






## Research Article

# Cryptic Marine Diversity in the Northern Arabian Gulf: An Integrative Approach Uncovers a New Species of Oyster (Bivalvia: Ostreidae), *Ostrea oleomargarita*

Daniele Salvi <sup>1</sup>, Manal Al-Kandari <sup>2</sup>, P. Graham Oliver <sup>3</sup>, Emanuele Berrilli <sup>1</sup>,  
and Matteo Garzia <sup>1</sup>

<sup>1</sup>Department of Health, Life & Environmental Sciences-University of L'Aquila, Via Vetoio snc, 67100 L'Aquila-Coppito, Italy

<sup>2</sup>Ecosystem-Based Management of Marine Resources, Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, Hamad Al-Mubarak Street, Building 900004, Area 1, Ras Salmiya, Kuwait

<sup>3</sup>Honorary Research Fellow, National Museum of Wales, Cardiff CF10 3NP, UK

Correspondence should be addressed to Daniele Salvi; [danielesalvi.bio@gmail.com](mailto:danielesalvi.bio@gmail.com)

Received 7 March 2022; Revised 24 May 2022; Accepted 25 May 2022; Published 4 July 2022

Academic Editor: Savel Daniels

Copyright © 2022 Daniele Salvi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Animal biodiversity is greatly underestimated in nontemperate marine regions, especially for intertidal benthic organisms such as oysters. Recent surveys in the northern Arabian Gulf suggest the presence of numerous unidentified species, some of which form shallow reef ecosystems while others are cryptic and found under rocks. In this study, we focused on small oysters from Kuwait, which show typical characteristics in common with the genus *Ostrea* except for the presence of lophine chomata that would link it to the genera *Lopha*, *Dendostrea*, and *Alectryonella*. Phylogenetic analyses based on mitochondrial and nuclear markers unambiguously placed the Kuwait oyster within the Ostreinae as a sister to the Japanese species *Ostrea futamiensis*. The hypothesis that the Kuwait oyster represents a new species was assessed with phylogenetic and species delimitation methods combined with a morphological assessment. Results corroborated the Kuwait oysters as a new species herein described as *Ostrea oleomargarita* Oliver, Salvi, and Al-Kandari, sp. nov. The phylogeny of the Ostreinae shows extensive disagreement between morphology-based genera and phylogenetic clades. The genus *Ostrea* is polyphyletic, and the form and distribution of taxonomic characters such as chomata are not as definitive as suggested in previous studies. This study, along with other recent investigations, confirmed the Arabian Gulf as a key region for discovering marine animal diversity and suggested a possible biogeographic divide between the Eastern and Western Indo-Pacific. A pattern that has been documented in a growing number of taxa and that warrants further research attention.

## 1. Introduction

Marine animal biodiversity is greatly underestimated, especially outside temperate regions [1]. This lack of knowledge is due to a variety of taxonomic impediments, including the difficulty of sampling in marine ecosystems, the lower availability of systematists, and poorer support infrastructures for documenting biodiversity in tropical and subtropical regions [2]. In addition, many intertidal benthic organisms, such as bivalve molluscs, show limited morphological complexity and high phenotypic plasticity driven by different environmental factors, making traditional taxo-

nomic assessment based on morphology inherently challenging [3]. This is particularly true for oysters (*Ostreidae* Rafinesque, 1815), a relatively small family of bivalves with 74 currently accepted species [4] that are widely distributed throughout world oceans and estuaries.

Despite the relatively low species diversity, oysters play an important role in marine ecosystems and are important fishery resources that support aquaculture industries worldwide. However, the diversity and distribution of living oysters are far from being fully understood (e.g., [5–7]) as focus has been given to commercially exploited species. The assessment of species diversity in oysters is hampered

by the lack of reliable morphological characters for species identification as well as for their assignment to higher taxa [8]. Historically, oysters have been described from few shell characters that are phenotypically plastic and phylogenetically unreliable [9–11]. Several molecular studies have shown how shell morphology is both unreliable and misleading for oyster classification up to the subfamily level [11–13]. Particularly emblematic in this respect are those cases where allopatric ecomorphs of a single species have been classified as distinct species in different subfamilies [10], or where syntopic populations of distinct species have long been considered as a single species due to their phenotypic convergence (e.g., [13, 14]). More recently, an integrative molecular and morphological approach has allowed a great leap forward in our knowledge of oyster diversity and phylogeny. This in turn has led to the characterization of local and endemic faunas (e.g., [15, 16]). However, while a molecular approach has great potential in the discovery of new species of oysters, these studies have been biased toward the clarification of the taxonomic status of cultured species and have been focused especially on the Eastern Indo-Pacific (e.g., [13, 17]). Far less is known on the oyster diversity of the Western Indo-Pacific, despite this region acting as a centre of origin for shallow water marine biota [18].

Recent surveys along the coast of Kuwait (northern Arabian Gulf) suggest that in these shallow waters, there are a number of unidentified oyster species including some that form prominent intertidal features. This is perhaps not surprising since serious biological investigation, in Kuwait, only dates from the 1980s [19]. A recent survey of the intertidal macrofauna of Kuwait, based on a morphological assessment, reported five species of Ostreidae [20]. These authors listed the ostreids *Alectryonella* cf. *crenulifera* (Sowerby, 1871) = *Dendostrea sandvichensis* (Sowerby, 1871), cf. *Booneostrea subucula* (Jousseaume in Lamy, 1925), cf. *Nanostrea deformis* (Lamarck, 1819), *Crassostrea* sp., and *Saccostrea cucullata* (Born, 1778). It is evident from this listing that there was considerable doubt with many of the morphological identifications and that many of these oysters might represent undescribed species. One oyster listed by Al-Kandari et al. [20] as *Crassostrea* sp. has now been described as a new species *Talonostrea salpinx* Oliver Salvi Al-Kandari 2021 based on molecular and morphological data [15]. That was the first record of the genus *Talonostrea* X.-X. Li and Z.-Y. Qi, 1994, in the Arabian region and the Indian Ocean as a whole.

In this study, we performed an integrative molecular and morphological assessment of specimens of a small cryptic, flat oyster (here referred to as “the Kuwait oyster”) that was tentatively referred to as the ostreine taxon *Nanostrea deformis* by Al-Kandari et al. [20]. While all specimens possessed typical ostreine anachomata and chomata, a number also had small pustular (lophine chomata) that are characteristic of lophine genera [21, 22]. The subfamily Lophinae Vialov, 1936, is no longer accepted, and lophine genera are now included in the subfamily Ostreinae Rafinesque, 1815, because they form a subclade that is nested within the Ostreinae clade [10, 11, 23]. However, it is still unclear whether their shared morphological characteristics do repre-

sent synapomorphies of this subclade or instead lack any phylogenetic and systematic value within the Ostreidae [9, 21, 22, 24]. In this respect, the taxonomic and phylogenetic assessment of this small oyster from Kuwait might provide some insights on the phylogenetic significance of lophine chomata. Here, we used mitochondrial and nuclear markers to estimate phylogenetic relationships of this taxon within the Ostreidae and we used cytochrome oxidase I (COI) data to assess species delimitation, intraspecific variation, and phylogeographic relationships with its closest relative. Combined with a morphological assessment, these results support the recognition of Kuwaiti specimens as a new species that is described herein. The aim of this study is to identify independent evolutionary lineages or cryptic taxonomic units within northern Arabian Gulf flat oysters and to contribute to our understanding of the biogeographic and biodiversity patterns of intertidal fauna of this region.

## 2. Materials and Methods

**2.1. Study Area.** Details of the oceanography and marine biology of Kuwait can be found in Al-Yamani et al. [25] and more recently revised by Al-Yamani [26]. These works contain an extensive synthesis of Kuwait’s marine environments, and we have extracted some pertinent characteristics relevant to the subject of this paper.

The intertidal of Kuwait features hard and soft substrate shores. In the inner Kuwait Bay and the northern regions, around Boubyan, soft sediment consisting of fine muds dominates. On the outer shores of Kuwait Bay, the soft sediments become sandier and this trend continues along the south coast. Hard substrate shores are present throughout the country and can consist of bedrock reefs or a mixture of boulders, and cobble intermixed with soft sediments. A similar assortment of substrates is also found on the outer islands of Failaka and Miskan.

The waters of Kuwait are shallow, much of the region being a flooded estuary rarely exceeding a few meters depth and with a maximum of 35 m offshore. The tidal range on average is 2 m but can reach 3.5 to 4 m during equinoxes. This, in combination with the shallow topography, results in extensive intertidal flats exposed for many hours. While sea water temperatures vary a little, air temperatures range from a possible 0°C on winter nights to 51°C at the height of summer. Consequently, the intertidal fauna is subjected to extreme variations when exposed at low tide. The shallowness of the water and predominance of fine muds offshore results in a high level of sediment resuspension especially in Kuwait Bay and around Boubyan. This is exacerbated around Boubyan through the input of riverine suspended solids from the Shatt el-Arab, such that the concentration can reach 510 mg/l. In Kuwait Bay, concentrations may reach 40 mg/l but drop off along the south coast to a recorded minimum of 0.3 mg/l.

The extremes and variation in temperature, salinity, and turbidity make the rocky intertidal a rather inhospitable environment for unprotected animals. On exposed surfaces, the molluscan fauna is limited to a small variety of gastropods, mainly thick-shelled Cerithiidae and Muricidae, and

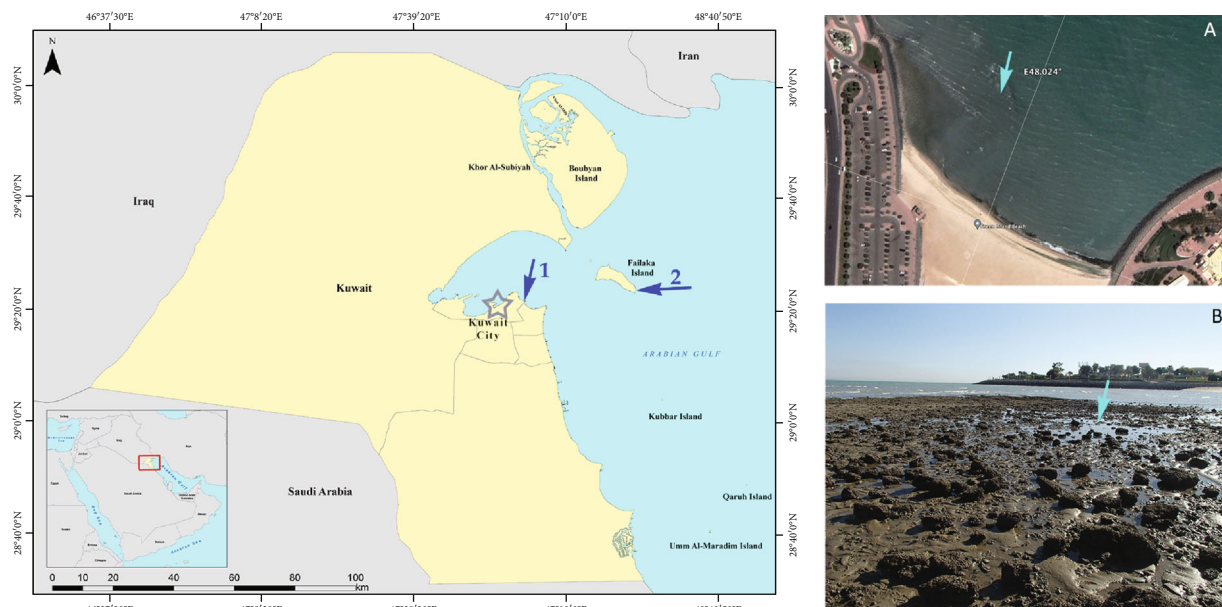


FIGURE 1: Map of Kuwait indicating known distribution of the Kuwait oyster under study. Blue arrows indicate collecting sites near Kuwait City (1: Al Sha'ab) and Failaka Island (2: Al Liwan). (a) Satellite image of the type locality, Al Sha'ab; (b) photograph of the beach at low tide at Al Sha'ab.

cemented bivalves such as larger oysters, spondylids, and plitaculids. However, in cryptic environments such as rock crevices and beneath rocks, there is a diverse fauna of smaller species [20].

**2.2. Sampling.** Samples were initially collected during the KISR intertidal survey of 2014–2017 [20], and in 2019, further samples were collected specifically for tissue extraction for molecular studies. Fifty-six specimens of the morphotype listed in cf. *Nanostrea deformis* by Al-Kandari et al. [20] were collected from two localities (Figure 1; Table 1). Twenty-three specimens selected for molecular analyses were levered open, and the adductor muscle and mantle were excised whole or in part and fixed in 100% ethanol. All specimens used for the taxonomic assessment are deposited in the National Museum of Wales (NMW.Z).

**2.3. Morphological Assessment.** Morphometric assessments of oyster taxa are of little taxonomic utility because of the wide and overlapping intraspecific and interspecific variability in shell size and shape that are strongly determined by habitat type, growth stage, and settlement condition (e.g., [8, 10, 27–29]). Therefore, a morphological assessment of collected specimens was performed based on qualitative characters traditionally used to discriminate oyster taxa. Harry [21] and Littlewood [9] gave lists of characters used to define five groups of oysters: Saccostreinae, Striostreini, Crassostreinae (Crassostreini sensu; [21]), Ostreinae, “lophinae” (Lophinae sensu; [21]; now considered as part of Ostreinae; [4, 10, 11]), and Pycnodonteinae (Gryphaeidae Vialov, 1936). The shell characters listed were as follows: plication of the upper valve, hyote spines, lamellation of the upper valve, chalky deposits, commissural shelf, shape of adductor muscle, and colour of adductor scar. These char-

acters were derived from Harry [21], and in that paper, the characters were further refined especially the form of the chomata, which are listed as vermicular, pustular, and ostreine (anachomata on the right valve and catachomata on the left valve). Vermicular chomata take the form of dense transverse ridges on either side of the ligament; ostreine take the form of single tubercles along the outer margin and where the anachomata and catachomata interlock; lophine or pustular chomata take the form of small raised pustules on the inner part of the margin and have no corresponding pits on the opposing valve (Figure 2). These characters will be used to compare species under consideration here.

#### 2.4. Molecular Assessment

**2.4.1. DNA Extraction, Amplification, and Sequencing.** Total genomic DNA was extracted from alcohol-preserved tissues of the 23 specimens following standard high-salt protocols [30]. Two mitochondrial gene fragments were amplified by polymerase chain reaction (PCR), the barcode fragment of the cytochrome oxidase subunit I (COI), and the 16S rRNA (16S), using primers and PCR protocols described in Salvi et al. [31] and Crocetta et al. [32]. Additionally, for selected specimens, we amplified a fragment of the nuclear 28S rRNA (28S) using published primers slightly modified to better match the available 28S sequences of oysters: the primer F4ostrd-OS (5'-TCCGATAGCAAACAAGTACCGTG-3') modified from 28S-F4-OSTRD by Mazón-Suástegui et al. [33] and the primer D6R-OS (5'-GCTATCCTGAGGGAACYTCAGAGG-3') modified from D6R by Park and Ó Foighil (2000). PCR protocol for the amplification of 28S followed Park and Ó Foighil (2000). Sequencing of PCR products was carried out by the company GENEWIZ

TABLE 1: GenBank accession number, COI haplotype, and GenSeq nomenclature (after Chakrabarty et al. [35]) for genetic sequences obtained from voucher specimens of *Ostrea oleomargarita* sp. nov. analysed in this study.

Species	Specimen catalog #	Locality	GenBank accession number		COI haplotype	GenSeq nomenclature
			COI	28S		
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/1 (paratype)	Kuwait City, Al Sha'ab	—	ON614105	—	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/2 (paratype)	Kuwait City, Al Sha'ab	ON614732	ON614106	h8	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/3 (paratype)	Kuwait City, Al Sha'ab	ON614733	ON614107	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/4 (paratype)	Kuwait City, Al Sha'ab	ON614734	ON614108	h9	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/5 (paratype)	Kuwait City, Al Sha'ab	ON614735	ON614109	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/6 (paratype)	Kuwait City, Al Sha'ab	ON614736	ON614111	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/7 (paratype)	Kuwait City, Al Sha'ab	ON614737	ON614112	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/8 (paratype)	Kuwait City, Al Sha'ab	ON614738	ON614113	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/9 (paratype)	Kuwait City, Al Sha'ab	ON614739	ON614114	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/10 (paratype)	Kuwait City, Al Sha'ab	ON614740	ON614116	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.009/11 (paratype)	Kuwait City, Al Sha'ab	ON614741	ON614117	h1	GenSeq-2 COI, 16S, 28S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/1 (paratype)	Ras Al Liwan, Failaka Island	ON614721	ON614100	h4	GenSeq-2 COI, 16S, 28S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/2 (paratype)	Ras Al Liwan, Failaka Island	ON614722	ON614104	h5	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/3 (paratype)	Ras Al Liwan, Failaka Island	ON614723	ON614110	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/4 (paratype)	Ras Al Liwan, Failaka Island	ON614724	ON614115	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/5 (paratype)	Ras Al Liwan, Failaka Island	ON614725	ON614118	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/6 (paratype)	Ras Al Liwan, Failaka Island	ON614726	ON614119	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/7 (paratype)	Ras Al Liwan, Failaka Island	ON614727	ON614120	h2	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/8 (paratype)	Ras Al Liwan, Failaka Island	ON614728	ON614121	h3	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/9 (paratype)	Ras Al Liwan, Failaka Island	ON614729	ON614122	h6	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/10 (paratype)	Ras Al Liwan, Failaka Island	ON614730	ON614101	h7	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/11 (paratype)	Ras Al Liwan, Failaka Island	ON614731	ON614102	h1	GenSeq-2 COI, 16S
<i>Ostrea oleomargarita</i>	NMW.Z.2021.009.011/12 (paratype)	Ras Al Liwan, Failaka Island	—	ON614103	—	GenSeq-2 COI, 16S



FIGURE 2: Types of chomata as defined by Stenzel [63] and Harry [21]. (a) Small ostreine chomata in *Ostrea edulis*. (b) Large tuberculous ostreine chomata in *Saccostrea cucullata*. (c) Dense pustular lophine chomata in *Lopho cristagalli*. (d) Irregular ostreine chomata close to the hinge and sparse lophine pustules around the margin in *Dendostrea sandvichensis*. (e) Vermiculate chomata in *Hyotissa inermis* (Gryphaeidae).

(<https://www.genewiz.com>), using the forward and reverse primers employed for amplification.

The obtained chromatograms of each sequence were manually edited and assembled into a consensus sequence using Geneious v. 11.0.12 (Biomatters Ltd., Auckland, New Zealand). The COI chromatograms did not show any double

peak, and the translated aminoacidic sequence did not have any stop codon, thus confirming that nuclear copies (pseudogenes) were not occurring in our mitochondrial COI sequence dataset. The 28S chromatograms did not show any heterozygote position. We used MEGA-X v.10.0.8 [34] to calculate the number of variable sites of each alignment

and to compute genetic distances between the Kuwait oyster and *Ostrea futamiensis* based on COI sequences.

We obtained 21 sequences of COI (600 base pair, bp), 23 sequences of 16S (446-451 bp), and two sequences of 28S (751 bp) of the Kuwait oyster. Details on sample data and GenBank accession numbers of sequences generated in this study are provided in Table 1 where we also indicated the GenSeq nomenclature for genetic sequences based on the taxonomic reliability of the source specimens following [35].

**2.4.2. DNA-Barcoding and Phylogenetic Analyses.** After verification that all the sequenced specimens belong to the same species (COI sequence identity  $\geq 99\%$ ), we used one sequence (h1) as a query to perform a preliminary molecular species-level identification with the DNA-barcoding approach implemented in the BOLD Identification System (IDS; [36]). IDS analyses based on all COI barcode records with a minimum sequence length of 500 bp did not find either a species-level (less than 1% divergence) or a genus-level (less than 3% divergence) match for the query sequence. The top 20 matches were sequences of *Ostrea futamiensis* from Japan with a similarity score of 91.9-92.5%. Therefore, we performed phylogenetic analyses to assess the systematic relationships of the Kuwait oyster.

The phylogenetic assessment was performed in two steps. First, to establish the affiliation of the Kuwait oyster with extant subfamilies of Ostreidae, newly generated sequences of the three genes of two specimens were aligned with homologous sequences of 20 oyster species obtained from GenBank representing all currently accepted genera within the subfamilies Striostreinae, Saccostreinae, and Crassostreinae, and six out of nine currently accepted genera of Ostreinae [4]. For this analysis, we used two species of Gryphaeidae as the outgroup based on previous phylogenetic studies [37–39]. Once the affiliation of the Kuwait oyster to the subfamily Ostreinae had been established, we built another dataset using 24 species of Ostreinae and two species of the subfamily Saccostreinae. The latter was used as the outgroup based on the results of the first analysis and previous phylogenetic studies [37, 39]. GenBank accession numbers and references for the DNA sequences used for phylogenetic analyses are reported in Table 2.

Multiple sequence alignments were performed with MAFFT v.7 on the web server (<https://mafft.cbrc.jp/alignment/server/>; [40]) using the E-INS-i iterative refinement algorithm. For each gene and dataset, details on alignment length and number of polymorphic sites and parsimony informative sites are provided in Supplementary Table S1. Single-gene alignments were concatenated in a single matrix that was used for downstream phylogenetic analyses using maximum likelihood (ML) and Bayesian Inference (BI) methods. ML analyses were performed in the W-IQ-TREE web server v.1.6.12 (<http://iqtree.cibiv.univie.ac.at/>; [41]) based on a partitioned model. For each gene partition, the best substitution model was determined by the ModelFinder module [42] using an edge-linked model and the cAIC criterion (the BIC criterion selected the same models). ML tree searches were based on the 20 best initial trees (out of 100 parsimony trees and the

BIONJ generated) and until W-IQ-TREE cannot find a new best tree in the last 100 iterations (nstop = 100). Node support was assessed with 1,000 pseudoreplicates of ultrafast bootstrapping (uBS). Bayesian analyses were carried out with MrBayes v.3.2.7 [43], using for each gene partition the same substitution models selected by ModelFinder. We ran two Markov chains of five million generations each, sampled every 1,000 generations. Consensus trees (50% majority rule) and posterior probability values (PP) were calculated on trees sampled after a burn-in of 25%. Tracer v.1.7 (Rambaut et al., 2018) was used to check for run convergence and ESS values  $> 200$ . FigTree v.1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used to visualize the tree.

The close phylogenetic relationship between the Kuwait oyster and *Ostrea futamiensis* recovered in ML and BI analyses was further investigated using a phylogenetic network approach based on COI sequence data. We used POPART v1.7 [44] to construct phylogenetic networks based on the median-joining network [45] and the statistical parsimony [46] methods.

**2.4.3. Species Delimitation.** The BOLD Identification System (IDS) uses a standard conservative threshold of  $<1\%$  sequence divergence for animal species identification as this method is designed to defend against “overdiagnosis” [36]. Therefore, a lack of species-level match does not guarantee an interspecific divergence for the COI sequence of our unidentified oyster. To aid discrimination between intraspecific and interspecific variation, we applied species delimitation methods. For this purpose, we built a nonredundant database of COI of Ostreinae, mining sequences from the public repositories of GenBank and BOLD, with accepted and synonymized genus names as search queries (data updated to 23/11/2021). Retrieved sequences not identified to species level or shorter than 300 base pairs were eliminated. Duplicated () function [47] of RStudio v.1.4.1103 [48] was used to dereplicate the dataset by removing sequences having identical GenBank accession numbers (see [49], for more details). This dataset was aligned with the newly generated COI sequences of oysters from Kuwait using the G-INS-i progressive method algorithm implemented in MAFFT. The alignment was trimmed to the standard COI barcoding fragment (5' portion of the gene) using Folmer's primers as reference [50]. The final alignment used for species delimitation analyses consisted of 456 sequences and 659 positions (among which 113 polymorphic sites and 93 parsimony informative sites).

We applied species delimitation (SD) on the generated dataset to infer species clusters using both distance-based and tree-based approaches. Assemble Species by Automatic Partitioning (ASAP) analyses were run using the ASAP web interface (<https://bioinfo.mnhn.fr/abi/public/asap>; [51]) with the Kimura-two parameter (K2P; [52]) substitution model and default parameters. ASAP delimitation was defined by evaluating both the partitions with the first and the second best ASAP scores according to Puillandre et al. [51]. As a tree-based method, we used the multirate Poisson Tree Processes model (mPTP v.0.2.4; <https://github.com/>

TABLE 2: GenBank accession number and reference for the DNA sequences used in this study for phylogenetic analyses.

Species	GenBank accession number		COI	DNA sequence data source	
	16S	28S		16S	28S
<i>Alectryonella plicatula</i>	—	AF137037	—	Jozefowicz et al., [89]	Park et al., [90]
<i>Dendostrea folium</i>	—	AF052069	—	Jozefowicz et al., [89]	Ó Foighil and Taylor [68]
<i>Dendostrea frons</i>	KP455014	AF137039	Pagenkopp Lohan et al., [91]	Jozefowicz et al., [89]	Ó Foighil and Taylor [68]
<i>Dendostrea sandwicensis</i>	KC847121	KC847142	Li and Wang, unpublished	Li et al., unpublished	Li et al., unpublished
<i>Lophia cristagalli</i>	AB076908	AF137038	Matsumoto, [38]	Jozefowicz et al., [89]	Ó Foighil and Taylor [68]
<i>Ostrea algoensis</i>	—	AF137041	—	Jozefowicz et al., [89]	Ó Foighil and Taylor [68]
<i>Ostrea angasi</i>	AF112287	AF137046	Ó Foighil et al., [92]	Jozefowicz et al., [89]	Ó Foighil and Taylor [68]
<i>Ostrea angelica</i>	KT317442	—	Raith et al., [10]	Jozefowicz et al., [89]	Ó Foighil and Taylor [68]
<i>Ostrea chilensis</i>	AF112286	AF137045	Ó Foighil et al., [92]	Raith et al., [10]	—
<i>Ostrea circumpecta</i>	AB898294	AB898280	Hamaguchi et al., unpublished	Jozefowicz et al., [89]	Ó Foighil and Taylor [68]
<i>Ostrea conchaphila</i>	KT317478	KT317155	Raith et al., [10]	Hamaguchi et al., [58]	Hu and Wang, unpublished
<i>Ostrea denselamellosa</i>	KP067907	AF052067	Kim et al., [93]	Raith et al., [10]	Ó Foighil and Taylor [68]
<i>Ostrea edulis</i>	JF274008	AF137047	Danic-Tchaleu et al., [94]	Jozefowicz et al., [89]	Ó Foighil and Taylor [68]
<i>Ostrea equestris</i>	AY376607	DQ242465	Kirkendale et al., [95]	Danic-Tchaleu et al., [94]	Ó Foighil and Taylor [68]
<i>Ostrea fluctigera</i>	LC149507	LC149503	Hamaguchi et al., [58]	Kirkendale et al., [95]	Shilts and Ó Foighil, unpublished
<i>Ostrea futamiensis</i>	AB898290	LC051605	Hamaguchi et al., unpublished	Hamaguchi et al., [58]	—
<i>Ostrea lurida</i>	KT317519	KT317226	Raith et al., [10]	Hamaguchi et al., [58]	—
<i>Ostrea megodon</i>	KX364276	KX364274	[6]	Raith et al., [10]	—
<i>Ostrea neostentina</i>	MK370330	MK370369	He and Wang, unpublished	[6]	[67]
<i>Ostrea permollis</i>	DQ226524	AF052075	Shilts and Ó Foighil, 2005, unpublished	[60]	—
<i>Ostrea puelchana</i>	DQ226518	AF052073	Shilts and Ó Foighil, unpublished	Jozefowicz et al., [89]	—
<i>Ostrea setoensis</i>	LC149514	LC149511	Hamaguchi et al., [58]	Jozefowicz et al., [89]	Ó Foighil and Taylor [68]
<i>Ostrea stentina</i>	DQ313183	DQ313180	Lapègue et al., [96]	Hamaguchi et al., [58]	—
<i>Planostrea pestigris</i>	—	KC847125	—	Lapègue et al., [96]	Shilts and Ó Foighil, unpublished
<i>Crassostrea virginica</i>	AY905542	AY905542	Milbury and Gaffney, [97]	Li et al., unpublished	Li et al., unpublished
<i>Crassostrea rhizophorae</i>	FJ717613	FJ478032	Lazoski et al., [98]	Milbury and Gaffney, [97]	Ó Foighil and Taylor [68]
<i>Magallana gigas</i>	KJ855241	KJ855241	Ren et al., [100]	Melo et al., [99]	Mazón-Suástegui et al., [33]
<i>Magallana hongkongensis</i>	FJ841963	FJ841963	Wu et al., [101]	Ren et al., [100]	Matsumoto, [38]
<i>Talostrea salpinx</i>	MZ126560	MZ099713	Al-Kandari et al., [15]	Wu et al., [101]	Wang et al., [13]
<i>Talostrea talonata</i>	KC683515	KC847134	Li et al., [102]	Al-Kandari et al., [15]	—
<i>Saccostrea scyphophilla</i>	MT293857	LM993882	Cui et al., [103]	Li et al., unpublished	Li et al., unpublished
<i>Saccostrea cucullata</i>	EU816078	AF458901	Xia et al., [104]	Salvi et al., [11]	Li et al., unpublished
<i>Striostrea margaritacea</i>	LT220873	LT220867	Salvi and Mariottini, [23]	Lam and Morton, [64]	Hammer, [105]
<i>Striostrea prismatica</i>	KP455045	KT317422	Pagenkopp Lohan et al., [91]	Salvi and Mariottini, [23]	Ó Foighil and Taylor [68]
<i>Hyotissa hyotis</i>	GQ166583	LM993886	Piazzi and Passamonti, [106]	Raith et al., [10]	—
<i>Neopycnodonte cochlear</i>	JF496772	JF496758	Piazzi et al., [39]	Salvi et al., [11]	Ó Foighil and Taylor [68]
		AF137034		Piazzi et al., [39]	Ó Foighil and Taylor [68]

Pas-Kapli/mptp; [53]) applied on the maximum likelihood tree obtained using the W-IQ-TREE web interface. *Saccostrea cucullata* (EU816078) was used as an outgroup and then removed from the SD analysis. mPTP analyses were performed using 10 runs of 100 million MCMC generations each, sampling every 10,000 (burn-in = 10%). Results from SD analyses were compared with the software LIMES v.1.3b [54] using the taxonomic index of congruence  $C_{\text{tax}}$  and the match ratios, which are two indexes that quantify the congruence between two partitions resulting from different SD approaches, and the relative taxonomic resolving power index  $R_{\text{tax}}$ , which quantifies the potential of an approach to capture a high number of species boundaries [55, 56].

### 3. Results

**3.1. Morphology and Patterns of Chomata.** Using the characters listed above, the morphology of the Kuwait oyster is compared with that of the five major groups of extant Ostreoidae (Table 3).

Considering all characters, the Kuwait oyster has most characters in common with the Ostreinae, and only in the presence of lophine chomata does it differ (Figure 2). Of the 56 specimens collected for this study including the 23 used for the molecular study in only 7 shells was the presence of lophine-like pustules expressed. There was no difference in numbers between Failaka and Al Sha'ab material. Given the confined sampling sites, no association between habitats can be inferred. Pustules are most often seen in thinner (perhaps less mature) shells, and in some thicker shells, they can be seen below the nacreous layer suggesting that they can be overgrown as the shell thickens.

**3.2. Phylogenetic Analyses.** Maximum likelihood and Bayesian analyses resolved the phylogenetic position of the Kuwait oyster by placing it within the subfamily Ostreinae with a high statistical support (uBS = 90; PP = 1.0; Figure 3). Phylogenetic trees of Ostreinae show three main clades (Figure 3): the *edulis* group including *Ostrea edulis*, *O. angasi*, and *O. chilensis* (uBS = 100; PP = 1.0) which is sister to *O. denselamellosa* (although this relationship received low support); the *stentina* group including *Ostrea stentina* and nine allied *Ostrea* species (uBS = 100; PP = 1.0); and the *algoensis* group including the Kuwait oyster, four remaining *Ostrea* species, and species of the genera *Nanostrea*, *Planostrea*, *Alectryonella*, *Dendostrea*, and *Lopha* (uBS = 74; PP = 0.99). The Kuwait oyster has a sister relationship with *O. futamiensis* that is well supported (uBS = 100; PP = 1.0), and this sister-pair forms a clade with *O. algoensis* (uBS = 84; PP = 1.0). Species previously included in the Lophinae form a clade (genera *Alectryonella*, *Dendostrea*, and *Lopha*), nested within the *algoensis* lineage, but with low support (uBS = 84; PP = 0.59).

The median-joining network shows a clear phylogeographic separation between haplotypes of the Kuwait oyster and haplotypes of *O. futamiensis* (Figure 4). The haplogroups corresponding to these two species are recovered as two separate networks in TCS analyses (result not shown).

TABLE 3: Matrix of shell characters of the Kuwait oyster and the main groups of the Ostreoidae.

Taxon		1	2	3	4	5	6	7	8
Fam. Gryphaeidae	Pycnodontinae	1	1	1	1	1	0	1	0
Fam. Ostreidae	Saccostreinae	1	1	2	2	0	1	2	1
Fam. Ostreidae	Crassostreinae	1	0	2	2	0	1	0	1
Fam. Ostreidae	Ostreinae	0	0	2	2	0	1	2	0
Fam. Ostreidae	“Lophinae”	1	1	0	0	0	1	3	0
Kuwait oyster		0	0	2	2	0	1	4	0

1: right valve not plicate (0); plicate (1); 2: hyote spines absent (0); present (1); 3: lamellae of right valve absent (0); erect (1); appressed (2); 4: chalk deposits absent (0); vesicular (1); nonvesicular (2); 5: commissural shelf absent (0); present (1); 6: muscle scar circular (0); reniform (1); 7: chomata absent (0); vermicular (1); ostreine (2); pustular (3); both ostreine and pustular (4); 8: muscle scar not distinctly coloured (0); distinctive (1).

Both species show a lack of geographic structure at the investigated geographical scale.

**3.3. Molecular Species Delimitation.** Results of species delimitation analyses based on ASAP and mPTP recovered the oyster from Kuwait as a species cluster distinct from the cluster of the closely related *O. futamiensis* (Figure 5). This result is confirmed in both partitions with the first (1.50) and second (4.50) best ASAP score and received maximum mPTP support (1.0). Overall, SD results by ASAP and the mPTP were highly congruent, with a  $C_{\text{tax}}$  value of 0.84 and a match ratio of 0.81. ASAP recovered 17 species groups, whereas mPTP recovered 20 groups. The latter results because of further splits in two of the groups recovered by ASAP: the group of the *O. stentina* splits into three clusters and the group of *O. lurida/O. concaphila* into two clusters. Both methods lumped *O. permollis/O. puelcana* and *O. edulis/O. angasi* sequences into a single cluster. Relative to the current taxonomy, as represented in MolluscaBase [4], the resolving power ( $R_{\text{tax}}$ ) of ASAP and mPTP was 0.84 and 1.0, respectively.

Based on morphological and molecular assessments, we assign the Kuwait oyster to a new species that is described in the following section.

#### 3.4. Systematics. Ostreoidae Rafinesque, 1815

Ostreidae Rafinesque, 1815

Ostreinae Rafinesque, 1815

*Ostrea* Linnaeus, 1758

**Generic Definition.** It is commonplace to give a generic definition when describing a new species. However, the molecular data presented here suggests that *Ostrea* is polyphyletic and that the new species described here and its sister taxon *O. futamiensis* do not cluster within the clade that includes *Ostrea edulis*, the type species of the genus *Ostrea* (see Discussion). While a systematic revision of the genus *Ostrea* is clearly needed, we have resorted to use *Ostrea* in its common understanding until a much more detailed phylogeny of the Ostreinae can be presented.



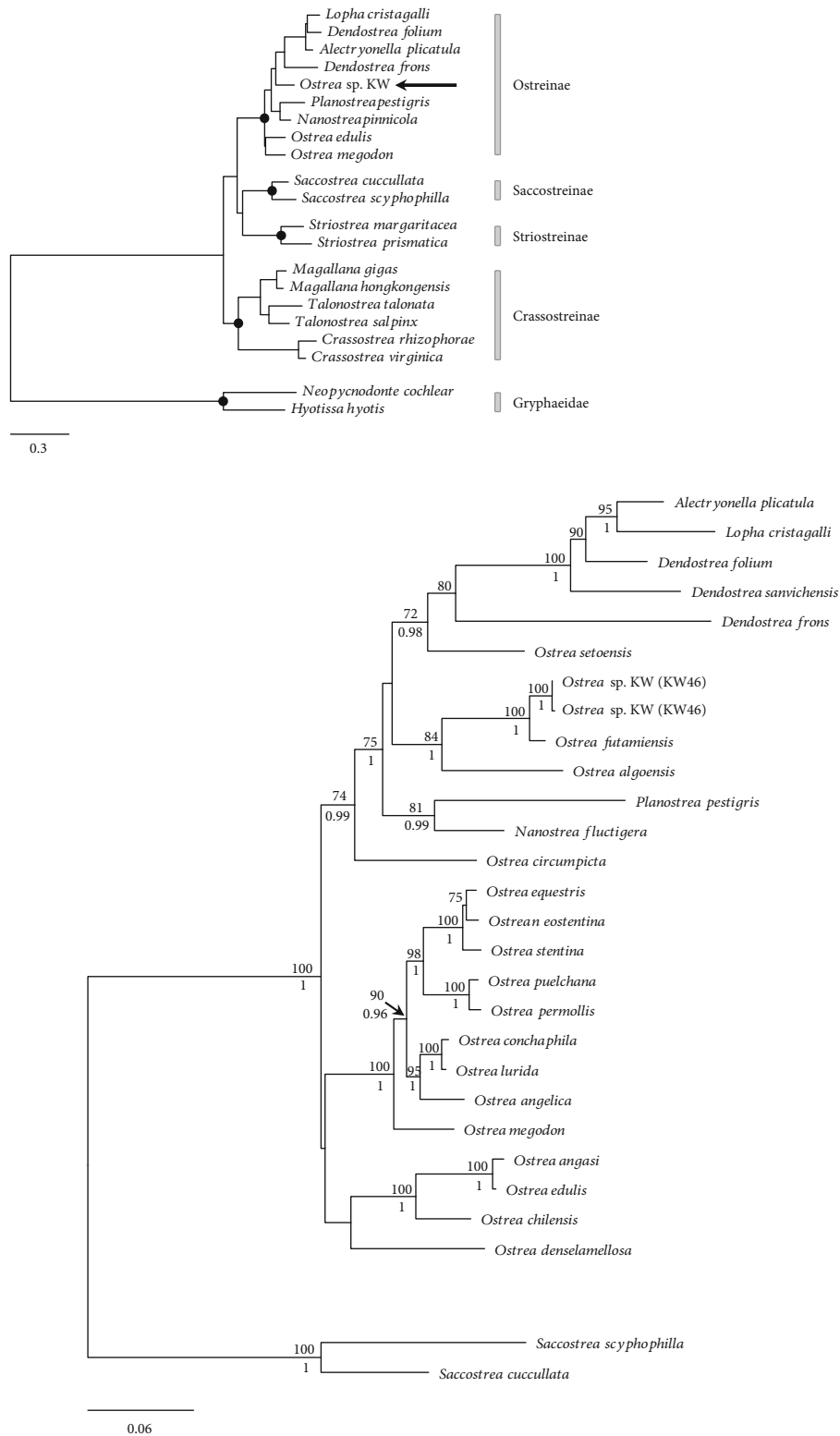


FIGURE 3: Maximum likelihood (ML) phylogenetic tree of Ostreidae (above) and Ostreinae (below) based on concatenated COI, 16S, and 28S sequences. The Ostreidae tree is rooted with Gryphaeidae species and represents the phylogenetic position of the Kuwait oyster relative to current subfamilies; black circles represent nodal support at main nodes (ultrafast bootstrap of maximum-likelihood analysis, uBS > 90; posterior probability of Bayesian analysis, PP > 0.95). The Ostreinae tree is rooted with *Saccostrea* species and represents the phylogenetic relationships between the Kuwait oyster (*Ostrea sp. KW*) and Ostreinae species and represents the phylogenetic relationships between the Kuwait oyster (*Ostrea sp. KW*) and Ostreinae species; nodal support is reported at nodes (uBS/PP).

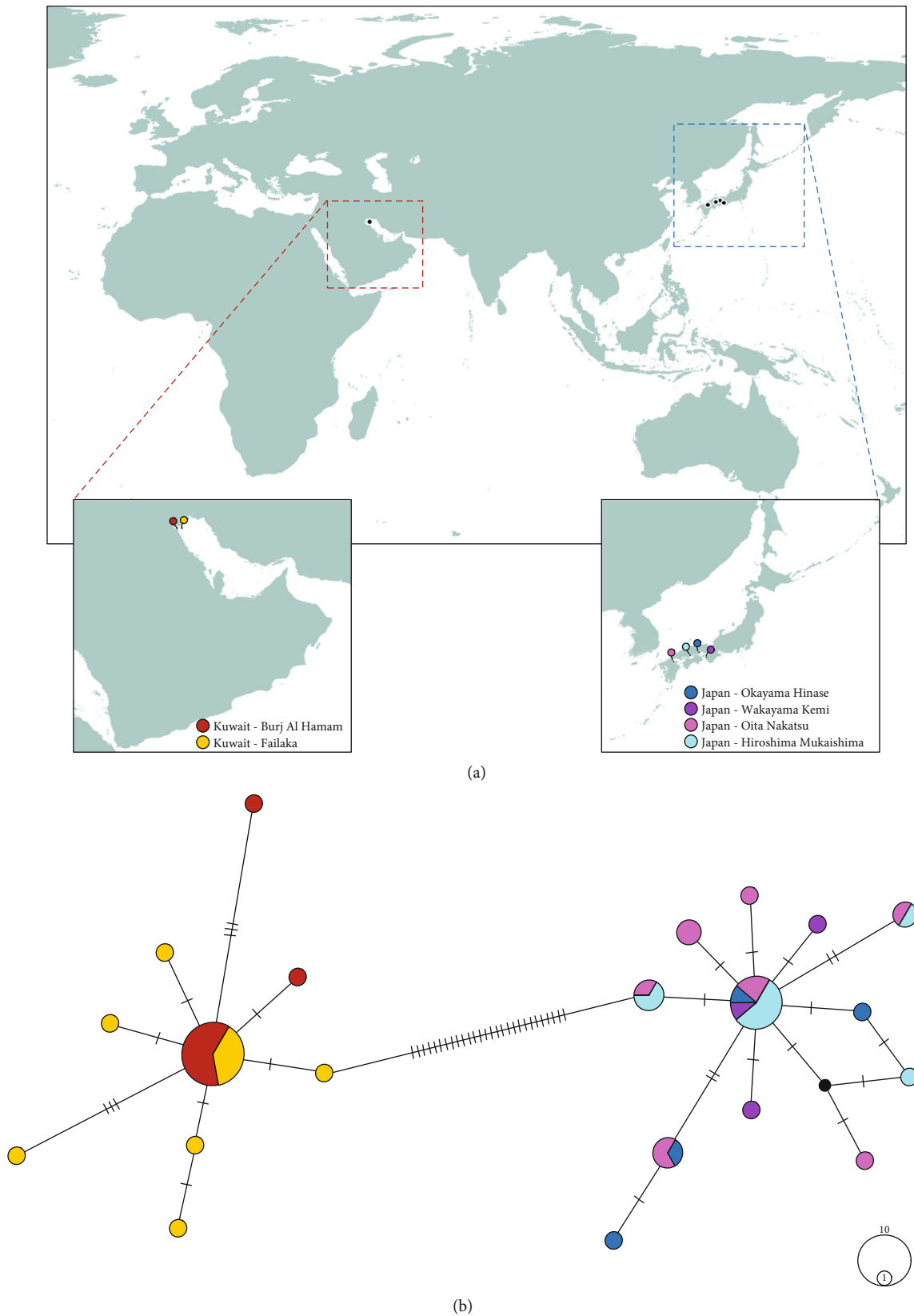


FIGURE 4: Phylogeography of *Ostrea* sp. KW from Kuwait and *Ostrea futamiensis* from Japan. (a) Maps represent collection localities. (b) Median-joining network based on COI sequences generated in this study (Kuwait samples) and Hamaguchi et al. [88] (Japan samples). Haplotypes in the network are represented by circles with size proportional to their frequencies and coloured according to locality of origin; small vertical bars represent nucleotide substitutions.

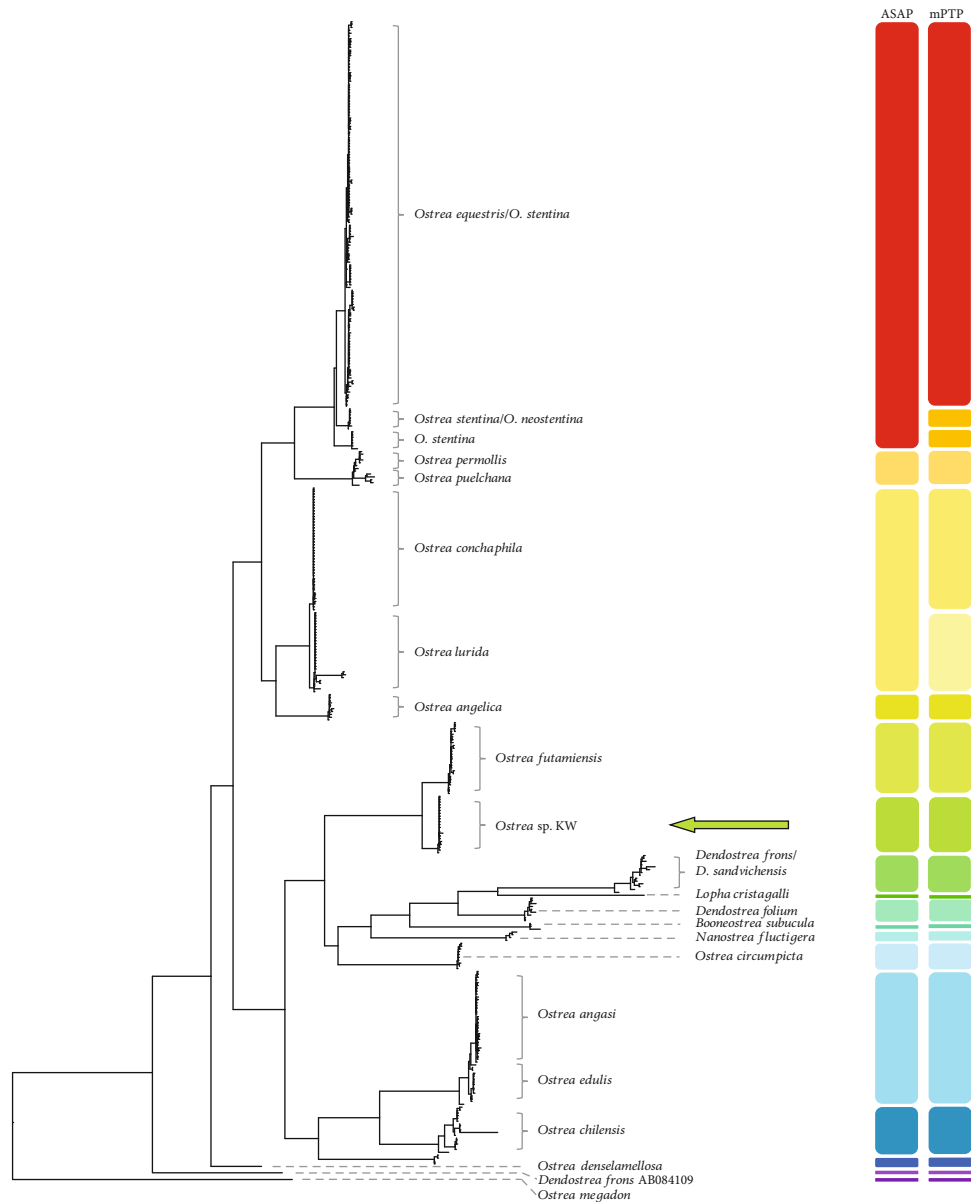


FIGURE 5: Rooted maximum likelihood tree based on the COI gene fragment (456 sequences) with information about the taxonomic assignment of sequences as in GenBank and BOLD records (morphospecies assignments). On the right, results of species delimitations based on Assemble Species by Automatic Partitioning (ASAP) and multirate Poisson Tree Process (mPTP) methods. Candidate species obtained with each analysis are depicted as coloured boxes arranged in columns. The green arrow highlights the clade representing sequences of the Kuwait oyster.

*Ostrea oleomargarita*. Oliver, Salvi, and Al-Kandari, sp. nov. (Figures 6–7)

**Type Material.** All type material is deposited in the National Museum of Wales (NMW.Z). Kuwait • holotype, 1 complete shell attached to a rock. Kuwait City, Al Sha'ab, 29.3675°N 48.0244°E. Low intertidal, attached under a rock on gravel. Coll. PG. Oliver, December 2019, not sequenced. NMW.Z.2021.009.008 (Figures 6(a), 6(b), and 7(a)). Paratypes, 11 spec. used for sequencing NMW.Z.2021.009.009/#1–#11.4 figured shells, data as holotype. NMW.Z.2021.009.010 (Figures 6(c)–6(f), 7(b), and 7(d)).

Kuwait • Paratypes, 12 spec. used for sequencing, NMW.Z.2021.009.011/#1–#12; #7 figured, Figures 6(g) and

7(c)). Ras Al Liwan, Failaka Island, 29.3902°N, 48.3988°E. Low intertidal, attached under rocks. Coll. PG Oliver, December 2019.5 shells +3 upper valves, not sequenced, NMW.Z.2021.009.012., as NMW.Z.2021.009.011.

**Other Material Examined.** Kuwait • 20 shells attached to a rock. Kuwait City, Al Sha'ab, 29.3675°N 48.0244°E. Low intertidal, attached under a rock on gravel. Coll. PG. Oliver, December 2019. NMW.Z.2021.009.013.

India • 1 shell +1v. Bombay (Mumbai), Bandra (approx. 19.06°N, 72.82°E). 5 April 1946. Annotated "Ranson 26." 3v. Bombay (Mumbai), Juhu (approx. 19.10°N, 72.8°E). Coll. Winckworth, 23 October 1936. Annotated "Ranson 27."

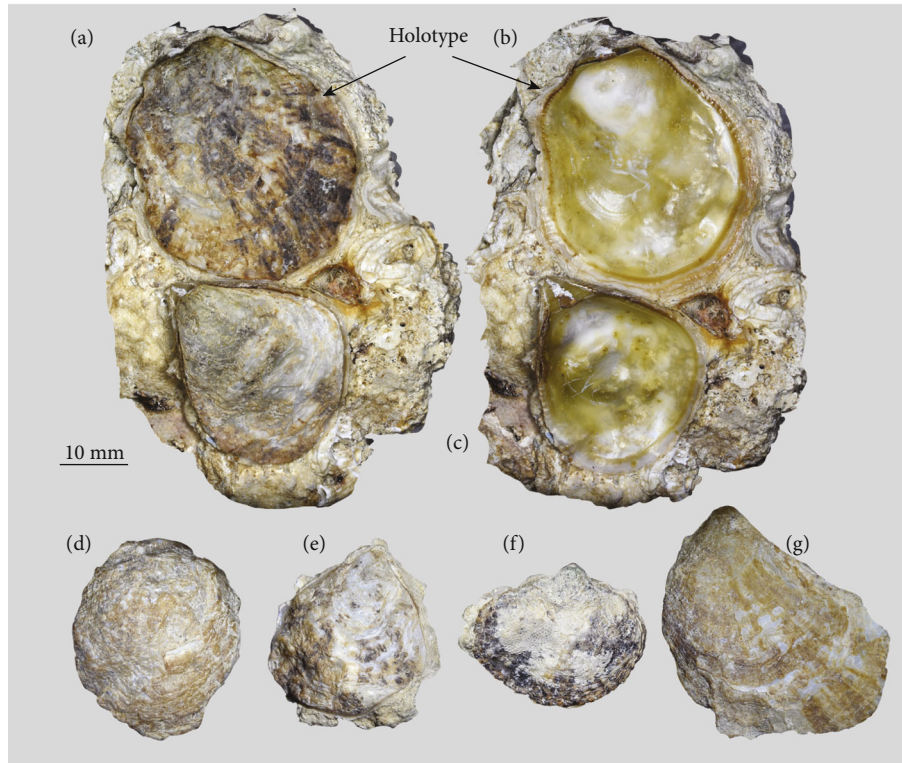


FIGURE 6: *Ostrea oleomargarita* sp. nov. (a) Holotype upper shell, Al Sha'ab specimens in situ. (b) Holotype upper shell, internal view of attached valves showing catachomata, greenish interior, and brown margin (NMW.Z.2021.009.008). (c-f) Paratypes, variations in external sculpture, and colouration, all Al Sha'ab; (d) brown tubercular; (e) black and beige, foliar; (f) Al Sha'ab typically encrusted with Bryozoa; (NMW.Z.2021.009.010). (g) Failaka, rayed foliar (NMW.Z.2021.009.011 #7).

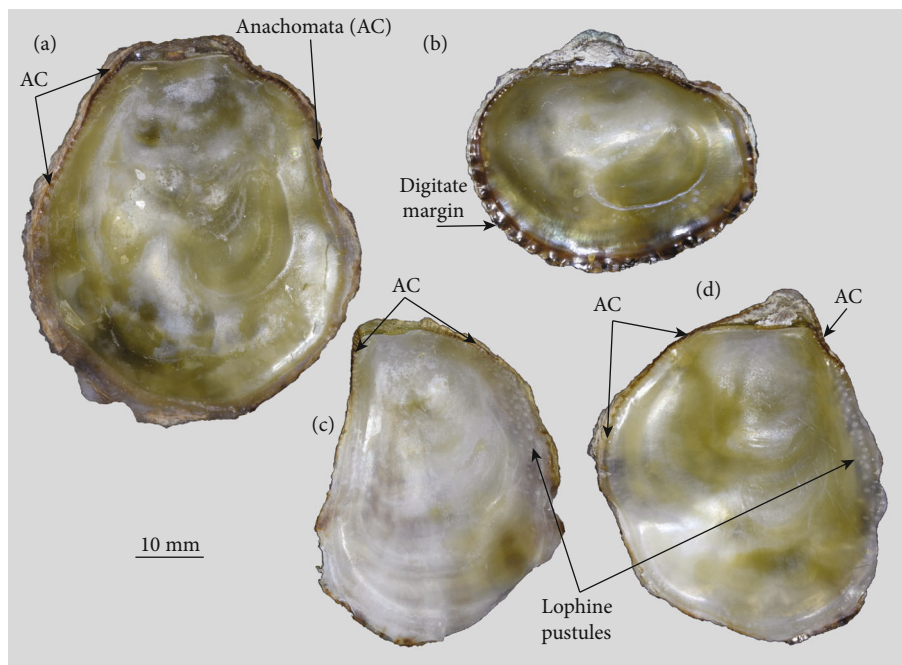


FIGURE 7: *Ostrea oleomargarita* sp. nov. Internal views of upper valves to show differences in marginal form and chomata. (a) Holotype Al Sha'ab, anachomata, and ventral smooth margin (NMW.Z.2021.009.008). (b) Al Sha'ab, digitate margin (NMW.Z.2021.009.010). (c), Failaka, weak anachomata, and lophine pustules. (d) Al Sha'ab anachomata and lophine pustules (NMW.Z.2021.009.010).

**Shell Description.** Small shells to 20 mm in diameter. Thin but robust. Roughly circular, oval to pyriform. Lower valve shallowly cupped to irregular; cemented for most or all of its attachment, margin smooth or if free then finely plicate. Nonnacreous margin, very narrow. Ligament area narrow, elongated in some. Prominent catachomata on both anterior and posterior dorsal margins. Adductor muscle large, reniform. Interior colour flushed olive-green, paler in some smaller shells, most with a distinct brown, narrow marginal band. Upper valve flat, irregular to domed. Anachomata corresponding to catachomata. Sparse elongate tubercles (lophine pustules) on posterior ventral edge in some. Internal colour as a lower valve. The outer surface is usually obscured by epifaunal growths, typically Bryozoa, calcareous algae, worm tubes, or other oysters. Sculpture mostly of flattened foliar scales, some areas finely tubercular, colour of radial bands of rust-brown to black on a grey-beige ground, some almost uniform ground colour. Thin chalky layers are present most visible in attached valves.

**Derivation of Name.** Derived from the combination *oleo* from *olivarius* (Latin) referring to the colour olive-green and *margarita* from *margarites* (Latin) referring to the pearly lustre of the inner surfaces of the valves. Thus, to the typical olive-green lustre of the nacreous inner surface of the valves.

**Type Locality.** Kuwait, Kuwait City, Al Sha'ab, 29.3675°N 48.0244°E. Intertidal, under rocks.

**Distribution.** In Kuwait, specimens from only two sites were verified as *O. oleomargarita* by molecular data. Similar shells were recorded from a number of other sites [20] under the name of *Nanostrea deformis* ranging from Khor Al-Subaya in the north of the country to other sites in Kuwait Bay and on Failaka. This species is probably widely distributed in the Northern Arabian Gulf but given the morphological plasticity, all records need confirmation by molecular data. Shells from the west coast of India were confirmed by shell morphology only.

**Remarks.** Comparison of the shells of *O. oleomargarita* with those of *O. futamiensis* can be made and distinctions listed, but the degree of variation seen in the former suggests that such differences may not be consistent. Perhaps, most significantly neither Seki [57] nor Hamaguchi et al. [58] note the presence of lophine pustules in *O. futamiensis* while these are present in some specimens of *O. oleomargarita*. Seki [57] gives the maximum size of *O. futamiensis* as 40 mm in height whereas *O. oleomargarita* reaches no more than 20 mm. Seki [57] states that *O. futamiensis* is circular or oval, and Hamaguchi states that it is circular while *O. oleomargarita* is much more variable. Seki [57] notes that the internal colour is greenish often tinged with dark blue, and this is seen in the photographs given by [59] (plate 8, Figures 1(b) and 1(c)); such blue colouration has not been observed in *O. oleomargarita*.

While these morphological differences may have been sufficient to differentiate the two species, they are not consistent enough, on their own, to warrant species recognition. Hamaguchi et al. [58] comment on the problematic shell-based taxonomy of five species of small Japanese flat oysters noting that molecular sequences were instrumental in separating them.

## 4. Discussion

The northern Arabian Gulf is confirmed as a key region for the discovery of marine animal diversity. This study documented a new overlooked oyster species with phylogenetic affinities to an Indo-West Pacific species and a peculiar combination of morphological characters among oysters. This result echoes the recent discovery of another enigmatic oyster species in the same waters, *Talonostrea salpinx*, also related to the Indo-West Pacific taxa [15]. The results are relevant to our comprehension of oysters' diversity and distribution and the biogeography of the marine biota of the Pacific Ocean, as discussed in the following.

**4.1. The New Ostreine from Kuwait: Insights into the Classification of Ostreinae and on the Taxonomic Value of Chomata.** The Kuwait oyster, *Ostrea oleomargarita* sp. nov., shows an unprecedented combination of morphological characters typical of different groups of Ostreinae. The shell morphology is most similar to *O. futamiensis*, but in none of the descriptions or figures of that species is there mention of the presence of lophine pustules on the inner margin [57–59]. These pustules are not seen on all specimens from Kuwait, and in itself, this character may not be enough to justify the raising of the Kuwait populations to new species level. However, the molecular data presented here provides strong evidence that the Japanese and Kuwaiti populations do represent distinct species.

The combination of a phylogenetic approach with species delimitation methods strongly supports an interspecific divergence between *O. oleomargarita* sp. nov. and *O. futamiensis* (Figures 4 and 5). The average genetic divergence between these two species at the COI (*p*-distance: 5.16%; K2P distance: 5.41%) is similar to, or higher than, values observed between closely related oysters (e.g., *O. conchaphila*/*O. lurida*, *O. angasi*/*O. edulis*, *O. equestris*/*O. stentina*, and *O. puelchana*/*O. permollis*; ([5, 60]; Raith et al., [10])). Remarkably, species delimitation methods, applied to oysters for the first time in this study, revealed a close match between current ostreine taxonomy [4] and species clusters inferred based on the barcoding marker COI (Figure 5). The only differences in species delimitations produced by distance-based and tree-based methods concern two well-known species complexes: the *O. stentina* complex and the *O. lurida*/*O. conchaphila* pair. These complexes are lumped into two species clusters by the distance-based method, whereas they are split into more species by the tree-based approach. These complexes have undergone several taxonomic changes following various phylogenetic assessments [5, 21, 60, 61] and are currently considered as composed of distinct species of recent diversification [60, 62]. On the other hand, both SD methods lumped *O. permollis*/*O. puelchana* and *O. edulis*/*O. angasi* sequences into two clusters, an issue that will require further taxonomic assessments.

From a morphological point of view, features of *Ostrea oleomargarita* have most in common with the Ostreinae and only in the presence of lophine-like pustules is there affinity with lophine genera (e.g., *Lopha* and *Dendostrea*). The flattened form with only weak plications, lack of hyote

spines, appressed lamella, and presence of chalky deposits are ostreine not lophine characters. The description and distribution of lophine pustular chomata are more variable than suggested by Stenzel [63] and Harry [21] who described them as “minute and numerous, they are located along margins and form from one to several lines.” This description fits well with *Lopha cristagalli* (Linnaeus, 1758) but not with many species assigned to the genus *Dendostrea* where the pustules are larger and variably present (see Figure 2). Interestingly, Harry [21] in Table 2 indicates that the ostreine genera *Ostrea* and *Ostreola* have lophine chomata but no ostreine chomata, but this must surely be an error and should be reversed. In that table, Harry also states that his ostreine *Booneostrea* lacks any chomata, but this is in error as in some shells, ostreine chomata are present on the posterior dorsal margin (pers. obs. PG Oliver). Therefore, there is an indication that the form and distribution of chomata are not as definitive as suggested in previous studies. The variable expression of pustules in *O. oleomargarita* appears to have no pattern of occurrence, although they are most often present in thinner (perhaps less mature) shells, and in some thicker shells, they can be seen below the surface suggesting they have been overgrown by later shell growth. The pattern and the dimension of these structures might drastically change within the life of an individual in lophine species too. The pustules as exhibited in *O. oleomargarita* are low and sparse unlike the dense pustules seen in *L. cristagalli* or some species of *Dendostrea* suggesting that they may not be analogous and a more considered review of ostreoid chomata is warranted.

Ambiguous classification based on morphological characters is commonplace in oysters (e.g., [16, 17, 62, 64, 65]), and in most cases, a molecular approach allowed a straightforward systematic assessment of debated species [10, 11]. Phylogenetic results unequivocally place *O. oleomargarita* within the subfamily Ostreinae. Ironically, also in the molecular phylogeny of Ostreinae, the position of *O. oleomargarita* and *O. futamiensis* is somehow in between the *Nanostrea/Planostrea* clade and the lophine clade. The phylogenetic trees based on ML and BI analyses of multilocus data (Figure 3) support three major clades within the Ostreinae, two of these consisting entirely of species currently recognised within the genus *Ostrea* (the *edulis* group and the *stentina* group), whereas in the third clade (the *algoensis* group), the lophine genera *Alectryonella*, *Lopha*, and *Dendostrea* cluster with *Nanostrea* and *Planostrea* and with four species of *Ostrea* (*O. circumpicta*, *O. algoensis*, *O. futamiensis*, and *O. oleomargarita*). The nonmonophyly of the genus *Ostrea*, as currently conceived, is not surprising and has been recovered in many molecular phylogenetic studies [5, 11, 15, 23, 58, 60, 66–69]. However, unlike other subfamilies such as the Crassostreinae for which the same main lineages have been recovered with high support in all phylogenetic studies (see the review by [8]), relationships among Ostreinae are poorly resolved and unstable across studies, especially among those using single genes. A better definition of the main lineages within Ostreinae is apparent in recent multilocus assessments based on mitochondrial and nuclear data and wider taxon sets ([11, 23, 67]; this

study), suggesting that more data are needed to robustly resolve the phylogeny and systematics of this subfamily.

Phylogenetic trees estimated in this study and previous studies suggest that careful consideration of the criteria used to define Ostreinae genera is particularly needed. Many taxonomic incongruences observed are perhaps only so because a number of genera based on morphology have been retained (for example, in the lophine subclade) contrasting with the other clades where at least five other available generic names have been regarded as junior synonyms of *Ostrea* (e.g., *Cryptostrea* [21], *Myrakeena* [21], *Ostreola* Monterosato, 1884 and *Undulostrea* [21]3). Indeed, some of these available generic names might be useful to resolve the extensive polyphyly of *Ostrea* as currently conceived. This implies that *O. oleomargarita*, *O. futamiensis*, and *O. algoensis* could be assigned to a different genus in the future. However, the molecular dataset of Ostreinae is far from complete and certainly not sufficient to begin to alter their taxonomy and nomenclature. A further example of this complexity is the situation with species previously assigned to the genus *Ostreola* (the *O. stentina* complex) but where the available data was not used to modify the nomenclature and bring the genus *Ostreola* back into acceptance [60]. This study and others such as Hu et al. [60] highlight the continuing difficulty of resolving the taxonomy of the Ostreidae, especially using morphological characters. Despite the increasing number of molecular studies, the current taxonomy remains based on morphological decisions. Firstly, the introduction of genera by Harry [21] was based entirely on morphology, and secondly, the current nomenclature used in MolluscaBase [4] resulted from the decisions made by Huber [70] again based on morphology. It is unfortunate that the decisions made by Huber are not supported by evidence so, for example, why have *Nanostrea* and *Booneostrea* been retained while other distinctive oysters such as *Undulostrea* have been synonymised. Of note here is that many of these more obscure genera, following synonymies by Huber [70] are now monospecific. It will probably become increasingly accepted to prioritise molecular data for oyster systematics; paradigmatic in this respect is the situation with *Crassostrea* and *Magallana*, a recent example where shell morphology is of no value in distinguishing these genera despite their remarkable evolutionary distinctiveness [8].

**4.2. Potential Geographic Distribution and Focus on Underestimated Biodiversity of Persian Gulf and East/West Indian Ocean.** The close phylogenetic relationship between *O. oleomargarita* and *O. futamiensis* and available sequences of these two species discloses a remarkably wide biogeographic gap. To date, we only have molecular data evidence for the presence of *O. oleomargarita* along the Kuwait coasts in the northern Persian Gulf. Shells in the NHMUK from the west coast of India agree morphologically with those from the Arabian Gulf but were identified as *O. futamiensis* by Ranson. ([71]: 236) does not cite these specimens of *O. futamiensis*; instead, he lists material from the Bay of Bengal, Malaya, Singapore, Thailand, Philippines, and Japan. Ramakrishna and Dey [72] cite *Saccostrea futamiensis* from Gujerat (WIO) and Orissa (Bay of Bengal), and

Subba Rao [73] does mention the species. The shell from Orissa is described by Subba Rao et al. [74], and in that, it states the interior is white, thus making the identification suspect. The distribution of *O. futamiensis* extends in a few scattered regions of the West Pacific Ocean [75] such as Hong Kong [76] and Korea [77]. Therefore, it may be inferred that *O. futamiensis* is an East Indo-West Pacific species, whereas *O. oleomargarita* is a West Indian Ocean and Arabian species.

This distribution pattern could be supported by the fact that currents usually form an effective barrier for larval dispersal [78]. In particular, Sivadas and Ingole [79] studied the biodiversity pattern of Indo-Pacific benthic organisms and found that the low similarity between the diversity patterns along the Western and Eastern Indian coasts is due to environmental factors such as habitat heterogeneity and spatio-temporal variability of coastal currents. Unfortunately, biogeographic patterns and genetic structures of marine species in the Western Indian Ocean are scarce, but studies start to highlight an effective allopatric distribution and cryptic diversity of benthic species between W and E Indian coasts [80–82]. This suggests that there may be many such examples to be discovered, especially if we consider that the biodiversity of the Western Indian Ocean is limited to some species or groups [83]. The underestimated number of marine species of the Arabian/Persian Gulf is also based on a low current understanding of the species diversity of some countries [83]. In addition, many intertidal benthonic organisms, such as bivalve molluscs, show high levels of phenotypic plasticity, challenging traditional taxonomic assessment based on morphology [3]. Currently, two of the authors here are preparing an Atlas to the Bivalvia of Kuwait, and within the recognised 210 species, 23 are currently without verified species names (Oliver PG, pers. comm). Oysters, in particular, are engendered with very wide geographical ranges throughout the Indo-West Pacific, and it is likely that none of the species cited by Al-Kandari et al. [20] were correctly identified or have been properly described. The work of Taylor and Glover, on the Lucinidae, over the period of 1997–2021 revealed many examples where assumed pan Indo-Pacific species were complexes of species with more restricted ranges [84]. Al-Kandari et al. [20] cited examples where some bivalve species (e.g., *Congestia chesneyi*; Oliver & Chesney, 1994) had disjunct distributions occurring in the northern Gulf and then on the Pakistan/North-West Indian coast, such species were also absent from Oman. Tsang et al. [82] using *Tetraclita* barnacles showed a pattern of regional species within the Western Ocean, and this pattern may be expected from a more detailed study of oysters. New efforts using a molecular approach are uncovering new marine groups and species in this region [15, 85, 86].

Given the inferred complexity of the malacofauna of the Western Indian Ocean fauna [87], a wide-ranging study of the Bivalvia is warranted. The Red Sea and Arabian upwelling regions have high levels of endemism but also share species with the Gulf, Indo-Pakistan, and wider north WIO area [87]. Not only are the relationships between the East and West Indian Oceans to be further elucidated but also

biodiversity patterns within the Arabian region itself remain to be documented.

## Data Availability

All genetic data analysed in this study, either original or from previous studies, is available on GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>), with respective accession numbers listed in the manuscript (Table 1).

## Conflicts of Interest

The authors declare that they have no conflict of interests.

## Acknowledgments

The authors wish to thank Harriet Wood for the documentation of the voucher and type material into the collection of the National Museum of Wales and also thank Henk Dekker for his invaluable help in the collection of specimens in Kuwait in 2019. The Kuwait Institute for Scientific Research (KISR) provided financial support for this study.

## Supplementary Materials

Table S1: number of sequences, multiple sequence alignment length, and number of variable positions are reported for each gene and dataset (\*parsimony informative sites are variable sites with the less frequent nucleotide occurring in at least two sequences). (*Supplementary Materials*)

## References

- [1] W. Appeltans, S. T. Ahyong, G. Anderson et al., “The magnitude of global marine species diversity,” *Current Biology*, vol. 22, no. 23, pp. 2189–2202, 2012.
- [2] C. M. Duarte, *The magnitude of marine biodiversity*, The Exploration of Marine Biodiversity, 2001.
- [3] M. S. Clark, L. S. Peck, J. Arivalagan et al., “Deciphering mollusc shell production: the roles of genetic mechanisms through to ecology, aquaculture and biomimetics,” *Biological Reviews*, vol. 95, no. 6, pp. 1812–1837, 2020.
- [4] MolluscaBase, “Ostreidae Rafinesque,” 2021, <http://molluscabase.org/aphia.php?p=taxdetails&id=215> on 2022-05-23.
- [5] X. Guo, C. Li, H. Wang, and Z. Xu, “Diversity and evolution of living oysters,” *Journal of Shellfish Research*, vol. 37, no. 4, pp. 755–771, 2018.
- [6] C. Li, H. Wang, and X. Guo, “Classification and taxonomic revision of two oyster species from Peru: *Ostrea megodon* (Hanley, 1846) and *Crassostrea talonata* (Li & Qi, 1994),” *Journal of Shellfish Research*, vol. 36, no. 2, pp. 359–364, 2017.
- [7] M. Sekino and H. Yamashita, “Mitochondrial and nuclear DNA analyses of *Saccostrea* oysters in Japan highlight the confused taxonomy of the genus,” *Journal of Molluscan Studies*, vol. 82, no. 4, pp. 492–506, 2016.
- [8] D. Salvi and P. Mariottini, “Revision shock in Pacific oysters taxonomy: the genus *Magallana* (formerly *Crassostrea* in part) is well-founded and necessary,” *Zoological Journal of the Linnean Society*, vol. 192, no. 1, pp. 43–58, 2021.

- [9] D. T. J. Littlewood, "Molecular phylogenetics of cupped oysters based on partial 28S rRNA gene sequences," *Molecular Phylogenetics and Evolution*, vol. 3, no. 3, pp. 221–229, 1994.
- [10] M. Raith, D. C. Zacherl, E. M. Pilgrim, and D. J. Eernisse, "Phylogeny and species diversity of gulf of California oysters (Ostreidae) inferred from mitochondrial DNA," *American Malacological Bulletin*, vol. 33, no. 2, pp. 263–283, 2016.
- [11] D. Salvi, A. Macali, and P. Mariottini, "Molecular phylogenetics and systematics of the bivalve family Ostreidae based on rRNA sequence-structure models and multilocus species tree," *PLoS One*, vol. 9, no. 9, pp. 19–21, 2014.
- [12] D. Salvi, E. Berrilli, M. Garzia, and P. Mariottini, "Yet another mitochondrial genome of the Pacific cupped oyster: the published mitogenome of *Alectryonella plicatula* (Ostreinae) is based on a misidentified *Magallana gigas* (Crassostreinae)," *Frontiers in Marine Science*, vol. 8, p. 1296, 2021.
- [13] H. Wang, X. Guo, G. Zhang, and F. Zhang, "Classification of Jinjiang oysters *Crassostrea rivularis* (Gould, 1861) from China, based on morphology and phylogenetic analysis," *Aquaculture*, vol. 242, no. 1–4, pp. 137–155, 2004.
- [14] B. L. Ignacio, T. M. Absher, C. Lazoski, and A. M. Solé-Cava, "Genetic evidence of the presence of two species of *Crassostrea* (Bivalvia: Ostreidae) on the coast of Brazil," *Marine Biology*, vol. 136, no. 6, pp. 987–991, 2000.
- [15] M. Al-Kandari, P. G. Oliver, and D. Salvi, "Molecular and morphological systematics of a new, reef forming, cupped oyster from the northern Arabian Gulf: *Talonostrea salpinx* new species," *ZooKeys*, vol. 1043, pp. 1–20, 2021.
- [16] X. Wu, S. Xiao, and Z. Yua, "Mitochondrial DNA and morphological identification of *Crassostrea zhanjiangensis* sp. nov. (Bivalvia: Ostreidae): a new species in Zhanjiang, China," *Aquatic Living Resources*, vol. 26, no. 4, pp. 273–280, 2013.
- [17] K. Lam and B. Morton, "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*, vol. 228, no. 1–4, pp. 1–13, 2003.
- [18] D. O. Obura, "An Indian Ocean Centre of origin revisited: Palaeogene and Neogene influences defining a biogeographic realm," *Journal of Biogeography*, vol. 43, no. 2, pp. 229–242, 2016.
- [19] D. Al-Bakri, M. Foda, M. Behbehani et al., "Environmental assessment of the intertidal zone of Kuwait," Kuwait Institute for Scientific Research, Kuwait, 1985.
- [20] M. Al-Kandari, P. G. Oliver, W. Chen et al., "Diversity and distribution of the intertidal Mollusca of the State of Kuwait, Arabian Gulf," *Regional Studies in Marine Science*, vol. 33, article 100905, 2020.
- [21] H. Harry, "Synopsis of the supraspecific classification of living oysters (Bivalvia: Gryphaeidae and Ostreidae)," *The Veliger*, vol. 28, no. 2, pp. 1121–1158, 1985.
- [22] H. B. Stenzel, "Oysters," *Journal of Paleontology*, vol. 51, no. 2, pp. 427–429, 1977.
- [23] D. Salvi and P. Mariottini, "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*, vol. 179, no. 2, pp. 263–276, 2016.
- [24] N. Malchus, "Multiple parallel evolution and phylogenetic significance of shell chambers and chomata in the Ostreoida (Bivalvia)," *Bivalves: An Eon of Evolution*, vol. 1998, pp. 393–407, 1998.
- [25] F. Y. Al-Yamani, J. Bishop, E. Ramadhan, M. Al-Husain, and A. N. Al-Ghadban, *Oceanographic Atlas of Kuwait's Waters*, Kuwait Institute for Scientific Research, 2004.
- [26] F. Y. Al-Yamani, *Fathoming the Northwestern Arabian Gulf: Oceanography and Marine Biology*, Kuwait Institute for Scientific Research, Safat, Kuwait, 2021.
- [27] S. T. Hsiao, S. C. Chuang, K. S. Chen, P. H. Ho, C. L. Wu, and C. A. Chen, "DNA barcoding reveals that the common cupped oyster in Taiwan is the Portuguese oyster *Crassostrea angulata* (Ostreoida; Ostreidae), not *C. gigas*," *Scientific Reports*, vol. 6, no. 1, pp. 1–11, 2016.
- [28] K. S. Reece, J. F. Cordes, J. B. Stubbs, K. L. Hudson, and E. A. Francis, "Molecular phylogenies help resolve taxonomic confusion with Asian *Crassostrea* oyster species," *Marine Biology*, vol. 153, no. 4, pp. 709–721, 2008.
- [29] H. G. Spencer, R. C. Willan, P. Mariottini, and D. Salvi, "Taxonomic consistency and nomenclatural rules within oysters: comment on Li et al. (2022)," *Molecular Phylogenetics and Evolution*, no. article 107437, 2022.
- [30] G. A. Evans, "Molecular Cloning: A Laboratory Manual. Second edition. Volumes 1, 2, and 3. Current Protocols in Molecular Biology. Volumes 1 and 2," *Cell*, vol. 61, no. 1, pp. 17–18, 1990.
- [31] D. Salvi, G. Bellavia, M. Cervelli, and P. Mariottini, "The analysis of rRNA sequence-structure in phylogenetics: an application to the family Pectinidae (Mollusca: Bivalvia)," *Molecular Phylogenetics and Evolution*, vol. 56, no. 3, pp. 1059–1067, 2010.
- [32] F. Crocetta, P. Mariottini, D. Salvi, and M. Oliverio, "Does GenBank provide a reliable DNA barcode reference to identify small alien oysters invading the Mediterranean Sea?," *Journal of the Marine Biological Association of the United Kingdom*, vol. 95, no. 1, pp. 111–122, 2015.
- [33] J. M. Mazón-Suástegui, N. T. Fernández, I. L. Valencia, P. Cruz-Hernández, and H. Latisnere-Barragán, "28S rDNA as an alternative marker for commercially important oyster identification," *Food Control*, vol. 66, pp. 205–214, 2016.
- [34] S. Kumar, G. Stecher, M. Li, C. Knyaz, and K. Tamura, "MEGA X: molecular evolutionary genetics analysis across computing platforms," *Molecular Biology and Evolution*, vol. 35, no. 6, pp. 1547–1549, 2018.
- [35] P. Chakrabarty, M. Warren, L. M. Page, and C. C. Baldwin, "GenSeq: an updated nomenclature and ranking for genetic sequences from type and non-type sources," *ZooKeys*, vol. 346, no. 346, pp. 29–41, 2013.
- [36] S. Ratnasingham and P. D. N. Hebert, "BOLD: the Barcode of life data system," *Molecular Ecology Notes*, vol. 7, no. 3, pp. 355–364, 2007.
- [37] S. Lemer, V. L. González, R. Bieler, and G. Giribet, "Cementing mussels to oysters in the pteriomorphian tree: a phylogenomic approach," *Proceedings of the Royal Society B: Biological Sciences*, vol. 283, no. 1833, 2016.
- [38] M. Matsumoto, "Phylogenetic analysis of the subclass Pteriomorphia (Bivalvia) from mtDNA COI sequences," *Molecular Phylogenetics and Evolution*, vol. 27, no. 3, pp. 429–440, 2003.
- [39] F. Plazzi, A. Ceregato, M. Taviani, and M. Passamonti, "A molecular phylogeny of bivalve mollusks: ancient radiations and divergences as revealed by mitochondrial genes," *PLoS One*, vol. 6, no. 11, 2011.



- [40] K. Katoh, J. Rozewicki, and K. D. Yamada, "MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization," *Briefings in Bioinformatics*, vol. 20, no. 4, pp. 1160–1166, 2019.
- [41] J. Trifinopoulos, L. T. Nguyen, A. von Haeseler, and B. Q. Minh, "W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis," *Nucleic Acids Research*, vol. 44, no. W1, pp. W232–W235, 2016.
- [42] S. Kalyaanamoorthy, B. Q. Minh, T. K. F. Wong, A. Von Haeseler, and L. S. Jermiin, "ModelFinder: fast model selection for accurate phylogenetic estimates," *Nature Methods*, vol. 14, no. 6, pp. 587–589, 2017.
- [43] F. Ronquist, M. Teslenko, P. Van Der Mark et al., "MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space," *Systematic Biology*, vol. 61, no. 3, pp. 539–542, 2012.
- [44] J. W. Leigh and D. Bryant, "POPART: full-feature software for haplotype network construction," *Methods in Ecology and Evolution*, vol. 6, no. 9, pp. 1110–1116, 2015.
- [45] H. J. Bandelt, P. Forster, and A. Röhl, "Median-joining networks for inferring intraspecific phylogenies," *Molecular Biology and Evolution*, vol. 16, no. 1, pp. 37–48, 1999.
- [46] M. Clement, D. Posada, and K. A. Crandall, "TCS: a computer program to estimate gene genealogies," *Molecular Ecology*, vol. 9, no. 10, pp. 1657–1659, 2000.
- [47] R. A. Becker, J. M. Chambers, and A. R. Wilks, *The New S Language : A Programming Environment for Data Analysis and Graphics*, Wadsworth & Brooks/Cole Advanced Books & Software, 1988.
- [48] R Core Team, *R: a language and environment for statistical computing*, R Foundation for Statistical Computing, Vienna, Austria, 2021.
- [49] D. Salvi, E. Berrilli, P. D'Alessandro, and M. Biondi, "Sharpening the DNA barcoding tool through a posteriori taxonomic validation: the case of Longitarsus flea beetles (Coleoptera: Chrysomelidae)," *PLoS One*, vol. 15, no. 5, pp. 1–20, 2020.
- [50] O. Folmer, M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek, "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates," *Molecular Marine Biology and Biotechnology*, vol. 3, no. 5, pp. 294–299, 1994.
- [51] N. Puillandre, S. Brouillet, and G. Achaz, "ASAP: assemble species by automatic partitioning," *Molecular Ecology Resources*, vol. 21, no. 2, pp. 609–620, 2021.
- [52] M. Kimura, "A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences," *Journal of Molecular Evolution*, vol. 16, no. 2, pp. 111–120, 1980.
- [53] P. Kapli, S. Lutteropp, J. Zhang et al., "Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo," *Bioinformatics*, vol. 33, no. 11, pp. 1630–1638, 2017.
- [54] J. Ducasse, V. Ung, G. Lecointre, and A. Miralles, "LIMES: a tool for comparing species partition," *Bioinformatics*, vol. 36, no. 7, pp. 2282–2283, 2020.
- [55] D. Ahrens, T. Fujisawa, H. J. Krammer, J. Eberle, S. Fabrizi, and A. P. Vogler, "Rarity and incomplete sampling in DNA-based species delimitation," *Systematic Biology*, vol. 65, no. 3, pp. 478–494, 2016.
- [56] A. Miralles and M. Vences, "New metrics for comparison of taxonomies reveal striking discrepancies among species delimitation methods in Madascincus lizards," *PLoS One*, vol. 8, no. 7, 2013.
- [57] H. Seki, "Description of a new species of oyster from Japan," *Proceedings of the Imperial Academy*, vol. 5, no. 10, pp. 477–479, 1929.
- [58] M. Hamaguchi, M. Manabe, N. Kajihara, H. Shimabukuro, Y. Yamada, and E. Nishi, "DNA barcoding of flat oyster species reveals the presence of *Ostrea stentina* Payraudeau, 1826 (Bivalvia: Ostreidae) in Japan," *Marine Biodiversity Records*, vol. 10, no. 1, pp. 1–10, 2017.
- [59] A. Inaba and K. Torigoe, "Oysters in the world, part 2: systematic description of the recent oysters," *Bulletin of the Nishinomiyama Shell Museum*, vol. 3, pp. 15–16, 2004.
- [60] L. Hu, H. Wang, Z. Zhang, C. Li, and X. Guo, "Classification of small flat oysters of *Ostrea stentina* species complex and a new species *Ostrea neostentina* sp. nov. (Bivalvia: Ostreidae)," *Journal of Shellfish Research*, vol. 38, no. 2, pp. 295–308, 2019.
- [61] B. Morton, K. Lam, and S. Slack-Smith, "First report of the European flat oyster *Ostrea edulis*, identified genetically, from Oyster Harbour, Albany, south-western Western Australia," *Molluscan Research*, vol. 23, no. 3, pp. 199–208, 2003.
- [62] M. P. Polson, W. E. Hewson, D. J. Eernisse, P. K. Baker, and D. C. Zacherl, "You SayConchaphila, i SayLurida: molecular evidence for restricting the *Olympia* oyster (*Ostrea lurida*-Carpenter 1864) to temperate Western North America," *Journal of Shellfish Research*, vol. 28, no. 1, pp. 11–21, 2009.
- [63] H. B. Stenzel, "Oysters," *Treatise on Invertebrate Paleontology*, vol. 3, pp. N953–N1224, 1971.
- [64] K. Lam and B. Morton, "Morphological and mitochondrial-DNA analysis of the Indo-West Pacific rock oysters (Ostreidae: Saccostrea species)," *Journal of Molluscan Studies*, vol. 72, no. 3, pp. 235–245, 2006.
- [65] C. R. Tureck, C. M. R. De Melo, C. H. A. M. De Gomes et al., "Use of artificial collectors to obtain oyster seeds in Babitonga bay, Santa Catarina, Brazil," *Boletim Do Instituto de Pesca*, vol. 46, no. 1, p. e541, 2020.
- [66] D. Ó. Foighil, P. M. Gaffney, A. E. Wilbur, and T. J. Hilbish, "Mitochondrial cytochrome oxidase I gene sequences support an Asian origin for the Portuguese oyster *Crassostrea angulata*," *Marine Biology*, vol. 131, no. 3, pp. 497–503, 1998.
- [67] C. Li, Q. Kou, Z. Zhang et al., "Reconstruction of the evolutionary biogeography reveal the origins and diversification of oysters (Bivalvia: Ostreidae)," *Molecular Phylogenetics and Evolution*, vol. 164, article 107268, 2021.
- [68] D. Ó. Foighil and D. J. Taylor, "Evolution of parental care and ovulation behavior in oysters," *Molecular Phylogenetics and Evolution*, vol. 15, no. 2, pp. 301–313, 2000.
- [69] M. H. Shilts, M. S. Pascual, and D. Ó. Foighil, "Systematic, taxonomic and biogeographic relationships of Argentine flat oysters," *Molecular Phylogenetics and Evolution*, vol. 44, no. 1, pp. 467–473, 2007.
- [70] M. Huber, "Compendium of Bivalves," in *A full-color guide to 3,300 of the world's marine bivalves. A status on Bivalvia after 250 years of research*, ConchBooks, 2010.
- [71] G. Ranson, "Les espèces d'huîtres vivant actuellement dans le monde, définies par leurs coquilles ou prodissconques," *Revue des Travaux de l'Institut des Pêches Maritimes*, vol. 31, no. 2, pp. 1–146, 1967.
- [72] D. A. Ramakrishna, "Annotated Checklist of Indian Marine Molluscs (Cephalopoda, Bivalvia and Scaphopoda) : Part-I,"

- Records of Zoological Survey of India (Occasional) Paper*, vol. 320, 2010.
- [73] N. V. Subba Rao, "Indian Seashells Part 2 Bivalvia," *Zoological Survey of India, Occasional Paper*, vol. 375, pp. 1–578, 2017.
- [74] N. V. Subba Rao, K. V. Suraya Rao, and S. Maitra, "Marine molluscs," *State Fauna Series*, vol. 1, pp. 1–175, 1991.
- [75] M. Bishop, R. Brumbaugh, M. Luckenbach, and J. Ruesink, *Ostrea futamiensis*, The IUCN Red List of Threatened Species, 2017.
- [76] K. Lam and B. Morton, "The oysters of Hong Kong (Bivalvia: Ostreidae and Gryphaeidae)," *Raffles Bulletin of Zoology*, vol. 52, no. 1, pp. 11–28, 2004.
- [77] Q. Hongying and L. Byung, "Five unrecorded Ostreacean species (Mollusca, Bivalvia) from Korean waters," *Animal Systematics, Evolution and Diversity*, vol. 16, no. 2, pp. 133–140, 2000.
- [78] M. A. Coleman, M. Feng, M. Roughan, P. Cetina-Heredia, and S. D. Connell, "Temperate shelf water dispersal by Australian boundary currents: implications for population connectivity," *Limnology and Oceanography: Fluids and Environments*, vol. 3, no. 1, pp. 295–309, 2013.
- [79] S. K. Sivadas and B. S. Ingole, "Biodiversity and biogeography pattern of benthic communities in the coastal basins of India," *Marine Biology Research*, vol. 12, no. 8, pp. 797–816, 2016.
- [80] M. M. M. Alam, K. M. Westfall, and S. Pálsson, "Mitochondrial DNA variation reveals cryptic species in *Fenneropenaeus indicus*," *Bulletin of Marine Science*, vol. 91, no. 1, pp. 15–31, 2014.
- [81] J. Trivedi, K. Patel, B. K. K. Chan, M. Doshi, and V. Padate, "Diversity of Indian barnacles in marine provinces and ecoregions of the Indian Ocean," *Frontiers in Marine Science*, vol. 8, p. 449, 2021.
- [82] L. M. Tsang, Y. Achituv, K. H. Chu, and B. K. K. Chan, "Zoogeography of intertidal communities in the West Indian Ocean as determined by ocean circulation systems: patterns from the Tetraclita barnacles," *PLoS One*, vol. 7, no. 9, article e45120, 2012.
- [83] M. Wafar, K. Venkataraman, B. Ingole, S. A. Khan, and P. LokaBharathi, "State of knowledge of coastal and marine biodiversity of Indian ocean countries," *PLoS One*, vol. 6, no. 1, article e14613, 2011.
- [84] J. Taylor and E. Glover, *Biology, Evolution and Generic Review of the Chemosymbiotic Bivalve Family Lucinidae*, Ray Society, 2021.
- [85] A. Gharahkhani, E. Pourjam, D. Leduc, and M. Pedram, "A molecular phylogenetic reappraisal of Richtersiidae Kreis, 1929 (Desmodorida: Desmodoroidea), with two new species of intertidal nematodes from the Persian Gulf," *Nematology*, vol. 22, no. 9, pp. 1045–1060, 2020.
- [86] S. Khatami, P. Tavakoli-Kolour, T. Valinassab, F. E. Anderson, and A. Farhadi, "Molecular identification and phylogenetic relationships of Coleoidea (Mollusca: Cephalopoda) from the Persian Gulf and Oman Sea reveals a case of cryptic diversity," *Molluscan Research*, vol. 38, no. 2, pp. 77–85, 2018.
- [87] G. Oliver, "Indian Ocean malacology with special reference to bivalves of the Indian subcontinent taxonomic revision of the Thyasiridae view project catalogue of type specimens of the Mollusca of George Montagu view project," 2019, <https://www.researchgate.net/publication/332158158>.
- [88] M. Hamaguchi, H. Shimabukuro, H. Usuki, and M. Hori, "Occurrences of the Indo-West Pacific rock oyster *Saccostrea cucullata* in mainland Japan," *Marine Biodiversity Records*, vol. 7, pp. 1–8, 2014.
- [89] C. J. Jozefowicz and D. Ó. Foighil, "Phylogenetic analysis of southern hemisphere flat oysters based on partial mitochondrial 16S rDNA gene sequences," *Molecular Phylogenetics and Evolution*, vol. 10, no. 3, pp. 426–435, 1998.
- [90] J. K. Park and D. Ó. Foighil, "Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments," *Molecular Phylogenetics and Evolution*, vol. 14, no. 1, pp. 75–88, 2000.
- [91] K. M. Pagenkopp Lohan, K. M. Hill-Spanik, M. E. Torchin, E. E. Strong, R. C. Fleischer, and G. M. Ruiz, "Molecular phylogenetics reveals first record and invasion of *Saccostrea* species in the Caribbean," *Marine Biology*, vol. 162, no. 5, pp. 957–968, 2015.
- [92] D. O. Foighil, B. A. Marshall, T. J. Hilbish, and M. A. Pino, "Trans-Pacific range extension by rafting is inferred for the flat oyster *Ostrea chilensis*," *Biological Bulletin*, vol. 196, no. 2, pp. 122–126, 1999.
- [93] J. Kim, D. Jung, W. S. Lee, and J. K. Park, "Development of a DNA microarray-based identification system for commercially important Korean oyster species," *Journal of Shellfish Research*, vol. 34, no. 3, pp. 841–847, 2015.
- [94] G. Danic-Tchaleu, S. Heurtebise, B. Morga, and S. Lapègue, "Complete mitochondrial DNA sequence of the European flat oyster *Ostrea edulis* confirms Ostreidae classification," *BMC Research Notes*, vol. 4, no. 1, p. 400, 2011.
- [95] L. Kirkendale, T. Lee, P. Baker, and O. D. Foighil, "Oysters of the Conch Republic (Florida Keys): a molecular phylogenetic study of *Parahyotissa mcgintyi*, *Teskeyostrea weberi* and *Ostreola equestris*," *Malacologia*, vol. 46, no. 2, pp. 309–326, 2004.
- [96] S. Lapègue, I. B. Salah, F. M. Batista, S. Heurtebise, L. Neifar, and P. Boudry, "Phylogeographic study of the dwarf oyster, *Ostreola stentina*, from Morocco, Portugal and Tunisia: evidence of a geographic disjunction with the closely related taxa, *Ostrea aupouria* and *Ostreola equestris*," *Marine Biology*, vol. 150, no. 1, pp. 103–110, 2006.
- [97] C. A. Milbury and P. M. Gaffney, "Complete mitochondrial DNA sequence of the eastern oyster *Crassostrea virginica*," *Marine Biotechnology*, vol. 7, no. 6, pp. 697–712, 2005.
- [98] C. Lazoski, J. Gusmão, P. Boudry, and A. M. Solé-Cava, "Phylogeny and phylogeography of Atlantic oyster species: evolutionary history, limited genetic connectivity and isolation by distance," *Marine Ecology Progress Series*, vol. 426, pp. 197–212, 2011.
- [99] C. M. R. Melo, F. C. Silva, C. H. A. M. Gomes, A. M. Solé-Cava, and C. Lazoski, "*Crassostrea gigas* in natural oyster banks in southern Brazil," *Biological Invasions*, vol. 12, no. 3, pp. 441–449, 2010.
- [100] J. Ren, Z. Hou, H. Wang et al., "Intraspecific variation in mitogenomes of five *Crassostrea* species provides insight into oyster diversification and speciation," *Marine Biotechnology*, vol. 18, no. 2, pp. 242–254, 2016.
- [101] X. Wu, X. Xu, Z. Yu, Z. Wei, and J. Xia, "Comparison of seven *Crassostrea* mitogenomes and phylogenetic analyses," *Molecular Phylogenetics and Evolution*, vol. 57, no. 1, pp. 448–454, 2010.
- [102] C. Li, H. Y. Wang, C. F. Liu, Y. W. Li, and X. M. Guo, "Classification and distribution of oysters from the coastal sea in

- Guangxi, China,” *Oceanologia et Limnologia Sinica*, vol. 44, pp. 1318–1324, 2013.
- [103] Z. Cui, L. Hu, C. Li, Z. Zhang, X. Guo, and H. Wang, “Identification of *Saccostrea mordax* and a new species *Saccostrea mordoides* sp. nov. (Bivalvia: Ostreidae) from China,” *Journal of Shellfish Research*, vol. 40, no. 1, pp. 63–75, 2021.
- [104] J. Xia, Z. Yu, and X. Kong, “Identification of seven *Crassostrea* oysters from the South China Sea using PCR-RFLP analysis,” *Journal of Molluscan Studies*, vol. 75, no. 2, pp. 139–146, 2009.
- [105] S. E. Hammer and M. D. L. Salvini-Plawen, *Molekulare Phylogenie der pteriomorphen Bivalvia (Mollusca)*, University Vienna, 2001.
- [106] F. Plazzi and M. Passamonti, “Towards a molecular phylogeny of mollusks: bivalves’ early evolution as revealed by mitochondrial genes,” *Molecular Phylogenetics and Evolution*, vol. 57, no. 2, pp. 641–657, 2010.