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DNA barcoding uncovered cryptic diversity in Seabob shrimp *Xiphopenaeus kroyeri*, Heller, 1862 (Family: Penaeidae) from the South Mediterranean off Port Said coast, Egypt

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ABSTRACT

This study investigated the phylogenetic relationship of *Xiphopenaeus kroyeri* in the Mediterranean and Atlantic regions. Total DNA was extracted from muscle tissue of frozen shrimp samples using the Tissue Extraction Kit (Qiagen). DNA integrity and quantity were assessed via agarose gel electrophoresis and NanoDrop. The COI gene was amplified using PCR with the LCO 1490 and HCO 2198 primer set. PCR reactions contained 12.5 µL 2x PCR Master Mix (GeneDireX), 1 µL of each primer (20 pmol), 2 µL DNA template (20 ng/µL), and 8.5 µL deionized water. Thermal cycling conditions included initial denaturation at 94°C for 4 min, followed by 30 cycles of 94°C for 45 sec, 53°C for 45 sec, and 72°C for 90 sec, with a final extension at 72°C for 7 min. COI products were purified using the QIAquick PCR Purification Kit (QIAGEN) and sequenced using a 3500 Genetic Analyzer (Applied Biosystems). Raw sequences were edited and uploaded to NCBI GenBank (accession numbers MW737452.1 and MW737453.1). Sequences were aligned using ClustalW, and a phylogenetic tree was constructed using the maximum likelihood method with 1000 bootstrap replicates under the Tamura 3-parameter substitution model. *Portunus pelagicus* COI sequences served as the outgroup. Phylogenetic analysis was performed using MEGA version 10.2.6. Phylogenetic analysis revealed a high degree of relatedness between the two successful *X. kroyeri* samples and those from the Atlantic-Pacific region. Two main COI haplotypes were identified, with the Egyptian samples belonging to one of them.

Keywords: *Xiphopenaeus kroyeri*, Atlantic seabob, DNA barcoding, Port Said, South Mediterranean,

1. INTRODUCTION

Xiphopenaeus kroyeri, commonly known as the Seabob shrimp, which is found in the western Atlantic Ocean, ranging from Cape Hatteras in North Carolina, USA (approximately 37°N) to Rio Grande do Sul in southern Brazil (approximately 30°S). It is typically found at depths of down to 25 meters, particularly in soft, muddy, and sandy bottoms [1-5]. The seabob shrimp was an important target species for artisanal

fishermen in southern Brazil. and is also one of the top ten penaeid species harvested worldwide [5, 6]. Unfortunately, southeastern and southern Brazil regions are subjected to overexploitation for this species [7].

Xiphopenaeus kroyeri is one of the bigger species in this genus [8], Like the majority of penaeid shrimps, *X. kroyeri* is a species with rapid growth, with females reaching maximum size after around 21 months and males after around 16 months It can grows to reach a length 10 cm (T.L.) and more [9, 10], Males are significantly smaller than females [11-13]. *X. kroyeri* size and abundance make it a key species in global shrimp fisheries, contributing significantly to the commercial shrimp market [4, 5]. *X. kroyeri* spends its entire life in marine waters, and unlike many penaeid shrimp species, it does not require an estuarine growth period for its development [14].

Xiphopenaeus kroyeri appeared for the first time at Port Said coast in October 2018. It was noticed during the routine scientific surveys for sampling and data collection. It recorded firstly in small quantity and then it came in abundance. At this time *X. kroyeri* was unknown species for us. Likely, ship ballast water is strongly implicated in the introduction of this organism to the Mediterranean Sea. Despite the prevalence of traditional methods for species identification and classification, the advent of molecular taxonomy, which employs genetic data for classification, has transformed conventional taxonomic approaches. Nonetheless, morphological and genetic characteristics can often display subtle variations, rendering species delimitation challenging.

DNA barcoding, a method utilizing specific DNA sequences for species identification, has become a robust tool in contemporary taxonomy [15]. The mitochondrial cytochrome oxidase subunit I (COI) gene was used as a universal DNA barcode to identify species, delineate species boundaries and facilitate taxonomic studies [16]. The mitochondrial COI gene was utilized to explore the phylogenetic relationships of endemic marine shrimp species in Egypt by Abbas , et al. [17] This study demonstrates the effectiveness of COI as a molecular marker for species identification and differentiation. Egyptian researchers have utilized the COI gene to enhance the accuracy of shrimp species identification, authentication, and morphological characterization [18, 19]. Abbas , et al. [17] .

This study represents the first genetic investigation of this novel species in Port Said, Egypt, utilizing data available from the GenBank.

2. STUDY AREA

Site Description: Port Said, situated in the northeastern region of Egypt, is bordered by the Suez Canal to the east, the Mediterranean Sea to the north, and the eastern portion of Lake Manzala to the west [20]. Port Said, situated in the northeastern region of Egypt, is bordered by the Suez Canal to the east, the Mediterranean Sea to the north, and the eastern portion of Lake Manzala to the west [20].

It’s a prominent fishing ground along Egypt's Mediterranean coast, accounts for approximately 19.85% of the total fish production in this region [21]. The sampling site is located on the Egyptian Mediterranean coast near the entrance of the Suez Canal and Boughaz El-Gamil (Figure 1). The catch was obtained at depths ranging from one to ten meters using beach seine netting, a widely employed fishing technique in the Port Said area for year-round capture of various economically valuable fish species.

The predominant fishing gears employed in this region consisted of trawling (223 vessels), purse seining (51 vessels), and various forms of lining, including longline and handline fishing (238 vessels). Additionally, a number of small-scale fishing methods were utilized, primarily involving 512 second- and third-class sailing vessels. Fishing activities in Port Said are conducted all the year-round [22]. During the current study, over 16 beach seine fishing vessels were observed, and this fleet was intensively studied through this work[23].

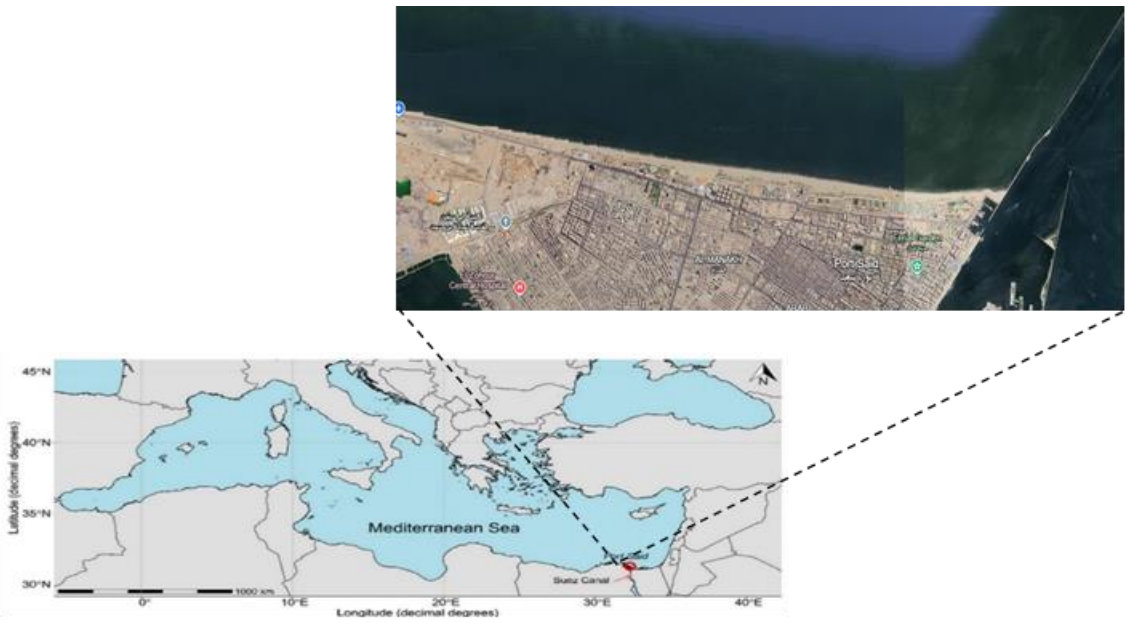


Fig. 1: Sampling site from the Mediterranean Sea, Egypt. Location of Port Said coast.

3. MATERIALS AND METHODS

3.1 Samples collection and analysis: Specimens of *X. kroyeri* were collected from the catch of beach seine off Port Said during a study period from 2018 to 2021. Fresh samples were immediately stored on ice and transported to the laboratory for dissection and collection of appropriate tissue samples for genetic identification.

3.2 DNA barcoding: Total DNA was extracted from muscles of the freezed samples (n= 24) using DNeasy Blood and Tissue Extraction Kit (Qiagen) following the manufacturing instructions. Both integrity and quantity of DNA were assessed using 1.5% agarose gel electrophoresis and NanoDrop (ND-1000), respectively. After that, COI from each sample was amplified via polymerase chain reaction (PCR) using:

LCO 1490 [5'- GGTCAACAAATCATAAAGATATTGG-3'] and HCO 2198 [5'-TAAACTTCAGGGTGACCAAAAAATCA-3'] primer set [24]

Each PCR reaction consisted of 12.5 µL of 2x PCR Master Mix (GeneDireX), 1 µL of each primer (20 pmol), 2 µL DNA template (20 ng/µL), and completed to a final volume of 25 µL with 8.5 µL deionized water. PCR samples were subjected to the following thermal conditions: initial denaturation at 94°C for 4 min followed by 30 cycles of 94°C for 45 secs, 53°C for 45 sec, and 72°C for 90 sec and final extension at 72°C for 7 min. COI products were then purified using QIAquick PCR Purification Kit (QIAGEN) and sequenced using 3500 Genetic Analyzer (Applied Biosystems) in the Applied Biotechnology CO. (Private lab in Ismailia).

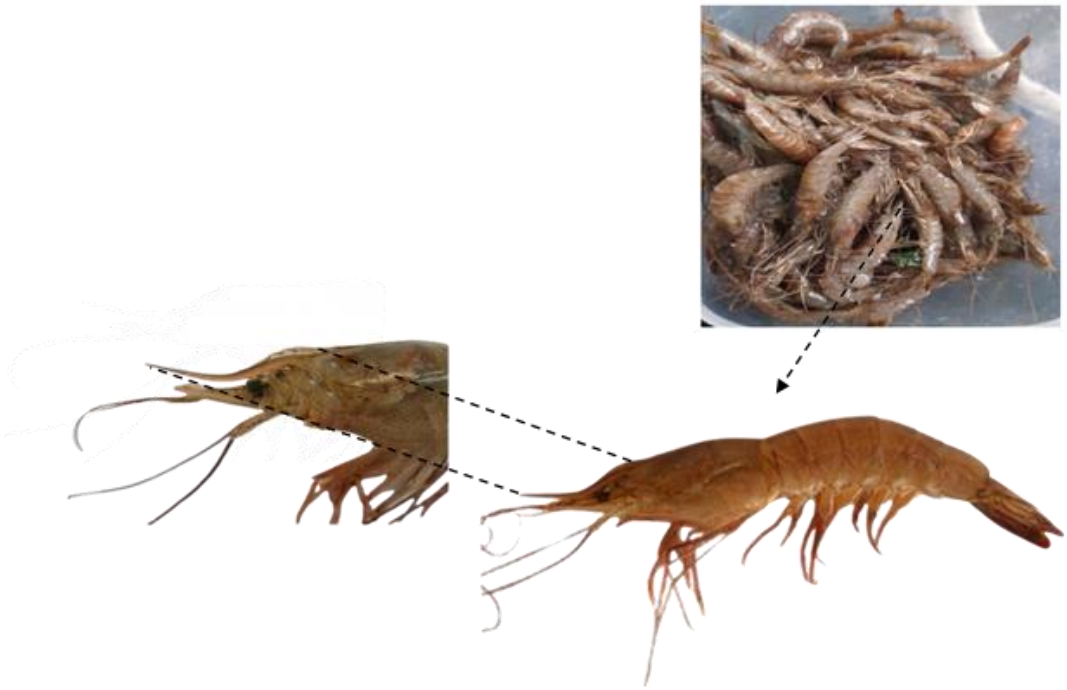
Sequences of COI were used to investigate the phylogenetic relationship between *X. kroyeri* recorded in the present study and that found in the Mediterranean and Atlantic region. To do that, raw sequences obtained by the current study were checked and edited, when required, and finally uploaded to the NCBI GenBank (accession numbers MW737452.1 and MW737453.1). Sequences obtained by the current study and those downloaded from NCBI system were then aligned using ClustalW. Phylogenetic tree was constructed based on the maximum likelihood method (ML) of 1000 bootstrap value under the Tamura 3-parameter substitution model [25]. *Portunus pelagicus* COI sequences were used to root the tree. The phylogenetic analysis and illustration were principally performed using MEGA version 10.2.6 [26].

4. RESULTS AND DISCUSSION

4.1. DNA barcoding: From the 24 samples analyzed, only two were successful in providing gDNA with sufficient purity and quantity. These samples were exclusively derived from the muscle tissue of the shrimp under investigation. Phylogenetic analysis showed that the successful examined samples (n= 2) are highly related to *X. kroyeri* inhabiting the Atlantic-Pacific region. Also, based on COI sequences, there were two main haplotypes, and the two samples collected from the Egyptian coast were belonging to one of those haplotypes.

Species identification and population discrimination were important in the conservation of biodiversity, natural resources, and fisheries management. Accurate identification of individual specimens was also necessary to investigate biological traits such as growth, mortality, fecundity, trophic relations, and parasite relationships [27].

In the present study, the role of genetic identification is indeed very important, as it provides information about the scientific name of this species, which has become economically significant and paves the way for conducting some biological studies that will be presented in later research. It also provides a basic understanding that this species likely entered the Mediterranean Sea through the ballast water of ships passing through the Strait of Gibraltar [23].



Fig, 2: *Xiphopenaeus kroyeri*

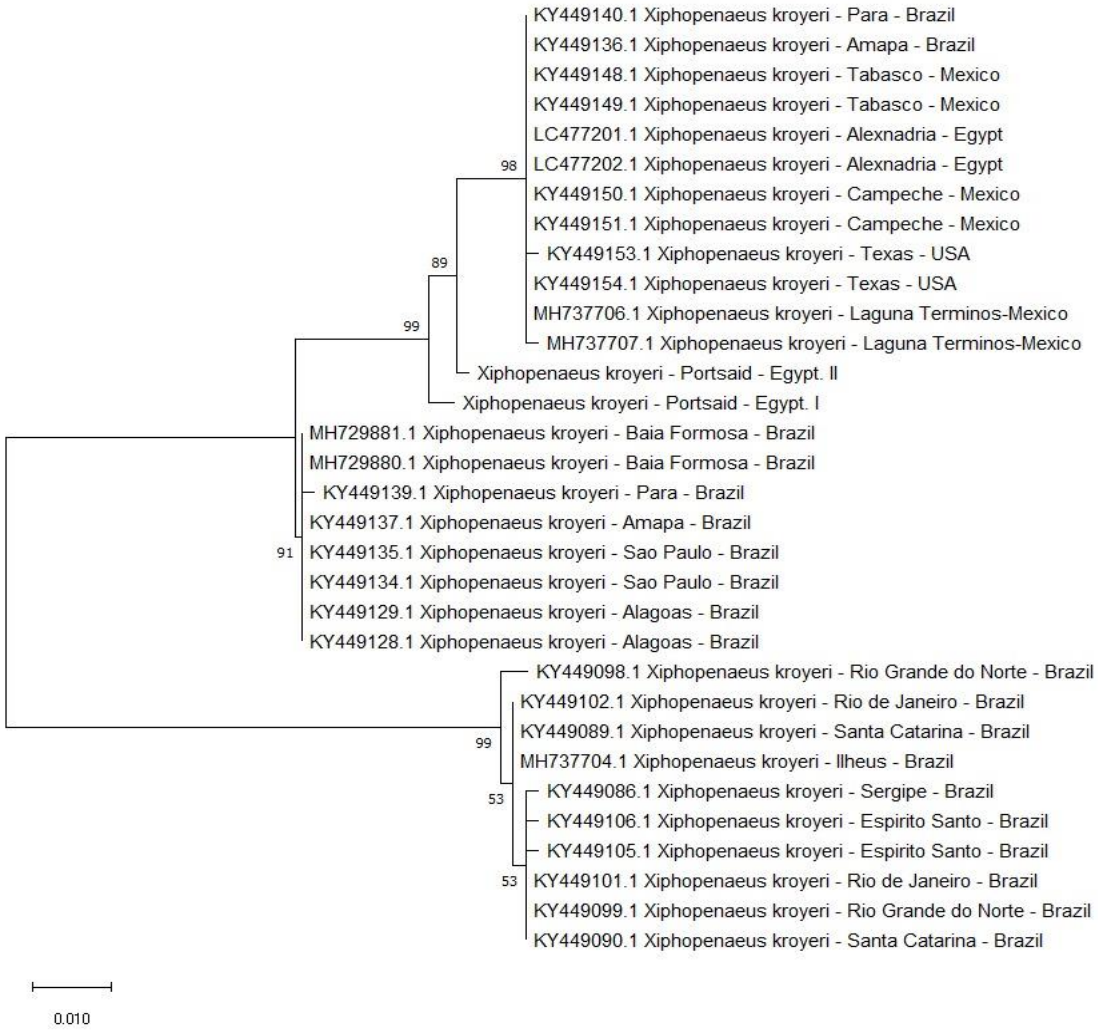
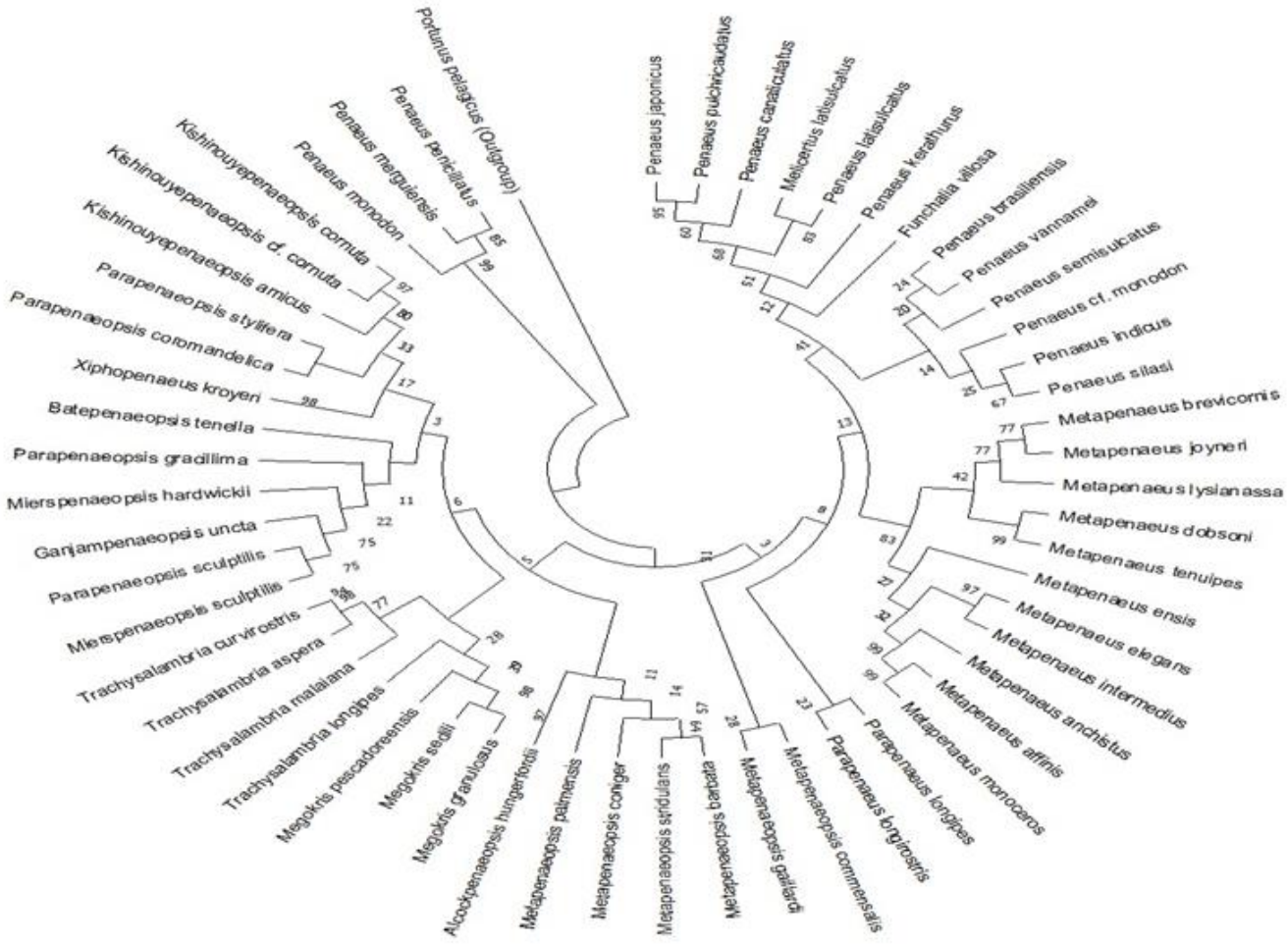


Fig. 3 : Phylogenetic relationship between *X. kroyeri* recorded in the present study and those retrieved from NCBI GenBank . Phylogenetic tree was constructed based on ML method of COI sequence alignment (right).



Fig,4: Phylogenetic relationship between *X. kroyeri* recorded in the present study and family Penaeidae from NCBI GenBank. Phylogenetic tree was constructed based on ML method of COI sequence alignment

5. CONCLUSION

The genetic study confirmed the genetic identity and species confirmation of this shrimp species. Additionally, the study indicated that this species has begun to establish itself in the Mediterranean Sea and has started to exhibit genetic diversity within the same population.

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