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Molecular identification of intertidal rock oyster species in north-eastern Australia reveals new candidates for aquaculture

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ABSTRACT

Oyster aquaculture in Australia is currently centred on two species, *Saccostrea glomerata* (Gould, 1850) and *Magallana gigas* (Thunberg, 1793) which are both susceptible to disease. Disease outbreaks have caused significant losses to the industry and there is currently an appetite for diversification to mitigate risk. Development of other, native oyster species for aquaculture is limited by a poor understanding of their biodiversity and distribution, which is primarily due to the difficulty in distinguishing species based on morphology alone. In this study we sampled intertidal rock oysters from 19 localities in north-eastern Australia and performed phylogenetic analyses based on partial COI and 16S mitochondrial markers. A total of 14 distinct oyster lineages (most likely representing distinct species) were identified from the 357 specimens collected and sequenced. In total, we report the presence of 8 *Saccostrea* lineages, 4 Ostreinae lineages, one *Magallana* lineage (*Magallana bilineata* (Röding, 1798), a recent introduction), and one *Talonostrea* lineage (likely undescribed). A number of these lineages have broad distributions and attain large sizes, and several are currently cultured in other parts of the world. Although morphological identification is challenging we argue that the large size of the tropical black-lip oyster, *Saccostrea lineage J*, enables it to be definitively identified as *Saccostrea spathulata* (Lamarck 1819), thus providing taxonomic certainty for this commercially important species. The identification of these oyster lineages and their distributions is a fundamental step towards development of viable alternatives for oyster aquaculture in the region.

1. Introduction

Ostreid oysters are of great commercial importance worldwide (Botta et al., 2020), with a number of species commercially farmed and many more harvested for consumption (Angell, 1986; Lam and Morton, 2003; Lau et al., 2020). They are also ecologically important, providing a number of ecosystem services (for example, water filtration, substrate stabilization, and habitat creation) that are amplified when individuals are present in large numbers (Grabowski et al., 2012; Richardson et al., 2022; van der Schatte Olivier et al., 2018), and are therefore prime candidates for sustainable seafood production (Hilborn et al., 2018; Naylor et al., 2000). Despite this oyster taxonomy remains poorly understood, and is made especially challenging due to highly variable shell morphologies that overlap across species, high larval dispersal, and sympatry of morphologically similar species (Guo et al., 2018; Lam and Morton, 2006; Wu et al., 2010; Xia et al., 2009). Molecular methods have been shown to be necessary for reliable species identification in several cases (Al-Kandari et al., 2021; Cui et al., 2021; Ferreira et al., 2023; Jozefowicz and Foighil, 1999; Lam and Morton, 2006; Raith et al., 2015; Sekino and Yamashita, 2016).

The oyster aquaculture industry in Australia is concentrated in the south-east and is dominated by two species, the Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) and the Pacific oyster *Magallana gigas* (Thunberg, 1793) (Myers and Stephens, 2020; Nell, 2001). The 'native oyster', *Ostrea angasi* (Sowerby, 1871), and the tropical black-lip oyster, '*Saccostrea* lineage J' (often referred to as *Saccostrea echinata* (Quoy and

Abbreviations: COI, cytochrome oxidase 1; 16S, 16S ribosomal RNA; mtDNA, mitochondrial DNA.

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Gaimard, 1835) by industry), are also farmed on a small scale (Crawford, 2016; Myers and Stephens, 2020; Nowland et al., 2018; Nowland et al., 2019a). Total oyster production currently does not meet domestic demand, with disease posing a major challenge in most existing farming regions (Myers and Stephens, 2020). Geographical expansion of the industry is theoretically possible (Gentry et al., 2017) and may mitigate disease risk through diversification to additional species that are unaffected by the prevalent disease agents. This expansion relies on the development of aquaculture techniques for subtropical and tropical species, however is hampered by a lack of understanding of species taxonomy and distributions (Nowland et al., 2019a). A recent study (Snow et al., 2023) used DNA barcoding to survey *Saccostrea* species in Western Australia and documented distributions of two known and two previously unknown oyster lineages, with a view to supporting aquaculture expansion. No similar comprehensive study has occurred on the east coast of Australia where the majority of the industry is located.

Early studies (Cox, 1883; Hedley, 1909; Iredale, 1939; Saville-Kent, 1891a, 1891b; Shirley, 1911) as well as popular books (Allan, 1950; Rippingale and McMichael, 1961) have attempted to document the ostreid species of Australia's east coast based on shell morphology. Several previously recognised species were synonymised by Thomson (1954), who was of the view that there were "10 native Australian species" distributed between *Ostrea* (four species), *Crassostrea* (six species) and *Pycnodonte* (one species; now in Gryphaeidae). Although he employed a combination of anatomical and shell features, and provided much useful information on habitat and distribution, his study has largely been superseded in terms of its taxonomic conclusions (see Huber, 2010; Lamprell and Healy, 1998; McDougall et al., 2020).

The persistent challenge of reliable identification from shell features has prevented any consensus being reached on taxonomic identities for numerous species of Ostreidae, but possibly no more so than in the genus *Saccostrea*. The well-known Sydney rock oyster for example (*Saccostrea glomerata* (Gould, 1850)) has been variously placed in *Ostrea*, *Crassostrea, Saxostrea* or *Saccostrea*, renamed (*Ostrea commercialis* Iredale, 1939), synonymised with *Saccostrea cucullata* (Born, 1778) (Harry, 1985; Stenzel, 1971) as part of a 'superspecies' concept of *S. cucullata*, before finally being returned to full species status as *Saccostrea glomerata* after molecular study (Anderson and Adlard, 1994).

In their molecular and morphometric study of Indo-Pacific *Saccostrea*, Lam and Morton (2006) demonstrated the presence of multiple, genetically distinct lineages within the '*S. cucullata*' complex (separate from the *'S. mordax'* complex), identifying these as *Saccostrea cucullata* lineages A-G, *Saccostrea glomerata*, and *Saccostrea kegaki* Torigoe and Inaba, 1981. The study also detected two genetic lineages (A and B) within the *'S. mordax'* complex (now considered to be *S. scyphophilla* (Péron and Lesueur, 1807, Snow et al., 2023). Most of these lineages (except lineages C, D, F and G) were reported from Australia, with *S. mordax A*, *S. mordax B*, *S. glomerata*, and *S. cucullata B* found on the east coast. Sekino and Yamashita (2016) later built upon this work while surveying *Saccostrea* in Japan, dropping '*cucullata*' from the name and adding *Saccostrea* lineages H, I, and J. They also argued that lineages G, I and J, at least, were distinct reproductively isolated species. Snow et al. (2023) again used this framework, demonstrating that *Saccostrea* lineages A, B, J, and *S. scyphophilla* naturally occur on Australia's west coast. The use of these molecular techniques has now enabled robust identification of oysters and comparisons between studies, however assigning these lineages to morphologically described species remains a major challenge (Sekino and Yamashita, 2016).

In this study we conducted a genetic survey of intertidal oysters in north-eastern Australia (from Moreton Bay to Cooktown) with the aim of documenting their diversity and distribution. Partial 16S and COI mtDNA markers were used to identify specimens and to facilitate comparisons with previous studies. Our findings are of critical importance for the consideration of species diversification within the Australian aquaculture industry, as native oyster species are likely to perform best, and be deemed environmentally suitable, when grown within their natural latitudinal range. The information is also essential for the legislative bodies who govern oyster aquaculture in Australia, to enable the development of appropriate biosecurity measures, and for the facilitation of effective management of the industry.

2. Materials and methods

2.1. Sample collection

Field trips were undertaken to 19 Queensland localities between March 2018 and September 2019 and were timed to coincide with spring low tides. Up to 30 oyster specimens were collected from the intertidal zone at each site, with care taken to sample a broad range of sizes, morphologies, and microhabitats. Oysters were generally collected from the wild, however 28 specimens were sourced from wild-caught spat at an oyster farm in Bowen. Three hatchery-produced tropical blacklip oysters were also included, generated from wild Bowen broodstock. One specimen, donated by a colleague, was sampled from the back of a flatback turtle. Oysters were generally stored whole in 70% ethanol, however larger individuals were shucked, soft tissues placed in 70% ethanol, and shells labelled and retained. Samples were stored at 4 °C until processed.

2.2. DNA extraction, PCR and sequencing

In the laboratory, each oyster was shucked, photographed, and a small piece of adductor muscle dissected. DNA was extracted from the adductor muscle using a DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's instructions. PCR amplifications were performed in 20 μL volume reactions containing 1× ThermoPol Polymerase Buffer and 1.25 U Taq DNA polymerase (New England Biolabs M0267), 0.5 μM of each primer, 0.2 mM dNTPs, and 30-50 ng template DNA. PCR amplification of partial mtDNA 16S genes was undertaken using the primers 5′- CGCCTGTTTATCAAAAACAT-3′ ((Banks et al., 1993), reported in (Lam and Morton, 2006)) and 5'-CCGGTCTGAACTCAGATCACGT-3′ (Palumbi et al., 1991), and the following thermoprofile: 94 °C for 2 min, 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 68 °C for 1 min, with a 10 min final extension at 68 °C. PCR amplification of partial mtDNA COI genes was undertaken using the primers 5'-GGTCAACAAATCATAAAGATATTGG-3′ and 5'-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer et al., 1994), and the following thermoprofile: 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 44 °C for 30 s, and 68 °C for 45 s, with a 10 min final extension at 68 °C. In some cases inhibitor removal was required to enable PCR amplification, this was performed using a Zymo OneStep PCR Inhibitor Removal Kit. Amplified products were gel purified and submitted for capillary electrophoresis sequencing at Macrogen, South Korea. Resulting sequencing traces were manually inspected, trimmed, and ambiguous bases converted to degenerate code within the program 4Peaks (Griekspoor and Groothuis, 2006).

2.3. Phylogenetic analysis

Partial 16S and COI sequences generated were aligned within AliView v1.18 (Larsson, 2014), and identical haplotypes collapsed. A comprehensive set of ostreid 16S and COI DNA sequences were downloaded from NCBI using the search terms "16S or LSrRNA or large subunit ribosomal RNA gene AND ostreidae[orgn] NOT complete NOT genome NOT predicted" and "cytochrome OR COI OR COX1 AND Ostreidae[orgn] NOT complete NOT predicted NOT scaffold NOT linkage NOT genome NOT similar NOT COII NOT COIII NOT cytb". These sequences were then aligned with the unique 16S and COI haplotypes generated for Queensland oysters. Taxa that were represented by a large number of sequences from NCBI were reduced to 10 representative sequences; short sequences were preferentially removed, otherwise selection was random. Maximum Likelihood trees were produced using

the IQ-TREE web server (Trifinopoulos et al., 2016) with automatic model selection (GTR+F+I+G4 selected for 16S; TPM3u+F+R5 selected for COI) and 1000 ultrafast bootstrap replicates. Resulting consensus trees were visualised and formatted in FigTree v1.4.4 (Rambaut, 2006).

3. Results

3.1. Phylogenetic identification of Queensland oyster species

Partial mitochondrial COI and/or 16S sequences were obtained for 357 specimens collected from 19 locations in Queensland (north-eastern Australia). It was not possible to obtain both COI and 16S sequences for all specimens due to sequencing reaction failure (Supplementary Table 1). 74 and 49 unique haplotypes were sequenced for COI and 16S, respectively (Supplementary Tables 2 and 3). Unique haplotype sequences have been deposited in GenBank (accessions OR339797-OR339867 and OR339988-OR340035). These sequences were aligned with a comprehensive set of ostreid COI and 16S sequences retrieved from GenBank, resulting in a 650 bp COI alignment and a 517 bp 16S alignment.

Phylogenetic trees resulting from Maximum Likelihood analyses of partial COI and 16S sequences differed in topology at deeper nodes but were largely congruent at shallower branches, resolving comparable lineages (Figs. 1 and 2). Queensland oyster mitochondrial sequences were placed in 14 distinct clades (note that only 16S sequences were obtained for *Dendostrea* sp. 1 and *Dendostrea sandvichensis* (Sowerby, 1871). The majority of the obtained sequences fell within eight distinct *Saccostrea* clades, each corresponding to a lineage previously identified by Lam and Morton (2006) and/or Sekino and Yamashita (2016); lineage B, lineage F, lineage G, lineage I, lineage J, *Saccostrea glomerata*, *Saccostrea 'mordax'* A/B (= *Saccostrea scyphophilla* (see Snow et al., 2023)), and *Saccostrea 'mordax'* C (= *Saccostrea mordoides* (Cui et al., 2021)). The remaining sequences fell within four Ostreinae clades (*Ostrea stentina/equestris complex*, *Dendostrea sandvichensis*, *Dendostrea* sp. 1, and *Dendostrea* sp. 2; the latter two not grouping closely with any Genbank sequences), one *Magallana* clade (*Magallana bilineata*), and one *Talonostrea* clade (in this case, forming a clade to the exclusion of other *Talonostrea* sequences). The sequence falling within the *Ostrea stentia/equestris* species complex grouped with sequences designated as *Ostrea equestris* in a recent revision (Hu et al., 2019). Representative specimens for each species have been deposited in the Queensland Museum (registration numbers are listed in Supplementary File 1).

3.2. Distributions

Several of the oyster lineages identified had broad distributions while others were more restricted (Fig. 3). Four of these, *Talonostrea* sp., *S. scyphophilla,* and *Saccostrea* lineages B and G, were found across the entire sampled distribution. *Saccostrea* lineages F, I, and J and *S. mordoides* were primarily detected in tropical locations (with the southern-most detection of lineages I and J at Pancake Creek, just south of the Tropic of Capricorn), whereas *S. glomerata* was restricted to the subtropics, with the most northern detection just north of the Tropic of Capricorn (Wop-pa; Great Keppel Island). The exotic *M. bilineata* was only detected at the northern-most sites investigated. Very few Ostreinae specimens were collected, therefore a true indication of their range cannot be determined.

3.3. Shell morphology

Morphology was highly variable within *Saccostrea* lineages (Fig. 4), and overlapping morphologies were particularly noted between *S. glomerata* (Fig. 4, 2a-c), larger *Saccostrea* lineage B (Fig. 4, 1f-i), *Saccostrea* lineage G (Fig. 4, 3a-g), and smaller *Saccostrea* lineage J (Fig. 4, 7c-d). Young specimens possessing hyote (tubular) spines invariably belonged to *Saccostrea* lineage B (although not all individuals were spined, e.g. Fig. 4, 1b and d), whereas larger specimens of *Saccostrea* lineage J (Fig. 4, 7a) were distinguishable due to their large, thick, and usually deeply cupped shells with a broad, black shell margin (sometimes marbled with white). *Saccostrea* lineage F (Fig. 4, 8a-c) was generally distinguishable by its lighter colouration and ribbed lower (left) valve, and *Saccostrea* lineage I was easily identified by broad but delicate purple lobe-like squamae (lamellae) on the lower (left) valve (Fig. 4, 6a-b). *S. scyphophilla* (Fig. 4, 4a-c) and *S. mordoides* (Fig. 4, 5a-c) were generally distinguishable from other *Saccostrea* due to their purple/pink external colouration, highly scalloped shell margins, and radial ribs on the upper (right) valve. *S. mordoides* was generally much smaller than *S. scyphophilla*.

Oysters from *Magallana* and *Talonostrea* were distinguished from *Saccostrea* by the complete absence of chomata (small ridges and grooves found around the inner margin of the shell). *Talonostrea* (Fig. 4, 9a-c) otherwise resemble young *S. glomerata*, with few distinguishing shell features. *M. bilineata* was distinct due to its large size, pale yellow or purple external colouration, lack of chomata, and distinctive black adductor muscle scar (see figures in Willan et al., 2021). Ostreinae specimens found in this study are not pictured; due to their small numbers generalisations cannot currently be made regarding their morphology.

4. Discussion

This study demonstrates that the intertidal oyster community in Australia's north-east is highly diverse. We identified 14 genetically distinct lineages, of which eight fall within *Saccostrea,* four within the *Ostrea/Dendostrea* complex, and one each within *Magallana* and *Talonostrea*. We detected all lineages reported from Queensland by Lam and Morton (2006), as well as several lineages recorded from Australia for the first time (including *Saccostrea* lineages F and I, *O. equestris* Say, 1834 (sensu Hu et al., 2019), and *D. sandvichensis* (sensu Sutton et al., 2020)). The *Talonostrea* lineage and two of the *Dendostrea* lineages do not cluster with any other previously sequenced specimens in the phylogenetic analysis and could potentially represent undescribed species. The overlapping shell morphologies of oysters from different lineages observed in this study demonstrates that molecular methods are required for unequivocal identification in most cases. Given that our survey was restricted to specific sites and did not extend to Cape York or the Torres Strait it is possible that additional lineages may be present in the region. The lineages identified here are therefore unlikely to be a complete catalogue of intertidal oyster biodiversity in north-eastern Australia.

We agree with other researchers (Guo et al., 2018; Snow et al., 2023) that, in most cases, the genetic lineages identified through phylogenetic analysis of partial mitochondrial markers in this study (and others) likely represent distinct species. Support for the specific status of several of these lineages has been obtained from nuclear DNA markers. Sekino and Yamashita (2016) demonstrated that *Saccostrea* lineages G, I and J were genetically distinct using ITS1 data, and argued that this merits their status as biological species. More recently, evidence for genetic distinctiveness of *S. glomerata* was provided in an analysis of the population structure of Australian specimens based on genome-wide SNP markers (O'Hare et al., 2021). The accidental inclusion of mis-identified specimens from two other lineages in the study (subsequently found to be *Saccostrea* lineage B and *Saccostrea* lineage G (O'Hare, 2023)) clearly showed that the three mitochondrial lineages are genetically distinct. The case is less clear for the newly discovered Australian *Talonostrea* sp., which forms a well-supported cluster closely related to *T. zhanjiangensis* specimens from China (Wu et al., 2013) in both COI and 16S analyses. Whether these two lineages simply represent geographically distant populations of the same species requires further investigation.

Our analysis resolves some existing taxonomic problems relating to cultured oyster species in Australia. We demonstrate that the mid-sized,

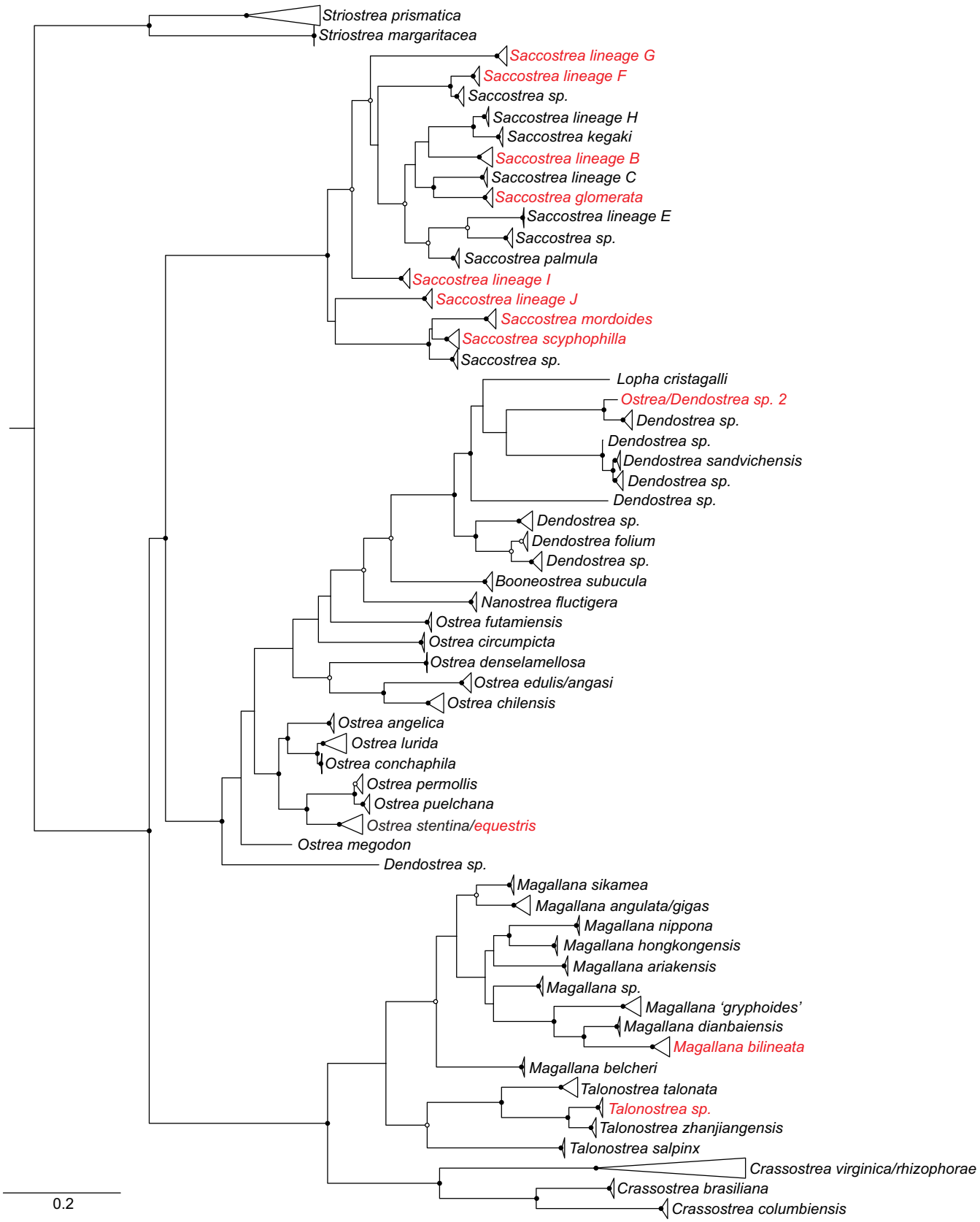

Fig. 1. Maximum Likelihood phylogenetic analysis using partial COI sequences. Clades have been collapsed for readability, and those containing north-eastern Australian specimens sequenced in this study are indicated in red. Clades with ultrafast bootstrap support values of 95 or over are indicated by black circles, clades with support values between 80 and 94 are indicated by open circles. The scale bar indicates the number of substitutions per site. Taxonomic nomenclature generally follows Huber (2010), with some variations due to recent works (Ferreira et al., 2023; Harzhauser et al., 2016) For a full version of this phylogenetic tree see Supplementary Fig. 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Maximum Likelihood phylogenetic analysis using partial 16S rRNA sequences. See Fig. 1 legend for details. For a full version of this phylogenetic tree see Supplementary Fig. 2.

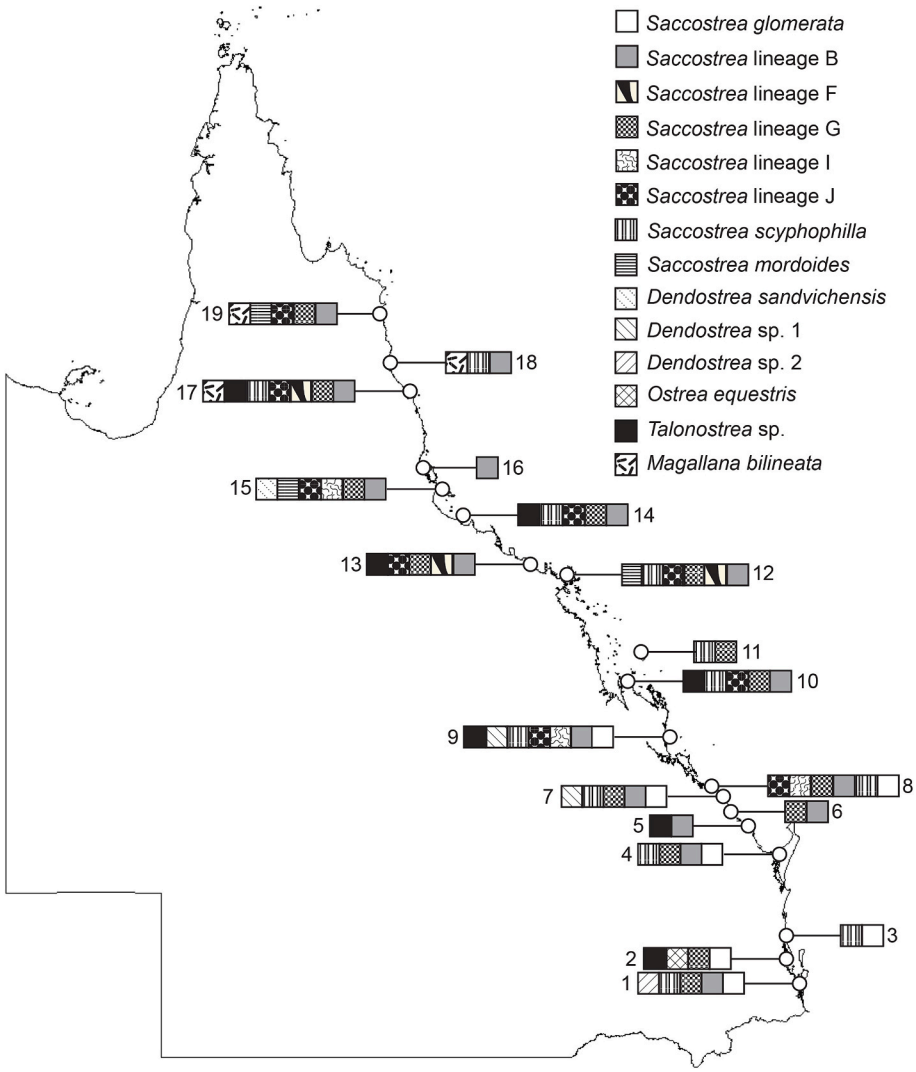

Fig. 3. Observed distribution of ostreid lineages in north-east Australia. General localities are as follows: 1 – Southern Moreton Bay, 2 – Northern Moreton Bay, 3 – Caloundra, 4 – Hervey Bay/K'gari (Fraser Island), 5 – Bundaberg, 6 – Miara, 7 – Seventeen Seventy, 8 – Turkey Beach/Pancake Creek, 9 – Yeppoon/Wop-pa (Keppel Islands), 10 – Stanage/Avoid Island, 11 – Percy Islands, 12 – Whitsunday Islands/Shute Harbour, 13 – Bowen, 14 – Magnetic Island, 15 – Orpheus Island, 16 – Cardwell, 17 – Cairns, 18 – Port Douglas, 19 – Cooktown. For more details on collection sites see Supplementary Table 1.

often spined oyster common on Queensland's coast (*Saccostrea* lineage B) is genetically distinct from the large, farmed, tropical blacklip oyster (*Saccostrea* lineage J). The name *S. echinata* has been used for *Saccostrea* lineage J on the assumption that the spined form is a juvenile morph of this species (Huber, 2010; Thomson, 1954); our study shows that this is not the case. All spined individuals sequenced in this study fell within *Saccostrea* lineage B, with smaller *Saccostrea* lineage J individuals tending to grow quite flat upon the substrate, generally possessing small, dark lamellae on the upper (right) valve (Fig. 4, 7b-c). Only larger individuals possessed the distinctive deeply-cupped shape. We are confident that the correct scientific name for *Saccostrea* lineage J is *S. spathulata* (Lamarck, 1819), as proposed by Sekino and Yamashita (2016). We reach this conclusion primarily due to the large size of the shell of the holotype (145 mm long, Fig. 5a) which appears unique amongst *Saccostrea* (maximum shell dimensions for *Saccostrea* specimens collected in this study are reported in Supplementary Table 4; also see Table 6, Sekino and Yamashita, 2016). Although the largest *Saccostrea* lineage J specimen collected in this study is smaller than the holotype (110 m long), the largest specimen recorded by Sekino and Yamashita (2016) measured 123 mm long, and the largest adult '*Saccostrea echinata*' reported from the Northern Territory, Australia, by Nowland et al. (2019b) measured 192.2 mm. The deep, widely spaced chomata observed within the holotype is also in accordance with *Saccostrea* lineage J specimens sequenced in this study (although these varied in extent around the shell margin), as is the broad, black pigmented band around the inner shell margin that gives this species its common name (Fig. 5, b-c). The holotype of *Ostrea echinata* Quoy and Gaimard, 1835 (= *Saccostrea echinata* (Quoy and Gaimard, 1835)) was a spined oyster described from Ambon in Indonesia (for photograph see Inaba and Torigoe, 2004). As several *Saccostrea* lineages have been reported to possess spines (*Saccostrea* lineages A, B, F, H and *S. kegaki*, Lam and Morton, 2006; Sekino and Yamashita, 2016), sequencing of specimens from the type locality will be required to determine whether a single lineage can be assigned to the name. We therefore recommend that the aquaculture industry refrain from using *S. echinata* for *Saccostrea* lineage J, as this name is certainly incorrect, and utilise the name *S. spathulata* henceforth.

Although several of the oyster species identified in this study have widespread distributions, others are restricted either to the tropics or the subtropics. Many have broad Indo-Pacific distributions, being found as far north as Japan (Sekino and Yamashita, 2016) and none (except possibly the *Talonostrea* sp.) are endemic to north-eastern Australia. New species detections often raise the suspicion of recent introductions, especially when the known range of the species is distant. However, the

Fig. 4. Indicative shell morphologies of ostreid oysters collected from north-eastern Australia. 1a-i, *Saccostrea* lineage B; 2a-c, *Saccostrea glomerata*; 3a-g, *Saccostrea* lineage G; 4a-c, *Saccostrea scyphophilla*; 5a-c, *Saccostrea mordoides*; 6a-b, *Saccostrea* lineage I; 7a-d, *Saccostrea* lineage J; 8a-c, *Saccostrea* lineage F; 9a-c, *Talonostrea* sp.

challenges of morphological oyster identification, coupled with the broad distribution of most of the species within Queensland, suggests that these species are native and have previously been misidentified. The one exception is *M. bilineata*; detection of this species in our survey coincided with three other detections in the region, including by commercial fishers and Indigenous rangers. This species is considered a recent introduction to north-eastern Australia (Willan et al., 2021).

Our survey revealed that the ranges of many of the lineages overlap, with multiple species detected at every site visited except one. This extensive sympatry implies that the various lineages are reproductively distinct, again indicating that they are true species (Harrison and Larson, 2014). We did not find *Saccostrea* lineage A (originally reported from Australia's west coast by Lam and Morton (2006), and subsequently by Snow et al. (2023)) in our survey, supporting the assertion that this may be a Western Australian endemic. The other three lineages detected in Western Australia (*S. scyphophilla* and *Saccostrea* lineages B and J) were also detected on the east coast, with *Saccostrea* lineages B and J found at much higher latitudes than in the west. This is surprising, as tropical and subtropical species tend to be found at higher latitudes on the west coast due to the influence of the Leeuwin current (Maxwell and Cresswell,

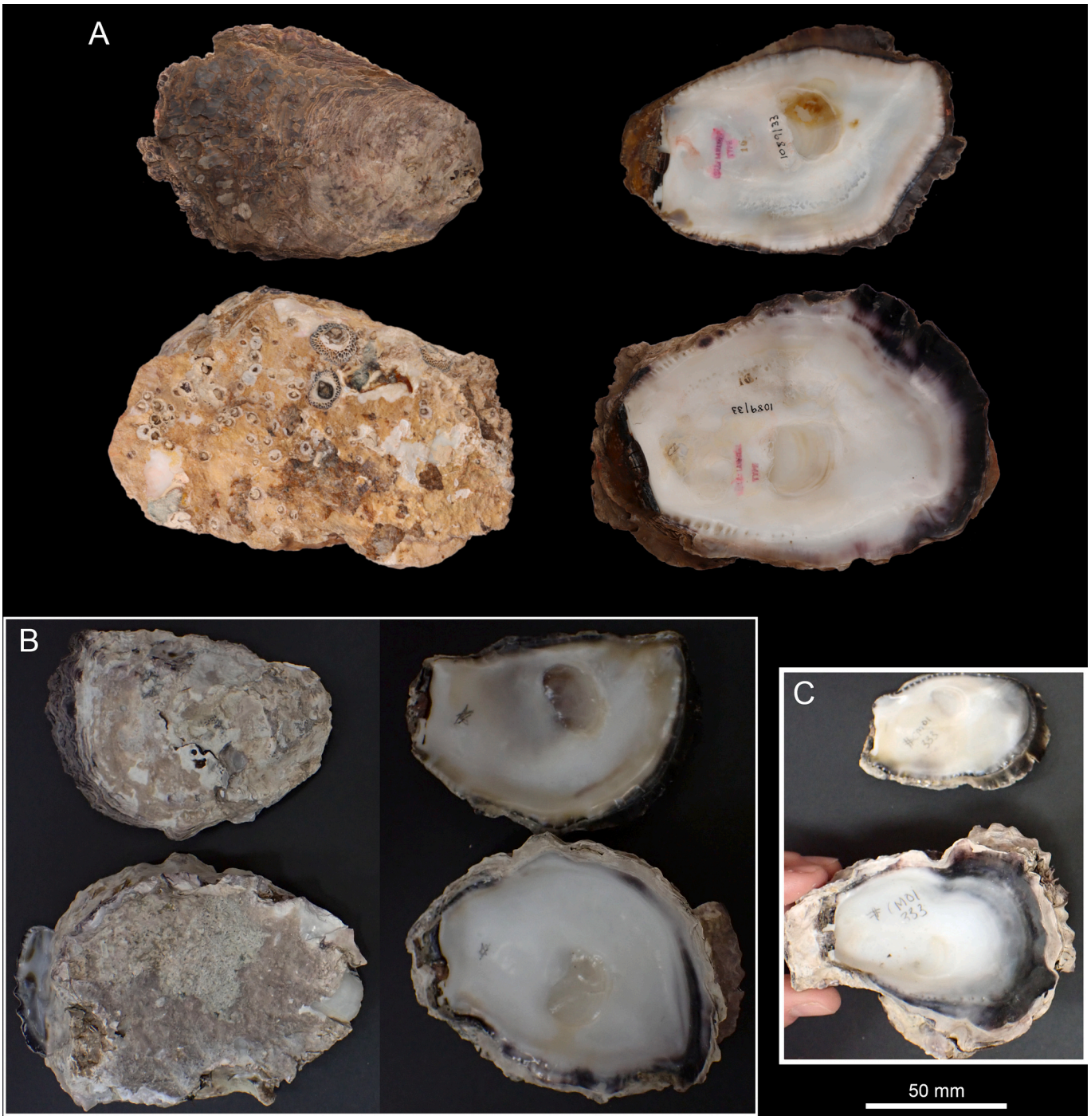

Fig. 5. The holotype of *S. spathulata*, with a comparison to *Saccostrea* lineage J. A. *Ostrea spathulata* (= *Saccostrea spathulata*) Lamarck, 1819, holotype lodged within the Museum de Geneve, MHNG-MOLL-50830. Collection locality is uncertain, and is given as 'Australie?'. Photographed by Emmanuel Tardy. B. Sequenced specimen of *Saccostrea* lineage J. C. A second sequenced *Saccostrea* lineage J showing similar chomata and black banding to the holotype.

1981). The restricted documented distribution in Western Australia could be due to several factors including lack of suitable habitat, relatively recent introduction of the species, or insufficient sampling. As both species have broad Indo-Pacific distributions the latter explanation may be the most likely.

Although not systematically assessed, it was clear that the different lineages were found in different habitats or environments. As noted by Sekino and Yamashita (2016), *Saccostrea* lineage I was almost exclusively found on the stems of *Rhizophora* mangroves, or, occasionally, on rock near *Rhizophora* trees, in sheltered oceanic or estuarine sites. *Saccostrea spathulata* tended to be found in the lower intertidal in similar sites, and was often co-located with *S. mordoides*. This points towards niche differentiation between *S. mordoides* and the morphologically and genetically similar *S. scyphophilla* that prefers exposed oceanic rocky shores (Snow et al., 2023) and is found higher in the intertidal. *S. glomerata* appears exclusively estuarine, and *Saccostrea* lineages B and G are euryhaline and favour sheltered sites, with *Saccostrea* lineage G found lower in the intertidal. The few *Ostrea/Dendostrea* specimens collected here were found very low in the intertidal on spring tides, indicating that they may in fact be subtidal species. The differences in habitat preferences of the species present in the region point towards distinct larval settlement processes or spat survival rates, and the

investigation of the physiological basis of their different tolerances will be important for the development of any of these species for aquaculture.

Of the 14 lineages detected in Australia's north-east, several possess characteristics that make them attractive candidates for aquaculture production. *Saccostrea* lineages B and G can attain reasonably large sizes and have broad distributions, meaning that they may be suitable for farming in both tropical and sub-tropical waters. Further, *Saccostrea* lineage G is morphologically very similar to *S. glomerata* and is therefore likely to be well received by consumers. *Saccostrea spathulata* is already farmed on a small scale in northern Australia and has significant potential for expansion. Hatchery trials have established successful production methodology for this species, and growth rates are comparable to other commercially farmed oyster species (Nowland et al., 2019c, 2019d, 2021; Nowland and Roberts, 2023). Another tropical candidate is *Saccostrea* lineage F (likely *S. malabonensis* (Faustino, 1932) or possibly *S. circumsuta* (Gould, 1850) now that the lineage has been detected in the South Pacific (Li et al., 2017; Sekino and Yamashita, 2016)), which is currently farmed in the Philippines (Angell, 1986; Nowland et al., 2019a). *M. bilineata* is also extensively farmed (Faustino, 1932; Kinch et al., 2019; Suja et al., 2020), but is unlikely to be suitable for Australian aquaculture given its exotic status (Willan et al., 2021). Hatchery trials will be required for each of these species to determine which show the most promise for aquaculture in the region.

5. Conclusions

This study has demonstrated that the intertidal oyster community in north-eastern Australia is highly diverse. The *Saccostrea* and some of the *Ostrea/Dendostrea* lineages detected have also been reported from other geographic locations, highlighting the broad Indo-Pacific distribution of multiple lineages within these genera. As the various lineages were clearly separated into distinct clades in phylogenetic analysis, and were frequently found in sympatry, we argue that they represent distinct species. Taxonomy of these species remains challenging as original descriptions are largely based on shell morphology alone. This survey revealed distinct tropical, subtropical-tropical or temperate-subtropical distributions of oyster species, alluding to key differences in their physiology and habitat preferences. Ultimately, the information gathered through the survey will inform future research and development efforts to expand oyster aquaculture in northern Australia.

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CRediT authorship contribution statement

Carmel McDougall: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Nikolina Nenadic:** Writing – review & editing, Investigation, Data curation. **Marina Richardson:** Writing – review & editing, Visualization, Investigation. **John M. Healy:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Carmel McDougall reports financial support was provided by Queensland Government Department of Science, Information Technology and Innovation. Additional funding was also received from the Fisheries Research and Development Corporation. The other authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

Data availability

DNA sequences have been deposited to GenBank (accessions OR339797-OR339867 and OR339988-OR340035)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.740838.

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