Iran. J. Ichthyol. (2022) 9(3): 158-164
P-ISSN: 2383-1561; E-ISSN: 2383-0964

Short Communication

Chromosomes number and mitosis time of Siamese fighting fish (*Betta splendens* Regan, 1910)

Budi Setiadi DARYONO*, Johan Putra KOENTJANA, Ayudha Bahana Ilham PERDAMAIAN

Laboratory of Genetics and Breeding, Faculty of Biology, Gadjah Mada University, Yogyakarta, Indonesia.

Correspondence
bs_daryono@mail.ugm.ac.id

Article history:
Accepted 21 November 2022

Abstract
Siamese fighting fish (*Betta splendens*) is originated from Southeast Asia and one of Indonesia's most profitable exports commodity. However, scientific studies on Siamese fighting fish chromosomes remain limited. Chromosomal information is very useful for disclosing the diversity and genetic relationships of animals and provides clues to the most appropriate conservation measures for a species. Moreover, affordable chromosome visualization procedures are also a main concern for animal research. This research aims to determine the optimal chromosome preparation time, the best method for observing prometaphase, and the chromosomal characteristics of different types of betta fish based on their diploid numbers. The results showed that the optimal time for chromosome preparation is between 07:00 am and 12:00 pm and that the best method for observing chromosomes is the splash method from Kligerman and Bloom modified by soaking samples in colchicine for 11-12 h. The chromosome numbers of Giant, Plakat, Crown tail, and Halfmoon bettas are identical, i.e., 2n=42.

Keywords: Siamese fighting fish, Chromosomes number, Cytogenetic, Mitosis time.

INTRODUCTION

Siamese fighting fish (*Betta splendens*) is originated from Southeast Asia (Purivirojkul 2012; Saekhow et al. 2018) and one of the most popular ornamental fish in Indonesia. Siamese fighting fish is recognized by its coloration, showoff tail, fins, and boldness. This species is known for its diverse breeds (e.g., Halfmoon, Double Tail, Crown tail, Plakat, and Giant) and is able to produce fertile offspring when crossed with other bettas. Siamese fighting fish or commonly known as Betta fish are classified as aggressive fish and like to show off their boldness, flowing tails and fins, and flaring gill. Siamese fighting fish possess short tails and fins, two features resulting from selective breeding (Ramos & Goncalves, 2019).

Siamese fighting fish has a wide range of colorations having unique reproductive and paternal care behavior. Fish exhibits its distinctive behaviors in laboratory setups making this fish valuable animal model for many purposes (Forsatkar et al. 2014; Purivirojkul 2012; HedayatiRad et al. 2017; Saekhow et al. 2018; Eisenreich et al. 2015; Eisenreich et al. 2017; Norazmi-Lokman et al. 2020). This species can be categorized into two groups: ornamental and fighting. Siamese fighting fish with long fins and tails, also known as Slayer, is the forerunner of ornamental bettas. Hobbyists then began to breed and develop ornamental Siamese fighting fish for aquariums (Mandal et al. 2010; Sipaúba-Tavares et al. 2016).

Ornamental bettas are often seen in various exhibitions, and contests to highlight the beauty of the fish. Ornamental Betta has attractive colors, beautiful scales, and proportionate body shapes and is divided into several breeds according to their morphological features, including Halfmoon, Double Tail, Crown tail, Plakat, and Giant (Zhan et al. 2022; Lichak et al. 2022).

The vast variety of Siamese fighting fish in the market is produced from mass cultivation since this fish is capable of producing fertile offspring when crossed with other Bettas. Chromosomal information is useful for disclosing the diversity and genetic relationships of animals and provides clues on the best preservation efforts for a species. This

research aims to investigate the chromosome numbers and the mitotic time of Siamese fighting fish Giant Plakat, Crown tail, and Halfmoon in Indonesia.

MATERIALS AND METHODS

The specimens in this research were obtained from an ornamental fish market (PASTY; Pasar Satwa dan Tanaman Hias Yogyakarta) in Yogyakarta Special Province, Indonesia. Chromosome preparation and observation, and photographs of the samples were taken at the Laboratory of Genetics and Breeding, Faculty of Biology UGM and Laboratory of Aquaculture, Faculty of Fisheries and Marine Science Institute Pertanian Bogor (IPB). The chromosome preparation methods reported by previous authors (Foresti et al. 1993; Tjio & Wang 1960; Klingerman & Bloom 1977) have been modified to improve the obtained results. For example, the speed of centrifugation has been increased to 1200 rpm, and centrifugation is repeated four times. A modification to the coloring process, which involves the use of 3% Giemsa solution for 45min, has been proposed. Modifications to the Kligerman method include incubation with 0.07% colchicine for 11h, increasing the KCl concentration to 0.075 M (by dissolving 5-6 g of KCl in 1 liter of distilled water), and two cycles of soaking for 30min each time.

The use of Carnoy solution with 2 repetitions of soaking glass paraphernalia in 70% alcohol and increasing concentrations of Giemsa to 10% in distilled water with a soaking time of 30-60min have also been suggested. This research was conducted to determine the optimal chromosome preparation time and method for observing the prometaphase of Betta fish. Samples of *B. splendens* gill tissue were collected and prepared for chromosomal observation between 07:00 am to 12:00 pm.

Explain Betta fish characteristics were analyzed, and number of chromosomes was counted. Adobe Photoshop CS5 and PhotoScape were used to edit images, and Image Raster 3 was used to count chromosome numbers.

RESULTS AND DISCUSSION

Siamese fighting fish is one of the most extensive breed of fish in the world (Prost et al. 2020). Many popular betta fish breeds were produced from cross-breeding mating which resulted in fading of the species' identity. The most affordable and inexpensive approach for preliminary genetics study was chromosome visualization through the squashing methods.

Protocols of Foresti et al. (1993) and Tjio et al. (1960) are techniques commonly applied in preparing the chromosomes of fish. The advantages of these methods include small starting materials, low cost, and short preparation time. However, cell clumping and poor visualization of chromosomes limit the applications of these methods. A modification of a third method, the Kligerman (Kligerman et al. 1977) method, is commonly used at the Institute of Aquaculture, Faculty of Fisheries and Marine Sciences IPB University and Center for Research and Development of Ornamental Fish Aquaculture, Depok. While this modified technique requires fewer reagents than the previous methods, it also requires a relatively longer preparation time as the fish must first be incubated in colchicine and glass objects should be soaked in 70% alcohol for 2h before use.

The results of betta fish chromosome preparation according to the Foresti et al. (1993), Tjio et al., (1960), and Kligerman et al. (1977) protocols are shown in Figure. 1. Observation of the chromosome number may be performed via the Kligerman (Kligerman et al. 1977) protocol. In this case, the fish is soaked for 11h, and the optimal preparation time is between 7:00am and 12:00pm.

The tissue samples of different betta breeds were soaked in colchicine at different times. Giant and Halfmoon betta samples were soaked for 11h (Tables 1a, b), and Crown tail betta samples were soaked for 12 h (Table 1c). The calculation results reveals that the diploid numbers of the chromosomes of Giant, Halfmoon, and Crown tail betta are identical at, 2n=42.

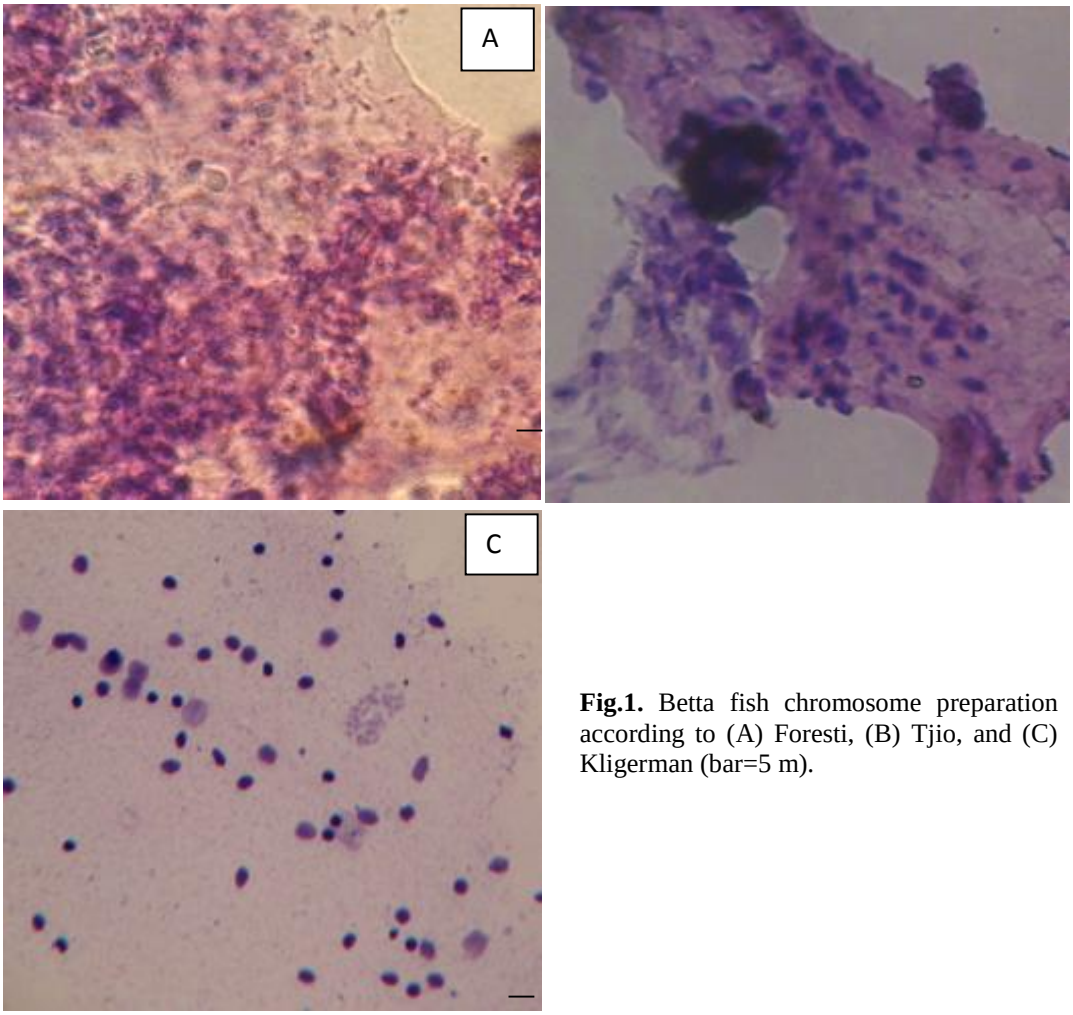


Fig.1. Betta fish chromosome preparation according to (A) Foresti, (B) Tjio, and (C) Kligerman (bar=5 m).

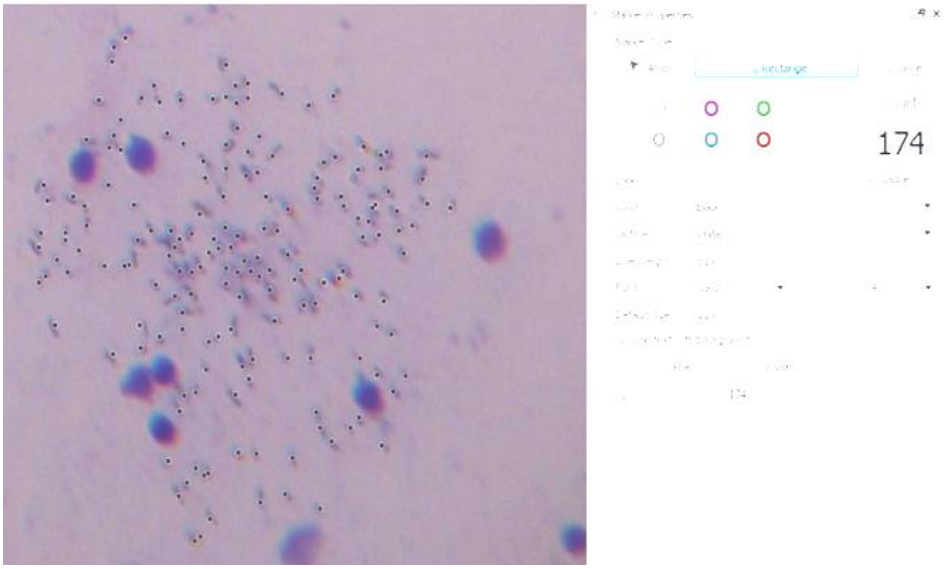


Fig.2. Plakat breed of betta fish (Betta splendens) showing a diploid number of 2n=174.

Plakat betta samples yielded chromosome number of 2n=174 (Fig. 2), which contrasts the chromosome number (i.e., 2n=42) observed in previous research (Ratanatham et al. 1978; Selezniow et al. 2008) (Fig. 3). The unusual chromosome number of Plakat bettas may be attributed to several reasons. Adjacent cell rupture may cause the number of chromosomes calculated

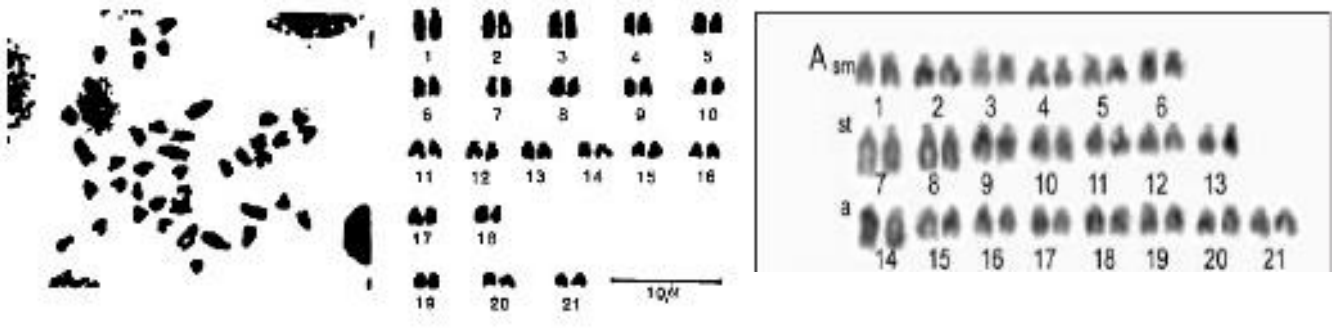
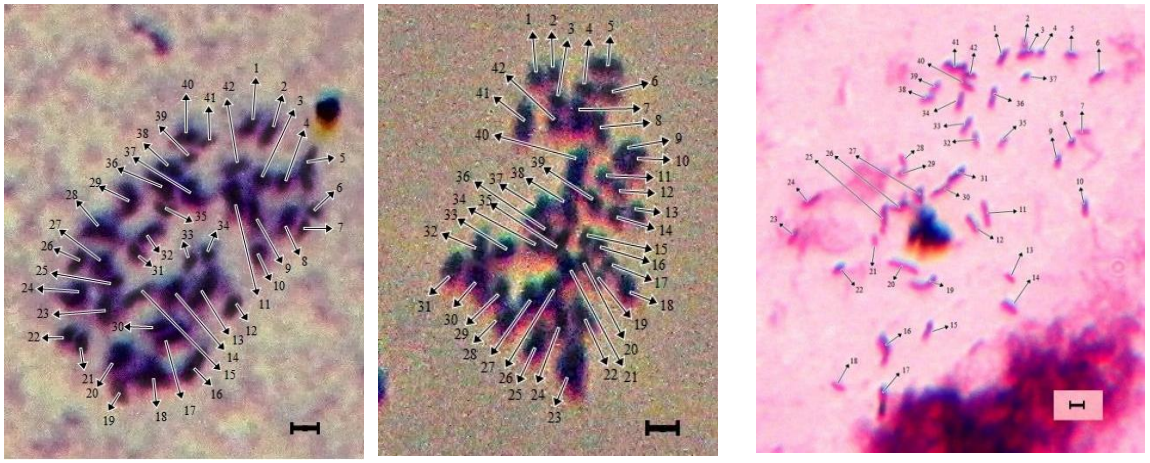


Fig.3. Karyotype of Plakat breed of betta fish (*Betta splendens*).

Table 1. Karyotype visualizations of three Betta fish (*Betta splendens*) breed according to the Kligerman protocol (bar=1µm)**.**

Variable	Colchicines soaking time 11 hours		12 hours
breeds	Giant	Halfmoon	Crown tail
Picture			
Chromosome numbers	42	42	42
			

to exceed the number of chromosomes in the reference. Polyploidy (i.e., tetraploidy) may also occur in this breed (Hu et al. 2019). Finally, the excessive number of chromosomes observed may be a result of excessive excision, which breaks cells.

The results reveal that *B. splendens* has a chromosome number of 2n=42. In another study, among fish from Osphronemidae, *Colisa fasciatus* (Osphronemidae) revealed a chromosome number of 2n=48 (Kaur & Srivastava 1965); *Trichogaster microlepis*, *T. pectoralis*, *T. trichopterus*, *T. leeri*, *Colisa chuna*, and *C. leeri*, have same chromosome number 2n=46 (Ali et al. 2017).

Chromosome preparation using Kligerman's technique has also been applied to African catfish (*Clarias gariepinus*) and zebrafish (*Danio rerio*). The number of chromosomes found in these cases was 2n=56 and 2n=50 (Karami et al. 2015). In the

present study, the karyotype was not presented because the findings are insufficient to construct its shape (the position of the centromere), which requires knowledge of the size and shape of chromosomes. Research on the morphological Karyotype of betta fish is necessary to better understand the format has been done (Ratanatham et al. 1978; Selezniow et al. 2008). Based on previous research (Ratanatham et al. 1978; Selezniow et al. 2008), *Betta splendens* features 14 submetacentric and 28 acrocentric chromosomes.

Other studies on the betta fish karyotype reveal that *B. splendens* has 12 submetacentric (Selezniow et al. 2008), 14 subtelocentric, and 16 acrocentric chromosomes. Differences in karyotype composition may be due to differences in the measurement level of spiralisation chromosomes and data analysis issues related to the small size of the chromosomes (Selezniow et al. 2008).

CONCLUSION

The results of this research reveal that Giant, Plakat, Crown tail, and Halfmoon Bettas have a chromosome number of 2n=42. The optimal time for Betta fish chromosome preparation is between 07:00am and 12:00pm. An effective method to obtain good results is the modified Kligerman's technique.

ACKNOWLEDGEMENTS

The authors would like to acknowledge colleagues from Faculty of Fisheries and Marine Science, IPB University which has provided a cytological analysis training, Dra. Tuty Arysuryanti, M.Sc. Ph.D. and Ganies Riza Aristya M.Sc. Ph.D. from the Faculty of Biology, UGM for the help and suggestions in this research.

REFERENCES

Abu-Almaaty, A.; Hassan, M.; Bahgat, I.M. & Suleiman, M.E. 2017. Inter Simple Sequence Repeat (ISSR) and cytogenetic analysis of three fish species of Family Osphronemidae. Egyptian Journal of Aquatic Biology and Fisheries 21(2): 1-15.

Amini, F. & Sokoot, A.S. 2013. Karyotyping and chromosomal Ag-NOR banding of siamese fighting fish (*Betta splendens*). Journal of Veterinary Research 68(3): 249-255

Eisenreich, B.R. & Szalda-Petree, A. 2015. Behavioral effects of fluoxetine on aggression and associative learning in Siamese fighting fish (*Betta splendens*). Behavioural Processes 121: 37-42.

Eisenreich, B.R.; Greene, S. & Szalda-Petree, A. 2017. Of fish and mirrors: Fluoxetine disrupts aggression and learning for social rewards. Physiology & Behavior 173: 258-262.

Fan, G.; Chan, J.; Ma, K.; Yang, B.; Zhang, H.; Yang, X.; Shi, C.; Chun-Hin Law, H.; Ren, Z.; Xu, Q.; Liu, Q.; Wang, J.; Chen, W.; Shao, L.; Gonçalves, D.; Ramos, A.; Cardoso, S.D.; Guo, M.; Cai, J.; Xu, X.; Wang, J.; Yang, H.; Liu, X. & Wang, Y. 2018. Chromosome-level reference genome of the Siamese fighting fish *Betta splendens*, a model species for the study of aggression. Gigascience 7(11): giy087.

Foresti, F.; Oliveira, C. & Foresti de Almeida-Toledo, L. 1993. A method for chromosome preparations from large fish specimens using in vitro short-term treatment with colchicine. Experientia 49: 810-813.

Forsatkar, M.N.; Nematollahi, M.A.; Amiri, B.M. & Huang, W.B. 2014. Fluoxetine inhibits aggressive behavior during parental care in male fighting fish (*Betta splendens* Regan). Ecotoxicology 23(9): 1794-802.

Hedayati Rad, M., Nematollahi, M.A., Forsatkar, M.N. & Brown, C. 2017. Prozac impacts lateralization of aggression in male Siamese fighting fish. Ecotoxicology and Environmental Safety 140: 84-88.

Hu, F.; Fan, J.; Qin, Q.; Huo, Y.; Wang, Y.; Wu, C.; Liu, Q.; Li, W.; Chen, X.; Cao, L.; Tao, M.; Wang, S.; Zhao, R.; Luo, K. & Liu, S. 2019. The sterility of allotriploid fish and fertility of female autotriploid fish. Frontiers in Genetics 10: 377.

Karami, A.; Eghtesadi Araghi, P.; Syed, M.A. & Wilson, S.P. 2015. Chromosome preparation in fish: effects of fish species and larval age. International Aquatic Research 7: 201-210.

Kligerman, A.D. & Bloom, S.E. 1977. Rapid chromosome preparations from solid tissues of fishes. Journal of Fishery Research Board of Canada 34: 266-269.

Lichak, M.R.; Barber, J.R.; Kwon, Y.M.; Francis, K.X. & Bendesky, A. 2022. Care and use of Siamese fighting fish (*Betta Splendens*) for Research. Comparative

Medicine 72(3):169-180.

Mandal, S.C.; Sahu, N.P.; Kohli, M.P.S.; Das, P.; Gupta, S.K. & Munilkumar, S. 2010. Replacement of live feed by formulated feed: effect on the growth and spawning performance of Siamese fighting fish (*Betta splendens* Regan, 1910). Aquaculture Research 41: 1707-1716.

Norazmi-Lokman, N.H.; Baderi, A.A.; Zabidi, Z.M. & Diana, A.W. 2020. Effects of different feeding frequency on Siamese fighting fish (*Betta splenden*) and Guppy (*Poecilia reticulata*) Juveniles: Data on growth performance and survival rate. Data Brief 32: 106046.

Prost, S.; Petersen, M.; Grethlein, M.; Hahn, S.J.; Kuschik-Maczollek, N.; Olesiuk, M.E.; Reschke, J.O.; Schmey, T.E.; Zimmer, C.; Gupta, D.K.; Schell, T.; Coimbra, R.; De Raad, J.; Lammers, F.; Winter. S. & Janke, A. 2020. Improving the chromosome-level genome assembly of the Siamese fighting fish (*Betta splendens*) in a University Master's Course. G3: Genes, Genomes, Genetics 10(7): 2179-2183.

Purivirojkul, W. 2012. Potential application of extracts from Indian almond (*Terminalia catappa* Linn.) leaves in Siamese fighting fish (*Betta splendens Regan*) culture. Communications in Agricultural and Applied Biological Science 77(4): 439-48.

Ramos, A. & Gonçalves, D. 2019. Artificial selection for male winners in the Siamese fighting fish *Betta splendens* correlates with high female aggression. Frontier in Zoology 16(1): 34.

Ratanatham, S. & Patinawin, S. 1978. Cytogenetic studies of Siamese fighting fish (*Betta splendens* Regan). Journal of the science society of Thailand 5: 17-26.

Saekhow, S.; Thongprajukaew, K.; Phromkunthong, W. &Sae-Khoo, H. 2018. Minimal water volume for intensively producing male Siamese fighting fish (*Betta splendens* Regan, 1910). Fish Physiology and Biochemistry 44(4): 1075-1085.

Selezniow, F.; Fopp-Bayat, G.D.; Jankun, M.; Krejszeff, S. & Mamcarz, A. 2008. Note on the karyotype and nor location of Siamese fighting fish *Betta splendens* (Perciformes, Osphronemidae). Caryologia 61(4): 349-353.

Sipaúba-Tavares, L.H.; Appoloni, A.M.; Fernandes, J.B.K. & Millan, R.N. 2016. Feed of Siamese fighting fish, *Betta splendens* (Regan, 1910) in open pond: live and formulated diets. Brazilian Journal of Biology 76(2): 292-299.

Song, Y.N.; Xiao, G.B. & Li, J.T. 2016. Complete mitochondrial genome of the Siamese fighting fish (*Betta splendens*). Mitochondrial DNA Part A 27(6): 4580-4581.

Tjio, J.H. & Wang, J. 1960. Chromosome preparations of bone marrow cells without prior in vitro culture or in vivo colchicine administration. Stain Technology 37(1): 17-20.

Vu, T.D.; Iwasaki, Y.; Shigenobu, S.; Maruko, A.; Oshima, K.; Iioka, E.; Huang, C.L.; Abe, T.; Tamaki, S.; Lin, Y.W.; Chen, C.K.; Lu, M.Y.; Hojo, M.; Wang, H.V.; Tzeng, S.F.; Huang, H.J.; Kanai, A.; Gojobori, T.; Chiang, T.Y.; Sun, H.S.; Li, W.H. & Okada, N. 2020. Behavioral and brain- transcriptomic synchronization between the two opponents of a fighting pair of the fish *Betta splendens*. PLOS Genetics 16(6): e1008831.

Zhang, W.; Wang, H.; Brandt, D.Y.C.; Hu, B.; Sheng, J.; Wang, M.; Luo, H.; Li, Y.; Guo, S.; Sheng, B.; Zeng, Q.; Peng, K.; Zhao, D.; Jian, S.; Wu, D.; Wang, J.; Zhao, G.; Ren, J.; Shi, W.; van Esch, J.H.M.; Klingunga, S.; Nielsen, R. & Hong, Y. 2022. The genetic architecture of phenotypic diversity in the Betta fish (*Betta splendens*). Science Advance 8(38): eabm4955.

Iran. J. Ichthyol. (2022) 9(3): 158-164
P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

doi: 10.22034/iji.v9i3.845
dor: 20.1001.1.23831561.2022.9.3.4.3

مقاله کوتاه

تعداد کروموزوم‌ها و زمان میتوز ماهی فایتر سیامی (*Betta splendens*)

بودی ستیادی داریونو *، یوهان پوترا کونتیانا، اودها بهانا الهام پردامایان

آزمایشگاه ژنتیک و اصلاح، دانشکده زیست شناسی، دانشگاه گدجه مادا، یوگیاکارتا، اندونزی.

چکیده: ماهی فایتر سیامی (*Betta splendens*) با منشاء آسیای جنوب شرقی، یکی از سودآورترین ماهیان صادراتی اندونزی محسوب می‌شود. با این حال، مطالعات بر روی کروموزوم‌های ماهی فایتر سیامی محدود است. اطلاعات کروموزومی برای نمایش تنوع و روابط ژنتیکی حیوانات بسیار مفید است و شواهدی مناسب در راستای اقدامات حفاظتی یک گونه ارائه می‌دهد. علاوه بر این، روش‌های مقرون به صرفه تصویرسازی کروموزومی یکی از نگرانی‌های اصلی در تحقیقات حیوانی است. این پژوهش، با هدف تعیین زمان بهینه آماده‌سازی کروموزوم، بهترین روش برای مشاهده مرحله پرومتافاز و خصوصیات کروموزومی انواع مختلف ماهی *B. splendens* براساس شمارش دیپلوئید آن‌ها انجام شده نتایج نشان داد که زمان بهینه برای آماده‌سازی کروموزوم بین ساعت ۰۷:۰۰ قبل از ظهر تا ۱۲:۰۰ بعد از ظهر بوده و بهترین روش برای مشاهده کروموزوم‌ها روش اسپلش کلیگرمن و بلوم اصلاح‌شده با غوطه‌ور کردن نمونه‌ها در کلشی‌سین به مدت ۱۲-۱۱ ساعت است. اعداد کروموزومی واریته‌های این گونه شامل بزرگ جثه، پلاکت، دم تاجدار و هلالی به‌صورت ۲n=۴۲ تشخیص داده شد.

کلمات‌کلیدی: ماهی مبارز سیامی، تعداد کروموزوم، سیتوژنتیک، زمان میتوز، اسپلش.