J Genet Resour 2023; 9(2): 166-173 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2023.25508.1357*

J|G|R

Molecular Analysis of the Mangrove Oysters (Mollusca: Bivalvia) in Lagos Lagoon, Nigeria Based on Mitochondrial Genome

Victoria Folakemi Akinjogunla[1*], Olufemi Olukolajo Soyinka[2] and Shamsudeen L Pedro[3]

[1]Department of Fisheries and Aquaculture, Faculty of Agriculture, Bayero University Kano, Nigeria
[2]Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
[3]Centre for Biotechnology Research, Bayero University Kano, Nigeria

ARTICLE INFO

Article history:
Received 17 April 2023
Accepted 18 June 2023
Available online 01 July 2023

Keywords:
Crassostrea gasar
COI gene
Mangrove swamps
Phylogenetic
Plasticity

****Corresponding authors:***
✉ VF Akinjogunla
vfakinjogunla.faq@buk.edu.ng

p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

The commercial and economic importance of the mangrove oysters in the Lagos Lagoon provokes a great deal of biotic investigation, which provides a convincing justification for sequencing an oyster genome. Differentiating oysters based on their morphological characteristics for species identification and taxonomy is highly challenging because of the high intensity of phenotypic changes they exhibit. The genomic resources available for the mangrove oysters are incomparable to resources for any other bivalve invertebrates. In this study, unidentified mangrove oysters were collected from three different mangrove swamps off the Lagos Lagoon, Nigeria. Molecular procedures were used to identify the oysters genetically while pairwise and multiple alignments of mitochondrial DNA gene sequences of representative oyster strains within the clusters were used to relate them phenotypically to other oysters from various locations. Genetic diversity present in the selected mangrove oyster samples based on cytochrome oxidase I (*COI*) gene sequences reveals that the unidentified species at the three locations are *Crassostrea gasar* (Adanson, 1757) and were shown to be more like Brazilian oysters (*Crassostrea brasiliana*) with 99.55% similarity but clustered in a different clade of mangrove oysters in the GenBank. Similarities in the genetic makeup can principally be accredited to high levels of constant gene flow that are aftermaths of dispersal facilitated by a relatively long pelagic larval stage while the morphological differences can be primarily attributed to ontogeny with environmental conditions. A phylogenetic tree was constructed. The significance of these existing resources for a broad range of evolutionary and environmental sciences will be critically leveraged by having a recent or current genome sequence. The information obtained from this report is crucial to the understanding of diversity, systematics, and population genetics of mangrove oyster species of the Lagos Lagoon.

Please cite this paper as: Akinjogunla, V. F., Soyinka, O. O., & Pedro, S. L. (2023). Molecular analysis of the mangrove oysters (Mollusca: Bivalvia) in Lagos Lagoon, Nigeria based on mitochondrial genome. *Journal of Genetic Resources*, *9*(2), 166-173. doi: 10.22080/jgr.2023.25508.1357

Introduction

Natural aquatic (fisheries) resources are abundant in the various water bodies in Nigeria (Akinjogunla *et al.*, 2017, Akinjogunla and Shuaibu, 2022). Resources derived from the aquatic environs vary in appearance in terms of shape, size, color, and taste (Akinjogunla *et al.*, 2021) and can be broadly grouped into finfishes and shellfishes. The two groups of shellfish that are important in human food are the mollusks (clams and oysters) and the arthropods (crabs, shrimps, prawns, crayfish, and lobsters) (Akinjogunla *et al.*, 2017).

Mangrove biomes are widely distributed as they are estimated to cover 100,000-200,000 km^2 of the world's tropical estuarine zones where sea and rivers mix (Blasco *et al*., 1998). The genus '*Crassostrea'* are bivalve mollusks of the Phylum Mollusca, Family Ostreidae, Order Dysonta, and Class Lamellibranchiate; these bivalves occur worldwide with over fifty-four (54) species (Abgrall *et al*., 2010).

In Japan, the Pacific oyster (*Crassostrea gigas*) is the most cultivated species while the Portuguese oyster (*Crassostrea angulate*), the Eastern oyster (*Crassostrea virginica*), the Brazilian oyster (*Crassostrea brasiliana*), South American mangrove oyster (*Crassostrea rhizophorae*), and *Crassostrea gasar* are the most cultivated in West African coastal zones (Leguerrier *et al*., 2004; Adite *et al*., 2013). Lapegue *et al*., (2002) established the survival of only one species of mangrove oyster on the West African Coast through the study of the 16S mitochondrial polymorphism using sequencing and PCR- RFLP analysis.

Morphological documentation of the *Crassostrea* to the species level is tough due to the extreme environmental impact on shell growth (Lam and Morton, 2003); consequently, other methods, such as karyological and molecular analyses must be used to differentiate the mangrove oyster species. With the advances in Science and Technology, methods of identifying or classifying new organisms such as biochemical identification and molecular approaches (Yamamoto, 1992) have been developed.

According to Boudry *et al*. (1998), Hedgecock *et al*. (1999), Ignacio *et al*. (2000), Lapegue *et al*. (2002), Leguerrier *et al*. (2004), and Amarakoon (2016), molecular procedures have been used to illustrate, classify, and study the varieties of mangrove oyster's species in Africa and Asia. While the allozyme study was used to determine two distinct species in the American coasts (Ignacio *et al*., 2000), the cytochrome oxidase C subunit I (*COI*) and mitochondrial noncoding region (MNR) methods were used to confirm the two species in Zhejiang coast in China (Sheng *et al*., 2021).

Molecular approaches can complement morphological and karyological studies in the oyster taxa. For example, such procedures have already been used to distinguish between closely related Asian *Crassostrea* species (Hedgecock, *et al*., 1999) and to better comprehend the close associations between *C. gigas* and *C. angulata* (Boudry *et al*., 1998). This procedure has also been used to extricate sympatric species of the *Saccostrea* (rock oyster) in Thailand (Day *et al*., 2000). Limited genetic procedures (allozyme data) have been carried out on *C. rhizophorae* (Ignacio *et al*., 2000), while nothing has earlier been published on the molecular identification of the mangrove oysters from the Lagos Lagoon, Nigeria.

The standard technique to distinguish between closely related species, detect new or invasive species, and review species assemblages in communities across many animal phyla centered on the mitochondrial cytochrome oxidase I (*COI*) fragment is through the DNA barcode (Amarakoon, 2016), using the conventional tenet that intraspecific *COI* disparity is < 1%, whereas interspecific divergence is normally > 2% (Hsiao *et al*., 2016).

Significant broadcast in the number of comprehensive mitochondrial sequences accessible for all aquatic species has been observed during the last few years. The number has more than doubled for mollusks in the last three years (Yu *et al*., 2008); therefore 98 complete mollusk mitochondrial genomes are now obtainable in the GenBank, predominantly from gastropods (Yamamoto, 1992) and bivalves (Hsiao *et al*., 2016).

According to Wu *et al*., (2010), the genus *Crassostrea* has been thoroughly studied with seven species: six Asian oysters- *C. nippona, C. hongkongensis, C. monophyly, C. gigas, C. angulate*, and *C. sikamea*, and one American oyster- *Crassostrea virginica*, but none from the African territories.

This research was aimed at ascertaining the nomenclature of the mangrove oysters collected from the mangrove swamps in Lagos Lagoon, Nigeria. The objective of this research was to establish the molecular characterization of the mangrove oysters found in the Lagos Lagoon using mitochondrial DNA sequencing for proper scientific identification of the species.

Materials and Materials

Sampling

Thirty oyster samples were randomly collected from three mangrove swamps: Agala, Ebute-Oko, and Tomaro (Table 1) in the Lagos Lagoon, but some strains were lost due to faulty extraction. The Lagos Lagoon lies between latitude 6° 26' - 6° 37' N and longitude 3° 23' - 4° 20' E in the South-Western part of Nigeria, covering a surface area of 208km^2 (Akinjogunla *et al.*, 2017) and is characterized by seasonal salinity fluctuation (Lawal- Are and Akinjogunla 2012) as it receives freshwater from Lekki Lagoon via Epe Lagoon and discharges via Majidun, Agboyi, and Ogudu creeks in the North-West as cited by Akinjogunla and Lawal-Are (2020).

Mangrove oysters (Fig. 1) collected from mangrove roots exposed at low tides representing the selected mangrove swamps (Table 1) were taken to the research laboratory of the Nigerian Institute of Oceanography and Marine Research (NIOMR) where the flesh was exposed from the shells and the adductor muscles were removed using a steel knife and immediately preserved in 100% ethanol in Eppendorf tubes before extraction.

Table 1. GPS Coordinates descriptions of sample locations in the Lagos Lagoon.

Locations	Coordinates
Agala	5.6872°N 7.1503°E
Ebute Oko	5.6981°N 7.1393°E
Tomaro	5.6819°N 7.1650°E

PCR amplification and DNA sequencing

Total genomic DNA was isolated from the adductor muscles using the standard DreamTag™ DNA Polymerase (Thermo Scientific, USA). The mitochondrial cytochrome mtDNA gene was amplified using species-specific primers according to Folmer *et al*. (1994) and subjected to *COI* gene sequencing in the forward and reverse direction on the ABI PRISM®™ 3500xl Genetic Analyser (Thermo Fisher Scientific, USA) in other to confirm their identities.

Gel electrophoresis

1.5% Agarose Gel Electrophoresis was done to detect the genomic DNA quantity and purity using the Zymo Research, Zymoclean™ Gel (Waltham, MA, USA) DNA Recovery Kit at absorbance reading of 260 nm (NanoDrop™1000 Spectrophotometer, Thermo Scientific, Waltham, MA, USA) and stored at -20 °C. Bands were observed at the predicted 1.4kb size spectrophotometry (Allsheng, Hangzhou, China).

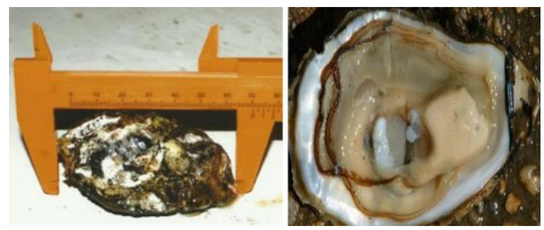

Fig. 1. Sample of the mangrove oyster from Lagos Lagoon. Magnification ×1000.

Sequence analysis and gene annotation

The PCR products were purified using the Zymo Research, ZR -96 DNA (Thermo Scientific, Waltham, MA, USA) sequencing clean-up kit and analyzed using CLC Main Workbench 7. Pairwise and multiple alignments of mitochondrial cytochrome mtDNA gene sequences of representative oyster strains within the formed clusters and sequences of related oyster species were compared with known gene sequences in the NCBI GenBank database by multiple sequence alignment using Clustal W program 2.0.12 (Thompson *et al.*, 1994) to determine closest relatives.

Phylogenetic analysis

A maximum-likelihood (ML) phylogenetic reconstruction (tree) approach was created using one representative mitochondrial genome from each *Crassostrea* species available on the NCBI database. Initial trees were determined using neighbor-joining with stochastic branch swapping and nearest-neighbor interchange used to identify the maximum-likelihood tree. Branch support was assessed using 1,000 bootstrap replicates and stochastic branch rearrangement. Determination of the best model of nucleotide evolution and tree construction (with 99% similarity) and bootstrapping (1000) was performed using MEGA 6.0 (Tamura *et al*., 2013).

Results

Pairwise Identity

The pairwise identity of mitochondrial cytochrome mtDNA gene sequences of representative oyster strains within the formed clusters is shown in Figure 2, from the apex, using the keys by the side. All sequenced species are 100% related. From the base, Samples B_1 and A_1 are 98% similar, and C_1 and A_3 are 97% similar. Their different locations might have necessitated their little genetic variations.

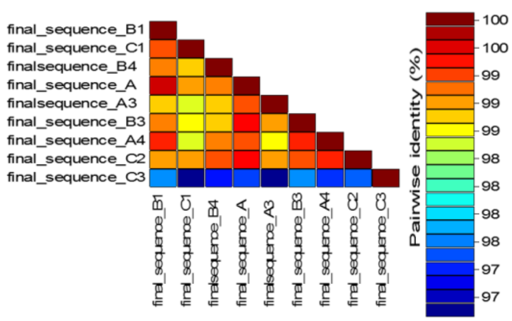

Fig. 2. Pairwise identity of the *Crassostrea* spp sequence. Oysters $A_{2,3,4}$= Ebute- Oko, $B_{1,3,4}$ = Agala, $C_{1,2,3}$= Tomaro.

Phylogenetic analysis

The BLAST search was aligned with previously published mt genomes from species of *Crassostrea*, *Saccostrea*, and other closely related mollusks and identified all nine (9) sequences as *Crassostrea gasar* with the total length of the mitogenome as 2-,030 bp (base pairs); but the results show that they were different from *Crassostrea gasar* obtained elsewhere, particularly *Crassostrea gasar* FJ717611 and *Crassostrea gasar* HM00352. The sequence analyses indicated that the oyster species in Lagos Lagoon were genetically closely related to *Crassostrea brasiliana* (*C. brasiliana*) with the highest percentage identity of 99.55% and accession number FJ717640.1. The cytochrome oxidase I (*COI* region) gene sequences aided the construction of a phylogenetic tree of oysters obtained from African (excluding Nigeria) and Asian countries, with other referenced oyster strains deposited from different collections while *Tilapia guinasana* was used as one out-group in the phylogenetic tree (Fig. 3). Finally, the nucleotide sequences were submitted to GenBank NCBI and received accession no. KR856227.1 and Gene Info Identifier number of HV8FZWNZ014

Discussion

In the current study, the mitochondrial DNA (mtDNA) *COI* gene sequence was used to characterize *Crassostrea spp*. in the Lagos Lagoon and it was confirmed to be widely distributed in the mangrove swamps of the Lagos Lagoon. The mangrove oyster species found in the Lagos Lagoon are mesohaline species that prefer the estuaries and intertidal habitats with sheltered mangrove trees, roots, and branches for attachment as these environments provide rich organic matter on which they feed and also give protection from storms (Blasco *et al*., 1998; Abgrall *et al*., 2010; Akinjogunla and Soyinka, 2022; Mahu *et al*., 2022). This report is consistent with reports from various researchers from different climes (Xu *et al*., 2009; Sheng *et al*., 2021).

Genetic diversity presented in the *Crassostrea gasar* selected samples based on mtDNA (*COI*) sequences specified that *C. gasar* populations in the Lagos Lagoon were fundamentally panmictic (homogenous) across the sampling locations. *C. gasar* from the Lagos Lagoon can be considered to comprise a single populace (homogenous population) as a consequence of high gene flow transfer among the species in the sampled sites as a result of fairly long-lived planktonic larval and spat phases. Many other bivalve species (scallops, mussels, and cockles) have also been reported to possess extensive dispersal and low genetic differentiation amongst the wild populations (Yu and Chu 2006). This means that the mangrove oyster (*C. gasar*) has propagated its populations naturally on various hard substrates (rocks, stones, bottles, branches, and roots) in the Lagos Lagoon to exist as a dominant species.

Fig. 3. Bayesian Inference phylogenetic relationships among *Crassostrea* spp. based on their mitochondrial genomes. The branch length is determined with BI analysis. BI/ML bootstrap values are given for each branch (** BI - Bayesian inference; ML- Maximum likelihood)

Although all sequenced strains are *Crassostrea gasar* (*C. gasar*) according to the BLAST result, they are grouped in a different clade of mangrove oysters in the GenBank. This study conforms with Leguerrier *et al*., (2004) who reported that *C. gasar* is the most common oyster on the West African Coasts, while this report disagrees with the reports of Lapegue *et al*., (2002), who documented that there is only one species of the mangrove oyster occurring in the coast of West Africa.

The variations observed in morphology (color and shape) of the shells (plasticity) of the mangrove oysters from the sampled sites could be said to be more correlated with local environmental factors like tidal and wave strength/exposure (Sánchez *et al*., 2011), prey substrate (Manríquez *et al*., 2009), predatory activities (Hollander and Butling 2010), and the nature of the substrates (Johannesson *et al*., 1993) than some genetic or phenotypic factors (Johannesson *et al*., 1993). These plastic responses of the shells (shell heritability) to environmental factors are a strong determinant of the oysters' ability to survive, reproduce, and colonize in these areas under diverse environmental conditions and expand their distribution range (Márquez *et al*., 2016) and has nothing to do with genetic contents.

The shells of the mangrove oysters sampled did not exhibit sexual dimorphism concerning formation (shape); this could be because they are protandrous (sequential) hermaphrodite mollusks that alternate sexes throughout their life span (Broquard *et al*., 2020). The difference can only be spotted in the morphometric (shell length, width, and weight) relationship measurements (Akinjogunla and Soyinka, 2022). This report, however, contradicts the reports of Márquez *et al*., (2013), Sawangproh *et al*., (2021), and Phung *et al*., (2022) who reported phenotypic sexual dimorphism in the organisms they studied. Using combined morphological, ecological, and genetic methods to quantify differentiation between populations of *C. gasar* from Lagos Lagoon, some level of variation was observed morphologically but genetically, they are the same as they were all confirmed to be mangrove oysters (*Crassotrea gasar*).

Conclusion

The findings of this study revealed that the shell morphology (size and shape) of the individual mangrove oysters varied between sampling sites, which necessitated the genetic analyses. This variation in the shell morphology could be explained primarily by plasticity, which suggests that the shell characteristics of *Crassostrea gasar* change through ontogeny (developmental history of an organism through its lifetime) according to environmental conditions. Despite the differences observed in the shell morphology of the oysters from the sampling sites, the Cytochrome b genomic identification revealed that the mangrove oysters in the Lagos Lagoon are *Crassostrea gasar* (*C. gasar*) and are unique because they are distinct from other *Crassostrea gasar* submitted to the GenBank from other parts of the world. Many of the relationships recovered in the present analysis through the constructed phylogenetic tree are consistent with previous publications.

Acknowledgment

The authors are grateful to Mr. Bamidele Williams (Nigerian Institute of Oceanography and Marine Research, Victoria Island, Lagos State, Nigeria) for his laboratory and technical assistance, Prof. A. O. Lawal-Are for her constructive input, and Dr. Olajide Joseph Akinjogunla for his financial support.

Conflicts of Interest

The authors declare no conflict of interest.

References

Abgrall, M. J., Bastien-Daigle, S., Miron, G., & Ouellette, M. (2010). Potential interactions between populations of Softshell Clams (*Mya arenaria*) and Eastern Oysters (*Crassostrea virginica*) in temperate estuaries, a literature review. Canadian Technical Report of Fisheries and Aquatic Sciences, No. 2892. https://publications.gc.ca/

Adite, A., Abou, Y., Sossoukpe, E., & Fiogbe, E. D. (2013). The oyster farming in the coastal ecosystem of southern Benin (West Africa): Environment, growth and contribution to sustainable coastal fisheries management. *International Journal of Development Research,* *3*(10), 087-094. https://www.journalijdr.com/

Akinjogunla, V. F, Lawal-Are, A. O., & Soyinka, O. O. (2017). Proximate composition and mineral contents of Mangrove Oyster (*Crassostrea gasar*) from Lagos Lagoon, Lagos, Nigeria. *Nigerian Journal of Fisheries,* *5*(2), 36-49. https://www.nijfaq.com.ng/

Akinjogunla, V. F., & Lawal-Are, A. O. (2020). Seasonal assessment of the impacts of heavy metal deposits in *Crassostrea gasar* (Adanson, 1757) from the mangrove swamp of the Lagos Lagoon, Lagos, Nigeria. *Journal of Experimental Research, 8*(2), 21-31. http://www.er-journal.com/

Akinjogunla, V. F., & Shu'aibu, U. (2022). Ichthyofauna composition and operative artisanal fishing activities in Ajiwa Irrigation Dam, Katsina State, Northern Nigeria. *Journal of Innovative Research in Life Sciences*, *4*(1), 45-53. https://jirlsonline.com/

Akinjogunla,V. F., & Soyinka, O. O. (2022). Morphometric assessment and condition factor of the mangrove oyster from a tropical mangrove swamp, off Lagos Lagoon, South-West, Nigeria. *Omni-Akuatika, 18*(1), 62-71. http://dx.doi.org/10.20884/1.oa.2022.18.1.957

Akinjogunla, V. F., Mudi, Z. R., Akinnigbagbe, O. R., & Akinnigbagbe, A. E. (2021). Biochemical profile of the mangrove oyster, *Crassostrea gasar* (Adanson, 1757) from the Mangrove Swamps, South-West, Nigeria. *Tropical Journal of Natural Product Research,* *5*(12), 2137-2143.https://www.tjnpr.org/

Amarakoon, A. G. U. (2016). Molecular identification of oyster (*Crassostrea* sp.) in Sri Lanka from mitochondrial DNA sequence data. *World Scientific News*, *57*, 116-121. http://www.worldscientificnews.com/

Blasco, F., Gauquelin, T., Rasolofoharinoro, M., Denis, J., Aizpuru, M., & Caldairou, V. (1998). Recent advances in mangrove studies using remote sensing data. *Marine and Freshwater Research*, *49*(4), 287-296. https://doi.org/10.1071/MF97153

Boudry, P., Heurtebise, S., Collet, B., Cornette, F., & Gérard, A. (1998). Differentiation between populations of the Portuguese oyster, Crassostrea angulata (Lamark) and the Pacific oyster, Crassostrea gigas (Thunberg), revealed by mtDNA RFLP analysis. *Journal of Experimental Marine Biology and Ecology*, *226*(2), 279-291. https://doi.org/10.1016/S0022-0981(97)00250-5

Broquard, C., Martinez, A. S., Maurouard, E., Lamy, J. B., & Degremont, L. (2020). Sex determination in the oyster Crassostrea gigas- A large longitudinal study of population sex ratios and individual sex changes. *Aquaculture*, *515*, 734555. https://doi.org/10.1016/j.aquaculture.2019.734555

Day, A. J., Hawkins, A. J. S., & Visootiviseth, P. (2000). The use of allozymes and shell morphology to distinguish among sympatric species of the rock oyster Saccostrea in Thailand. *Aquaculture*, *187*(1-2), 51-72. https://doi.org/10.1016/S0044-8486(00)00301-X

Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*(5), 294-9. https://pubmed.ncbi.nlm.nih.gov/7881515/

Hedgecock, D., Li, G., Banks, M. A., & Kain, Z. (1999). Occurrence of the Kumamoto oyster *Crassostrea sikamea* in the Ariake Sea, Japan. *Marine Biology*, *133*, 65-68. https://doi.org/10.1007/s002270050443

Hollander, J., & Butlin, R. K. (2010). The adaptive value of phenotypic plasticity in two ecotypes of a marine gastropod. *BMC Evolutionary Biology*, *10*, 1-7. https://doi.org/10.1186/1471-2148-10-333

Hsiao, S. T., Chuang, S. C., Chen, K. S., Ho, P. H., Wu, C. L., & Chen, C. A. (2016). DNA barcoding reveals that the common cupped oyster in Taiwan is the Portuguese oyster Crassostrea angulata (Ostreoida; Ostreidae), not C. gigas. *Scientific Reports*, *6*(1), 34057. https://doi.org/10.1038/srep34057

Ignacio, B. L., Absher, T. M., Lazoski, C., & Solé-Cava, A. M. (2000). Genetic evidence of the presence of two species of Crassostrea (Bivalvia: Ostreidae) on the coast of Brazil. *Marine Biology*, *136*, 987-991. https://doi.org/10.1007/s002270000252

Johannesson, K., Johannesson, B., & Rolán-Alvarez, E. (1993). Morphological differentiation and genetic cohesiveness over a microenvironmental gradient in the marine snail Littorina saxatilis. *Evolution*, *47*(6), 1770-1787. https://doi.org/10.1111/j.1558-5646.1993.tb01268.x

Lam, K., & Morton, B. (2003). Mitochondrial DNA and morphological identification of a new species of Crassostrea (Bivalvia: Ostreidae) cultured for centuries in the Pearl River Delta, Hong Kong, China. *Aquaculture*, *228*(1-4), 1-13. https://doi.org/10.1016/S0044-8486(03)00215-1

Lapègue, S., Boutet, I., Leitão, A., Heurtebise, S., Garcia, P., Thiriot-Quiévreux, C., & Boudry, P. (2002). Trans-Atlantic distribution of a mangrove oyster species revealed by 16S mtDNA and karyological analyses. *The Biological Bulletin*, *202*(3), 232-242. https://doi.org/10.2307/1543473

Lawal-Are, A. O., & Akinjogunla, V. F. (2012). Penaeus notialis (pink shrimps): length-weight relationships and condition factor in Lagos Lagoon, South West, Nigeria. https://doi.org/10.5923/j.scit.20120203.02

Leguerrier, D., Niquil, N., Petiau, A., & Bodoy, A. (2004). Modeling the impact of oyster culture on a mudflat food web in Marennes-Oléron Bay (France). *Marine Ecology Progress Series*, *273*, 147-162. https://doi.org/10.3354/meps273147

Mahu, E., Sanko, S., Kamara, A., Chuku, E. O., Effah, E., Sohou, Z., ... & Marchant, R. (2022). Climate resilience and adaptation in West African oyster fisheries: An expert-based assessment of the vulnerability of the oyster crassostrea tulipa to climate change. *Fishes*, *7*(4), 205. https://doi.org/10.3390/fishes7040205

Manríquez, P. H., Lagos, N. A., Jara, M. E., & Castilla, J. C. (2009). Adaptive shell color plasticity during the early ontogeny of an intertidal keystone snail. *Proceedings of the National Academy of Sciences*, *106*(38), 16298-16303. https://doi.org/10.1073/pnas.0908655106

Márquez, E. D. N. A., Landínez-García, R. M., Ospina-Guerrero, S. P., Aicardo Segura, J., Prada, M., Castro, E. R. I. C. K., ... & Borda, C. A. R. L. O. S. (2013). Genetic analysis of queen conch *Strombus gigas* from the Southwest Caribbean. Proceedings of the 65th Gulf and Caribbean Fisheries Institute, November 5-9, 2012 Santa Marta, Colombia. http://hdl.handle.net/1834/36343

Márquez, E. J., Restrepo-Escobar, N., & Montoya-Herrera, F. L. (2016). Shell shape variation of queen conch Strombus gigas (Mesograstropoda: Strombidae) from Southwest Caribbean. *Revista de Biología Tropical*, *64*(4), 1585-1595. https://doi.org/10.15517/rbt.v64i4.21468

Phung, C. C., Choo, M. H., & Liew, T. S. (2022). Sexual dimorphism in shell size of the land snail Leptopoma perlucidum (Caenogastropoda: Cyclophoridae). *PeerJ*, *10*, e13501. https://doi.org/10.7717/peerj.13501

Sánchez, R., Sepúlveda, R. D., Brante, A., & Cárdenas, L. (2011). Spatial pattern of genetic and morphological diversity in the direct developer Acanthina monodon (Gastropoda: Mollusca). *Marine Ecology Progress Series*, *434*, 121-131. https://doi.org/10.3354/meps09184

Sawangproh, W., Phaenark, C., Chunchob, S., & Paejaroen, P. (2021). Sexual dimorphism and morphometric analysis of Filopaludina martensi martensi (Gastropoda: Viviparidae). *Ruthenica, Russian Malacological Journal*, *31*(2), 87-92. https://doi.org/10.35885/ruthenica.2021.31(2).4

Liu, S., Xue, Q., Xu, H., & Lin, Z. (2021). Identification of main oyster species and comparison of their genetic diversity in Zhejiang coast, South of Yangtze river estuary. *Frontiers in Marine Science*, *8*, 662515. https://doi.org/10.3389/fmars.2021.662515

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725-2729. https://doi.org/10.1093/molbev/mst197

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673-4680. https://doi.org/10.1093/nar/22.22.4673

Wu, X., Xu, X., Yu, Z., Wei, Z., & Xia, J. (2010). Comparison of seven Crassostrea mitogenomes and phylogenetic analyses. *Molecular Phylogenetics and Evolution*, *57*(1), 448-454. https://doi.org/10.1016/j.ympev.2010.05.029

Xu, F., Zhang, G., Liu, X., Zhang, S., Shi, B., & Guo, X. (2009). Laboratory hybridization between Crassostrea ariakensis and C. sikamea. *Journal of Shellfish Research*, *28*(3), 453-458. https://doi.org/10.2983/035.028.0305

Yamamoto, H. (1992). Detection and identification of Legionella species by PCR. *Nihon Rinsho*, *50*, 394-399. https://pubmed.ncbi.nlm.nih.gov/1404930/.

Yu, D. H., & Chu, K. H. (2006). Species identity and phylogenetic relationship of the pearl oysters in Pinctada Röding, 1798 based on ITS sequence analysis. *Biochemical Systematics and Ecology*, *34*(3), 240-250. https://doi.org/10.1016/j.bse.2005.09.004

Yu, Z., Wei, Z., Kong, X., & Shi, W. (2008). Complete mitochondrial DNA sequence of oyster Crassostrea hongkongensis-a case of" Tandem duplication-random loss" for genome rearrangement in Crassostrea?. *BMC Genomics*, *9*, 1-13. https://doi.org/10.1186/1471-2164-9-477