



The bisexual natural gynogenetic blunt snout bream lineage derived from the distant hybridization of female blunt snout bream and male Bleeker's yellow tail

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ABSTRACT

Distant hybridization often results in the generation of naturally gynogenic offspring. However, there are currently few reports about fertile natural gynogenetic bream. In this study, we established a natural gynogenetic blunt snout bream (NGBSB-YT, F₁) through distant hybridization from female blunt snout bream (*Megalobrama amblycephala*, BSB) × male Bleeker's yellow tail (*Xenocypris davidi* Bleeker, YT), and further obtained NGBSB₁-YT (F₂) by self-cross. Except for the morphological characteristics, the DNA content, chromosome numbers, and 5 S rDNA sequences of NGBSB₁-YT had no significant difference compared to those of BSB. The results of gonad sections showed that NGBSB₁-YT was bisexual. The NGBSB₂-YT (F₃) obtained by self-cross further proved the fertility of NGBSB₁-YT. Interestingly, the results of microsatellite (prime XD007) analysis and sox9 sequence analysis confirmed that paternal DNA fragments were inserted in NGBSB₁-YT. This study has established a lineage of natural gynogenetic bream (F₁-F₃), that provides a reference for improvement of the theory of natural gynogenesis in fish. The NGBSB-YT lineage can be used as a new type of germplasm resource of bream.

1. Introduction

Distant hybridization can combine the genomes of diverse species that are genetically distant or have a distant interspecific relationship, resulting in significant changes in the phenotype and genotype of the hybrid offspring, and may produce offspring with multiple ploidies (Liu, 2010, Wang et al., 2019, Zhang et al., 2014). For example, in the hybrid combination of red crucian carp (*Carassius auratus* red var.) and common carp (*Cyprinus carpio* L.), the F₁ and F₂ generations are diploid, whereas triploids, tetraploids and amphiprotic allotetraploid hybrids are found in the F₃ generation (Liu et al., 2001, Liu et al., 2016). Additionally, distant hybridization can also lead to natural gynogenesis, where the activation of sperm from distantly related species triggers ovum development without the fusion of male and female pronuclei, resulting in an autotriploid offspring (Chen et al., 2018, Paschos et al., 2001, Xiao et al., 2011). This phenomenon has been observed in several distant hybrid combinations. For instance, Delomas et al. found that the females of second generation (F₂) of koi Carp (*C. carpio haematopterus*) ×

goldfish (*C. auratus*) were gynogens or gynogenetic offspring, which repeated complete maternal genotype (Delomas et al., 2017); and Wu et al. obtained a natural gynogenetic offspring of grass carp through distant hybridization from grass carp (*Ctenopharyngodon idellus*) × top-mouth culter (*Erythroculter ilishaeformis*) (Wu et al., 2019a). Wang et al. obtained a natural gynogenetic blunt snout bream through distant hybridization from blunt snout bream × Chinese perch (*Siniperca chuatsi*). Wu et al. conducted a study on the meat quality (Wang et al., 2022, Wu et al., 2022). Additionally, red crucian carp × blunt snout bream (Liu et al., 2010), koi Carp × blunt snout bream (Wang et al., 2021), and other distant hybrid combinations have obtained natural gynogenesis offspring. These naturally occurring autotriploid fish include both females and a few male individuals. Increasing evidence suggests that the manifestation of natural gynogenesis in distant hybridization is a universal phenomenon rather than a chance event.

Currently, there are some relevant studies on the males of naturally gynogenetic fish. In 1959, Hubbs et al. accidentally discovered a natural gynogenetic male of Amazon molly (*Poecilia formosa*), and subsequent

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studies have demonstrated that these males may have resulted from the micro-chromosomes derived from the male parent during interspecific hybridization (Hubbs et al., 1959, Schar et al., 1995, Lamatsch et al., 2004, Nanda et al., 2007). Wild gibel carp has the ability to reproduce unisexually, and contains male individuals (Zhou et al., 2000). Previous studies isolated a male-specific genetic marker and identified several extra microchromosomes in males, which were revealed to be closely related to the male determination of the gibel carp (Li et al., 2016, Ding et al., 2021). In a previous study, we obtained surviving natural gynogenetic red crucian carps, both males and females, by crossing the red crucian carp with blunt snout bream, demonstrating that male gynogenetic individuals resulted from paternal genetic leakage (Liu et al., 2010). Generally, the gynogenetic offspring induced by traditional artificial heterologous sperm produces only female individuals and no male individuals (Sun et al., 2006, Xu et al., 2015). Contrarily, in autotriploid fish with natural gynogenesis, both female and male individuals can be found, marking a significant difference between the two (Ding et al., 2021). However, there is currently a lack of systematic research on the causes and mechanisms underlying gender formation in naturally gynogenetic fish.

Blunt snout bream (*M. amblycephala*; BSB, $2n = 48$) belongs to the subfamily Cultrinae, characterized by herbivory, fast growth rate, and strong disease resistance (Gong et al., 2023). Bleeker's yellow tail (*X. davidi* Bleeker; YT, $2n = 48$) belongs to the subfamily Xenocyprininae, with a long, round body and excellent flesh. Both are economically important freshwater fish in China. By distant hybridization between blunt snout bream (♀) and Bleeker's yellow tail (♂) (Hu et al., 2012), we established a natural gynogenetic blunt snout bream (NGBSB-YT, F_1), and further obtained NGBSB₁-YT (F_2) and NGBSB₂-YT (F_3) by self-crossing. In this study, the morphological features, DNA content, chromosome number, 5S rDNA sequence, sox gene sequence, and microsatellite DNA of NGBSB₁-YT were analyzed.

2. Materials and methods

2.1. Ethics statement

The Administration of Affairs Concerning Animal Experimentation Guidelines states that approval from the Science and Technology Bureau of China and the Department of Wildlife Administration is unnecessary when the fish in question are not rare or near extinction (first-class or second-class state protection level). Therefore, approval was not required for the experiments conducted in this study.

2.2. Animals and crosses of NGBSB-YT generations

BSB and YT were obtained from the State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, China. During the freshwater fish reproductive seasons (from April to June) in 2017, 30 adult female BSBs and 30 adult male YTs were chosen as the parents. This experiment was repeated thrice.

As there is no obvious difference in the reproductive behavior between BSB and YT, artificial insemination was used to complete the cross. Oxytocin was injected into the female BSB and male YT. The drug doses were 600 units of chorionic gonadotropin for injection I (HCG), 8 units of luteinizing hormone releasing hormone A_2 for injection (A_2), and 2 units of domperidone injection (DOM) and 300 units of HCG, 4 units of A_2 , 1 unit of DOM. They were then placed in a $2\text{ m} \times 3\text{ m}$ pool to ensure water flowing and sufficient dissolved oxygen. The effect time was 7–9 hours when the water temperature was 24°C . After this period, the fish were removed for artificial insemination. The specific process was as follows. The female BSB eggs were squeezed into a clean basin, followed by squeezing the male YT semen into the basin. We used feathers for gentle and even stirring and even spreading on a gauze in water to complete fertilization. The ratio of the parents used was BSB:YT = 1:1. Fertilized eggs developed in a pool with a water temperature

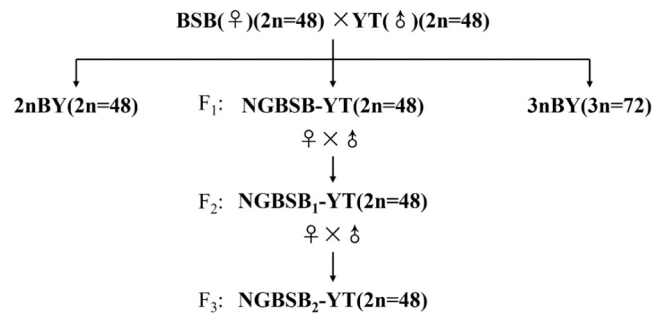


Fig. 1. Crossing procedure and formation of NGBSB-YT generation.

of $23\text{--}26^\circ\text{C}$, maintaining water flow and sufficient dissolved oxygen. Hatching took 2–3 days, and the hatched fry were transferred to ponds for further cultivation.

Randomly, 1000 offsprings were selected for screening. A small number of bream type offsprings, including male and female individuals, were identified as NGBSB-YT (F_1), and their proportions were calculated. The collected F_1 groups were raised individually in a $5\text{ m} \times 10\text{ m}$ pond for 2 years. During this period, feed was provided twice a day to ensure necessary nutrients needed for growth, and a 24-hour uninterrupted water flow was maintained to keep the water normal. In 2019, the second generation (NGBSB₁-YT, F_2) was successfully obtained by self-crossing of the mature males and females of NGBSB-YT (at the age of 2 years). In 2022, the third generation (NGBSB₂-YT, F_3) was successfully obtained through self-crossing the second generation. The feeding processes of the F_2 and F_3 generations were similar to those of F_1 . The mating procedure and formation of NGBSB-YT generation are illustrated in Fig. 1.

2.3. Morphological traits of NGBSB₁-YT

At the age of 2 years, 30 NGBSB₁-YT, 30 BSB, and 30 YT were selected for morphological examinations, respectively. The measurable traits included the whole length to body length (WL/BL), body length to body height (BL/BH), body length to head length (BL/HL), head length to head height (HL/HH), caudal peduncle length to caudal peduncle height (CPL/CPH), and body height to head height (BH/HH) ratios. The countable traits included the number of dorsal fins, abdomen fins, anal fins, lateral scales, and upper and lower lateral scales (Zou et al., 2008).

The above data were averaged, and the analysis of variance (ANOVA) and multiple comparison tests were used to evaluate the data of the three fish species in NGBSB₁-YT, BSB, and YT employing SPSS Statistics version 22.0 (Kirkpatrick and Feeney, 2011).

2.4. Observation of sexes and gametes

The testes and ovaries of NGBSB₁-YT (10 individuals, 2 years old) were moved and fixed in Bouin's solution to prepare tissue sections. Paraffin embedding and HE (hematoxylin and eosin) staining were conducted according to previous reports (Gong et al., 2021). The testicular and ovarian structures were observed and photographed under a light microscope (Leica DM2500 LED). The sperm or oocyte type of each gonad sample was identified according to the early standards for BSB and common carp (Hu et al., 2012, Liu, 1993).

2.5. Measurement of DNA content and examination of the chromosomal metaphases

To determine NGBSB₁-YT ploidy, we analyzed DNA content and prepared chromosomal metaphase spreads. Blood samples and kidney tissues were treated following the methods described in previous reports (Liu et al., 2007, Gong et al., 2019).

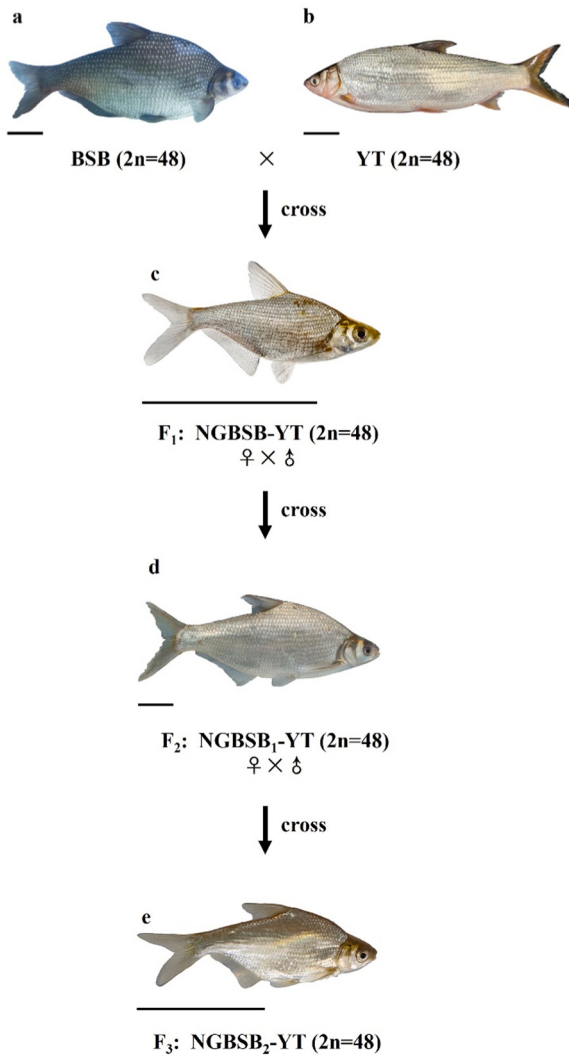


Fig. 2. The formation procedure and the appearance of NGBSB- YT generation. Bar = 5 cm.

Blood samples (1 mL) were collected from the caudal vein of 10 BSBs, 10 YTs, and 10 NGBSB₁-YTs using syringes containing 400 units of anticoagulant. The samples were then treated with DAPI DNA staining solution (Partec) in the dark for 10–15 min and filtered. The average DNA content of each sample was measured using flow cytometry (Partec PA) (Liu et al., 2001). Additionally, we randomly selected 10 BSB, 10 YT, and 10 NGBSB₁-YT individuals at 1 year of age for kidney tissue chromosome preparation (Gold et al., 1990).

We used a light microscope (Leica DM2500 LED) to observe and evaluate the chromosome shape and number using oil lenses at 100X magnification.

2.6. Examination of the DNA markers of 5 S rDNA and sox genes

Blood samples were collected from BSB, YT, and NGBSB₁-YT (10 samples per group) and total genomic DNA was extracted using a San-Prep Column DNA Gel Extraction Kit (Sangon Biotech, Shanghai, China). The DNA concentration and quality were assessed using agarose gel electrophoresis and ultraviolet spectrophotometer, and the experiments were conducted as per previous descriptions (Wang et al., 2017). The specific primers of 5 S rDNA (5S-F: 5'-GCTATGCCCGATCTCGTCTGA-3' and 5S-R: 5'-CAGGTTGGTATGGCCGTAAGC-3') and sox gene (Sox-F, 5'-TGAAGCGACCATGAA(C/T)G-3' and Sox-R, 5'-AGGTCG(A/G)TACTT(A/G)TA(A/G)T-3') were selected based on previous reports

Table 1

Comparison of the morphological traits of BSB, YT and NGBSB₁-YT.

Phenotypes	Types of fish		
	YT	BSB	NGBSB ₁ -YT
WL/BL	1.22±0.03 ^b	1.22±0.03 ^b	1.25±0.03 ^a
BL/HL	5.10±0.15 ^a	4.61±0.22 ^b	4.67±0.28 ^b
BL/BH	3.69±0.11 ^a	2.41±0.08 ^b	2.30±0.09 ^c
HL/HH	1.26±0.04 ^b	1.30±0.07 ^a	1.16±0.09 ^c
BH/HH	1.74±0.06 ^c	2.50±0.13 ^a	2.34±0.14 ^b
CPL/CPH	1.33±0.03 ^a	1.02±0.07 ^b	1.06±0.12 ^b
No. of lateral scales	60–67	52–57	47–56
No. of upper lateral scales	10–12	11–14	10–13
No. of lower lateral scales	6–8	8–11	7–10
No. of dorsal fins	III+7–9	III+7–8	III+7–8
No. of abdominal fins	8–9	9–10	9–10
No. of anal fins	III+11–13	III+23–31	III+22–29

^a, ^b and ^c represent significantly different between NGBSB₁-YT, BSB and YT group fish ($p < 0.05$). The Roman numerals means the number of spine fins.

respectively (Gong et al., 2021, Wang et al., 2020, Chen et al., 2009).

The amplification, purification, and DNA sequencing methods of target sequences were based on previous reports (Gong et al., 2021). The DNA sequencer used was the Applied Biosystems 3730xl Genetic Analyzer.

2.7. Microsatellite DNA cloning and analysis

PCR conditions and two pairs of specific microsatellite primers for BSB and YT, namely XD001 (F: 5'-GTCCAGACTGTCATCAGGAG-3', R: 5'-GAGGTGTACTGAGTCACGC-3') and XD007 (F: 5'-CAGCCGCTG-GATCCCAACTG-3' R: 5'-TGCAGATGCGTAGCAATGTAAACC-3'), were based on published studies (Guo et al., 2020, Zeng et al., 2011). Electrophoresis and silver staining of microsatellite DNA were conducted as per previous descriptions (Liu et al., 2010).

2.8. Data analysis

SPSS Statistics version 22.0 was used to analyze the data presented as mean ± standard deviation (SD), and analysis of variance (ANOVA) was used to determine significant differences among BSB, YT, and NGBSB₁-YT. Statistical significance among the groups was set at $p < 0.05$.

3. Results

3.1. Formation and morphological traits of NGBSB₁-YT

Among the offsprings of BSB × YT, only 0.3 % were NGBSB-YT, whereas the rest were hybrids. The procedure for the formation of NGBSB-YT and its offsprings is indicated in Fig. 1. The appearance traits of BSB (Fig. 2a), YT (Fig. 2b), NGBSB-YT (Fig. 2c), NGBSB₁-YT (Fig. 2d), and NGBSB₂-YT (Fig. 2e) is depicted in Fig. 2. The polyploid hybrids and NGBSB₁-YT were easily distinguishable. The body shape of the triploid and diploid hybrids was between that of BSB and YT (Hu et al., 2012), while that of NGBSB₁-YT was similar to the body shape of BSB.

Table 1 presents the measurable and countable traits in NGBSB₁-YT, BSB, and YT. For measurable traits, the WL/BL in NGBSB₁-YT exceeded that of BSB or YT, showing a significantly difference that of with BSB and YT. The BH/HH in NGBSB₁-YT was immediate to that in BSB and YT and was significantly different from that in BSB and YT. BL/BH and HL/HH in NGBSB₁-YT were lower than those in either BSB or YT. They were significantly different from those in BSB and YT. BL/HL and CPL/CPL in NGBSB₁-YT were similar to those in BSB and were significantly different from those in YT. For the countable traits, the number of lateral scales, lower lateral scales, and number of anal fins in NGBSB₁-YT were close to those in BSB and significantly different from those in YT. However, the number of upper lateral scales, dorsal fins, and abdominal fins were

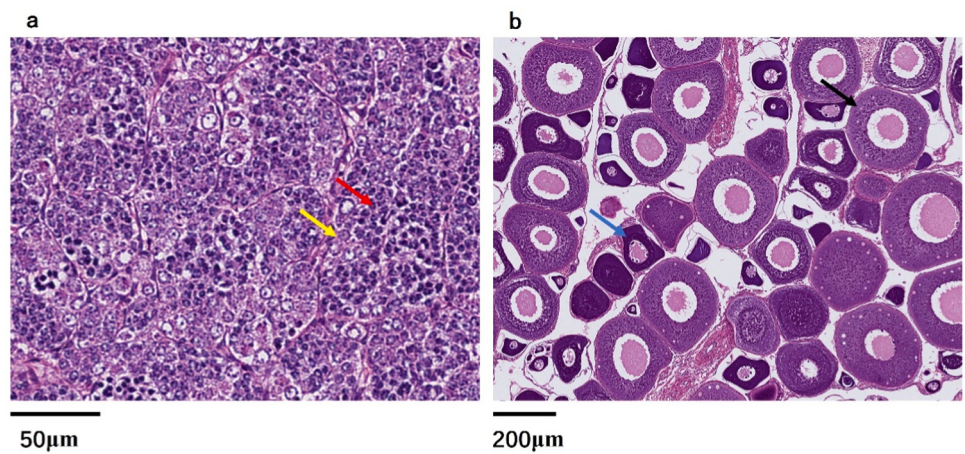


Fig. 3. Structures of the mature testis and ovary of NGBSB₁-YT. **a** The testis with many comprising numerous spermatids and some spermatogonia; **b** The ovary predominantly comprised oocytes in phases II and III. Yellow arrow: spermatogonia, red arrow: spermatids, blue arrow: oocytes in phase II, black arrow: oocytes in phase III.

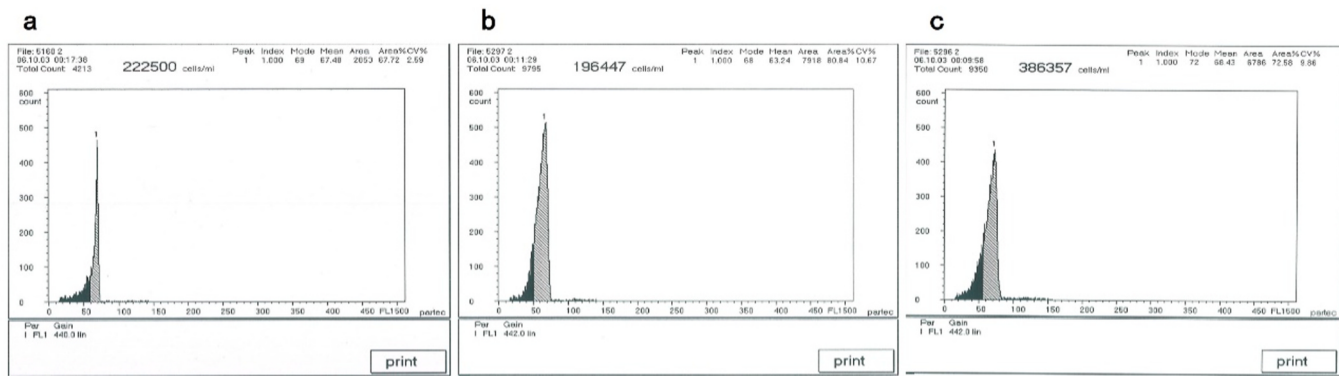


Fig. 4. Cytometric Histogram of DNA fluorescence in three fish. **a** BSB; **b** YT; **c** NGBSB₁-YT.

Table 2
Chromosome numbers in BSB, YT and NGBSB₁-YT.

Types of fish	No. of metaphase photographs	Distribution of chromosome numbers	
		<48	48
BSB	48	15	85
YT	48	10	90
NGBSB ₁ -YT	48	15	85

similar among the three groups of fish.

3.2. Fertility

Seven of the ten individuals were female and three were male. The gonadal microstructure of NGBSB₁-YT (2 years old) is depicted in Fig. 3. The ovaries of the NGBSB₁-YT females developed normally and predominantly comprised oocytes in phases II and III (Fig. 3b). The testis of NGBSB₁-YT males contained several lobules, comprising numerous spermatozoa and some spermatogonia (Fig. 3a). Furthermore, during the reproductive season in 2022, white sperm or mature eggs were

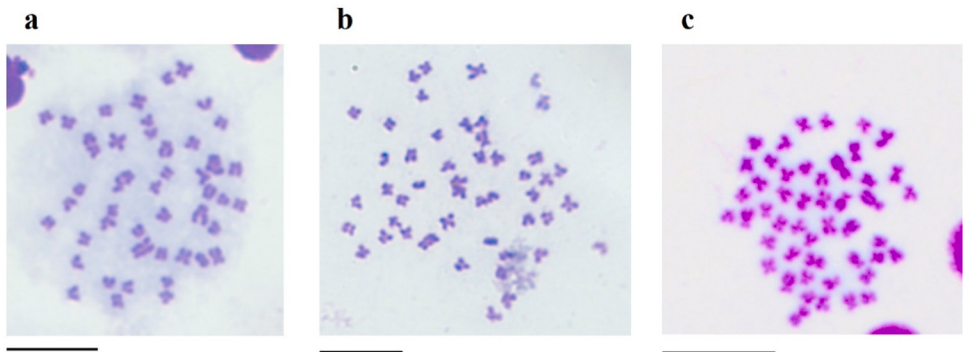


Fig. 5. Chromosome spreads at metaphase in three fish. **a** BSB; **b** YT; **c** NGBSB₁-YT. Bar = 5μm.

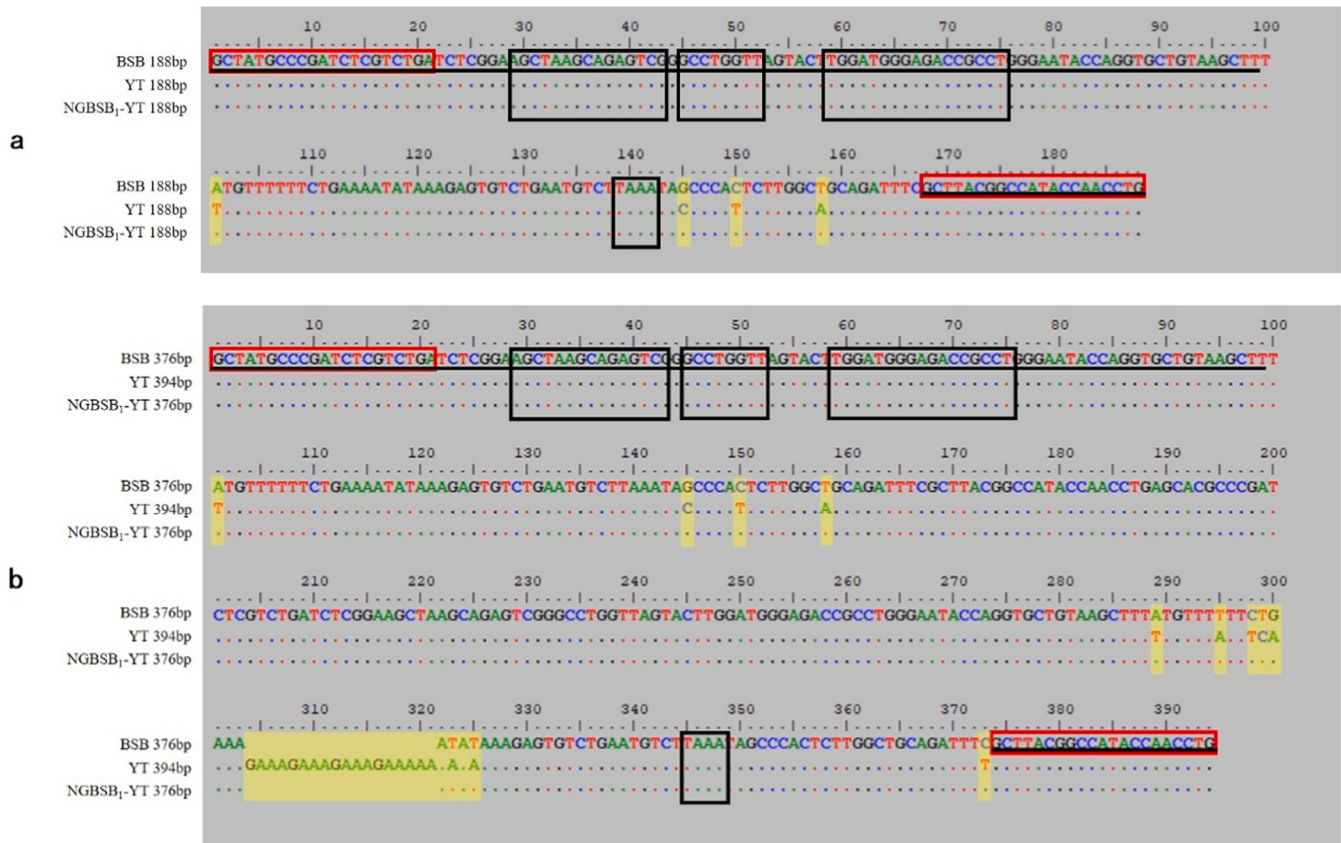


Fig. 6. Nucleotide sequence alignment of sequenced 5 S rDNA fragments in BSB, YT and NGBSB₁-YT. **a** nucleotide sequence alignment of 5 S rDNA fragments in BSB (188 bp), YT (188 bp) and NGBSB₁-YT (188 bp). **b** nucleotide sequence alignment of 5 S rDNA fragments in BSB (376 bp), YT (394 bp) and NGBSB₁-YT (376 bp).

squeezed from 2-year-old NGBSB₁-YT individuals, and many NGBSB₁-YT offspring (NGBSB₂-YT) were obtained through self-crossing (Fig. 2e). These results suggest that NGBSB₁-YT individuals were fertile.

3.3. DNA content and chromosome numbers

The DNA contents was detected by flow cytometry, and the average relative fluorescence intensities of BSB, YT, and NGBSB₁-YT in the DNA histogram were 67.48, 63.24, and 68.43, respectively (Fig. 4a, b, and c). Chromosome preparations were performed from kidney tissue, wherein 100 metaphase phases of each sample were counted. In total, 82 % of the metaphases showed 48 chromosomes in NGBSB₁-YT (Table 2). The largest submetacentric chromosome was present in the metaphase chromosome set of NGBSB₁-YT (Fig. 5c), which is a marker chromosome for BSB (Fig. 5a). The distribution of chromosome numbers indicated that NGBSB₁-YT individuals were diploid.

3.4. Molecular organization of 5 S rDNA and sox genes

The 5 S rDNA sequences of BSB, YT, and NGBSB₁-YT were analyzed. The results demonstrated that BSB and NGBSB₁-YT contained two 5 S rDNA fragments (188 bp and 376 bp), YT contained two 5 S rDNA fragments (188 bp and 394 bp) (Fig. 6). Herein, each 5 S rDNA molecule contained two fragmented coding regions (5'-99 bp and 3'-21 bp) and a separated NTS sequence. The 376 bp fragment of BSB and NGBSB₁-YT comprised two 188 bp repeats. The sequence similarity of the two 5 S rDNA fragments between BSB and NGBSB₁-YT was 100 %. These results suggested that the 5 S rDNA of NGBSB₁-YT was inherited completely from BSB. Furthermore, there were base differences between the 5 S rDNA of BSB and YT, because the 5 S rDNA of BSB and YT were species-specific (Fig. 6).

The PCR results were based on the primers for the high mobility group (HMG)-box of sox genes, and the sequencing results showed that there were three DNA fragments (215, 215, and 714 bp) in NGBSB₁-YT (Fig. 7). By comparing the sequences, we verified that the two 215 bp DNA fragments existed in NGBSB₁-YT belonged to sox1 and sox11. The 714 bp DNA fragment in NGBSB₁-YT represented sox9 gene. Table 3 indicates the percentage of nucleotide similarities in the HMG-box of sox genes in NGBSB₁-YT, BSB, and YT. sox1 sequences in NGBSB₁-YT, BSB, and YT demonstrated 100 % similarity between NGBSB₁-YT and BSB and 99.53 % similarity between NGBSB₁-YT and YT. In sox11 sequences in NGBSB₁-YT, BSB, and YT, there existed 99.07 % similarity between NGBSB₁-YT and BSB and 98.6 % similarity between NGBSB₁-YT and YT. In sox9 sequences in NGBSB₁-YT, BSB, and YT, the similarity between NGBSB₁-YT and BSB was 99.58 % and that between NGBSB₁-YT and YT was 93.5 %. These results indicated that the sequences of the HMG-box of sox genes in NGBSB₁-YT were highly homologous to those of BSB. On comparing the sequences of sox9 gene of NGBSB₁-YT, BSB, and YT, we found that the three sox9 sites of NGBSB₁-YT had the same bases as those of YT.

3.5. Microsatellite DNA analysis

Two pairs of microsatellite DNA primers, XD001 and XD007, were used to amplify the DNA fragments from BSB, YT, and NGBSB₁-YT (Fig. 8). In primer XD001, NGBSB₁-YT expression pattern was the same as that of BSB and is completely different from that of YT. DNA fragments in NGBSB₁-YT were similar with those in BSB, suggesting that NGBSB₁-YT inherited those DNA fragments from BSB.

For primer XD007, seven distinct bright bands were amplified from one NGBSB₁-YT individual. Conversely, only five bands were observed in BSB. The two fragments indicated by the arrows (arrows in Fig. 8b)



Fig. 7. Nucleotide sequence alignment of sequenced Sox9 fragments in BSB (714 bp), YT (710 bp) and NGBSB₁-YT (714 bp).

Table 3
Nucleotide similarities of separate regions of the DNA fragments produced by PCR with the primers of the HMG-box of Sox genes in NGBSB₁-YT, BSB and YT.

DNA fragment	NGBSB ₁ -YT and BSB	NGBSB ₁ -YT and YT
<i>sox1</i>	100	99.53
<i>sox11</i>	99.07	98.6
<i>sox9</i>	99.58	93.05

were similar to those in YT. This suggested that part of NGBSB₁-YT also inherited these DNA fragments from YT.

4. Discussion

Distant hybridization normally results in polyploid hybrid progeny, including diploids, triploids, and tetraploids. It can also lead to the generation of naturally gynogenic offspring (Liu, 2010). In this study, we

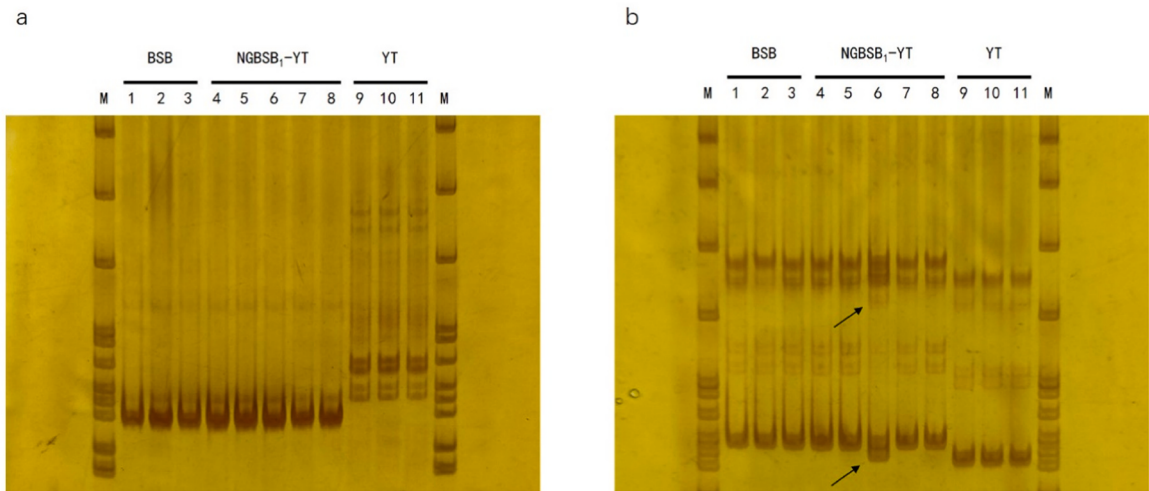


Fig. 8. Microsatellite patterns of XD001 and XD007 in BSB, YT and NGBSB₁-YT. a primer XD001; b primer XD007. The black arrow indicates the DNA bands shared in NGBSB₁-YT and YT.

obtained sexually fertile NGBSB-YT (F₁) through distant crosses between BSB and YT, and further obtained NGBSB₁-YT (F₂) by self-crossing, which does not require UV irradiation of male sperm, unlike artificial gynogenesis (Pang et al., 2022, Wang et al., 2023). Several biological characteristics of NGBSB₁-YT were studied, including the appearance, DNA content, chromosomes, 5 S rDNA, *sox* genes, and fertility.

Previous studies have found that there were no significant differences in morphological traits, DNA content, chromosome number, and 5 S rDNA sequences between gynogenetic mrigal carp (*Cirrhinus mrigala*) and maternal mrigal carp (Li et al., 2023). In this study, the results demonstrated that NGBSB₁-YT offsprings were not significantly different from the original female parent BSB, but were significantly different from the original male parent YT. Their DNA content and chromosome results were not significantly different from those of BSB. At the genetic level, the 5 S rDNA sequence analysis revealed similarity with BSB. These results prove that NGBSB₁-YT is a naturally occurring gynogenetic offspring of distant hybrids. However, the results of appearance results showed that NGBSB₁-YT was different from BSB, which was consistent with the result of gynogenetic olive flounder (*Paralichthys olivaceus*) (Wu et al., 2021). We speculate that this difference may be attributed to the influence of heterologous sperm on the gynogenetic offspring.

According to a previous study, distant hybridization between BSB and YT can produce allodiploid and allotriploid hybrids (Hu et al., 2012). How did we differentiate and identify them? In appearance, both hybrids demonstrated obvious phenotypic divergence from BSB and YT, whereas NGBSB-YT and NGBSB₁-YT were very similar to BSB, so they could be clearly distinguished in appearance. At the molecular level, several 5 S rDNA fragments were detected in the hybrid offspring, originating from BSB and YT. This is because the hybrid offspring contain two sets of genomes: maternal and paternal. In NGBSB₁-YT, we found only the 5 S rDNA sequence consistent with BSB. This demonstrates that during NGBSB₁-YT embryo development, the genetic material mainly comes from the maternal BSB.

Typically, the offsprings of artificial gynogenesis are female (Xu et al., 2015). Tabata et al. used gynogenesis and sex reversal technology to establish an all-female flounder colony, which exhibited faster growth compared to males (Tabata et al., 1986). Previous studies used UV-irradiated largemouth bass (*Micropterus salmoides*) sperm to successfully prepare artificial gynogenetic mandarin fish (*S. chuatsi*), and the offsprings were all females through sex-specific markers (Wu et al., 2023). However, in this study, we found that NGBSB₁-YT included males. Similarly, The male natural gynogenesis has also been observed in gynogenetic red crucian carp (Liu et al., 2010) and gynogenetic grass carp (Wu et al., 2019a). In this study, the gonad section results demonstrated that the NGBSB₁-YT gonads, including the testis and ovary structures, developed normally. In 2022, we obtained NGBSB₂-YT (F₃) through NGBSB₁-YT selfing (Fig. 2; data not yet published). These results demonstrated that NGBSB₁-YT is fertile for both sexes, which is one of the biggest differences between natural gynogenesis and artificial gynogenesis. All-female artificial gynogenetic groups can continue to produce offspring by mating with pseudo-male groups formed using sex reversal technology (Chen et al., 2009). Compared to gynogenetic offspring of a single sex, gynogenetic offspring of both sexes possesses better reproductive potential. In previous studies, natural gynogenetic offspring of both sexes was obtained from the distant hybrid between blunt snout bream and Chinese perch. The growth rate, muscle texture, and antioxidant capacity of the offspring were significantly better than those of BSB (Wang et al., 2022, Wu et al., 2022). However, no lineages have been reported yet. In difference to this, the F₁-F₃ generations of the NGBSB-YT lineages were successfully obtained. This study further indicates that natural gynogenesis in distant hybrids is a common phenomenon, and NGBSB-YT lineage (F₁-F₃) may become a new improved bream germplasm resource.

Previous studies have discovered a carryover of paternal genetic material fragments in gynogenetic red crucian carp (*C. auratus* red var.) and gibel carp (*C. gibelio*) (Liu et al., 2010, Li et al., 2016). Similarly,

microsatellite experiments revealed that some NGBSB₁-YT individuals had the same fragment as those of YT. *sox* gene sequence analysis also found that *sox9* of NGBSB₁-YT had the same base as that of YT. These results suggest the introgression of paternal genetic material in the NGBSB₁-YT. The increase in genetic material can lead to changes in the characteristics of the host species, including sex determination (Noletto et al., 2012). Previous studies have identified a male-specific sequence in gibel carp that plays an important role in male determination (Li et al., 2016). It is speculated that the insertion of YT genetic material fragments may be the reason for the appearance of male NGBSB₁-YT gynogenetic offspring. It is noteworthy that not every NGBSB₁-YT sample could be amplified with the same specific sequence as YT using primer XD007. A previously reported that the amount of paternal genetic material in gibel carp varies between individuals (Li et al., 2016). Similarly, a special microsatellite DNA fragment was found in 10 % gynogenetic allodiploid hybrid lineage of BSB × topmouth culter (*Culter alburnus*) (Wu et al., 2019b). Moreover, a special microsatellite DNA fragment was found in 30 % gynogenetic BSB (inactivated koi carp sperm stimulate) (Gong et al., 2019). This may be due to the randomness of the insertion of fragments. However, it is unclear how the random insertion of fragments affects NGBSB₁-YT sex determination. The underlying mechanisms require further investigation.

Based on the above results, we obtained NGBSB₁-YT with sexual reproductive ability, which provides a reference value for the study of natural gynogenesis in fish. Simultaneously, the advantages demonstrated by the NGBSB-YT lineage (F₁-F₃) make it an excellent novel variety of bream.

Author statement

M.T, S.L, and S.F conceived and designed the experiments. S.F, Y.W, C.W, K.L, J.H, and D.G performed the experiments including parent screening, hybridization and feeding. M.T, S.F, Z.T, Z.Z and X.D performed the fish experiments and data analysis. All authors were involved in preparing and writing the manuscript and approved the final version.

CRediT authorship contribution statement

Min Tao: Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Shaojun Liu:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Siyu Fan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Zhong Tang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology. **Yuequn Wang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology. **Zhifeng Zhou:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology. **Chang Wu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology. **Kaikun Luo:** Writing – review & editing, Writing – original draft, Methodology. **Jie Hu:** Writing – review & editing, Writing – original draft, Methodology. **Dingbin Gong:** Writing – review & editing, Writing – original draft, Methodology. **Shengnan Li:** Writing – review & editing, Writing – original draft.

Declaration of Competing Interest

The authors declare no competing interests.

Data Availability

The data that has been used is confidential.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.aqrep.2024.102206](https://doi.org/10.1016/j.aqrep.2024.102206).

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