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A symbiotic oyster in a shrimp burrow: phylogenetic position of *Anomiostrea* within the Ostreidae (Bivalvia)

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ABSTRACT

The ostreid genus *Anomiostrea* Habe & Kosuge, 1966, is monotypic for *A. coralliophila* Habe, 1975, which is known as a symbiont inhabiting the burrow of the ghost shrimp *Neocallichirus jousseaumei* (Nobili, 1904), but despite this unusual habit among oysters its phylogenetic position within the Ostreidae remained unknown. Using specimens collected from two distant localities of the Indo-Pacific, Oman and Japan, we compared shell morphology of these specimens with the holotype and assessed their phylogenetic relationships based on DNA sequence data from two mitochondrial and two nuclear genes. The genetic distance in cytochrome *c* oxidase subunit I between the specimens from Japan and Oman was 5.5%, indicating substantial geographic differentiation. Our molecular phylogenetic results suggest that *A. coralliophila* is sister to *Ostrea algoensis* G. B. Sowerby II, 1871, an oyster from rocky shores in South Africa, and both are closely related to other *Ostrea* species. This confirms assignment of *Anomiostrea* to Ostreinae and suggests that this burrow-wall symbiont evolved from typical rocky-shore oysters. Moreover, *A. coralliophila* was not monophyletic with another symbiotic ostreid *Ostrea permollis* G. B. Sowerby II, 1871, nor with other symbiotic oysters, indicating that the symbiotic habit evolved multiple times in the Ostreinae.

INTRODUCTION

The bivalve family Ostreidae Rafinesque, 1815, currently comprises 75 recognized, extant species (MolluscaBase, 2024) including several commercially important oysters. Members of this family inhabit intertidal and shallow subtidal zones worldwide and are assigned to four subfamilies, Crassostreinae Scarlato & Starobogatov, 1979, Ostreinae, Saccostreinae Salvi & Mariottini, 2016, and Striostreinae Harry, 1985. Two monotypic genera are currently unassigned to any subfamily: *Nicaisolopha* Vyalov, 1936, and *Anomiostrea* Habe & Kosuge, 1966.

Anomiostrea is monotypic for *Anomiostrea coralliophila* Habe, 1975. The species name *coralliophila* is a replacement name for *Ostrea pyxidata* Adams & Reeve, 1850, a junior homonym of *O. pyxidata* Born, 1778 (Habe, 1975). The type specimen was collected from the Philippines during the voyage of the "Samarang" (Adams & Reeve, 1848). The species has since been reported across the Indo-West Pacific region, including Kuwait, Sri Lanka, Java, Borneo, the Maluku Islands, the Philippines, and the Ryukyu Islands (Inaba & Torigoe, 2004; Goto, Ohsuga & Kato, 2014; GBIF, 2024; Oliver *et al.*, 2023). No habitat data were given in the original description of *O. pyxidata* but Habe & Kosuge (1966: 338) stated, "found on the corals in the Philippines and rather commonly collected at Samboanga, Mindanao by Mr. Ichiro Yamamura". The coral association was repeated by Huber (2010, Chapter 5 CD) "subtidal 2–30 m; coral reefs, cemented on corals and coral branches". In our study *Anomiostrea* specimens were found attached to dead coral fragments lining the burrow walls of the ghost shrimp *Neocallichirus jousseaumei* (Nobili, 1904) as reported by Goto *et al.* (2014). Considering the observations in Goto *et al.* (2014), it is probable that the specimens in Habe & Kosuge (1966) were attached to dead corals that were embedded in the burrow walls of the ghost shrimp, a hypothesis that can be rejected if, in the future, the genus is clearly documented from coral. However, in extensive sampling across the Indo-West Pacific (G. Paulay, personal observations), including numerous collections of coral-associated oysters [*Dendostrea sandvichensis* (Sowerby, 1871), *Alectryonella plicatula* (Gmelin, 1791) and other undescribed and unidentified species], we have never seen *Anomiostrea* associated with coral.

The symbiotic (i.e. 'live together' *sensu*; de Bary, 1879) association of *A. coralliophila* with its hosts is likely an example of commensalism. The oyster probably benefits through better protection from predators or environmental stress by living inside the shrimp burrows and may also utilize the water currents created by hosts for filter feeding or respiration (Goto *et al.*, 2014). The host shrimps may benefit from the existence of *A. coralliophila* in that the shells of the oyster may stabilize the loose sand or rubble walls of the burrow. This may reduce the time and energy of the host shrimps for burrow maintenance (Goto *et al.*, 2014). In addition, the host shrimps may gain benefits of better water circulation by *A. coralliophila*. If the shrimps actually gain some of these benefits from *A. coralliophila*, their association can be considered one of mutualism.

It is an intriguing question how such a symbiotic habit evolved in the Ostreidae. Several other Ostreinae oysters have symbiotic relationships with other marine invertebrates. *Ostrea permollis* (Sowerby, 1871) lives embedded in the sponge *Stelletta grubii* Schmidt, 1862 (Forbes, 1964, 1966). *Alectryonella plicatula* (Gmelin, 1791) and two other undescribed/unidentified oysters are obligate associates of living scleractinian corals, while *Dendostrea sandvichensis* (G. B. Sowerby II, 1871) appears to be a facultative associate (G. Paulay, personal observations). *Booneostrea subucula* (Jousseaume, 1925) was recorded from the burrows of the axiid shrimp *Axiopsis serratifrons* (A. Milne-Edwards, 1873) (Kneer *et al.*, 2013), as similar to *A. coralliophila*. However, phylogenetic relationships among Ostreinae having a symbiotic habit remain uninvestigated.

Harry (1985) placed *Anomiostrea* in the Lophinae (Harry, 1985), whereas Huber (2010) tentatively placed it in the Ostreinae. Several molecular phylogenetic analyses of the Ostreidae have been conducted (Foighil & Taylor, 2000; Salvi, Macali & Mariottini, 2014; Salvi & Mariottini, 2017, 2021; Li *et al.*, 2021), but none included *Anomiostrea*, thus the phylogenetic position of this lineage remained unclear.

In this work, we studied morphologically identified specimens of *A. coralliophila* taken from shrimp burrows at two distant localities of the Indo-Pacific—Oman and Japan. We performed the first molecular phylogenetic assessment of *Anomiostrea*, based on mitochondrial and nuclear markers, to infer its affiliation and also to assess the relationships between Omani and Japanese populations. The main aim of this study is to clarify the systematic position of *Anomiostrea* and to gain insights on the evolution of the symbiotic habit in oysters.

MATERIAL AND METHODS

Sampling and morphological observation

We collected individuals of *Anomiostrea coralliophila* from the burrows of the ghost shrimp *Neocallichirus jousseaumei* in Edateku, Amami-Oshima Island, Kagoshima, Japan, in May 2012 (Goto *et al.*, 2014). At this site, the burrows of *N. jousseaumei* were often constructed under rocks and thus we turned over rocks embedded in sediments to find the shrimp burrows. In addition, we collected two individuals of *A. coralliophila* from the burrows of *N. jousseaumei* in Qurum, Oman. Specimens were levered open and the adductor muscle and mantle excised and fixed in 100% ethanol. Specimens from Japan used for the taxonomic assessment are retained by the first author in the Seto Marine Biological Laboratory, Kyoto University, and those from Oman are deposited in the Florida Museum of Natural History, University of Florida (UF). The holotype of *A. coralliophila* and specimens from Oman, Japan and Kuwait are illustrated in Fig. 1. The internal anatomy of a specimen from Japan is illustrated in Fig. 2.

Molecular methods

Genomic DNA was extracted following standard (Evans, 1990) or modified (Sokolov, 2000) high-salt protocols. Two mitochondrial and two nuclear gene fragments were amplified by polymerase chain reaction (PCR): the barcode fragment of cytochrome *c* oxidase subunit I (COI), and 16S, 18S and 28S ribosomal RNA (rRNA) genes. Amplifications were performed in 25.23 μl mixtures consisting of 0.3 μl forward and reverse primers (10 μM each), 2.5 μl 10xExTaq buffer or reaction buffer (Bioline), 2.0 μl dNTP mixture, 0.13 μl TaKaRa ExTaq polymerase (TaKaRa, Otsu, Japan) or BioTaq (Bioline), 2.5 μl template DNA (at a concentration of ~20 ng/μl) and 17.5 μl distilled water. The primers used are shown in Table 1. Thermal cycling was performed with an initial denaturation for 3 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at a gene-specific annealing temperature (COI: 42 °C, 16S rRNA, 18S rRNA: 50 °C, 28S rRNA: 50 °C), and 2 min at 72 °C, with a final 3 min extension at 72 °C. PCR products were purified with ExoSAP-IT™ Express PCR Cleanup Reagent (Thermo Fisher Scientific K.K., Tokyo, Japan). The sequencing reaction was performed using the same PCR primers by Eurofins Genomics (Japan) and Genwiz (Germany). Sequences obtained are deposited in the DDBJ/EMBL/GenBank with the accession numbers PP580508-PP580514 and PP582338-PP582339 (Table 2).

Phylogenetic analyses

Chromatograms were edited and assembled using 4Peaks (Griekspoor & Groothuis, nucleobytes.com) or Geneious v. 11.0.12 (Biomatters Ltd, Auckland, New Zealand). The COI chromatograms did not show any double peak and the translated amino acid sequence did not have any stop codons.

To assess the affiliation of *A. coralliophila*, we analysed newly generated sequences with homologous sequences of 19 oyster species obtained from GenBank representing all currently accepted genera within the Striostreinae, Saccostreinae and Crassostreinae, six of nine currently accepted genera of Ostreinae (MolluscaBase, 2024), plus two outgroup species from the sister family Gryphaeidae (Matsumoto, 2003; Plazzi *et al.*, 2011; Lemer *et al.*, 2016). Phylogenetic analyses based on this 'family dataset' placed *A. coralliophila* in the Ostreinae. To further assess the relationship of *A. coralliophila*, we created a second, Ostreinae dataset including 25 species of Ostreinae plus two outgroup species from the Saccostreinae (Plazzi *et al.*, 2011; Lemer *et al.*, 2016). GenBank accession numbers and references for the DNA sequences used are reported in Table 2.

Multiple sequence alignments were performed with MAFFT v. 7 on the web server (Katoh, Rozewicki & Yamada, 2019) using the E-INS-i iterative refinement algorithm. 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 using the IQ-TREE v. 1.6.12 (Nguyen *et al.*, 2015) based on the best substitution model determined for each gene fragment by the ModelFinder module (Kalyaanamoorthy *et al.*, 2017) under the corrected Akaike information criterion (cAIC). ML tree searches were based on 20 initial trees until a new best tree could not be found in the last 100 iterations (nstop = 100). Node support was assessed with 1,000 ultrafast bootstrapping pseudoreplicates (uBS). IQ-TREE analyses were performed using the W-IQ-TREE web server (Trifinopoulos *et al.*, 2016). Bayesian analyses were performed using MrBayes v. 3.2.7 (Ronquist *et al.*, 2012), using the same substitution models as for the ML analyses. We ran two Markov chains of 5 million generations each, sampled every 500 generations. Consensus trees (50%

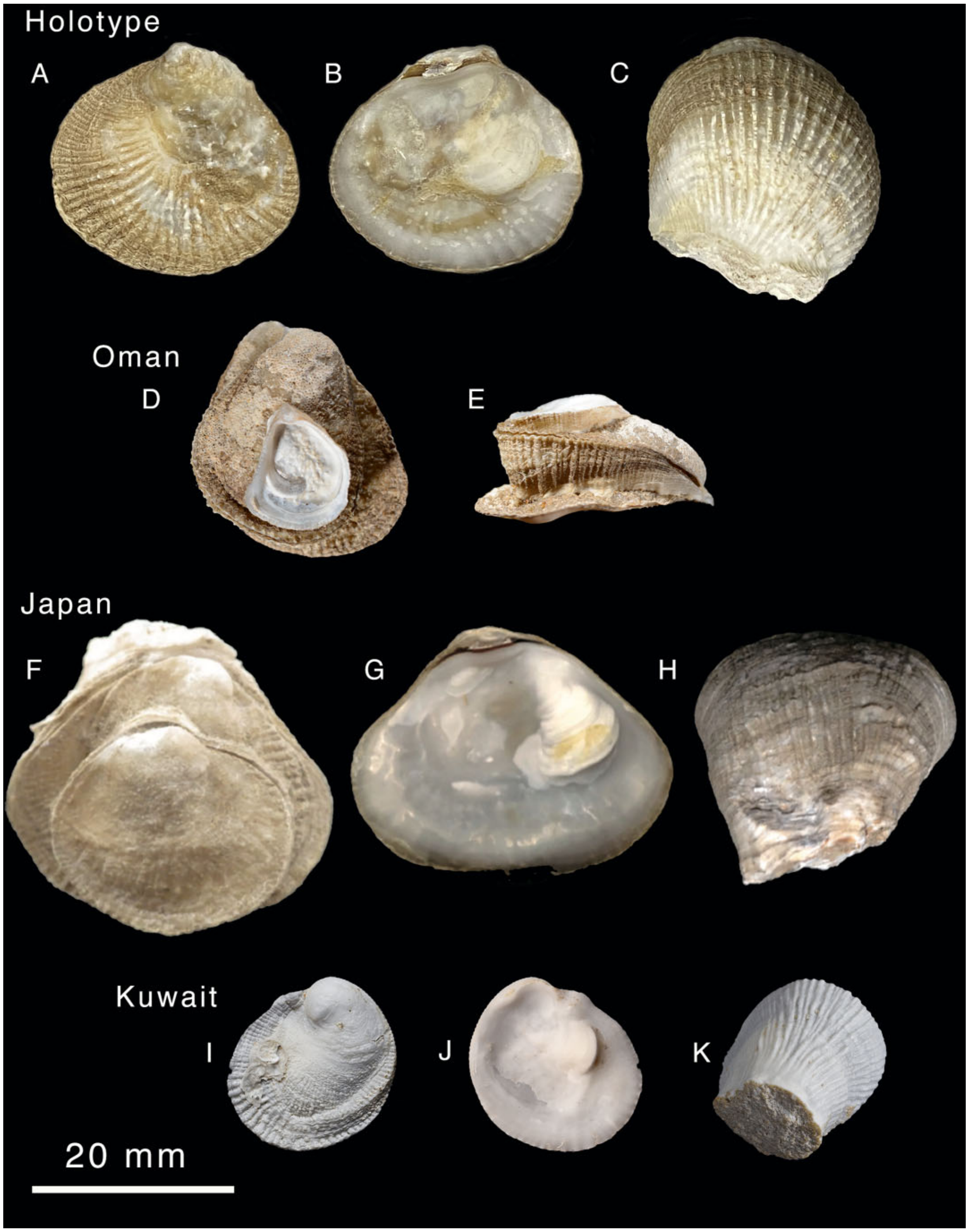

Figure 1. *Anomiostrea coralliophila* Habe, 1975. **A–C.** Holotype of *Ostrea pyxidata* Adams & Reeve, 1850. NHMUK 1965114, Philippines. **A, B.** External and internal surfaces of upper valve. **C.** Lateral view of external surface of lower valve. **D, E.** Oman, UF Mollusca 576703. **D.** External surface of upper valve with juvenile attached. **E.** Lateral view of whole specimen attached to older upper valve and juvenile attached on top. **F–H.** Japan, coll, at Seto Marine Biological Laboratory, Kyoto University, not registered. **F, G.** External and internal surfaces of upper valve with smaller specimen attached. **H.** Lateral view of external surface of lower valve. **I–K.** Kuwait, An Niggalayat, ex Smythe coll, NHMUK not registered. **I, J.** External and internal surfaces of upper valve. **K.** lateral view of external surface of lower valve.

majority rule) and Bayesian posterior probability values (BPP) were calculated on 7,500 trees sampled after burn-in (25%). Run convergence and effective sample size (ESS) values >200 were assessed using Tracer v. 1.7 (Rambaut *et al.*, 2018).

Phylogenetic trees were visualized and edited using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). We used MEGA-X v. 10.0.8 (Tamura, Stecher & Kumar, 2021) to compute genetic distances between specimens of *Anomiostrea* from Oman and Japan based on COI and 16S rRNA sequences.

RESULTS

Molecular phylogenetic analyses

ML and BI analyses based on the family dataset placed *Anomiostrea coralliophila* within the Ostreinae with strong support (uBS = 98; BPP = 1.0; Fig. 3A). ML and BI trees had identical topology with four main clades corresponding to the Ostreinae, Crassostreinae, Striostreinae and Saccostreinae, and generic limits matching current systematics of oysters except for the paraphyletic *Ostrea* and *Dendostrea* (Salvi & Mariottini, 2017; MolluscaBase, 2024).

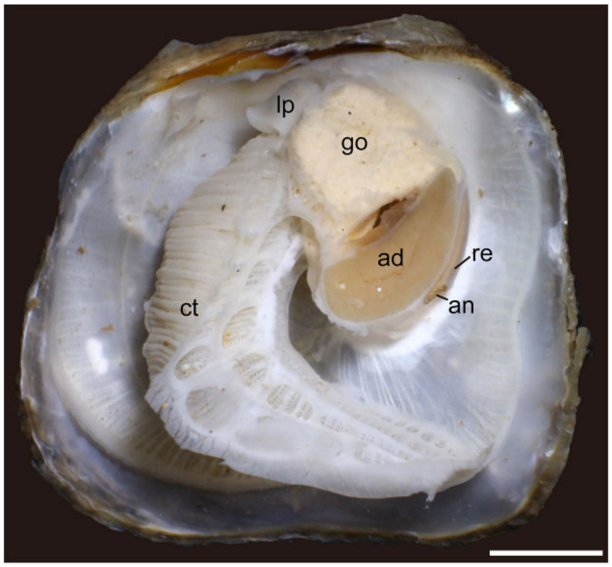

Figure 2. Internal anatomy of *Anomiostrea coralliophila* from Japan. Abbreviations: ad, adductor muscle; an, anus; ct, ctenidia; go, gonad; lp, labial palp; re, rectum. Scale bar = 5 mm.

The phylogenetic trees based on the Ostreinae dataset placed *A. coralliophila* sister to *Ostrea algoensis* (uBS = 90; BPP = 1.0; Fig. 3B), with this lineage sister to a clade comprised of *O. futamiensis* and *O. oleomargarita* (uBS = 100; BPP = 1.0), the four species forming a well-supported group (uBS = 83; BPP = 0.98). This clade in turn is sister to a clade that includes the genera *Alectryonella*, *Lopha*, *Dendostrea*, *Planostrea* plus *O. setoensis* and *O. fluctigera* (uBS = 71; BPP = 0.99). This large clade corresponds to the *algoensis* group described in Salvi *et al.* (2022). The average genetic divergence (*p*-distance) between specimens of *A. coralliophila* collected in Oman and Japan is 5.5% for COI and 0.5% for 16S rRNA.

SYSTEMATIC DESCRIPTION

Superfamily OSTREOIDEA Rafinesque, 1815

Family OSTREIDAE Rafinesque, 1815

Subfamily OSTREINAE Rafinesque, 1815

Genus *Anomiostrea* Habe & Kosuge, 1966

Type species: Anomiostrea coralliophila Habe, 1975. Replacement name for *Ostrea pyxidata* Adams & Reeve, 1850.

Original diagnosis of Anomiostrea: "The shell is small, thin, orbicular, dark green to pale yellow, inequivalve. The upper valve is flat, divergently ribbed and has the ovate white muscular scar situated near the centre of its inner surface. The lower valve attached to the coral is deeply concaved, forming a cup-shape and crenulated at the margin by the ribs on the surface and its muscular scar is distinctly elevated by the white callus. The ligament is small and short."

Diagnosis based on present study: Small oysters to over 35 mm, strongly inequivalve, lower valve deeply cup-shaped, upper valve flat. Juvenile shell more or less smooth, abruptly transitioning to adult sculpture of dense, radial, partly divaricating riblets. Inner margins finely crenulate, aligned with external sculpture. Few sparse, indistinct, ostreine chomata present on dorsolateral margins. Adductor scar raised and thickened toward the posterior.

Remarks: *Anomiostrea* appears to have been distinguished by the deep, cup-shaped lower valve, flat upper valve, and dense radial, sometimes bifurcating, sculpture. This morphological distinction remains diagnostic, as no other living oysters show such an extreme inequivalve condition.

Anomiostrea coralliophila Habe, 1975

Ostrea pyxidata Adams & Reeve, 1850: 72, pl. 21, fig. 19 (holotype NHMUK 1965114).

Ostrea coralliophila Habe & Kosuge, 1966: 323, 338, pl. 29, fig. 1.

Anomiostrea coralliophila Habe, 1975: 184. Inaba & Torigoe, 2004: 62, pl. 13, figs 2a–d. Huber, 2010: 179. Goto *et al.*, 2014: 201–205, fig. 2.

Material studied: UF Mollusca 576703, Oman, Muscat, Qurum beach and mangroves (23.6249, 58.47861), from *Neocallichirus jousseaumei* burrows, BOMAN-10623, Lasley, Robert; Paulay, Gustav; Uehling, Abby; Anker, Arthur; Maslakova, Svetlana; Cherneva, Mira; live specimen attached to dead right valve of second specimen. NHMUK, unregistered, Kuwait ex Smythe coll, cited as *Ostrea pyxidata* in Glayzer, Glayzer & Smythe (1984), illustrated shells from An Niggalayat. Seto Marine Biological Laboratory, Kyoto

Table 1. Primers used in this study.

Gene	Primer	Direction	Sequence 5′–3′	References
COI	LCO1490	Forward	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. (1994)
	HCO2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	
16S rRNA	16SarL	Forward	CGCCTGTTTATCAAAAACAT	Palumbi *et al.* (1991)
	16SbrH	Reverse	CCGGTCTGAACTCAGATCACGT	
28S rRNA	D1	Forward	ACCCSCTGAAYTTAAGCAT	Colgan *et al.* (2003)
	D3	Reverse	GACGATCGATTTGCACGTCA	Vonnemann *et al.* (2005)
	D2F	Forward	CCCGTCTTGAAACACGGACCAAGG	Vonnemann *et al.* (2005)
	C2R	Reverse	ACTCTCTCTTCAAAGTTCTTTTC	Dayrat *et al.* (2001)
18S rRNA	1F	Forward	TACCTGGTTGATCCTGCCAGTAG	Giribet *et al*. (1996)
	5R	Reverse	CTTGGCAAATGCTTTCGC	
	3F	Forward	GTTCGATTCCGGAGAGGGA	
	18Sbi	Reverse	GAGTCTCGTTCGTTATCGGA	
	18Sa2.0	Forward	ATGGTTGCAAAGCTGAAAC	
	9R	Reverse	GATCCTTCCGCAGGTTCACCTAC	

All primers were used both for the amplification and sequencing except for the 28S rRNA gene for which primers D1 and D3 were used for amplifications and primers D2F and C2R for sequencing.
For the 18S rRNA gene, we produced contigs using fragments sequenced with three primer combinations (1F + 5R, 3F + 18Sbi and 18Sa2.0 + 9R).

Table 2. GenBank accession numbers for the DNA sequences used for phylogenetic analysis.

Family	Organism	16S rRNA	COI	28S rRNA	18S rRNA
Ostreidae	*Alectryonella plicatula*	AF052072	–	AF137037	–
Ostreidae	*Anomiostrea coralliophila* (Japan)	PP580510	PP582338	PP580512	PP580508
Ostreidae	*Anomiostrea coralliophila* (Oman)	PP580511	PP582339	PP580513	PP580509
Ostreidae	*Dendostrea folium*	AF052069	–	AF137040	–
Ostreidae	*Dendostrea frons*	AF052070	KP455014	AF137039	KX713287
Ostreidae	*Dendostrea sandvichensis*	KC847121	KC683511	KC847142	MZ239437
Ostreidae	*Lopha cristagalli*	AF052066	AB076908	AF137038	AJ389635
Ostreidae	*Ostrea algoensis*	AF052062	–	AF137041	–
Ostreidae	*Ostrea angasi*	AF052063	AF112287	AF137046	–
Ostreidae	*Ostrea angelica*	KT317129	KT317442	–	–
Ostreidae	*Ostrea chilensis*	AF052065	AF112285	AF137045	KX977494
Ostreidae	*Ostrea circumpicta*	AB898280	AB898294	MG560201	KM460887
Ostreidae	*Ostrea conchaphila*	KT317155	KT317478	AF137044	–
Ostreidae	*Ostrea denselamellosa*	AF052067	KP067907	AF137043	MZ239433
Ostreidae	*Ostrea edulis*	JF274008	JF274008	AF137047	L49052
Ostreidae	*Ostrea equestris*	AY376603	AY376607	DQ242465	–
Ostreidae	*Ostrea fluctigera*	LC149503	LC149507	–	–
Ostreidae	*Ostrea futamiensis*	LC051605	AB898290	–	–
Ostreidae	*Ostrea lurida*	KT317226	KT317519	–	–
Ostreidae	*Ostrea megodon*	KX364274	KX364276	MZ231933	MZ239438
Ostreidae	*Ostrea neostentina*	MK370369	MK370330	–	–
Ostreidae	*Ostrea oleomargarita*	ON614100	ON614721	ON614127	–
Ostreidae	*Ostrea permollis*	AF052075	DQ226524	–	–
Ostreidae	*Ostrea puelchana*	AF052073	DQ226518	AF137042	–
Ostreidae	*Ostrea setoensis*	LC149511	LC149514	–	–
Ostreidae	*Ostrea stentina*	DQ313180	DQ313183	DQ242464	MZ239439
Ostreidae	*Planostrea pestigris*	KC847125	–	KC847146	MZ239440
Ostreidae	*Crassostrea virginica*	AY905542	AY905542	AF137050	KC429335
Ostreidae	*Crassostrea rhizophorae*	FJ478032	FJ717613	KF370366	–
Ostreidae	*Magallana gigas*	KJ855241	KJ855241	AB102757	AB064942
Ostreidae	*Magallana hongkongensis*	FJ841963	FJ841963	AY632552	MZ239431
Ostreidae	*Talonostrea salpinx*	MZ099713	MZ126560	PP580514	–
Ostreidae	*Talonostrea talonata*	KC847134	KC683515	KC847154	–
Ostreidae	*Saccostrea scyphophilla*	LM993882	MT293857	KC847153	–
Ostreidae	*Saccostrea cucullata*	AF458901	EU816078	AJ344329	MZ239428
Ostreidae	*Striostrea margaritacea*	LT220867	LT220873	AF137048	–
Ostreidae	*Striostrea prismatica*	KT317422	KP455045	–	–
Gryphaeidae	*Hyotissa hyotis*	LM993886	GQ166583	AF137036	KX713300
Gryphaeidae	*Neopycnodonte cochlear*	JF496758	JF496772	AF137034	KX713325

University, not registered, Japan, Kagoshima, Edateku, Amami-Oshima Island, intertidal sand flats (28.29, 129.22), from *Neocallichirus jousseaumei* burrows; live specimen.

Original description of Ostrea pyxidata *(translated from the Latin)*: Shell 25 mm. Ostr. shell orbicular, inequivalve, left valve flat, radially ribbed, nodular ribs, right convex, radially strongly ribbed, nodular ribs often duplicated, ventral margin crenulated; dirty brown.

Description based on present study: Japanese and Omani specimens (molecularly validated): 21.6 mm (Oman) to 36.3 mm (Japan). Thin, brittle shells. Umbos coiling. Strongly inequivalve. Lower, left valves deeply cupped, depth greatest at the ventral edge, attachment area proportionately large, sides vertical. Upper, right valve more or less flat, umbonal region a little raised. Outline of upper valves subcircular to trigonal, some wider than high, others higher than wide. Sculpture of upper valves initially smooth then abruptly developing radial riblets, increasing through bifurcations to up to 50 at margin, weakly imbricated. Lower valve similar to upper. Inner margin finely crenulate corresponding to external sculpture. Few ostreine chomata on dorsolateral inner margins, most apparent in smaller shells. Adductor scar subcentral, crescentic, becoming swollen and elevated toward posterior ventral edge. Ligament small on a triangular resilifer. Shell colour tan to brown. Juveniles with tendency to attach to adults forming stacks. The gross anatomy is dominated by the large gills with fully reflected paired ctenidia that are attached to the mantle at their distal ends, adductor muscle kidney shaped, mantle has a complex of pallial muscles, labial palps are small, roughly triangular in shape, rectum attached to the posterior of adductor muscle, anus is simple.

Kuwaiti specimens (not molecularly validated): Illustrated shells from An Niggalayat, Kuwait ex Smythe coll, cited as *O. pyxidata* in Glayzer *et al.* (1984). Disarticulated valves only. Small, not exceeding 20 mm in length. Thin, rather brittle. Lower valve

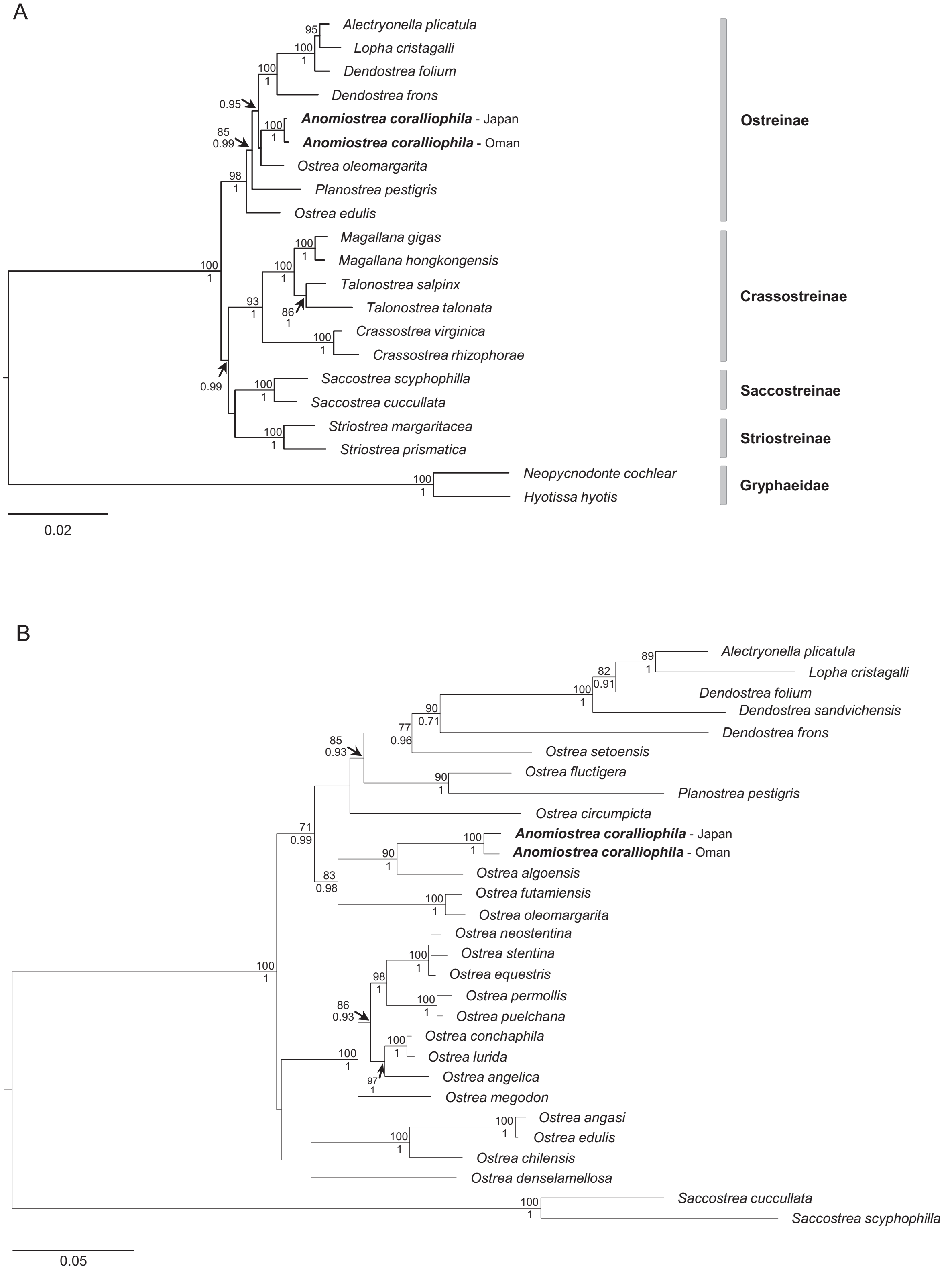

Figure 3. Phylogenetic tree of Ostreidae (**A**) and Ostreinae (**B**) based on concatenated DNA sequences of COI, 16S rRNA, 28S rRNA and 18S rRNA genes. The topologies of ML and BI trees are identical and only the ML trees are shown. Node support values for ML (ultrafast bootstrapping pseudoreplicates >70) and BI (BPP >0.90) analyses are indicated above and below nodes, respectively.

deeply cupped, asymmetric with ventral side elevated to 13 mm in 20 mm length, sculptured with fine vertical riblets. Upper valve flat, sculptured by dense narrow riblets, some bifurcating, all slightly imbricated.

Remarks: As would be expected with any oyster species, there is variation in the shell with the outline of the upper valve varying from subcircular to trigonal, lower valve rather shallow (Oman) to almost as deep as long (Kuwait). The expression of the radial riblets also varies. In both Japanese and Oman populations the animals settle upon each other. This may reflect shortage of substrata for attachment but may also provide a reproductive advantage or reflect a direct mode of development.

DISCUSSION

Anomiostrea coralliophila is recorded across the Indo-West Pacific Oceans from Arabia (Kuwait & Oman) in the west to the Maluku Islands, Philippines, and Ryukyu Islands (Japan) in the east. We sequenced Omani and Japanese specimens, i.e. from these endpoints of its distribution and found them to be 5.5% divergent in COI. This suggests modest allopatric differentiation across the Indo-West Pacific, a common pattern in marine organisms. The level of differentiation found within *A. coralliophila* is lower than observed in most interspecific comparisons in the Crassostreinae, Saccostreinae and Striostreinae but similar to, or higher than, values observed between closely related Ostreinae such as *Ostrea conchaphila*/*O. lurida*, *O. angasi*/*O. edulis*, *O. equestris*/*O. stentina* or *O. puelchana*/*O. permollis* (Raith *et al.*, 2015; Guo *et al.*, 2018; Hu *et al.*, 2019). Such allopatric differentiation is challenging to interpret in a taxonomic framework as they often represent actively diverging lineages that have not completed the speciation process (see Meyer, Geller & Paulay, 2005; Lasley *et al.*, 2023). We thus refrain from making taxonomic decisions on this lineage pending availability and study of more extensive samples.

Our molecular phylogenetic analyses resolved the position of *A. coralliophila* within the subfamily Ostreinae. The placement of *Anomiostrea* within Ostreinae was tentatively proposed by Huber (2010). Guo *et al.* (2018) also placed *Anomiostrea* in the Ostreinae without providing evidence. In this study, beside molecular evidence, the presence of ostreine chomata along the dorsolateral margins of *A. coralliophila* provides further support for its placement within Ostreinae. Although the soft parts were not fully dissected, the gross anatomy as described above conforms with that of other Ostreinae as described by Torigoe (1981). There were no apparent modifications of the anatomy that might relate to the small size or unusual habitat of *Anomiostrea*. Further anatomical comparison between this species and its sister *O. algoensis* would improve our understanding of this ostreid lineage.

Knowledge on the ecology of *A. coralliophila* is very limited. Although Habe & Kosuge (1966) reported *A. coralliophila* attached to reef-building corals, the habitat of this species has been clarified by Goto *et al.* (2014) as a burrow-associated symbiont of the ghost shrimp *Neocallichirus jousseaumei*. The Omani sample was also taken from the burrows of this ghost shrimp indicating a potentially specific symbiotic relationship. We found *A. coralliophila* to be sister to *O. algoensis* described from South Africa where it has been reported to attach to the underside of intertidal rocks (Kilburn & Rippey, 1982; Haupt *et al.*, 2010). *Anomiostrea coralliophila* is nested within the paraphyletic genus *Ostrea* (Fig. 3B) suggesting that this burrow-symbiont oyster evolved from a group of oysters with a usual lifestyle, sessile on rock surfaces. Our phylogenetic results show that neither *O. permollis* and *A. coralliophila* or they with other symbiotic oysters (e.g. *Alectryonella plicatula* and *Dendostrea sandvichensis*) form a monophyletic group (Fig. 3B), suggesting a multiple origin of the symbiotic habit in the Ostreinae.

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CONFLICT OF INTEREST

The authors have no conflict of interests to declare.

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