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The genome organization of repetitive sequences in the Golden Damselfish, *Amblyglyphidodon aureus* (Cuvier, 1830) (Family Pomacentridae): insights from extensive pericentric inversions

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Abstract. The genus *Amblyglyphidodon* (damselfishes) is one of the most distinctive fish groups in the Indo-Pacific coral reef ecosystem. It consists of 11 recognized species, which display complex taxonomic relationships and cryptic lineages across their extensive range, often inhabiting coral environments. The timing of evolutionary divergences among these species varies greatly, and the cytogenetic events that accompanied their diversification remain poorly understood. In this regard, chromosomal divergence analysis from a phylogenetic viewpoint provides valuable insights into karyoevolutionary trends. In this study, we employed conventional cytogenetic and cytogenomic techniques to investigate the Golden Damselfish, *Amblyglyphidodon aureus*, focusing on mapping repetitive DNA elements and transposable elements, including 18S rDNA, 5S rDNA, $(CA)_{15}$, $(GA)_{15}$, and $(CAA)_{10}$. The result show that *A. aureus* has a distinct karyotype (2n=48, FN=96), characterized by numerous bi-armed chromosomes, with pericentric inversions playing a key role in its karyotypic structure. Pericentric inversions often function as postzygotic reproductive barriers in many species, and this may also be the case for *A. aureus*. These chromosomal differences illustrate the contrasting evolutionary pathways within the *Amblyglyphidodon* genus, shedding light on the karyotypic characteristics that define the group.

Keywords: Damselfishes, chromosome, repetitive sequences, repetitive DNA

INTRODUCTION

The Pomacentridae family, commonly known as damselfishes, is among the most abundant and characteristic families of coral reef fishes (Bone and Moore, 2008). Due to the strong interactions between damselfishes and their reef habitats, they represent a crucial lineage for biodiversity conservation (Litsios et al., 2012) and evolutionary research. With 399 species (Eschmeyer and Fong, 2016), Pomacentridae ranks as one of the most species-rich fish families. The family exhibits a variety of sex-related patterns, including gonochoric, hermaphroditic, protogynous, and protandrous species (Fishelson, 1998; Fricke and Holzberg, 1974; Warner, 1984). Additionally, the Pomacentridae family exhibits phylogeographic patterns that range from minimal genetic structuring across vast oceanic regions (Rocha et al., 2008) to notable divergences between populations in close proximity (Drew and Barber, 2012; Planes et al., 2011). This considerable genetic variation is also evident in the family's diverse chromosomal characteristics (Getlekha et al., 2016a,b; Kashiwagi et al., 2005; Molina, 2007; Molina and Galetti, 2002; 2004a,b).

The subfamily Pomacentrinae is the most diverse among the five subfamilies of Pomacentridae, representing half of all damselfish species. Some of these species exhibit complex taxonomic patterns (Jang-Liaw et al., 2002; Quenouille and Bermingham, 2004). One of its genera, *Amblyglyphidodon* Bleeker, 1877, which includes 11 species primarily found in the Central and Western Pacific and Indian Oceans, is the second most species-rich group of damselfishes. These damselfishes are typically found swimming alone, in pairs, or in small groups, often among coral reefs. The clades within this genus display distinct evolutionary histories, with some lineages having been separated for extended periods, while others have diverged recently (Frédérich et al., 2013; Sorenson et al., 2014).

Damselfishes are among the few reef fish species that have benthic eggs and, in some cases, relatively short pelagic larval stages, as observed in certain *Amblyglyphidodon* species (Wellington and Victor, 1989). Their limited mobility restricts gene flow, which promotes local adaptation and leads to differentiation processes, the formation of polytypic species, and the emergence of cryptic or incipient speciation (Drew and Barber, 2012; Steinke et al., 2009). In fact, several species complexes within the Pomacentridae family, which share similar meristic and morphological characteristics, including chromatic body patterns, have been identified in the Indo-Pacific (Allen and Randall, 2002; Bernardi and Crane, 1999; Sorenson et al., 2014). Specifically, *Amblyglyphidodon* contains multiple species complexes with recent diversification (Allen et al., 2017; Liu et al., 2012), making them ideal models for studying chromosomal evolution in post-diversification processes. Indeed, many biological traits of Pomacentridae have been linked to chromosomal diversification (Getlekha et al., 2016a,b; Molina, 2007; Molina and Galetti Jr., 2004b).

Although there is a substantial amount of cytogenetic information available for the Pomacentridae family (Arai, 2011), chromosomal data on species complexes remain limited (Getlekha et al., 2016a). Cytogenetic studies are also taxonomically restricted for *Amblyglyphidodon*, often focusing mainly on karyotype descriptions. However, the available data generally show considerable variation in chromosomal structure (Arai, 2011; Molina & Galetti, 2004b). Several damselfish species complexes exhibit significant genetic differentiation (Allen et al., 2015; Liu et al., 2013), and cytogenetic analyses could provide valuable insights into their diversification and evolutionary processes, as well as their phylogenetic and taxonomic classification. These studies could also clarify the role of chromosomal changes as postzygotic barriers in lineage divergence. In the case of the *Amblyglyphidodon* genus, *A. aureus* from the Indian and Pacific Oceans, along with potential cryptic species yet to be described, has shown diversification linked to the Indo-Pacific barrier and sea level fluctuations during the Pleistocene (Liu et al., 2012, 2013; Sorenson et al., 2014).

Repetitive DNAs play a crucial role in the evolutionary dynamics of karyotype changes in several Pomacentridae species (Getlekha et al., 2016a,b; Molina and Galetti, 2002). In the genus *Abudefduf*, where species exhibit highly conserved karyotypes, different classes of repetitive DNAs show minimal variation across species (Getlekha et al., 2016b). In contrast, the genus *Dascyllus*, which displays significant karyotype modifications due to Robertsonian translocations, shows notable interpopulation variation in certain repetitive DNA sequences (Getlekha et al., 2016a). Mobile elements, known to be associated with chromosomal rearrangements (Dobigny et al., 2004; Lim and Simmons, 1994), may contribute to these changes. Additionally, in *Pomacentrus*, chromosomal alterations such as pericentric inversions are known to drive speciation and evolutionary divergence, and the organization and content of repetitive DNA sequences likely influence the genomic landscape and evolutionary trajectories of these species (Getlekha et al., 2018). The highly diversified karyotypes of *Amblyglyphidodon* further offer an opportunity to investigate the role of transposable elements in the diversification process.

In this study, we examined the chromosomal patterns and organization of five repetitive DNA classes [18S rDNA, 5S rDNA, $(CA)_{15}$, $(GA)_{15}$, and $(CAA)_{10}$] in *Amblyglyphidodon aureus*. Our results indicate that the species possess cryptic karyotypes with a high proportion of bi-armed chromosomes, suggesting that their evolution has largely been driven by extensive chromosomal inversions within this genus.

MATERIAL AND METHODS

Sampling and chromosome preparations and conventional methods

Cytogenetic analyses were performed on the Golden damselfish, *Amblyglyphidodon aureus* (Cuvier, 1830), with a sample consisting of 9 males and 10 females, collected from the Andaman Sea (Indian Ocean) near the Gulf of Thailand (Fig. 1). The specimens were captured using a hand-net, placed in sealed plastic bags containing oxygenated clean water, and transported to the research station for further analysis.

Mitotic chromosomes were obtained from cell suspensions of the anterior kidney following the conventional air-drying method (Bertollo et al., 2015). The C-banding technique was used to identify the distribution of C-positive heterochromatin (Sumner, 1972), while silver staining helped locate the Ag-NOR sites on the chromosomes (Howell and Black, 1980). The specimens were then deposited in the fish collection of the Cytogenetic Laboratory, Department of Biology, Faculty of Science and Technology, Muban Chombueng Rajabhat University.

Chromosome probes and FISH experiments

Two DNA sequences, arranged in tandem and isolated from the genome of the Erythrinidae fish *Hoplias malabaricus* (Bloch, 1794), were used as probes. The first probe contained a 5S rDNA repeat copy, including 120 base pairs (bp) of the transcribed 5S rRNA gene and 200 bp of the non-transcribed spacer (NTS) region (Martins et al., 2006). The second probe consisted of a 1400 bp segment from the 18S rRNA gene, which was amplified by PCR from the nuclear DNA. These 5S and 18S rDNA probes were then cloned into plasmid vectors and propagated in DH5α *Escherichia coli* competent cells (Invitrogen, San Diego, CA, USA). The probes were labeled with Spectrum Orange-dUTP and Spectrum Green-dUTP, respectively, using nick translation according to the manufacturer's guidelines (Roche, Mannheim, Germany).

Figure 1. General characteristic of the Golden damselfish, *Amblyglyphidodon aureus* (Cuvier, 1830).

The microsatellites $(CA)_{15}$, $(GA)_{15}$, and $(CAA)_{10}$ were synthesized according to Kirkpatrick (2010). During synthesis, these sequences were directly labeled with Cy3 at the 5' terminus by Sigma (St. Louis, MO, USA).

Fluorescence *in situ* hybridization (FISH) was carried out under high stringency conditions (Yano et al., 2017). Metaphase chromosome slides were treated with RNAse (40 µg/ml) for 1.5 hours at 37°C. Chromosomal DNA was denatured in a solution of 70% formamide/2x SSC at 70°C for 4 minutes. Then, 20 µl of the hybridization mixture (containing 2.5 ng/µl probes, 2 µg/µl salmon sperm DNA, 50% deionized formamide, and 10% dextran sulfate) was applied to the slides, and hybridization was carried out overnight at 37°C in a moist chamber with 2x SSC. The first post-hybridization wash was done with 2x SSC at 65°C for 5 minutes, followed by a final wash at room temperature in 1x SSC for 5 minutes. Finally, the slides were counterstained with DAPI and mounted in an antifade solution (Vectashield, Vector Laboratories).

Image processing

Around 20 metaphase spreads were examined per individual to verify the diploid chromosome number, karyotype structure, and FISH results. Images were captured using an Olympus BX50 microscope (Olympus Corporation, Ishikawa, Japan) equipped with a CoolSNAP camera and Image Pro Plus 4.1 software (Media Cybernetics, Silver Spring, MD, USA). Chromosomes were classified based on their arm ratios as metacentric (m), submetacentric (sm), acrocentric (a), or telocentric (t).

RESULTS

All Golden damselfish, *Amblyglyphidodon aureus* (Cuvier, 1830), exhibit a consistent diploid chromosome number of 2n=48. The karyotype of *A. aureus* (6m+22sm+20a; FN=96) is mainly composed of bi-armed chromosomes (Fig. 2). C-positive heterochromatic blocks are primarily located in the centromeric and telomeric regions of specific chromosome pairs in all specimens. Additionally, faint heterochromatic regions are present in the interstitial areas of chromosome pair No. 18 (Fig. 2).

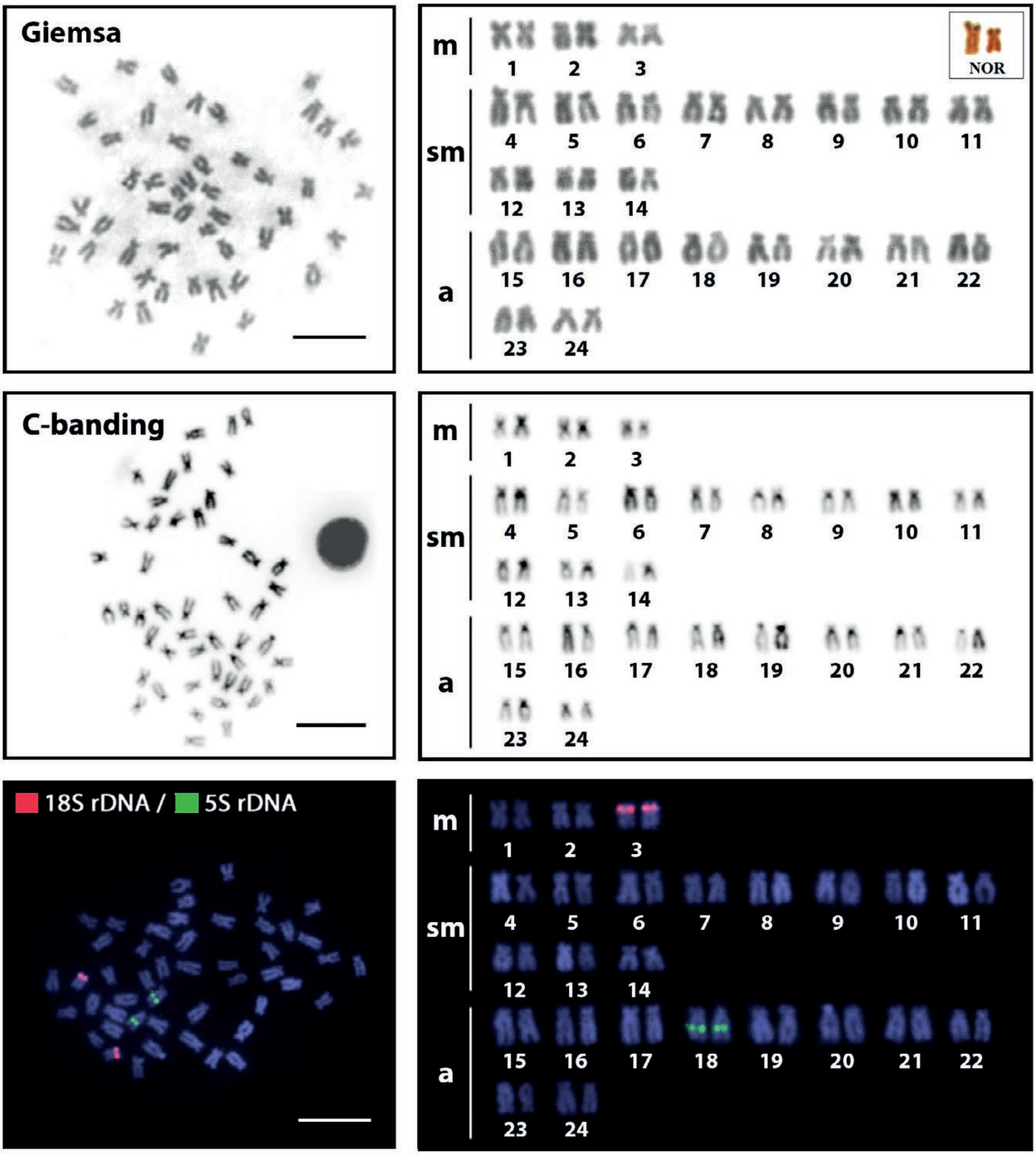

Figure 2. The metaphase and karyotypes of the Golden damselfish, *Amblyglyphidodon aureus* (Cuvier, 1830), were organized using several techniques, including Giemsa staining, Ag-NOR banding (indicated within the boxes), C-banding, and fluorescence *in situ* hybridization with 5S and 18S rDNA probes. Scale bar: 5 µm.

The Ag-NOR sites in all specimens are positioned in the subtelomeric portion of the short arms of the submetacentric chromosomes (Fig. 2). The 5S rDNA sites are not adjacent to the 18S rDNA sites. Instead, they are found in the interstitial regions of acrocentric chromosome pair 18, whereas the 18S rDNA sites are located at the distal ends of the short arms of metacentric chromosome pair 3 (Fig. 2).

The mapping of the $(CA)_{15}$ microsatellite revealed a broad distribution across most chromosomes, with prominent clusters present in certain chromosomal pairs. These clusters are located in the centromeric regions and occur with decreasing frequency across all specimens (Fig. 3). In contrast, the $(GA)_{15}$ and $(CAA)_{10}$ repeats are more sparsely distributed on the chromosomes, showing less distinct clusters in some chromosomal pairs, including in the interstitial regions (Fig. 3).

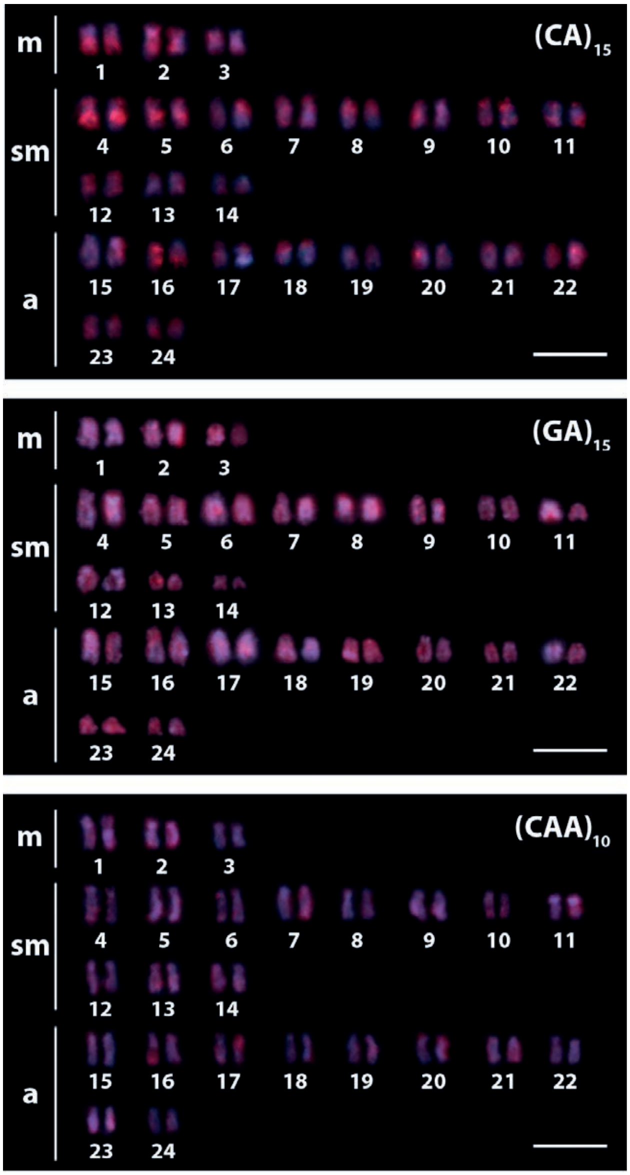

Figure 3. Fluorescence *in situ* hybridization was employed to map the chromosomes of the Golden damselfish, *Amblyglyphidodon aureus* (Cuvier, 1830), using di- and tri-nucleotide microsatellites. The distribution patterns of the (CA)15, (GA)15, and (CAA)10 microsatellites were analyzed as probes. Scale bar = 5 µm.

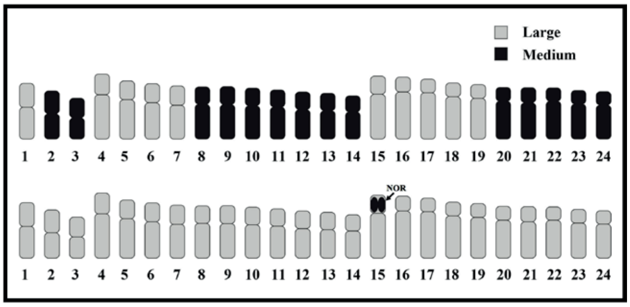

Figure 4. A standardized idiogram illustrating the chromosomal lengths and shapes of the Golden damselfish, *Amblyglyphidodon aureus* (Cuvier, 1830), with a diploid number of 48 (2n=48), created using conventional staining and Ag-NOR banding techniques, with arrows indicating the NOR regions.

The idiogram of the Golden damselfish, *Amblyglyphidodon aureus* (Cuvier, 1830), shows chromosomes with progressively decreasing lengths (Fig. 4). The karyotype of this species is marked by an asymmetrical chromosome arrangement, consisting of metacentric (m), submetacentric (sm), and acrocentric (a) chromosomes. The karyotype formula for *Amblyglyphidodon aureus* is expressed as: $2n\ (48) = L^{m}_{2}+L^{sm}_{8}+ L^{a}_{10}+ M^{m}_{4}+M^{sm}_{14}+ M^{a}_{10}$ or 6m+22sm+20a

This formula offers a detailed depiction of the chromosome composition and structure in *Amblyglyphidodon aureus*, highlighting the variety in chromosome types and sizes within its genome.

DISCUSSION

Phylogenetic divergence, karyotypic evolution, and repetitive DNA organization across Amblyglyphidodon species

The varying periods of divergence among some *Amblyglyphidodon* clades, resulting from multiple radiations under different scenarios, create an ideal setting for studying chromosomal evolution and diversification mechanisms in this genus. The distribution of *Amblyglyphidodon* species, primarily concentrated in the Indo-Malay-Philippines Archipelago with approximately 80% of the species (Cooper et al., 2009), has been shaped by several historical events, such as sea level fluctuations during glacial cycles (Naish, 2009), significantly impacting their evolution and phylogenetic diversification (Allen, 2008; Gaither and Rocha, 2013). It has been estimated that the stem lineages of *Amblyglyphidodon* originated approximately 30 million years ago. Previously, the Indo-Pacific pomacentrid genus *Amblyglyphidodon* was believed to consist of several distinct geographic color variations. However, the current study reveals that it is actually a complex of four species: *A. leucogaster* (Bleeker, 1847), *A. orbicularis* (Hombron & Jacquinot, 1853), *A. indicus* Allen & Randall, 2002, and *A. melanopterus* Allen & Randall, 2002. The species within the *Amblyglyphidodon* complex are primarily distinguished by a combination of color patterns and gill-raker counts (Allen and Randall, 2002).

The previously reported phylogenetic analysis of *Amblyglyphidodon curacao* (Bloch, 1787), along with the currently available mitogenome data for the Pomacentridae family (Kumar et al., 2016), revealed that *A. curacao* clusters with species in the subfamily Pomacentrinae, such as *Stegastes flavilatus* (Gill, 1862) and *Abudefduf vaigiensis* (Quoy & Gaimard, 1825), distinguishing it from species in the subfamily Amphiprioninae. However, *A. curacao* showed the highest sequence identity (87%) with *Amphiprion frenatus* Brevoort, 1856. This suggests that the fish genera *Amblyglyphidodon*, *Stegastes*, *Abudefduf*, and *Amphiprion* are closely related.

A species key for *Amblyglyphidodon* is provided, and the extended phylogenetic divergence within these clades may help explain their unique karyotype patterns. Specifically, *Amblyglyphidodon curacao* and *A. aureus* (Table 1) exhibit karyotypes predominantly consisting of bi-armed chromosomes (2n=48; FN=76 and 96, respectively) (Takai and Ojima, 1986; Ojima and Yamamoto, 1990; Ojima, 1983; Hardie and Hebert, 2004; present study). The *Stegastes* complex, comprising at least seven species, displays karyotypes with 2n=48 and NF=78-90, while the *Amphiprion* complex, with at least three species, shows karyotypes with 2n=48 and NF=78-86. In contrast, the *Abudefduf* complex, consisting of at least five species, exhibits cryptic karyotypes exclusively made up of acrocentric chromosomes (2n=48, FN=50-52) (Arai, 2011).

A karyotype with 2n=48a chromosomes, considered a foundational trait for Perciformes, is commonly found across several Clupeocephala families (Arai, 2011; Molina, 2007). While many Percomorpha families preserve a stable karyotype structure, the karyotype evolution in Pomacentridae is notably dynamic (Molina & Galetti Jr., 2004b). In Pomacentrinae, pericentric inversions are the most common karyotype alteration mechanism. These events are particularly evident in *Amblyglyphidodon* clades, where the gradual accumulation of inversions supports an in-depth analysis of a possible coordinated pericentric inversion process. This pattern has also been observed in other Pomacentrinae genera, where it occurs recurrently and leads to significant karyotype diversification (Kasiroek et al., 2014; Molina & Galetti, 2004a,b). The stabilization of specific chromosomal rearrangements in damselfishes seems not to be random but is likely driven by common orthoselective pressures across multiple lineages (Molina, 2007; Molina and Galetti Jr., 2004a,b).

The presence of C-positive heterochromatin in the centromeric and terminal regions of chromosomes, as observed in the species under investigation, appears to represent a basal condition previously reported in other Pomacentridae species (Molina and Galetti Jr., 2004a,b; Takai, 2012). In contrast, the data for *A. aureus* reveal interstitial C-positive blocks, indicating potential chromosomal rearrangements, likely caused by paracentric inversions, which are often considered cryptic events in fish chromosomes.

In *A. aureus*, the Ag-NOR sites are located at the terminal position on the short arms of acrocentric chromosome pair 15, while the 18S rDNA sites are positioned on the short arms of pair 3. The 5S rDNA sites are found in an interstitial position on the long arms of pair 18. The non-syntenic arrangement of these genes is common and likely represents a plesiomorphic condition in Pomacentridae (Cunha et al., 2014; Getlekha et al., 2016b, 2018; Molina and Galetti, 2002).

The role of repetitive DNAs in driving karyotype variation in damselfishes has been extensively studied (Getlekha et al, 2016a,b, 2018). The high evolutionary dynamics observed in Pomacentridae chromosomes (Molina et al., 2014b) may be closely linked to their organization and composition, as demonstrated by the repetitive DNA arrangements in *A. aureus*. Transposable elements (TEs), which are among the most abundant elements in fish genomes, have been shown to play a significant role in genome evolution and chromosomal rearrangements (Belyayev et al., 2010; Ferreira et al., 2011). However, the physical organization of these elements in Pomacentridae chromosomes remains largely unexplored. In *A. aureus*, the scattered distribution of repetitive DNA elements in the chromosomes has been associated with low evolutionary dynamism (Getlekha et al, 2016b, 2018). This is similar to the pattern observed in *Dascyllus* and *Pomacentrus* species, where microsatellite sequences are evenly distributed across most chromosomes without any significant accumulation. In contrast, *Abudefduf* species show varied and inconsistent distribution patterns, with $(CA)_{15}$ and $(GA)_{15}$ microsatellites concentrated in the nucleolar organizer regions, and $(CAA)_{10}$ repeats being highly accumulated across all chromosomes (Getlekha et al., 2016a).

Chromosomes and speciation in Amblyglyphidodon

Conspicuous evolutionary changes in chromosome number, karyotype formula, and heterochromatin structure are well-documented among damselfishes (Getlekha et al. 2016a,b, 2018; Molina and Galetti, 2002, 2004a). These structural karyotypic differences are clearly visible in the species analyzed (Table 1). This raises the question of whether alterations in chromosome structure and the organization of repetitive DNA may have actively contributed to reproductive isolation and speciation in this family (Getlekha et al. 2016a,b, 2018; Molina, 2007; Molina and Galetti, 2004a; Takai and Ojima, 1999). While speciation is typically a gradual process, it can occur rapidly in cases of ecological divergence. The resulting genomic differentiation tends to be concentrated in regions with low recombination rates, often clustered around genes that control ecotype-specific phenotypic traits (Marques et al., 2016).

In allopatric fish species, the time required for complete reproductive isolation is estimated to be around 11.6 million years (Russell, 2003). In this context, the early stages of speciation within species complexes represent a critical period of evolution that is not well understood in terms of cytogenetic factors. Although hybridization is considered rare in species with benthic nesting, several instances have been reported across different subfamilies (Coleman, 2014; Johansen et al., 2017; Maruska and Peyton, 2007; Van Herwerden and Doherty, 2006). These cases suggest that, during phyletic diversification, the genetic and cytogenetic differentiation in some damselfishes may have been insufficient to maintain genetic integrity and cohesion within certain species. Hybridization events have occurred in the evolutionary history of some damselfish species, particularly during the early phases of their divergence, when reproductive barriers are often less effective (Montanari et al., 2016).

In Pomacentrinae, a significant number of pericentric inversions, and possibly paracentric inversions as well, are primary mechanisms of karyotype differentiation, as demonstrated in some *Pomacentrus* species (Getlekha et al, 2018). These chromosomal rearrangements, along with structural modifications such as changes in centromere position, can have additional consequences, such as alterations in gene expression, meiotic pairing abnormalities, and reduced recombination (Kirkpatrick, 2010). The reduction in recombination helps increase the frequency of adaptive alleles in local populations (Hoffmann and Rieseberg, 2008), driving genomic differentiation and evolutionary divergence of lineages. These rearrangements have been extensively observed in closely related Pomacentrinae species, indicating their direct involvement in the diversification process (Molina WF, Galetti Jr PM, 2004a).

In contrast, certain chromosomal rearrangements appear to have limited impact as postzygotic reproductive barriers. For example, in Chrominae, the karyotypes of the Whitetail dascyllus, *Dascyllus aruanus* (Linnaeus, 1758) (2n=28-32), and the Reticulate dascyllus, *D. reticulatus* (Richardson, 1846) (2n=34-36), diverge significantly from each other due to Robertsonian rearrangements (Getlekha et al., 2016a; Kashiwagi et al., 2005). Notably, despite belonging to separate species complexes (Bernardi and Crane, 1999) and having diverged for a long period (around 15 Mya) (Frédérich et al., 2013, 2014), natural hybridization has been observed between these species (Johansen et al., 2017).

Some species of pomacentrid fish, such as *Pomacentrus auriventris* Allen, 1991 and *P. coelestis* Jordan & Starks, 1901, have a relatively recent divergence time (~0.3 Mya) (Sorenson et al., 2014), raising questions about the extent of reproductive isolation they have achieved. Two species from this complex, *P. similis* Allen, 1991 and *P. alleni* Burgess, 1981, coexist sympatrically in the northeastern Indian Ocean. Interestingly, *P. alleni* exhibits one of the most pronounced color differences within the "coelestis" complex, suggesting that color morphs may have evolved through assortative mating, thereby maintaining their genetic isolation even after secondary contact (Sorenson et al., 2014).

The high cytogenomic similarity observed in recently radiated allopatric species is likely shared, at least partially, with other species within the pomacentrid complex. This suggests that the genetic cohesion in these incipiently divergent species may be maintained by pre-zygotic barriers rather than post-zygotic ones. In fact, the structural and organizational patterns of genes and repetitive sequences in the karyotypes indicate that chromosomal changes play a limited role as key postzygotic barriers. Consequently, cytogenetic data imply potential risks of reverse speciation due to secondary contact between these species, highlighting the importance of this information for their genetic conservation.

Overall, the available data highlight the significant role that chromosomal rearrangements have played in the evolutionary development of *Amblyglyphidodon* species, as well as the genome distribution of certain repetitive DNA classes. We were able to identify distinct chromosomal pathways in the phyletic diversification of *Amblyglyphidodon aureus*, with a detailed characterization of its chromosomal features. *Amblyglyphidodon aureus* was most recently evaluated by The IUCN Red List of Threatened Species in 2010, where it was classi-

Table 1. Reviews of papers on cytogenetics in the Subfamily Pomacentrinae (2n = diploid number, NF = fundamental number, NORs = nucleolar organizer regions, m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric, – = not available).

Species	2n	NF	Formula	NORs	Reference
Abudefduf bengalensis	48	52	2m+2sm+44t	2	Getlekha et al. (2016b)
A. notatus	48	52	2m+2a/sm+44t	-	Arai and Inoue (1976)
A. saxatilis	48	52	2m+2sm+44t	2	Getlekha et al. (2016b)
A. sexfasciatus	48	50	2m+46t	2	Getlekha et al. (2016b)
A. sordidus	48	54	2m+2sm+2a+42t	-	Hardie and Hebert (2004)
A. vaigiensis	48	52	2m+2sm+2a+42t	2	Arai and Inoue (1976)
			2m+2sm+44t	2	Getlekha et al. (2016b)
Amblyglyphidodon curacao	48	76	6m+22sm+20t	2	Takai and Ojima, 1986 Ojima and Yamamoto 1990 Ojima, 1983 Hardie and Hebert, (2004)
A. aureus	48	96	6m+22sm+20a	2	*Present study*
Cheiloprion labiatus	48	74	26sm+22t	2	Takai and Ojima (1987)
Chrysiptera cyanea	42	64	6m+16sm+2a+18t	2	Takai and Ojima (1995) Ojima and Yamamoto (1990) Hardie and Hebert (2003)
	48	48	48st/a	-	Ojima (1983)
C. hemicyanea	48	78	30sm+10a+18t	2	Takai and Ojima (1991, 1999) Ojima and Yamamoto (1990)
	48	80	32sm+16t	-	Ojima (1983)
C. leucopoma	48	74	4m+22sm+6a+16t	2	Takai and Ojima (1995a)
C. rex	36	58	12m+10sm+14t	2	Takai and Ojima (1995a)
	48	78	8m+22sm+18t	-	Ojima (1983)
C. starcki	48	60	2m+10sm+36t	2	Takai and Ojima (1986, 1987)
Microspathodon chrysurus	48	54	6m+10sm+32t	2	Galetti et al. (2006) Molina and Galetti (2004a)
Neoglyphidodon melas	48	82	8m+26sm+2a+12t	-	Takai (2010)
N. nigroris	48	82	8m+26sm+2a+12t	2	Takai and Ojima (1991)
N. oxyodon	48	70	2m+20sm+12a+14t	2	Takai and Ojima (1991)
Plectroglyphidodon leucozonus	48	52	4a+44t	-	Arai and Inoue (1976)
Pomacentrus auriventris	48	48	48t	2	Getlekha et al. (2018)
P. chrysurus	48	90	8m+22sm+12a+6t	2	Hardie and Hebert (2004)
P. coelestis	48	48	48t	2	Takai and Ojima (1999)
	48	48	48t	–	Hardie and Hebert (2004)
P. cuneatus	48	86	8m+16sm+14a+10t	2	Getlekha et al. (2018)
P. moluccensis	48	94	10m+26sm+10a+2t	2	Hardie and Hebert (2004)
	48	94	10m+26sm+10a+2t	2	Getlekha et al. (2018)
P. cf. nagasakiensis	48	50	2m+46t	2	Takai and Ojima (1987)
P. philippinus	48	90	8m+24sm+10a+6t	2	Takai and Ojima (1991)
P. trilineatus	50	58	8m/sm+42t	–	Rishi (1973)
P. similis	48	48	48t	2	Getlekha et al. (2018)
Stegastes fuscus	48	90	20m+22sm+6t	2	Galetti et al. (2006)
S. insularis	48	86	14m+24sm+6a+4t	2	Nagpure et al. (2006)
S, leucostictud	48	88	18m+22sm+8t	2	Galetti et al. (2006) Molina and Galetti (2004b)
S. lividus	48	78	6m+24sm+18t	-	Ojima (1983)
S. nigricans	48	52	2m+2sm+24t	-	Ojima (1983) Hardie and Hebert (2004)
S. pictus	48	90	14m+28sm+2a+4t	2	Galetti et al. (2006) Molina and Galetti (2004b)
S. variabilis	48	88	18m+22sm+8t	2	Galetti et al. (2006)

fied as Least Concern. This species is found in the tropical marine waters of the Eastern Indian and Western Pacific oceans, native to the central Indo-Pacific. It typically inhabits outer reef areas, deep lagoons, and regions with strong ocean currents, often living among gorgonians, either alone or in small groups. Its range extends from Thailand to Indonesia, across New Guinea, north to the Philippine Islands and Taiwan, and as far east as the Marshall Islands and Fiji. In Australia, it is found in the offshore reefs of north-western Western Australia and the Great Barrier Reef in Queensland. In the context of glacial cycles, where sea levels have risen and fallen, *Amblyglyphidodon* species may have experienced repeated isolation and reconnection events, making them an ideal model for studying chromosomal patterns related to the speciation process.

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