeys.365.6287

**Apreshgi, K. P.; Dhaneesh, K. V.; Radhakrishnan, T. and Kumar, A.** (2016). DNA barcoding of fiddler crabs *Uca annulipes* and *U. perplexa* (Arthropoda, Ocypodidae) from the southwest coast of India. *J. Mar. Biol. Ass. India*, *58*(1): 101-104.

**Arlyza, I. S.; Shen, K. N.; Solihin, D. D.; Soedharma, D.; Berrebi, P. and Borsa, P.** (2013). Species boundaries in the *Himantura uarnak* species complex (Myliobatiformes: Dasyatidae. *Mol. Pylo. Evol., 66*(1): 429-435. doi:10.1016/j. ympev.2012.09.023

**Bariche, M.; Torres, M.; Smith, C.; Sayar, N.; Azzurro, E.; Baker, R. and Bernardi, G.** (2015). Red Sea fishes in the Mediterranean Sea: a preliminary investigation of a biological invasion using DNA barcoding. *J. Biogeography., 42*(12): 2363-2373.doi:10.1111/jbi.12595

**Barrett, R. D. and Hebert, P. D.** (2005). Identifying spiders through DNA barcodes. *Can. J. zool., 83*(3): 481-491. doi:10.1139/z05-024

**Bilgin, R.; Utkan, M. A.; Kalkan, E.; Karhan, S. U. and Bekbölet, M.** (2015). DNA barcoding of twelve shrimp species (Crustacea: Decapoda) from Turkish seas reveals cryptic diversity. *Med. Mar. Sci.*, *16*(1): 36-45. doi:10.12681/mms.548

**Bingpeng, X.; Heshan, L.; Zhilan, Z.; Chunguang, W.; Yanguo, W. and Jianjun, W.** (2018). DNA barcoding for identification of fish species in the Taiwan Strait. *PloS one*, *13*(6): e0198109. doi:10.1371/journal.pone.0198109

**Blagoev, G. A.; Nikolova, N. I.; Sobel, C. N.; Hebert, P. D. and Adamowicz, S. J.** (2013). Spiders (Araneae) of Churchill, Manitoba: DNA barcodes and morphology reveal high species diversity and new Canadian records. *BMC Ecology, 13*(1): 1-17. doi:10.1186/1472-6785-13-44

**Carpenter, K. E. and Niem, V. H. (1998).** FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific, Vol. 2. *FAO Library, Rome. Cephalopods, crustaceans, holothurians, and sharks*, pp. 687-1396.

**Cawthorn, D. M.; Steinman, H. A. and Witthuhn, R. C.** (2012). DNA barcoding reveals a high incidence of fish species misrepresentation and substitution on the South African market. *Food Res. Inter. J.*, *46*(1): 30-40. doi:10.1016/J.FOODRES.2011.11.01

**Chan, T. Y.; Tong, J.; Tam, Y. K. and Chu, K. H.** (2008). Phylogenetic relationships among the genera of the Penaeidae (Crustacea: Decapoda) revealed by mitochondrial 16S rRNA gene sequences. *Zootaxa*, *1694*(3): 38-50.

**Chanda, A.** (2013). A systematic study on genus Metapenaeus wood-mason, 1891 with special reference to extended distribution of four species from Indian water. *Inter. J. Fish. Aquat. Studies,* **2**(1): 5-16.

**Chang, C. H.; Shao, K. T.; Lin, H. Y.; Chiu, Y. C.; Lee, M. Y.; Liu, S. H. and Lin, P. L.** (2017). DNA barcodes of the native ray-finned fishes in Taiwan. *Mol. Eco. Resource, 17*(4): 796-805. doi:10.1111/1755-0998.12601

**Chu, K. H.; Ho, H. Y.; Li, C. P. and Chan, T. Y.** (2003). Molecular phylogenetics of the mitten crab species in *Eriocheir, sensu lato* (Brachyura: Grapsidae). *J. Crust. Biol.*, *23*(3): 738-746. doi:10.1651/C-2347

**Dall, W.; Hill, B. J.; Rothlisberg, P. C. and Sharples, D. J.** (1990) The biology of the Penaeidae. *Adv. Mar. Biol.,* **27**: 282-306.

**Ferrari, A.; Di Crescenzo, S.; Cariani, A.; Crobe, V.; Benvenuto, A.; Piattoni, F.; Mancusi, C.; Bonnici, L.; Bonello, J. J.; Schembri, P. J.; Serena, F.; Massi, D.; Titone, A and Tinti, F.** (2021). Puzzling over spurdogs: molecular taxonomy assessment of the Squalus species in the Strait of Sicily. *Eur. Zool. J., 88*(1): 181-190. Doi:10.1080/24750263.2020.1849436

**Folmer, O.; Hoeh, W. R.; Black, M. B. and Vrijenhoek, R. C.** (1994). Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. *Mol. Mari. Biol. Biotechnology. J.*, *3*(5): 294-299.

**Gokoglu, M. and Kaya, Y.** (2008). First record of *Melicertus hathor* (Penaeidae) from the Gulf of Antalya (Mediterranean Sea). *Mari. Biodiversity Rec. J.*, *1*. doi:10.1017/S1755267206001771

**Hebert, P. D. N.; Stoeckle, M. Y.; Zemlak, T. S.; Francis, C. M. and Godfray, C.** (2004). Identification of birds through DNA barcodes. *PLoS biology, 2*(10): e312. doi:10.1371/journal.pbio.0020312

**Hebert, P. D.; Cywinska, A.; Ball, S. L. and DeWaard, J. R.** (2003). Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B Biol. Sci.*, *270*(1512): 313-321. doi:10.1098/rspb.2002.2218

**Hogg, I. D. and Hebert, P. D.** (2004). Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Can. J. Zool., 82*(5): 749-754. doi:10.11646/zootaxa.2639.1.6

**Holthuis, L. B.** (1980). Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries, FAO species catalogue. *FAO Fisheries Synopsis, 125*: 1-271.

**Hubert, N.; Hanner, R.; Holm, E.; Mandrak, N. E.; Taylor, E.; Burridge, M.; Watkinson, D.; Dumont, P.; Curry, A.; Bentzen, P.; Zhang, J.; April., J. and Bernatchez, L.** (2008). Identifying Canadian freshwater fishes through DNA barcodes. *PLoS One, 3*(6): e2490. doi:10.1371/journal.pone.0002490

**Hurzaid, A.; Chan, T. Y.; Mohd Nor, S. A.; Muchlisin, Z. A. and Chen, W. J.** (2020). Molecular phylogeny and diversity of penaeid shrimps (Crustacea: Decapoda) from South-East Asian waters. *Zool. Scripta J., 49*(5): 596-613. doi:10.1111/zsc.12428

**Janzen, D. H.; Hajibabaei, M.; Burns, J. M.; Hallwachs, W.; Remigio, E. and Hebert, P. D.** (2005). Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. *Philos. Trans. R. Soc. Lond. B Biol. Sci., 360*(1462): 1835-1845. doi:10.1098/rstb.2005.1715

**Johnston, I. G. and Williams, B. P.** (2016). Evolutionary inference across eukaryotes identifies specific pressures favoring mitochondrial gene retention. *Cell Sys. J., 2*(2): 101-111. doi:10.1016/j.cels.2016.01.013

**Karuppasamy, P. K.; Logeshwaran, V.; Priyadarshini, R. and Ramamoorthy, N.** (2020). Molecular and Systematic Identification of Food Marine Shrimps Using mtCOI Marker from Southeast Coast of India. *Thalassas: Inter. J. Mar. Sci., 36*(2): 487-495. doi:10.1007/s41208-020-00201-3

**Kimura, M.** (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol., 16*(2): 111-120. Doi:10.1007/BF01731581

**Kumar, S.; Stecher, G.; Li, M.; Knyaz, C. and Tamura, K.** (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol., 35*(6): 1547. doi:10.1093/molbev/msy096

**Kundu, S.; Rath, S.; Tyagi, K.; Chakraborty, R. and Kumar, V.** (2018). Identification of penaeid shrimp from Chilika Lake through DNA barcoding. *Mitochondrial DNA Part B, 3*(1): 161-165. doi:10.1080/23802359.2018.1437794

**Liu, X. F.; Yang, C. H.; Han, H. L.; Ward, R. D. and Zhang, A. B.** (2014). Identifying species of moths (Lepidoptera) from Baihua Mountain, Beijing, China, using DNA barcodes. *Ecol. Evol. J., 4*(12): 2472-2487. doi:10.1002/ece3.1110

**Matzen da Silva, J.; Creer, S.; Dos Santos, A.; Costa, A. C.; Cunha, M. R.; Costa, F. O. and Carvalho, G. R.** (2011). Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Crustacea: Malacostraca). *PLoS One, 6*(5): e19449. doi:10.1371/journal.pone.0019449

**Perez Farfante, I. S. A. B. E. L. and Kensley, B.** (1997). *Penaeoid and sergestoid shrimps and prawns of the world. Keys and diagnoses for the families and genera*. Editions du Museum national d'Histoire naturelle, pp. 175-223.

**Porco, D.; Bedos, A.; Greenslade, P.; Janion, C.; Skarżyński, D.; Stevens, M. I. and Deharveng, L.** (2012). Challenging species delimitation in Collembola: cryptic diversity among common springtails unveiled by DNA barcoding. *Invert. System., 26*(6): 470-477. doi:10.1071/IS12026

**Rajkumar, G.; Bhavan, P. S.; Udayasuriyan, R. and Vadivalagan, C.** (2015). Molecular identification of shrimp species, *Penaeus semisulcatus*, *Metapenaeus dobsoni*, *Metapenaeus brevicornis*, *Fenneropenaeus indicus*, *Parapenaeopsis stylifera* and *Solenocera crassicornis* inhabiting in the coromandel coast (Tamil Nadu, India) using MT-COI gene. *Inter. J. Fish. Aqua. Studies*, *2*(4): 96-106.

**Samadi, S.; Ghavam Mostafavi, P.; Rezvani Gilkolaii, S.; Fatemi, M. and Fazli, H.** (2016). Phylogenetic relationships of the commercial marine shrimp family Penaeidae from Persian Gulf. *Iran. J. Fish. Sci.* 15(1): 333-346.

**Sambrook, J.; Fritsch, E. F. and Maniatis, T.** (1989). Molecular Cloning: A Laboratory Manual. New York (NY): Cold Spring Harbor Laboratory Press.

**Samuels, D. C.** (2005). Life span is related to the free energy of mitochondrial DNA. *Mech. Ag. Dev. J., 126*(10): 1123-1129. doi:10.1016/j.mad.2005.05.003

**Sharawy, Z. Z.; Abbas, E. M.; Khafage, A. R.; Galal-Khallaf, A.; Ismail, R. F.; Ahmed, H. O.; Mohammed-Geba, K. and Kato, M.** (2017). Descriptive analysis, DNA barcoding and condition index of Penaeids (Crustacea: Decapoda) from the Egyptian Mediterranean coast. *Fish. Res., 188*: 6-16. doi:10.1016/j.fishres.2016.12.007.

**Sharawy, Z.; Abbas, E. M.; Desouky, M. G. and Kato, M.** (2016). Descriptive analysis and molecular identification of the green tiger shrimp *Penaeus semisulcatus* (De Haan, 1844) in Suez Gulf, Red Sea. *Inter. J. Fish. Aqua. Studies*, *4*(5): 426-432.

**Wang, J. F. and Qiao, G. X.** (2009). DNA barcoding of genus *Toxoptera Koch* (Hemiptera: Aphididae): identification and molecular phylogeny inferred from mitochondrial COI sequences. *J. Insect Sci., 16*(6): 475-484. doi:10.1111/j.1744-7917.2009.01270.x

**Ward, R. D.; Holmes, B. H.; White, W. T. and Last, P. R.** (2008). DNA barcoding Australasian chondrichthyans: results and potential uses in conservation. *Mari. Freshwater Rese., 59*(1): 57-71. doi:10.1071/MF07148

**Ward, R. D.; Zemlak, T. S.; Innes, B. H.; Last, P. R. and Hebert, P. D.** (2005). DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. Lond. B Biol. Sci., 360*(1462): 1847-1857. doi:10.1098/rstb.2005.1716

**Wilson, J. J. and Sing, K. W.** (2013). DNA barcoding can successfully identify Penaeus monodon, associate life cycle stages, and generate hypotheses of unrecognised diversity. *Sains Malaysiana*, *42*(12): 1827-1829.