Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(2): 2347 – 2356 (2025)
www.ejabf.journals.ekb.eg

Molecular Identification of the Bivalves *Corbicula fluminea* and *Sinanodonta woodiana* in Thi-Qar Province, Iraq, Using Mitochondrial COX-I Gene Sequencing

Zainab Mashjel and Qasim Al-Taher*
Department of Sciences, College Basic of Education, University of Sumer, Iraq
*Corresponding Author: qasim20020@gmail.com

ARTICLE INFO
Article History:
Received: Jan. 26, 2025
Accepted: March 31, 2025
Online: April 17, 2025

Keywords:
Molecular characterization,
Bivalvia species,
Thi-Qar province,
Iraq

ABSTRACT

The present study was conducted to identify and characterize two bivalve species, *Sinanodonta woodiana* and *Corbicula fluminea*, collected from Thi-Qar Province, southern Iraq, based on sequence analysis of the mitochondrial cytochrome oxidase subunit 1 (COX1) gene. Samples of *S. woodiana* and *C. fluminea* were collected from the Al-Gharraf River in Thi-Qar Province from October 2023 to September 2024. Genomic DNA was extracted from 10 samples—five individuals each of *S. woodiana* and *C. fluminea*. The mitochondrial COX1 gene was amplified by PCR, and the amplicons (710 bp) were subsequently sequenced. Sequence analysis revealed that the identified *S. woodiana* sequences showed 99–100% similarity compared to international samples available in the NCBI database. Two COX1 sequences of *S. woodiana* obtained in this study have been deposited in GenBank with accession numbers LC833751.1 and LC833752.1, along with three sequences of *C. fluminea* (LC833753.1, LC833754.1, and LC833755.1). Phylogenetic analysis placed the Iraqi specimens of both species in clusters with their counterparts from various global regions. The findings of this study also highlight genetic variations within these two bivalve species.

INTRODUCTION

Bivalve molluscs represent an important class of organisms that significantly impact human society, serving as vital food sources and sentinel organisms indicating environmental pollution. Accurate species identification is crucial for effective aquaculture management, biodiversity studies, and safe human consumption **(Mathew & Ampili, 2022)**. Invertebrate morphological patterns are largely governed by gene regulatory networks—groups of genes that collaboratively control organismal development, directing cellular communication and differentiation during early developmental stages. Traditional species identification methods rely predominantly on morphological observations, often resulting in overly broad classifications and species units that are not optimal for conservation. Recently, high-throughput sequencing (HTS) technologies have revolutionized approaches to defining species boundaries, enabling the detailed analysis of intra-species diversity through genome-wide data. Molecular techniques can uncover cryptic species undetectable through morphology alone

(Hosegood *et al.*, 2018). Both morphological and molecular methods have previously been employed successfully for resolving identification challenges, such as distinguishing *Fasciola* species in Thi-Qar Province **(Hansh, 2024)**. Molecular diagnostics in animals involves employing molecular methodologies to identify and characterize animal species, populations, or diseases—a practice increasingly significantly in conservation biology. Molecular diagnostics serve as powerful tools that enhance biodiversity assessments and strengthen conservation efforts. Specifically, genetic markers in molluscs, such as mitochondrial COI and nuclear ITS2, play pivotal roles in species identification, assessing environmental health, and managing aquaculture practices. These genetic tools are fundamental for ensuring food safety and understanding evolutionary relationships within this diverse group of organisms **(Ampili & Mathew, 2022)**.

MATERIALS AND METHODS

Sample collection

Samples for molecular analysis were collected from four sites along the Gharraf River in Thi-Qar Province, Iraq, during the period from September 2023 to October 2024. A total of ten bivalve samples were selected for molecular examination: five specimens belonging to *Sinanodonta woodiana* and five to *Corbicula fluminea*.

Molecular study

DNA extraction

A small tissue sample from the foot region of each of the two bivalve species was obtained using sterilized scissors for genomic DNA extraction. Genomic DNA was extracted using the WizPrep™ gDNA Mini Kit (Cell/Tissue) according to the manufacturer's instructions and stored at -20°C until subsequent analysis.

Polymerase chain reaction (PCR)

PCR amplification of the mitochondrial COX-I gene was conducted on genomic DNA samples from both bivalve species using primers described by **Folmer *et al.* (1994)**: the forward primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and the reverse primer HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). Each PCR reaction was prepared to a final volume of 25µL containing 5µL PCR PreMix (AccuPower® PCR PreMix, Bioneer, Korea), 1µL of each primer (forward and reverse, 10 pmol each), 5µL genomic DNA, and 13µL nuclease-free water. The PCR amplification was performed in a thermal cycler using the following conditions: initial denaturation at 95°C for 10 minutes; followed by 30 cycles consisting of denaturation at 95°C for 35 seconds, annealing at 51°C for 35 seconds, and extension at 72°C for 35 seconds; with a final extension step at 72°C for 10 minutes. PCR products were analyzed using electrophoresis on a 1.5% agarose gel prepared with TBE buffer and stained with ethidium bromide. Visualization and imaging of PCR products were conducted using a UV transilluminator.

DNA sequencing and phylogenetic analysis

Ten PCR products (five from each species) corresponding to the mitochondrial COX-I gene were sent to Macrogen Inc. (South Korea) for sequencing. The resulting sequences were submitted to the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov), and accession numbers were obtained for five sequences. Sequence comparisons were conducted using the Basic Local Alignment Search Tool (BLAST) to align sequences from this study with previously published sequences for the two bivalve species in the GenBank database. Phylogenetic analysis was performed using MEGA 11 software **(Tamura *et al.*, 2021)**, employing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) for phylogenetic tree construction. Evolutionary distances were calculated using the Maximum Composite Likelihood method **(Tamura *et al.*, 2004)**.

RESULTS

1. Molecular study

In the current study, a total of 10 bivalve samples representing two species (five samples each of *Sinanodonta woodiana* and *Corbicula fluminea*) were analyzed. PCR amplification targeting the mitochondrial COX-I gene was performed on genomic DNA extracted from all samples. A single PCR product of approximately 710 bp was successfully amplified using mitochondrial COX-I primers. PCR products were confirmed by electrophoresis on a 1.5% agarose gel.

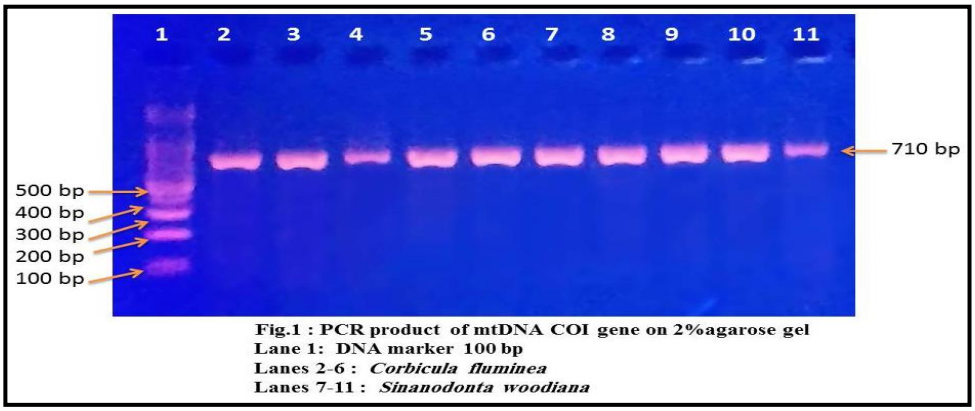

Fig. 1 Electrophoresis of mt *COI* gene. 1 lane: DNA Ladder. Lane 2-6 *Sinanodonta woodiana* species. Lane 5-8 *Corbicula fluminea* species

1.1. Molecular analysis

Partial mitochondrial COX-I (mtCOX-I) gene fragments (710bp) from 10 PCR samples were directly sequenced in this study and analyzed for sequence similarity. The resulting mtCOX-I gene sequences demonstrated high similarity with reference sequences of *Sinanodonta woodiana* and *Corbicula fluminea* available in the GenBank database, as revealed by BLASTn alignment. Two sequences of *Sinanodonta woodiana* obtained in this study were deposited in GenBank for the first time, under accession

numbers LC833751 and LC833752. Additionally, three sequences from *Corbicula fluminea* were also newly submitted to GenBank under accession numbers LC833753, LC833754, and LC833755. All sequences exhibited 99–100% similarity with the reference sequences. Alignment analysis of the newly recorded mtCOX-I sequences revealed specific variable sites within each species. In *S. woodiana*, transversion mutations were detected at positions g.179 G>T and g.187 T>A (LC833751), and a deletion mutation was observed at position g.126 del A (LC833752). For *C. fluminea*, transition mutations were observed at positions g.378 T>C (LC833753), g.300 T>C (LC833754), and g.377 G>A (LC833755) (Table 1 & Figs. 2, 3).

Table 1. New accession numbers submission obtained from the current study and nucleotide diversity

Species	Mutation	Type	Frequency	Accession numbers
Sinanodonta woodiana	**g.179 G>T**	Transversion	1 (10%)	LC833751
	g.187 T>A	Transversion	1 (10 %)	
Sinanodonta woodiana	**g.126 *del* A**	Deletion	1 (10%)	LC833752
Corbicula fluminea	**g.378T>C**	Transition	1 (10%)	LC833753
Corbicula fluminea	**g.300 T>C**	Transition	1 (10%)	LC833754
Corbicula fluminea	**g. 377G>A**	Transition	1 (10 %)	LC833755

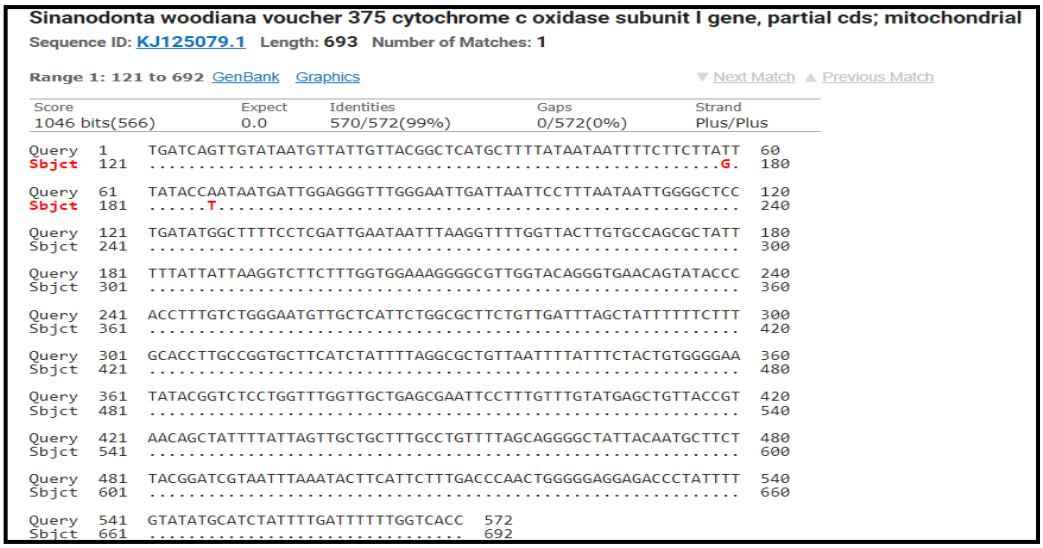

Fig. 2. Genetic analysis of mutation in *COX-1* gene in *Sinanodonta woodiana* species according to Web BLAST (nucleotide BLAST)

Fig. 3. Genetic analysis of mutation in *COX-1* gene in *Corbicula fluminea* species according to Web BLAST (nucleotide BLAST)

1.2. Phylogenetic analysis

Phylogenetic trees were constructed using the UPGMA method based on mitochondrial COX-I gene sequences obtained from *Sinanodonta woodiana* and *Corbicula fluminea* in the present study, along with reference sequences from GenBank originating from various countries, to analyze phylogenetic relationships. The phylogenetic analysis revealed a close relationship between the two *S. woodiana* samples with accession numbers LC833751 and LC833752 and three additional, unpublished sequences from the current study. These sequences clustered closely with *S. woodiana* from countries including Russia (OM698347), Poland (KJ125079.1), Iran (OP279027.1), and Romania (KF731775.1). However, sequences LC833751 and LC833752 formed a separate branch within the phylogenetic tree due to observed genetic variations and showed the highest identity with a reference sequence from Malaysia (MG591512.1) (Fig. 4).

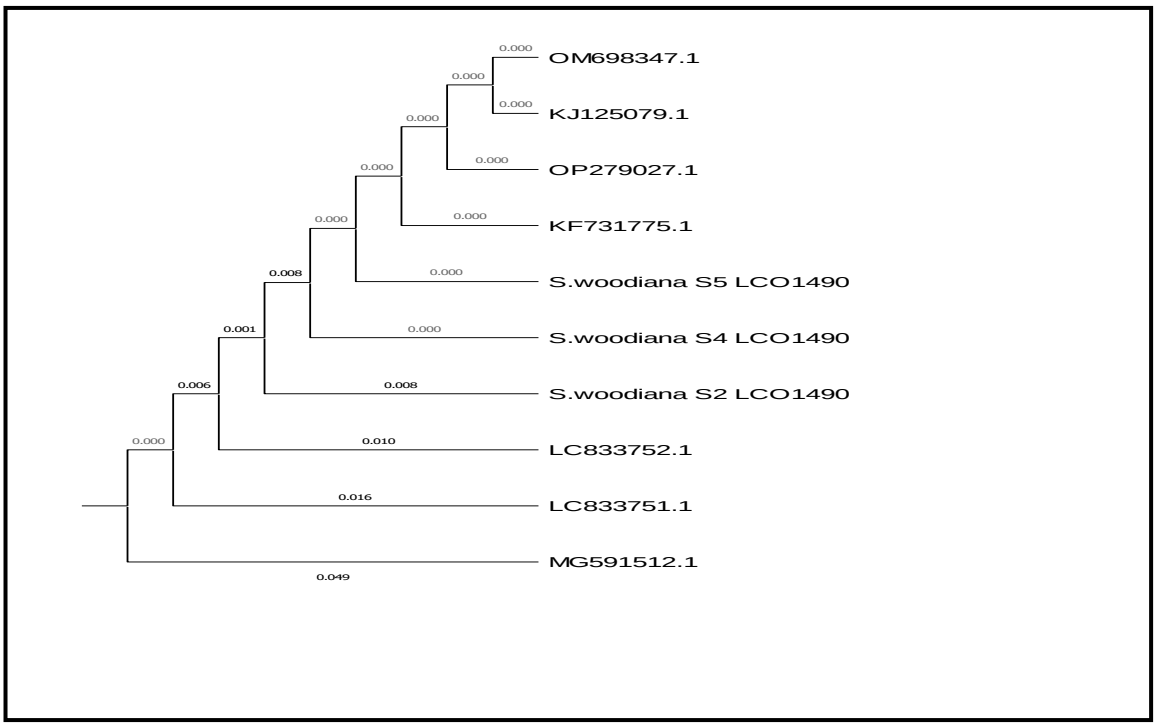

Fig. 4. Phylogenetic tree of *COX-1 gene* sequences of *Sinanodonta woodiana* in the current study with other sequences listed in GenBank data. Russia (OM698347.1), Poland (KJ 125079.1), Iran (OP279027.1), Romania (KF731775.1) and Malaysia (MG591512.1)

The phylogenetic relationships of *C. fluminea* sequences in this study indicated that sequences LC833753, LC833754, and LC833755 clustered within the same clade. BLAST analyses revealed that sequence LC833753 showed the highest similarity to sequences from the USA (AF196269) and Japan (KC211283 and LC763711), although it diverged slightly, forming an independent branch due to minor genetic variation. Additionally, sequence LC833755 showed high similarity to a Belgian sequence (GU721084). Sequence LC833754, along with two other unpublished samples from this study, diverged further, forming distinct branches on the phylogenetic tree and displayed closest similarity to a different Belgian reference sequence (OM912137) (Fig. 5).

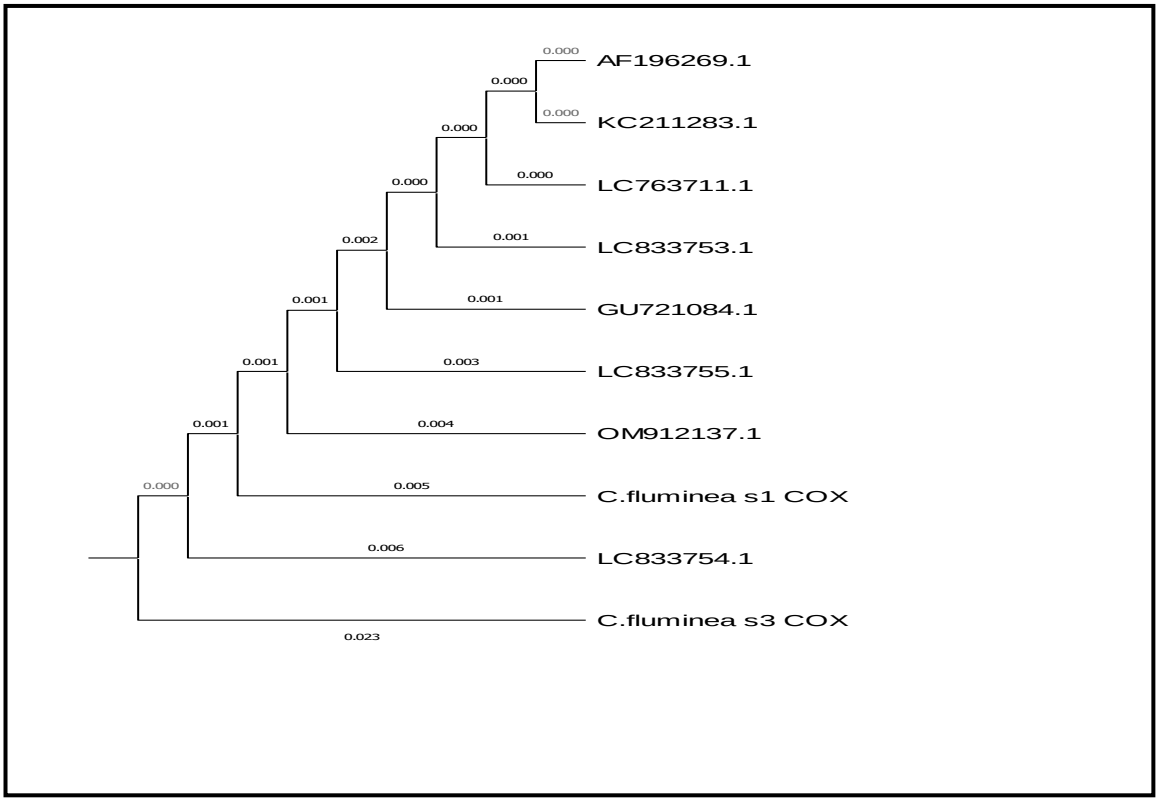

Fig. 5. Phylogenetic tree of *COX-1 gene* sequences of *Corbicula fluminea* in this study with other sequences listed in GenBank data. USA (AF196269), Japan (KC211283 and LC763711), Belgium (GU721084 and OM912137)

DISCUSSION

The mitochondrial cytochrome c oxidase subunit 1 (COX-1) gene is commonly used in evolutionary studies due to its relative stability across diverse animal species, making it a powerful molecular marker for investigating evolutionary relationships among different taxa **(Hebert *et al.*, 2003)**. This gene has a low mutation rate, rendering it suitable for genetic analyses at taxonomic levels such as families and species **(Folmer *et al.*, 1994)**.

In the current study, primers previously described by **Folmer *et al.* (1994)** were employed to amplify a portion of the COX-1 gene. These primers are widely utilized in mitochondrial DNA analyses of various organisms, particularly invertebrates **(Simon *et al.*, 1994)** and have been demonstrated to effectively distinguish species and determine genetic relationships **(Hajibabaei *et al.*, 2006)**.

PCR (polymerase chain reaction) was employed to amplify the target gene, and PCR products were subsequently analyzed using 2% agarose gel electrophoresis—a concentration suitable for separating DNA fragments of approximately 710 bp **(Sambrook & Russell, 2001)**. Samples from *Corbicula fluminea* and *Sinanodonta woodiana* exhibited matching fragment sizes, indicating high genetic similarity, as

expected for closely related taxa **(Clarke *et al.*, 2012)**. These results align well with previous studies utilizing the COX-1 gene for molluscan phylogenetic analyses, demonstrating its efficacy in species classification and measuring genetic divergence among related species **(MolluscaBase, 2020)**.

Mutational analysis revealed genetic stability in *C. fluminea*, with only transition mutations detected. Transition mutations may significantly impact protein structure and function, depending on their location and the resulting alteration of protein stability **(Sotomayor-Vivas *et al.*, 2022)**. In contrast, *S. woodiana* exhibited more diverse mutations, including transversions and deletions, potentially reflecting genetic responses to environmental pressures or evolutionary adaptations **(Moran, 1992)**. The presence of deletion mutations suggests an elevated rate of adaptation and genetic change, possibly resulting from exposure to varying aquatic conditions **(Avise, 2004)**.

The sequences obtained in this study were compared with existing data in the GenBank database, one of the largest repositories of genetic sequences. Sequence comparisons indicated shorter COX-1 gene sequences in *S. woodiana* relative to *C. fluminea*, potentially reflecting evolutionary divergence between these two taxa. Previous studies have shown that *C. fluminea* possesses a comparatively stable genome, which facilitates its rapid dispersal across various habitats **(Gomes *et al.*, 2016)**. These findings are valuable for environmental adaptation studies aiming to understand species responses to environmental stressors such as pollution, climate change, and water scarcity **(Strayer, 2017)**.

Sequence lengths for *C. fluminea* ranged between 577 and 581 nucleotides, closely matching lengths previously reported for this species **(Pigneur *et al.*, 2011)**. For *S. woodiana*, sequences ranged from 527 to 578 nucleotides. This species belongs to the family Unionidae, recognized for its high genetic diversity and adaptive potential across different habitats **(Froufe *et al.*, 2016)**.

Phylogenetic analysis was conducted using the UPGMA method, a technique suitable for constructing phylogenetic trees with equal branch lengths from the root to each leaf. However, it should be noted that UPGMA may be less accurate when mutation rates differ significantly between lineages **(Yoshida *et al.*, 2022)**.

CONCLUSION

Studies concerning the molecular identification of the bivalve species *Corbicula fluminea* and *Sinanodonta woodiana* in Thi-Qar Province, southern Iraq, are limited. Therefore, the current study aims to perform molecular characterization of these two species based on sequence analysis of the mitochondrial COX-I gene, contributing essential genetic data for species identification and biodiversity assessment in this region.

REFERENCES

Avise, J. C. (2004). Molecular Markers, Natural History, and Evolution

Clarke, A.; Smith, J. P.; Zhang, L. and Thompson, R. D. (2012). Phylogenetic analysis of mollusks using mitochondrial genes. Molecular Biology and Evolution, 29(4).

Felsenstein, J. (2004). 'Confidence limits on phylogenies: An approach using the bootstrap.' Evolution.

Folmer, O.; Black, M.; Hoeh, W.; Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. Oct;3(5):294-9. PMID: 7881515.

Froufe, E.; Prié, V.; Faria, J.; Ghamizi, M.; Gonçalves, D. V.; Gürlek, M. E. and Lopes-Lima, M. (2016). Phylogeny, phylogeography, and evolution in the Mediterranean region: news from a freshwater mussel (Potomida, Unionida). *Molecular Phylogenetics and Evolution*, *100*, 322-332.

Gomes, C.; Dreher Mansur, M. C.; **Pie, M. R.**; **Vilardo, P. J.**; **Uliano-Silva, M.**; **de Almeida, M. G. and Cataldo, D. H.** (2016). Genetic diversity of Corbicula fluminea in South America. Freshwater Science.

Hajibabaei, M.; Janzen, D. H.; Burns, J. M.; Hallwachs, W. and Hebert, P. D. (2006). DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences*, *103*(4), 968-971.

Hansh, W. J. (2024). Morphological and phylogenetic characterization of fasciola species isolated from cows and buffaloes in Thi-Qar province. *Iraq. J. Anim. Health Prod*, *12*(1),40-47.

Hosegood, J.; Humble, E.; Ogden, R.; De Bruyn, M.; Creer, S.; Stevens, G. M. and Carvalho, G. (2020). Phylogenomics and species delimitation for effective conservation of manta and devil rays. *Molecular ecology*, *29*(24), 4783-4796.

Mathew, A. A. and Ampili, M. (2022). Molecular identification tools for Bivalves: a review.

MolluscaBase (2020). "A global taxonomy database for mollusks." Available online.

Moran, N. A. (1992). The evolutionary maintenance of alternative phenotypes. The American Naturalist, 139(5), 971–989. https://doi.org/10.1086/285369

NCBI (https://www.ncbi.nlm.nih.gov).

Pigneur, L.; Marescaux, J.; Roland, K.; Etoundi, E.; Descy, J. and Van Doninck, K. (2011). Phylogeny and androgenesis in the invasive Corbicula clams (Bivalvia, Corbiculidae) in Western Europe. BMC Evolutionary Biology, 11(1). https://doi.org/10.1186/1471-2148-11-147

Sambrook, J. and Russell, D. W. (2001). "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor Laboratory Press.

Simon, C.; Frati, F.; Beckenbach, A.; Crespi, B.; Liu, H. and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the entomological Society of America*, *87*(6), 651-701.

Sneath P.H.A. and Sokal R.R. (1973). *Numerical Taxonomy*. Freeman, San Francisco.

Sotomayor-V. C.; Hernández-L. E. and Dorantes-G. R. (2022). Linking protein structural and functional change to mutation using amino acid networks. PLOS ONE, 17(1), e0261829. https://doi.org/10.1371/journal.pone.0261829

Strayer, D. L. (2017). Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance.

Tamura, K.; Nei, M. and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.

Tamura, K.; Stecher, G. and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.

Van der Geer, J.; Hanraads, J.A.J. and Lupton, R.A. (2010). The art of writing a scientific article. J. Sci. Commun., 163: 51–59.

Yoshida, R.; Paul, L. and Nesbitt, P. E. (2022). Stochastic Safety Radius on UPGMA. Algorithms, 15(12), 483. https://doi.org/10.3390/a15120483