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First report of the European flat oyster *Ostrea edulis*, identified genetically, from Oyster Harbour, Albany, south-western Western Australia

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Abstract

Ostrea angasi is the only large native ostreine species in southern Australian waters (i.e. New South Wales, Victoria, Tasmania, South Australia and Western Australia (WA)). It has also been recorded as a fossil from Late Pliocene calcarenites of the Roe Plain along the south-eastern coast of WA. Wild populations were harvested for food before and after European settlement. A sample of flat oysters from Oyster Harbour, Albany, WA, was typed for *16S* and cytochrome oxidase 1 (*CO1*) mitochondrial (mt) DNA markers previously shown to be diagnostic for species of Ostreidae. *Ostrea edulis* was identified in the sample at an approximate 30% occurrence with *O. angasi*. Interspecific partial *16S* and *CO1* mtDNA sequence divergences, estimated using Kimura's two-parameter model, were 0.83% and 1.45%, respectively. The occurrence of *O. edulis* among native *O. angasi* populations has not hitherto been suspected and, thus, there has not been a legitimate morphological separation of the two. These results emphasise the value of molecular markers in: (*1*) discriminating morphologically plastic and closely related species; and, thus (*2*) the monitoring of species introduced into morphologically similar stocks. We caution against such introductions because of the possibility of the importation of oyster diseases (e.g. bonamiasis) and of this and other adverse impacts upon native species.

Additional keywords: Bonamia, cytochrome oxidase 1 (*CO1*) gene, *16S* gene, introduced species, *Ostrea angasi*.

Introduction

Wild populations of native flat oysters were harvested for food in many parts of southern Australia before and after European settlement. Dakin (1952) noted that: '…to judge by the remains found in their coastal kitchen-middens the aborigines evidently appreciated it, and it must at one time have been relatively common even around Sydney'.

The identity of the southern Australian flat oyster has been debated for many years. Tenison-Woods (1877–1878) considered that both *Ostrea edulis* (Linnaeus, 1758) and *Ostrea angasi* (Sowerby, 1871) occurred in Tasmania. Saville-Kent (1895) considered that the so-called mud-oyster abundant in the vicinity of Albany, Western Australia (WA), was *O. edulis* being '…identical with the British 'Native". The common flat oyster of South Australia (SA) and New South Wales (NSW) was considered by Allan (1950) to be *Ostrea sinuata* (Lamarck, 1819), although this author also noted that, in 1947 and 1948, '…seeds of the large Japanese edible oyster, *Ostrea gigas*, were transported from Japan to beds in Tasmania and Western Australia'. This suggests that, at least prior to this date, it was common for oysters to be introduced from elsewhere without significant hindrance and this may have some relevance to the present study. Cotton and Godfrey (1938), Dakin (1952) and Cotton (1961) also considered that *O. sinuata* was the common flat oyster of SA.

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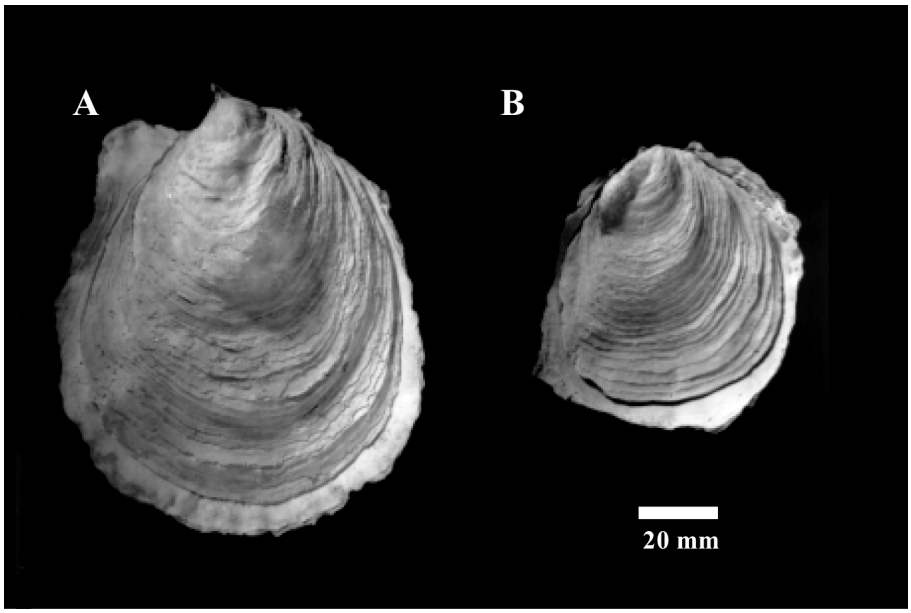

Fig. 1. Native fossil flat oysters in Western Australia (WA) are *Ostrea angasi*. *A*, Fossil *O. angasi* from an emergent Middle Holocene shell bed at Cervantes, near the coast, northern Perth Basin, Western Australia; *B*, fossil *O. angasi* from the Late (though not latest) Pliocene of the Roe Calcarenite of the Eucla Basin, WA. See Fig. 2 for collection sites. These specimens are from the collections of the Western Australian Museum with reference numbers WAM 82.2891.b and WAM 69.536.a, respectively.

However, Thomson (1954) considered that the correct name for the native Australian oyster was *Ostrea angasi*, with *O. sinuata* as a junior synonym, and Ludbrook and Gowlett-Holmes (1989) also describe *O. angasi* as the only flat oyster present in southern Australia. Edgar (1997) considers that *O. angasi* occurs from Fremantle, WA, to NSW and around Tasmania and is '…virtually indistinguishable from the European oyster *Ostrea edulis*'. Roberts and Wells (1981) also give the same distribution for *O. angasi*, whereas Lamprell and Healy (1998) are the most recent authors to consider that *O. sinuata* (Cotton and Godfrey, 1938, non-Lamarck, 1819) is a junior synonym of *O. angasi* and also give it a wide, southern distribution in WA, SA, Tasmania, Victoria and NSW. Jozefowicz and O' Foighil (1998) sequenced the *16S* gene of eight flat oyster individuals from St Helens, Tasmania, and Pambula, NSW, and all were genetically identified as *O. angasi*.

Thus, *O. angasi* is considered to be the only large ostreine species in southern Australian waters. It has also been recorded as a fossil from the mid-Holocene of Cervantes, north of Perth, and the Late Pliocene calcaranites of the Roe Plain along the southern coast of WA (Slack-Smith 2000; Fig. 1).

In (Albany) WA, in 1791, Vancouver records that he:

> '…found a passage, narrow and shoal for some distance, into the north-eastern harbour; where a bar was found to extend across its entrance, on which there was only three fathoms [6 m] of water…In our way out of this harbour, the boats grounded on a bank we had not before perceived; this was covered with oysters of a most delicious flavour, on which we sumptuously regaled; and, loading in about half an hour, the boats for our friends on board, we commemorated the discovery by calling it Oyster Harbour' (Vancouver 1798).

Twelve years later, in 1803, Baudin prepared to go and visit the head of the port (Oyster Harbour) and reconnoitre the oyster-beds spoken of by Vancouver:

> 'We finally reached the head of the port and stood off the edge of a shoal, where we collected a reasonable number of oysters 1½ to 2 feet down on a bed of weeds [actually seagrasses]. They were found to have a good flavour and to be extremely big' (Baudin 1809).

As with the flat oysters in all of southern Australia, in WA the species attracted attention as a fisheries object from the earliest days and its name thereat followed the fortunes of its conspecifics in other parts of the country.

Saville-Kent (1894) considered '…*Ostrea edulis*…is common to the colonies of Victoria, Tasmania and South Australia' and '…was abundant in the vicinity of Albany'. Saville-Kent (1895) reiterated the above, but pointed out that the oyster had been '…so exhaustively fished as to have been almost exterminated' and that '…steps are now being taken by Government…to re-establish the oyster beds of King's Sound [i.e. King George Sound]'. The same proposals were reiterated in 1897 and 1898 (Anonymous 1897, 1898) but, thereafter, it seems no identified action was taken and the industry in Albany was allowed to die, the market for local oysters being taken up by crassostreine rock oysters of the genus *Saccostrea* being sent down to Perth and Fremantle from the neighbourhoods of Shark Bay and Geraldton with '…supplementary importation from Queensland and New South Wales' (Anonymous 1999).

With the decline of *O. angasi* in Oyster Harbour, Albany, rock oysters (*Saccostrea glomerata*) introduced from more western coasts of WA are farmed there today (Anonymous 1999). The numbers of wild individuals of *O. angasi* in Oyster Harbour are still now so low that this species is no longer a significant fisheries product. However, in 2002, samples of flat oysters living in Oyster Harbour were obtained and their identities studied using mitochondrial (mt) DNA sequences and shell characteristics.

Materials and methods

In 2002, samples of flat oysters from Oyster Harbour, Albany, WA (Fig. 2), were typed for the *16S* and cytochrome oxidase 1 (*CO1*) mtDNA markers previously shown to be diagnostic for the Ostreidae (Jozefowicz and O'Foighil 1998). Genomic DNA was extracted from 10 individual oysters using Dneasy™ Tissue Kit (Qiagen, Venlo, the Netherlands). The partial *16S* and *CO1* segments were amplified using primer pairs of 16sar/16sbr (Banks *et al.* 1993) and LCO1490/HCO2198 (Folmer *et al.* 1994), respectively. Polymerase chain reaction (PCR) conditions followed those of Jozefowicz and O'Foighil (1998) and Lam *et al.* (2003). The PCR products were purified using Sephaglas™ BandPrep Kit (Amersham Pharmacia Biotech, Buckinghamshire, England) and then sequenced using an ABI Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Buckinghamshire, England) and an ABI 377 Perkin Elmer (Buckinghamshire, England) DNA sequencer. The PCR primers were used as sequencing primers. Sequences of Albany individuals and other ostreines from GenBank (http://www.ncbi.nlm. nih.gov/, accessed May 2002) were aligned using ClustalX 1.81 (Thompson *et al.* 1997). Phylogenetic analysis and sequence divergence values were obtained using PAUP* 4.0b8 (Swofford 1998).

Comparisons were made between the shell characteristics of genetically identified individuals to clarify conchological distinctions. Shell characters involved were: (*1*) shape and surface sculpture; (*2*) external and internal shell colour; (*3*) hinge line and ligament position and extent; (*4*) attachment area of the left valve; (*5*) presence and pattern of chomata; and (*6*) position, colour and relative size of the adductor muscle scar.

Voucher specimens have been lodged in the Western Australian Museum, Perth (S10871 to S10874).

Results

Partial *16S* (approximately 445 nucleotides (nt)) and *CO1* (approximately 700 nt) sequences of 10 individuals (identified as Albany 1–10) and two individuals (Albany 1 and

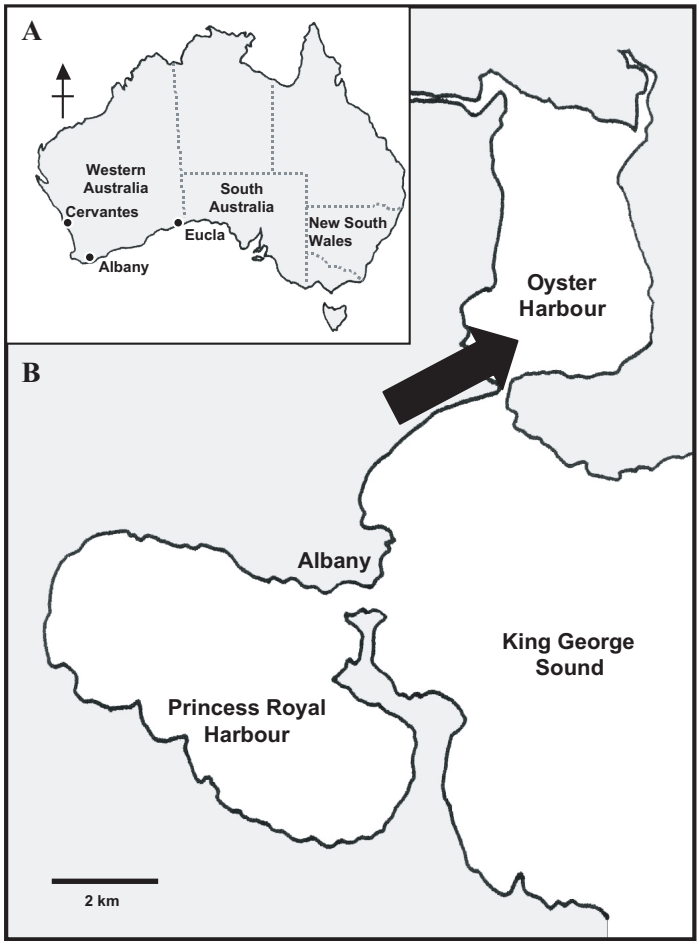

Fig. 2. *A*, Map of Australia showing the locations of the fossil *Ostrea angasi*. *B*, Detailed map of Albany, Western Australia, showing the collection site (arrow) for flat oysters.

Albany 2), respectively, of Albany flat oysters, locally identified as *O. angasi*, were obtained. Four and two haplotypes were obtained from the *16S* and *CO1* datasets, respectively. When these sequences of Albany flat oysters were compared with those published on GenBank using a BLAST network service, two taxa were identified. One was similar to *O. angasi* (for *16S*, GenBank accession number = AF052063 (Jozefowicz and O'Foighil 1998); for *CO1*, GenBank accession number = AF112287 (O'Foighil *et al.* 1999)) and the other was similar to *O. edulis* (for *16S*, GenBank accession number = AF052068 (Jozefowicz and O'Foighil 1998); for *CO1*, GenBank accession number = AF120651 (Giribet and Wheeler 2002)). For *16S*, one of the haplotypes is similar to the sequence of *O. angasi* from GenBank with one transition. The other three are similar to the sequence of *O. edulis* from GenBank with ranges from zero to one transition. The sequences reported upon herein have been deposited in GenBank under accession numbers AF540594–AF540599.

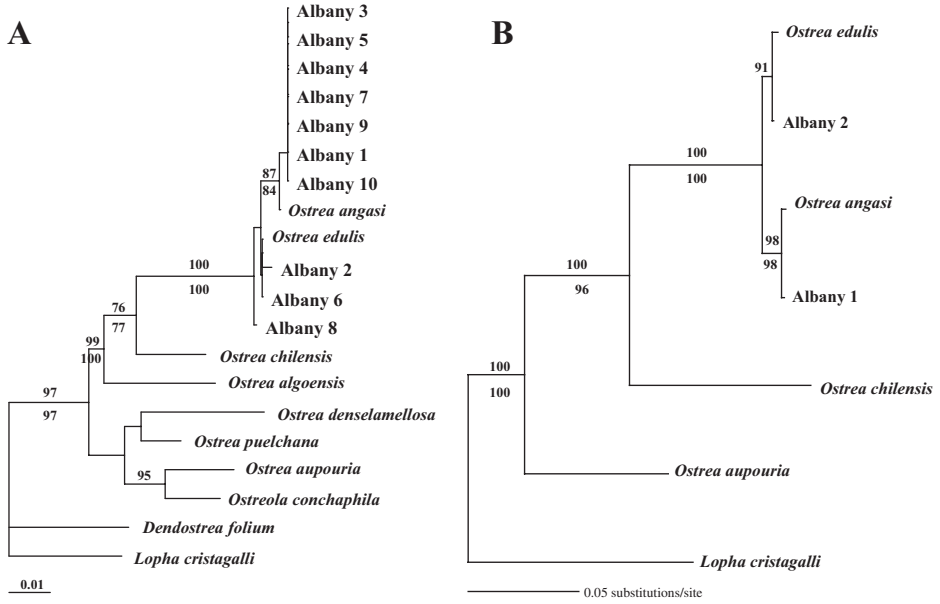

Fig. 3. *A*, Neighbour-joining (NJ) tree constructed using sequence divergence values based on the Kimura two-parameter model obtained from mitochondrial (mt) *16S* gene fragment (approximately 445 nucleotide) sequences for flat oysters of the Ostreinae. Members of the Lophinae were used as outgroups. Albany 1–10 represent ten individuals from Albany, Western Australia. Other oyster sequences were derived from Jozefowicz and O'Foighil (1998) (GenBank accession numbers AF052062–AF052069, AF052071 and AF052073). The tree topology of the 24 most parsimonious (MP) trees (131 steps; consistency index (*CI*) = 0.756; retention index (*RI*) = 0.822), obtained by heuristic search from the same dataset, is similar to that of the NJ tree. Bootstrap values (number of replicates = 1000) for NJ and MP trees are indicated above and below the branches, respectively. Only values >75 are shown. The scales represent the percentage of substitution. *B*, An NJ tree calculated using sequence divergence values based on the Kimura two-parameter model obtained from mt cytochrome oxidase I gene fragment (approximately 700 nucleotide) sequences for flat oysters of the Ostreinae. Albany 1 and Albany 2 represent two individuals from Albany. *Lopha cristagalli* was used as an outgroup (Matsumoto 2001; direct GenBank submission, GenBank accession number AB076908). Sequences of *O. chilensis*, *O. angasi*, and *O. aupouria* were derived from O'Foighil *et al.* (1999) (GenBank accession numbers AF112285, AF112287 and AF112288, respectively). The tree topology of the MP tree (248 steps; *CI* = 0.891, *RI* = 0.738), obtained by an heuristic search, is the same as that of the NJ tree.

The identities of *O. angasi* and *O. edulis* in Albany were confirmed further by molecular phylogenetic analysis of the two taxa and comparison with conspecifics from elsewhere and with other ostreine species (Fig. 3). Flat oysters, named Albany 1, 3, 4, 5, 7, 9 and 10, were identified genetically as *O. angasi*, whereas those named Albany 2, 6 and 8 were identified as *O. edulis*. Mean interspecific partial *16S* and *CO1* mtDNA sequence divergences, estimated using Kimura's two-parameter model, were 0.83% and 1.45%, respectively. Figures 4 and 5 show external and internal views of the valves of flat oysters from Albany that have been identified genetically as *O. angasi* and *O. edulis*.

Ostrea angasi shells are approximately 50 mm in dorsoventral height, subtriangular to oval in shape and slightly prosogyrous. Individuals are unattached and reside on the left valve. Shells are heavily bored by polychaetes and encrusted with balanoid barnacles. The right valve is nearly flat and heavily eroded dorsally, such that the chalky calcareous shell

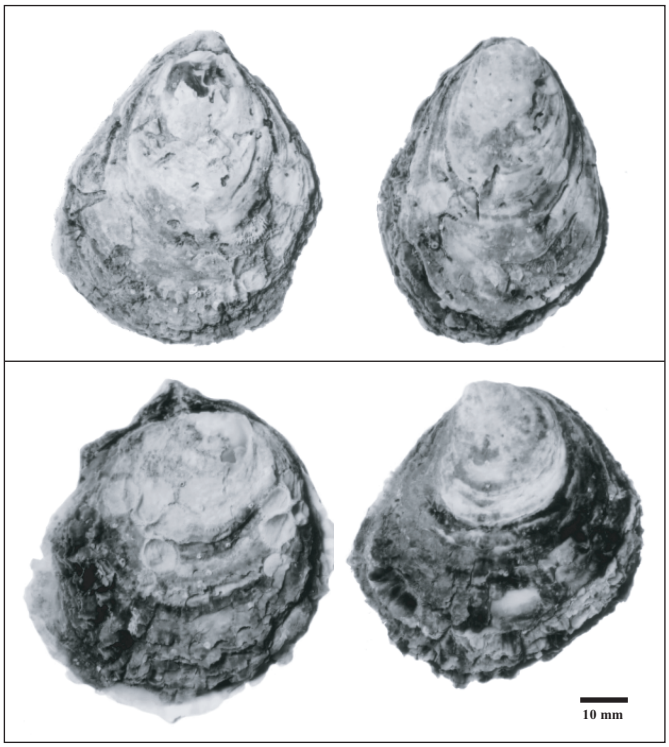

Fig. 4. Genetically identified shells of *Ostrea angasi* (top row) and *Ostrea edulis* (bottom row) collected from Oyster Harbour, Albany, Western Australia. External surfaces of the right valves are shown.

layer is exposed. The ventral margin is covered by dense layers of brown, overlapping, thin and brittle lamellae or scales arising from growth lines. The left valve is more inflated than the right and white. Chomata are absent. The hinge line is straight and short. The ligament area is slightly extended in both valves. The interior of the shell has white and bluish–green patches with a pearly nacre. Small patches of chalky deposits are prominent on the interiors of both the left and right valves. The adductor muscle scar is white with lightly coloured growth lines. It is anteroposteriorly elongate, crescentic and positioned in the posterior ventral one-third of the pallial area.

Ostrea edulis shells are also approximately 50 mm in dorsoventral height, but have a subcircular outline and are distinctively opisthogyrous. Because the right valve is less eroded, fouled and bored, layers of brown growth lamellae with dark radial rays are obvious. The layer of lamellae in *O. edulis* is less dense than that in *O. angasi*. The left valve is more inflated than the right and is white with low, widely spaced radial ribs. Individuals are unattached and, like *O. angasi*, also reside on the left valve. Chomata are absent. The hinge line is straight and short and the ligament area is slightly extended in both valves. The interior of the shell is white with lightly coloured patches on the ventral side. Large patches of chalky deposits are prominent on the interiors of both left and right valves. The adductor muscle scar is white with lightly coloured growth lines. This scar is anteroposteriorly elongate, crescentic and tapers posteriorly. It is centrally positioned, slightly near the posterior.

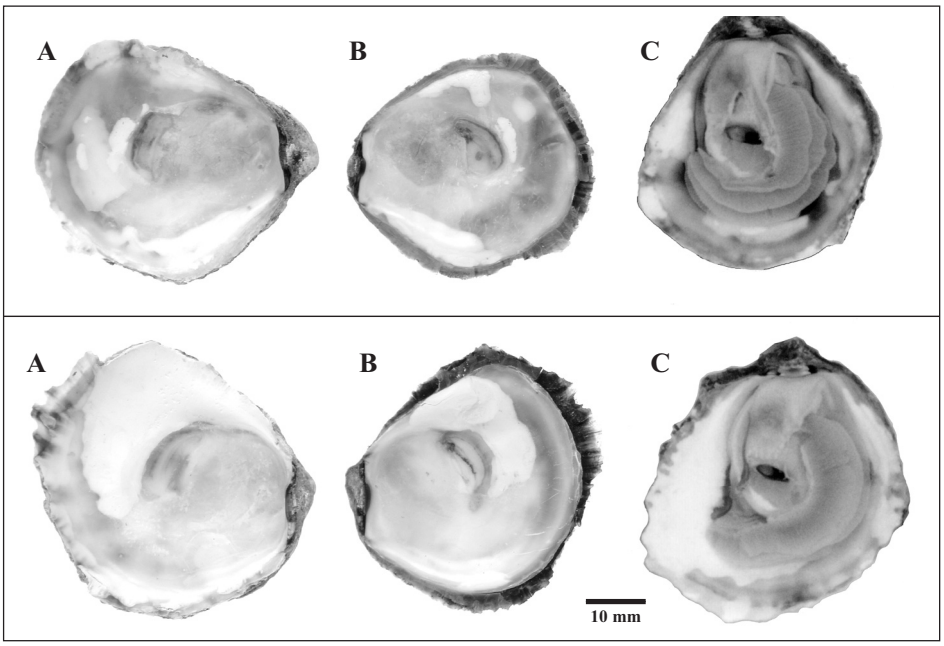

Fig. 5. Genetically identified shells of *Ostrea angasi* (top row; WAM S10872) and *Ostrea edulis* (bottom row; WAM S10874) collected from Oyster Harbour, Albany, Western Australia. *A*, Internal views of the right valves; *B*, internal views of the left valves; *C*, internal views of the left valves showing the organs of the mantle cavity.

Anatomical structures, such as the shape and position of the adductor muscle and the arrangement, relative size and colour of various organs in the mantle cavity are similar in the two species. Therefore, the only moderately reliable morphological characteristic useful in distinguishing these two species is the direction of shell growth.

Discussion

The occurrence in Australia of *O. edulis* among a native *O. angasi* population has not hitherto been generally suspected, possibly because of similarities in shell characters. However, Tenison-Woods (1877) did think two species may be present and described briefly the external shell appearance of the Tasmanian *O. edulis* and *O. angasi*, but did not provide a morphological separation of the two. Stenzel (1971) and Harry (1985) also mentioned both species in their taxonomic studies of the genus *Ostrea* without identifying morphological differences between them. Sowerby (1871) considered that, of the two species, the sculpture of *O. angasi* was much less coarse and the upper valve more convex. Among the samples collected by us, the two sympatric species are difficult to differentiate individually in terms of shell characteristics. For example, *O. edulis* individuals from Oyster Harbour, when compared with sympatric *O. angasi*, do not have a more convex right valve, as suggested by Sowerby (1871). However, in general, *O. angasi* is slightly prosogyrous, whereas *O. edulis* is distinctively opisthogyrous. The former also has denser flaky lamellae on the margin of the right valve.

Previous studies have also shown it to be difficult to distinguish between *O. edulis* and *O. angasi* in terms of allozyme genetics and karyology. Blanc and Jaziri (1990)

compared 17 enzyme loci between the two species, since *O. angasi* was introduced experimentally into France from Australia. These authors showed that no locus was diagnostic and that no single locus could be considered specific for *O. angasi*. The patterns of karyotype, C-band and silver-stained nucleolus organizer regions of *O. angasi* from Port Lincoln, SA, are also very similar to those of European *O. edulis* (Li and Havenhand 1997). Not until recently has *O. angasi* from NSW and Tasmania been differentiated from French and Irish *O. edulis* in terms of a *16S* sequence divergence value of 0.67% (Jozefowicz and O'Foighil 1998). This genetic difference value was the lowest compared with other species pairs of the genus *Ostrea*. These results are confirmed herein (0.83%).

The *16S O. edulis* dataset obtained in the present study suggests an introduction of this species from Europe. The studied Albany 6 individual has a partial *16S* sequence identical to that of *O. edulis* from France and Ireland (Jozefowicz and O'Foighil 1998). However, comparisons of population genetics (e.g. *CO1* sequences of *O. edulis* individuals from the north-eastern Atlantic and the Mediterranean) are required to identify the origin of the introduced Albany *O. edulis*. Partial *16S* sequences of *O. angasi* from Albany have one consistent transition site compared with individuals from St Helens, Tasmania, and Pambula, NSW (Jozefowicz and O'Foighil 1998). This shows that the *O. angasi* populations in south-western and south-eastern Australian waters are distinct in terms of molecular genetics.

The present discovery of the European flat oyster *O. edulis* among native *O. angasi* stocks in Albany, WA, opens up the possibility of the former being introduced either accidentally or unofficially into Australia. There have been recent outbreaks of the parasitic protist *Bonamia ostreae* in *O. angasi* from Australia and, because this is usually considered to be specific to *O. edulis* in Europe and North America, is it possible that this pest was also brought into Australia with the non-native species?

Adlard (2000) reports that the first recorded instance of bonamiasis in the flat oysters of French waters was in 1979 (Comps *et al.* 1980). Significant mortalities of *O. edulis* have since been attributed to that disease in France, Spain, England, Ireland, Denmark, The Netherlands and North America (Balouet *et al.* 1983; Bucke and Feist 1985; Van Banning 1985; Elston *et al.* 1986). The disease is thought to have originated in North America, from where large numbers of oyster seed (*O. edulis*) were transferred from California to France prior to 1979 and, thence, spread to other European countries (Elston *et al.* 1986). Adlard (2000) also records that:

> '…in the summer of 1991, an ultrastructurally identical and genetically similar parasite to that found in New Zealand, was identified in the haemocytes of *O. angasi* in Port Phillip Bay and along the coast of Victoria, in southern and eastern Tasmania, and in south-western Western Australia, and has been associated with large scale mortalities of the host oyster. Thus, two distinct species of *Bonamia* are currently known, *Bonamia ostreae* in Europe and *Bonamia* sp. in Australasia.'

Adlard (2000) also considered the Australian species of *Bonamia* to be different from but ultrastructurally 'identical' to *B. ostreae* in Europe. Moreover, Bougrier *et al.* (1986), in France, showed that *O. angasi* is susceptible to *B. ostreae*. The earliest report, in late 1985, of bonamiasis in New Zealand was from *Tiostrea chilensis*. However, the disease was not reported from Australia (in *O. angasi*) until 1991. Therefore, it is possible that the initial outbreak of bonamiasis, in the case of Australia, may be correlated with the introduction of infected *O. edulis* sometime prior to 1991.

The results presented herein emphasise the value of molecular markers in: (*1*) discriminating morphologically plastic and closely related species; and (*2*) monitoring the consequences of species introduced into morphologically similar native stocks.

We caution against such introductions because of the likelihood of the importation of oyster diseases, such as bonamiasis, and of such impacts (as well as others) on native species.

Acknowledgments

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