

Occurrence of golden Portuguese Oysters (*Crassostrea angulata*) in farmed populations in Taiwan

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Abstract. Although selective breeding for shell colour has been established in oysters, the prevalence of golden oysters in wild or farmed populations remains poorly known. In the present study, we investigated the occurrence of the golden Portuguese Oyster, *Crassostrea angulata* (Lamarck, 1819), in aquaculture areas in Taiwan. Our results showed no significant difference in the proportion of golden oysters among the Taixi, Qigu, and Kinmen sites (one-way ANOVA, $p > 0.05$), with respective averages of $9.7 \pm 5.2\%$, $9.5 \pm 5.4\%$, and $8.4 \pm 5.8\%$. The consistent presence of approximately 10% golden oysters across all three locations may be attributed to the year-round availability of oyster spat and the absence of site-specific selective pressures.

Key words. *Magallana angulata*, shell colour, COI

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INTRODUCTION

Oyster aquaculture is a traditional industry in Taiwan, and the cultured species has been classified as the Pacific Oyster, *Crassostrea gigas* (Thunberg, 1793) (Lin & Liang 1982). In the late 20th century, based on mtDNA restriction fragment length polymorphism (RFLP) analyses, the Portuguese Oyster, *Crassostrea angulata* (Lamarck, 1819), was identified as distinct from *C. gigas* (Boudry *et al.* 1998). Asian and European cultured oysters have been classified as *C. angulata* based on analyses of mitochondrial cytochrome c oxidase subunit I (COI) sequences (O’Foighil *et al.* 1998), and, possibly, Asian populations were the origin of European *C. angulata*. Yu (2002) found minimal differences (0–1 bp) in the 16S rRNA gene between *C. angulata* from Taiwan and those from Portugal and France. Subsequent studies have also confirmed that *C. angulata* is the cultured species in Taiwan, as demonstrated by Wang *et al.* (2010) using 16S rRNA analysis and by Hsiao *et al.* (2016) using COI sequences.

Globally, oysters represent the leading farmed species of mollusc by production volume, with most consump-

tion in Asia (Botta *et al.* 2020). To improve product segmentation, golden Pacific oysters were developed in Australia and China for the Asian market (Nell 2001; Cong *et al.* 2014; Wang *et al.* 2016). Ge *et al.* (2015) reported that shell colour in Pacific oysters is determined by background colour and foreground pigmentation. A single genetic locus with two alleles governs the background colour, with the golden allele dominating the white one. The epistatic effect of background colour influences foreground pigmentation. Xu *et al.* (2019) investigated shell pigmentation and found that black and purple pigments contribute to the variation of shell colour. In China, established colour strains include white, black, orange, and gold (Zhang *et al.* 2023).

Studies on the Atlantic Bay Scallop, *Argopecten irradians* (Lamarck, 1819), have reported that background colour is controlled by a single gene, with orange and yellow dominant over white (Adamkewicz & Castagna 1988; Elek & Adamkewicz 1990). The epistasis effect of the background colour on foreground pigmentation produces a variety of shell patterns, including striped, mottled, or solid colours. In two *A. irradians* populations from Martha’s Vineyard, Massachusetts, background shell colours were 94% white,

5% yellow, and 1% orange (Elerk & Adamkewicz 1990). In comparison, the proportion of yellow individuals of the Chilean Scallop, *Argopecten purpuratus* (Lamarck, 1819), in natural versus hatchery populations was 1–3% and 8–15% in Herradura Bay, Peru (Wolff & Garrido 1991).

To date, few studies have focused on shell-colour strains in cultured oysters in Taiwan, despite the significance of the oyster farming industry. To address this gap in the literature, the present study investigated the occurrence of golden Portuguese oysters in three oyster-farming areas in Taiwan. The identity of these golden oysters was also confirmed through COI sequence analysis.

MATERIALS AND METHODS

The experiments were conducted from July to September 2019 in oyster farming areas in Taixi (23°43'09.3"N, 120°10'03.2"E), Qigu (23°07'22.9"N, 120°03'14.9"E), and Kinmen (24°28'32.1"N, 118°20'06.4"E). Three strings of oysters with locally settled spat were collected from each site. From each string, three or four oyster clumps were gathered and stored at –20 °C for subsequent analysis. Individual oysters were separated from the clumps and cleaned with a brush to allow examination of the shell colour.

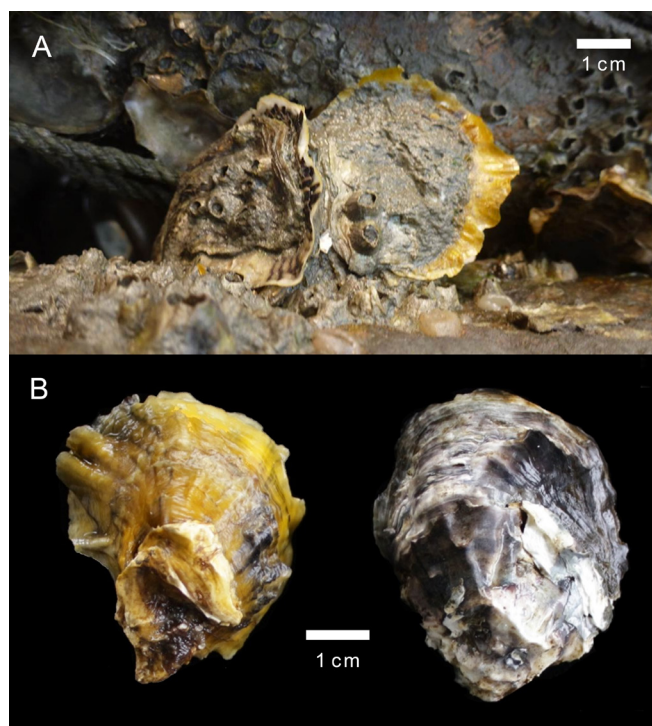


Figure 1. Golden and typical Portuguese oysters (*Crassostrea angulata*) in the field (A) and the samples with fouling organisms eliminated (B).

Based on shell colour, specimens were classified as golden or non-golden (typical) oysters (Fig. 1). Data analysis was performed using IBM SPSS Statistics v. 29.0 (IBM Corp., Armonk, NY, USA). Prior to conducting the one-way ANOVA, the assumptions of normality and homogeneity of variances were examined. A one-way ANOVA was used to determine whether significant differences existed in the proportion of golden oysters among the farming populations.

Species identification of cultivated *Crassostrea* species was previously conducted by Hsiao *et al.* (2016) using COI sequences. Their analysis confirmed that all samples collected from 12 locations—New Taipei City, Hsinchu, Changhua, Chiayi, Tainan, Pingtung, Yilan, Taitung, Wenzhou, Matsu, Kinmen, and Beihai—along the coastlines of Taiwan and southern China belonged to *C. angulata*. Notably, this study included specimens from our sampling sites in Qigu (Tainan) and Kinmen. Accordingly, COI-based species identification in the current study was limited to farmed oysters collected from Taixi, comprising six individuals each from the golden and non-golden (general) morphotypes.

Crude DNA was extracted from the adductor muscle of oysters using the Tissue & Cell Genomic DNA Purification Kit (GeneMark, DP021-150). PCR primers and experimental procedures for COI analysis followed the methods described by Hsiao *et al.* (2016). For comparative purposes, additional COI sequences were obtained from GenBank, including those of *C. angulata* (JQ027306, JQ027308) and *C. gigas* (HM626169, HQ661002). COI sequences were aligned and trimmed using MAFFT v. 7.505 (Katoh & Standley 2013) and ClipKIT v. 2.6.1 (Steenwyk *et al.* 2020), respectively. The HKY+F model was determined to be the optimal model for sequence evolution using ModelFinder (Kalyaanamoorthy *et al.* 2017). A maximum-likelihood tree with 1,000 ultrafast bootstrap replicates (Hoang *et al.* 2018) was reconstructed using IQ-TREE v. 2.3.5 (Minh *et al.* 2020).

RESULTS AND DISCUSSION

In Taixi, Qigu, and Kinmen, the number of *Crassostrea angulata* per clump ranged from 17 to 51, with an average of 27 individuals/clump (Table 1). Shell lengths ranged from 4 to 10 cm. Golden oysters accounted for $9.7 \pm 5.2\%$, $9.5 \pm 5.4\%$, and $8.4 \pm 5.8\%$ of the total samples at Taixi, Qigu, and Kinmen, respectively. The Kolmogorov–Smirnov test indicated that the residuals were normally distributed ($p = 0.200 > 0.05$), confirming that the normality assumption was satisfied. Levene's test further showed no significant difference in variances among groups ($p = 0.905$), satisfying

Table 1. The number of golden and typical Portuguese Oysters (*Crassostrea angulata*) in Taixi, Qigu, and Kinmen. The same letter indicates no significant differences by one-way ANOVA $p > 0.05$.

Site	Taixi	Qigu	Kinmen
Clump (n)	11	11	10
Oyster (no./clump)	27.4 ± 9.5 (17–51)	26.9 ± 5.2 (20–36)	27.1 ± 6.2 (19–36)
Total oyster sample (N)	301	296	271
Golden shell rate (%)	9.7 ± 5.2 ^a (0–17.7)	9.5 ± 5.4 ^a (0–17.4)	8.4 ± 5.8 ^a (0–18.2)

the assumption of homoscedasticity. No significant differences of colour ratio were observed among the three sites (one-way ANOVA, $p > 0.05$). The ratio of golden-to-general oyster was approximately 1:9.

The COI sequence length obtained from golden and general Portuguese oysters was 589 bp nucleotides ($N = 12$). The 12 sequences were deposited in GenBank with the accession numbers of PX242748–PX242759 (Table 2; Appendix 1). Phylogenetic analysis using the maximum-likelihood method grouped all samples within the *C. angulata* clade (Fig. 2).

Bivalves exhibit a variety of shell colours, which are often genetically determined. In *Argopecten irradians* the yellow and orange background colours of shells were dominant over recessive white (Elek & Adamkewicz 1990). In populations from Lagoon and Nashaquitsa Ponds in Massachusetts, the juvenile shell colour ratios of yellow and orange to white were 13:87 and 2:98. In contrast, adult ratios at both sites were 5:95. It has been suggested that vertebrate predators such as teleost fish and birds exert selection pressure on juveniles with brighter colouration in

exposed habitats. In Herradura Bay, Peru, yellow individuals of *Argopecten purpuratus* comprised 1–3% of the natural population, but 8–15% in hatchery settings (Wolff & Garrido 1991). The survival rate of the yellow versus other colour morphs of *A. purpuratus* (20–30 mm) was compared from August 1987 to July 1988 in the Bay. The monthly survival rate of yellow *A. purpuratus* was significantly lower than that of other colour morphs (88.3% vs 96.8%). This suggests a genetic association between yellow colour and lower general performance in the wild.

Through selective breeding, white, purple, gold, and black colour strains were developed in *Crassostrea gigas* (Cong *et al.* 2014). Spat from these four strains were cultured at Shuangdao Bay and Haiyangsuo Sea in Shandong, China. After 420 days, both growth and survival rates were affected by shell colour and culture site. At Shuangdao Bay, the gold strain exhibited significantly greater shell length and total weight compared to the control group (38.2 mm vs 30.2 mm and 17.6 g vs 13.4 g, respectively). Similar trends were observed at Haiyangsuo Sea, with measurements of 45.9 mm vs 42.0 mm and 35.7 g vs 30.0 g. In contrast, the survival rates were no different in the gold strain and control but differed between sites, that is, 25 vs 35%, respectively. These results suggest that the influence of culture sites on oyster growth and survival was more pronounced than that of shell colour. According to Jiang *et al.* (2024), heritability estimates for shell colour in *C. gigas* were 0.156 ± 0.08 for white, 0.270 ± 0.09 for gold, and ranged from 0.52 to 0.69 for black. Notably, Jiang *et al.*'s study showed no positive correlation between shell colour traits and most growth traits.

In contrast, some studies argue that shell colour may not be an essential trait for the evolutionary success of particular species (e.g. Williams 2017). For example, infaunal bivalves such as *Meretrix lusoria* (Röding, 1798) exhibit various colours despite living buried in sediments. Similarly, oysters often accumulate fouling organisms or sediments on their shells, which can obscure underlying shell colouration.

Additionally, all three studied populations contained

Table 2. The GenBank accession numbers for the 12 COI sequences of golden and general Portuguese oysters (*Crassostrea angulata*) collected from Taixi, Taiwan. G: golden oysters; W: typical oysters.

Oyster code	Accession number
G1	PX242748
G2	PX242749
G3	PX242750
G4	PX242751
G5	PX242752
G6	PX242753
W1	PX242754
W2	PX242755
W3	PX242756
W4	PX242757
W5	PX242758
W6	PX242759

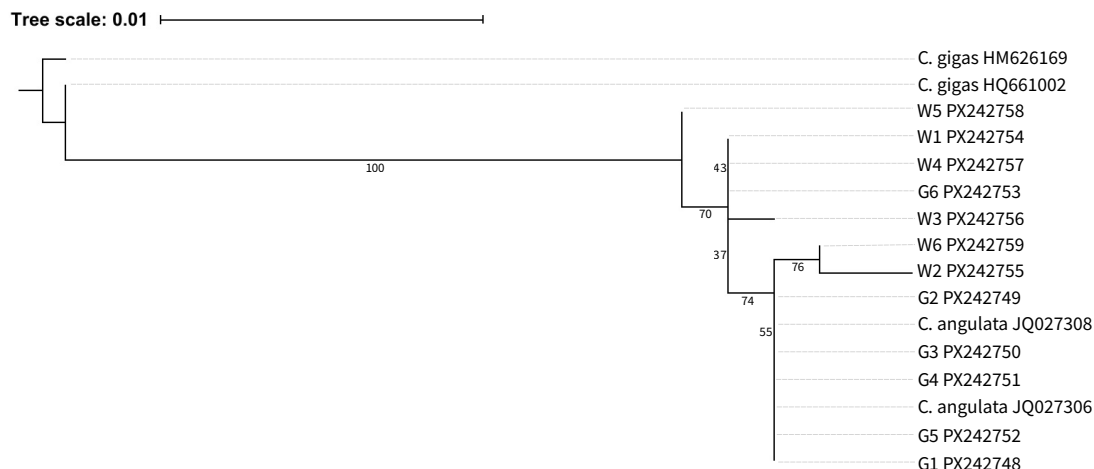


Figure 2. The maximum-likelihood tree based on COI sequences obtained from golden and general Portuguese oysters (*Crassostrea angulata*). G: golden oyster; W: typical oyster.

approximately 10% golden oysters, which may be associated with the year-round occurrence of oyster spat and the absence of differential selective forces among sites. At the northwestern study site (Shunsun) of Taiwan, the spawning season of Portuguese oysters was limited to the summer months (July–September) (Wu & Yang 1999). In contrast, spawning occurred in spring (February–April) and summer (July–September) at the southwestern sites (Taixi and Budai). In a study conducted from March 2012 to July 2014, oyster spat was collected year-round in Taixi (Ueng *et al.* 2020). Oyster veligers, the final planktonic larval stage, is reached in 2–3 weeks at 28 °C in laboratory conditions (Lin 2014). Such a long period also facilitates high gene flow among oyster populations.

Moreover, seasonal variations in the surface circulation in the Taiwan Strait of western Taiwan also contribute to larval dispersal. The primary surface currents in the Taiwan Strait vary by season (Jan *et al.* 2002). The China Coastal Current (CCC) dominates in winter. The Kuroshio Branch Current and CCC are significant in spring, and the South China Sea Current is predominant during summer and fall. The consistent presence of approximately 10% golden oysters across the three farming sites may be attributable to the year-round occurrence of oyster spat and the complex seasonal ocean currents in the Taiwan Strait, which promote high gene flow among populations.

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The COI sequences from golden and general Portuguese oysters (*Crassostrea angulata*) with GenBank accession numbers. G: golden oysters; W: typical oysters. G1: PX242748; G2: PX242749; G3: PX242750; G4: PX242751; G5: PX242752; G6: PX242753; W1: PX242754; W2: PX242755; W3: PX242756; W4: PX242757; W5: PX242758; W6: PX242759. Shade character: different nucleotides from other individuals.

	510		520		530		540		550		560		570		580	
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G2	GACTACTCTC	CCAGTGTTAAG	CTGGAGGCTC	TACTATGCTT	TTGACTGATC	GTCATTTTAA	TACCTCTTTT	TTCGACCCGT	TCGGAGGGG							
G3	GACTACTCTC	CCAGTGTTAAG	CTGGAGGCTC	TACTATGCTT	TTGACTGATC	GTCATTTTAA	TACCTCTTTT	TTCGACCCGT	TCGGAGGGG							
G4	GACTACTCTC	CCAGTGTTAAG	CTGGAGGCTC	TACTATGCTT	TTGACTGATC	GTCATTTTAA	TACCTCTTTT	TTCGACCCGT	TCGGAGGGG							
G5	GACTACTCTC	CCAGTGTTAAG	CTGGAGGCTC	TACTATGCTT	TTGACTGATC	GTCATTTTAA	TACCTCTTTT	TTCGACCCGT	TCGGAGGGG							
G6	GACTACTCTC	CCAGTGTTAAG	CTGGAGGCTC	TACTATGCTT	TTGACTGATC	GTCATTTTAA	TACCTCTTTT	TTCGACCCGT	TCGGAGGGG							
W1	GACTACTCTC	CCAGTGTTAAG	CTGGAGGCTC	TACTATGCTT	TTGACTGATC	GTCATTTTAA	TACCTCTTTT	TTCGACCCGT	TCGGAGGGG							
W2	GACTACTCTC	CCAGTGTTAAG	CTGGAGGCTC	TACTATGCTT	TTGACTGATC	GTCATTTTAA	TACCTCTTTT	TTCGACCCGT	TCGGAGGGG							
W3	GACTACTCTC	CCAGTGTTAAG	CTGGAGGCTC	TACTATGCTT	TTGACTGATC	GTCATTTTAA	TACCTCTTTT	TTCGACCCGT	TCGGAGGGG							
W4	GACTACTCTC	CCAGTGTTAAG	CTGGAGGCTC	TACTATGCTT	TTGACTGATC	GTCATTTTAA	TACCTCTTTT	TTCGACCCGT	TCGGAGGGG							
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