

Table S1. Comparison of morphometric measurements for blue catfish, channel catfish, and their reciprocal hybrids in the tank environment.

	Channel	Blue	B×C hybrid	C×B hybrid	F-value	Heterosis		Heterobeltiosis	
						B×C	C×B	B×C	C×B
Total length (cm)	17.25±1.03 ^c	12.06±0.25 ^a	14.15±0.66 ^b	14.03±0.92 ^b	40.88**	-3.45	-4.26	-17.97	-18.67
Standard length (cm)	14.40±0.96 ^b	10.66±0.21 ^a	11.58±0.71 ^a	11.58±0.87 ^a	25.78**	-7.58	-7.58	-19.58	-19.58
Head length (cm)	3.27±0.39 ^b	2.44±0.09 ^a	2.40±0.15 ^a	2.51±0.14 ^a	18.77**	-15.94	-12.08	-26.61	-23.24
Head width (cm)	2.15±0.20 ^b	1.70±0.12 ^a	1.67±0.09 ^a	1.68±0.15 ^a	14.77**	-13.25	-12.73	-22.33	-21.86
Head depth (cm)	2.27±0.20 ^b	1.70±0.12 ^a	1.77±0.10 ^a	1.89±0.17 ^a	15.02**	-10.83	-4.79	-22.03	-16.74
Body depth (cm)	2.83±0.65	2.38±0.08	2.43±0.10	2.43±0.33	1.79	-6.72	-6.72	-14.13	-14.13
Caudal depth (cm)	1.26±0.10 ^b	0.92±0.04 ^a	0.94±0.05 ^a	1.02±0.08 ^a	25.59**	-13.76	-6.42	-25.4	-19.05
Weight (g)	41.33±8.31 ^c	14.40±1.52 ^a	19.50±3.62 ^{ab}	23.33±4.37 ^b	29.01**	-30.02	-16.27	-52.82	-43.55

Significance of one-way ANOVA: *: $P < 0.05$; **: $P < 0.01$. Values in the same column having the same superscripts are not significantly different (Tukey's test, $P > 0.05$). Channel: channel catfish; Blue: blue catfish; B×C hybrid: female blue catfish × male channel catfish; C×B hybrid: female channel catfish × male blue catfish.

$$\text{Heterosis} = \frac{\bar{F}_1 - \text{MPV}}{\text{MPV}} \times 100$$

$$\text{Heterobeltiosis} = \frac{\bar{F}_1 - \text{BPV}}{\text{BPV}} \times 100$$

Where: \bar{F}_1 average of F1 hybrid; BPV: Better Parent Value; MPV: Mid Parent Value (P1+P2)/2.

Table S2. Biochemical and immunological assays in blue catfish, channel catfish, and their reciprocal hybrids cultured in the tank environment.

	Blue catfish	Channel catfish	C×B hybrid	B×C hybrid
Lactate (mmol/L)	3.599 ± 0.811 ^a	6.263 ± 1.173 ^b	7.168 ± 1.375 ^b	8.104 ± 1.444 ^b
Glucose (mg/dl)	98.385 ± 18.741 ^a	96.788 ± 25.282 ^a	172.462 ± 35.578 ^b	133.615 ± 19.666 ^a
Lysozyme (µg/mL)	3.872 ± 0.7 ^a	0.160 ± 0.086 ^b	0.378 ± 0.102 ^b	0.935 ± 0.427 ^b
ACH50 (units/mL)	76.179 ± 22.325 ^{ac}	6.820 ± 1.496 ^b	43.979 ± 13.584 ^c	46.596 ± 9.410 ^c

Note: values in the same row having the same superscripts are not significantly different (Tukey's test, $P > 0.05$). B×C hybrid: female blue catfish × male channel catfish; C×B hybrid: female channel catfish × male blue catfish.

Table S3. Liver sample RNA sequencing yield, quality control, and alignment statistics.

Sample ID	Sample name	Body weight at collection	Raw reads	Filtered reads	Mapping rate to channel genome	Mapping rate to blue genome
CxC_C58_indiv1	Channel catfish liver	47.6 g	74,645,394	72,939,430	79.6%	37.4%
CxC_C58_indiv2	Channel catfish liver	52.5 g	79,057,946	76,881,484	79.3%	29.7%
BxB_C57_indiv1	Blue catfish liver	20.2 g	89,934,258	87,650,476	37.3%	84.1%
BxB_C57_indiv2	Blue catfish liver	18.6 g	67,639,738	66,215,758	42.0%	81.9%
BxC_C66_indiv1	B×C hybrid liver	29.0 g	92,917,484	90,536,414	57.6%	68.1%
BxC_C66_indiv2	B×C hybrid liver	32.2 g	91,926,868	90,204,272	65.2%	71.2%
CxB_C56_indiv1	C×B hybrid liver	23.5 g	100,242,864	97,806,146	66.9%	55.6%
CxB_C56_indiv2	C×B hybrid liver	26.8 g	93,576,928	90,995,214	50.6%	60.4%

Table S4. Numbers of significantly differentially expressed genes (DEGs) in pairwise comparisons among four genetic backgrounds (PB, PC, F₁BC, and F₁CB.).

	Number of DEGs	Upregulated	Downregulated
PC vs. PB	2,307	1,346	961
PB vs. F ₁ BC	665	326	339
PB vs. F ₁ CB	598	266	332
PC vs. F ₁ BC	458	289	169
PC vs. F ₁ CB	810	484	326
F ₁ BC vs. F ₁ CB	98	50	48

Table S5. List of primer sequences for quantitative reverse transcription PCR validation.

Gene name	5' - 3' primer sequence	PCR product size (bp)
<i>Cyp2k19</i> -F	GGCCAAGTACCCTCACATACAGG	154
<i>Cyp2k19</i> -R	ACTCATGGGTACTATATTTGCCAG	
<i>Fgflb</i> -F	ATCATCAGAGGTCACGAGGC	166
<i>Fgflb</i> -R	CGTACCAGACCCCGTGCTC	
<i>Hmox</i> -F	GTCGCACCAATTTACTTCCCT	234
<i>Hmox</i> -R	GGTAATTCGGCCCAACACC	
<i>Irf7</i> -F	TATGATTTGCCAAACCACATGC	170
<i>Irf7</i> -R	ACAGTCATGTTATTCGGAAGG	
<i>Cregl</i> -F	CAAGCACAAAGGTTACGACCC	210
<i>Cregl</i> -R	CCTCCGAAGTAGTCCAACACC	
<i>Tm4sf4</i> -F	AGCTGGTTATTCTTTTCATCGT	217
<i>Tm4sf4</i> -R	CAGAGCCATTTGAACCATGC	

Figure S1. Morphometric measurements of channel catfish (C), *Ictalurus punctatus*, blue catfish (B), *I. furcatus*, and their reciprocal F1 hybrids raised in the tank environment, standardized by total length (TL).

Morphometric traits normalized by total length for the four genetic cross types: channel catfish (C) parental cross (PC), blue catfish (B) parental cross (PB), blue catfish female \times channel catfish male hybrids (F₁BC), and channel catfish female \times blue catfish male hybrids (F₁CB). (A), body length; (B), body depth; (C), head length; (D), head width; (E), head depth; (F), caudal depth. Statistical significance was assessed by the nonparametric Mann-Whitney U test. (*, adjusted P -value < 0.05).

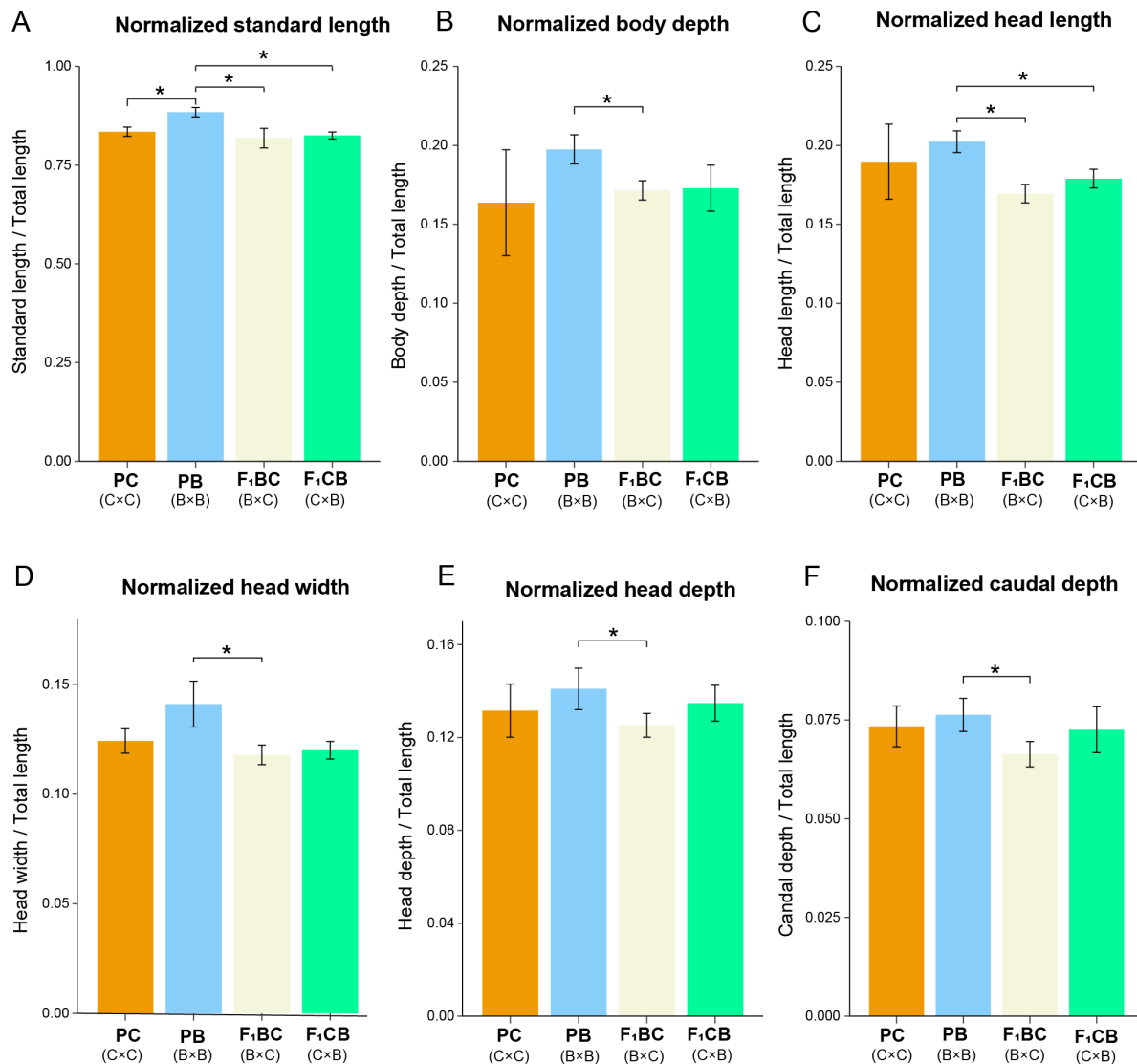


Figure S2. Radar plot of seven body metrics in channel catfish (C), *Ictalurus punctatus*, blue catfish (B), *I. furcatus*, and their reciprocal F₁ hybrids raise in the tank environment.

Radar chart for seven body measurements (total length, body length, head length, head width, head depth, caudal depth) in channel catfish (C) parental cross (PC), blue catfish (B) parental cross (PB), blue catfish female \times channel catfish male hybrids (F₁BC), and channel catfish female \times blue catfish male hybrids (F₁CB). The channel measurement area is shaded in orange.

