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Deep-Sea Rose: Decoding the Genetics and Preliminary Fishery of the Giant Red Shrimp *Aristaeomorpha foliacea* (Risso, 1827)

Taha Soliman[1], Eman M. Abbas[1], Zaki Sharawy[1]*, Engy T. Megahed[1], Hamdy O. Ahmed[1], Ola AbdelHady[2]

[1]National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt
[2]Faculty of Fish and Fisheries Technology, Aswan University, Egypt
*Corresponding Author: zaki_sharawy@yahoo.com

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ABSTRACT

This study investigated *Aristaeomorpha foliacea*, the exclusive species of Aristaeomorpha in the Mediterranean, Egypt, employing morphometric, molecular, and phylogenetic analyses. Morphometric assessments unveiled notable size variations between males and females, with males exhibiting a negative allometric growth pattern during spring. Utilizing *COI* and *16S rRNA* genes, molecular analysis demonstrated congruence between morphometrically and molecular taxonomy. Phylogenetic insights revealed genetic relationships between *A. foliacea* populations from Egypt, Portugal, Italy, Malta, and China, suggesting a migratory pattern from the Atlantic to the Mediterranean. The study identified a moderate genetic diversity in *A. foliacea*, with six haplotypes in both *COI* and *16S rRNA* regions, providing evolutionary insights. The integration of morphometric identification and phylogenetic study proved instrumental in elucidating the evolutionary relationships of this shrimp species. This interdisciplinary approach contributes valuable morphometric information for fisheries management, emphasizing the need for combined morphometric and DNA-based assessments for effective species identification and conservation. The research enhanced our understanding of *A. foliacea* in the Mediterranean, emphasizing the necessity for comprehensive approaches to species characterization and conservation.

INTRODUCTION

The giant red shrimp, *A. foliacea* (Risso 1827; Decapoda: Dendrobranchiata: Aristeidae), is a crustacean inhabiting muddy bottoms of the continental slope. It is typically found at depths up to 600 meters, especially in areas where canyons intersect the seabed; there have been observations of it at depths between 123 and 1145m **(Fernández *et al.*, 2013)**. This species holds an economic importance, drawing attention from fisheries since 1959 in the Western Mediterranean Sea, with additional fisheries established in Northwestern Australia and the Mozambique Channel **(Campillo, 1994; Wadley, 1994; Sobrino *et al.*, 2009)**.

Red shrimp exhibit a broad geographical range, as evidenced by several studies **(Guijarro *et al.*, 2019; Aydın & Tıraşın, 2023)**. In addition to the Mediterranean Sea, they existed in the Indian Ocean, the western and eastern Atlantic, the western and

eastern Pacific, and along South Africa's coastline. Abundance varies, with higher concentrations in the south and east of the Mediterranean **(Guillen *et al.*, 2012; Guijarro *et al.*, 2019)**. Notably, this tends to favor the eastern basin, which is characterized by warmer and more saline waters, as observed by **Politou *et al.* (2004)**.

The Mediterranean's hydrographical features, influenced by a complex marine circulation system, create oceanographical barriers impacting species migration and potentially fostering genetic diversity **(Sarà, 1985; Kritzer & Sale, 2006)**. Despite being first caught in Egypt in 2014, the giant red shrimp has gained recognition for its high market value in the European countries due to its nutritional value **(Bono *et al.*, 2012)**.

Molecular markers, particularly mitochondrial DNA (mtDNA), play a crucial role in understanding genetic composition and evolutionary history **(Avise, 2004; Waples *et al.*, 2008)**. Previous molecular studies on *A. foliacea*, utilizing ISSRs, revealed minimal genetic differentiation across the Mediterranean and Mozambique Channel populations **(Fernández *et al.*, 2011)**. **Fernández *et al.* (2013)** employed the mitochondrial *COI* gene to assess mtDNA variation and regional population structure, revealing medium to high levels of genetic differentiation, consistent with other shrimp species **(Reuschel *et al.*, 2010; Fernández *et al.*, 2011)**.

Various researchers conducted morphometric and genomic investigations to confirm decapod species in Egypt **(Sharawy *et al.*, 2017; Abbas *et al.*, 2018a, 2021, 2022)**. This study represented the initial examination of *A. foliacea* from the Mediterranean Sea in Egypt, combining morphometric and molecular identification techniques. Utilizing *COI* and *16S rRNA* mitochondrial barcoding genes, alongside morphometric characteristics, provides valuable insights into the economic aspects of producing this species. These findings support an immediate stock evaluation and effective management strategies, serving as a resource for identifying suitable stocks for profitable hatcheries and enhancing the economic benefits of shrimp aquaculture in Egypt.

MATERIALS AND METHODS

1. Sampling and measurements

The samples of *A. foliacea* were collected in the spring of 2022 at Abo Qir, a fish landing site in Alexandria, Egypt. Individual lengths were measured to the closest 0.1cm using a Matlab program to reduce biases from various sources and assure an improved precision. The images were taken with a USB camera that has a 1:2.8-4916.3-25.2 ASPH lens (Fig. 1). Furthermore, the photos were saved for additional measurements or verification when necessary **(Sharawy *et al.*, 2017, 2019)**.

To eliminate the natural curvature of the *A. foliacea* body, individual *A. foliacea* specimens that had entire rostrums were manually straightened on millimeter paper. Carapace length (CL, cm) is the distance between the rear margins of the orbit to the posterior border of the carapace. Additionally, wet weight (Wt, g) was measured using an electronic digital balance. These measurements were taken to determine the various characteristics of the carapace.

Before conducting morphometric measures, the sex of *A. foliacea* was determined based on the presence or absence of the petasma. During the sample period, we used a

binomial test to look for discrepancies between the observed and predicted sex ratios (1:1) of *A. foliacea* **(Wilson & Hardy, 2002)**.

Fig. 1. *Aristaeomorpha foliacea* - Photomicrograph capturing an individual specimen

2. Carapace length- weight relationship (CLWR)

The carapace length- weight relationship (CLWR) was determined using the log transformation technique developed by **Le Cren (1951)**. The equation $Wt = aL^b$ was used to predict the correlation between the weight and carapace length of the shrimp. Linear regression was applied to log(Wt) = log(a) + b log(CL) to get the intercept (a) and slope (b) of the relationship. CLWRs were created to address periodic fluctuations that could impact b **(Zargar *et al.*, 2012)**. When the value of b differed from the optimal value of 3, it signifies an isometric growth as described by **Ricker and Carter (1958)**. When b is less than 3, *A. foliacea* exhibits a negative allometric growth, becoming narrower as its length increases. When b is more than 3, the shrimp's weight increases, indicating a positive allometric development and implying ideal growth conditions.

3. Condition factor (K)

K was determined to evaluate the current condition of various *A. foliacea* individuals, distinguishing between males and females in the research. K is calculated by dividing the weight of *A. foliacea* by the product of a; CL raised to the power of b, where CL is the carapace length, and a and b are constants from the CLWRs **(Le Cren, 1951)**. An organism is in good growth condition when $K \geq 1$, and in bad growth condition when $K < 1$.

4. Molecular analysis

4.1. DNA extraction and PCR amplification

DNA extraction (fifteen samples) was carried out using the HotSHOT DNA extraction method **(Montero-Pau *et al.*, 2008)**. An alkaline lysis buffer (pH 12) containing 25mM NaOH and 0.2mM Na2EDTA was employed, and the neutralizing solution (pH 5) comprised 40mM Tris-HCl, explicitly discouraging the use of TRIS base. The process involved aliquoting 50µl of the lysis buffer into 0.2 PCR tubes. In case of the shrimp *A. foliacea*, 50mg of shrimp flesh was utilized, individually transferred to each tube, and crushed with a pipette tip. Subsequently, incubation at 95 ºC for 30 minutes was performed, followed by placing the samples on ice for 4- 5 minutes, with an optional centrifugation. The addition of 50µl of neutralizing solution, vortexing, and subsequent

centrifugation concluded the extraction process. Subsequently, 2µl of the extracted DNA was used for PCR and stored at either 4 or -20ºC for long-term preservation. For PCR amplification, specific regions targeting the *16S rRNA* region used the primers *16S rRNA* region utilized primers 16SARLpan-T (5'-TGCCTGTTTATCAAAAACAT-3') and 16SBRHpan (5'-CCGGTCTGAACTCAAATCATGT-3'), following the protocol outlined by **Roldán *et al.* (2009)**. The *COI* region was amplified and sequenced using the universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') according to **Folmer *et al.* (1994)**.

PCR conditions involved an initial denaturation at 95°C for 5 minutes, followed by denaturation at 98°C for 20 seconds, annealing at 54°C for 20 seconds, and extension at 72°C for 30 seconds. A final extension at 72°C for 5 minutes concluded the PCR process, with a holding temperature set at 4°C indefinitely.

Electrophoresis on a 1.4% agarose gel stained with 25µg of ethidium bromide was used to find the amplified products. By employing the Isolate II PCR and Gel Extraction Kit obtained from Bioline in London, UK, distinct bands of the anticipated dimensions were isolated from each sample. Utilizing the USA Applied Biosystems ABI3730 Sequencer and the Big Dye Terminator Cycle Sequencing Kit, DNA fragments were sequenced. The sequencing PCR procedure commenced with a two-minute incubation at 96°C, which was followed by twenty-five cycles of certain temperature and time intervals **(Abbas *et al.*, 2011)**.

4.2. Sequencing, phylogenetic, and statistical analysis

The COI and *16S rRNA* gene sequences were modified using Chromas Lite software version 2.5 from Technelysium Pty Ltd and analyzed with BioEdit 7.2.6.1. The Clustal W program was utilized for nucleotide gap analysis and alignment. The partial coding sequences of the *COI* gene and 16S rRNA sequences from the Egyptian *A. foliacea* samples were analyzed against the GenBank database using BLAST for verification of identity.

The study's sequence data were submitted to GenBank with the following accession numbers: PP230861-PP230875 for *COI* and PP230845-PP230859 for the *16S rRNA* regions, creating a detailed reference for future research and examination. The *16S rRNA* and *COI* sequences of *A. foliacea* from the GenBank database were retrieved and aligned using Clustal W software **(Thompson *et al.*, 1994)**, a feature of the MEGA X program **(Kumar *et al.*, 2018)**. The dataset comprised 24 *COI* sequences (15 from the present study and 9 from GenBank), with a selected region of 476 base pairs. A total of 15 sequences from this study were combined with 7 sequences obtained from GenBank for analysis, with a selected region of 470 base pairs.

The pairwise genetic distances were computed utilizing the MEGA X software in conjunction with the FASTA format for the sequences. Maximum likelihood (ML) trees were generated for the crustacean species using 1000 bootstrap replicates and the Kimura 2-parameter method **(Kimura, 1980)**. Haplotype networks were inferred using PopART **(Leigh & Bryant, 2015)**. Genetic diversity and polymorphism were assessed using DnaSP v6 **(Rozas *et al.*, 2003)**, providing measures of haplotype diversity, nucleotide diversity, and Tajima's D.

RESULTS

1. Morphometric characteristics

The characteristics related to the sample size, length, and weight are outlined in Table (1). The total number of *A. foliacea* individuals analyzed was 317 (184 males and 133 females). The mean CL± SD and Wt± SD were 3.27± 0.46cm and 6.26± 2.64g for males and 3.31± 0.45cm and 6.30± 2.13g for females, respectively. The minimum and maximum CL and Wt for males were 1.9- 4.4cm and 1.49- 15.44g, respectively, while for females, they were 2.1- 4.2cm and 1.68- 11.65g, respectively. Males and females showed a noticeable variation in both carapace length and weight, with a male-to-female ratio of 1.38. In the current study, the K values of the evaluated males and females fluctuated from 0.71 to 1.44 and from 0.88 to 1.19, as shown in Table (1); these values indicate that the examined species are in a state of well-being.

Table 1. Carapace length- weight relationship (CLWR) for *A. foliacea* collected during spring 2022

Parameter	Male	Female
Sample size	184	133
CL range	1.9-4.4cm	2.1-4.2cm
Wt range	1.49-15.44g	1.68-11.65g
a	0.2106	0.2524
b±SE	2.81±0.04	2.65±0.08
r^2	96.85	92.23
P*	0.000	0.000
t-test**	0.000	0.000
K range	0.71-1.44	0.88-1.19
Growth behavior	Negative allometric	Negative allometric

*Significance of regression with $P< 0.05$.

**A *t-test* was performed to see whether the value of b was significantly different from 3.

2. Phylogenetic analysis and pairwise distance

Thirty mitochondrial region sequences were obtained from *A. foliacea* for *COI* and *16S rRNA* genes. All sequences were simple and clear, without any insertions, deletions, or stop codons. BLAST was used to analyze a similarity search by comparing the obtained sequences with their respective counterparts. The results revealed a substantial degree of resemblance (≤ 99%) between the examined sequences and those found in the GenBank database. This was further supported by aligning the obtained *COI* and *16S rRNA* sequences (which showed a 99% similarity with *A. foliacea*) and conducting a morphometric assessment. According to the GenBank database references, ML trees made with both *COI* and *16S rRNA* displayed distinct clusters for *A. foliacea*. Due to variable positions between the sequences from Egypt in some nucleotides, only four haplotypes (*COI* gene) and two (*16S rRNA* gene) out of fifteen sequences were used to construct the trees. The *COI*-based ML tree (for the sequences from Egypt and different countries) grouped the studied species into three distinct clades. The first clade included *A. foliacea* from China, with one sequence from Portugal separated in the second clade. The third clade was divided into two subclades; the first subclade included one of the

Egyptian sequences and the other from Malta. While, the second subclade represented the sequences from Egypt clustering with the sequences from the Mediterranean individuals and the Atlantic Ocean (Fig. 2). For *COI*-based pairwise distances (Table 2), the Kimura 2-parameter (K2P) model was used to assess genetic distances. The analysis of genetic distances among *A. foliacea* from different countries (Egypt, Portugal, China, Malta, and Italy) showed the maximum genetic distance (0.050) between the samples from China (accession no. NC_039153) and Italy (acc. No. JQ305887). However, the genetic distances were 0.00 between the samples from Egypt and each of these countries: Malta, Italy, and Portugal. Despite the limited number of sequences in the GenBank database, phylogenetic analysis for the *16S rRNA* gene of *A. foliacea* showed a clear clustering for the Egyptian sequences (acc. no. LC310708, PP230845) with their counterparts from Spain (acc. no. MN816694, MN816693) (Fig. 3), and the genetic distances between these sequences were 0.000 (Table 3).

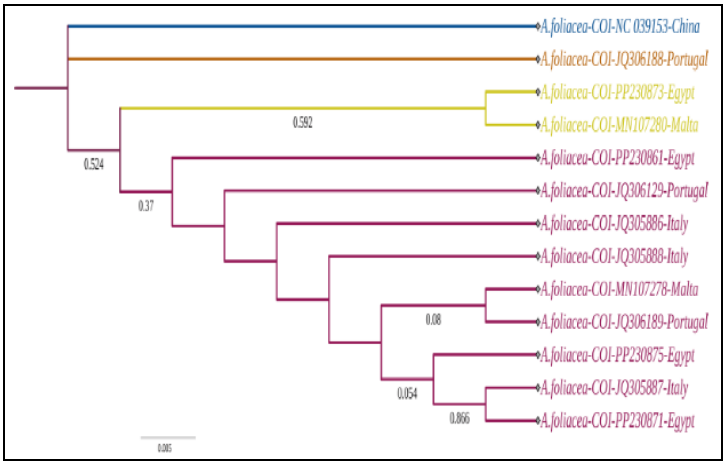

Fig. 2. Phylogenetic tree based on ML analysis of *COI* gene sequences of *A. foliacea* from the Mediterranean Sea and sequences from the GenBank database for the same species

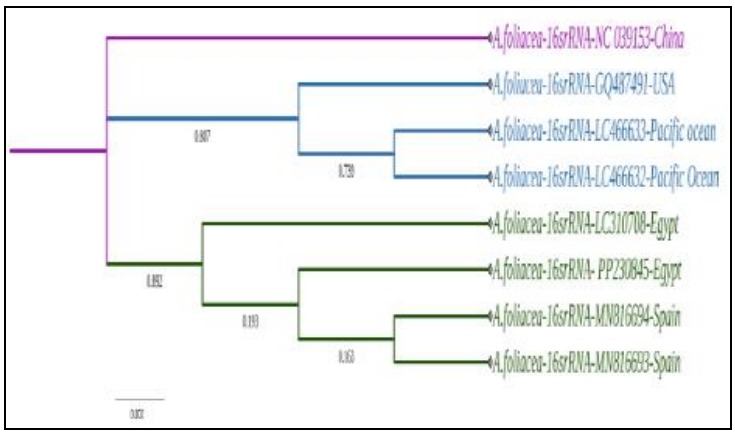

Fig. 3. ML phylogenetic tree generated based on *16S rRNA* gene sequences of *A. foliacea* obtained from the GenBank database and samples collected in the Mediterranean Sea

Table 2. Pairwise distances based on the *COI* gene between Egyptian *A. foliacea* samples and other GenBank-accessible species that are related

	JQ305886-Italy	JQ305887-Italy	JQ305888-Italy	JQ306129-Portugal	JQ306188-Portugal	JQ306189-Portugal	MN107278-Malta	MN107280-Malta	NC_039153-China	PP230861-Egypt	PP230871-Egypt	PP230873-Egypt	PP230875-Egypt
JQ305886-Italy													
JQ305887-Italy	0.004												
JQ305888-Italy	0.000	0.004											
JQ306129-Portugal	0.000	0.004	0.000										
JQ306188-Portugal	0.002	0.006	0.002	0.002									
JQ306189-Portugal	0.000	0.004	0.000	0.000	0.002								
MN107278-Malta	0.000	0.004	0.000	0.000	0.002	0.000							
MN107280-Malta	0.002	0.006	0.002	0.002	0.004	0.002	0.002						
NC_039153-China	0.046	0.051	0.046	0.046	0.044	0.046	0.046	0.044					
PP230861-Egypt	0.000	0.004	0.000	0.000	0.002	0.000	0.000	0.002	0.046				
PP230871-Egypt	0.004	0.000	0.004	0.004	0.006	0.004	0.004	0.006	0.051	0.004			
PP230873-Egypt	0.002	0.006	0.002	0.002	0.004	0.002	0.002	0.000	0.044	0.002	0.006		
PP230875-Egypt	0.002	0.006	0.002	0.002	0.004	0.002	0.002	0.004	0.048	0.002	0.006	0.004	

Table 3. Pairwise distances between Egyptian *A. foliacea* samples and other closely related species as determined by the *16S rRNA* gene and the GenBank database

	PP230845-Egypt	GQ487491-USA	LC310708-Egypt	LC466632-Pacific Ocean	LC466633-Pacific Ocean	MN816693-Spain	MN816694-Spain	NC_039153-China
PP230845-Egypt								
GQ487491-USA	0.010							
LC310708-Egypt	0.000	0.010						
LC466632-Pacific Ocean	0.010	0.000	0.011					
LC466633-Pacific Ocean	0.010	0.000	0.011	0.000				
MN816693-Spain	0.000	0.010	0.000	0.010	0.011			
MN816694-Spain	0.000	0.010	0.002	0.010	0.013	0.002		
NC_039153-China	0.010	0.020	0.013	0.020	0.015	0.013	0.015	

3. Genetic diversity and haplotype distribution

For the *COI* gene region, 24 variable sites were identified, comprising 20 singletons and 4 parsimony informative sites. Six haplotypes were detected, with a haplotype diversity (Hd) of 0.500 and nucleotide diversity (Pi) of 0.005 (Fig. 4). The *16S rRNA* gene region revealed 10 variable sites, with 5 singletons and 5 parsimony informative sites (Fig. 5). Six haplotypes were determined, indicating a moderate genetic diversity (Hd of 0.407 and Pi of 0.004). Tajima's D for the *COI* gene was -2.338 ($P< 0.01$) and -1.231 ($P> 0.10$) for the *16S rRNA* gene. The average number of nucleotide differences (k) was 2.373 for *COI* and 1.753 for *16S rRNA*.

In the analysis of the mitochondrial *COI* gene, six diverse haplotypes were identified across five geographic locations: Egypt, Malta, Portugal, Italy, and China. Haplotype 1 (Hap1) was the most dominant, found in all plotted locations except China. Hap2 was present in Egypt and Italy, while Hap3 was shared between Egypt and Malta. Hap4 was unique to Egypt, Hap5 to Portugal, and Hap6 exclusively to China. The analysis of the *16S rRNA* gene revealed six haplotypes distributed among Egypt, Spain, the Pacific Ocean, China, and the USA. Hap 1 was dominant in Egypt. Furthermore, Hap2 and Hap6 were unique to China, USA, the Pacific Ocean, Spain, and Egypt, respectively.

The phylogenetic analysis of the *COI* gene showed significant divergence between the Chinese haplotype (Hap6) and those from other regions, with 20 nucleotide differences. For the *16S rRNA* gene, the network analysis revealed that Hap1 was closely related to both Hap5 and Hap6 with a single nucleotide difference, while there was a more noticeable departure between Hap6 and Hap2, with six nucleotide differences. Hap3 (USA) and Hap4 (Pacific Ocean) were separated by only one nucleotide difference.

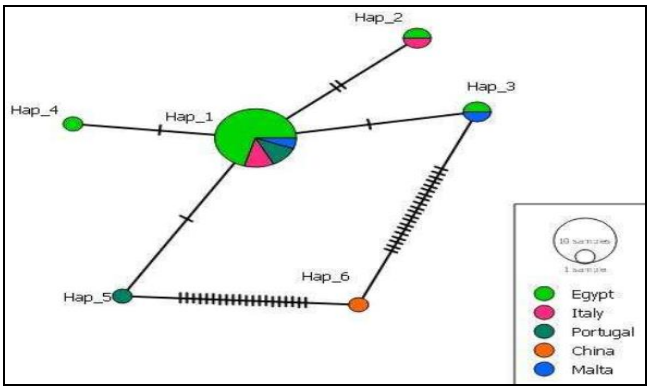

Fig. 4. The most parsimonious network for haplotypes of *COI* gene in *A. foliacea* species illustrating 6 haplotypes

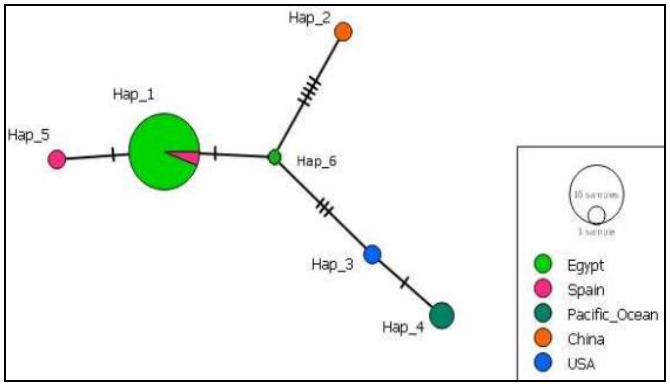

Fig. 5. The most parsimonious network for haplotypes of *16S rRNA* gene of *A. foliacea* species illustrating 6 haplotypes

DISCUSSION

This study presents *A. foliacea* as the sole species of Aristaeomorpha found in the Mediterranean Sea of Egypt. Despite being executed within a comparatively brief timeframe of three months, this research provides a morphometric description, molecular characterization, and phylogenetic relationship of the giant red shrimp *A. foliacea*.

The high coefficient of determination values (r^2) and significant correlation ($P>$ 0.05) during the spring season indicate an accurate prediction of the regression for *A. foliacea*. This suggests that catches can be reliably extrapolated in such specific condition areas for this size range. The negative allometric growth seen in males and females of *A. foliacea* (b< 3) indicates a slow growth rate with a typically slender shape. A statistically significant variation was observed in the estimated b values of the CLWRs between male and female of red *A. foliacea* specimens. According to our findings, males and females

displayed a negative allometric growth during the entire spring season. **Aydın and Tıraşın (2023)** documented that males demonstrated an isometric growth during January, May, June, and July 2017, in addition to August and October 2016. Nonetheless, the males showed no allometric development even for the remaining months. On the other hand, a negative allometric growth was the feature of female growth. **Righini and Abella (1994)** revealed that the Tyrrhenian Sea female *A. foliacea* exhibited a negative allometric development, whereas males exhibited an isometric growth. Furthermore, **Spedicato *et al.* (1994)** found a negative allometric growth in the Tyrrhenian Sea for three seasons and only determined CLWR for females. **Ragonese *et al.* (1997)** observed a negative allometric development in the Strait of Sicily for both sexes of *A. foliacea*. In the Ionian Sea, **Kapiris *et al.* (1998)** and **Kapiris (2005)** reported that females exhibited higher b values in comparison to males. On the contrary, the current study corroborates the results reported by **Deval (2019)**, **Righini and Abella (1994)** and **Aydın and Tıraşın (2023)**.

The sex ratios of *A. foliacea* have been observed to differ across regions, seasons, and depths, according to several studies **(Fiorentino *et al.*, 2013; Aydın & Tıraşın, 2023)**. A recent study found that *A. foliacea* had a sex ratio of 1:1.38, favoring males. This is applied to some water bodies as for example, the Tyrrhenian Sea **(Leonardi & Ardizzone, 1994)**, the Aegean Sea **(Cau *et al.*, 2002)**, the eastern Ionian Sea **(Politou *et al.*, 2004)**, and the Mediterranean Sea **(Can & Aktaş, 2005; Aydın & Tıraşın, 2023)**. On the other hand, several studies have shown a higher proportion of females in various regions, such as the Tyrrhenian Sea, Sicilian Channel, Ionian Sea, and western and central Mediterranean Sea.

The molecular-based approach entails sequencing a small gene region that has been standardized to identify and recognize various species of organisms discovered over the past two decades **(Hebert *et al.*, 2003)**. This method does not intend to dismiss the morphometries, and its overarching goal is to establish a partnership between morphometric and molecular information to facilitate the identification of species in a manner that is both speedy and unambiguous **(Sharawy *et al.*, 2017; Abbas *et al.*, 2021)**. Several shrimp species have been examined to confirm a high degree of agreement between morphometric and DNA-based crustacean identifications utilizing *COI* and *16S rRNA* **(Mata *et al.*, 2009; Abbas *et al.*, 2022; Hairani *et al.*, 2023)**. It is important to note that *COI* is a reliable marker for identifying shrimp species **(Abbas *et al.*, 2022)**. Additionally, the *16S rRNA* gene has been proposed as an even better identifier, particularly for higher crustacean species **(Galal-Khallaf *et al.*, 2016; AL-Qurashi & Saad, 2022)**. Right now, there has been a complete absence of genetically connected morphometric clues to authenticate species that belong to this species. To the best of our knowledge, this research is the first to give morphometric as well as genetic signs of *A. foliacea*. Thus, two molecular markers, *COI* and *16S rRNA*, were used to characterize *A. foliacea* genetically. The discovered sequences' lack of stop codons provided an evidence considering that the whole coding region had been amplified. The similarity between the reference sequences in the GenBank databases and the acquired *COI* sequences was used to verify that our data set was devoid of nuclear mitochondrial DNA **(Ward *et al.*, 2005)**.

Based on *COI* phylogenetic analysis, the Egyptian *A. foliacea* was found to have a close relationship with its Italian and Maltese counterparts, which placed them in a sub-clade with the Egyptian specimen. The other *A. foliacea* specimens were found to be

from Portugal, Italy, Malta, and China. On the other hand, the ML tree classified the Chinese and Portuguese individuals into different clades. Based on these phylogenetic relationships, it was postulated that *A. foliacea* may have migrated from the Atlantic Ocean to the Mediterranean Sea, which explained its wide regional range **(Guijarro *et al.*, 2019)**. Despite the natural barrier that separates these distant regions, this may explain why there is such a high degree of similarity. Following the fact that *A. foliacea* is widely dispersed throughout the Mediterranean Sea, it has a higher abundance observed in the southern and eastern regions **(Guillen *et al.*, 2012; Guijarro *et al.*, 2019; Aydın & Tıraşın, 2023)**.

The examination of the *16S rRNA*-based tree allows for the determination of the phylogenetic relationship between *A. foliacea* from Egypt and other countries. This analysis reveals various clustering patterns that provide valuable information. The *16S rRNA* sequences of *A. foliacea* samples from Egypt were found to be directly aligned with their corresponding GenBank references in the same subclade. This alignment confirms the findings obtained from the morphometric description. Remarkably, the clustering pattern deduced from the study of *16S rRNA* closely corresponds to the pattern deduced from the analysis of *COI* gene segments. Genetic distance and phylogenetic relationships between *A. foliacea* in Egypt and other countries' populations demonstrated a close relationship between the Egyptian and non-Egyptian *A. foliacea* individuals, who belonged to the same clades and subclades, with no genetic distances between them. The phylogenetic analysis showed an extensive association between three Egyptian shrimp species and those from other countries since they were grouped in the same clades **(Sharawy *et al.*, 2017)**.

The current study on *A. foliacea* has revealed a moderate genetic diversity, demonstrated by the identification of six haplotypes in both the *COI* and *16S rRNA* gene regions. This finding is reliable with other species, where *COI* and *16S rRNA* often reveal genetic diversity. For instance, **Kim *et al.* (2023)** study on *Nodularia breviconcha* demonstrated considerable genetic variation across different populations using these markers. This is equivalent to the study of **Abbas *et al.* (2018b)**, where the *COI* marker is used to explore a genetic diversity in *Diplodus sargus* and *Diplodus vulgaris*. These studies highlighted the role of mitochondrial DNA in the detection of genetic diversity.

Moreover, similar patterns of haplotype distribution and differentiation have occurred in various studies, emphasizing the influence of geographical barriers in determining genetic diversity **(Kim *et al.*, 2023)**. In contrast, the study by **Wei *et al.* (2023)**, which examined *Pseudaspius leptocephalus*, detected only five haplotypes among 85 individuals across four populations, with nucleotide diversity ranging from 0.00027 to 0.00065. This is notably less than the diversity found in *A. foliacea* in this study, which displayed a higher haplotype diversity (Hd) of 0.500 and nucleotide diversity (Pi) of 0.00499. The research by **Matzen da Silva *et al.* (2011)** on a wide selection of decapods, contributing *COI* data for 528 species, highlighted significant taxonomic ambiguities. They also noted diverse nucleotide compositions. This stands in contrast to our intensive analysis of *A. foliacea*, where we observed distinct haplotype distribution and moderate genetic diversity within a single species across various regions. This proposes the importance of genetic drift in prompting genetic diversity within a species. Studies on diverse species like *Holothuria atra* and cultivable carp have also employed these genetic

markers, yielding significant insights into their genetic structure and diversity **(Mohanty *et al.*, 2015; Hamamoto *et al.*, 2021)**.

Molecular identification techniques accurately confirmed the morphometrically identified species. The fusion of genetic and morphometric identification has produced a robust approach to shrimp species identification. Additionally, this method can assist in resolving questions about molecular identification.

CONCLUSION

In this study, *A. foliacea*, identified as the sole species of Aristaeomorpha in the Mediterranean Sea off the coast of Egypt, underwent a comprehensive morphometric, molecular, and phylogenetic analysis. The investigation lasted three months, offering valuable insights into the giant red shrimp's characteristics and its population dynamics. Morphometric analysis revealed significant differences in size between male and female *A. foliacea*, with males exhibiting a negative allometric growth pattern during the spring season. This finding aligns with previous studies, emphasizing the variability in growth patterns across different regions and seasons.

The molecular approach, utilizing *COI* and *16S rRNA* genes, provided a reliable means of species identification. The congruence between morphometric and molecular taxonomic methods highlighted the effectiveness of combining these approaches for an accurate and efficient species identification. Phylogenetic analysis unveiled the genetic relationships of *A. foliacea* populations from Egypt with those from Portugal, Italy, Malta, and China. The clustering patterns suggested a close association between the Egyptian specimens and their counterparts from Italy and Malta, supporting the hypothesis of *A. foliacea*'s migration from the Atlantic Ocean to the Mediterranean Sea.

The study demonstrated a moderate genetic diversity in *A. foliacea*, evidenced by the identification of six haplotypes in both *COI* and *16S rRNA* gene regions. This genetic diversity, as indicated by haplotype distribution and nucleotide diversity, provides valuable information about the species' evolutionary history and adaptation. In conclusion, morphometric identification combined with a phylogenetic study can serve as a collaborative tool to verify the evolutionary relationships of these significant shrimp species. The present study has offered essential morphometric relationship information for managing the examined shrimp species from the Mediterranean Sea for fisheries applications. Thereby, utilizing both morphometric and DNA-based species evaluation methods may be implemented to monitor and conserve the native species in this ecosystem and avoid the introduction of invasive species. This interdisciplinary study not only enhances our knowledge of *A. foliacea* in the Mediterranean but also underscores the significance of integrating morphometric and molecular approaches for robust species characterization and conservation management.

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