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Karyological Analysis and Nucleolar Organizer Region of Tropical Oyster, *Crassostrea iredalei* (Ostreoida, Ostreidae) in Thailand

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Summary Conventional karyotyping and detection of nucleolar organizer region (NOR) were performed on a tropical oyster, *Crassostrea iredalei* from Nakhon Si Thammarat province, Thailand. Chromosome preparations were made from gill tissues. Conventional Giemsa's staining and Ag-NOR banding were performed. The results revealed that the chromosome number was $2n=20$ and fundamental number (NF) was 40, the karyotype consisting of 12 large metacentric and eight medium metacentric chromosomes. The NORs were located terminally on the long arms of the chromosome pair 10.

Key words *Crassostrea iredalei*, Tropical oyster, Karyotype, Chromosome, Nucleolar organizer region.

The family Ostreidae is belonged to Bivalves that are source of food all over the world and commercially important marine species form ancient time. This family is consists about 75 species (Salvi *et al.* 2014) which distributed throughout the world along the shores, shallow water and estuary water is richer nutrients. It has significant role in near shore ecosystems (Ruesink *et al.* 2005) and oysters are one of the extensively studied marine animals. Nine species of oysters in the superfamily Ostreoidea were found in Thai waters. However, only three species of *C. belcheri*, *C. iredalei* and *Saccostrea cucullata* were reared commercially in Thailand (Jarayabhand and Thavornyutikarn 1995, Klinbunga *et al.* 2005).

C. iredalei is commonly called the black scar oyster or Takrom-Kram Dum due to the spices having black of adductor muscle scar (Fig. 1). The species is commercially important in Thailand but less than the big oyster or *C. belcheri*. The habitats of the species are many substrates such as stilts, roots of mangrove, and rocks area in estuaries in southern Thailand both the gulf of Thailand and the Andaman Sea (Visootiviseth *et al.* 1998, Zainal Abidin *et al.* 2014).

In the previous study morphological descriptions and guidelines for basic identification of oyster group were provided. However, taxonomists have been challenging oyster systematics for a long time. Attributable oysters are one of the most variable animals with respect to shell shape because it has shell plasticity (Lam and Morton 2003). Classification and identification based on morphological characters led to more errors and confusion in oyster taxonomy (Liu *et al.* 2011). Because oyster species have sensitive to influence of substrate and environmental factor, the shape, size, texture, and color of shell contains large variation. Moreover, anatomy of soft tissue is difficult to identification. Therefore, the resulting in classification and phylogenetic analysis are problematic in the oyster (Wang *et al.* 2004).

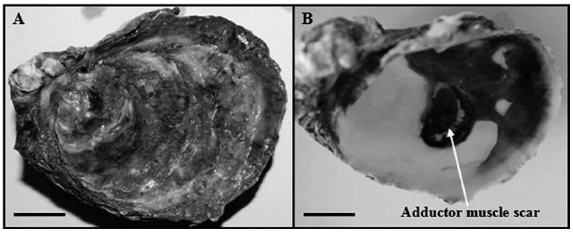

Fig. 1. General characteristics (A) and black adductor muscle scar (B) of *C. iredalei*. Scale bars=2 cm.

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The studies on chromosomes of marine mollusks are significant, especially for those as oysters. The first study was concerned about chromosome number and gross morphology (Leitao *et al.* 1999b). Later, morphometric analyses of chromosomes provided the characterization of karyotype. Cytogenetics of oyster has been reported in 22 species of Ostreidae (Leitao *et al.* 2002). All previous studies in oyster species have shown same diploid chromosome number of $2n=20$, which was commonly characteristic of this family. Moreover, the most of karyotype of this family were consists of only metacentric and submetacentric chromosomes (Chooseangjaew *et al.* 2017).

Many banding techniques such as G-banding, C-banding, Ag-NOR banding and FISH have been applied for the identification of individual chromosomes and also for particular regions of chromosomes. These techniques were applied to several oyster species such as *C. gigas*, *C. belcheri*, *Ostrea edulis*, *O. denselamellosa*, *O. puelchana* and *Tiostrea chilensis* (Insua and Thiriot-Quievreux 1991, 1993, Thiriot-Quiévreux and Insua 1992, Chooseangjaew *et al.* 2017). Moreover, three species *C. angulata*, *C.gigas* and *C. virginiga* have been investigated by G-banding (Leitao *et al.* 1999b). Five species *C. angulata*, *O. conchaphila*, *O. angasi*, *O. stentina* and *C. gigas* have been studied by C-banding (Li and Havenhand 1997, Leitao *et al.* 2002, Cross *et al.* 2005, Pereira *et al.* 2011). Seven species, *C. gigas*, *C. virginiga*, *C. angulata*, *C. plicatula*, *C. ariakensis*, *C. rhizophorae* and *S. mordax* have been applied to chromosome analysis using FISH technique (Xu *et al.* 2001, Cross *et al.* 2003, 2005, Wang *et al.* 2004, 2005, Lu *et al.* 2011).

Ag-NOR banding technique is applied in many oyster species and analysis of NOR positions has been used for inferring phylogenetic relationships (Amemiya and Gold 1990) and the polymorphisms of NORs which comprise number and locations are appeared often in species specific (Cross *et al.* 2005). Normally, in haploid karyotype of family Ostreidae one to three-NORs were presented at the terminal region of chromosome arms (Thiriot-Quiévreux 2002).

In this study, conventional karyotype and NOR positions were studied in *C. iredalei*.

Materials and methods

Samples collection

Ten mature *C. iredalei* were collected from Nakhon Si Thammarat province, southern Thailand. The samples were then taken to the Marine Shellfish Breeding Research Unit, Department of Marine Science, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang campus.

Chromosome preparation and stainings

The specimens of oysters were incubated in 0.1% colchicine in sea water for 10–12 h. The gills were then removed by dissection, gently minced and treated for 30 min in 0.075 M KCl. Subsequently, the sample was centrifuged at 1500 rpm for 8 min and fixed in a freshly prepared mixture of methanol and acetic acid (3 : 1) with three time changes for 20 min. Finally, cell suspension was dropped onto clean warmed glass slides, and air-dried (Leitao *et al.* 2002, Khrueanet *et al.* 2013). For conventional karyotyping, chromosome preparations were stained with 20% Giemsa for 40 min. The glass slides were gently rinsed by water and the chromosomes were counted in each individual under a microscope (CH30, Olympus, Japan). The complete 20 metaphase plates were selected to perform karyotype analysis. Two drops of 2% gelatin and four drops of 50% silver nitrate were added on samples on glass slides prior to seal by coverslips and incubated at 60°C for 8 min. The glass slides were soaked in distilled water until the coverslips were separated (Howell and Black 1980).

Chromosome measurements

Twenty complete metaphase plates in each individual were counted and photographed under a microscope. The length of short arm (Ls) and long arm (Ll) were measured by using the Adobe Photoshop and were used to calculate for the length of chromosome (LT=Ls+Ll). The relative length (RL), centrometric index (CI), and standard deviation (S.D.) of RL and CI were estimated follow by the method according Chaiyasut (1989). The CI=Ls/LT as 0.50–0.59, 0.60–0.69, 0.70–0.89 and 0.90–0.99 are described as metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively. All values were used for karyotyping follows that of Turpin and Lejeune (1965).

Results and discussion

The diploid chromosome number of $2n=20$ were confirmed by a representative metaphase spreads from gill tissue of ten individuals of *C. iredalei* (Fig. 2). The diploid chromosome number consistent with all previous reports of oyster in the family Ostreidae and revealed that is commonly characteristic of the Ostreidae (Leitao *et al.* 1999a, Leitao *et al.* 2002, Thiriot-Quiévreux 2002). For karyotyping, the metaphase chromosomes were paired on the basis of chromosome size and centromere position. Our study showed 10 pairs of metacentric chromosomes (Fig. 2B) which corresponds with other species in the Ostreidae such as *C. belcheri* (Chooseangjaew *et al.* 2017, Koedprang and Wattanakul 1996), *C. gigas* (Koedprang and Wattanakul 1996, Leitao *et al.* 1999a, Thiriot-Quiévreux and Insua 1992), *S. mordax* (Lu *et al.* 2011) and *C. angulata* (Cross *et al.* 2003), while differed from Leitao *et al.* (1999a) that showed 18 metacentric chromosome and two submetacentric chromosomes in *C. angulata* species.

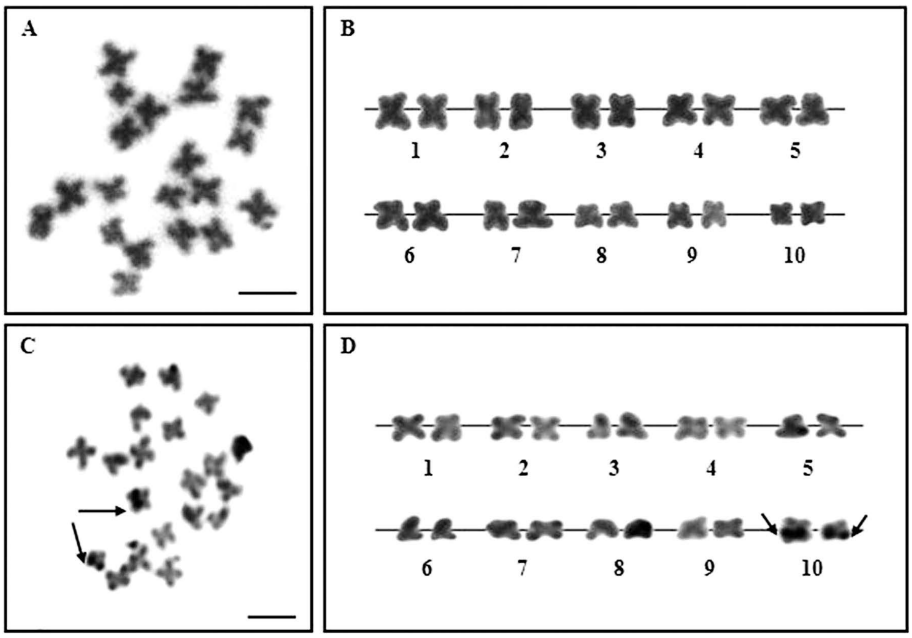

Fig. 2. Metaphase chromosome plates (A, C) and karyotypes (B, D) of *C. iredalei*, 2*n*=20 by conventional straining (A, B) and Ag-NOR banding (C, D). Arrow indicates NORs. Scale bars=5 μm.

Table 1. Mean length of short arm (Ls), long arm (Ll), chromosomes lenght (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL and CI from 20 metaphase cells of *C. iredalei*.

Chromosome pair	Ls (μm)	Ll (μm)	LT (μm)	RL±S.D.	CI±S.D.	Chromosome size	Chromosome type
1	2.83	3.22	6.05	0.121±0.006	0.532±0.022	L	Metacentric
2	2.63	2.99	5.62	0.112±0.004	0.533±0.028	L	Metacentric
3	2.56	2.84	5.40	0.108±0.004	0.526±0.016	L	Metacentric
4	2.51	2.85	5.36	0.107±0.004	0.531±0.024	L	Metacentric
5	2.42	2.71	5.13	0.102±0.004	0.527±0.020	L	Metacentric
6	2.37	2.67	5.05	0.101±0.003	0.530±0.018	L	Metacentric
7	2.28	2.54	4.82	0.096±0.003	0.527±0.016	M	Metacentric
8	2.12	2.50	4.62	0.093±0.004	0.540±0.025	M	Metacentric
9	1.99	2.22	4.21	0.084±0.004	0.529±0.026	M	Metacentric
10*	1.79	2.03	3.82	0.076±0.006	0.531±0.033	M	Metacentric

L=large chromosome (LT>4.93 μm), M=medium chromosome (LT=3.02–4.93 μm), *=NOR-bearing chromosome.

The NF or number of chromosome arms was 40. All the chromosomes of *C. iredalei* were regard as bi-arm chromosome. The karyotype was comprised of six large and four medium metacentric chromosomes pairs (Table 1). However, difference number of submetacentric chromosome in karyotype among the reports (see Table 1 of Chooseangjaew *et al.* 2017) might be caused by condition of pretreatment as concentrations or duration of colchicine (Leitao *et al.* 1999a). Moreover, type variation of metacentric to submetacentric might be attributable to pericentric inversion, re-ciprocal translocation, or centromere reposition (Ahmed 1973, Pereira *et al.* 2011). It is possible that subspecies of oysters might have been differentiated by inversions (Ahmed 1973). Furthermore, the karyotype of *C. angulata*, *C. gigas*, *C. belcheri*, *O. chilensis*, *S. commercialis*, *S. mordax* and *C. iredalei* were with symmetrical karyotype attribute showing only metacentric and submetacentric chromosome pairs (Ahmed 1973, Leitao *et al.* 1999a). In *C. iredalei* the karyotype composed of metacentric chromosome was considered as primitive karyotype (Leitao *et al.* 1999a).

Previous studies of oyster species by Ag-NOR banding revealed specific chromosomal location of NORs (see Table 1 of Chooseangjaew *et al.* 2017). Our result showed that one NORs was located on the terminal region of long arm of chromosome pair 10 (Fig. 2D) which was similar to number and locations of NORs with other oyster species; *C. belcheri* (Chooseangjaew *et al.* 2017), *C. angulata* (Cross *et al.* 2003), *C. gigas* (Thiriot-Quiévreux and Insua 1992). The oyster in the Ostreidae have NORs on one or two chromosome pairs except for *O. angasi* has three locations of NORs (Pereira *et al.* 2011). According by Amemiya and Gold (1990) shown that one pair of NORs in the oyster was considered to more primitive characteristic than with two pairs of NORs in *C. angulata*, *C. belcheri*, *C. gigas*, *C. gasar*, *C. rhizophorae* and *C. sikamea*. Cytogenetic characterization of both the karyotype and NORs in oyster of Ostreidae will certainly useful and help clarify to taxonomy

and evolutionary relationships of oyster group.

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