

Chromosomal Characteristics of the Pink Ear Emperor, *Lethrinus lentjan* (Perciformes, Lethrinidae) by Conventional Staining Technique

Piwpuen, N.¹, Tanomtong, A.¹, Getlekha, N.¹, Supiwong, W.²,
& Pinthong, K.^{3*}

บทคัดย่อ

การศึกษาค้างนี้มีจุดมุ่งหมายเพื่อศึกษาแคโรไทป์และอติโอแกรมของปลาหมูสีแก้มแดง (*Lethrinus lentjan* Lacepède, 1802) เตรียมโครโมโซมโดยวิธีทางตรงจากเนื้อเยื่อไตจากปลาเพศผู้และเพศเมียอย่างละ 5 ตัว จากศูนย์วิจัยและพัฒนาทรัพยากรทางทะเลและชายฝั่งทะเลอันดามัน จังหวัดภูเก็ต ด้วยเทคนิคโคลชิซิน-ไฮโปโทนิค-ฟิกเซชัน-แอร์ตรายอิง ย้อมสีโครโมโซมแบบธรรมดาด้วยสีจิมซ่า ผลการศึกษาพบว่า ปลาหมูสีแก้มแดงมีจำนวนโครโมโซมดิพลอยด์เท่ากับ 48 แห่ง โครโมโซมพื้นฐานเท่ากับ 50 แคโรไทป์ ประกอบด้วยโครโมโซมชนิดอะโครเซนทริกขนาดใหญ่ 2 แห่ง เทโลเซนทริกขนาดใหญ่ 32 แห่ง และเทโลเซนทริกขนาดกลาง 14 แห่ง ตรวจไม่พบความแตกต่างของโครโมโซมระหว่างปลาเพศผู้และเพศเมีย ปลาหมูสีแก้มแดงมีสูตรแคโรไทป์ ดังนี้ $2n(48) = L^3_2 + L^1_{32} + M^1_{14}$

คำสำคัญ: ปลาหมูสีแก้มแดง, โครโมโซม, แคโรไทป์, อติโอแกรม

¹ Toxic Substances in Livestock and Aquatic Animals Research Group, Department of Biology, Faculty of Science, Khon Kaen University, Mueang, Khon Kaen. 40002

² Faculty of Applied Science and Engineering, Khon Kaen University, Nong Kai Campus, Mueang, Nong Kai. 43000

³ Department of Fundamental Science, Faculty of Science and Technology, Surindra Rajabhat University, Mueang, Surin. 32000

* Corresponding Author E-mail: k_pinthong@yahoo.com

Abstract

The present study aims to characterize the karyotype and idiogram of pink ear emperor (*Lethrinus lentjan* Lacepède, 1802). Fish chromosome were prepared from kidney tissues using colchicine-hypotonic-fixation-air drying technique and stained with Giemsa dye. Conventional technique was applied to stain the chromosomes. Five males and five females of *L. lentjan* which were reared at Phuket Marine Biological Center, Phuket province were sampled. The results showed that *L. lentjan* has $2n=48$, and the fundamental number (NF) was 50. The karyotype comprised 2 large acrocentric, 32 large telocentric and 14 medium telocentric chromosomes. The chromosomes of male and female of *L. lentjan* were not difference in both sexes. The karyotype formula is as follows: $2n (48) = L^a_2 + L^t_{32} + M^t_{14}$.

Keyword: *Lethrinus lentjan*, Chromosome, Karyotype, Idiogram

1. Introduction

Emperor fish, *Lethrinus* spp. (Family Lethrinidae) are indigenous to the tropical and subtropical Indo-Pacific region, encompassing the Indian Ocean and Western Pacific (Johnson, 1980; Springer, 1982; Carpenter & Allen, 1989). The family Lethrinidae comprises approximately 39 species in 5 genera of emperors and large-eye breams. Lethrinids comprise two subfamilies, the Monotaxinae with *Gnathodentex*, *Gymnocranius*, *Monotaxis*, and *Wattsia*, and the Lethrininae with *Lethrinus* (Carpenter & Johnson, 2001).

The pink ear emperors, *Lethrinus*

lentjan (Lacepède 1802) are common fish of Andaman Sea, Phuket, Thailand (Nateewathana, Aungtonya, & Sirivejabandhu, 1993; Sirimon traporn, Bussarawit, & Nateewathana, 1993; Satapoomin, 1993; Hylleberg & Aungtonya, 2013). It is very common fish for consumption in southern Thailand. However, one species, *L. miniatus*, is sometimes discarded due to the risk of ciguatera poisoning (Carpenter & Allen, 1989; Laboute & Grandperrin, 2000), but most other species are consumed.

Only two species of the *Lethrinus* spp. have been cytogenetically studied, each having the same diploid chromosome number: *L. nebulosus*, $2n=48$ (NF= 48) and *L. xanthochilus*, $2n=48$ (NF=48) (Bo-

chun, Hu, Feng, Xiao-yue, Jia-huan, & Hai-fa, 2009; Arai, 2009) were reported. In this study, we report the standardization of karyotype and idiogram of *L. lentjan*. Due to the external morphology of adult *Lethrinus* species is very similar, the cytotaxonomy of this fish species can be used in species identification and evolutionary study.

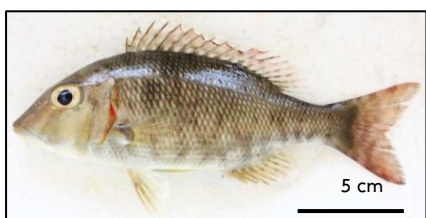


Figure 1 General characteristic of the pink ear emperors (*Lethrinus lentjan*) from Andaman Sea, Phuket of Thailand.

2. Materials and Methods

2.1 Chromosome Preparation

Five males and five females of *L. lentjan* were obtained from the Institute of Phuket Marine Biological Center, in Phuket province, Thailand. The 0.01% colchicine was injected into the fish abdomen (1 mL/100 g body weight) and left for one hour. Chromosomes were prepared from the kidney cells by the squash technique (Chen & Ebeling, 1968; Nanda, Schartl, Feichtinger, Schlupp, Parzefall, & Schmid, 1995). Kidney tissues

were cut into small pieces then mixed with hypotonic solution (0.075 M KCl). After discarding all large pieces of tissues, 7 mL of cell sediments were transferred to a 15 mL centrifuge tube and incubated for 30 min. Hypotonic solution was obtained from cell supernatant after subsequent centrifugation at 1,200 rpm for 10 min. Cells were fixed in a fresh cool fixative (3 absolute methanol: 1 glacial acetic acid) to which up to 7 mL were gradually added before centrifuging again at 1,200 rpm for 10 min, at which time the supernatant was discarded. The fixation was recurrent until the supernatant was cleared and the pellet was mixed with 1 mL fixative. The mixture was dropped onto a clean slide by a plastic pipette followed by air-dry technique.

2.2 Chromosome Staining

Conventional staining with 20% stock Giemsa's solution for 15 min.

2.3 Karyotyping and idiogramming

Standardized karyotypes and idiograms of this fish were constructed. Chromosome counting was performed on mitotic metaphase cells under a light

microscope. Twenty cells of each both sexes with clearly observable and well-spread chromosome were selected for karyotyping.

The length of the short arm (Ls) and long arm (Ll) chromosome were measured and calculated to the total arm length of chromosome (LT, $LT=Ls+Ll$), further the relative length (RL), the Centromeric Index (CI) and Standard Deviations (SD) of RL and CI were calculated. The CI ($q/p+q$) was also estimate to classify the types of chromosome between 0.50-0.59, 0.60-0.69, 0.70-0.89, and 0.90-0.99 were representing the metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively (Chaiyasut, 1989). The fundamental number

(number of chromosome arm, NF) was established by assigning a value of 2 to metacentric, submetacentric, acrocentric chromosomes, and one to telocentric chromosome. All parameters were used in karyotyping and idiogram.

3. Results and Discussion

This study showed the somatic chromosome number of *L. lentjan* was $2n=48$ in both sexes. It was in concordance with the prior study of other fish in the family Lethrinidae (genus *Lethrinus*), *L. nebulosus*, $2n=48$ (Bo-chun *et al.*, 2009) and *L. Xanthochilus*, $2n=48$ (Arai, 2011) (Table 1).

Table 1

Review of cytogenetic publications in the genus *Lethrinus* (Lethrinidae).

Species	2n	Chromosome Formula	NF	Reference
<i>Lethrinus lentjan</i>	48	2a+46t	50	present study
<i>L. nebulosus</i>	48	48t	48	Bo-chun <i>et al.</i> (2009)
<i>L. xanthochilus</i>	48	48t	48	Arai (2011)

Remarks: 2n = diploid chromosome number, NF = fundamental number

The chromosome morphology of *L. Lentjan* was 2 acrocentric and 46 telocentric (2 large acrocentric 32 large telocentric and 14

medium telocentric chromosomes) (Figure 2 and 3).

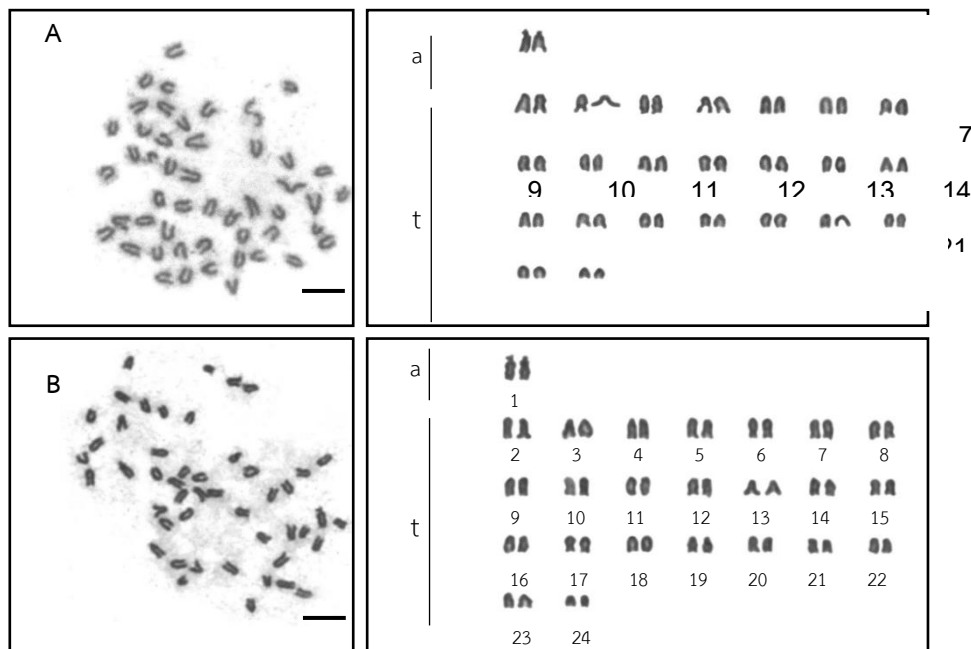


Figure 2 The metaphase chromosome plates and karyotypes of female (A.) and male (B.) pink ear emperors (*Lethrinus lentjan*), $2n=48$ by conventional staining technique. Scale bar indicates 5 micrometers (a = acrocentric chromosome and t = telocentric chromosome)

The chromosome features of *L. Lentjan* was shared among most of the Lethrinidae species previously analyzed, such as *L. nebulosus*, 48t (Bo-chun *et al.*, 2009) and *L. xanthochilus*, 48t (Arai, 2011). Consequently, this is the first report on its chromosome size.

The fundamental number of *L. lentjan* was 50 in both male and female. In comparison with other fish in the family Lethrinidae, *L. lentjan* showed the dissimilar in NF as those

found in *L. nebulosus* and *L. xanthochilus*, $NF=48$ (Bo-chun *et al.*, 2009; Arai, 2011). The differences in NF and karyotype formula might be the result from pericentric inversions of telocentric chromosome (mono-arm) which can be transformed to a new bi-arm chromosome. In addition, the difference in NF values may be due to the variability of fish in this group (Galletti, Aguilar, & Molina, 2000)

Table 2.

Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from 20 metaphase cells of the male and female pink ear emperors (*Lethrinus lentjan*), $2n=48$.

Chro. pair	Ls	Ll	LT	RL±SD	CI±SD	Chro. size	Chro. type
1	0.331	2.215	2.546	0.060±0.004	0.87±0.078	Large	Acrocentric
2	0.000	2.109	2.109	0.057±0.002	1.00±0.000	Large	Telocentric
3	0.000	2.052	2.052	0.056±0.001	1.00±0.000	Large	Telocentric
4	0.000	2.003	2.003	0.054±0.002	1.00±0.000	Large	Telocentric
5	0.000	1.955	1.955	0.053±0.002	1.00±0.000	Large	Telocentric
6	0.000	1.953	1.953	0.052±0.002	1.00±0.000	Large	Telocentric
7	0.000	1.944	1.944	0.052±0.002	1.00±0.000	Large	Telocentric
8	0.000	1.891	1.891	0.051±0.001	1.00±0.000	Large	Telocentric
9	0.000	1.876	1.876	0.051±0.002	1.00±0.000	Large	Telocentric
10	0.000	1.845	1.845	0.050±0.001	1.00±0.000	Large	Telocentric
11	0.000	1.818	1.818	0.049±0.002	1.00±0.000	Large	Telocentric
12	0.000	1.817	1.817	0.048±0.003	1.00±0.000	Large	Telocentric
13	0.000	1.799	1.799	0.048±0.002	1.00±0.000	Large	Telocentric
14	0.000	1.789	1.789	0.048±0.002	1.00±0.000	Large	Telocentric
15	0.000	1.762	1.762	0.047±0.001	1.00±0.000	Large	Telocentric
16	0.000	1.697	1.697	0.046±0.002	1.00±0.000	Large	Telocentric
17	0.000	1.671	1.671	0.045±0.002	1.00±0.000	Large	Telocentric
18	0.000	1.654	1.654	0.045±0.001	1.00±0.000	Medium	Telocentric
19	0.000	1.614	1.614	0.044±0.002	1.00±0.000	Medium	Telocentric
20	0.000	1.564	1.564	0.042±0.002	1.00±0.000	Medium	Telocentric
21	0.000	1.533	1.533	0.036±0.002	1.00±0.000	Medium	Telocentric
22	0.000	1.479	1.479	0.035±0.002	1.00±0.000	Medium	Telocentric
23	0.000	1.335	1.335	0.031±0.003	1.00±0.000	Medium	Telocentric
24	0.000	1.212	1.212	0.026±0.003	1.00±0.000	Medium	Telocentric

Sex chromosome systems was also similar to other species in the family Lethrinidae, no cytologically distinguishable sex chromosome was observed (Bo-chun *et al.*, 2009; Arai, 2011). It is possible that the fish's sex chromosomes are depending on an initiation of differentiation (Na-Naknon, 2000). Thus, chromosomes which contained the sex-determination gene could not be detected by conventional cytogenetic analyses (Na-Naknon, 2000). The karyotype formula for *L. lentjan* is as follows: $2n$ (diploid) $48 = L_2^a + L_{32}^t + M_{14}^t$.

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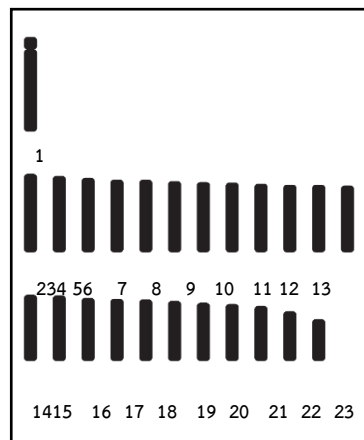


Figure 3 Idiogram showing lengths and shapes of chromosome of the pink ear emperor, (*L. lentjan*), $2n$ (diploid) = 48, by conventional staining technique.

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