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Unveiling the Chromosomal Blueprint of the Pearl Oyster (*Pintada maxima*): A First Comprehensive Karyotype Analysis in Indonesia

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Abstract

The pearl oyster (*Pinctada maxima*) is a high-value commodity in the pearl aquaculture industry. However, aquaculture development in Indonesia is hindered by the lack of genetic information, particularly on its chromosomal characteristics. This study aimed to characterize the chromosomes and analyze the karyotype of *P. maxima* for the first time in Indonesia. Chromosome preparations were made from gill tissues of pearl oyster juveniles using a modified solid tissue technique. Karyotype analysis was performed by observing and measuring chromosomes at the mitotic metaphase stage. *P. maxima* has 28 diploid chromosomes (2n) with sizes ranging from 0.419 to 2.077 microns. The karyotype consists of two pairs of metacentric, three pairs of submetacentric, five pairs of subtelocentric, and four pairs of telocentric chromosomes, with the chromosomal formula 2m + 3sm + 5st + 4t. No physical chromosomal abnormalities were found. This study provides valuable information on the genome organization of *P. maxima*, which can be used as a basis for comparative studies, development of breeding strategies, and aquaculture management programs.

Keywords: Pearl Oyster; *Pinctada maxima*; Chromosome Characterization; Karyotype Analysis; Genome Organization

Introduction

The pearl oyster (*Pinctada maxima*) is a highly valuable aquatic commodity due to its ability to produce high-value and rare pearls [1]. However, the development of pearl oyster aquaculture in Indonesia, particularly in Maluku, still faces various challenges. One of the main obstacles is the lack of experts in the pearl industry and the necessary capital, which results in the dominance of foreign companies in this sector [1]. Pearl production is also limited because it relies heavily on diving activities in the seabed, which do not always yield successful results [2]. Additionally, the slow growth rate of pearl oysters and the decline in their natural populations are serious concerns. Hatchery-based pearl oyster cultivation presents a promising alternative but is hindered by limitations in technology and human resources.

An in-depth understanding of the genetics of pearl oysters is crucial to overcoming these challenges. Accurate genetic information can be used to develop more effective breeding programs, enhance disease resistance, and optimize cultivation conditions [2]. One important aspect of understanding the genetics of an organism is karyotype analysis, which provides a complete picture of the number and morphology of chromosomes in a cell [3,4]. Karyotype analysis can offer information about ploidy (the number of chromosome sets), the size and shape of chromosomes, and the position of centromeres (the attachment point for

spindle fibers during cell division). This information can be used to identify species, detect chromosomal abnormalities, and study the evolutionary relationships between different species [5-7].

Cytogenetic research on pearl oysters in Indonesia is still very limited, and information regarding the chromosome number and genetic characteristics of *P. maxima* is scarce [2]. However, this information is crucial for understanding genetic diversity, relationships, and developing effective conservation strategies. Therefore, this research aims to address several key questions: What is the diploid chromosome number (2n) of the pearl oyster *P. maxima*?; What are the characteristics of the chromosomes (size, arm ratio, relative length, centromere position, and morphology) of the pearl oyster *P. maxima*?; and What is the karyotype and chromosomal formula of *P. maxima*?

Although cytogenetic studies have been conducted on various organisms, similar research on pearl oysters, particularly *P. maxima*, is still very limited. Information on the chromosome number, characteristics, and karyotype of *P. maxima* is not yet fully available. Previous studies have focused more on the cultivation and ecology of pearl oysters, while genetic studies, especially cytogenetics, have been relatively neglected.

Several studies have been conducted on other species of pearl oysters, such as *Pinctada fucata* and *Pinctada margaritifera*. However, each species of pearl oyster has unique genetic characteristics, so the findings from other species cannot be directly generalized to *P. maxima*. Therefore, this study will be the first in Indonesia to comprehensively characterize the chromosomes and analyze the karyotype of *P. maxima*.

The general objective of this research is to provide basic information about the chromosomes of *P. maxima* that can be used in understanding the diversity, relationships, and genetic conservation of the species. The specific objectives of this study are: To determine the diploid chromosome number (2n) of *P. maxima*; to analyze the characteristics of the chromosomes (size, arm ratio, relative length, centromere position, and morphology) of *P. maxima*; and to construct the karyotype and chromosomal formula of *P. maxima*.

Materials and Methods

The samples of pearl oysters (*P. maxima*) used were juveniles measuring 4-5 cm. This size was chosen because they are young (spat), easy to transport, and easy to maintain in the laboratory. The samples were collected in a live and healthy condition from the aquaculture center. Chromosome preparations were made using a modified and optimized solid tissue technique from previous methods [8-12]. The gill tissue of the pearl oyster was used as a source of chromosomes because this tissue actively divides and has a high mitotic index. The steps for preparing the slides included: a) Acclimatization and immersion in a colchicine solution to arrest cell division at the metaphase stage; b) Fixation of the tissue using Carnoy's solution to preserve cell structure; c) Preparation of cell suspensions with acetic acid and spreading the cells on glass slides; d) Staining with Giemsa solution for chromosome visualization; e) Washing and drying the slides.

Chromosome preparations were observed under a light microscope at magnifications of 10x, 40x, and 100x. Chromosomes were counted and photographed using a digital camera. Chromosome images were processed using ImageJ software version 1.54i to obtain a clear count and image of the chromosomes. Micrometric measurements were performed to obtain chromosome arm lengths. Chromosome characteristics data, such as relative chromosome length (RCL), arm ratio (AR), and centromeric index (CI), were calculated using formulas proposed by Brown [5] and Levan, et al. [13]. Chromosome types were determined based on CI and AR according to Levan, et al. [13] classification.

Statistical analyses, such as homogeneity tests and ANOVA, were used to determine whether the pearl oyster individuals studied belonged to the same or different populations (species). Software such as Microsoft Excel 2013, Minitab version 19, ImageJ version 6, and Genstat release 7 were used for data processing and statistical analysis.

Results and Discussion

Chromosomal Distribution of *Pinctada maxima*

The chromosomes of *P. maxima* were clearly observed in juvenile individuals measuring 4-5 cm using a 0.075% colchicine treatment for 7-8 hours, a hypotonic treatment for 100 minutes, and Giemsa staining at 2.5% for 25-30 minutes. These results differ from previous studies that used different organisms. Carman, et al. [14] reported that warm-water fish larvae required a shorter colchicine soaking time, specifically 3-4 hours in a 0.07% solution. This discrepancy may be due to differences in species and developmental stages of the organisms being observed. Warm-water fish larvae in their early developmental stages may have a faster cell division rate compared to juvenile pearl oysters, thus requiring a shorter colchicine soaking time to halt cell division at the metaphase stage.

Said [15] reported that 10-day-old Irian rainbowfish larvae required a 9-hour colchicine soaking time in a 0.07% solution. This difference could be attributed to species

differences and rearing conditions. Irian rainbowfish larvae might have a different sensitivity to colchicine compared to juvenile pearl oysters. Additionally, environmental rearing conditions such as temperature and water quality could affect the organism's response to colchicine.

These varying results suggest that the optimal colchicine soaking time can differ depending on species, developmental stage, and environmental conditions. Therefore, it is essential to optimize the chromosome preparation method for each organism studied. In this research, a colchicine soaking time of 7-8 hours proved effective in arresting cell division at the metaphase stage in juvenile *P. maxima*, allowing for clear chromosome observation and accurate karyotype analysis.

Chromosome Morphology of *Pinctada maxima*

This study utilized gill tissue from juvenile pearl oysters (*Pinctada maxima*) measuring 4-5 cm to observe chromosomes. The selection of gill tissue was based on several considerations:

(a) Gill tissue is easily accessible and relatively easy to isolate from pearl oysters, facilitating the sample collection and chromosome preparation process.

(b) Gill tissue in pearl oysters is actively involved in mitotic division due to its crucial role in respiration and food filtration, requiring constant cell regeneration.

(c) Gill tissue has a high mitotic index, meaning there are many dividing cells in this tissue, increasing the likelihood of finding cells in the metaphase stage of mitosis, which is the best stage for observing and analyzing chromosomes.

This study also involved modifying and optimizing previously established chromosome preparation methods [8-12]. These modifications were made to tailor the methods to the characteristics of pearl oyster gill tissue and to improve the quality of the resulting chromosome preparations.

The use of gill tissue and the optimization of chromosome preparation methods contributed to the success of this study in clearly observing the chromosomes of *P. maxima*. This facilitated accurate karyotype analysis and provided valuable information about the genomic organization of this species.

Chromosome Number of *Pinctada maxima*

An analysis of 675 cells from five individuals of *P. maxima* revealed that the diploid chromosome number (2n) varied between 26 and 30, with a mode of 28. Statistical tests (Levene and ANOVA) confirmed that this variation is not significant and that all five individuals belong to the same population (species). Although the variation in chromosome number is not statistically significant, it can be explained from several perspectives: polyploidy, aneuploidy, and intraspecific variation.

Polyploidy is a condition in which an organism has more than two sets of chromosomes. This phenomenon is common in mollusks, including pearl oysters. The observed variation in chromosome number in *P. maxima* may be due to the presence of polyploid individuals in the studied sample. Aneuploidy is a condition where the number of chromosomes in a cell is not an exact multiple of the haploid number. The variation in chromosome number in *P. maxima* could also be due to aneuploidy, which can occur due to errors during cell division. Chromosome number variation within a species, although not common, can occur naturally. This might be due to genetic or environmental factors. Further research is needed to determine the exact cause of this variation in *P. maxima*.

Wada conducted karyotype analysis on several species of pearl oysters, including *P. fucata* and *P. maxima*, and found that the diploid chromosome number varied between 28 and 30. This indicates that variation in chromosome number is a common phenomenon in pearl oysters. This study provides new evidence supporting the hypothesis that chromosome number variation is a common characteristic in pearl oysters. Moreover, this study highlights the importance of conducting karyotype analysis on different populations to gain further insight into the genetic variation and chromosomal evolution in *P. maxima*.

Chromosome Characteristics

Chromosome Size of *Pinctada maxima*

This study successfully measured the chromosome sizes of pearl oysters (*Pinctada maxima*) at the metaphase stage of mitosis. The results showed that the chromosome sizes of *P. maxima* varied from 0.419 to 2.077 microns, with an average size of 1.436 ± 0.503 microns. There were 12 pairs of large chromosomes measuring more than 1 micron and 2 pairs of small chromosomes measuring less than 1 micron. This variation in chromosome size is a common phenomenon in eukaryotic organisms. McIntosh, et al. [16] stated that chromosomes within a genome can differ in size and arm ratio. For instance, in the human genome, there is a 3-4 fold difference in size between the largest chromosome (chromosome 1) and the smallest chromosome (chromosome 21).

The study also found that there is no dominant chromosome in the *P. maxima* genome, as the size difference between the largest and smallest chromosomes is relatively small (8.246% or 1.558 microns). This is different from some other organisms that have one or several chromosomes significantly larger than the others. The variation in chromosome size in *P. maxima* can have important implications for genome organization and the evolution of this species. Larger chromosomes tend to contain more

genes, so chromosome size variation may reflect differences in the number and types of genes possessed by different individuals or populations. Furthermore, chromosome size variation can also affect genome stability and susceptibility to mutations.

Further research is needed to understand the implications of chromosome size variation in *P. maxima*. Comparative analysis with other pearl oyster species could provide insights into how chromosome size variation contributes to genetic diversity and the species' adaptation to different environments.

Chromosome Arm Ratio (CAR) of *Pinctada maxima*

The Chromosome Arm Ratio (CAR) is the ratio between the length of the longer arm of the chromosome and the shorter arm. This study found that the CAR in *Pinctada maxima* varied widely, ranging from 1.469 to 8.500, with an average of 4.344. This wide variation in CAR indicates that the chromosomes of *P. maxima* exhibit diverse shapes and sizes.

The average CAR value of 4.344 suggests that most chromosomes in *P. maxima* tend to be subtelocentric. Subtelocentric chromosomes have a centromere located near one end of the chromosome, resulting in one very short arm and one very long arm. This study aligns with the findings of McIntosh, et al. [16] who stated that chromosomes within a single genome can differ in size and arm ratio. The wide variation in CAR observed in *P. maxima* indicates genetic diversity within this species. This diversity can form the basis for species adaptation to different environments and play a crucial role in the evolutionary process.

Further research is necessary to understand the implications of CAR variation in *P. maxima*. Comparative analysis with other pearl oyster species could provide insights into how CAR variation contributes to genetic diversity and the adaptation of this species to different environments.

Relative Chromosome Length of *Pinctada maxima*

This study successfully measured the relative chromosome length (RCL) in pearl oysters (*Pinctada maxima*). Relative chromosome length is defined as the percentage of the total length of a single chromosome relative to the total length of the entire haploid chromosome set. The results showed that the RCL in *P. maxima* varied between 2.084% and 10.330%, with an average of 7.143%.

One of the significant findings of this study is the absence of a dominant chromosome in the *P. maxima* genome. This means that no single chromosome is significantly larger or smaller than the others. The size difference between the largest and smallest chromosomes is 8.246% or 1.558 microns. This finding contrasts with some other organisms that have one or several chromosomes much larger than the others. For example, in humans, chromosome 1 is the largest, while chromosome 21 is the smallest, with a significant size difference.

The absence of a dominant chromosome in *P. maxima* may have important implications for the evolution and genetic diversity of this species. Larger chromosomes tend to contain more genes. Without a dominant chromosome, the distribution of genes in *P. maxima* may be more even among its chromosomes. This can contribute to genome stability and reduce the risk of losing important genes due to mutations or other genetic changes.

Relative chromosome length is crucial information in karyotype analysis. Measuring RCL allows researchers to order the chromosomes in a genome based on their size, from largest to smallest. This information can be used to identify species, compare karyotypes between different species, and study chromosome evolution. In this study, RCL was used to construct the karyotype of *P. maxima* and determine its chromosomal formula. These results provide valuable information about the genomic organization of *P. maxima* and can serve as a basis for further research on the genetics, evolution, and management of this species.

Centromere Position of *Pinctada maxima*

This study successfully analyzed the centromere position on the chromosomes of the pearl oyster (*Pinctada maxima*). The centromere position is a critical characteristic in karyotype analysis as it determines the shape and classification of chromosomes. The study found that the centromere position on *P. maxima* chromosomes varied, with numerical values ranging from 10.523% to 40.506%. The centromere position numerical value (CPNV) is the percentage of the distance from the end of the short arm of the chromosome to the centromere relative to the total chromosome length. The varied distribution of centromere positions indicates that the centromeres are not randomly distributed on the chromosomes of *P. maxima*. This means there are certain centromere positions that appear more frequently than others. Although this study does not provide detailed explanations about specific centromere distribution patterns, these results pave the way for further research into the factors influencing centromere positions in *P. maxima*.

Centromeres are crucial parts of chromosomes that play a role in chromosome segregation during cell division [17]. They serve as attachment sites for spindle fibers that pull chromosomes toward opposite poles of the cell. The position of the centromere on a chromosome can affect chromosome stability and segregation during cell division. The centromere position also determines the morphology

of the chromosome. Based on the centromere position, chromosomes can be classified as metacentric (centromere in the middle), submetacentric (centromere slightly off-center), subtelocentric (centromere near the end), and telocentric (centromere at the end).

This study found that *P. maxima* has all four types of chromosomes, with subtelocentric chromosomes being the most dominant. The variation in centromere positions in *P. maxima* may have important evolutionary implications. Changes in centromere position can lead to changes in chromosome structure and function, which in turn can affect species evolution. Further research into the relationship between centromere position and chromosome evolution in *P. maxima* could provide new insights into the mechanisms of evolution at the chromosomal level.

Chromosomal Karyotype of *Pinctada maxima*

This study successfully analyzed the karyotype of the pearl oyster (*Pinctada maxima*), which is a complete visual representation of the chromosomes in a cell. The karyotype was arranged based on the size and morphology of the chromosomes. The results indicated that *P. maxima* has a karyotype consisting of two pairs of metacentric chromosomes (m), three pairs of submetacentric chromosomes (sm), five pairs of subtelocentric chromosomes (st), and four pairs of telocentric chromosomes (t).

The karyotype pattern found in *P. maxima* differs from the karyotypes found in some fish species, such as the Irian rainbowfish (Melanotaeniidae) and *Telmatherina ladigesi* [12,18]. These differences can be explained by several factors: (a) each species has a unique karyotype, reflecting the genetic diversity present among organisms. The karyotype differences between *P. maxima* and these fish indicate that they have different genetic makeups; (b) the karyotype of an organism can change over time through evolutionary processes. The karyotype differences between *P. maxima* and these fish may reflect different evolutionary paths they have undergone; (c) The karyotype of an organism can be influenced by adaptations to its environment. The karyotype differences between *P. maxima* and these fish may be related to differences in their habitats and lifestyles.

Karyotypes are characteristics that can be used to identify species and study evolutionary relationships between different species [5,13,19]. The unique karyotype of *P. maxima* can be used as a marker to distinguish this species from other pearl oyster species. Additionally, comparing the karyotype of *P. maxima* with related species can provide insights into their evolutionary relationships [20-80].

Chromosomal Formula of *Pinctada maxima*

This study successfully identified the karyotype and formulated the chromosomal formula of the pearl oyster (*Pinctada maxima*) as 2m+3sm+5st+4t [81,82]. This indicates the presence of two pairs of metacentric chromosomes (m), three pairs of submetacentric chromosomes (sm), five pairs of subtelocentric chromosomes (st), and four pairs of telocentric chromosomes (t).

The karyotype pattern differs from that of several fish species previously studied [83-90]. For instance, the Irian rainbowfish has 48 chromosomes (24 pairs), with seven pairs of subtelocentric and 17 pairs of telocentric chromosomes [91]. Similarly, *Telmatherina ladigesi* has 48 chromosomes (24 pairs), with three pairs of submetacentric, seven pairs of subtelocentric, and 14 pairs of telocentric chromosomes [92,93]. The differences in karyotype patterns between *P. maxima* and these fish species highlight that each species possesses a unique genetic arrangement, reflecting differences in evolutionary processes and adaptations to different environments [94-96].

The chromosomal formula 2m + 3sm + 5st + 4t in *P. maxima* provides a concise representation of the species' karyotype. This formula can be used as a tool for identification and differentiation between *P. maxima* and other pearl oyster species. This aligns with the views of Brown [5] and Levan, et al. [13] who stated that the arrangement and morphology of chromosomes can be used as a key for species identification within a genus [97-104].

This research contributes significantly to understanding the genetic characteristics of *P. maxima* and can serve as a foundation for further studies on the evolution, taxonomy, and management of this species. The findings provide essential insights into the chromosomal characteristics and karyotype of *P. maxima*. This information can be used as a basis for comparative studies with other pearl oyster species, aiding in understanding evolutionary relationships and developing better management strategies for this species. Additionally, this study offers foundational information useful for developing more effective pearl oyster breeding programs.

Conclusions and Recommendations

This study successfully characterized the chromosomes and analyzed the karyotype of the pearl oyster (*Pinctada maxima*), revealing important new information about the genomic organization of this species. The results indicate that *P. maxima* possesses 28 diploid chromosomes (2n) with varying sizes and centromere positions. These chromosomes comprise two pairs of metacentric, three

pairs of submetacentric, five pairs of subtelocentric, and four pairs of telocentric chromosomes, resulting in the karyotype formula 2m + 3sm + 5st + 4t. No physical abnormalities such as secondary constrictions or satellite chromosomes were detected.

The researchers recommend several further steps to deepen the understanding of the genetics and evolution of *P. maxima*: (a) Conduct comparative karyotype analysis of *P. maxima* with other pearl oyster species from various regions to uncover broader genetic variation and evolutionary relationships. This study will help identify karyotypic patterns specific to each species and elucidate chromosomal evolutionary mechanisms within this group; (b) Perform karyotype analysis using tissues other than gills, such as epithelial cells from the mantle or gonadal cells, to obtain a more comprehensive picture of the chromosomal characteristics of *P. maxima*. Analysis from multiple tissues will help ensure the consistency of results and evaluate chromosomal variations in different cell types; and (c) Apply more advanced chromosome staining techniques, such as banding techniques, to facilitate the identification of homologous chromosome pairs and study chromosome structures in greater detail. These techniques will provide higher resolution in examining chromosomal details and help identify chromosomal changes that may not be detected with conventional methods. These further recommended studies will provide more comprehensive information on the genetics of *P. maxima*, which in turn can support conservation efforts, aquaculture management, and the development of more effective breeding programs for this species. A deeper understanding of karyotypes and genetic variation will be crucial in developing sustainable and effective management strategies for *P. maxima*.

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