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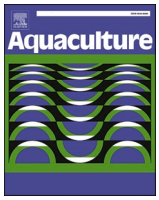

A new type of triploid fish derived from female *Carassius auratus* red var. × male *Gobiocypris rarus*

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ABSTRACT

Distant hybridization can combine the genomes of the parental species, leading to changes in both genotypes and phenotypes in the hybrids, often accompanied by the appearance of polyploid progeny. In this study, we obtained an allotriploid hybrid (RR, 3n = 125) through inter-subfamily hybridization between female red crucian carp (*Carassius auratus* red var., RCC, 2n = 100) and male rare gudgeon (*Gobiocypris rarus*, RG, 2n = 50). We investigated the fertilization rate, hatching rate, morphological traits, chromosome number, DNA content, 5S rDNA, mitochondrial DNA (mtDNA), growth rate and muscle fibers in RR. The results indicated that RR is an allotriploid fish with 125 chromosomes. RR exhibited a fertilization rate of 70.30 %, a hatching rate of 52.70 %, and displayed morphological traits that were intermediate between those of its parents. The 5S rDNA of RR inherited specific bands from both parents, with recombination and mutation. Through sequence alignment and the NJ evolutionary tree analysis, the mtDNA of RR showed a higher similarity to that of RCC, with some partial base insertions observed, indicating that the mitochondria of RR follows maternal inheritance. At 12 months of age, the mean weight of RR was significantly increased by 18.43 % compared to RCC. Furthermore, the total area and mean area of muscle fiber of RR increased significantly than those of RCC, indicating muscle hypertrophy and hyperplasia. This study provided a new approach to create allotriploid fish.

1. Introduction

Distant hybridization is a common and effective method used in fish genetic breeding. Distant hybridization can alter both the phenotype and genotype of offspring, and the offspring often have advantage traits (Liu, 2010; Xu et al., 2015; Zhang et al., 2014). For instance, distant hybridization between white crucian carp (*Carassius cuvieri*) and red crucian carp (*Carassius auratus* red var., RCC) has successfully produced an allodiploid lineage with heterosis, and some chimeric genes and mutated genes related to advantage traits were found in this lineage. (Liu et al., 2024; Liu et al., 2019a; Liu et al., 2019b; Liu et al., 2018; Liu et al., 2017; Wang et al., 2015). Additionally, distant hybridization was accompanied by the appearance of polyploids, especially the triploid hybrid fish (Wang et al., 2020; Zhong et al., 2012). In previous studies, many triploid hybrid fish were produced by distant hybridization (Liu et al., 2021a; Tiwary et al., 2004). However, these triploid hybrid fish mostly with even number of chromosomes; there were few reports about triploid hybrid fish with odd number of chromosomes, and triploid offspring from distinctly different parents have also rarely been reported.

Red crucian carp and rare gudgeon (*Gobiocypris rarus*, RG) are from distinct subfamilies and exhibit markedly different biological traits. In the catalog, the RCC has 100 chromosomes and is classified under the *cyprinidae* subfamily, Cyprinidae (Liu et al., 2019a), while the RG with 50 chromosomes belongs to the *gobioninae* subfamily, Cyprinidae (Liu et al., 2021b). So, the hybridization between RCC and RG was inter-subfamily hybridization. The RCC is characterized by red body color, spindle-shaped body (the body length of adult fish is 15–20 cm and the body weight is 180–250 g), strong stress resistance, excellent meat quality, reaching sexual maturity at one year (sexual maturity occurs from March to July each year) (Wang et al., 2015). In contrast, the RG is characterized by light-yellow color, spindle-shaped body (the body length of adult fish is 3.8–4.5 cm and the body weight is 4.0–6.5 g), sexual maturity at four months, and ability to breed year-round (Hong

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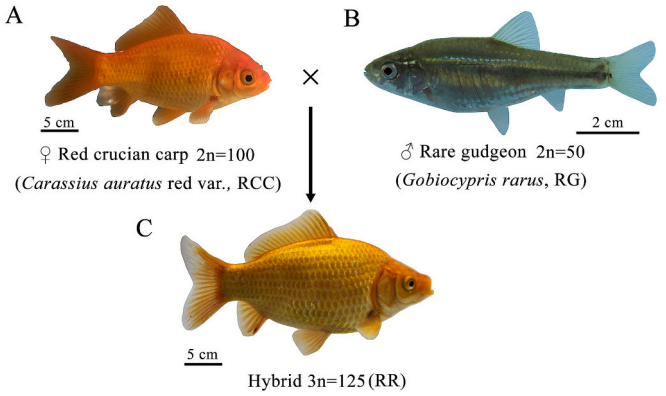

Fig. 1. The crossing procedure and the phenotypes of RR and its parents. (A) The appearance of RCC, (B) the appearance of RG, and (C) the appearance of the RR.

Table 1
Morphological assessment of RCC, RG, and RR.

Fish type	Lateral line scales	Upper lateral line scales	Lower lateral line scales	Dorsal fins	Abdominal fins	Anal fins
RCC	28–30[b]	5–6	6–7	III + 18–20[b,c]	8–9	III + 6–7
RG	34–35[a]	4–5	5	III + 7–8[a,c]	8–9	III + 6–7
RR	28–30[b]	6–7	5–6	III + 14–17[a,b]	6–8	III + 6–7

[a] Represents a significant difference from RCC in the same column.
[b] Represents a significant difference from RG in the same column.
[c] Represents a significant difference from RR in the same column ($P < 0.05$).

Table 2
The ratios of measurable traits of RCC, RG, and RR.

Fish type	WL/BL	BL/BH	BL/HL	HL/HH	CPL/CPH
RCC	1.23 ± 0.04	2.20 ± 0.09[b,c]	3.72 ± 0.01[b,c]	1.18 ± 0.01[b,c]	0.82 ± 0.02[b,c]
RG	1.27 ± 0.02	3.79 ± 0.08[a,c]	4.73 ± 0.02[a,c]	0.96 ± 0.01[a,c]	1.69 ± 0.03[a,c]
RR	1.21 ± 0.02	2.46 ± 0.06[a,b]	4.24 ± 0.11[a,b]	0.82 ± 0.02[a,b]	1.03 ± 0.05[a,b]

[a] Represents a significant difference from RCC in the same column.
[b] Represents a significant difference from RG in the same column.
[c] Represents a significant difference from RR in the same column ($P < 0.05$).

et al., 2016; Zhong et al., 2005). In previous studies, researchers reported the genetic rules at the chromosome level of fish distant hybridization, which including that triploid fish can be produced when the number of the maternal chromosomes is larger than that of the paternal chromosomes (Liu et al., 2020). So, the combination of RCC and RG may result in a triploid offspring with hybridization traits and odd number chromosomes.

In this study, we obtained an allotriploid fish (RR, 3n = 125) by the distant hybridization derived from female RCC and male RG. The genetic characteristics and growth traits of RR were investigated by studying the morphological characters, ploidy, fertilization rate, hatchability, 5S rDNA, mtDNA, growth rate and muscle fiber. This study provides new genetic resources for fish breeding and establishes an ideal model for research on triploid fish.

2. Materials and methods

2.1. Ethics statement

Fish species that are not rare or endangered do not require approval from the National Science and Technology Bureau or the Wildlife Management Department. Therefore, the experiments mentioned in this manuscript do not require such approval. All fish were caught and anesthetized with 80 mg/L MS-222 and then dissected.

2.2. Material and generation of hybrid populations

The RCC and RG used in this study were sourced from the Engineering Research Center of Polyploid Fish Reproduction and Breeding of the State Education Ministry at Hunan Normal University, China. From March to June 2020, 40 mature female RCC and 60 male RG were selected. By injecting the female RCC with hormones to encourage it to produce eggs, male RG does not require hormonal injections. The hybrid offspring were obtained by artificial insemination (Liu, 2022). The hybrid embryos were placed in culture dishes at a water temperature of 22–24 °C for development. Approximately 4000 embryos were randomly selected from the hybrids to assess the fertilization rate (number of gastrula-stage embryos/total number of eggs fertilized × 100 %) and the hatching rate (number of hatched fries/numbers of fertilized eggs × 100 %). The hatched hybrid larvae were transferred to 200-m^2 ponds for rearing, and 200 larvae were placed in each pond. The larvae were fed with soybean milk for the first 1–2 days of rearing. When the fish is about 2 cm long, feed the fish with powdered feed 3–4 times a day and increase the amount of feed according to the size of the fish until it is fed for one year.

2.3. Measurement of morphological traits

We randomly selected 20 one-year-old fish from each of the RCC, RG, and RR groups (total = 60) for morphological measurements. The measurable traits such as whole length (WL), body length (BL), body height (BH), caudal peduncle length (CPL), caudal peduncle height (CPH), head length (HL), and head height (HH) were recorded to 0.1 cm and converted into proportional values. Additionally, countable traits like the number of lateral line scales, upper and lower lateral line scales, dorsal fin rays, ventral fin rays, and anal fin rays were tallied for each group.

2.4. Examination of the ploidy level

The ploidy level of RR was assessed by analyzing DNA content and chromosome number. DNA content in erythrocytes from RCC, RG, and RR was measured using a flow cytometer (Cell Counter Analyzer, Partec, Germany), with 50 fish (1 year of age) from each group. Approximately 0.5–1 mL of blood was collected from the caudal vein using a syringe with 100–200 units of sodium heparin. Samples were processed according to the previously published methods (Liu et al., 2007), and DNA content was measured under identical conditions, with RCC and RG as controls. A χ^2 test with Yates' correction was applied to determine if the RR DNA content ratio deviated from the expected. The number of chromosomes was measured by cell suspension prepared from kidney tissue of RCC, RG and RR (ten-month-old), each sample included 10 fishes. The preparations were made according to the method described by Luo et al. (Luo et al., 2011). For each fish sample, 100 metaphase spreads (10 spreads per sample) were examined. The chromosome shape and numbers were analyzed under a light microscope.

2.5. Genomic DNA extraction, PCR and sequencing

Total genomic DNA was extracted from the peripheral blood cells of RCC, RG, and RR using the Universal Genomic DNA Extraction Kit

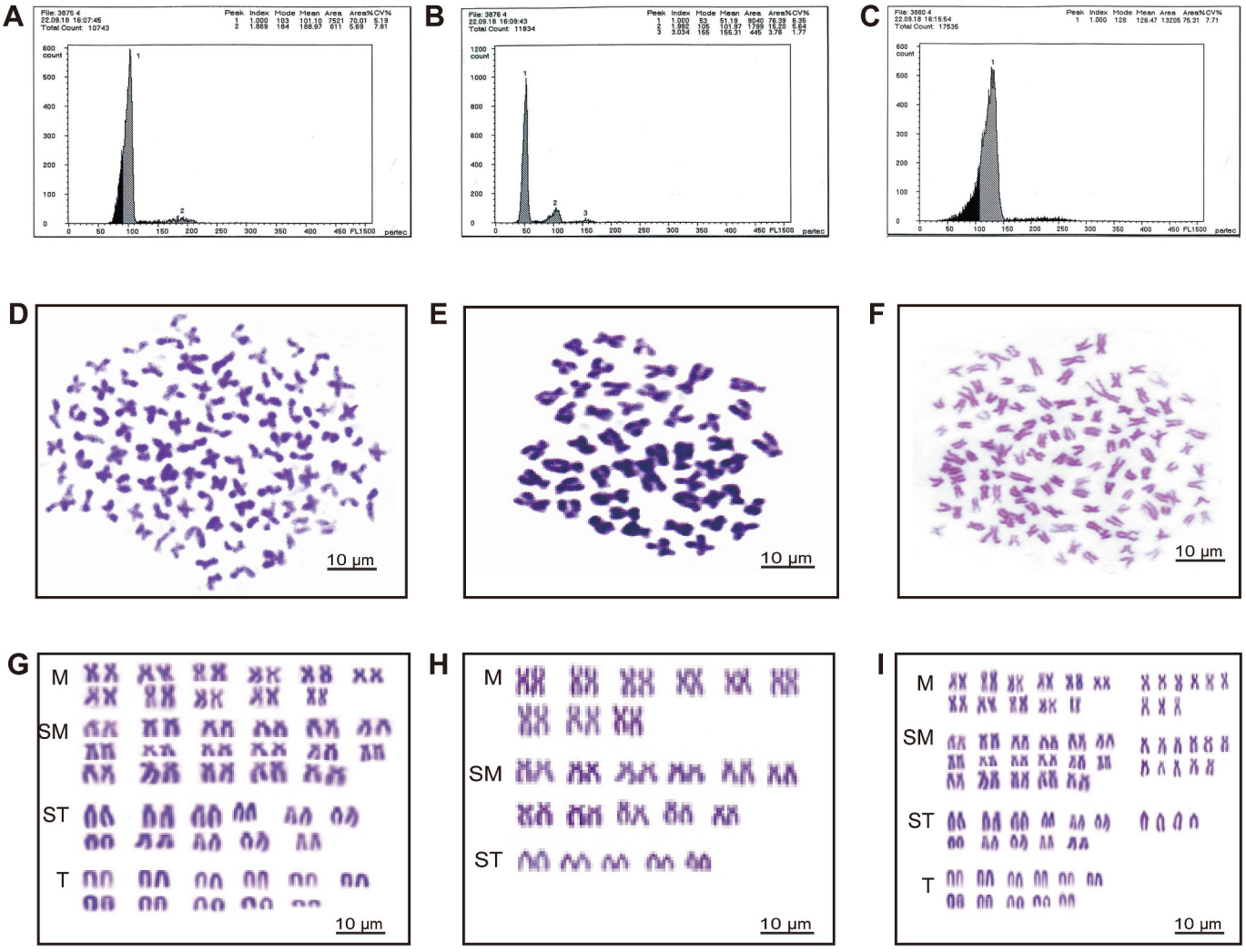

Fig. 2. Cytometric histograms of DNA fluorescence, metaphase chromosome spreads and karyotypes of RCC, RG, and RR. (A) The mean DNA content of RCC (peak 1:101.10), (B) the mean DNA content of RG (peak 1:51.19), (C) the mean DNA content of RR (peak 1:126.47), (D) the metaphase chromosome spreads of RCC (2n = 100), (E) the metaphase chromosome spreads of RG (2n = 50), (F) the metaphase chromosome spreads of RG (3n = 125), (G) the karyotype of RCC: 22m + 34sm + 22st +22t, (H) the karyotype of RG: 18m + 22sm + 10st, and (I) the karyotype of RR: 31m + 45sm + 27st + 22t.

(TaKaRa). Amplification primers for 5S rDNA (F: 5′-GCTATGCCC-GATCTCGTCTGA-3′ and R: 5′-CAGGTTGGTATGGCCGTAAGC-3′) and mtDNA primers (as shown in Table S1) were designed using NCBI and synthesized. PCR amplification conditions and DNA fragment sequencing followed the methods described in published articles (Liu et al., 2021b). We randomly selected three samples from each type of fish to clone the 5S rDNA, sequencing 10 clones from each band in every sample, resulting in a total of 240 clones. Similarly, we randomly selected three samples from the RR for mtDNA cloning and sequenced 10 clones for each band of each sample, yielding a total of 380 clones. Sequences amplified from RCC, RG, and RR were analyzed using BioEdit and Clustal W. The mtDNA sequences of RR, RCC (AY714387.1), RG (NC_018099.1), *Carassius cuvieri* (AB045144.1), *Carassius auratus auratus* isolate J1 (AB111951.1), *Carassius auratus auratus* (MF443758.1), *Cyprinus carpio* (KU050703.1), and *Danio rerio* (AC024175.3) were used to construct an NJ evolutionary tree in Mega 5.05.

2.6. Body weight and muscle fiber analyses

In a pond with an area of 400 m^2, 150 RCC and 150 RR were selected and reared separately. After two months of feeding, the body weights (BWs) of both RCC and RR groups were measured over a period of one year (n = 50 for each group). Because RG is a kind of small fish, we selected 50 RG (1 year old) to measure their BWs. For the body weight analysis, 50 fish each from the RCC and RR groups were selected. Additionally, 5 RCC (1 year old) and 5 RR (1 year old) were selected for muscle fiber analysis. Dorsal muscle tissue was used for muscle samples and paraffin sections were made. Then, after staining with hematoxylin-eosin (H&E), the sections were 5 μm in thick and observed using a microscope. The contour, number and fiber area of individual muscle fibers were analyzed using ImageJ. Statistical data were collected from twenty cross-sections of both RCC and RR.

3. Results

3.1. Formation and morphological traits of RR

The fertilization and hatching rates of RR are 70.30 % and 52.70 %, respectively (Table S2). The hybridization process and the phenotypic characteristics of RCC, RG, and RR are illustrated in Fig. 1. The body color of RCC was red, while RG had a light-yellow color, and RR exhibited a light red body color. Countable traits for RCC, RG and RR are presented in Table 1. Measurable traits are listed in Table 2. The lateral line scales of RR (28–30) was significantly lower than RG (34–35) and was the same with that of RCC (28–30). The dorsal fin of RR (III + 14–17) was significantly lower than that of RCC (III + 18–20) and

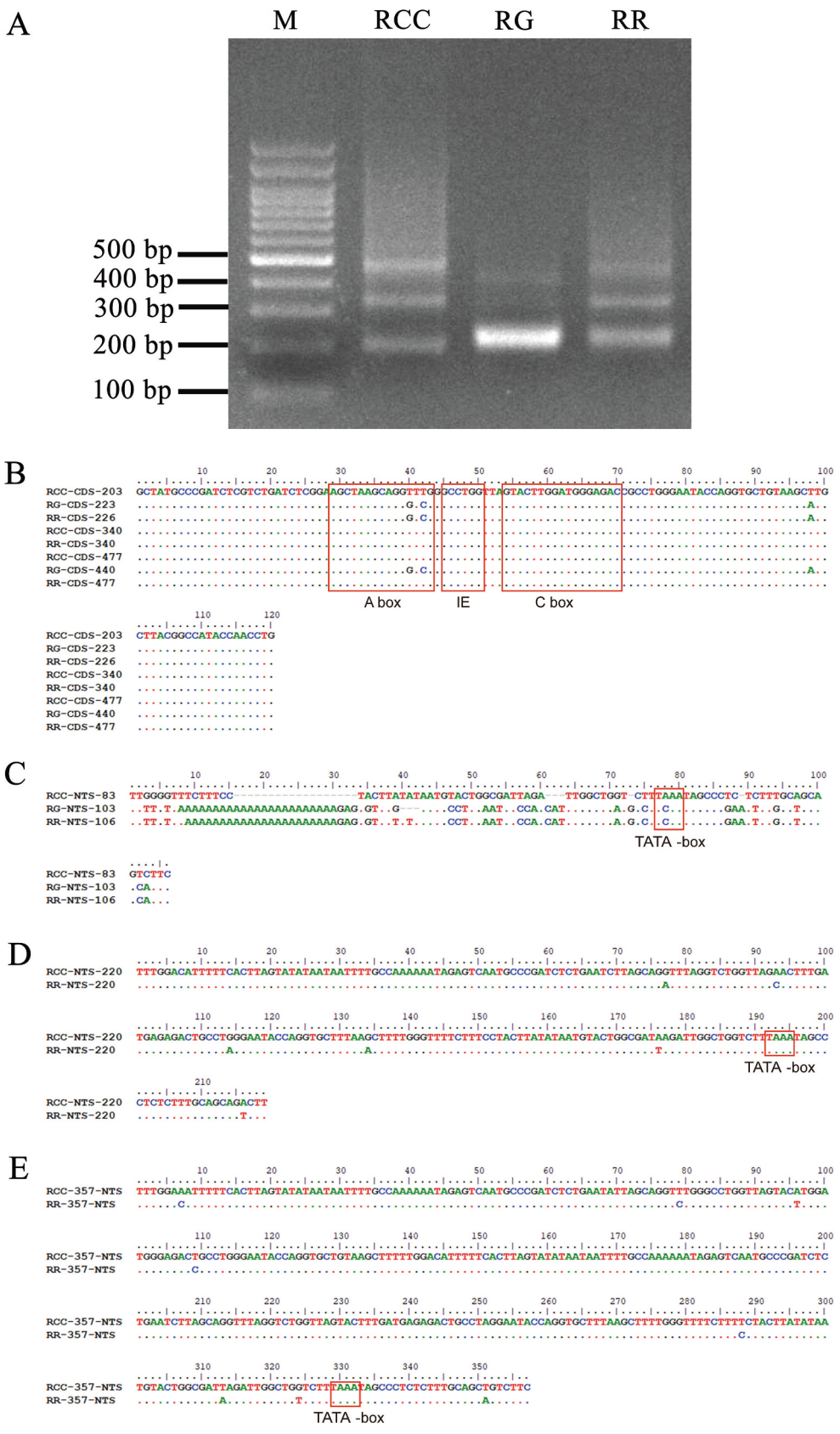

Fig. 3. Comparative analysis of 5S rDNA from RCC, RG and RR. (A) DNA bands amplified from the RCC, RG and RR, (B) comparison of the 5S rDNA coding regions from RCC, RG, and RR. ICRs are included in the boxes. (C–E) Comparison of the NTS sequences from RCC, RG, and RR. The NTS upstream TATA-like sequences are included in the boxes.

Table 3
The specific structural features of the mtDNA of RR.

	From	To	Size		Codons		Spacer/overlap	Strand
			bp	aa	Start	Stop		
tRNA-Phe	1	69	69					H
12s rRNA	70	1023	954					H
tRNA-Val	1024	1096	73					H
16s rRNA	1097	2777	1681					H
tRNA-Leu	2778	2853	76				1-base spacer	H
NADH 1	2855	3829	975	325	ATG	TAA	4-base spacer	H
tRNA-Ile	3834	3905	72				2-Base overlap	H
tRNA-Gln	3904	3974	71				1-base spacer	L
tRNA-Met	3976	4044	69					H
NADH2	4045	5089	1045	348	ATG	T–		H
tRNA-Trp	5090	5160	71				2-base spacer	H
tRNA-Ala	5163	5231	69				1-base spacer	L
tRNA-Asn	5233	5305	73				33-base spacer	L
tRNA-Cys	5339	5407	69				1-base overlap	L
tRNA-Tyr	5407	5477	71				1-base spacer	L
COI	5479	7041	1562	521	GTG	TAA		H
tRNA-Ser	7042	7112	71				2-base spacer	L
tRNA-Asp	7116	7187	72				12-base spacer	H
COII	7200	7890	691	230	ATG	T–		H
tRNA-Lys	7891	7966	76				1-base space	H
ATPase8	7968	8132	165	55	ATG	TAA	7-base overlap	H
ATPase6	8126	8808	683	227	ATG	TA-		H
COIII	8809	9593	785	261	ATG	TA-		H
tRNA-Gly	9594	9665	72					H
NADH3	9666	10,014	349	116	ATG	T–		H
tRNA-Arg	10,015	10,084	70					H
NADH4L	10,085	10,381	297	99	ATG	TAA	7-base overlap	H
NADH4	10,375	11,755	1381	460	ATG	T–		H
tRNA-His	11,756	11,824	69					H
tRNA-Ser	11,825	11,893	69				1-base space	H
tRNA-Leu	11,895	11,967	73				3-base space	H
NADH5	11,971	13,794	1824	608	ATG	TAA	6-base overlap	H
NADH6	13,789	14,310	522	174	ATG	TAG		L
tRNA-Glu	14,311	14,378	68				6-base space	L
Cytb	14,385	15,525	1141	380	ATG	T–		H
tRNA-Thr	15,526	15,597	72				1-base overlap	H
tRNA-Pro	15,597	15,666	70					L
D-loop	15,667	16,590	924					H

significantly higher than that of RG (III + 7–8) ($P < 0.05$). The BL/BH, BL/HL, and CPL/CPH of RR were significantly higher than those of RCC and significantly lower than those of RG. The HL/HH of RR (0.82 ± 0.02) was significantly lower than those of RCC (1.18 ± 0.01) and RG (0.96 ± 0.01). In conclusion, these results indicate that RR is more similar in appearance to RCC, and at the same time exhibits unique hybrid traits.

3.2. DNA content and chromosome number of RR

We compared the DNA content of RR with that of its parents (as shown in Table S3), using the DNA content of RCC and RG as controls. The results showed that the DNA content of all RR individuals was equal to the sum of the DNA content of RCC and half of the DNA content of RG ($P > 0.05$), suggesting that RR is an allotriploid fish. Metaphase spreads from 100 RR chromosomes were further examined, and the chromosome numbers of RR are listed in Table S4. The results showed that 86 metaphase spreads contained 125 chromosomes, 12 metaphase spreads contained 124 chromosomes, and 2 metaphase spreads contained 123 chromosomes. The karyotype formula for the RCC chromosomes was 22m + 34sm + 22st + 22t, and for the RG chromosomes was 18m + 22sm + 10st. The karyotype formula for RR was 31m + 45sm + 27st + 22t, indicating that RR contained 125 chromosomes, of which 100 were from RCC and 25 were from RG (Fig. 2).

3.3. Nucleotide sequence of 5S rDNA in RR

We amplified and sequenced RR and its parental species using 5S primers, which showed three bands (203, 340 and 477 bp) for RCC, two bands (223 and 477 bp) for RG, and three bands (226, 340 and 477 bp) for RR (Fig. 3A). BLASTn analysis showed that all the bands corresponded to the 5S rDNA repeating units, and each repeating unit consisted of 3′ coding region, NTS region and 5′ coding region. The nucleotide homology and sequence information of the 5S rDNA between RCC, RG and RR are shown in Tables S5 and S6. The results showed that the 226 bp fragment in RR was inherited from the 223 bp fragment of RG (99.10 % similarity), while the 340 bp and 477 bp fragments were inherited from the corresponding 340 bp and 477 bp fragments of RCC (98.24 % and 98.32 % similarity, respectively). The 5S rDNA fragments in RR (designated 226, 340, and 477 bp) were classified into different NTS types: NTS-106, NTS-220, and NTS-357, corresponding to fragments of 106, 220, and 357 bp, respectively. The A box, internal elements, and C box were identified within the coding region (Fig. 3B). The TATA box was identified in all NTS sequences and was modified to TAAA (Fig. 3C, D, and E). In conclusion, the coding region and NTS sequence data from RR inherited genetic traits from both parent species, with some recombination and mutation.

3.4. Mitochondrial DNA structure of RR

Sequencing analysis showed that the complete mtDNA length of RR was 16,590 bp, which included 2 rRNA genes, 13 protein-coding genes, 22 tRNA genes, and non-coding regions (D-loop region and light chain replication initiation region). The mtDNA sequence of RR is shown in Table S7. The specific structural features of the mtDNA of RR are summarized in Table 3. The proportions of bases in RR mtDNA are shown in

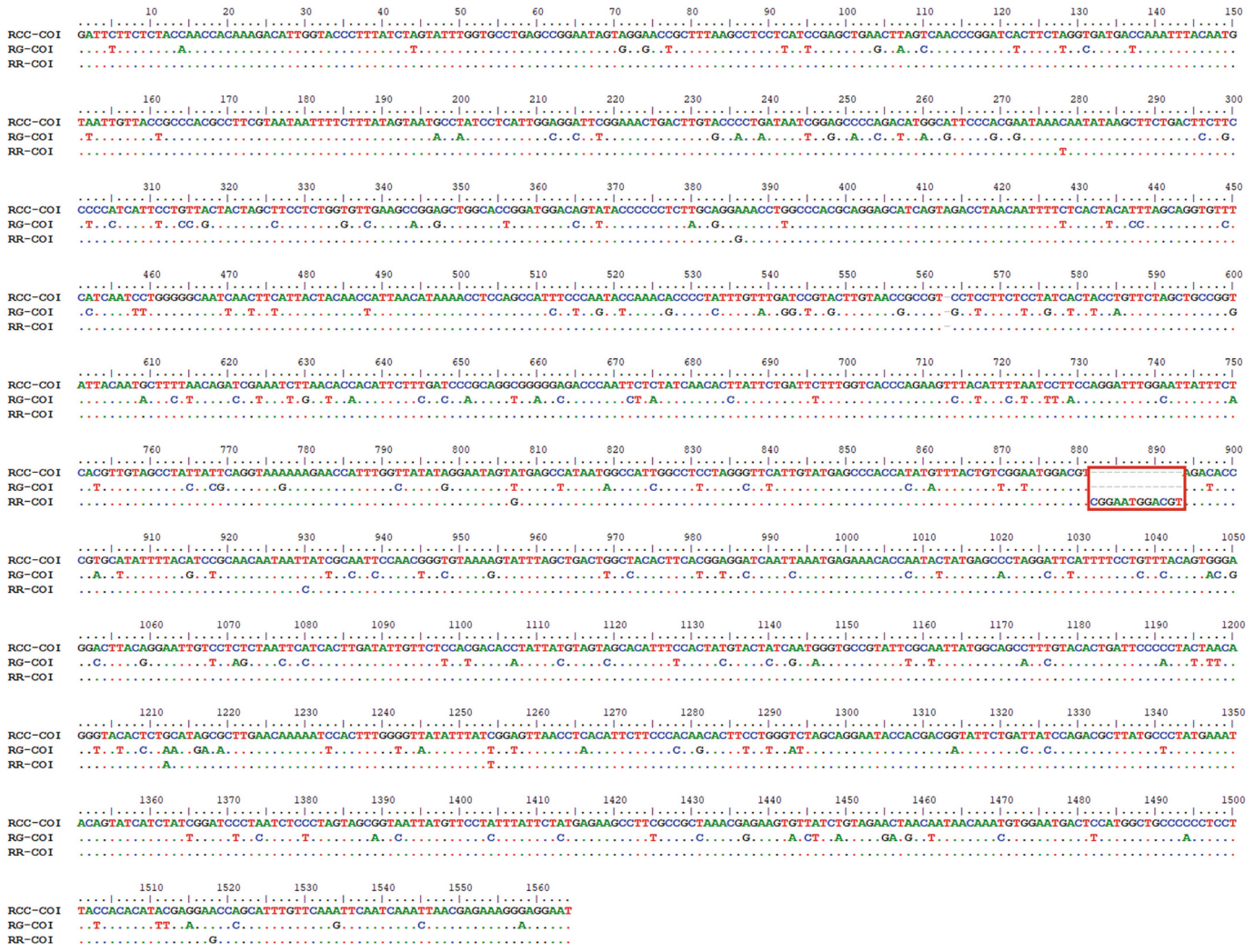

Fig. 4. *COI* gene sequences for RCC, RG and RR. The insertion sequence is included in the box.

Table S8, where the proportion of A + T (57.65 %) is higher than that of G + C (42.35 %), indicating a strong AT preference. The mtDNA similarity between RR and RCC was 99.4 %, and between RR and RG was 82.1 %, indicating that the mtDNA of RR strictly follows maternal inheritance (Table S9). The mtDNA sequence of RR showed a partial base insertion compared to its parents, with an insertion sequence of CGGAATGGACGT, located in the cytochrome *c* oxidase subunit I (*COI*) gene (Fig. 4). The D-loop region of RR mtDNA revealed that its sequence inherited the genetic characteristics of its parent species, with some mutated bases and indels. The core sequence (TACAT), the reverse complement of the core sequence (ATGTA), and conserved sequences (including CSB-D, CSB-E, CSB-F, CSB1, CSB2, and CSB3) were identified in the D-loop (Fig. 5). The results from the NJ evolutionary tree analysis indicated that the genetic distance between RR and the maternal RCC was 0.0046, whereas the genetic distance between RR and the paternal RG was 0.1664, and the genetic distance between RR and *Carassius auratus auratus* isolate J1 and *Carassius auratus auratus* was relatively close (as shown in Table S10) (Fig. 6). Comparison of the evolutionary tree with the genetic distances revealed that the mtDNA of RR followed maternal inheritance.

3.5. The growth rate and muscle fiber of RR

We measured the body weights of RR and RCC under the same rearing conditions. The results indicated that the average body weight of RR (251.9 ± 16.3 g) was significantly higher than that of RCC (212.7 ± 15.6 g) and RG (5.5 ± 0.3 g). By 12 months of age, the weight of RR was 18.43 % greater than that of RCC (Fig. 7A). Additionally, muscle fiber analysis showed that RR exhibited both hypertrophy and hyperplasia of muscle fibers compared to RCC (Fig. 7B). The total fiber area of RR (445,450 ± 19,080 μm^2) was significantly larger ($P < 0.05$) than that of RCC (377,567 ± 16,800 μm^2) (Fig. 7C). The mean muscle fiber area of RR (9898.8 ± 51.2 μm^2) was also significantly greater ($P < 0.05$) than that of RCC (2904.3 ± 34.7 μm^2) (Fig. 7D). Furthermore, the total number of fibers in RR (45 ± 2.1) was significantly lower ($P < 0.05$) than that in RCC (100 ± 5.3) (Fig. 7E).

4. Discussion

Distant hybridization is an important technique for preventing genetic degradation and improving varieties (Wang et al., 2019). Previous studies have shown that distant hybridization usually leads to polyploidy production (Chen et al., 2018). For example, triploid and tetraploid hybrid fish (3n = 124, 4n = 148) were produced by crossing red crucian carp (♀) with blunt snout bream (*Megalobrama amblycephala*, ♂) (Qin et al., 2014); triploid hybrids (3n = 72) were obtained by crossing grass carp (*Ctenopharyngodon Idella*, ♀) with topmouth culter (*Culter alburnus*, ♂) (Wu et al., 2019) or blunt snout bream (♂) (He et al., 2013); and the allotetraploid lineage produced by red crucian carp (♀) × common carp (*Cyprinus carpio*, ♂) (Liu et al., 2001; Ren et al., 2019; Xiao

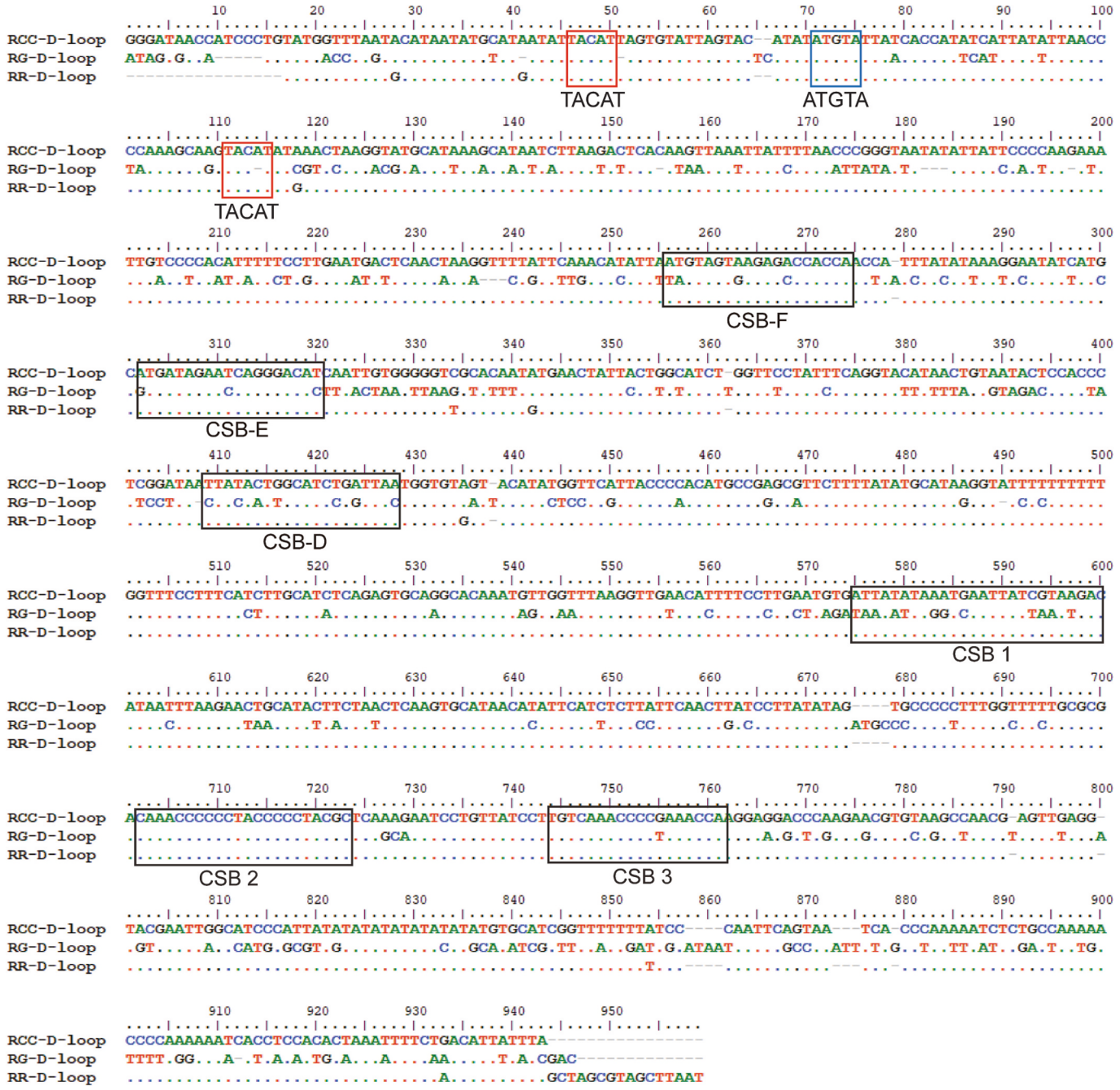

Fig. 5. Comparative analysis of mitochondrial D-loop region of RCC, RG and RR. The core sequence of the TAS element in the termination-binding sequence region (TACAT) is shown in the red box, and its reverse complementary sequence (ATGTA) is shown in the blue box. The central conserved sequence region (CSB-D, CSB-E, CSB-F) and the conserved sequence regions (CSB 1, CSB 2, CSB 3) are included in the black boxes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

et al., 2014). Extensive and systematic studies of distant hybridization in fish have provided valuable insights into the genetic characteristics of these hybrids (Gong et al., 2021; Hu et al., 2020; Liu et al., 2020; Wang et al., 2019; Robertson et al., 2010; Wang et al., 2013; Liu, 2010). In the pattern of inheritance of distant hybridization, polyploid offspring can be produced when the number of chromosomes in the maternal parent was greater than the number of chromosomes in the paternal parent (Liu et al., 2020). In this study, allotriploid hybrid progeny (RR, 3n = 125) were obtained through distant hybridization between RCC (♀) and RG (♂), which is consistent with the genetic patterns observed in distant hybridization across subfamilies (Liu et al., 2020).

In distant hybridization, the genomes of parent species combine, leading to changes in gene structure and gene expression within the hybrids (Li et al., 2019). Previous studies have shown that hybrid fish often contain chimeric and mutant genes, as revealed through genome or transcriptome sequencing (Liu et al., 2018; Liu et al., 2016). Additionally, 5S rDNA undergoes base recombination in many hybrid fish species and is an important marker for understanding variation, organization, and evolution in fish species (Campo et al., 2009; Martins and Galetti Jr., 2001; Merlo et al., 2012). In this study, the 5S rDNA of RR also showed recombination and mutation, which is consistent with previous studies (Liu et al., 2019a). Fish mtDNA is an effective tool for studying phylogeny and population genetics (Bogenhagen, 2012; Johansen et al., 1990), and its arrangement is highly conserved across

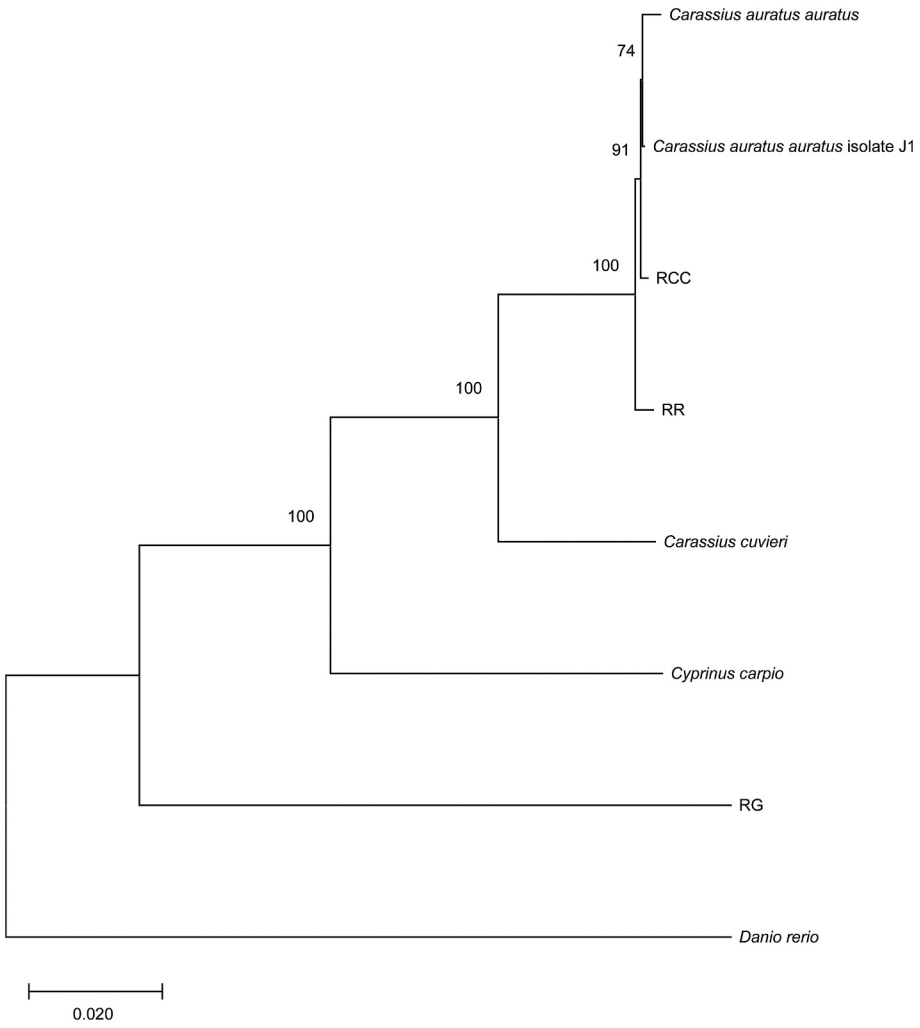

Fig. 6. Comparative analysis of the evolutionary trees of RCC, RG, RR and five fish species.

fish species, with mitochondrial genomes generally maintaining the same order (Chang et al., 1994; Satoh et al., 2016). Mt. DNA usually follows maternal inheritance, the mtDNA of RR are also inherited maternally. Furthermore, the mtDNA of RR showed a partial base insertion compared to the parental species, with the insertion sequence (CGGAATGGACGT) located in the *COI* gene. Numerous studies have validated the use of mitochondrial *COI* gene sequences as DNA barcodes for fish species identification and for phylogenetic analysis at the species level (Ward et al., 2008; Ward et al., 2009; Ward et al., 2005). Therefore, the *COI* gene can be used to analyze the proximity of RR to its parental species. In conclusion, the 5S rDNA and mtDNA of RR showed mutation and recombination compared with the parental species, and they are reliable molecular markers for RR.

Distant hybridization is often associated with changes in phenotype. Our results showed that the number of dorsal fin strips and several other countable traits differed significantly ($P < 0.05$) among RR, RCC, and RG (Tables 1 and 2), with RR displaying clear hybrid characteristics. Interestingly, the body size and color of RR were more similar to those of RCC, as RR was larger and exhibited a lighter body color compared to RCC, displaying a light red coloration. Overall, RR exhibited distinct hybrid characteristics in its appearance. Based on these results, we hypothesize that the prominent traits in RR may stem from the significant genetic distance and biological differences between the parents. At 12 months of age, the mean body weight of RR was significantly higher than that of RCC ($P < 0.05$) (Fig. 7A). Muscle fiber analysis revealed that the total fiber area and mean fiber area in RR were significantly greater than those in RCC, indicating that RR exhibited muscle fiber hypertrophy and hyperplasia. We speculate that the faster growth of RR may be due to the hypertrophy of its muscle fibers. In previous studies, many hybrid offspring with advantage traits have been produced through distant hybridization. For example, triploid fish with fast growth rates were produced by the distant hybridization between diploid fish and tetraploid fish (Liu et al., 2021a), and hybrids with faster growth, stronger disease resistance, and good meat quality were obtained by the crossing of white crucian carp and red crucian carp (Liu et al., 2024; Zeng et al., 2024; Zhang et al., 2024). Combining the results of this study, we found that distant hybridization can indeed produce economically beneficial traits for aquaculture, particularly rapid growth traits. In future studies, we plan to employ genome sequencing, transcriptome analysis, and gene editing to explore the molecular mechanisms underlying these hybrid traits.

In summary, we have successfully created a novel allotriploid fish (RR) by distant hybridization. RR has 125 chromosomes, which 100 chromosomes from RCC and 25 chromosomes from RG. RR showed morphological traits that were intermediate between those of its parents. The 5S rDNA and mtDNA of RR showed recombination or mutation, and the mitochondria of RR follows maternal inheritance. Furthermore, RR exhibited rapid growth and showed muscle hypertrophy and hyperplasia than RCC. The rapid growth advantage of RR is most beneficial to aquaculture. This study contributes to the understanding of the genetic characteristics of progeny from distant hybridization in fish, and the RR provides valuable material for investigating

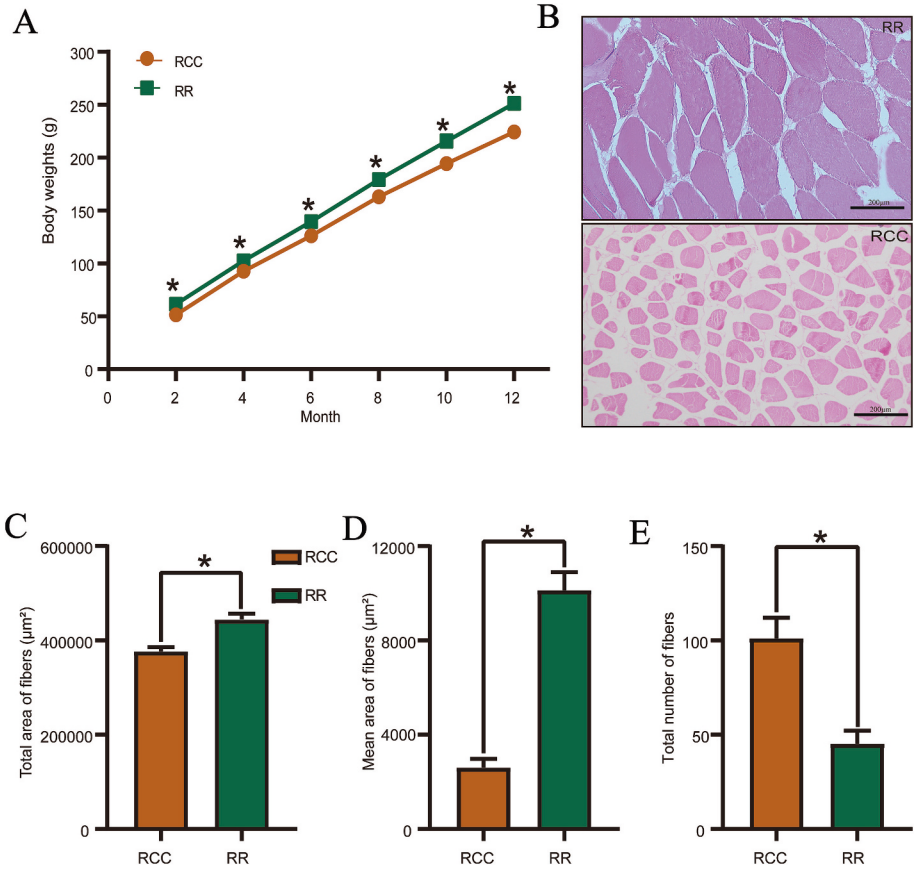

Fig. 7. Muscle fiber hypertrophy and average body weight were higher in the hybrid progeny RR than in RCC. (A) Average body weights of RCC and RR from 1 to 12 months of age. (B) Paraffin sectioning and H&E staining of 12-month-old RCC and RR. (C–E) Quantitative analysis of muscle fibers from RCC and RR, including calculation of the total number of fibers.

hybrid traits, particularly those related to growth in hybrid fish species.

CRediT authorship contribution statement

Fanglei Liu: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Ziyi Huang:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Xuanyi Zhang:** Investigation, Data curation. **Bei Li:** Investigation, Data curation. **Lujiao Duan:** Methodology, Formal analysis. **Jianming Yu:** Methodology, Formal analysis. **Qizhi Liu:** Software, Methodology. **Siyang Huang:** Software. **Hongwen Liu:** Resources. **Qiuli Liang:** Data curation. **Qingfeng Liu:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Shaojun Liu:** Writing – review & editing, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2025.742442.

Data availability

Data will be made available on request.

References

Bogenhagen, D.F., 2012. Mitochondrial DNA nucleoid structure. Biochim. Biophys. Acta 1819 (9–10), 914–920. https://doi.org/10.1016/j.bbagrm.2011.11.005.

Campo, D., Machado-Schiaffino, G., Horreo, J.L., Garcia-Vazquez, E., 2009. Molecular organization and evolution of 5S rDNA in the genus Merluccius and their phylogenetic implications. J. Mol. Evol. 68 (3), 208–216. https://doi.org/10.1007/s00239-009-9207-8.

Chang, Y.S., Huang, F.L., Lo, T.B., 1994. The complete nucleotide sequence and gene organization of carp (Cyprinus carpio) mitochondrial genome. J. Mol. Evol. 38 (2), 138–155. https://doi.org/10.1007/bf00166161.

Chen, J., Luo, M., Li, S., Tao, M., Ye, X., Duan, W., Zhang, C., Qin, Q., Xiao, J., Liu, S., 2018. A comparative study of distant hybridization in plants and animals. Sci. China Life Sci. 61 (3), 285–309. https://doi.org/10.1007/s11427-017-9094-2.

Gong, D., Xu, L., Liu, Q., Wang, S., Wang, Y., Hu, F., Wu, C., Luo, K., Tang, C., Zhou, R., 2021. A new type of hybrid bream derived from a hybrid lineage of *Megalobrama amblycephala* (♀)× Culter alburnus (♂). Aquaculture 534, 736194.

He, W., Xie, L., Li, T., Liu, S., Xiao, J., Hu, J., Wang, J., Qin, Q., Liu, Y., 2013. The formation of diploid and triploid hybrids of female grass carp × male blunt snout

bream and their 5S rDNA analysis. BMC Genet. 14, 110. https://doi.org/10.1186/1471-2156-14-110.
Hong, X., Qin, J., Chen, R., Yuan, L., Zha, J., Wang, Z., 2016. Identification and Characterization of Novel and Conserved microRNAs in Several Tissues of the Chinese Rare Minnow (Gobiocypris rarus) Based on illumina Deep Sequencing Technology, 17. Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa City, Chiba, 277-8654, Japan, pp. 1–11 sato.takashi.4a@kyoto-u.ac.jp. Collection Center, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba City.
Hu, F., Zhong, H., Fan, J., Wu, C., Wang, S., Wang, Y., Luo, K., Zhao, R., Liu, J., Qin, Q., 2020. A new type of tetraploid fish derived via female autotetraploid × male allotetraploid hybridization. Aquaculture 524, 735244.
Johansen, S., Guddal, P.H., Johansen, T., 1990. Organization of the mitochondrial genome of Atlantic cod, *Gadus morhua*. Nucleic Acids Research 18 (3), 411–419. https://doi.org/10.1093/nar/18.3.411.
Li, W., Tan, H., Liu, J., Hu, J., Cui, J., Wang, S., Liu, Q., Hu, F., Ren, L., Tao, M., Zhao, R., Yang, C., Qin, Q., Liu, S., 2019. Comparative analysis of testis transcriptomes associated with male infertility in triploid cyprinid fish. Reprod. Fertil. Dev. 31 (2), 248–260.
Liu, S., 2010. Distant hybridization leads to different ploidy fishes. Sci. China Life Sci. 53, 416–425.
Liu, S., 2022. Fish Distant Hybridization. Springer.
Liu, S., Liu, Y., Zhou, G., Zhang, X., Luo, C., Feng, H., He, X., Zhu, G., Yang, H., 2001. The formation of tetraploid stocks of red crucian carp × common carp hybrids as an effect of interspecific hybridization. Aquaculture 192 (2–4), 171–186.
Liu, S., Qin, Q., Xiao, J., Lu, W., Shen, J., Li, W., Liu, J., Duan, W., Zhang, C., Tao, M., 2007. The formation of the polyploid hybrids from different subfamily fish crossings and its evolutionary significance. Genetics 176 (2), 1023–1034.
Liu, S., Luo, J., Chai, J., Ren, L., Zhou, Y., Huang, F., Liu, X., Chen, Y., Zhang, C., Tao, M., 2016. Genomic incompatibilities in the diploid and tetraploid offspring of the goldfish× common carp cross. Proc. Natl. Acad. Sci. U. S. A. 113 (5), 1327–1332.
Liu, Q., Wang, J., Xiao, J., Chen, X., Qi, Y., Li, W., Tao, M., Zhang, C., Qin, Q., Luo, K., 2017. Muscle nutrient of *Carassius auratus cuvieri* (♀)× *Carassius auratus* red var.(♂) and its parents. J. Fish. China 41 (7), 1133–1139.
Liu, Q., Qi, Y., Liang, Q., Xu, X., Hu, F., Wang, J., Xiao, J., Wang, S., Li, W., Tao, M., 2018. The chimeric genes in the hybrid lineage of *Carassius auratus cuvieri* (♀)× *Carassius auratus* red var.(♂). Sci. China Life Sci. 61, 1079–1089.
Liu, Q., Liu, J., Liang, Q., Qi, Y., Tao, M., Zhang, C., Qin, Q., Zhao, R., Chen, B., Liu, S., 2019a. A hybrid lineage derived from hybridization of *Carassius cuvieri* and *Carassius auratus* red var. and a new type of improved fish obtained by back-crossing. Aquaculture 505, 173–182.
Liu, Q., Qi, Y., Liang, Q., Song, J., Liu, J., Li, W., Shu, Y., Tao, M., Zhang, C., Qin, Q., 2019b. Targeted disruption of tyrosinase causes melanin reduction in *Carassius auratus cuvieri* and its hybrid progeny. Sci. China Life Sci. 62, 1194–1202.
Liu, Q., Liu, J., Yuan, L., Li, L., Tao, M., Zhang, C., Qin, Q., Chen, B., Ma, M., Tang, C., 2020. The establishment of the fertile fish lineages derived from distant hybridization by overcoming the reproductive barriers. Reproduction 159 (6), R237–R249.
Liu, Q., Luo, K., Zhang, X., Liu, F., Qin, Q., Tao, M., Wen, M., Tang, C., Liu, S., 2021a. A new type of triploid fish derived from the diploid hybrid crucian carp (♀) × autotetraploid fish (♂). Reproduction and Breeding 1 (2), 122–127.
Liu, Q., Zhang, X., Liu, J., Liu, F., Shi, F., Qin, Q., Tao, M., Tang, C., Liu, S., 2021b. A new type of allodiploid hybrids derived from female *Megalobrama amblycephala* × male *Gobiocypris rarus*. Front. Genet. 12, 685914.
Liu, Q., Duan, L., Li, B., Zhang, X., Liu, F., Yu, J., Shu, Y., Hu, F., Lin, J., Xiong, X., Liu, S., 2024. The key role of myostatin b in somatic growth in fishes derived from distant hybridization. Sci. China Life Sci. 67 (7), 1441–1454.
Luo, K., Xiao, J., Liu, S., Wang, J., He, W., Hu, J., Qin, Q., Zhang, C., Tao, M., Liu, Y., 2011. Massive production of all-female diploids and triploids in the crucian carp. Int. J. Biol. Sci. 7 (4), 487.
Martins, C., Galetti Jr., P.M., 2001. Two 5S rDNA arrays in neotropical fish species: is it a general rule for fishes? Genetica 111 (1–3), 439–446. https://doi.org/10.1023/a:1013799516717.
Merlo, M.A., Pacchiarini, T., Portela-Bens, S., Cross, I., Manchado, M., Rebordinos, L., 2012. Genetic characterization of Plectorhinchus mediterraneus yields important clues about genome organization and evolution of multigene families. BMC Genet. 13, 33. https://doi.org/10.1186/1471-2156-13-33.
Qin, Q., Wang, Y., Wang, J., Dai, J., Xiao, J., Hu, F., Luo, K., Tao, M., Zhang, C., Liu, Y., Liu, S., 2014. The autotetraploid fish derived from hybridization of *Carassius auratus* red var. (female) × *Megalobrama amblycephala* (male). Biol. Reprod. 91 (4), 93. https://doi.org/10.1095/biolreprod.114.122283.
Ren, L., Li, W., Qin, Q., Dai, H., Han, F., Xiao, J., Gao, X., Cui, J., Wu, C., Yan, X., 2019. The subgenomes show asymmetric expression of alleles in hybrid lineages of *Megalobrama amblycephala*× *Culter alburnus*. Genome Res. 29 (11), 1805–1815.
Robertson, A., Rich, T.C., Allen, A.M., Houston, L., Roberts, C., Bridle, J.R., Harris, S.A., Hiscock, S.J., 2010. Hybridization and polyploidy as drivers of continuing evolution and speciation in Sorbus. Mol. Ecol. 19 (8), 1675–1690. https://doi.org/10.1111/j.1365-294X.2010.04585.x.
Satoh, T.P., Miya, M., Mabuchi, K., Nishida, M., 2016. Structure and Variation of the Mitochondrial Genome of Fishes, 17. Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa City, Chiba, 277-8654, Japan, p. 719. https://doi.org/10.1186/s12864-016-3054-y, 1. sato.takashi.4a@kyoto-u.ac.jp. Collection Center, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba City.
Tiwary, B.K., Kirubagaran, R., Ray, A.K., 2004. The biology of triploid fish. Rev. Fish Biol. Fish. 14, 391–402.
Wang, A., Zhang, X., Yang, C., Song, Z., Du, C., Chen, D., He, Y., Cai, D., 2013. Development and characterization of synthetic amphiploid (AABB) between Oryza sativa and Oryza punctata. Euphytica 189, 1–8.
Wang, J., Xiao, J., Zeng, M., Xu, K., Tao, M., Zhang, C., Duan, W., Liu, W., Luo, K., Liu, Y., 2015. Genomic variation in the hybrids of white crucian carp and red crucian carp: evidence from ribosomal DNA. Sci. China Life Sci. 58, 590–601.
Wang, S., Tang, C., Tao, M., Qin, Q., Zhang, C., Luo, K., Zhao, R., Wang, J., Ren, L., Xiao, J., 2019. Establishment and application of distant hybridization technology in fish. Sci. China Life Sci. 62, 22–45.
Wang, S., Xu, X., Luo, K., Liu, Q., Chen, L., Wei, Z., Zhou, P., Hu, F., Liu, Z., Tao, M., 2020. Two new types of triploid hybrids derived from *Cyprinus carpio* (♀)× *Megalobrama amblycephala* (♂). Aquaculture 528, 735448.
Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D., 2005. DNA barcoding Australia's fish species. Philos. Trans. R. Soc. Lond. B Biol. Sci. 360 (1462), 1847–1857. https://doi.org/10.1098/rstb.2005.1716.
Ward, R.D., Costa, F.O., Holmes, B.H., Steinke, D., 2008. DNA barcoding of shared fish species from the North Atlantic and Australasia: minimal divergence for most taxa, but Zeus faber and Lepidopus caudatus each probably constitute two species. Aquat. Biol. 3 (1), 71–78.
Ward, R.D., Hanner, R., Hebert, P.D., 2009. The campaign to DNA barcode all fishes, FISH-BOL. Journal of Fish Biology 74 (2), 329–356. https://doi.org/10.1111/j.1095-8649.2008.02080.x.
Wu, C., Huang, X., Hu, F., Ouyang, Y., Zhao, L., Wang, S., Li, W., Fan, J., Zhang, C., Ren, L., 2019. Production of diploid gynogenetic grass carp and triploid hybrids derived from the distant hybridization of female grass carp and male topmouth culter. Aquaculture 504, 462–470.
Xiao, J., Kang, X., Xie, L., Qin, Q., He, Z., Hu, F., Zhang, C., Zhao, R., Wang, J., Luo, K., 2014. The fertility of the hybrid lineage derived from female *Megalobrama amblycephala*× male *Culter alburnus*. Anim. Reprod. Sci. 151 (1–2), 61–70. https://doi.org/10.1016/j.anireprosci.2014.09.012.
Xu, K., Duan, W., Xiao, J., Tao, M., Zhang, C., Liu, Y., Liu, S., 2015. Development and application of biological technologies in fish genetic breeding. Sci. China Life Sci. 58, 187–201.
Zeng, Q., Liu, X., Tang, Y., Li, Z., Yang, Y., Hu, N., Liu, Q., Zhou, Z., 2024. Evolutionarily conserved IL-22 participates in gut mucosal barrier through its receptors IL-22BP, IL-10R2 and IL-22RA1 during bacterial infection in teleost. Developmental & Comparative Immunology 152, 105110.
Zhang, Z., Chen, J., Li, L., Tao, M., Zhang, C., Qin, Q., Xiao, J., Liu, Y., Liu, S., 2014. Research advances in animal distant hybridization. Sci. China Life Sci. 57, 889–902.
Zhang, X., Liu, F., Li, B., Yu, J., Duan, L., Huang, Z., Zhou, Z., Shu, Y., Lin, J., Xiong, X., Liu, Q., Liu, S., 2024. Comparative analysis of nutrients in muscle and ovary between an improved fish and its parents. Reproduction and Breeding 4 (1), 16–21.
Zhong, X., Xu, Y., Liang, Y., Liao, T., Wang, J., 2005. The Chinese rare minnow (Gobiocypris rarus) as an in vivo model for endocrine disruption in freshwater teleosts: a full life-cycle test with diethylstilbestrol. Aquat. Toxicol. 71 (1), 85–95.
Zhong, H., Zhou, Y., Liu, S., Tao, M., Long, Y., Liu, Z., Zhang, C., Duan, W., Hu, J., Song, C., Liu, Y., 2012. Elevated expressions of GH/IGF axis genes in triploid crucian carp. Gen. Comp. Endocrinol. 178 (2), 291–300. https://doi.org/10.1016/j.ygcen.2012.06.006.