 Cytologia **78**(4): 345–351

Standardized Karyotype and Idiogram of Titan Triggerfish, *Balistoides viridescens* (Tetraodontiformes, Balistidae) in Thailand

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Received July 23, 2012; accepted March 18, 2013

Summary The standardized karyotype and idiogram of the titan triggerfish, *Balistoides viridescens* (Bloch & Schneider 1801) from Phuket Province, Thailand, were obtained from the present study. Kidney cell samples were taken from five male and five female fish. The mitotic chromosome preparations were conducted directly from the kidney cells. Conventional staining and Ag-NOR banding techniques were applied to stain the chromosomes. The results indicated that the diploid chromosome number of *B. viridescens* was $2n$=44, and the fundamental number (NF) was 60 in both sexes. The chromosome types consist of 2 large metacentric, 4 large acrocentric, 2 large telocentric, 8 medium acrocentric, 10 medium telocentric, 2 small acrocentric, and 16 small telocentric chromosomes. No strange-sized chromosomes related to sex was observed. The region adjacent to the subtelomeric of short arm of chromosome pair 3 showed clearly observable secondary constriction/NORs. The karyotype formula for *B. viridescens* is as follows:

$2n$ (diploid) $44=L_2^m+L_4^a+L_2^t+M_8^a+M_{10}^t+S_2^a+S_{16}^t$

Key words Titan triggerfish, *Balistoides viridescens*, Karyotype, Idiogram.

The titan triggerfish (*Balistoides viridescens*) is a member of the class Actinopterygii (ray-finned fishes), subclass Neopterygii, order Tetraodontiformes, and family Balistidae (Fig. 1). The order Tetraodontiformes has about 428 species in nine families: Triacanthodidae, Triacanthidae, Balistidae, Monacanthidae, Ostraciidae, Triodontidae, Tetraodontidae, Diodontidae, and Molidae. They are widely distributed in tropical and temperate freshwater and marine environments (Nelson 2006). In the family Balistidae, cytogenetic studies have been performed on 22 species: *Balistapus undulates*, *Sufflamen fraenatus* (Takai and Ojima 1987), *Balistes capriscus* (Vitturi *et al.* 1988), *Balistes carolinensis* (Vitturi *et al.* 1992, Thode *et al.* 1994), *Balistes vetula* (Gustavo and Molina 2005), *Balistoides conspicillus* (Takai and Ojima 1987, Gustavo and Molina 2004), *Balistoides viridescens* (Takai and Ojima 1988), *Cantherhines pardalis*, *Oxymonacanthus longirostris*, *Pseudobalistes flavimarginatus*, *Rhinecanthus verrucosus*, *Rucanus arcodas*, *Sufflamen chrysopterus* (Arai and Nagaiwa 1976), *Rhinecanthus aculeatus* (Arai and Nagaiwa 1976, Kitayama and Ojima 1984), *Rhinecanthus echarpe* (Kitayama and Ojima 1984), *Carolinensis gmelin* (Thode *et al.* 1994), *Melichthys niger* (Gustavo and Molina 2005), *Melichthys vidua*, *Odonus niger* (Kitayama

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DOI: 10.1508/cytologia.78.345

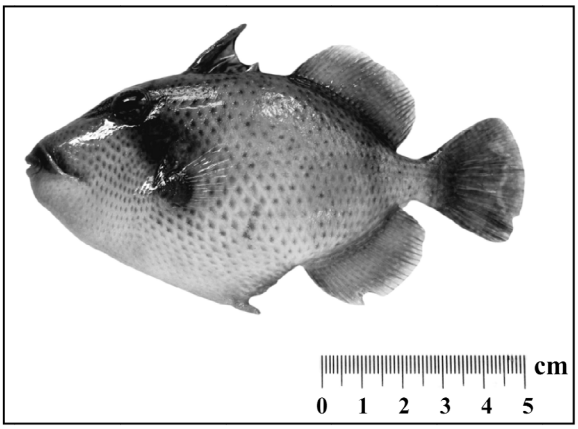

Fig. 1. General characteristics of the titan triggerfish, *Balistoides viridescens* (Bloch & Schneider, 1801).

Table 1. Review of cytogenetic reports of triggerfish in the family Balistidae (15 genera).

Species	2*n*	Karyotype formula	NF	NOR banded	References
Balistapus undulates	42	42a/t	42	2	Takai and Ojima (1987)
Balistes capriscus	44	44t	44	2	Vitturi *et al.* (1988)
Balistes carolinensis	44	44t	44	2	Vitturi *et al.* (1992)
	44	44t	44	2	Thode *et al.* (1994)
Balistes vetula	44	44t	44	2	Gustavo and Molina (2005)
Balistoides conspicillus	44	44t	44	2	Takai and Ojima (1987)
	44	44a/t	44	2	Gustavo and Molina (2004)
Balistoides viridescens	44	2m+2sm+40a/t	48	2	Takai and Ojima (1988)
	44	2m+14a+28t	60	3	Present study
Cantherhines pardalis	40	44a/t	40	—	Arai and Nagaiwa (1976)
Carolinensis gmelin	44	44t	—	—	Thode *et al.* (1994)
Melichthys niger	40	40t	40	2	Gustavo and Molina (2005)
Melichthys vidua	40	40a/t	40	2	Kitayama and Ojima (1984)
Novodon modestus	40	40a/t	40	—	Murofushi and Yosida (1979)
Odonus niger	42	42a/t	—	2	Kitayama and Ojima (1984)
Oxymonacanthus longirostris	36	36a/t	36	—	Arai and Nagaiwa (1976)
Paramonacanthus japonicus	34	34a/t	34	—	Murofushi and Yosida (1979)
Parika scaber	40	40a/t	40	—	Murofushi *et al.* (1989)
Pseudobalistes flavimarginatus	44	2m+42a/t	—	—	Arai and Nagaiwa (1976)
Rhinecanthus aculeatus	44	44t	44	2	Arai and Nagaiwa (1976)
	44	44t	44	2	Kitayama and Ojima (1984)
Rhinecanthus echarpe	44	44a/t	—	2	Kitayama and Ojima (1984)
Rhinecanthus verrucosus	44	44t	44	2	Arai and Nagaiwa (1976)
Rucanus arcodas	36	36a/t	36	—	Arai and Nagaiwa (1976)
Sufflamen chrysopterus	46	46a/t	46	—	Arai and Nagaiwa (1976)
Sufflamen fraenatus	46	46a/t	46	2	Takai and Ojima (1987)

Remarks: 2*n* = diploid chromosome number, NF = fundamental number (number of chromosome arm), m = metacentric chromosome, a = acrocentric chromosome, t = telocentric chromosome, and – = not available.

and Ojima 1984), *Novodon modestus*, *Paramonacanthus japonicas* (Murofushi and Yosida 1979), and *Parika scaber* (Murofushi *et al.* 1989). The members of the family Balistidae have 2*n* ranging from 36 to 46, and most species have the karyotype present as acrocentric and telocentric chromosomes except *B. viridescens* and *P. flavimarginatus*, which are comprised of metacentric and submetacentric chromosomes (Table 1).

Although a large number of 13,000 marine fish species have been recorded (Nelson 2006), fewer than 2% of these have been studied cytogenetically (Brum 1996). According to previous reports, most of them have a diploid complement of 48 acrocentric chromosomes (Sola *et al.* 1981, Klinkhardt *et al.* 1995, Brum 1996). Karyological studies of fishes can contribute significantly to a better understanding of many problems in areas of research ranging from taxonomy, systematics, and genetics to phylogenetics and environmental toxicology (Al-Sabti 1985). The present study is an analysis of the karyotype and chromosomal characteristics of the nucleolar organizer region (NOR) (satellite chromosome) in *Balistoides viridescens* by conventional staining and Ag-NOR banding techniques. Only three previous cytogenetic studies of the genus *Balistoides* exist, showing a diploid chromosome number of 2*n*=44 (Takai and Ojima 1987, 1988, Gustavo and Molina 2004). The results obtained from this study will increase our basic knowledge of the cytogenetics of *B. viridescens*, which could form the basis for future research and provide data to ensure their survival.

Materials and methods

The *B. viridescens* samples were obtained from Phuket Marine Biological Center and Phang Nga Coastal Research and Development Center, Thailand. The fish, five males and five females of *B. viridescens*, were transferred to laboratory aquaria and were kept under standard conditions for 7 days prior to the experiments. Chromosome preparation was conducted by the squash technique from kidney cells (Chen and Ebeling 1968, Nanda *et al.* 1995). The chromosomes were stained with 10% Giemsa's for 30 min and identified for NORs by Ag-NOR staining (Howell and Black

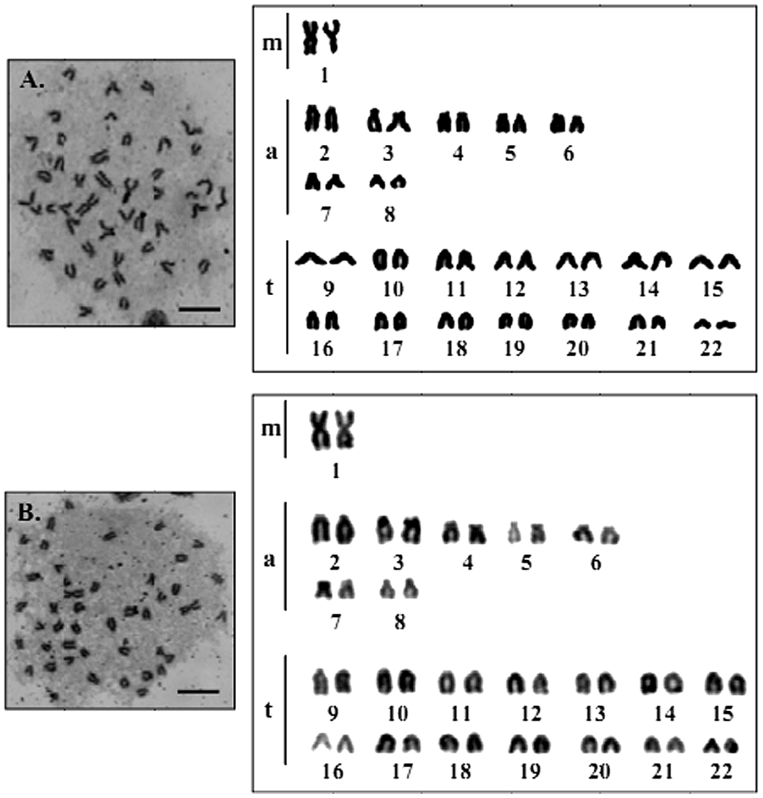

Fig. 2. Metaphase chromosome plates and karyotypes of male (A.) and female (B.) titan triggerfish (*Balistoides viridescens*), 2*n*=44, by conventional straining technique. Scale bars indicate 10 μm.

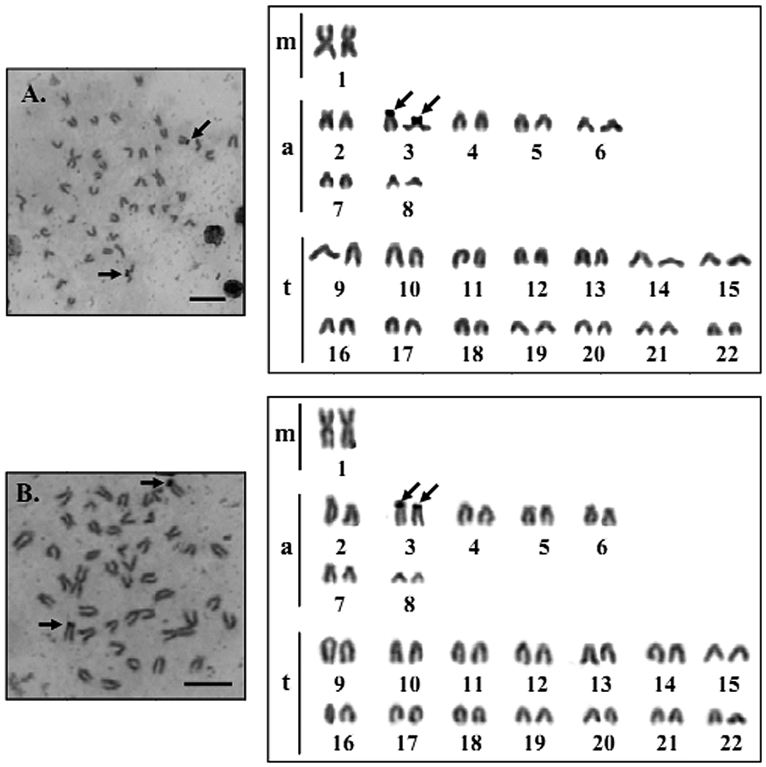

Fig. 3. Metaphase chromosome plates and karyotypes of male (A.) and female (B.) titan triggerfish (*Balistoides viridescens*), 2*n*=44, by Ag-NOR banding technique. The arrows indicate satellite chromosomes/NOR, and scale bars indicate 10 μm.

1980). The short arm chromosome length (Ls) and long arm chromosome length (Ll) were measured, and the length of the total arm chromosome (LT, LT=Ls+Ll) was calculated. The relative length (RL) and centromeric index (CI) were estimated. The CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiograming.

Results and discussion

Our results showed that the diploid chromosome number of *B. viridescens* was 2*n*=44. This is in agreement with the earlier studies conducted in the Indo-West Pacific region (Takai and Ojima 1988). The fundamental number (NF, chromosome arm number) was 60 in both males and females, which is different from the report by Takai and Ojima (1988) demonstrating the NF of *B. viridescens* from the Indo-West Pacific region to be 48. Our results exhibited 2 large metacentric, 4 large acrocentric, 2 large telocentric, 8 medium acrocentric, 10 medium telocentric, 2 small acrocentric, and 16 small telocentric chromosomes. Accordingly, this is the first report on its chromosome size.

We suggest here that no cytologically distinguishable sex chromosome was observed, which is consistent with *B. viridescens* (Takai and Ojima 1988) and other fishes in the order Tetraodontiformes (Gustavo and Molina 2005). It may be possible that the fish's sex chromosomes are at the initiation of differentiation, and hence these chromosomes which contain the sex determination gene cannot be detected by cytogenetic analyses (Na-Nakron 2000). The origin and development of sex chro-

mosomes have been reported for Neotropical fish in Brazil (Bertollo *et al.* 2004).

Among the order Tetraodontiformes, the karyotype of the families Balistidae, Diodontidae, and Tetraodontidae have been studied, derived, and compared to the more basal members of the family Triacanthidae (Brum 1995). The different Balistidae species underwent an extremely diversified karyotype evolution, considering the numerical and structural aspects of their complements, with diploid chromosome number varying from 2*n*=36 to 46, and marked differences in the NF that varied from 36 to 60. The family Balistidae is of special interest, having both the lowest number of chromosomes in *Oxymonacanthus longirostris* and *Rucanus arcodas* (2*n*=36) and the highest in *Sufflamen chrysopterus* and *Sufflamen fraenatus* (2*n*=46) of any fish order. Analyses performed highlight the combined importance of the different chromosome rearrangements in the evolutionary modelling of their karyotypes, such as centric fission (Arai and Nagaiwa 1976), fusion, and especially, pericentric inversions (Gustavo and Molina 2005).

The family Balistidae has 2*n* values lower than 2*n*=48, varying from 2*n*=36 to 46 with most of their representatives presenting acrocentric and telocentric chromosomes. However, the two exceptions are the species *B. viridescens* (Takai and Ojima 1988) and *Pseudobalistes flavimarginatus* (Arai and Nagaiwa 1976), which have been reported to possess metacentric and submetacentric chromosomes. We noticed that this karyotypic pattern was also observed in the present study in *B. viridescens* (2*n*=44). The origin of the reduced diploid chromosome numbers in these species seems to be centric fusions or in tandem followed by pericentric inversions, which seems to be common in other species of the family (Arai and Nagaiwa 1976).

The objective of Ag-NOR banding technique is to reach out determine the nucleolar organizer region (NOR), which represents the location of genes (loci) that function in ribosome synthesis (18S and 28S ribosomal RNA) (Sharma *et al.* 2002). In addition, the subtelomeric of the large acro-

Table 2. Mean short arm chromosome length (Ls), long arm chromosome length (Ll), total arm chromosome length (LT), relative length (RL), centromeric index (CI), and standard deviations (SD) of RL and CI from 20 metaphases of the Titan triggerfish (*Balistoides viridescens*), 2*n*=44.

Chromosome pairs	Ls	Ll	LT	RL±SD	CI±SD	Chromosome sizes	Chromosome types
1	1.811	2.181	3.992	0.083±0.003	0.546±0.016	Large	Metacentric
2	0.573	2.369	2.943	0.061±0.006	0.805±0.026	Large	Acrocentric
3*	0.539	2.176	2.715	0.056±0.004	0.801±0.022	Large	Acrocentric
4	0.476	2.039	2.515	0.052±0.004	0.810±0.022	Medium	Acrocentric
5	0.414	1.913	2.326	0.048±0.005	0.822±0.028	Medium	Acrocentric
6	0.383	1.746	2.128	0.044±0.007	0.820±0.064	Medium	Acrocentric
7	0.346	1.713	2.059	0.042±0.003	0.831±0.033	Medium	Acrocentric
8	0.267	1.557	1.824	0.038±0.004	0.853±0.035	Small	Acrocentric
9	0.000	2.675	2.675	0.055±0.005	1.000±0.000	Large	Telocentric
10	0.000	2.471	2.471	0.051±0.004	1.000±0.000	Medium	Telocentric
11	0.000	2.339	2.339	0.048±0.003	1.000±0.000	Medium	Telocentric
12	0.000	2.252	2.252	0.047±0.003	1.000±0.000	Medium	Telocentric
13	0.000	2.142	2.142	0.044±0.002	1.000±0.000	Medium	Telocentric
14	0.000	2.035	2.035	0.042±0.002	1.000±0.000	Medium	Telocentric
15	0.000	1.942	1.942	0.040±0.002	1.000±0.000	Small	Telocentric
16	0.000	1.875	1.875	0.039±0.001	1.000±0.000	Small	Telocentric
17	0.000	1.817	1.817	0.037±0.001	1.000±0.000	Small	Telocentric
18	0.000	1.739	1.739	0.036±0.002	1.000±0.000	Small	Telocentric
19	0.000	1.677	1.677	0.035±0.002	1.000±0.000	Small	Telocentric
20	0.000	1.628	1.628	0.034±0.002	1.000±0.000	Small	Telocentric
21	0.000	1.525	1.525	0.031±0.002	1.000±0.000	Small	Telocentric
22	0.000	1.344	1.344	0.028±0.003	1.000±0.000	Small	Telocentric

Remarks: *=NOR-bearing chromosome (satellite chromosome).

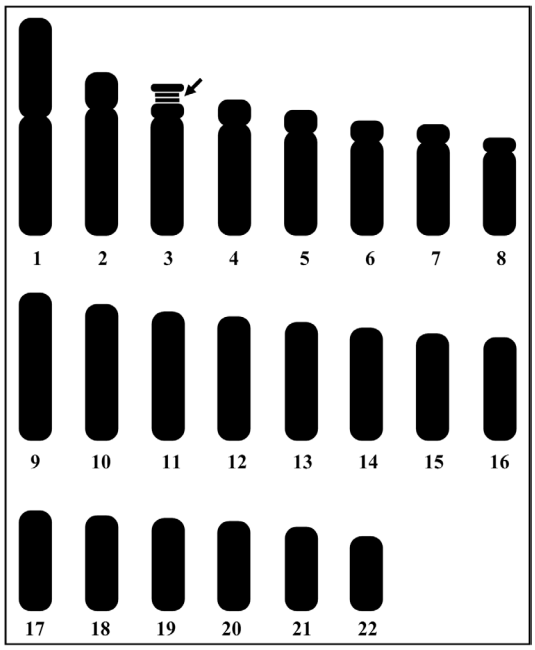

Fig. 4. Idiogram showing lengths and shapes of chromosomes of the titan triggerfish (*Balistoides viridescens*), 2*n*=44 by conventional staining technique. The arrow indicates NOR-bearing chromosome pair 3.

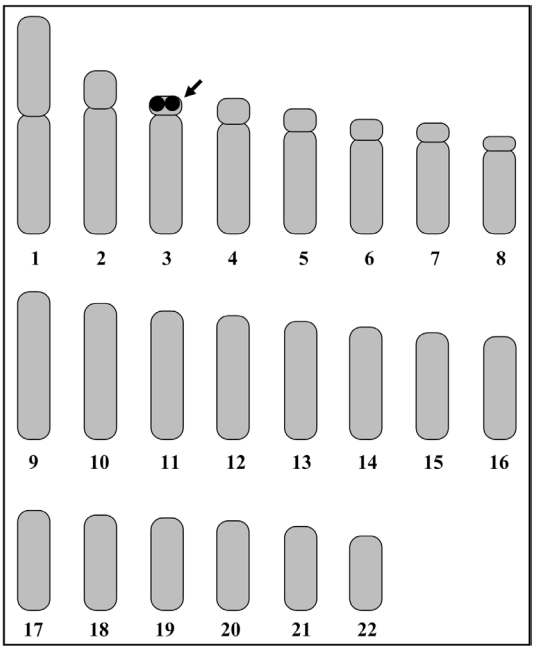

Fig. 5. Idiogram of chromosomes of the titan triggerfish (*Balistoides viridescens*), 2*n*=44 by Ag-NOR banding technique. The arrow indicates NOR-bearing chromosome pair 3.

centric chromosome pair 3 showed clearly observable NORs (satellite chromosomes). This is quite consistent with the reports of Takai and Ojima (1988), which reported the karyotype of *B. viridescens* showing a clearly observable pair of NORs on acrocentric chromosomes. Normally, most fishes have only one pair of small NORs on chromosomes. Only some fishes have more than two NORs, which may be caused by the translocation between some parts of the chromosomes that have NOR and another chromosome (Sharma *et al.* 2002). Our present study showed that the species analyzed presented NOR site on a single chromosome pair in a subtelomeric position. This is considered a simple isomorphic condition in fish (Almeida-Toledo 1985). Another peculiar cytogenetic aspect of Tetraodontiformes is the small quantity of heterochromatic regions, localized in telomeric or centromeric positions on most of the chromosome pairs (Gustavo and Molina 2005).

The chromosomes of the mitotic metaphase cells and the karyotypes of *B. viridescens* by conventional staining and Ag-NOR banding techniques are shown in Figs. 2 and 3, respectively. The chromosome lengths in centimetres of 20 cells (males and females) in the mitotic metaphase were measured. The Ls, Ll, LT, RL, CI, standard deviations of RL and CI, and size and type of chromosome are presented in Table 2. The idiogram of *B. viridescens* showed the gradually decreasing length of the chromosomes. Our results indicate that the chromosome markers of *B. viridescens* are the chromosome pair 1, which is the largest metacentric chromosome, and the chromosome pair 22, which is the smallest telocentric chromosome. The important karyotype feature is the asymmetrical karyotype, which has three types of chromosomes (metacentric, acrocentric, and telocentric). An approximately three-fold difference in size between the largest and smallest chromosomes was detected. The idiograms of the *B. viridescens* from conventional staining and Ag-NOR banding techniques are shown in Figs. 4 and 5, respectively. The karyotype formula of the species could be deduced as:

$2n$ (diploid) $44=L_2^m+L_4^a+L_2^t+M_8^a+M_{10}^t+S_2^a+S_{16}^t$

Acknowledgments

This work was supported by the Applied Taxonomic Research Center (ATRC), Khon Kaen University grant; ATRC-R5304 and the Faculty of Science, Khon Kaen University.

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