***J. Ocean Univ. China*** (Oceanic and Coastal Sea Research)
https://doi.org/10.1007/s11802-023-5641-2
ISSN 1672-5182, 2023 22 (6): 1669-1676
*http://www.ouc.edu.cn/xbywb/*
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# Contraction of Heat Shock Protein 70 Genes Uncovers Heat Adaptability of *Ostrea denselamellosa*

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(Received January 6, 2023; revised March 17, 2023; accepted May 23, 2023)


**Abstract** The milin oyster *Ostrea denselamellosa* is a live-bearing species with a sharp decline in the natural population. Unlike other oysters, *O. denselamellosa* lives in the subtidal zone and its adaptability to heat, salinity, *etc*. is different from most other oysters. Heat shock proteins 70 (HSP70) are a family of conserved ubiquitously expressed heat shock proteins which are produced in response to stressful conditions, especially heat. In this study, we identified *Hsp70* genes through bioinformatic analysis in five species of oyster. Among them, *O. denselamellosa* holds the fewest number of *Hsp70* genes, which may be one of the reasons why *O. denselamellosa* cannot tolerate high temperatures. The conserved motifs and gene structures of *Hsp70B2* sub-family and other types of *Hsp70* in *O. denselamellosa* were different from that of *Hspa12* sub-family, which may be due to performing necessary multiple physiological functions. Transcription profile analysis for *Hsp70* genes of *O. denselamellosa* indicated that gills play an important role in responding to multiple external challenges. In addition, synteny analysis of *Hsp70* genes among *O. denselamellosa*, *O. edulis* and *Crassostrea ariakensis* showed that *Hsp70* genes in genus of *Ostrea* genome might have evolved from a common ancestor with genus of *Crassostrea*. In short, our results lay the foundation for further investigation of the evolution of *O. denselamellosa Hsp70* genes and heat adaptability.

**Key words** *Ostrea denselamellosa*; oysters; *Hsp70*; heat; adaptability

## 1 Introduction

Milin oyster *Ostrea denselamellosa* is a potential economically important species, which generally inhabits the subtidal zone with high salinity along the coasts of China, Japan and Korea (Xu *et al*., 2008; Chen *et al*., 2011). However, previous studies showed that oysters such as *Crassostrea sikamea* and *C. nippona* had a survival rate of more than 50% at 32℃ (Wang and Li, 2017; Hu *et al*., 2020), while *O. denselamellosa* couldn not survive at 32℃ (Yang *et al*., 2003), which means that milin oyster is relatively poorly adapted to high temperatures. Moreover, *O. denselamellosa* is a kind of live-bearing oyster, which differs significantly from oysters *Crassostrea*, *Saccostrea* and most other bivalves. During reproduction, the eggs are fertilized and grow to D-shaped larvae in the female mental cavity within 1-3 days (Buroker, 1985; Foighil and Taylor, 2000; Yang *et al*., 2001). Previous study showed that species are more likely to be ovovivivipparous when they live in lower-temperature environments, and the female keep the embryos in their body to make sure they can grow in a suitable temperature (Webb *et al*., 2006). To date, previous studies on *O. denselamellosa* mainly focused on its mitochondrial genome, seed production and biological characteristics (Insua and Thiriot-Quievreux, 1991; Chen *et al*., 2011; Yu *et al*., 2016; Han *et al*., 2022), while the genomic-level research on their ecological adaptation remains limited (Xu *et al*., 2008).

*Hsp70*, also known as *Hspa*, has functions in a wide range of housekeeping and stress-related activities (Rosenzweig *et al*., 2019). In previous studies, several members of *Hsp70* have been cloned in *C. sikamea* and *C. virginica*, and these genes were significantly up-regulated when the oysters were under heat stress (Nagata *et al*., 2017; Casas and La Peyre, 2020). In addition, with the quick development of genome sequencing, the genome-wide *Hsp70* gene family has been studied in oysters based on their genomes. These genes were found to be expanded in *C. gigas*, *C. hongkongensis* and *S. glomerata*, which indicated that *Hsp70* genes play important roles in adaptation to heat in dynamic environments with a wide variety of stress factors (Zhang *et al*., 2012; Powell *et al*., 2018; Peng *et al*., 2020). Although we have generated the genome assembly of *O. denselamellosa* (Dong *et al*., 2023), no detailed analysis of the *Hsp70* family has been performed in *O. denselamellosa*.

In this study, in order to provide a comprehensive understanding of the *Hsp70* gene family in the *O. denselamellosa*, the number of gene copies, chromosomal locations, tissue specific expression pattern, structure and motifs were

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examined based on the genome of *O. denselamellosa* and other oysters. We also analyzed the evolution of *Hsp70* genes among oysters by performing phylogenetic trees and synteny analyses, and carried out a preliminary study on the ecological adaptability of *O. denselamellosa* by comparative genomics and gene family enrichment.

## 2 Material and Methods

### 2.1 Data Preparation

The genomes, the longest peptides sequences and gff3 files of *C. gigas* (GCA_902806645.1) (Zhang *et al*., 2012), *C. virginica* (GCA_002022765.4) (Gomez-Chiarri *et al*., 2015) and the genome of *Saccostrea glomerata* (GCA_003671525.1) (Powell *et al*., 2018) were downloaded from NCBI website. The longest peptides sequences and gff3 file of *S. glomerata* were obtained from http://soft.bioinfo-minzhao.org/srog/. All these three data sets of *C. ariakensis* (CNA0022698) (Wu *et al*., 2022) were obtained from the China National GeneBank DataBase (CNGBdb). In addition, we have generated the genomic data of *O. denselamellosa* (Dong *et al*., 2023) and *O. edulis* (Li *et al*., 2023), which can be found in Figshare https://doi.org/10.6084/m9.figshare.19801705 and https://doi.org/10.6084/m9.figshare.20013503, respectively. To identify *Hsp70* genes in oysters, HSP70 protein sequences from *C. gigas* were downloaded from UniProt (https://www.uniprot.org/). The RNA-Seq data of *O. denselamellosa* was derived from NCBI website with SRA numbers: SRR19238441, SRR19238440, SRR19238438 and SRR19238439.

### 2.2 Identification of *Hsp70* Genes

To identify *Hsp70* genes in *C. ariakensis*, *S. glomerata*, *O. denselamellosa* and *O. edulis*, HSP70 protein sequences in *C. gigas* were used as query database. First, basic local alignment search tool algorithm program (BLASTP) (Altschul *et al*., 1990) was used to get the initial candidate genes of *Hsp70* in each species with e-value $\leq e^{-5}$ and identity $\geq 30\%$. To make the results more accurate, these candidate genes were then filtered by conserved domain. The hidden Markov model (HMM) (Eddy, 1996) profile of the HSP70 domain (PF00012) was downloaded from the Pfam protein family database http://pfam-legacy.xfam.org/family/HSP70. We filtered the candidate genes of the previous step by running 'hmmsearch --cut_tc' algorithm. Finally, we selected the candidate genes with corresponding amino acid length $>300$ as *Hsp70* genes.

### 2.3 Sequence Alignment and Phylogenetic Analysis

To investigate the evolutionary relationship of *Hsp70* family, the *Hsp70* genes of *C. gigas*, *O. denselamellosa* and *O. edulis* were used to build a phylogenetic tree. First, the protein sequences of these genes were aligned using MUSCLE (v3.6) (Edgar, 2004) and then the tree was constructed using FASTTREE (Price *et al*., 2009). The tree was finally decorated and displayed with Interactive Tree of Life (ITOL, https://itol.embl.de/).

### 2.4 Gene Structure and Conserved Motif

PEPTIDES (2.4.4) (Osorio *et al*., 2015) was used to calculate the molecular weight and isoelectric point (PI). We used MEME (4.11.2) (Bailey *et al*., 2015) with parameters '-mod anr -protein -nmotifs 10 -minw 6 -maxw 200' to detect the conserved motif. In addition, the structure of each gene was analyzed based on the coding sequence (CDS) and untranslated region (UTR) data from the gff3 file. Both the conserved motif and gene structure were visualized by TBtools (Chen *et al*., 2020).

### 2.5 Chromosomal Distribution and Synteny Analysis

*Hsp70* genes of *O. denselamellosa* were mapped to the chromosomes according to the gff3 file, and the results were processed and visualized by MAPCHART (Voorrips, 2002). To figure out what chromosomes-level changes occurred in *Hsp70* genes in oysters during evolution, DIAMOND (Buchfink *et al*., 2015) was used to two-way align the protein sequences of *O. denselamellosa* against *O. edulis* and *C. ariakensis* with parameters '--max-target-seqs 5 --evalue 1e-10'. Then the synteny of genes of these three species was identified based on MCscanX (Wang *et al*., 2012). The corresponding chromosome was determined by the fraction of genes in a block of approximately 25 genes. JCVI (Sleator, 2016) was used to visualize the final result and *Hsp70* genes were marked in red.

### 2.6 Expression Profiles of *OdeHsp70* Genes in Different Tissues

We generated twelve sets of RNA-seq data for tissue specific expression analysis, including gonads, gills, adductor and mantle from each of the three female *O. denselamellosa* in stage of ovulation. These data have already been uploaded to NCBI with SRA numbers SRR19238441, SRR19238440, SRR19238438 and SRR19238439. First, the RNA-Seq raw data was mapped to *O. denselamellosa* genome by HISAT2 (v2-2.2.1) (Kim *et al*., 2015). Then the quantity of transcriptomes was detected by FEATURECOUNTS (v2.0.1) (Liao *et al*., 2014). The result was standardized using TPM and TMM in order to balance the differences between tissues and individuals. Based on the standardized quantification results, we plotted a heat map of *Hsp70* genes of *O. denselamellosa* using PHEATMAP in R.

## 3 Results and Discussion

### 3.1 Identification of *Hsp70* Genes

With the strict filtering standard, a total of 401 *Hsp70* genes were identified, including 59 in *O. denselamellosa*, 84 in *C. ariakensis*, 83 in *S. glomerata*, 84 in *O. edulis* and 88 in *C. gigas* (Table 1). Compared to other species of oysters and previous studies of bivalves, including 133 *Hsp70* genes in hard calm *Mercenaria mercenaria* (Hu *et al*., 2022), 61 *Hsp70* genes in *Mizuhopecten yessoensis* (Cheng *et al*., 2016) and 65 *Hsp70* genes in *Chlamys farreri* (Hu *et al*., 2019), *O. denselamellosa* holds the fewest *Hsp70* genes.

The number of *Hsp70* genes among bivalves varied greatly, which might be a reflection of regulatory physiological variation that can help the bivalves adapt to the changing environment, especially temperature (Zhang *et al*., 2012). Therefore, the decrease in heat shock protein gene may affect *O. denselamellosa* to regulate heat-adaptability.

Moreover, we found that the *O. denselamellosa Ode-Hsp70* genes included 6 Heat shock protein70 B2 (*Hsp-70B2*) genes, which was the same as in *O. edulis*, and one copy more than that in *C. gigas*; 47 Heat Shock Protein Family A Member 12 (*Hspa12*) genes and 6 other types of *Hsp70* genes. Compared to *Hsp70* gene family in other species of oysters and scallops (Cheng *et al*., 2016; Hu *et al*., 2019), the contraction of the *Hsp70* gene family in *O. denselamellosa* was mainly due to the decrease in the number of *Hspa12* genes (Table 1).

Table 1 Copy numbers of *Hsp70* sub-families among mollusk genomes

| Gene name | *M. yessoensis* | *C. farreri* | *C. gigas* | *C. ariakensis* | *S. glomerata* | *O. edulis* | ***O. denselamellosa*** |
|---|---|---|---|---|---|---|---|
| *Hsp70B2* | 6 | 9 | 5 | 5 | 8 | 6 | **6** |
| *Hspa12* | 57 | 47 | 73 | 63 | 67 | 69 | **47** |
| Other types | 2 | 9 | 10 | 16 | 8 | 9 | **7** |
| Total | 65 | 65 | 88 | 84 | 83 | 84 | **59** |

### 3.2 Phylogenetic Analysis of the *Hsp70* Genes

In order to figure out the evolutionary relationships of *Hsp70* genes among oysters, four phylogenetic trees with maximum likelihood were constructed based on the longest protein sequences. Because the *Hsp70* genes of *C. gigas* are well studied and examined, we used its protein sequences as a reference. These four trees had similar topological structures, and the *Hsp70* gene family in the five species of oysters all mainly had two clusters, the *Hspa12* sub-family (black branches) and other sub-families (red and green branches), as shown in Fig.1. The *O. denselamellosa Hsp70B2* genes were first clustered together. The other sub-families like *Hspa13* and *Hspa14* gene pairs were also clustered together. The other branch consisted of 47 *Hspa12* sub-family members. Compared with *C. gigas*, the copy number of *Hspa12* in *O. denselamellosa* is significantly reduced (Fig.1). This condition also occurred in a small

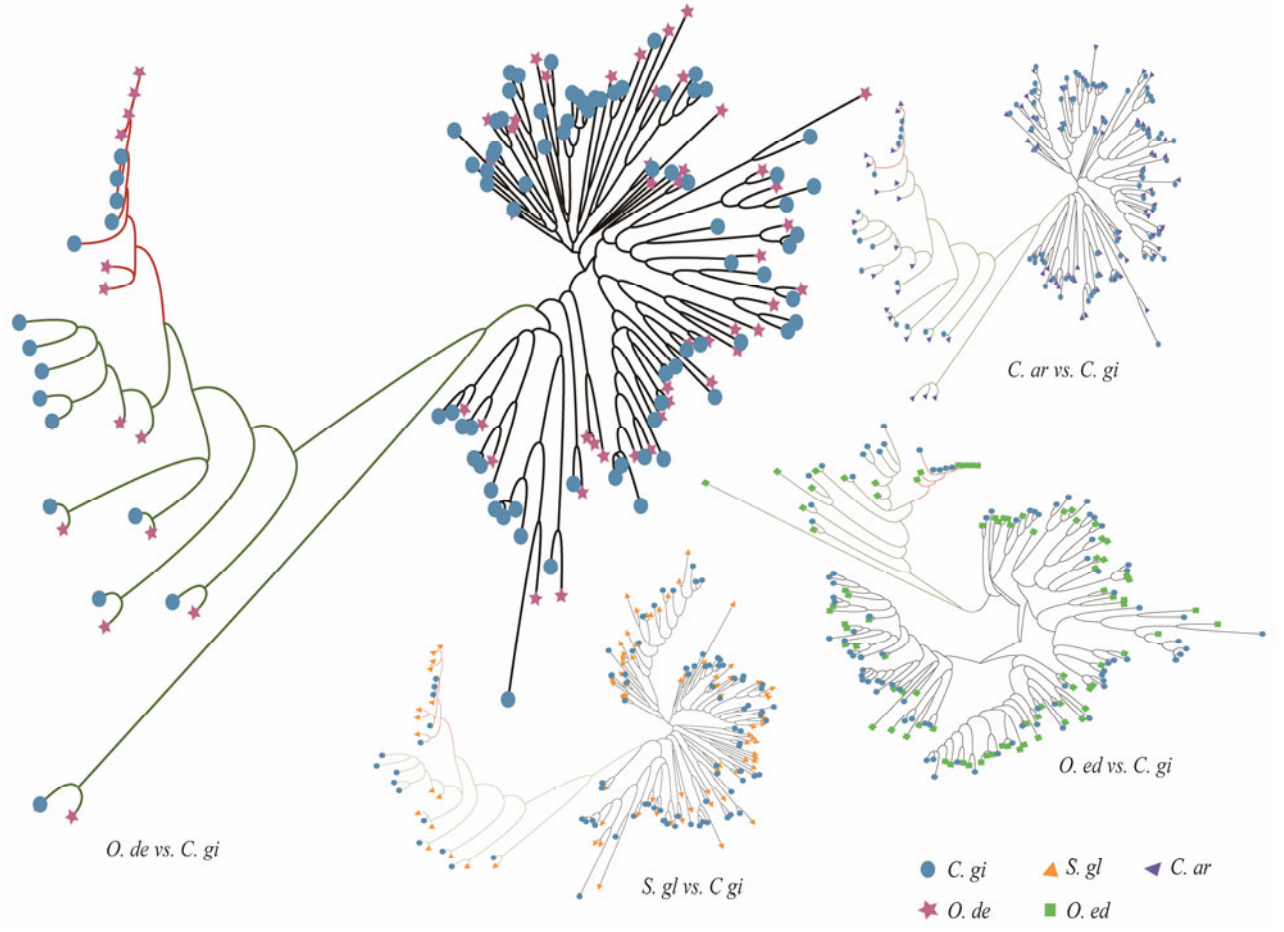


Fig.1 Phylogenetic relationships of *Hsp70* genes in oysters. The phylogenetic tree was constructed using the maximum likelihood method by Fasttree. Different labels represent different species, blue for *C. gigas* (*C. gi*, reference), pink for *O. denselamellosa* (*O. de*), purple for *C. ariakensis* (*C. ar*), green for *O. edulis* (*O. ed*), and orange for *S. glomerata* (*S. gl*). Different branch colors represent different sub-families, red for *Hsp70B2*, green for other types, and black for *Hspa12*.

subset of regions in *O. edulis* and *S. glomerata*. For oysters, not only the total number of *Hsp70* genes was changing, but the copy number of *Hsp70* sub-family genes was also different. These differences between species may be explained by duplicated genes having independent origins or there may have existed some genomic rearrangement in this region (Metzger *et al*., 2016). This situation in *O. denselamellosa* may be due to the subtidal living environment which is different from the environments of other oysters.

### 3.3 *OdeHsp70* Gene Structure and Conserved Motifs

The information about *Hsp70* genes' location, amino acid length and MolWts is summarized in https://doi.org/10.6084/m9.figshare.24152763.v1. The predicted MolWts of the *OdeHsp70* proteins varied from 46.04 kDa (ode_0040 25-RB) to 171.14 kDa (*ode_006273-RB*). In addition, *OdeHsp70s* encoded proteins varying from 421 aa (*ode_0004 67-RA*) to 1508 aa (*ode_006273-RB*). Compared to other oysters (*CarHsp70* proteins from 384 aa to 2290 aa, *OdeHsp70* proteins from 347 aa to 2460 aa and *SglHsp70* proteins from 316 aa to 2595 aa), the protein length range of *OdeHsp70s* in *O. denselamellosa* was relatively small. This may be due to the evolution of *Hsp70* genes themselves and the different genome assembly-annotation methods of each species.

A total of ten conserved motifs were detected in *OdeHsp70* genes. The conserved motifs and gene structures of *OdeHsp70B2* sub-family (red) and other types of *OdeHsp70* (green) were different from that of *Hspa12* sub-family. This may be due to performing necessary multiple physiological functions, according to the conjecture that different motifs may indicate different functions or functional divergence (Liu *et al*., 2016). Except *ode_006806-RA*, the conserved motifs and gene structures of the genes in each of the above three sub-families were highly similar (Fig.2).

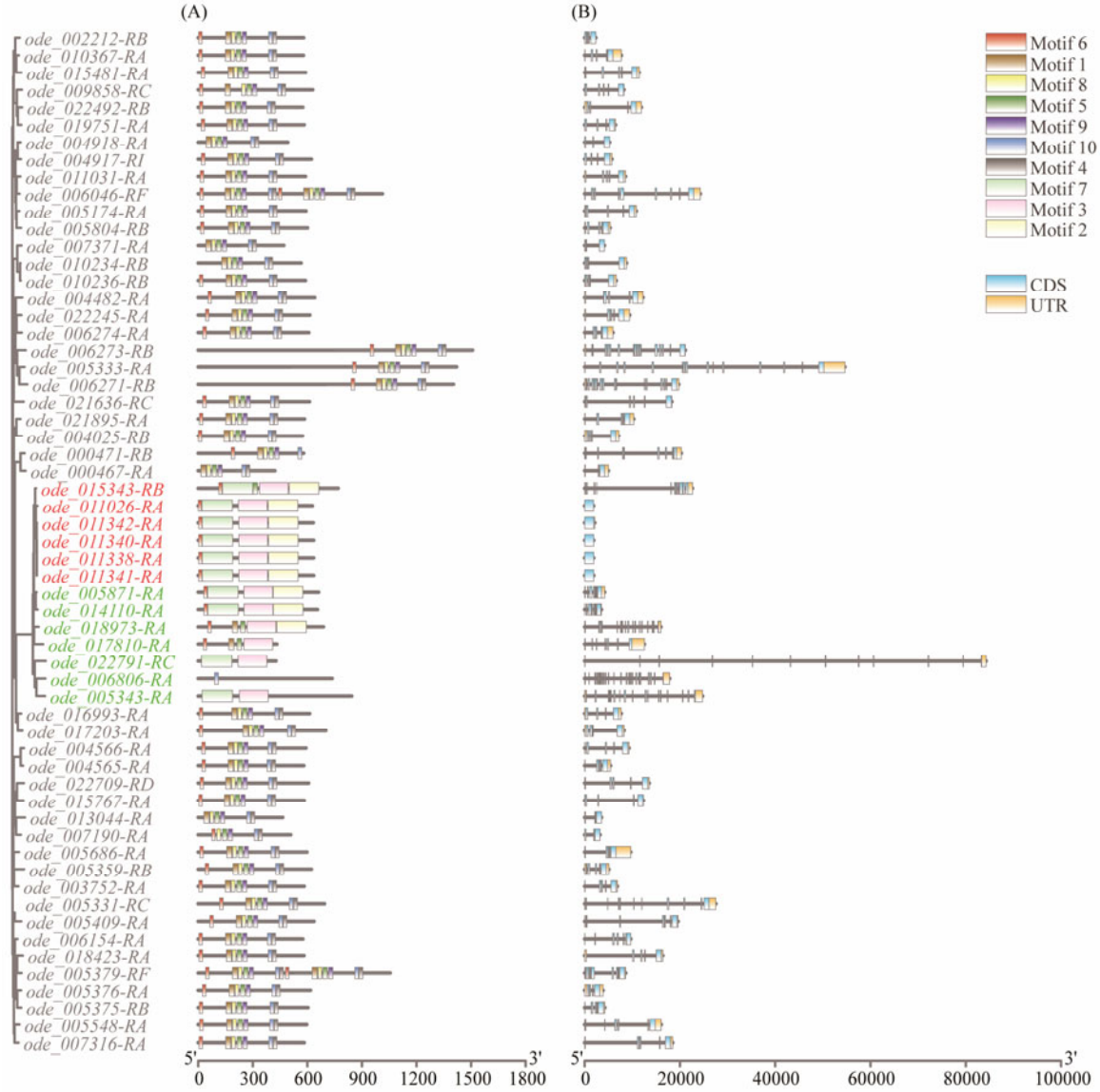


Fig.2 Architecture of conserved protein motifs and gene structure of *Hsp70* genes in *O. denselamellosa*. Different branch colors represent different sub-families, red for *Hsp70B2*, green for other types, and black for *Hspa12*. (A) The motifs of *Hsp70* proteins in *O. denselamellosa*. The ten motifs were displayed with different colors. The length of the protein can be estimated using the scale at the bottom. (B) Exons and introns of *Hsp70* proteins in *O. denselamellosa*. Blue boxes indicated untranslated regions; orange boxes indicated exons.

### 3.4 Chromosomal Distribution of *OdeHsp70* Genes

A total of 59 *OdeHsp70* genes were finally distributed among 8 *O. denselamellosa* chromosomes (Fig.3). Among all these *OdeHsp70* genes, 28 (48.27%) were located on chromosome 4. The other chromosomes have fewer than 7 genes on each one. Only one *Hsp70* gene was observed on chromosome 9. Similarly, *OedHsp70* genes were also located on 8 chromosomes and 42 of 84 (50.00%) were distributed on chromosome 2 (https://doi.org/10.6084/m9.figshare.24152763.v1). Only one *OedHsp70* gene was observed on chromosome 1 and two *OedHsp70* genes were observed on chromosome 6. Unlike the European oyster *O. edulis*, in which one *OedHsp70* gene was distributed on a scaffold, the *Hsp70* genes of *O. denselamellosa* were all found on chromosomes. It might be a common phenomenon for members of large gene families to be unequally distributed on chromosomes. For example, only 19 of 66 transient receptor potential channel genes were located on chromosome 2 in *C. gigas* (Fu *et al*., 2021).

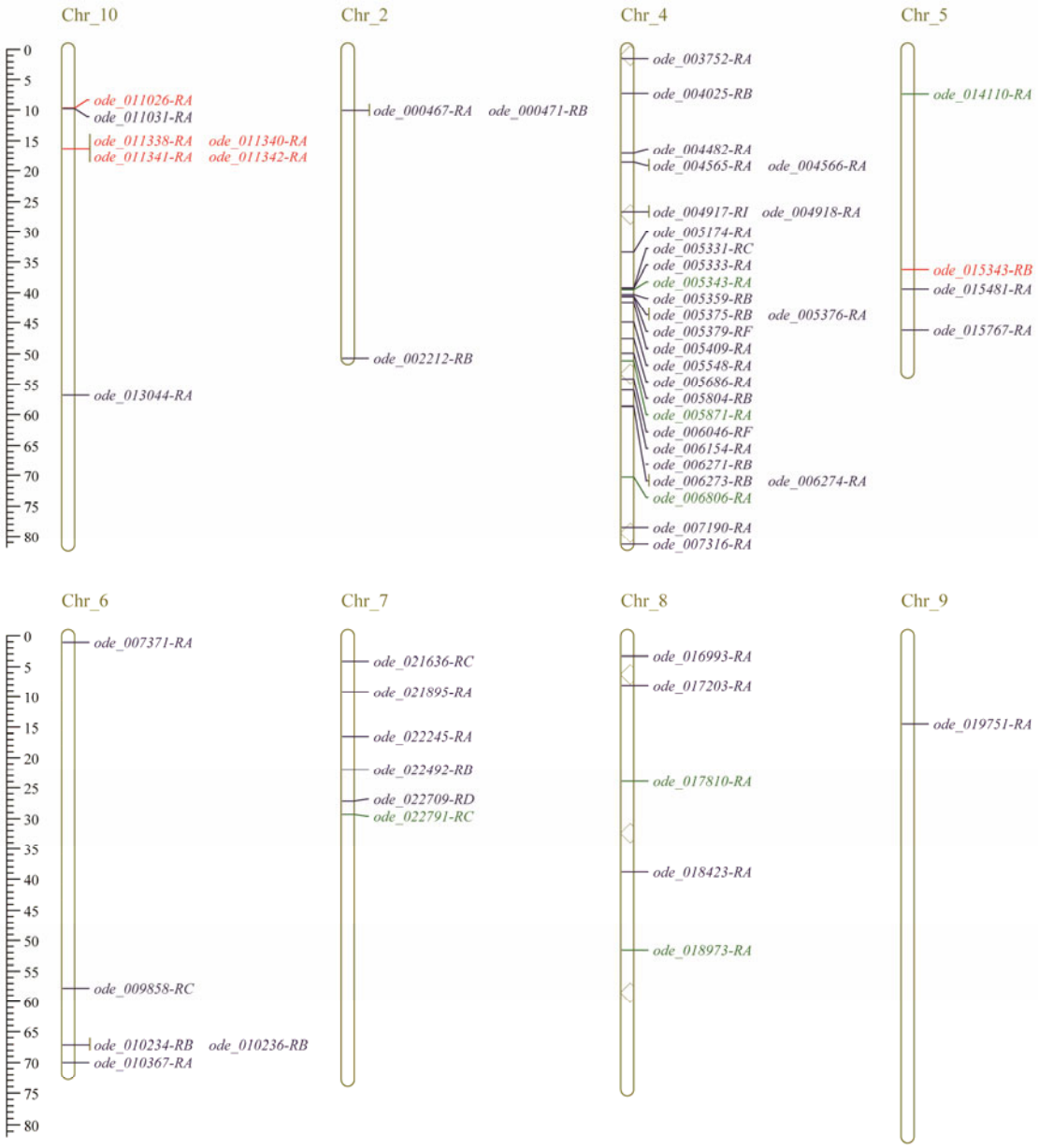


Fig.3 Chromosomal distribution of *Hsp70* genes in *O. denselamellosa*. The position of each gene on the chromosome can be estimated using the scale on the right. Red labels for *Hsp70B2*, green labels for other types of *Hsp70*, and blue labels for *Hspa12*.

### 3.5 Synteny Analysis of *OdeHsp70* Genes Between *O. edulis* and *C. ariakensis*

Synteny analysis of the *Hsp70* genes was performed among *O. denselamellosa*, *O. edulis* and *C. ariakensis*. There are many syntenic blocks between the genomes of these oysters, with 616 between *O. denselamellosa* and *C. ariakensis*, 396 between *O. denselamellosa* and *O. edulis* (Fig.4). The *Hsp70* genes were mainly distributed on chromosome 4 of *O. denselamellosa*, chromosome 2 of *O. edulis* and chromosome 5 of *C. ariakensis*. Interestingly, most *OdeHsp70* genes located on chromosomes 10 were *Hsp-*

*70B2* genes. For *Ostrea*, in addition to chromosomes Ode4 and Oed2, *Hsp70* genes were also more or less distributed on other chromosomes, and eight out ten chromosomes of each species have the distribution of this gene family. But the situation was different in *C. ariakensis*, only 6 chromosomes have the distribution of *Hsp70* genes, other than chromosomes Car3 and Car5. There are distributions on chromosomes Car6, Car7, Car8 and Car9, but just in small quantities. These results indicated that the *C. ariakensis Hsp70* gene family may be more conserved and that the *Hsp70* genes widely distributed on more chromosomes of *Ostrea* genome might have evolved from those of a common ancestor with *Crassostrea*.

### 3.6 Expression Patterns of *OdeHsp70* Genes in Different Tissues

RNA-seq data of four tissues, including mantle, gill, adductor muscle and gonad, from an ovulating female *O. denselamellosa* were used to characterize the expression profiles of *OdeHsp70*s. Based on TPM+TMM values, a heat-

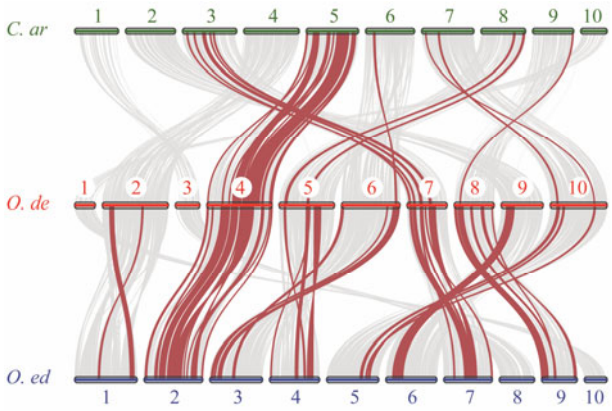


Fig.4 Synteny analyses between the *Hsp70* genes of the *C. ariakensis* (*C. ar*), *O. denselamellosa* (*O. de*), and *O. edulis* (*O. ed*). Grey lines in the background indicated Synteny blocks among these genomes, while red lines highlight syntenic *Hsp70* gene pairs.

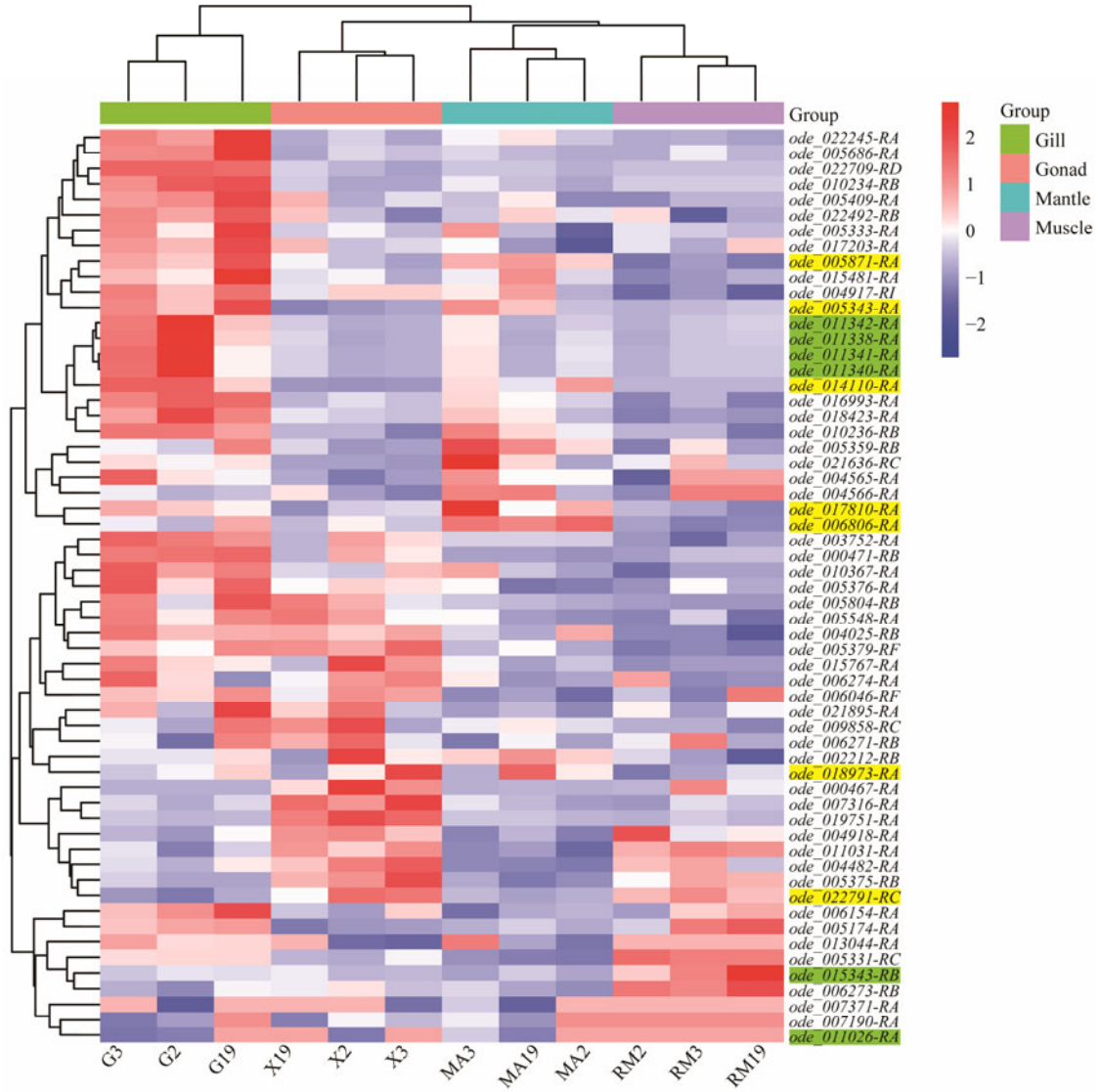


Fig.5 Expression pattern analysis of *Hsp70* genes in four tissues of ovulating female *O. denselamellosa* based on TPM and TMM analyses. G, gill; X, gonad; MA, mantle; RM, muscle. The labels of *Hsp70B2* genes are highlighted with green, the labels of *Hspa12* genes are not highlighted and the labels of other types genes are highlighted with yellow. The color scale represents Z-score.

map of *OdeHsp70* genes in various tissues was created (Fig.5). *Hsp70* genes were highly expressed in gills and relatively lowly expressed in mantle and adductor muscle. The same expression pattern has also been reported in hard clam *M. mercenaria* and Manila clam *Venerupis philippinarum* (Liu *et al*., 2015; Nie *et al*., 2017; Hu *et al*., 2022). In Bivalve, gills are considered to be sensitive to environmental changes, and high expression of *Hsp70* genes will promote the regulation of the environmental changes response (Cheng *et al*., 2019). Interestingly, some *Hsp70* genes showed highly tissue-specific expression. For example, four *Hsp70B2* genes, including *ode_011342-RA*, *ode_011338-RA*, *ode_011341-RA* and *ode_011340-RA*, had high expression in the gills. Genes *ode_007316-RA* and *ode_019751-RA* showed high expression levels in the gonad. Genes *ode_017810-RA*, *ode_006806-RA* were highly expressed in the mantle. Genes *ode_015343-RB*, *ode_006273-RB* had higher expression in adductor muscle. In addition to a small part of genes like *ode_021895-RA*, *ode_009858-RC*, *ode_004025-RB* and *ode_005379-RF*, most *OdeHsp70* genes were highly expressed in only one tissue.

## 4 Conclusions

In summary, a genome-wide analysis of *Hsp70* gene family in 5 oyster species identified 401 genes including 59 in *O. denselamellosa*, 84 in *C. ariakensis*, 83 in *S. glomerata*, 84 in *O. edulis* and 88 in *C. gigas*. The fewest number of *Hsp70* genes in *O. denselamellosa* is mainly due to the decrease in the number of *Hspa12* genes, possibly explaining why *O. denselamellosa* cannot tolerate high temperature. The gene structure and conserved motif investigation revealed that the conserved motifs and gene structures of *OdeHsp70B2* genes and other types of *OdeHsp70* were different from that of *Hspa12*, which may be due to performing necessary multiple physiological functions. Transcription profile analysis for *Hsp70* genes of *O. denselamellosa* support that gills play an important role in responding to multiple external challenges. In addition, synteny analysis of *OdeHsp70* genes between *O. edulis* and *C. ariakensis* demonstrated that *Hsp70* genes in *Ostrea* genome might have evolved from those of a common ancestor with *Crassostrea*. Taken together, the information obtained in this study lay the foundation for further investtigation of the evolution of *Hsp70* genes and heat adaptability of *O. denselamellosa*.

## Acknowledgements

This work was supported by grants from the National Key R&D Program of China (No. 2022YFD2400305), the China Agriculture Research System Project (No. CARS-49), and the Key R&D Project of Shandong Province (Nos. 2021ZLGX03, 2021LZGC027).

## References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J., 1990. Basic local alignment search tool. *Journal of Molecular Biology*, **215** (3): 403-410, DOI: 10.1016/S0022-2836(05)80360-2.

Bailey, T. L., Johnson, J., Grant, C. E., and Noble, W. S., 2015. The MEME Suite. *Nucleic Acids Research*, **43** (W1): W39-W49, DOI: 10.1093/nar/gkv416.

Buchfink, B., Xie, C., and Huson, D., 2015. Fast and sensitive protein alignment using DIAMOND. *Nature Methods*, **12** (1): 59-60, DOI: 10.1038/nmeth.3176.

Buroker, N. E., 1985. Evolutionary patterns in the family Ostreidae: Larviparity *vs*. Oviparity. *Journal of Experimental Marine Biology and Ecology*, **90** (3): 233-247, DOI: 10.1016/0022-0981(85)90169-8.

Casas, S. M., and La Peyre, J. F., 2020. Heat shock protein 70 levels and post-harvest survival of eastern oysters following sublethal heat shock in the laboratory or conditioning in the field. *Cell Stress Chaperones*, **25** (2): 369-378, DOI: 10.1007/s12192-019-01056-1.

Chen, C., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y., *et al*., 2020. TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Molecular Plant*, **13** (8): 1194-1202, DOI: 10.1016/j.molp.2020.06.009.

Chen, L., Li, Q., Wang, Q., Kong, L., and Zheng, X., 2011. Techniques of artificial breeding of the oyster *Ostrea denselamellosa*. *Periodical of Ocean University of China*, **41** (3): 43-46 (in Chinese with English abstract).

Cheng, D., Liu, H., Zhang, H., Soon, T. K., Ye, T., Li, S., *et al*., 2019. Differential expressions of *Hsp70* gene between golden and brown noble scallops *Chlamys Nobilis* under heat stress and bacterial challenge. *Fish & Shellfish Immunology*, **94**: 924-933, DOI: 10.1016/j.fsi.2019.10.018.

Cheng, J., Xun, X., Kong, Y., Wang, S., Yang, Z., Li, Y., *et al*., 2016. *Hsp70* gene expansions in the scallop *Patinopecten yessoensis* and their expression regulation after exposure to the toxic dinoflagellate *Alexandrium catenella*. *Fish & Shellfish Immunology*, **58**: 266-273, DOI: 10.1016/j.fsi.2016.09.009.

Dong, Z., Bai, Y., Liu, S., Yu, H., Kong, L., Du, S., *et al*., 2023. A chromosome-level genome assembly of *Ostrea denselamellosa* provides initial insights into its evolution. *Genomics*, **115** (2): 110582, DOI: 10.1016/j.ygeno.2023.110582.

Eddy, S. R., 1996. Hidden Markov models. *Current Opinion in Structural Biology*, **6** (3): 361-365, DOI: 10.1016/S0959-440X(96)80056-X.

Edgar, R. C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32** (5): 1792-1797, DOI: 10.1093/nar/gkh340.

Foighil, D. O., and Taylor, D. J., 2000. Evolution of parental care and ovulation behavior in oysters. *Molecular Phylogenetics and Evolution*, **15** (2): 301-313, DOI: 10.1006/mpev.1999.0755.

Fu, H., Jiao, Z., Li, Y., Tian, J., Ren, L., Zhang, F., *et al*., 2021. Transient receptor potential (TRP) channels in the Pacific oyster (*Crassostrea gigas*): Genome-wide identification and expression profiling after heat stress between *C. gigas* and *C. angulata*. *International Journal of Molecular Sciences*, **22** (6): 3222, DOI: 10.3390/ijms22063222.

Gomez-Chiarri, M., Warren, W., Guo, X., and Proestou, D., 2015. Developing tools for the study of molluscan immunity: The sequencing of the genome of the eastern oyster, *Crassostrea virginica*. *Fish & Shellfish Immunology*, **46** (1): 2-4, DOI: 10.1016/j.fsi.2015.05.004.

Han, J., Kim, H. J., Oh, S. Y., and Choi, Y. U., 2022. Reproductive characteristics of the flat oyster *Ostrea denselamellosa* (Bivalvia, Ostreidae) found on the southern coast of South Korea. *Journal of Marine Science and Engeineering*, **10**: 1326, DOI: 10.3390/jmse10091326.

Hu, B., Li, M., Yu, X., Xun, X., Lu, W., Li, X., *et al*., 2019. Diverse expression regulation of *Hsp70* genes in scallops after exposure to toxic *Alexandrium* dinoflagellates. *Chemosphere*, **234**: 62-69, DOI: 10.1016/j.chemosphere.2019.06.034.

Hu, Y. M., Li, Q., Liu, S. K., and Kong, L. F., 2020. Effects of acute temperature and salinity stress on the survival and immune indexes of Iwagaki oysters, *Crassostrea nippona*. *Journal of Fishery Sciences of China*, **27** (3): 286-294 (in Chinese with English abstract).

Hu, Z., Song, H., Feng, J., Zhou, C., Yang, M, J., Shi, P., *et al*., 2022. Massive heat shock protein 70 genes expansion and transcriptional signatures uncover hard clam adaptations to heat and hypoxia. *Frontiers in Marine Science*, **9**: 898669, DOI: 10.3389/fmars.2022.898669.

Insua, A., and Thiriot-Quievreux, C., 1991. The characterization of *Ostrea denselamellosa* (Mollusca, Bivalvia) chromosomes: Karyotype, constitutive heterochromatin and nucleolus organizer regions. *Aquaculture*, **97** (4): 317-325, DOI: 10.1016/0044-8486(91)90324-z.

Kim, D., Landmead, B., and Salzberg, S. L., 2015. HISAT: A fast spliced aligner with low memory requirements. *Nature Methods*, **12** (4): 357-360, DOI: 10.1038/nmeth.3317.

Kumar, S., Stecher, G., Suleski, M., and Hedges, S. B., 2017. Time tree: A resource for timelines, timetrees, and divergence times. *Systematic and Applied Microbiology*, **34** (7): 1812-1819, DOI: 10.1093/molbev/msx116.

Li, X., Bai, Y., Dong, Z., Xu, C., Liu, S., Yu, H., *et al*., 2023. Chromosome-level genome assembly of the European flat oyster (*Ostrea edulis*) provides insights into its evolution and adaptation. *Comparative Biochemistry and Physiology−Part D: Genomics and Proteomics*, **45** (1): 101045, DOI: 10.1016/j.cbd.2022.101045.

Liao, Y., Smyth, G. K., and Shi, W., 2014. featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, **30** (7): 923-930, DOI: 10.1093/bioinformatics/btt656.

Liu, T., Pan, L., Cai, Y., and Miao, J., 2015. Molecular cloning and sequence analysis of heat shock proteins 70 (*Hsp70*) and 90 (*Hsp90*) and their expression analysis when exposed to benzo (a) pyrene in the clam *Ruditapes philippinarum*. *Gene*, **555** (2): 108-118, DOI: 10.1016/j.gene.2014.10.051.

Liu, X., Tang, S., Jia, G., Schnable, J. C., Su, H., Tang, C., *et al*., 2016. The C-terminal motif of SiAGO1b is required for the regulation of growth, development and stress responses in foxtail millet (*Setaria italica* (L.) P. Beauv). *Journal of Experimental Botany*, **67** (11): 3237-3249, DOI: 10.1093/jxb/erw135.

Metzger, D. C., Hemmer-Hansen, J., and Schulte, P. M., 2016. Conserved structure and expression of *Hsp70* paralogs in teleost fishes. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, **18**: 10-20, DOI: 10.1016/j.cbd.2016.01.007.

Nagata, T., Sameshima, M., Uchikawa, T., Osafune, N., and Kitano, T., 2017. Molecular cloning and expression of the heat shock protein 70 gene in the Kumamoto oyster *Crassostrea sikamea*. *Fisheries Science*, **83**: 273-281, DOI: 10.1007/s12562-017-1064-6.

Nie, H., Liu, L., Huo, Z., Chen, P., Ding, J., Yang, F., *et al*., 2017. The *Hsp70* gene expression responses to thermal and salinity stress in wild and cultivated Manila clam *Ruditapes philippinarum*. *Aquaculture*, **470**: 149-156, DOI: 10.1016/j.aquaculture.2016.12.016.

Osorio, D., Rondon-Villarreal, P., and Torres, R., 2015. Peptides: A package for data mining of antimicrobial peptides. *The R Journal*, **7** (1): 4-14, DOI: 10.32614/RJ-2015-001.

Peng, J., Li, Q., Xu, L., Wei, P., He, P., Zhang, X., *et al*., 2020. Chromosome-level analysis of *Crassostrea hongkongensis* genome reveals extensive duplication of immune-related genes in bivalves. *Molecular Ecology Resources*, **20** (4): 980-994, DOI: 10.1111/1755-0998.13157.

Powell, D., Subramanian, S., Suwansaard, S., Zhao, M., O'Connor, W., Raftos, D., *et al*., 2018. The genome of the oyster *Saccostrea* offers insight into the environmental resilience of bivalves. *DNA Research*, **25** (6): 655-665, DOI: 10.1093/dnares/dsy032.

Price, M. N., Dehal, P. S., and Arkin, A. P., 2009. FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, **26** (7): 1641-1650, DOI: 10.1093/molbev/msp077.

Rosenzweig, R., Nillegoda, N. B., Mayer, M. P., and Bukau, B., 2019. The Hsp70 chaperone network. *Nature Reviews Molecular Cell Biology*, **20** (11): 665-680, DOI: 10.1038/s41580-019-0133-3.

Sleator, R. D., 2016. JCVI-syn3.0−A synthetic genome stripped bare. *Bioengineered*, **7** (2): 53-56, DOI: 10.1080/21655979.2016.1175847.

Voorrips, R. E., 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. *The Journal of Heredity*, **93** (1): 77-78, DOI: 10.1093/jhered/93.1.77.

Wang, T., and Li, Q., 2017. Effects of salinity and temperature on growth and survival of juvenile of kumamoto oyster (*Crassostrea sikamea*). *Oceanologia et Limnologia Sinica*, **48** (2): 297-302 (in Chinese with English abstract).

Wang, Y., Tang, H., Debarry, J. D., Tan, X., Li, J., Wang, X., *et al*., 2012. MCScanX: A toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Research*, **40** (7): e49, DOI: 10.1093/nar/gkr1293.

Webb, J. K., Shine, R., and Christian, K. A., 2006. The adaptive significance of reptilian viviparity in the tropics: Testing the maternal manipulation hypothesis. *Evolution*, **60** (1): 115-122, DOI: 10.1111/j.0014-3820.2006.tb01087.x.

Wu, B., Chen, X., Yu, M., Ren, J., Hu, J., Shao, C., *et al*., 2022. Chromosome-level genome and population genomic analysis provide insights into the evolution and environmental adaptation of Jinjiang oyster *Crassostrea ariakensis*. *Molecular Ecology Resources*, **22** (4): 1529-1544, DOI: 10.1111/1755-0998.13556.

Xu, F., and Zhang, S., 2008. *An Illustrated Bivalvia Mollusca Fauna of China Seas*. Science Press, Beijing, 336pp (in Chinese).

Yang, M. H., Bong, S. H., and Han, C. H., 2003. Growth and survival rates of flat oyster larvae, *Ostrea denselamellosa*, by condition of larvae cultivation. *Korean Journal of Malacology*, **19** (2): 133-142.

Yang, M. H., Kim, H. S., Lee, J. Y., and Han, C. H., 2001. Artificial mass culture of flat oyster larvae, *Ostrea denselamellosa*, and collection rates according to various spat collection methods. *Korean Journal of Malacology*, **17** (1): 35-44.

Yu, H., Kong, L., and Li, Q., 2016. Complete mitochondrial genome of *Ostrea denselamellosa* (Bivalvia, Ostreidae). *Mitochondrial DNA Part A*, **27** (1): 711-712, DOI: 10.3109/19401736.2014.913154.

Zhang, G., Fang, X., Guo, X., Li, L., Luo, R., Xu, F., *et al*., 2012. The oyster genome reveals stress adaptation and complexity of shell formation. *Nature*, **490** (7418): 49-54, DOI: 10.1038/nature11413.

**(Edited by Qiu Yantao)**