Int. J. Aquat. Biol. (2022) 10(5): 364-369
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

New distribution record of Crassostreine oyster *Magallana gryphoides* (Schlotheim, 1820) in Kerala, India

Vineetha Vijayan Santhi, Smrithi Sreekanth, Mugdha Sukumaran, Mano Mohan Antony*

Department of Zoology, (Research Centre, University of Kerala), University College, Palayam, Thiruvananthapuram, Kerala, India.

Abstract: The Crassostreine oyster *Magallana gryphoides* (Bivalvia: Ostreidae) has been recorded for the first time on the Kerala coasts from Dharmadom estuary, Kannur, Kerala, India. The report indicates the range extension of *M. gryphoides* on the South-west coast of India. The external morphological characters were phenotypic and insufficient for species identification as it resembles *Magallana bilineata*. However, internal shell characters gave important information, especially the adductor muscle scar. The accurate species determination was achieved from the mitochondrial COI and 16S gene sequencing, followed by molecular phylogenetic analysis. The native oyster *M. bilineata* and *M. gryphoides* were found to co-exist in the same habitat sharing similar ecological conditions, sharing a sister group relationship.

Article history:
Received 12 June 2022
Accepted 14 August 2022
Available online 25 October 2022

Keywords:
Morphology
Dharmadom estuary
Magallana bilineata
Gene sequencing
Phylogeny

Introduction

The true oysters of the family Ostreidae Rafinesque, 1851 have a cosmopolitan distribution and encompass species with high economic importance. They are considered the most efficient 'keystone niches' among all the keystone species known for making unique micro-ecosystems (Sanjeeva, 2008). In India, oysters are widely distributed in estuaries, bays, harbours, and backwaters. However, comprehensive data on the distribution of Oysters dated way back to 1987 by Rao (1987), who reported *Crassostrea* (*Magallana*) *gryphoides* from Gujarat, Maharashtra and Goa. Subsequently, *M. gryphoides* had been reported from different regions on Indian coasts, from Konkan coast (Sawant, 1997), Maharashtra (Tibile and Singh, 2003), Sunderbans (Trivedi et al., 2015), Goa (Reece et al., 2008; Nagi et al., 2010) and from nearby coasts of Karachi (Siddiqui and Ahmed, 2002), Myanmar (Li et al., 2017) and Bangladesh (Chowdury et al., 2021).

Initially, the Indian oysters were assigned to the genus *Ostrea* by Awati and Rai (1931), later revised and re-assigned under the genus *Crassostrea* by Rao (1956, 1958) and Durve (1967). Recently, Salvi et al. (2014) and Salvi and Mariottini (2017) proposed a new genus, *Magallana* Salvi & Mariottini, 2016, for the Asian-Pacific clade of true oysters of the subfamily Crassostreinae, thereby, Asian-Pacific species of *Crassostrea* were re-assigned into a new genus *Magallana* gen. nov (Salvi et al., 2014). The genus *Magallana* was valid based on the 2016 description and all the species in the genus were included in WoRMS in 2017, which makes *M. gryphoides* (Schlotheim, 1820), the accepted name for the Asian-Pacific species of *C. gryphoides* (Schlotheim, 1820) and *M. bilineata* (Roding, 1798) for the native Indian backwater oyster, *C. madrasensis*, Preston 1916.

However, it is noteworthy to observe the molecular data deficiency of oysters from the Indian sub-continent in the above-mentioned papers. Therefore, in the current study, we attempted to establish the new distribution record of *M. gryphoides* in the South-West coast of India

*Correspondence: Mano Mohan Antony
E-mail: manomohanantony@universitycollege.ac.in
DOI: https://doi.org/10.22034/ijab.v10i5.1672
DOR: 20.1001.1.23830956.2022.10.5.2.3

based on the mitochondrial COI and 16S gene sequencing and phylogenetic analysis along with ecological and morphological information.

Materials and Methods

Oysters were collected from Dharmadom Estuary (11.796918N, 75.462153E), Kannur, Kerala, India, by hand-picking from shallow coastal waters. Tissue from the adductor muscle was used for molecular analysis. The ecological factors such as depth, nature of Substrate, salinity, pH, hardness, dissolved oxygen, temperature, and population density were recorded (APHA, 1989; Peters et al., 2017).

The collected species were primarily identified based on published papers (Siddiqui and Ahmed, 2002; Li et al., 2017; Chowdhury et al., 2021). The classification and Scientific names followed WoRMS (www.marinespecies.org). For species confirmation, the mitochondrial gene sequencing method was employed using mitochondrial cytochrome oxidase I (COI) and 16S ribosomal RNA (16S rRNA). The genomic DNA was isolated from muscle tissue using NucleoSpin® Tissue Kit (Macherey-Nagel). Partial COI and 16S rRNA was amplified via Polymerase Chain Reaction using the primers of LCO1490 and HCO2198 (Folmer et al., 1994) for COI and 16Sar and 16Sbr (Palumbi et al., 1991) for 16S rRNA. All the retrieved sequences were deposited in NCBI-GenBank.

For both 16S and COI datasets, Multiple Sequence Alignment (MSA) was done in MEGA 7 (Kumar et al., 2016) and the phylogenetic tree (Bayesian Inference tree) was constructed in MrBayes 3.2.7 (Ronquist et al., 2012). Bayesian analysis (GTR+G+I model as best fitting model for both 16S and COI dataset) was done and two independent runs were performed for 2×10^6 generations sampling per 1000 generations. The first 25% of the trees acquired were discarded as burn-in, and a 50% majority-rule consensus tree with posterior probability (PP) values were generated from the leftover trees. The phylogenetic tree was constructed using FigTree v1.4.2. *Talonostrea salpinx* was taken as the outgroup.

Results

The studied site showed a depth of 0.8-1.2 m with a muddy substratum. Individuals of *M. gryphoides* were collected from the beds of *M. bilineata.* Solitary individuals along with gregarious forms with *M. bilineata* were observed. Compared to *M. bilineata* (11-18/m^2), the population density of *M. gryphoides* was very fewer ranges of 2-5/m^2. The pH of the studied site was 7.55, surface water temperature 32°C, salinity 30.14 ppt, hardness-284 mg/L and dissolved oxygen 7.33 mg/L.

All the external shell characters were similar for both *M. gryphoides* and *M. bilineata* since they are phenoplastic and share a common habitat. However, characteristic differences were observed in the internal shell characters, especially in the adductor muscle scar. *Magallana gryphoides* exhibited characteristic pearly white colouration, reniform or crescent-shaped adductor muscle scar, nearly straight dorsally and close to the posterodorsal margin and the shell interior was whitish and shiny devoid of any purplish or blackish markings. For *M. bilineata*, the adductor muscle scar was characteristically dark purplish-black in both valves. The white umbo cavity always had purple markings around the whole or some parts of the margin. In the ventral margin, deeper colouration was observed facing the posterior portion of the gill, especially in the lower valve (Fig. 1). The mean and standard deviation of shell length (SL), shell height (SH), shell width (SD), and total weight (TW) in *M. gryphoides* was 3.64±4.751, 79.68±6.215, 32.86±2.572, and 128.19±2.08, and for *M. bilineata* as 74.4±13.682, 100.74±14.504, 35.026±10.607, and 191.56±31.735, respectively.

A total of eight gene sequences were obtained from *M. gryphoides* and *M. bilineata* and submitted to the NCBI-GenBank and acquired accession numbers (Table 1). The phylogenetic relationship of *M. gryphoides* with the other Asian-Pacific species was inferred from COI and 16S gene datasets. The BI tree was constructed for each gene dataset, using four original sequences and other sequences of the Asian-Pacific species retrieved from NCBI-

Table 1. Gene sequence data of collected oysters.

Species	Species ID	GenBank Accession numbers	
		16S	COI
Magallana gryphoides	DB1	ON926958	ON920920
	DB2	ON926959	ON921053
Magallana bilineata	DU1	ON926953	ON912074
	DU2	ON926954	ON920843

GenBank (Figs. 2 and 3). The topology of both the trees was similar with *M. belcheri* in the upper clade, *M. dianbeinsis*, *M. bilineata* and *M. gryphoides* placed in the middle clade and *M. gigas*, *M. sikamea*, *M. hongonenesis*, *M. nippona*, *M. ariakensis* and *M. angulata* in the bottom clade. The original sequences of the *M. gryphoides* shared the same clade with the other 3 sequences of *M. gryphoides* in the COI tree and showed more similarity with the sequences from Goa, India (EU007488 and

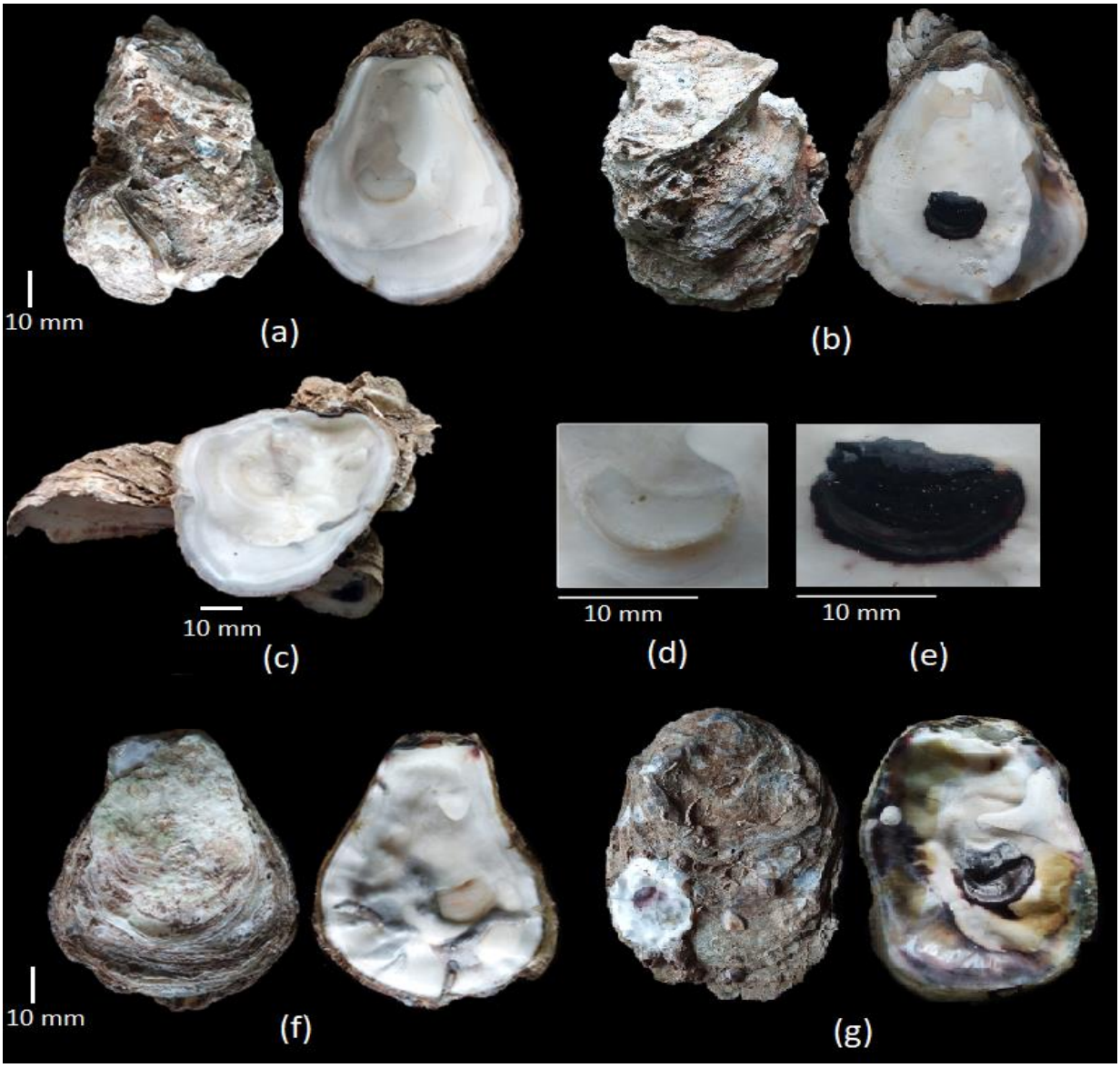

Figure 1. Shell characteristics of *Magallana gryphoides* and *M. bilineata*. (a) Lower valve of *M. gryphoides* (b) Lower valve of *M. bilineata* (c) *M. gryphoides* among the cluster of *M. bilineata* (d) Adductor muscle scar of *M. gryphoides* (e) Adductor muscle scar of *M. bilineata* (f) Upper valve of *M. gryphoides* (g) Upper valve of *M. bilineata*.

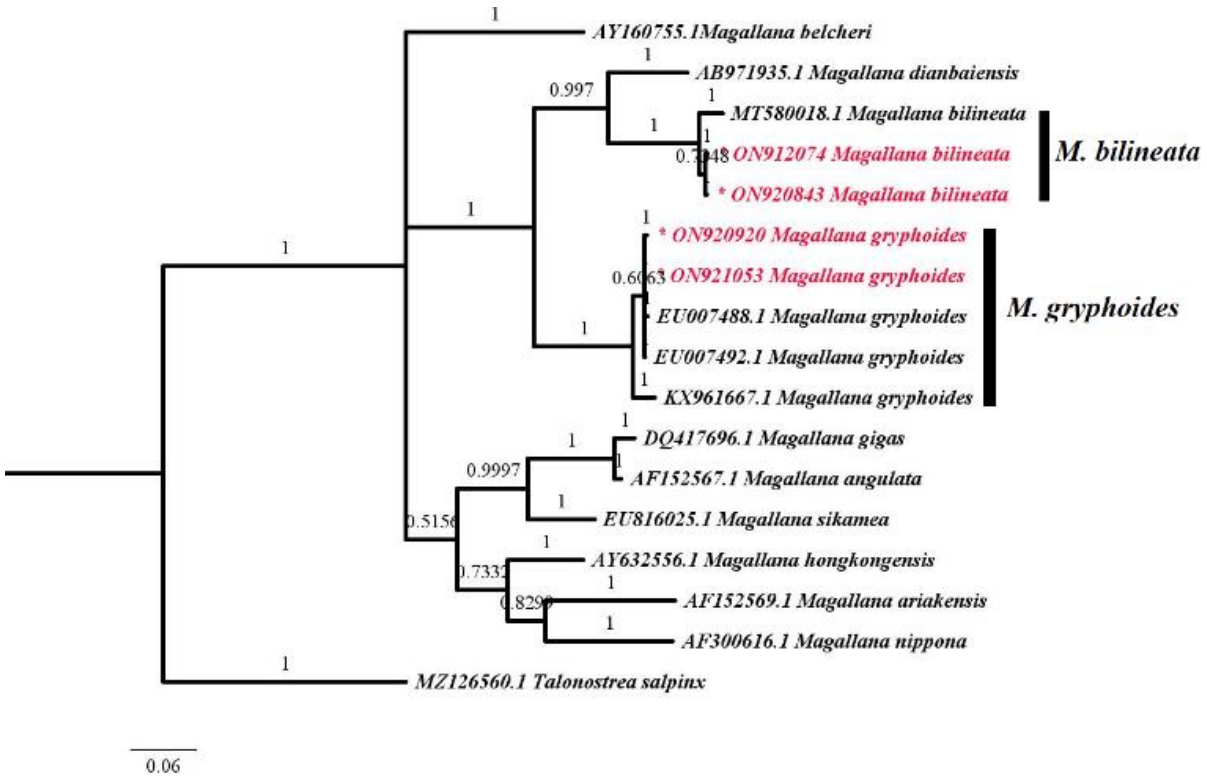

Figure 2. Phylogenetic tree based on BI analysis of COI sequences. Bayesian posterior probability is shown at the nodes. Original sequences are marked in Red.

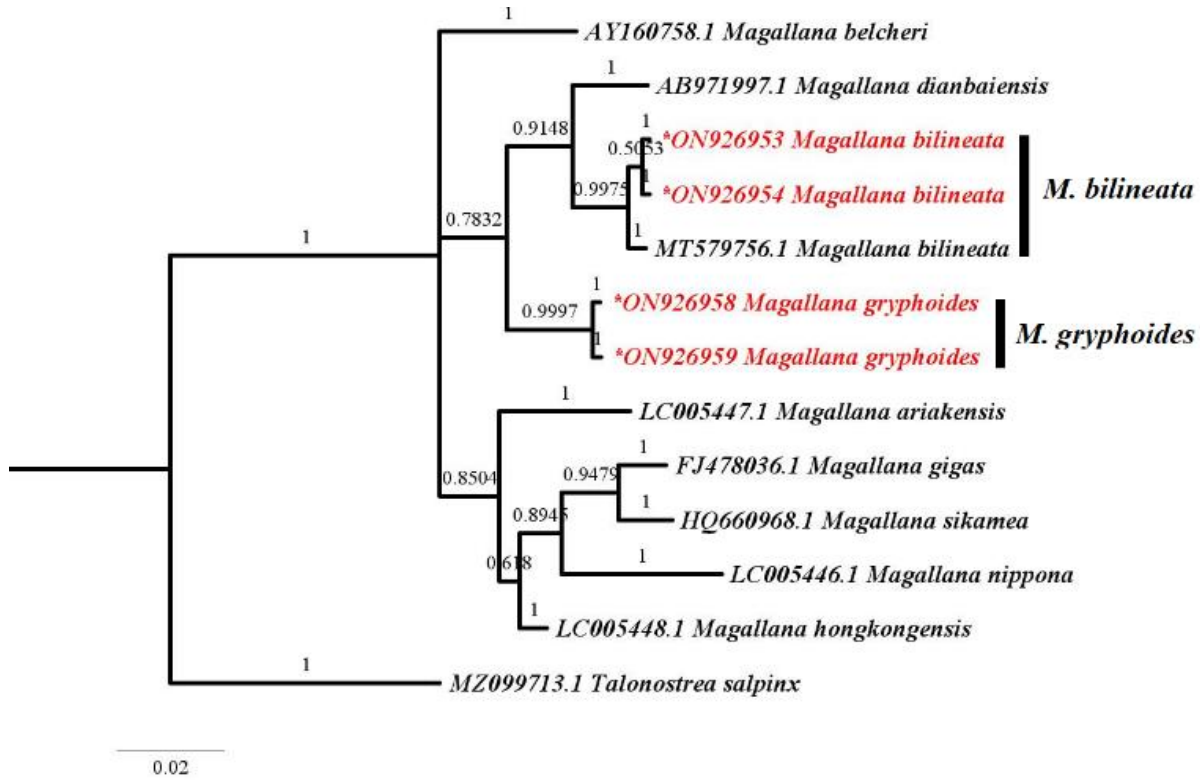

Figure 3. Phylogenetic tree based on BI analysis of 16S rRNA sequences. Bayesian posterior probability is shown at the nodes. Original sequences are marked in Red.

EU007492) (Reece et al., 2008). The clade of *M. gryphoides* exhibited a sister group relationship with *M. bilineata* and *M. dianbaiensis* in COI and 16S trees. However, 16S sequences were scarce in NCBI-GenBank.

Discussion

The new report of *M. gryphoides* from Dharmadom estuary represents the range extension of the species on the South-west coast as it was earlier reported from the coasts of Gujarat, Maharashtra and Goa (Rao, 1987; Tibile and Singh, 2003; Reece et al., 2008). Since, the Indian oyster taxonomy is mostly dependent on the morphological shell traits, which are highly plastic, the presence of *M. gryphoides* on the South-west coast might be masked earlier. Therefore, the presence of more oyster species and their distribution is highly anticipated on the Indian coasts. However, molecular identification methods should be encouraged and given priority as they can provide decisive outcomes in oyster taxonomy.

All the ecological conditions observed were optimum for the growth and distribution of oysters, highlighting the scope of oyster culture in the Dharmadom estuary. Being benthic, shells cope with the external environmental conditions that result in ecophenotypic plasticity (Lam and Morton, 2004; Huber, 2010; Liu et al., 2011; Santhi et al., 2021). Therefore, species identification could not be achieved based on external shell characteristics. However, it is the adductor muscle scar that gave important information for species identification. A similar finding was also made by Siddiqui and Ahmed (2002) in *C. madrasensis* (=*M. bilineata*) and *C. gryphoides (=M. gryphoides*) with respect to the white colour of the adductor muscle scar in *C. gryphoides (=M. gryphoides*) and purpleness in *C. madrasensis* (=*M. bilineata*). However, molecular markers had proved to be efficient markers for species delineation. Nevertheless, the molecular database of Indian oysters is still lacking and only a few sequences are available in NCBI-GenBank. Though, accurate identification was achieved by mitochondrial COI gene sequencing and thereby BLAST analysis.

The previous name of *Crassostrea gryphoides* was also used for a European fossil species as *Crasssostrea gryphoides* von Schlotheim, 1813 that existed during the Miocene and Pliocene, and became extinct over three million years ago (Harzhauser et al., 2016). However, it has been considered as not conspecific with the extant *M. gryphoides* (Harzhauser et al., 2016) differing with respect to the shape of adductor muscle scar (Durve, 1974), shell outline (Durve and Bal, 1961), and size (Chatterji et al., 1985; Nagi et al., 2011). Therefore, according to Huber (2010), Harzhauser et al. (2016), and Li et al. (2017), the same name should not be used also for an extant species. After the recent revision of Asia-Pacific oysters into the newly assigned genus *Magallana* Salvi and Mariottinni, 2016, the extant species of *C. gryphoides* from the India and Pakistan region were re-assigned as *M. gryphoides* (Schlotheim, 1820).

The current study also provides molecular phylogenetic data that agrees with the recent revision in the Crassostreinae subfamily by Salvi and Mariottini (2017). The BI tree showed the monophyletic origin of Asia-Pacific true oysters (Subfamily: Crassostreinae) recently assigned to the genus *Magallana* in COI and 16S trees. Moreover, *M. bilineata* and *M. gryphoides* exhibited a sister group relationship with *M. dianbaiensis* in both trees with a high Bayesian probability of 1. This is in accordance with Melo et al. (2010), Wu et al. (2013), Xia et al. (2014), Li et al. (2017), Chowdhury et al. (2021), and Ghaffari et al. (2022). The sister group relationship of *M. bilineata* and *M. dianbeiensis* was also demonstrated by Salvi and Mariottini (2017, 2021), Al-Kandari et al. (2021), and Willan et al. (2021). However, the gene data of *M. gryphoides* is still scarce in NCBI-GenBank. Therefore, more molecular studies are required on this species and other *Magallana* species of the Indian waters to resolve their taxonomic uncertainties.

Since, the systematics of true oysters is critical to developing the sustainable use of species and understanding the diversity of Oysters worldwide

(Sigwart et al., 2021), the gap areas should be immediately dealt with to document and conserve the biodiversity of Indian oysters. The aid of molecular taxonomy and evolutionary analyses via a phylogenetic approach would unmask the biodiversity and evolutionary reactions of Indian oysters that remained unnoticed and unknown to the world. Being the Keystone species, any conservation measures to this taxon will ultimately benefit the ecosystem. As the oyster population of India is radically decreasing (Laxmilatha, 2022), it has become an emergency issue to be dealt with.

References

Al-Kandari M., Oliver P.G., Salvi, D. (2021). Molecular and morphological systematics of a new, reef forming, cupped oyster from the northern Arabian Gulf: *Talonostrea salpinx* new species. ZooKeys, 1043: 1-20.

APHA. American Public Health Association, Water Pollution Control Federation. (1989). Standard methods for the examination of water and wastewater. 17 p.

Awati P.R., Rai H.S. (1931). *Ostrea cucullata* (The Bombay Oyster). Indian Zoological Memoir, 3: 107.

Chatterji A., Ansari Z.A., Ingole B.S., Parulekar A.H. (1985). Length-Weight relationship of giant oyster, *Crassostrea gryphoides* (Schlotheim). Mahasagar, 18(4): 521-524.

Chowdhury M.S., Reece K.S., Joy S.S., Coen L.D., Smaal A.C. (2021). Morphological and mitochondrial DNA analyses of oysters in the northern Bay of Bengal, Bangladesh. Journal of Shellfish Research, 40(2): 213-229.

Durve V.S. (1974). Malacological differences between the oysters *Crassostrea gryphoides* (Schlotheim) and *Crassostrea madrasensis* Preston. Indian Journal of Fisheries, 20(2): 624-625.

Durve V.S., Bal D.V. (1961). Some observations on shell-deposits of the oyster *Crassostrea gryphoides* (Schlotheim). Proceedings of the Indian Academy of Sciences-Section B. Springer India. pp: 45-55.

Durve V.S. (1967). On the nomenclature of two Indian backwater oysters. Journal of Marine Biological Association of India, 9: 173-178.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3: 294-299.

Ghaffari H., Ahmadzadeh F., Saberi-Pirooz R., Abtahi B. (2022). Molecular and morphological evidence for the presence of a non-native oyster (genus *Crassostrea*) in the Persian Gulf. Zoologischer Anzeiger, 296: 120-128.

Hall T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Nucleic Acids Symposium Series, 41: 95-98.

Harzhauser M., Djuricic A., Mandic O., Neubauer T.A., Zuschin M., Pfeifer N. (2016). Age structure, carbonate production and shell loss rate in an Early Miocene reef of the giant oyster *Crassostrea gryphoides*. Biogeosciences, 13(4): 1223-1235.

Huber M. (2010). A status on Bivalvia after 250 years of research. Compendium of bivalves. A full-color guide to 3,300 of the world's marine bivalves. Conch Books.

Kumar S., Stecher G., Tamura K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7): 1870-1874.

Lam K., Morton B. (2004). The oysters of Hong Kong (Bivalvia: Ostreidae and Gryphaeidae). Raffles Bulletin of Zoology, 52: 11-28.

Laxmilatha P. (2022). Molluscan Fisheries Present Status. CMFRI- Kochi Manual. 444 p.

Li C., Haws M., Wang H., Guo X. (2017). Taxonomic classification of three oyster (Ostreidae) species from Myanmar. Journal of Shellfish Research, 36(2): 365-371.

Liu J.U.N., Li Q.I., Kong L., Yu H., Zheng X. (2011). Identifying the true oysters (Bivalvia: Ostreidae) with mitochondrial phylogeny and distance-based DNA barcoding. Molecular Ecology Resources, 11(5): 820-830.

Melo A.G.C.D., Varela E.S., Beasley C.R., Schneider H., Sampaio I., Gaffney P.M., Tagliaro C.H. (2010). Molecular identification, phylogeny and geographic distribution of Brazilian mangrove oysters (*Crassostrea*). Genetics and Molecular Biology, 33: 564-572.

Nagi H.M., Jagtap T. (2010). Condition index of *Crassostrea madrasensis* Preston (Ostreoida, Ostreidae) and *C. gryphoides* Schlotheim (Ostreoida, Ostreidae) and its percentage edibility in populations associated with selected mangrove habitats from Goa, India. Yemeni Journal of Biological Sciences, 6(1): 107-116.

Nagi H.M., Shenai-Tirodkar, P.S., Jagtap T.G. (2011).

Dimensional relationships in *Crassostrea madrasensis* (Preston) and *C. gryphoides* (Schlotheim) in Mangrove ecosystem. Indian Journal of Geo-Marine Sciences, 40(4): 559-566.

Palumbi S.R., Martin A., Romano S., McMillan W.O., Stice L., Grabowski G. (1991). The simple fool's guide to PCR. Department of Zoology special publication, University of Hawaii, Honolulu. 46 p.

Peters J.W., Eggleston D.B., Puckett B.J., Theuerkauf S.J. (2017). Oyster demographics in harvested reefs vs. no-take reserves: implications for larval spillover and restoration success. Frontiers in Marine Science, 4: 326.

Rao K.S. (1987). Taxonomy of Indian oysters. CMFRI Bulletin, 38: 1-6.

Rao K.V. (1956). Seasonal gonadal changes in the adult backwater oyster, *Ostrea* (*Crassostrea*) *madrasensis* Preston from Ennur near Madras. In Proceedings of the Indian Academy of Sciences, 44(6): 332-356.

Rao K.V. (1958). Molluscan fisheries. In: S. Jones (Ed.). Fisheries of the West Coast of India. Mandapam Camp, India: Centre of Marine Fisheries Research Statistics. pp: 55-59.

Reece K.S., Cordes J.F., Stubbs J.B., Hudson K.L., Francis E.A. (2008). Molecular phylogenies help resolve taxonomic confusion with Asian *Crassostrea* oyster species. Marine Biology, 153(4): 709-721.

Ronquist F., Teslenko M., Van Der Mark P., Ayres D.L., Darling A., Höhna S., Huelsenbeck J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61(3): 539-542.

Salvi D., Mariottini P. (2017). Molecular taxonomy in 2D: a novel ITS2 rRNA sequence-structure approach guides the description of the oysters' subfamily Saccostreinae and the genus *Magallana* (Bivalvia: Ostreidae). Zoological Journal of the Linnean Society, 179(2): 263-276.

Salvi D., Mariottini P. (2021). Revision shock in Pacific oyster's taxonomy: the genus *Magallana* (formerly *Crassostrea* in part) is well-founded and necessary. Zoological Journal of the Linnean Society, 192(1): 43-58.

Salvi D., Macali A., Mariottini P. (2014). Molecular phylogenetics and systematics of the bivalve family Ostreidae based on rRNA sequence-structure models and multilocus species tree. PloS One, 9(9): e108696.

Sanjeeva Raj P.J. (2008). Oysters in a new classification of keystone species. Resonance, 13(7): 648-654.

Santhi V.V., Antony M.M., Lopez L., Vasanthi L., Joseph J. (2021). Evolution of ecophenotypic plasticity in Indian Oyster, *Crasssostrea madrasensis* (Preston) population from Ashtamudi Lake, Kerala, India. International Journal of Aquatic Biology, 9(5): 290-296.

Sawant M.S. (1997). Studies on edible oysters of the genus *Crassostrea*. MFSc Thesis. Submitted to Dr Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. 105 p.

Siddiqui G., Ahmed M. (2002). Oyster species of the sub-tropical coast of Pakistan (northern Arabian Sea). Indian Journal of Marine Sciences, 31(2): 108-118.

Sigwart J.D., Wong N.L. W., Esa Y. (2021). Global controversy in oyster systematics and a newly described species from SE Asia (Bivalvia: Ostreidae: Crassostreinae). Marine Biodiversity, 51(5): 1-16.

Tibile R.M., Singh H. (2003). Larval rearing and spat production of edible oyster *Crassostrea gryphoides* (Schlotheim). Aquaculture Research, 34(10): 785-792.

Trivedi, S., Aloufi, A. A., Ansari, A. A., Ghosh S.K. (2015). Molecular phylogeny of oysters belonging to the genus *Crassostrea* through DNA barcoding. Journal of Entomology and Zoology Studies, 3(1): 21-26.

Willan R.C., Nenadic N., Ramage A., McDougall C. (2021). Detection and identification of the large, exotic, Crassostreine oyster *Magallana bilineata* (Röding, 1798) in northern Queensland, Australia. Molluscan Research, 41(1): 64-74.

Wu X., Xiao S., Yu Z. (2013). Mitochondrial DNA and morphological identification of *Crassostrea zhanjiangensis* sp. nov. (Bivalvia: Ostreidae): a new species in Zhanjiang, China. Aquatic Living Resources, 26(4): 273-280.

Xia J., Wu X., Xiao S., Yu Z. (2014). Mitochondrial DNA and morphological identification of a new cupped oyster species *Crassostrea dianbaiensis* (Bivalvia: Ostreidae) in the South China Sea. Aquatic Living Resources, 27(1): 41-48.