Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
https://riviste.fupress.net/index.php/caryologia

OPEN ACCESS

Citation: Getlekha, N. & Sribenja, K. (2024). Robertsonian rearrangements in the genome of the azure damselfish, *Chrysiptera hemicyanea* (Perciformes, Pomacentridae). *Caryologia* 77(4): 33-42. doi: 10.36253/caryologia-3148

Received: November 1, 2024

Accepted: March 31, 2025

Published: July 15, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Robertsonian rearrangements in the genome of the azure damselfish, *Chrysiptera hemicyanea* (Perciformes, Pomacentridae)

Nuntaporn Getlekha[1], Kamika Sribenja[2,*]

[1] *Department of Biology, Faculty of Science and Technology, Muban Chombueng Rajabhat University, Chombueng, Ratchaburi 70150, Thailand*
[2] *Department of General Science, Faculty of Science and Technology, Muban Chombueng Rajabhat University, Chombueng, Ratchaburi 70150, Thailand*
*Corresponding author. E-mail: kamikasri@mcru.ac.th

Abstract. Cytogenetic studies on the azure damselfish, *Chrysiptera hemicyanea* (Weber, 1913), revealed a karyotype with 2n = 31 chromosomes (17 metacentric, 2 submetacentric, and 14 telocentric; FN = 52). The study found Robertsonian polymorphisms, which involve small heterochromatic regions at the centromeres. Nucleolar organizer regions (NORs) were observed near the ends of the long arms on the large metacentric chromosome pair (pair 2). FISH analysis, which detects specific DNA sequences, revealed notable variability in the distribution of ribosomal DNA (5S and 18S rDNAs) along the chromosomes. Specifically, the 18S rDNA was located at the ends of the long arms on the large metacentric chromosomes (pair 2), while the 5S rRNA genes were found near the centromere of another large metacentric chromosome pair (pair 3). The analysis also showed that repetitive DNA sequences $(CA)_{15}$, $(GA)_{15}$, and $(CAA)_{10}$ were spread across the subtelomeric and telomeric regions of various chromosomes. The study suggests that the structure of the karyotype and chromosome number are linked to the Robertsonian rearrangements observed, highlighting their significant role in the evolutionary changes in the karyotype of the genus *Chrysiptera*.

Keywords: azure damselfish, Chromosome, ribosomal DNA, Robertsonian rearrangements.

INTRODUCTION

When comparing the karyotypic (chromosomal) diversity of freshwater and marine fish, freshwater species exhibit more chromosomal changes. This increased diversity in freshwater environments is due to the numerous physical barriers present in continental waters, which limit gene flow and lead to greater karyotypic variation. In contrast, marine environments present complex and harder-to-define barriers, shaped by dynamic factors like ocean currents and winds, which can facilitate wider dispersal of species and lead to less distinct karyotypic differentiation. Genetic connectivity among marine fish popula-

tions, linked to changes in their karyotypes, is influenced not only by physical and ecological conditions but also by the pelagic larval stage, which is crucial for their dispersal (Brum & Galetti, 1997; Molina & Galetti, 2004; Martinez et al., 2015; Santos et al., 2024). Among Perciformes, the Pomacentridae family, which includes damselfish and clownfish, is particularly notable due to its close association with coral reefs (Allen and Werner 2002; Bellwood and Wainwright 2002; Tang et al., 2021). This family displays significant diversity in forms and biological traits, making it a useful model for studying how the pelagic (open water) period of larvae affects karyotypic evolution (Molina and Galetti, 2004; McCord et al., 2021).

As we advance into the era of genomic natural history, where genomic technologies offer significantly greater detail and statistical power, a reference genome will be crucial for enhancing our understanding of animal biology (Hotaling et al., 2021). Currently, there are 14 reference genomes available for Pomacentrids (Roberts et al., 2023), genetic variation in reef fish populations has been well-documented through studies of allozymes (variant forms of enzymes) and mitochondrial DNA (Knowlton et al., 1993; Shulman and Bermingham, 1995; Molina and Galetti, 2002; Limon et al., 2023). Despite this variation at the genetic level, many reef fish species maintain relatively stable chromosomal structures, even over extensive geographical ranges. A key factor in karyotypic differentiation among fish is Robertsonian chromosomal rearrangements, such as centric fusion (where two acrocentric chromosomes fuse at their centromeres) and fission (where a single chromosome splits into two). These rearrangements contribute significantly to chromosomal diversity and differentiation in various fish groups (Molina and Galetti, 2002; Getlekha et al., 2017).

In Pomacentridae reef fish, Robertsonian chromosomal rearrangements, particularly centric fusion, have been observed in various contexts. For example: *Dascyllus*: Centric fusion appears as a polymorphic trait, meaning different individuals or populations within the genus may exhibit this rearrangement (Ojima and Kashiwagi, 1981; Takai A., 2012; Getlekha et al., 2016a; Getlekha et al., 2017). *Chromis*: This genus also shows centric fusion, indicating its role in karyotypic diversity (Molina and Galetti, 2002). *Chrysiptera*: Centric fusion has been established in the derived karyotypes of specific species, highlighting its role in evolutionary changes (Takai and Ojima, 1995; Galetti et al. 2000; Hardie and Hebert, 2004; Molina and Galetti 2004). Moreover, new fish sex chromosomal systems have been associated with Robertsonian rearrangements (Brum et al., 1992). These rearrangements might contribute to the formation of genetically distinct populations by impeding gene flow.

In this study, we investigated the karyotype, heterochromatin pattern, and nucleolar organizer regions of the fish species *C. hemicyanea*. We employed several methods to analyze and identify chromosomal features: Microsatellite Sequences: Specific microsatellite markers, including $(CA)_{15}$, $(GA)_{15}$, and $(CAA)_{10}$, were used to examine chromosomal patterns and identify specific chromosomes involved in the rearrangements. Repetitive DNA Probes: Fluorescence *in situ* hybridization (FISH) with probes for 18S rDNA and 5S rDNA was used to localize ribosomal RNA genes and further characterize chromosomal structures. These techniques helped identify chromosomes involved in Robertsonian rearrangements, contributing to a deeper understanding of the chromosomal evolution and structure in *C. hemicyanea*.

MATERIAL AND METHODS

For the cytogenetic study, ten male and ten female azure damselfish (*Chrysiptera hemicyanea*) samples were collected from the Gulf of Thailand (Pacific Ocean). Chromosomes were prepared using the techniques outlined by Getlekha et al. (2016a). The method for detecting nucleolar organizer regions (Ag-NORs) was based on Howell and Black (1980), while the visualization of heterochromatin bands (C-bands) followed the method described by Sumner (1972).

Fluorescence *in situ* hybridization (FISH) was carried out on the chromosomes of *C. hemicyanea* following the procedure described by Martins and Galetti (1998). For this, two DNA sequences, 5S rDNA and 18S rDNA, were used as probes. The sequences were amplified from nuclear DNA using PCR and derived from the genome of the fish species *Hoplias malabaricus*, which belongs to the Erythrinidae family. The probes were cloned into plas-

Figure 1. General characteristic of the azure damselfish (*Chrysiptera hemicyanea* (Weber, 1913)).

mid vectors using competent *Escherichia coli* DH5α cells (Invitrogen, San Diego, CA, USA). After cloning, the 5S and 18S rDNA probes were labeled with Spectrum Green-dUTP and Spectrum Orange-dUTP, respectively, following Roche's protocols (Mannheim, Germany) for nick translation. Additionally, Sigma (St. Louis, MO, USA) synthesized the microsatellites $(CA)_{15}$, $(GA)_{15}$, and $(CAA)_{10}$, which were directly tagged with Cy3 at the 5' end.

High stringency conditions were used for fluorescence *in situ* hybridization (FISH) to ensure precise results. Initially, metaphase chromosomal slides were treated with 40 µg/ml RNase for 1.5 hours at 37°C to eliminate background interference and remove RNA. The hybridization solution was prepared with 10% dextran sulfate to enhance probe penetration, 2.5 ng/µl of labeled probes (microsatellites and rDNA), 2 µg/µl salmon sperm DNA to block non-specific binding, and 50% deionized formamide to minimize non-specific interactions. This mixture was then applied to the slides. To prepare the chromosomes for hybridization, the chromosomomal DNA was denatured in a solution of 70% formamide/2x SSC at 70°C for 4 minutes to prepare them for hybridization. The slides were then incubated overnight at 37°C in a moist chamber with 2x SSC buffer to allow the probes to bind to their target sequences. To reduce non-specific binding, excess unbound probes were removed by washing the slides first at 65°C for 5 minutes with 2x SSC and then at room temperature for 5 minutes with 1x SSC. The slides were mounted in Vectashield antifade solution to prevent photobleaching and enhance fluorescence, and counterstained with DAPI to visualize the DNA. This careful preparation ensured that the FISH analysis could detect microsatellite and rDNA sequences with high specificity and clarity.

After that, metaphase spreads were captured with a CoolSNAP camera and analyzed using Image Pro Plus 4.1 software. Imaging was performed with an Olympus BX50 microscope.

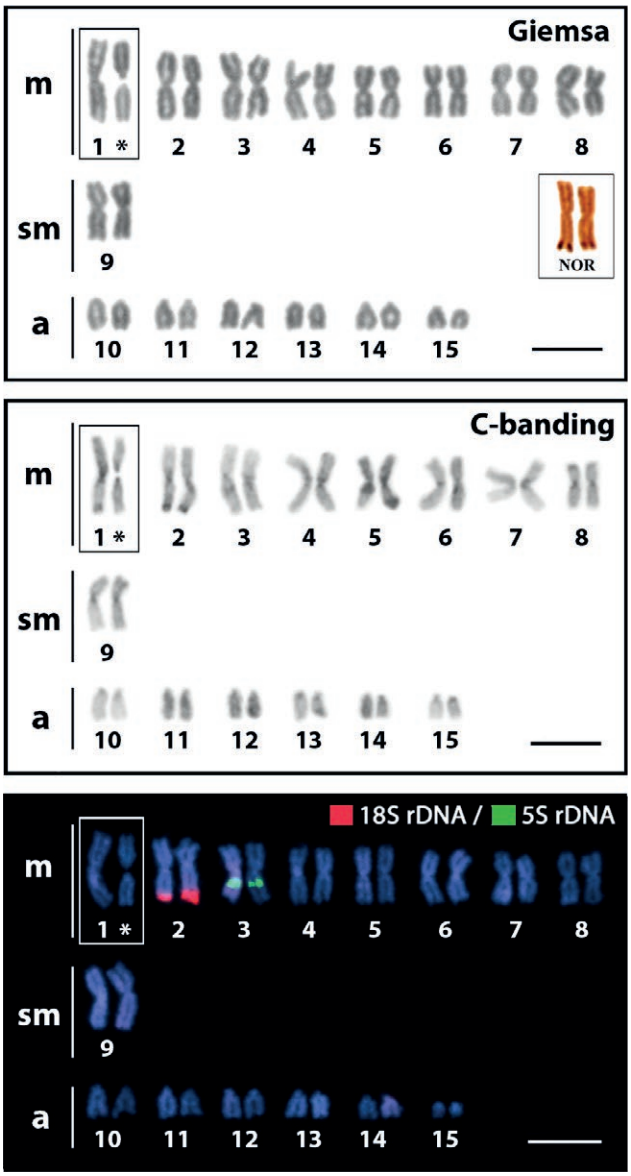

Figure 2. Karyotypes of *Chrysiptera hemicyanea* were analyzed using several methods: Giemsa staining, Ag-NOR banding (highlighted in the boxes), C-banding, and fluorescence *in situ* hybridization with 5S and 18S rDNA probes. Chromosomes involved in centric fusions are indicated in the larger boxes. Scale bar = 5 µm.

RESULTS

The azure damselfish (*Chrysiptera hemicyanea*) has a karyotype with 31 chromosomes and a fundamental number of 52 in both male and female, including 17 metacentric, 2 submetacentric, and 14 acrocentric chromosomes. Notably, the karyotype features large metacentric chromosomes that are nearly twice as large as the others. Nucleolar organizer regions (Ag-NORs) were located at the ends of the long arms on one pair of these large metacentric chromosomes. The C-banding analysis showed small blocks of heterochromatin primarily at the centromeres of most chromosomes.

FISH analysis showed that 18S rDNA clusters were located at the telomeric ends of two large metacentric chromosome pairs (pair No. 2). On the other hand, 5S rDNA genes were exclusively present in the pericentromeric regions of two different large metacentric chromosomes (pair No. 3). These results offer valuable information about the genomic arrangement of the azure dam-

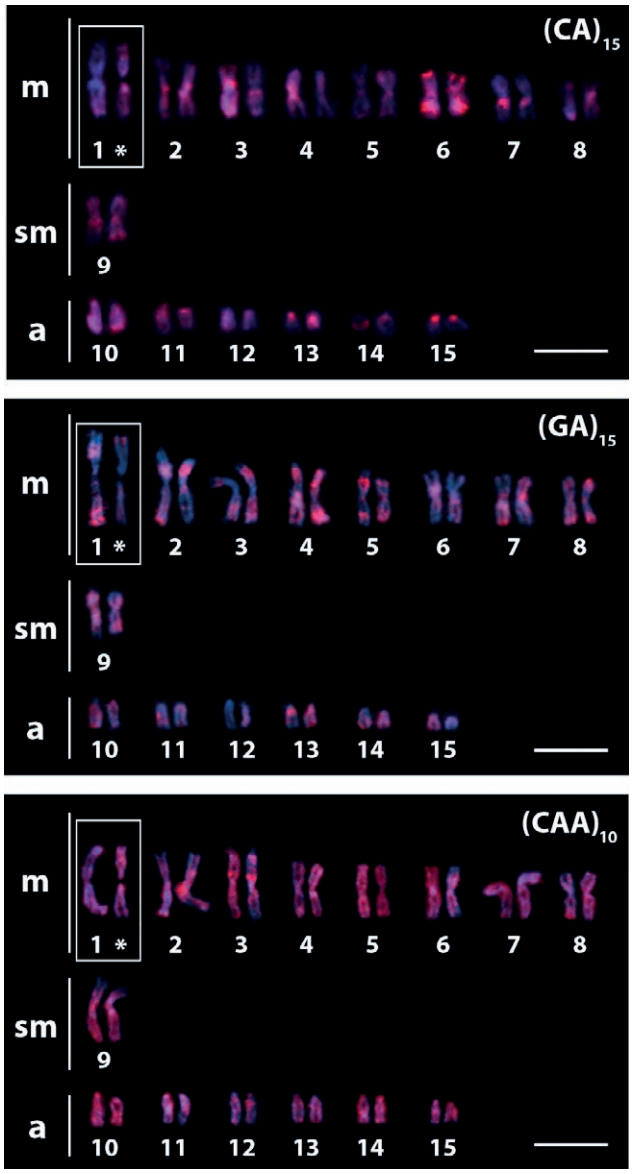

Figure 3. Fluorescence *in situ* hybridization was used to map the chromosomes of *Chrysiptera hemicyanea* with di- and tri-nucleotide microsatellites. The distribution patterns for (CA)15, (GA)15, and (CAA)10 microsatellites as probes are illustrated. Scale bar = 5 μm.

selfish, particularly in relation to the placement of ribosomal DNA sequences on its chromosomes.

Microsatellite sequences in *C. hemicyanea* are distributed unevenly across its chromosomes, as revealed by chromosomal mapping. Strong hybridization signals were observed in both the telomeric and interstitial regions of many chromosomes, indicating a broad distribution. Specifically, the $(CA)_{15}$ microsatellite sequence was primarily found on metacentric chromosome No. 6. Conversely, the $(GA)_{15}$ sequence, though weaker than $(CA)_{15}$, was distributed throughout the interstitial regions of all chromosomes. The $(CAA)_{10}$ sequence showed strong signals on several chromosomes. No significant differences were observed between males and females, suggesting that microsatellite distribution is similar in both sexes. These findings, which highlight the varied and uneven distribution of microsatellite sequences, provide valuable insights into the genomic organization and diversity of *C. hemicyanea*.

DISCUSSION

Recent genetic research on marine fish has shed light on how speciation occurs, emphasizing the importance of chromosome rearrangements in this process (King, 1987; Crandall et al., 2019). These chromosomal changes are thought to play a key role in the formation of new species. In freshwater fish, species with limited mobility and smaller reproductive populations often show greater karyotypic diversity, with a wider range of chromosome structures (Bertollo et al., 1979; Moreira-Filho and Bertollo, 1991; Sribenja and Getlekha, 2024a,b). In contrast, marine fish generally display more stable karyotypes due to fewer physical barriers, higher mobility, larger populations, and more consistent environmental conditions (Brum, 1995; Liggins et al., 2016).

In some families of Perciformes, such as the Gobiidae, distinct cytogenetic traits can help differentiate populations, with notable chromosome polymorphism linked to centric fusions and fissions (Giles et al., 1985; Vitturi and Catalano, 1989; Amores et al., 1990). However, in other fish groups, cytogenetic data may not provide clear markers for species identification, even in widely distributed species (Rossi et al., 1996). This lack of clear differentiation due to significant chromosomal rearrangements might be countered by internal changes within linkage groups, which can contribute to post-zygotic barriers essential for speciation (Molina et al., 2002).

The patterns of chromosomal rearrangements between Pomacentridae genome assemblies remain unclear. Numerous cytogenetic studies have explored how variations in chromosome number influence fish mobility, revealing an inverse relationship between chromosome diversity and mobility. Furthermore, chromosome rearrangements have been found to either facilitate or inhibit recombination events (Galetti et al., 2000; Molina and Galetti, 2004; Kirkpatrick and Barton, 2006; Martinez et al., 2015). Robertsonian polymorphisms play a significant role in karyotype variation across different Perciformes families, including Pomacentridae, Gobiidae, and Cichlidae. In addition to verifying variations in chromosome number, the dot-plot

comparison between this genome and that of the closest relative with a chromosome-scale assembly uncovered multiple rearrangements across all corresponding chromosomes (Roberts et al., 2023). For instance, in the Pomacentridae family, most species have a relatively stable karyotype with 48 chromosomes. However, Chromosome formulae vary widely (FN = 48–90), often due to pericentric inversions that contribute to karyotype diversification (Takai and Ojima, 1987). In contrast, the Chrominae subfamily, which includes genera such as *Acanthochromis*, *Azurina*, *Chromis*, and *Dascyllus*, exhibits notable Robertsonian polymorphisms. Diploid numbers within this subfamily vary considerably: *D. trimaculatus* (47–48), *D. reticulatus* (34–37), *D. aruanus* (27–33), *C. insolata* (46–47), and *C. flavicauda* (39) (Ojima and Kashiwagi, 1981; Molina and Galetti, 2002; Getlekha et al., 2016a,2017; Yuan et al., 2018). Although asynchronic hermaphroditism is prevalent among these species, the existence of multiple sexual chromosomes does not seem to correlate with the karyotypic variations observed (Ojima and Kashiwagi, 1981; Getlekha and Tanomtong, 2020). This assembly will provide a crucial basis for examining how genome structure varies at a metapopulation level and how these variations influence recombination and adaptation.

Gene flow among Pomacentridae populations mainly occurs through the drifting or active migration of pelagic larvae, as the adult fish are generally nonmigratory (Allen, 1991; Robitzch et al., 2016). Current evidence does not indicate a direct relationship between the length of the larval stage and the geographic range of these fish. Some researchers suggest that the complex behaviors of fish larvae (Leis and Carson-Ewart, 1998) might result in hydrodynamic movements that could actually limit their dispersal rather than facilitate it (Doherty et al., 1994; Salas et al., 2020).

Previous research on the genus *Chrysiptera* have revealed differences in the diploid chromosome number and fundamental number among species, which are caused by chromosomal rearrangements (Table 1). Centric fusions likely account for the species with fewer chromosomes and distinctive metacentric chromosomes. This chromosomal variation could be a transient phenomenon, reflecting a specific stage in the evolutionary process within the *Chrysiptera* species, potentially indicative of karyotypic orthoselection (White, 1973; Artoni et al., 2015; Santos et al., 2024).

In *C. hemicyanea*, the nucleolar organizer regions (NORs) reflect their evolutionary connections. Although the NORs are positioned at the ends of metacentric chromosomes in *C. hemicyanea*, their distribution pattern is quite similar to that observed in other Pomacentrid species, despite being located at a subterminal position on the metacentric chromosome (2nd pair) (Takai and Ojima, 1987; Takai and Ojima, 1995; Artoni et al., 2015).

Chromosomal regions that are differentially stained using C-banding techniques, known as C-bands, highlight localized areas of constitutive heterochromatin. In fish, C-bands are primarily found in centromeric regions, and occasionally in telomeric and interstitial regions (Gold et al., 1986; Takai and Ojima, 1988; Kashiwagi et al., 2005). Although there is considerable variation in the distribution of C-banded heterochromatin among chromosomes and species, many fish species exhibit C-bands as small dot-like formations predominantly in centromeric regions, with often weak staining. This relatively simple distribution of C-bands appears to be a fundamental characteristic in fish. In *C. hemicyanea*, the distribution patterns of centromeric C-bands, appearing as small dot-like spots in centromeric and telomeric regions, are consistent with previous cytogenetic studies. However, *C. hemicyanea* also displays distinctive C-bands in the terminal regions of the long arms of NOR-bearing chromosomes (pair no. 2). These observations suggest that *C. hemicyanea* has a notably differentiated karyotype with respect to the distribution of constitutive heterochromatin.

Table 1. Karyotype data of some genus *Chrysiptera*.

Species	2n	NF	Formula	NORs	Reference
Chrysiptera cyanea	42	66	6m+16sm+2st+18a	2	Takai and Ojima, 1995
C. leucopoma	48	80	4m+22sm+6st+16a	2	Takai and Ojima, 1995
C. rex	36	58	12m+10sm+14st-a	2	Takai and Ojima, 1995
C. rollandi	46	50	2sm+2st+42a	2	Kasiroek et al., 2014
C. starckii	48	60	2m+10sm+36a	2	Takai and Ojima, 1987
C. hemicyanea	48	78	30sm+10st+8a	2	Takai and Ojima, 1999
	31	56	8m+10sm+32a	2	present study

2n = diploid number, NF = fundamental number, NORs = nucleolar organizer regions, m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric chromosome.

The distribution of C-bands in pomacentrids varies widely, ranging from simple to complex patterns (Takai and Ojima, 1999). These variations highlight the significance of constitutive heterochromatin in chromosome evolution. The C-band distribution in *C. hemicyanea*, which exhibits G-band-like patterns (Takai and Ojima, 1999), appears to be a rare phenomenon among fish chromosomes. This pattern represents one of the most differentiated conditions not only within Pomacentridae but also among teleostean fish.

The alignment of 18S rDNA probes with the Ag-NOR clusters in *C. hemicyanea* suggests that having ribosomal DNA clusters on a single chromosomal pair is a fundamental trait for the Pomacentridae family (Molina, 2000; Getlekha et al., 2016a, b). In contrast, 5S rDNA clusters were located on a different pair of metacentric chromosomes, specifically subterminally on the long arm, and did not overlap with the chromosome pair containing the NORs. Previous research on 5S rRNA gene mapping has been conducted in genera like *Chromis*, *Dascyllus*, *Abudefduf*, and *Pomacentrus* (Molina and Galetti, 2002; Getlekha et al., 2016a, b; 2018; Zhang et al., 2021). The 5S rDNA loci appear to be more conserved in Pomacentridae, usually positioned in the pericentromeric region of a chromosome. In summary, various FISH applications using 18S and 5S ribosomal genes have proven effective in establishing phylogenetic relationships, distinguishing species, and understanding historical population dynamics in both freshwater and marine environments (Artoni et al., 2015).

Microsatellite sequences like $(CA)_{15}$, $(GA)_{15}$, and $(CAA)_{10}$ show significant variation in their distribution across the chromosomes of *C. hemicyanea*. Most of these sequences are concentrated in telomeric regions, which are rich in DNA repeats. Their clustering on specific chromosomes suggests that repetitive DNA dynamics could influence divergence among pomacentrid fish species. Research has shown that repetitive DNAs are crucial in the evolution of genomes across different fish species (Cioffi and Bertollo, 2012; Moraes et al., 2017; Saenjundaeng et al., 2018, 2020; Sassi et al., 2019; Yano et al., 2014; Yüksel and Gaffaroglu, 2008). In this study, microsatellites $(CA)_{15}$, $(GA)_{15}$, and $(CAA)_{10}$ are present on all chromosomes of *C. hemicyanea*, with notable clusters showing strong signals in specific regions (Figure 3). Recent and previous studies indicate that microsatellites are commonly located in heterochromatic regions of fish genomes (Getlekha et al., 2016a, b; 2018).

Investigating evolutionary relationships within the genus *Chrysiptera* necessitates analyzing these data. Despite differences in chromosomal architecture, similar microsatellite distribution patterns might indicate shared evolutionary histories. Conversely, variations in these patterns could highlight rapid changes in microsatellite sequences (Cioffi et al., 2011; Molina et al., 2014a, b). Analyzing microsatellite sequence structures provides valuable insights into the functional diversification and organization of repeated DNAs across species. However, studies examining how chromosome structure influences ecology, population dynamics, and adaptive evolution are facilitated by chromosome-scale genomes, which provide more detailed gene sequences and their locations (Roberts et al., 2023). Understanding the distribution and evolution of microsatellite sequences in *C. hemicyanea* enhances our knowledge of genomic dynamics and evolutionary processes in pomacentrid fishes. This insight also aids in more thorough investigations of genetic diversity and speciation.

Our focus is on Robertsonian rearrangements in the karyotypes of *C. hemicyanea*. According to Ojima (1983), in higher teleostean groups, including Pomacentridae, the average number of subtelo- and acrocentric (A-type) chromosomes is 38.3, while meta- and submetacentric (M-type) chromosomes average 7.5. In contrast, the pomacentrids studied show an average of 30.2 A-type chromosomes and 17.1 M-type chromosomes. This indicates that Pomacentridae has undergone significant structural changes, with a notable transition from A-type to M-type chromosomes.

CONCLUSION

Teleostean fishes frequently display a notable karyotypic characteristic: many species possess 48 diploid chromosomes, with a majority being acrocentric. This observation suggests a conservative pattern in the evolution of fish karyotypes (Ohno, 1974; Ojima, 1983). Gosline (1971) and Ojima (1983) noted that this karyotypic feature is especially prominent in intermediate and higher teleostean groups. Ohno (1974) proposed that a karyotype consisting of 48 acrocentric chromosomes (48A karyotype) might represent the ancestral form in fish evolution, as it appears across various fish families and orders.

In the Pomacentridae family, diploid chromosome numbers vary from 26 to 48, and fundamental numbers range from 48 to 84. Some species within this family also exhibit the 48A karyotype. It is hypothesized that the 48A karyotype could have been ancestral for Pomacentridae, with subsequent diversification occurring primarily through pericentric inversions and Robertsonian rearrangements (Takai and Ojima, 1987, 1991, 1995). Furthermore, this study proposes that the karyotypic diversification in pomacentrids has been influenced not

only by structural chromosomal changes but also by variations in the amount and placement of constitutive heterochromatin. In the future, telomeric probes will be tested to further enhance the understanding characterization of the karyotype of *Chrysiptera hemicyanea*.

ACKNOWLEDGEMENTS

The authors appreciate the correctness verification of the paper and the invaluable assistance provided by the Toxic Substances in Livestock and Aquatic Animals Research Group at Khon Kaen University and and Department of Biology, Faculty of Science and Technology, Muban Chombueng Rajabhat University that provided support for this study. We thank the Thailand Science Research and Innovation (Fundamental Fund; FF) for the financial support.

REFERENCES

Allen GR, Werner TB. 2002. Coral reef fish assessment in the 'coral triangle' of Southeastern Asia. Environ Biol Fishes. 65(2): 209–214. https://doi.org/10.1023/A:1020093012502

Allen GR. 1991. Damselfishes of the World. Mergus Publishers, Melle, Germany.

Amores A. Giles V. Thode G. Alvarez MC. 1990. Adaptative character of a Robertsonian fusion in chromosomes of the fish *Gobius paganellus* (Pisces, Perciformes). Heredity. 65: 150–155.

Artoni RF, Castro JP, Jacobina UP, Lima-Filho PA, da Costa GWWF, Molina, WF. 2015. Inferring diversity and evolution in fish by means of integrative molecular cytogenetics. The Scientific World Journal, 2015, 365787. https://doi.org/10.1155/2015/365787

Bellwood DR, Wainwright PC. 2002. Chapter 1: the history and biogeography of fishes on coral reels. In: Coral Reef Fishes. Elsevier Science.

Bertollo LAC, Takahashi CS, Moreira-Filho O. 1979. Karyotypic studies of two allopatric populations of the genus *Hoplias* (Pisces, Erythrinidae). Rev. Bras. Biol. 2: 17–37.

Brum MJI. Galetti PM. 1997. Teleostei ground plan karyo- type. J Comp Biol. 2: 91–102.

Brum MJI, Galetti PM, Corrêa MMO, Aguilar CT. 1992. Multiple Sex chromosomes in South Atlantic fish, *Brevoortia aurea*, Clupeidae. Braz J Genet. 15: 547–553.

Brum MJI. 1995. Correlation between phylogeny and cytogenetics of teleost fishes. Se. Monogr.-Soc. Bras. Genet. 2: 5–42.

Cioffi MB, Bertollo LAC. 2012. Distribution and evolution of repetitive DNAs in fish. In: Garrido- Ramos MA (Ed), Repetitive DNA. Karger, Genome Dyn. 7: 197–221.

Cioffi MB, Molina WF, Moreira-Filho O, Bertollo LAC. 2011. Chromosomal distribution of repetitive DNA sequences high- lights the independent differentiation of multiple sex chromosomes in two closely related fish species. Cytogenet Genome Res. 134: 295–302.

Crandall ED, Riginos C, Bird CE, Liggins L, Treml E, Beger M, Barber PH, Connolly SR, Cowman PF, DiBattista JD. 2019. The molecular biogeography of the Indo-Pacific: testing hypotheses with multispecies genetic patterns. Global Ecol Biogeogr. 28(7): 943–960. https://doi.org/10.1111/geb.12905.

Doherty PJ, Mather P. Planes S. 1994. *Acanthochromis polyacanthus*, a fish lacking larval dispersal, has genetically differentiated populations at local and regional scales on the Great Barrier Reef. Mar. Biol. 121: 11–21.

Galetti PM, Aguilar CT, Molina WF. 2000. An overview of marine fish cytogenetics In: Solé-Cava AM, Russo CAM, Thorpe JP, editors. Marine Genetics. Dordrecht: Springer Netherlands 55–62. https://doi.org/10.1007/978-94-017-2184-4_6.

Getlekha N, Cioffi MB, Maneechot N, Bertollo LAC, Supiwong W, Tanomtong A, Molina WF. 2018. Contrasting Evolutionary Paths Among Indo-Pacific Pomacentrus Species Promoted by Extensive Pericentric Inversions and Genome Organization of Repetitive Sequences. Zebrafish. 15(1): 45–54.

Getlekha N, Cioffi MB, Yano CF, Maneechot N, Bertollo LAC, Supiwong W, Tanomtong A, Molina WF. 2016b. Chromosome mapping of repetitive DNAs in sergeant major fishes (Abudefdufinae, Pomacentridae): a general view on the chromosomal conservatism of the genus. Genetica. 144: 567–576.

Getlekha N, Molina WF, Cioffi MB, Yano CF, Maneechot N, Bertollo LAC, Supiwong W, Tanomtong A. 2016a. Repetitive DNAs highlight the role of chromosomal fusions in the karyotype evolution of *Dascyllus* species (Pomacentridae, Perciformes). Genetica. 144: 203–211.

Getlekha N, Supiwong W, Yeesin P, Pengseng P, Kasiroek W, Tanomtong A. 2017. Chromosomal Characteristics of the Three-Spot Damselfish, *Dascyllus trimaculatus* (Perciformes, Pomacentridae) in Thailand. Cytologia. 82: 51–57.

Getlekha N, Tanomtong A. 2020. First Cytogenetic Study of Green Chromis, *Chromis viridis* (Perciformes, Pomacentridae) by Conventional Staining and Ag-

NOR Banding Techniques. CMU J. Nat. Sci. 19(2): 164–175.

Giles V, Thode G, Alvarez MC. 1985. A new Robertsonian fusion in the multiple chromosome polymorphism of a mediterranean population of *Gobius paganellus* (Gobiidae, Perciformes). Heredity. 55: 255–260.

Gold JR, Amemiya CT, Ellison JR. 1986. Chromosomal heterochromatin differentiation in North American cyprinid fishes. Cytologia. 51: 557–566.

Gosline WA. 1971. Functional Morphology and Classification of Teleostean Fishes. Univ. Press of Hawaii, Honolulu.

Hardie DC, Hebert PD. 2004. Genome-size evolution in fishes. Can J Fish Aquatic Sci. 61(9): 1636–1646. https://doi.org/10.1139/f04-106.

Hotaling S, Kelley JL, Frandsen PB. 2021. Toward a genome sequence for every animal: where are we now? Proc Natl Acad Sci USA. 118(52): e2109019118. https://doi.org/10.1073/pnas.2109019118.

Howell WM, Black A. 1980. Controlled silver staining of nucleolus organizer regions with protective colloidal devel- oper: 1- Step Method. Experientia. 36: 1014–1015.

Kashiwagi E, Takai A, Ojima Y. 2005. Chromosomal distribution of constitutive heterochromatin and nucleolus organizer regions in four *dascyllus* fishes (Pomacentridae, Perciformes). Cytologia. 70(3): 345–349. https://doi.org/10.1508/ cytologia.70.345.

Kasiroek W, Luangoon N, Getlekha N, Saowakoon S, Phinrub W, Tanomtong A. 2014. First report on heteromor- phic NORs and chromosome analysis of Rolland's demoiselle, *Chrysiptera rollandi* (Perciformes, Pomacentrinae) by conventional and Ag-NOR staining techniques. Cytologia. 79: 1–10.

King M. 1987. Chromosomal rearrangements, speciation and the theoretical approach. Heredity. 59: 1–6.

Kirkpatrick M, Barton N. 2006. Chromosome inversions, local adaptation and speciation. Genetics. 173(1): 419–434. https://doi.org/10.1534/genetics.105.047985.

Knowlton N, Weith LA, Solórzano LA, Mills DEK, Bermingham E. 1993. Divergence in proteins, mitochondrial DNA and reproductive compatibility across the Isthmus of Panama. Science 260: 1629–1632.

Leis JM, Carson-Ewart BM. 1998. Complex behaviour by coral-reef fish larvae in open-water and near-reef pelagic environments. Environ. Biol. Fisches. 53: 259–266.

Liggins L, Treml EA, Possingham HP, Riginos C. 2016. Seascape features, rather than dispersal traits, predict spatial genetic patterns in codistributed reef fishes. J Biogeogr. 43(2): 256–267. https://doi.org/10.1111/jbi.12647.

Limon J, Roberts MB, Schultz DT, Bernardi G. 2023. The complete mitochondrial genome of *Dascyllus trimaculatus*. Mitochondrial DNA Part B. 8(1): 105–106. https://doi.org/10.1080/23802359.2022.2161838.

Martinez PA, Zurano JP, Amado TF, Penone C, Betancur-R R, Bidau CJ, Jacobina UP. 2015. Chromosomal diversity in tropical reef fishes is related to body size and depth range. Mol Phylogenet Evol. 93: 1–4. https://doi.org/10.1016/j.ympev.2015.07.002.

Martins C, Galetti PM. 1998. Chromosomal localization of 5S rDNA genes in Leporinus fish (Anostomidae, Characiformes). Genetics and Molecular Biology. 21(2), 205–210. https://doi.org/10.1590/S1415-47571 998000 200007

McCord CL, Nash CM, Cooper W, Westneat MW. 2021. Phylogeny of the damselfishes (Pomacentridae) and patterns of asymmetrical diversification in body size and feeding ecology. PLoS One. 16(10): e0258889. https://doi.org/10.1371/journal.pone.0258889.

Molina WF, Galetti PM. 2004. Karyotypic changes associated to the dispersive potential on Pomacentridae (Pisces, Perciformes). J. Exp. Mar. Biol. Ecol. 309: 109–119.

Molina WF, Galetti PM. 2002. Robertsonian rearrangements in the reef fish *Chromis* (Perciformes, Pomacentridae) involving chromosomes bearing 5S rRNA genes. Genet. Mol. Biol. 25: 373–377.

Molina WF, Maia-Lima FA, Affonso PRAM. 2002. Divergence between karyotypical pattern and speciation events in Serranidae fish (Perciformes). Caryologia. 55: 299–305.

Molina WF, Martinez PA, Bertollo LAC, Bidau CJ. 2014a. Evidence for meiotic drive as an explanation for karyotype changes in fishes. Mar Genomics. 15: 29–34.

Molina WF, Martinez PA, Bertollo LAC, Bidau CJ. 2014b. Preferential accumulation of sex and Bs chromosomes in biarmed karyotypes by meiotic drive and rates of chromosomal changes in fishes. An Acad Bras Ciênc. 86(4): 1801–1812.

Molina WF. 2000. Analysis of genetic diversity in Pomacentridae (Perciformes) through the combined use of cytogenetics, molecular markers and multivariate morphometry. Ph.D. Thesis. Universidade Federal de São Carlos, São Carlos.

Moraes RLR, Bertollo LAC, Marinho MMF, Yano CF, Hatanaka T, Barby FF. 2017. Evolutionary relationships and cytotaxonomy considerations in the genus *Pyrrhulina* (Characiformes, Lebiasinidae). Zebrafish. 14(6): 536–546.

Moreira-Filho O, Bertollo LAC. 1991. *Astyanax scabripinnis* (Pisces, Characidae): a species complex. Rev. Bras. Genet. 14: 331–357.

Ohno, S. (1974). *Protochordata, Cyclostomata, and Pisces*. In: Animal Cytogenetics, Vol. 4, Chordata 1, pp. 1-92. Born-traeger, Berlin.

Ojima Y, Kashiwagi E. 1981. Chromosomal evolution associated with Robertsonian fusion in the genus *Dascyllus* (Chromininae, Pisces). Proc Jpn Acad. 57b: 368.

Ojima Y. 1983. *Fish cytogenetics*. In: Sharma AK and Sharma A (eds) Chromosomes in evolution of eukaryotic groups. CRC Press, Boca Raton.

Roberts MB, Schultz DT, Gatins R, Escalona M, Bernardi, G. 2023. Chromosome-level genome of the three-spot damselfish, *Dascyllus trimaculatus*. G3. 13(4): jkac339

Robitzch VSN, Lozano-Cortés D, Kandler NM, Salas E, Berumen ML. 2016. Productivity and sea surface temperature are correlated with the pelagic larval duration of damselfishes in the Red Sea. Mar Pollut Bull. 105(2): 566–574. https://doi.org/10.1016/j.marpolbul.2015.11.045.

Rossi AR, Crosetti D, Gornung E, Sola L. 1996. Cytogenetic analysis of global populations of Mugilcephalus (striped mullet) by different staining techniques and fluorescent *in situ* hybridization. Heredity. 76: 77–82.

Saenjundaeng P, Kaewmad P, Supiwong W, Pinthong K, Pengseng P, Tanomtong A. 2018. Karyotype and characteristics of nucleolar organizer regions in longfin carp, *Labiobarbus leptocheilus* (Cypriniformes, Cyprinidae). Cytologia. 83(3): 265–269.

Saenjundaeng P, Supiwong W, Sassi FMC, Bertollo LAC, Rab P, Kretschmer R. 2020. Chromosomes of Asian cyprinid fishes: Variable karyotype patterns and evolutionary trends in the genus *Osteochilus* (Cyprinidae, Labeoninae,"Osteochilini"). Genet. Mol. Biol. 43: e20200195.

Santos ÉW, Cioffi MB, Costa GWWF, Amorim KDJ, Oliveira VCS, Bertollo LAC, Molina WF. 2024. The diploid number decrease in cardinalfishes (Apogonidae, Kurtiformes): Chromosomal rearrangements and related biological features. The Nucleus. 67, 419–430. https://doi.org/10.1007/ s13237-023-00438-2

Salas EM, Hobbs JA, Bernal MA, Simison WB, Berumen ML, Bernardi G, Rocha LA. 2020. Distinct patterns of hybridization across a suture zone in a coral reef fish (*Dascyllus trimaculatus*). Ecol Evol. 10(6): 2813–2837. https://doi.org/10.1002/ece3.6068.

Sassi FMC, Oliveira EA, Bertollo LAC, Nirchio M, Hatanaka T, Marinho MMF. 2019. Chromosomal evolution and evolutionary relationships of *Lebiasina* species (Characiformes, Lebiasinidae). Int. J. Mol. Sci. 20(12): 2944.

Shulman MA, Bermingham E. 1995. Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution. 49: 897–910.

Sribenja K, Getlekha N. 2024a. The chromosomal distribution of ribosomal DNA and repetitive DNAs in the genome of the red cheek barb, *Systomus rubripinnis* (Valenciennes, 1842). The Nucleus. 67(2), https://doi.org/10.1007/s13237-024-00504-3.

Sribenja K, Getlekha N. 2024b. Mapping of five classes of repetitive DNAs and microsatellite repeats in the genome of the Rainbow Shark, *Epalzeorhynchos frenatum* (Fowler, 1934). Caryologia. 77(2): 57-64. https://doi.org/10.36253/caryologia-2596

Sumner AT. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res. 75: 304–306.

Takai A, Ojima Y. 1988. Chromosomal distribution of C-banded heterochromatin in cyprinid fishes. Proc. Japan Acad. 64B: 49–52.

Takai A, Ojima Y. 1995. Chromosome evolution associated with Robertsonian rearrangements in Pomacentrid fish (Perciformes). Cytobios. 84: 103–110.

Takai A, Ojima Y. 1987. Comparative studies of karyotypes and distribution of nucleolus organizer regions in Pomacentrid fish. Proc Jpn Acad. 63B: 17–20.

Takai A, Ojima Y. 1991. Comparative studies of karyotypes and distributions of nucleolus organizer regions in pomacentrid fish. Cytobios. 65: 199–205.

Takai A, Ojima Y. 1999. Constitutive heterochromatin distribution in the chromosomes of pomacentrid fishes (Perciformes). Cytologia. 64: 87–91.

Takai A. 2012. Chromosomal distribution of C-band-positive heterochromatin in eight species of Pomacentridae (Perciformes). Chromosome Science. 15: 39–42.

Tang KL, Stiassny MLJ, Mayden RL, DeSalle R. 2021. Systematics of damselfishes. *Ichthyol* Herpetol. 109(1): 258–318. https://doi.org/10.1643/i2020105.

Vitturi R. Catalano E. 1989. Multiple chromosome polymorphism in the Gobiid fish *Gobius nigerjozo* L. 1758 (Pisces, Gobiidae). Cytologia. 54: 23–235.

White MJD. 1973. *Animal Cytology and Evolution*. Cambridge University Press, Cambridge.

Yano CF, Poltronieri J, Bertollo LAC, Artoni RF, Liehr T, Cioffi MB. 2014. Chromosomal mapping of repetitive DNAs in *Triportheus trifurcatus* (Characidae, Characiformes): insights into the differentiation of the Z and W chromosomes. PLoS One. 9(3): e90946.

Yuan Z, Liu S, Zhou T, Tian C, Bao L, Dunham R, Liu Z. 2018. Comparative genome analysis of 52 fish Species suggests differential associations of repetitive elements with their living aquatic environments. BMC Genomics 19(1): 141. https://doi.org/10.1186/s12864-018-4516-1.

Yüksel E, Gaffaroglu M. 2008. The analysis of nucleolar organizer regions in *Chalcalburnus mossulensis* (Pisces: Cyprinidae). J. fish. Sci. 2(4): 587–591.

Zhang H, Song L, Wang X, Cheng H, Wang C, Meyer CA, Liu T, Tang M, Aluru S, Yue F. 2021. Fast alignment and preprocessing of chromatin profiles with chromap. Nat Commun. 12(1): 6566. https://doi.org/10.1038/s41467-021-26865-w.