

# Ecological adaptation of an F1 hybrid cross of carnivorous and herbivorous Cyprinidae fishes

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## Research

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18

19 **Abstract:**

20 **Background:** Whether hybridization plays a positive or negative role in  
21 speciation remains a controversial issue to date. Genetic factors have been  
22 widely studied, but ecological factors also play an important role. Although  
23 studies on the ecological adaptation of hybrids between different niche parents  
24 have been widely reported, cases of extreme niche parental hybridization have  
25 not been documented, which may show more ecological phenomena in the  
26 fields of hybrid speciation and ecological species isolation.

27 **Results:** Taking Cyprinidae fish parents (*Schizothorax wangchiachii* and  
28 *Percocypris pingi*) with extreme ecological niches (herbivorous and  
29 carnivorous) and their F1 hybrids as research objects, fish, shrimp, blood  
30 worms and periphytic algae were selected as food correspond to four different  
31 ecological niches. Morphologically, most external and skeletal traits in the F1  
32 hybrids were balanced between the parents, but digestive traits were closer to  
33 those of herbivorous parents. In terms of diet, the F1 hybrids weakly foraged  
34 for parental food resources, but can more effectively forage for intermediate  
35 food resources. In foraging abilities, the F1 hybrids showed low foraging  
36 enthusiasm and abilities for parent resources, although the former was the  
37 more important factor. Interestingly, the F1 hybrids showed high foraging  
38 enthusiasm and success rates when they first foraged for fish, but then they  
39 vomited fish debris as a result of mechanical difficulty in chewing rather than  
40 taste, and the reason was a contradiction between the genetic behaviours and

41 intermediate morphology. This behaviour was harmful and was persistent in  
42 some individuals, representing a new mechanism in ecological species  
43 isolation. However, the F1 hybrids have also shown evidence of new  
44 ecological niche formation in favour of hybrid speciation by abandoning  
45 foraging parent resources and focusing more on foraging intermediate foods.

46 **Conclusions:** (1) Low foraging enthusiasm is an important reason for the  
47 fitness decrease of F1 hybrids to parent food. (2) The contradiction between  
48 genetic behavior and intermediate traits is reported for the first time. (3) F1  
49 hybrids may form an intermediate ecological niche between parents proved  
50 experimentally.

51

52 Key-words: Hybrid speciation, ecological isolation, new ecological niche,  
53 foraging behavior, hybrid fitness

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## 55 **1. Introduction**

56 What role does hybridization play in speciation? Some researchers  
57 thought that hybridization was an evolutionary dead end [1], because hybrids  
58 were more often observed to be less healthy and ecologically adapted than  
59 either parent species, and tended to be sterile [2-7]. However, others indicated  
60 that hybridization can provide an important source of genetic variation on  
61 which selection might act and that its adaptive role was more widespread  
62 [8-16]. The survival and adaptation of F1 hybrids is the first and most important

63 step in hybrid speciation and is affected by both genetic and ecological  
64 challenges. Genetic challenges refer to the low fitness of hybrids due to the  
65 prevalence of genetic incompatibility between the genomes of different  
66 populations [4, 7, 17]. Ecological challenges refer to reduced hybrid fitness due  
67 to the maladaptive intermediacy of their ecologically relevant genotypes and  
68 phenotypes in parental environments [3, 18, 19]. Genetic challenges have  
69 been widely reported, but ecological challenges also play an important role [17,  
70 20].

71 Morphology is often determined by quantitative traits, therefore the  
72 morphology of F1 hybrids are general between parents [19, 21]. If no  
73 intermediate ecological niche exists between the parents of the F1 hybrids,  
74 they often show low fitness for parent resources. For example, in  
75 zooplankton-feeding and benthic-feeding sticklebacks [18], and poplar and  
76 willow leaf beetles [20], intermediate niches are often lacking between the  
77 monotrophic parents because identifying transitional species between benthic  
78 species and zooplankton and between different plants is difficult. However,  
79 when intermediate ecological niches exist between hybrid parents, the results  
80 may be different, and the hybrids may develop new ecological niches [15, 22,  
81 23].

82 Of course, species foraging type is determined not only by traits but also  
83 by foraging behaviour, with a significant correlation between the two [24].  
84 Foraging traits are often quantitative, and are therefore frequently additive

85 between parents in F1 hybrids. However, many unique behaviours develop  
86 among species, and these unique parental genetic behaviours of F1 hybrids  
87 may be codominant [25] or dominant [26] rather than additive. Therefore,  
88 unique parental foraging behaviour is usually dominant rather than additive in  
89 F1 hybrids, and the traits of the parents are often additive in the F1 hybrids,  
90 which is obviously contradictory.

91 Due to the primitive evolutionary status of fish and in vitro fertilization,  
92 more cases of hybridization are observed in fish than in higher vertebrates [12].  
93 Moreover, similar to plants, fish have many polyploids, especially Cyprinidae,  
94 and this trait is often associated with the formation of allopolyploids by ancient  
95 distant hybridization that can instantly create new species that are  
96 reproductively isolated from their parents [27-31] indicating that hybridization  
97 between parents with two different ecological niches will be more likely, and  
98 genetically stable new species may form in Cyprinidae.

99 The species used in this study were cold-water Cyprinidae fishes from the  
100 upper Yangtze River basin in the south-eastern Tibetan Plateau. *Schizothorax*  
101 *wangchiachii* (SW) has a sharp horny front jaw and mainly scrapes and eats  
102 periphytic algae from rocks. *Percocypris pingi* (PP) is a typical carnivorous fish  
103 with a sub-superior mouth. Morphologically, the *Schizothorax* genus and  
104 *Percocypris* genus were once thought to belong to two different subfamilies  
105 [32]. However, molecularly, they were shown to be sister genera in a recent  
106 study [28, 33]. They are homogeneously distributed and have similar breeding

107 periods, and we have successfully bred healthy F1 hybrids (*P. pingi* ♂ × *S.*  
108 *wangchiachii* ♀, PS) through artificial hybridization, but backcrossing has failed  
109 [34]. Compared with PP, SW has a unique foraging behaviour of scraping and  
110 eating algae from rocks (Supplementary Movie 1). Similarly, compared with  
111 SW, PP has a unique foraging behaviour of hunting and ambushing prey  
112 (Supplementary Movie 2). Although we did not find their natural hybrids, such  
113 hybridization between parents with two extreme ecological niches is more  
114 likely to yield some valuable and distinct ecological phenomena than parents  
115 with small ecological niche differences in the fields of hybrid speciation and  
116 ecological species isolation.

117 In this study, carnivorous fish, herbivorous fish and their F1 hybrids were  
118 used to explore the ecological adaptability of the F1 hybrids through  
119 comparative behavioural and morphological studies to provide new theoretical  
120 results for related studies on the isolation of ecological species and speciation  
121 by hybridization.

122

## 123 **2. Materials and methods**

### 124 2.1 Experimental fish acquisition

125 In March 2017 and 2019, a hybridization experiment and parental  
126 reproduction were carried; for details on the methods, refer to the research by  
127 [34]. Age-two fishes (PP (122.03±1.78 mm, 25.2±1.05 g), SW (106.78±1.41  
128 mm, 18.43±0.74 g) and PS (125.84±2.71 mm, 29.22±1.85 g)) were used to

129 quantify both external and skeletal characteristics, and age-one fishes (PP  
130 (9.08±0.34 mm, 12.07±0.90 g), SW (9.23±0.14 mm, 13.03±1.50 g) and PS  
131 (9.17±0.48 mm, 14.02±3.76 g)) were used to quantify digestive characteristics,  
132 foraging and behavioural features.

## 133 2.2 Morphology

134 The external morphology of age-two SW (n=30), PP (n=30) and PS (n=30)  
135 was studied, and the examination standards were referenced from [35]. Then,  
136 we selected 10 fish individuals for quantification of skeletal morphology. Their  
137 opercular bone, pharyngeal bone, dentary bone and skull were obtained by  
138 boiling, and the examination standards are described in Supplementary Fig. 1.  
139 Next, the digestive characteristics of age-one SW (n=6), PP (n=6) and PS (n=6)  
140 were studied. This study quantified the anatomy and histology  
141 (Hematoxylin-eosin staining) of the digestive organs, and the examination  
142 standards are described in Supplementary Fig. 2. Finally, 19 external  
143 morphological indicators, 19 skeletal morphological indicators and 23 digestive  
144 indicators were quantified in this study, as shown in Supplementary Tables 1-2.  
145 To visually show the comprehensive morphological differences of the three  
146 fishes, we conducted principal component analysis (PCA) of the Z-scores of  
147 three categories of indicators in SPSS 21.0.

148 The body shapes were photographed using an SLR camera (Canon EOS  
149 100D, Japan). The details of the heads fixed by Bouin's fixative and bones  
150 were photographed (Figs. 1 and 3) by a stereomicroscope (Nikon SMZ25).

151 Slices of the digestive organs were photographed (Fig. 2) under a microscope  
152 (Nikon ECLIPSE 80i). Age-two PP, SW and PS were scanned (Fig. 3) using a  
153 MicroCT Skyscan 1176 (Bruker, Belgium) to obtain the holistic bone structure;  
154 specific methods are described in [36], and it were slightly modified in this  
155 study.

### 156 2.3 Comparison of foraging habit

157 We fed PP, SW and PS with small fish (*Sinibrama taeniatus*,  
158  $0.0507\pm 0.0043$ , a cyprinid fish that can breed in our lab year round (Fig. 4a)),  
159 small shrimp (*Neocaridina denticulate*,  $0.1093\pm 0.0227$ , which is widely  
160 distributed in China's rivers (Fig. 4c)), Tubificidae worms (an aquatic mollusc  
161 (Fig. 4b)) and periphytic algae (*Spirogyra*, a filamentous algae (Fig. 4d)), which  
162 correspond to foods in different ecological niches. Specific experimental  
163 methods can be found in Supplementary method 1. We compared each fish  
164 species' foraging level (FL) using the following formula:

$$FL = M2 / (M1 - M2)$$

165 where  $M1$  represents body weight, and  $M2$  represents chyme weight.

### 166 2.4 Hybrid vs *P. pingi* in foraging fish

167 We compared the foraging capacity of PP (n=15) and PS (n=18) for small  
168 fishes (*S. taeniatus*) (Fig. 5a). Specific experimental methods are described in  
169 Supplementary method 2. We observed experimental fishes by video and  
170 quickly replayed the video and counted the following indicators: first attack  
171 time (FAT), first success time (FST), the success rate of the first attack (SRFA),

172 first attack time after the first successful capture (FAT2), attack frequency (AF),  
173 the success rate of the total attacks (SRTA), and the rate of vomiting fish (RVF).

174 Details are as follows:

175 FAT: The time when an experimental fish first attacked the small fishes. To  
176 exclude the influence of irritability, only the experimental fishes that launched  
177 the first attack within 5 min were included in all statistical comparisons.

178 FST: The time when an experimental fish first successfully caught a small  
179 fish. If it did not succeed within 30 min, a value of 30 min was used as its first  
180 success time.

181 SRFA: The success rate when an experimental fish first attacked the small  
182 fishes.

183 FAT2: The time when an experimental fish first attacked after the first  
184 successful capture.

185 AF: The average number of attacks per minute of an experimental fish; this  
186 value was calculated using the following formula:

$$AF = N/T$$

187 SRTA: The success rate of the total attacks; this value was calculated  
188 using the following formula:

$$SRTA = N'/N$$

189 RVF: The rate of vomiting fish; some individuals catch fish and then vomit  
190 them out; this value was calculated using the following formula:

$$RVF = N''/N'$$

191 where  $N$  represents the total number of attacks;  $T$  represents the time at the  
192 end of the experiment;  $N'$  represents the total catch before the end of the  
193 experiment; and  $N''$  represents the number of fish vomited.

#### 194 2.5 Hybrid vs *S. wangchiachii* in foraging periphytic algae

195 We compared the abilities of SW (n=16) and PS (n=20) to forage  
196 periphytic algae (Fig. 5b). Specific experimental methods are described in  
197 Supplementary method 3. We quickly replayed the video and evaluated the  
198 following indicators: The FAT, AF, and foraging efficiency (FE). Details are as  
199 follows:

200 FAT: The time when an experimental fish first scraped periphytic algae from the  
201 rocks.

202 AF: The average number of scrapings per hour of experimental fish; this value  
203 was calculated using the following formula:

$$TAF = (N2 + N5 + N8)/3$$

204 FE: The average weight of a single scrape of periphytic algae per unit weight  
205 of experimental fish; this value was calculated using the following formula:

$$EF = M2/(TAF \times 8 \times (M1 - M2))$$

206 where  $N2$ ,  $N5$ , and  $N8$  represent the number of attacks in the second, fifth  
207 and eighth hours, respectively,  $M1$  represents the body weight of the  
208 experimental fish; and  $M2$  represents the chyme weight of the experimental  
209 fish.

#### 210 2.6 Whether the behaviour of hybrid fish vomiting fish is persistent

211 In the previous experiments, we observed that PS had obvious behaviour  
212 of vomiting fish (Fig. 4e and Supplementary Movie 3). This behaviour is very  
213 interesting and important but is this behaviour persistent? We set up a feeding  
214 experiment using small fish (*Carassius auratus*,  $0.0748 \pm 0.0023$  g (Fig. 6a)) for  
215 nine days, and PS still had obvious vomiting behaviour after catching the small  
216 *C. auratus* fishes (Fig. 6c)). For nine days, we fed not only fish, but we also fed  
217 the blood worms ( $0.0171 \pm 0.0006$ , Chironomidae larvae, a soft-bodied aquatic  
218 insect that is easier to count and preserve than Tubificidae worms (Fig. 6b)), to  
219 simulate a palatable food shortage in the natural environment but not a  
220 complete absence. Specific experimental methods are described in  
221 Supplementary method 4. We counted the daily catch, intake, and vomiting of  
222 each PS for small fish.

## 223 2.7 Mechanism explaining why hybrid fish vomited fish

224 Two mechanisms may explain why PS vomited small fish: the small fish  
225 tasted bad or they were difficult to chew. To explore this mechanism, we  
226 selected approximately 50 g of *C. carp* (Fig. 7a) and cut the back muscle into  
227 small pieces (Fig. 7b) without bone, instead of using small fish. We took PS  
228 that had the obvious behaviour of vomiting small fish in the last experiment as  
229 the experimental fishes ( $n=7$ ). Other than the small fishes that were replaced  
230 with small pieces of *C. carp* muscle, the other feeding and statistical schemes  
231 were the same as those in Section 2.6. However, the experiment lasted only  
232 three days. We counted the average number of daily foraging (ANDF) and the

233 vomiting rate (VR) of the 7 experimental fishes used in Section 2.6 and this  
234 experiment, which was equivalent to the former serving as a control group for  
235 the latter, by the following formulas:

$$ANDF = N/T$$

$$VR = N'/N$$

236 where  $N$  represents the total number of preys captured by PS during the  
237 experiment.  $T$  represents the number of days of the experiment, and  $N'$   
238 represents the total number of these fishes that vomited.

239 Then we compared the pharyngeal teeth details of PP, SW and PS, and  
240 quantified the foraging-related traits (Supplementary Table 9, 20 measured  
241 traits and 17 standardized traits) of all fishes ( $n=32$ ) in Section 2.6 to explore  
242 whether a correlation exists between these traits, and these indicators  
243 included the TNC (total number of captures), TNI (total number of ingestions),  
244 NVF (total number of vomiting fish) and RVF by Spearman's correlation in  
245 SPSS 21.0.

## 246 2.8 New ecological niche formation of hybrid fish

247 In previous experiments, we found that PS was unable to efficiently forage  
248 for both small fish and periphytic algae. Therefore, we questioned whether PS  
249 focused more on foraging intermediate foods, thereby forming a new  
250 ecological niche different from its parents. We tested effective attacks on blood  
251 worms per species in a mixed breeding experiment under conditions of only  
252 blood worms or mixed foods. Specific experimental methods are described in

253 Supplementary method 5. To eliminate differences in each parallel group, we  
254 standardized the foraging weight of each fish in each tank and calculated the  
255 foraging proportion (FP) by the following formula:

$$FP = N' / N$$

256 where  $N$  represents the total number of effective attacks on blood worms by  
257 three fishes in a tank, and  $N'$  represents the total number of effective attacks  
258 on blood worms by a fish species in a tank.

### 259 **3. RESULTS**

#### 260 **3.1 Morphology**

261 Regarding the external and skeletal morphology, most PS traits were  
262 between PP and SW. However, for digestive morphology, PS was closer to SW  
263 (Figs. 1-3 and Supplementary Table 2). Direct observation, Tukey test or PCA  
264 all supported the above data. Specific morphological descriptions are provided  
265 in Supplementary result 1.

#### 266 **3.2 Comparison of foraging habits**

267 In Fig. 4, for small fish, the FL of PP ( $0.0521 \pm 0.0170$ ) was significantly  
268 higher ( $P < 0.05$ ) than those of SW ( $0.0012 \pm 0.0014$ ) and PS ( $0.0038 \pm 0.0070$ ).  
269 The latter two ingested very few small fishes, and no significant difference  
270 ( $P \geq 0.05$ ) was found between them. For shrimp, the FL of PP ( $0.0563 \pm 0.0197$ )  
271 was significantly higher ( $P < 0.05$ ) than those of SW ( $0.0126 \pm 0.0099$ ) and PS  
272 ( $0.0239 \pm 0.0225$ ). PS and SW ingested more shrimp than fish, and the former  
273 ingested more shrimp than the latter, but no significant difference was noted

274 between them ( $P \geq 0.05$ ). For Tubificidae worms, the FLs of PP  
275 ( $0.0297 \pm 0.0144$ ), SW ( $0.0290 \pm 0.0196$ ) and PS ( $0.0327 \pm 0.0213$ ) were similar,  
276 with no significant difference between them ( $P \geq 0.05$ ). For periphytic algae, the  
277 FL of SW ( $0.0110 \pm 0.0046$ ) was significantly ( $P < 0.05$ ) higher than those of PP  
278 ( $0.0000 \pm 0.0000$ ) and PS ( $0.0035 \pm 0.0051$ ). PP did not ingest periphytic algae,  
279 and some PS individuals may have ingested a small amount of periphytic  
280 algae, but no significant difference was observed between them ( $P \geq 0.05$ ). SW  
281 had a low intake of periphytic algae, possibly because this study used only  
282 *Spirogyra* instead of more palatable diatoms.

283 Interestingly, we found a large amount of small fish debris in the PS  
284 aquarium tank (Fig. 4e), while less debris was noted in the tanks with SW and  
285 PP, suggesting that one of the reasons why fish intake by PS intake was low  
286 was vomiting of fish.

287

### 288 3.3 Hybrid vs parents in foraging fish or periphytic algae

289 In the PS vs SW experiment, the FAT of PS was extremely significantly  
290 higher ( $P < 0.01$ ) than that of SW (Fig. 5b), the AF was significantly lower  
291 ( $P = 0.02$ ) than that of SW (Fig. 5c), and the FE was significantly lower ( $P = 0.037$ )  
292 than that of SW (Fig. 5e). In summary, PS showed low interest in foraging for  
293 periphytic algae and had low foraging efficiency.

294 In the PS vs PP experiment, the SRFA ( $P = 0.219$ ) and the SRTA ( $P = 0.167$ )  
295 of PS were not significantly different from those of PP; the RVF of PS was

296 extremely significantly higher ( $P<0.01$ ) than that of PP (Fig. 5e); the FAT  
297 ( $P=0.459$ ) and the FST ( $P=0.161$ ) of PS were not significantly different from  
298 those of PP; the FAT2 was extremely significantly higher ( $P<0.01$ ) than that of  
299 PP (Fig. 5f); and the AF of PS was extremely significantly lower ( $P<0.01$ ) than  
300 that of PP (Fig. 5g). In summary, PS showed greater interest in first foraging for  
301 fish but had a high RVF, which caused it to be negative in later predation.

### 302 3.4 Whether the behaviour of hybrid fish vomiting fish is persistent

303 As shown in Fig. 6, at the beginning of the experiment, most PS had the  
304 behaviours of catching, vomiting and ingesting small fish. However, as the  
305 experiment proceeded, the number of PS with these behaviours decreased,  
306 and only a few fish retained these persistent behaviours by the end of the  
307 experiment (Figs. 6d, 6e, 6f and 6j); thus, this pattern was the main reason for  
308 the decline in the average number of daily captures, vomiting and ingestion  
309 (Figs. 6g, 6h and 6i). In summary, the behaviour of catching, vomiting and  
310 ingesting small fish by most PS were not persistent.

### 311 3.5 Mechanism of hybrid fish vomiting fish

312 No significant difference ( $P=0.702$ ) was found between the ANDF of fish  
313 meat and small fish in the individuals exhibiting persistent capture behaviours.  
314 However, the VR of fish meat was significantly lower ( $P<0.01$ ) than that of  
315 small fish, suggesting that the vomiting behaviour was not caused by bad taste  
316 but by chewing difficulty, which may be caused by pharyngeal tooth structure.  
317 Therefore, the details of the pharyngeal teeth were compared, and we found

318 that the pharyngeal bone of PP was long and narrow, with widely spaced  
319 well-developed conical hooked pharyngeal teeth, and the space was larger  
320 between the two pharyngeal bones in the closed mouth. These features are  
321 useful for piercing and hooking prey. In contrast, the pharyngeal bone of SW  
322 was short and thick, with closely spaced grinding pharyngeal teeth, which were  
323 curved and flat at the top, forming a grinding surface, and the space was  
324 smaller between the two pharyngeal bones in the closed mouth. These  
325 features are useful for grinding periphytic algae. The morphology of the  
326 pharyngeal bone in PS was balanced between that of the parents, and it had  
327 hooked grinding pharyngeal teeth, which were also intermediate between the  
328 parents.

329 Specific correlation analysis descriptions can be found in Supplementary  
330 result 2. The results of the correlation analysis can be summarized as follows:  
331 (1) as the number of fishes caught by PS increased, more were ingested and  
332 vomited. (2) The larger the PS was, the more fishes it caught. (3) The  
333 behaviour of vomiting fish by PS was not correlated with the size and shape of  
334 its characteristics.

### 335 3.6 New ecological niche formation of hybrid fish

336 In the first 3 days of feeding using only blood worms, PS and SW showed  
337 no significant difference ( $P \geq 0.05$ ) in the FP of blood worms, but they  
338 significantly differed ( $P < 0.05$ ) from PP (except that PS showed no significant  
339 difference ( $P \geq 0.05$ ) compared to SW and PP on the second day, Fig. 8d). After

340 3 days of feeding with three kinds of food, the FP of PP with 3 days of blood  
341 worm feeding decreased significantly ( $P=0.036$ ), and that of SW decreased  
342 nonsignificantly ( $P=0.168$ ); however, that of PS increased significantly ( $P=0.01$ ,  
343 Fig. 8e). Moreover, the FP was significantly ( $P<0.05$ ) different among PS and  
344 both parents in the first two days of feeding on the three kinds of food, but PS  
345 and SW showed no significant difference ( $P\geq 0.05$ ) on the third day (Fig. 8d).

#### 346 **4. Discussion**

347 Hybrid speciation needs to break through multiple isolation barriers, which  
348 are generally divided into prezygotic and postzygotic barriers [17]. Random  
349 external fertilization by medium makes it easier for plants and fishes to cross,  
350 which is beneficial to break through the pre-zygotic barrier [12]. Polyploid  
351 speciation also facilitates post-zygotic breakthroughs [10]. However, all above  
352 breakthroughs are genetic, for hybrid speciation, ecological challenges must  
353 be faced and foraging performance is the first barrier.

354 The feeding habits of a species are closely related to their foraging traits  
355 [37]. PP and SW have opposite feeding habits and foraging traits. With the  
356 exception of a few epistatic traits, most foraging traits of PS are somewhere  
357 between the traits of their parents (Figs. 1-3). In general, hybrids will produce  
358 intermediate foraging performance for parent resources [18-20, 23, 38].  
359 However, intermediate kinematics have also been shown produce more  
360 inferior foraging performance than intermediate morphology [39]. In this study,  
361 the intermediate foraging morphology of PS did not result in intermediate

362 foraging performance for parental resources, and PS could hardly forage for  
363 parental resources (Figs. 4f and 5). The above phenomenon was caused not  
364 only by the decline in PS foraging ability but, more importantly, by the decrease  
365 in foraging activity (Fig. 5). The diet of a species depends not only on heredity  
366 and environment, but also experience [40-45]. PS showed less interest in  
367 foraging for periphytic algae from the beginning of the experiment, which may  
368 be genetically negative. Interestingly, however, PS showed interest in foraging  
369 small fishes at the beginning of the experiment, while after the first successful  
370 capture, PS had difficulty ingesting the fish, which led to subsequent negative  
371 predation (Fig. 5). This result may be empirically negative.

372       The behaviour of PS vomiting fish is one of the highlights of this study.  
373 Some vomiting events are caused by an individual's ingestion of stimulating  
374 foods, which may be due not only to unpalatably or toxicity [46] but also to  
375 mechanical issues, such as dolphins ingesting hard squid beaks, resulting in  
376 vomiting [47]. Our experiments proved that the vomiting behaviour of PS was  
377 caused not by bad taste but rather by chewing difficulty, which may be caused  
378 by mechanical difficulties encountered due to the bones of small fish (Fig. 7).  
379 In the correlation analysis, we found no correlation between the structure of  
380 any trait in PS and this behaviour (Supplementary Table S9). However, the  
381 structure of pharyngeal teeth of PS was balanced between that of its parents,  
382 but its puncture ability was not as good as that of PP; thus, PS may not reach  
383 the threshold of normal chewing (Fig. 7). Of course, most PS individuals

384 abandoned foraging fish, but some showed persistent vomiting behaviour (Fig.  
385 6). Clearly, the behaviour itself is harmful as it requires energy that PS could  
386 expend on foraging, and PS consequently does not receive the intended  
387 source of energy. In short, PS must engage in hard work with no gain. Thus,  
388 we hypothesized that these contradictory foraging behaviours may dilute the  
389 energy of PS intended for foraging intermediate ecological niche prey, and a  
390 supplementary experiment was therefore carried out (Supplementary method  
391 6 and Supplementary result 3). However, we did not find a correlation between  
392 the amount of vomited fish and the total food intake of PS (Supplementary  
393 Table 11), which contradicts the above hypothesis. Interestingly, both SW and  
394 SP showed reduced predation of shrimps and worms in the food resource  
395 environment with fishes, suggesting that small fish were interfering with their  
396 predation. However, SP showed greater reductions than SW, which may be  
397 due to the negativity brought by vomiting fish (Fig. 5 and Supplementary Fig. 3).  
398 The periphytic algae will not affect the foraging of PS for intermediate  
399 ecological niche prey because PS is less interested in foraging on such algae  
400 and has less interference (Fig. 5 and Supplementary Fig. 3). In summary, the  
401 behaviour of PS vomiting fish is a typical paradoxical phenomenon between  
402 intermediate morphology and genetic behaviour, which led directly or indirectly  
403 to the ecological disadvantage of PS.

404 PS almost abandoned foraging on parental resources and could only  
405 choose intermediate ecological niche prey, which may indicate that PS forms a

406 middle ecological niche. We experimentally verified the above hypothesis that  
407 the foraging preference of PP and SW for intermediate ecological niche prey  
408 decreases after the addition of suitable parental food resources, whereas that  
409 of PS increases (Fig. 8). If no transitional food resources exist between  
410 parents, hybrids will be eliminated [3, 20]. Instead, hybrids may form a new  
411 ecological niche [15, 23]. Through a mixed breeding experiment, this study  
412 showed that the increase in the FP of PS to intermediate ecological niche prey  
413 was not due to the adaptation of new traits [23] and was more likely due to the  
414 decrease in the FP of the parents to such prey. However, this is a type of  
415 passive ecological niche formation, indicating that more competitors are likely  
416 to participate.

417       When there are transitional ecological niches exist between different  
418 ecological niche parents, the hybridization between them may be a  
419 double-edged sword for hybrid speciation, implying that the fate of hybrids is  
420 complicated in this process. The experimental period of this study was short,  
421 but the formation of a stable niche is long. Therefore, large-scale and  
422 long-term bionic breeding experiments and stable isotope methods [19] should  
423 be carried out in subsequent studies to verify whether hybrids form new niches  
424 or are eliminated.

425

426 **Ethics approval and consent to participate**

427 The authors claim that none of the material in the paper has been  
428 published or is under consideration for publication elsewhere. The submission  
429 is original, and all authors are aware of the submission and agree to its  
430 publication in *Frontiers in Zoology*. We declare that there is no conflict of  
431 interests regarding the publication of this paper.

432 **Consent for publication**

433 Written informed consent for publication was obtained from all participants.

434 **Availability of data and materials**

435 The datasets used or analysed during the current study are available from  
436 the corresponding author on reasonable request.

437 **Competing interests**

438 All authors declare that they have no competing interests.

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445 **Authors' contributions**

446 H.R. Gu and Z.J. Wang conceived the ideas and designed the  
447 methodology; Y. He took the microCT images; S.H. Deng, X.H. He, Y. Wu, K.Y.  
448 Xing and X. Gao contributed to the breeding and feeding of the experimental

449 fishes used in this experiment; H.R. Gu completed all the experiments and  
450 data processing and analysis in this study; X.F. He provided constructive  
451 guidance to H.R. Gu in morphology; H.R. Gu and Z.J. Wang led the writing of  
452 the manuscript. All authors contributed critically to the drafts and provided final  
453 approval for publication.

454

## 455 References

- 456 1. Mayr E: *Animal species and evolution*. Cambridge: Belknap Press; 1963.
- 457 2. Orr HA, Turelli M: **The evolution of postzygotic isolation: Accumulating**  
458 **Dobzhansky-Muller incompatibilities**. *Evolution* 2001, **55**:1085-1094.
- 459 3. Schluter D: **Adaptive Radiation In Sticklebacks - Trade-Offs In Feeding Performance**  
460 **And Growth**. *Ecology* 1995, **76**:82-90.
- 461 4. Powell DL, Garcia-Olazabal M, Keegan M, Reilly P, Du K, Diaz-Loyo AP, Banerjee S,  
462 Blakkan D, Reich D, Andolfatto P, et al: **Natural hybridization reveals incompatible alleles**  
463 **that cause melanoma in swordtail fish**. *Science* 2020, **368**:731-+.
- 464 5. Schluter D: **Evidence for Ecological Speciation and Its Alternative**. *Science* 2009,  
465 **323**:737-741.
- 466 6. Satokangas I, Martin SH, Helanterä H, Saramäki J, Kulmuni J: **Multi-locus interactions and**  
467 **the build-up of reproductive isolation**. *Philosophical Transactions Of the Royal Society*  
468 *B-Biological Sciences* 2020, **375**.
- 469 7. Brideau NJ, Flores HA, Wang J, Maheshwari S, Wang X, Barbash DA: **Two**  
470 **Dobzhansky-Muller genes interact to cause hybrid lethality in Drosophila**. *Science* 2006,  
471 **314**:1292-1295.
- 472 8. Dowling TE, Secor CL: **The role of hybridization and introgression in the diversification**  
473 **of animals**. *Annual Review Of Ecology And Systematics* 1997, **28**:593-619.
- 474 9. Rius M, Darling JA: **How important is intraspecific genetic admixture to the success of**  
475 **colonising populations?** *Trends In Ecology & Evolution* 2014, **29**:233-242.
- 476 10. Arnold ML: *Natural hybridization and evolution*. New York: Oxford University Press; 1997.
- 477 11. Pfennig KS: **ECOLOGY How to survive in a human-dominated world**. *Science* 2019,  
478 **364**:433-434.
- 479 12. Montanari SR, Hobbs JPA, Pratchett MS, van Herwerden L: **The importance of ecological**  
480 **and behavioural data in studies of hybridisation among marine fishes**. *Reviews In Fish*  
481 *Biology And Fisheries* 2016, **26**:181-198.
- 482 13. St John ME, Holzman R, Martin CH: **Rapid adaptive evolution of scale-eating kinematics**  
483 **to a novel ecological niche**. *Journal Of Experimental Biology* 2020, **223**.
- 484 14. Selz OM, Thommen R, Maan ME, Seehausen O: **Behavioural isolation may facilitate**  
485 **homoploid hybrid speciation in cichlid fish**. *Journal Of Evolutionary Biology* 2014,  
486 **27**:275-289.
- 487 15. Masello JF, Quillfeldt P, Sandoval-Castellanos E, Alderman R, Calderon L, Cherel Y, Cole TL,  
488 Cuthbert RJ, Marin M, Massaro M, et al: **Additive Traits Lead to Feeding Advantage and**  
489 **Reproductive Isolation, Promoting Homoploid Hybrid Speciation**. *Molecular Biology And*  
490 *Evolution* 2019, **36**:1671-1685.
- 491 16. Pfennig KS: **Facultative mate choice drives adaptive hybridization**. *Science* 2007,  
492 **318**:965-967.
- 493 17. Coyne JA, Orr HA: *Speciation*. Sunderland, MA: Sinauer Associates; 2004.
- 494 18. Hatfield T, Schluter D: **Ecological speciation in sticklebacks: Environment-dependent**  
495 **hybrid fitness**. *Evolution* 1999, **53**:866-873.
- 496 19. Arnegard ME, McGee MD, Matthews B, Marchinko KB, Conte GL, Kabir S, Bedford N,  
497 Bergek S, Chan YF, Jones FC, et al: **Genetics of ecological divergence during speciation**.

- 498 *Nature* 2014, **511**:307-+.
- 499 20. Egan SP, Funk DJ: **Ecologically dependent postmating isolation between sympatric host**  
500 **forms of *Neochlamisus bebbianae* leaf beetles.** *Proceedings Of the National Academy Of*  
501 *Sciences Of the United States Of America* 2009, **106**:19426-19431.
- 502 21. Douglas JF: *Evolution. Third Edition.* Sunderland, Massachusetts: Sinauer Associates; 2013.
- 503 22. Seehausen O: **Hybridization and adaptive radiation.** *Trends In Ecology & Evolution* 2004,  
504 **19**:198-207.
- 505 23. Selz OM, Seehausen O: **Interspecific hybridization can generate functional novelty in**  
506 **cichlid fish.** *Proceedings Of the Royal Society B-Biological Sciences* 2019, **286**.
- 507 24. Lopez-Fernandez H, Arbour J, Willis S, Watkins C, Honeycutt RL, Winemiller KO:  
508 **Morphology and Efficiency of a Specialized Foraging Behavior, Sediment Sifting, in**  
509 **Neotropical Cichlid Fishes.** *Plos One* 2014, **9**.
- 510 25. York RA, Patil C, Abdilleh K, Johnson ZV, Conte MA, Genner MJ, McGrath PT, Fraser HB,  
511 Fernald RD, Streebman JT: **Behavior-dependent cis regulation reveals genes and pathways**  
512 **associated with bower building in cichlid fishes.** *Proceedings Of the National Academy Of*  
513 *Sciences Of the United States Of America* 2018, **115**:E11081-E11090.
- 514 26. Wheatcroft D, Qvarnstrom A: **Genetic divergence of early song discrimination between**  
515 **two young songbird species.** *Nature Ecology & Evolution* 2017, **1**.
- 516 27. Song C, Liu SJ, Xiao J, He WG, Zhou Y, Qin Q, Zhang C, Liu Y: **Polyploid organisms.**  
517 *Science China-Life Sciences* 2012, **55**:301-311.
- 518 28. Yang L, Sado T, Hirt MV, Pasco-Viel E, Arunachalam M, Li JB, Wang XZ, Freyhof J, Saitoh  
519 K, Simons AM, et al: **Phylogeny and polyploidy: Resolving the classification of cyprinine**  
520 **fishes (Teleostei: Cypriniformes).** *Molecular Phylogenetics And Evolution* 2015, **85**:97-116.
- 521 29. Wang XZ, Gan XN, Li JB, Chen YY, He SP: **Cyprininae phylogeny revealed independent**  
522 **origins of the Tibetan Plateau endemic polyploid cyprinids and their diversifications**  
523 **related to the Neogene uplift of the plateau.** *Science China-Life Sciences* 2016,  
524 **59**:1149-1165.
- 525 30. Oellermann LK, Skelton PH: **Hexaploidy In Yellowfish Species (Barbus, Pisces,**  
526 **Cyprinidae) From Southern Africa.** *Journal Of Fish Biology* 1990, **37**:105-115.
- 527 31. Xu P, Xu J, Liu GJ, Chen L, Zhou ZX, Peng WZ, Jiang YL, Zhao ZX, Jia ZY, Sun YH, et al:  
528 **The allotetraploid origin and asymmetrical genome evolution of the common carp**  
529 ***Cyprinus carpio*.** *Nature Communications* 2019, **10**.
- 530 32. Yue PQ: *FAUNA SINICA, Osteichthyes Cypriniformes III.* Beijing, China: Science Press; 2000.
- 531 33. Wang M, Yang JX, Chen XY: **Molecular Phylogeny and Biogeography of Percocypris**  
532 **(Cyprinidae, Teleostei).** *Plos One* 2013, **8**.
- 533 34. Gu HR, Wan YF, Yang Y, Ao Q, Cheng WL, Deng SH, Pu DY, He XF, Jin L, Wang ZJ:  
534 **Genetic and morphology analysis among the pentaploid F-1 hybrid fishes (*Schizothorax***  
535 ***wangchiachii* female x *Percocypris pingi* male) and their parents.** *Animal* 2019,  
536 **13**:2755-2764.
- 537 35. Zou SP, Fang YL, Zhou RQ: **Measurement of characters. Inspection of germplasm for**  
538 **cultured fishes, part 3.,** vol. GB/T 18654.3-2008. China: Ministry of agriculture of the  
539 People's Republic of China; 2008.
- 540 36. He Y, Chen XY, Xiao TQ, Yang JX: **Three-dimensional morphology of the**  
541 ***Sinocyclocheilus hyalinus* (Cypriniformes: Cyprinidae) horn based on synchrotron**

- 542 **X-ray microtomography.** *Zoological Research* 2013, **34**:E128–134.
- 543 37. Manning CG, Foster SJ, Vincent ACJ: **A review of the diets and feeding behaviours of a**  
544 **family of biologically diverse marine fishes (Family Syngnathidae).** *Reviews In Fish*  
545 *Biology And Fisheries* 2019, **29**:197-221.
- 546 38. Kirkpatrick M: **Reinforcement during ecological speciation.** *Proceedings Of the Royal*  
547 *Society B-Biological Sciences* 2001, **268**:1259-1263.
- 548 39. Mcgee MD, Reustle JW, Oufiero CE, Wainwright PC: **Intermediate Kinematics Produce**  
549 **Inferior Feeding Performance in a Classic Case of Natural Hybridization.** *American*  
550 *Naturalist* 2015, **186**:807-814.
- 551 40. Jaenike J: **Genetic And Environmental Determinants Of Food Preference In**  
552 **Drosophila-Tripunctata.** *Evolution* 1985, **39**:362-369.
- 553 41. Bolivar VJ, Flaherty L: **Genetic control of novel food preference in mice.** *Mammalian*  
554 *Genome* 2004, **15**:193-198.
- 555 42. Utsumi S, Ando Y, Ohgushi T: **Evolution of feeding preference in a leaf beetle: the**  
556 **importance of phenotypic plasticity of a host plant.** *Ecology Letters* 2009, **12**:920-929.
- 557 43. Sotka EE: **Genetic control of feeding preference in the herbivorous amphipod Ampithoe**  
558 **longimana.** *Marine Ecology Progress Series* 2003, **256**:305-310.
- 559 44. Finestone E, Bonnie KE, Hopper LM, Vreeman VM, Lonsdorf EV, Ross SR: **The interplay**  
560 **between individual, social, and environmental influences on chimpanzee food choices.**  
561 *Behavioural Processes* 2014, **105**:71-78.
- 562 45. Turrovincent I, Launay F, Mills AD, Picard M, Faure JM: **Experiential And Genetic**  
563 **Influences on Learnt Food Aversions In Japanese-Quail Selected for High Or**  
564 **Low-Levels Of Fearfulness.** *Behavioural Processes* 1995, **34**:23-41.
- 565 46. Johnson EC, Hill E, Cooper MA: **Vomiting in wild bonnet macaques.** *International Journal*  
566 *Of Primatology* 2007, **28**:245-256.
- 567 47. AU Silva-Jr. JMP, Lizete Jardim; Sazima, Ivan: **Vomiting behavior of the spinner dolphin**  
568 **(Stenella longirostris) and squid meals.** *Aquatic Mammals* 2004, **30**:271-274.
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- 570

571 **Figure legends**

572 **Figure 1.** External characters comparison. (a) The full view of PP. (b-d) The  
573 head characters of PP. The full view of SW. (f-h) The head characters of SW. (i)  
574 The full view of PS) (j-i) The head characters of SP. (m) The PCA of external  
575 characters. The white scale is 1 mm; the black scale is 10 mm. We have used  
576 some figures in previous articles (Gu et al. 2019), including (a), (c), (d), (e), (g),  
577 (h), (i), (k) and (l).

578 **Figure 2.** Digestive characters comparison. (a) The anatomic observation of  
579 midgut mucosal folds of PP. (b) The histological observation of liver of PP. (c)  
580 The histological observation of foregut of PP. (d) The histological observation  
581 of midgut of PP. (e) The histological observation of Hindgut of PP. (f-j) The  
582 same description of SW as PP. (k-o) The same description of SP as PP. (p)  
583 The PCA of digestive characters. The scale in (a), (f) and (k) is 0.5 mm, in (b),  
584 (g) and (l) is 10  $\mu\text{m}$ , in (c), (d), (e), (h), (i), (j), (m), (n) and (o) is 25  $\mu\text{m}$ .

585 **Figure 3.** Osteal characters comparison. (a-c) The MicroCT image of head  
586 characters of PP. (d) The pharyngeal bone of PP. (e-g) The MicroCT image of  
587 head characters of SW. (h) The pharyngeal bone of SW. (i-k) The MicroCT  
588 image of side head of PS. (l) The pharyngeal bone of SP. (m) The PCA of  
589 osteal characters. The scale of MicroCT images is 6 mm, and the scale of  
590 pharyngeal bones is 1mm.

591 **Figure 4.** Comparison of foraging habit. (a) Small fish (*S. taeniatus*). (b)  
592 Tubificidae worms. (c) Small shrimp (*N. denticulate*). (d) Periphytic algae

593 (Spirogyra). (e) Small fish debris. (f) The FL of different foods among PP, SW  
594 and PS. The scale of all figures is 1 mm. The different superscripts (a, b)  
595 above the boxes differ significantly at  $P < 0.05$  based on Tukey test. The boxes  
596 give the first and third quartiles, the thick lines give the medians and whiskers  
597 indicate means  $\pm$  SD.

598 **Figure 5.** Hybrid vs parents in foraging little fish or periphytic algae. (a) Small  
599 fish (*S. taeniatus*). (b) Rock with periphytic algae (Spirogyra). (c) PS vs SW in  
600 the FAT. (d) PS vs SW in the AF. (e) PS vs SW in the FE. (f) PS vs PP in the  
601 SRFA, SRTA and RVF. (g) PS vs PP in the FAT, FST and FAT2. (h) PS vs PP in  
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604 and whiskers indicate mean  $\pm$  SE.

605 **Figure 6.** The changes of the related indicators of foraging fish in hybrid fish  
606 with time. (a) Small fish (*C. auratus*). (b) Blood worm (Chironomidae larvae). (c)  
607 Little fish debris. (d) The trends of vomiting of every SP with time. (e) The  
608 trends of ingestion of every SP with time. (f) The trends of captures of every  
609 SP with time. (g) The mean trend of captures of SP with time. (h) The mean  
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611 time. (j) The trends in the number of SP involved in capture, ingestion and  
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613 figures is 1 mm. The different superscripts (a, b) above the lines differ  
614 significantly at  $P < 0.05$  based on Tukey test, and whiskers indicate mean  $\pm$

615 SE.

616 **Figure 7.** Mechanism of hybrid fish vomiting small fish. (a) Small fish (*C.*  
617 *auratus*). (b) A small piece muscle in the back of *C. auratus*. (c) The ANDF for  
618 small fish or meat by these SP with a persistent vomiting-fish behavior. (d)  
619 Compare the VR of SP between foraging small fish and meat. (e) The MicroCT  
620 image of pharyngeal bones of PP. (f-g) The detail image of pharyngeal bones  
621 of PP. (h) The MicroCT image of pharyngeal bones of SW. (i-j) The detail  
622 image of reverse pharyngeal bones of SW. (k) The MicroCT image of  
623 pharyngeal bones of SP. (l-m) The detail image of pharyngeal bones of SP. (m)  
624 The scale in (a) is 1 mm, in (b), the meat is 1mm and the fish is 10mm, in (e), (h)  
625 and (k) is 2mm, in (f), (g), (i), (j), (l) and (m) is 0.5mm. The numbers above  
626 these columns give the P-value based on Tukey test, the height give the mean,  
627 the thick lines give the medians, and whiskers indicate mean  $\pm$  SE.

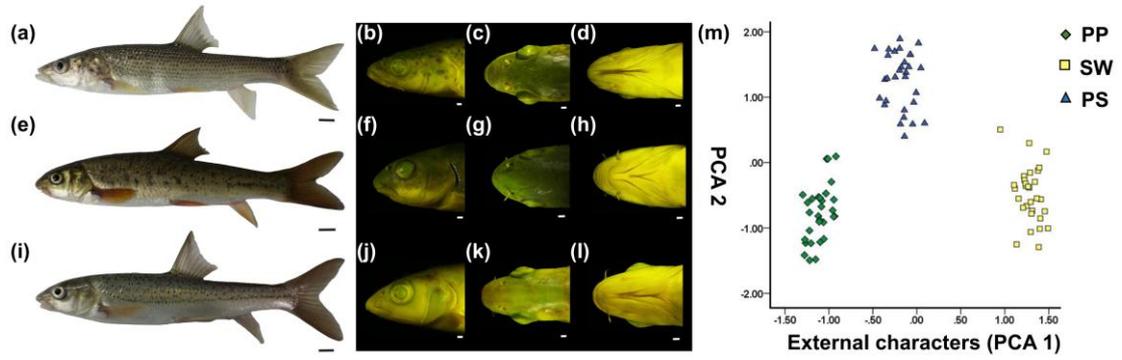
628 **Figure 8.** New ecological niche formation of hybrid fish. (a) Small fish (*C.*  
629 *auratus*). (b) Blood worm (Chironomidae larvae). (c) Rock with periphytic algae  
630 (*Spirogyra*). (d) The trends of daily FP of PP, SW and SP in an environment  
631 with only worms or fishes, worms and periphytic algae. (e) Comparison of total  
632 FP of PP, SW and PS between an environment with only worms or with fishes,  
633 worms and periphytic algae. The scale of all figures is 1 mm. The different  
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635 based on Tukey test. The numbers above the columns give the P-value based  
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637 medians and whiskers indicate mean  $\pm$  SE.

638

639 **Figures**

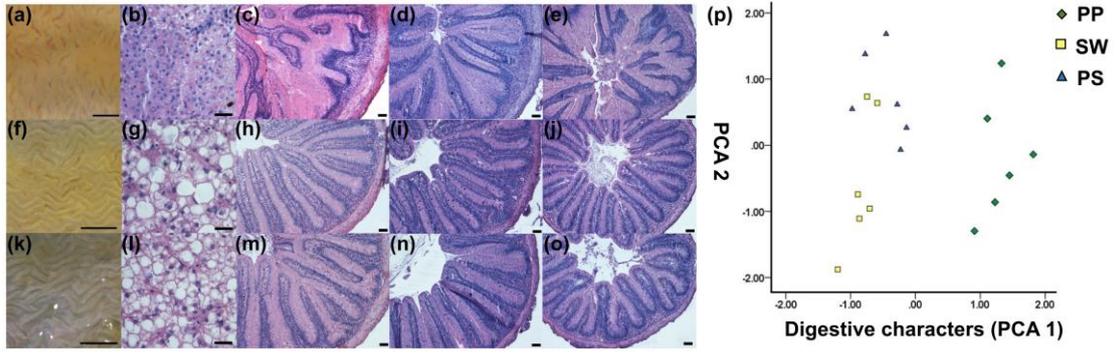
640 **Fig. 1**



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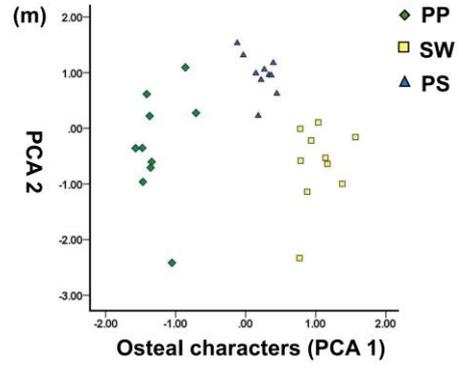
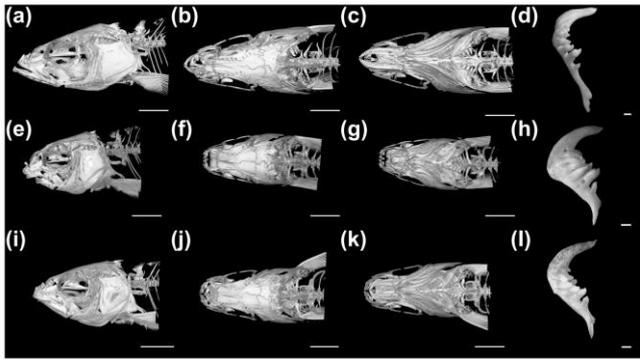
643 **Fig. 2**



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646 **Fig. 3**



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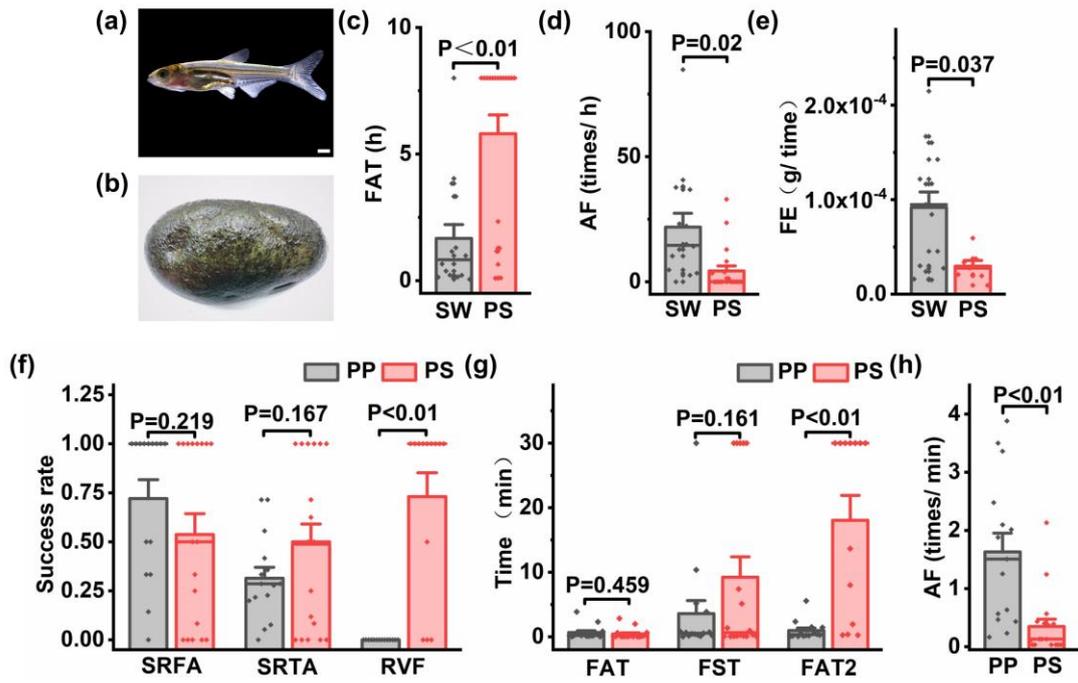
649 **Fig. 4**



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652 **Fig. 5**

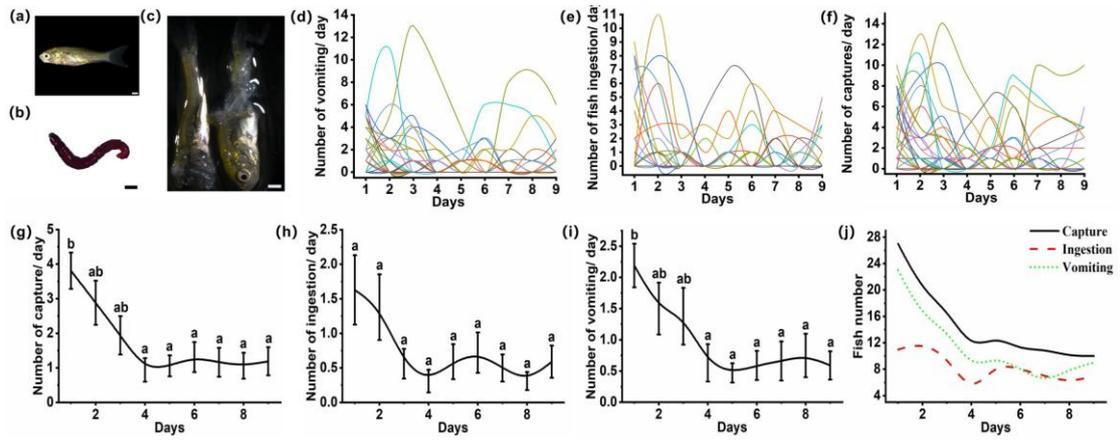


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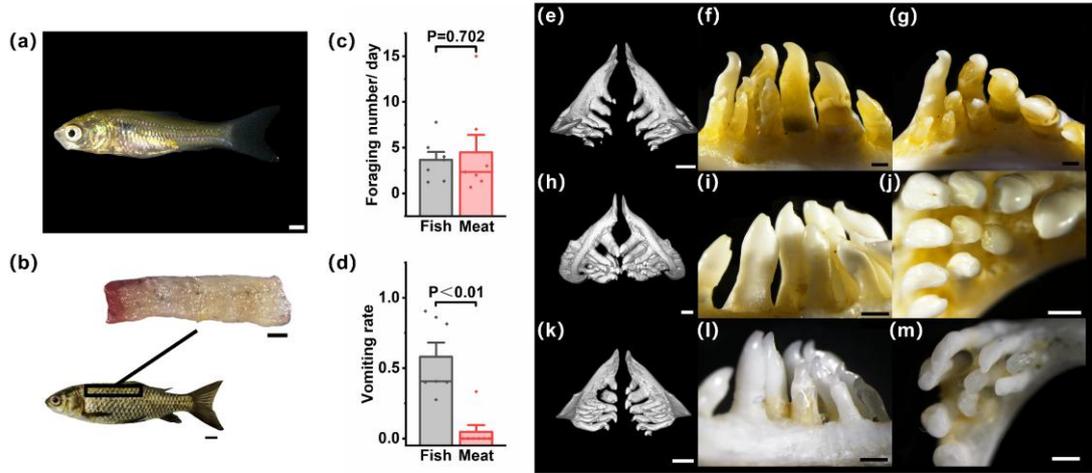
Fig. 6



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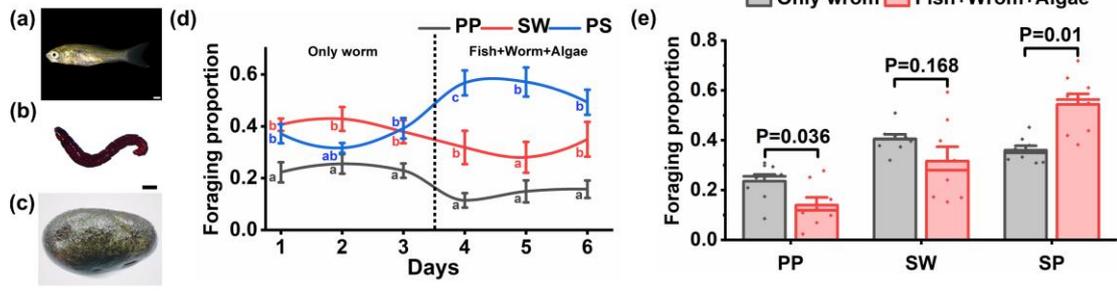
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658 **Fig. 7**



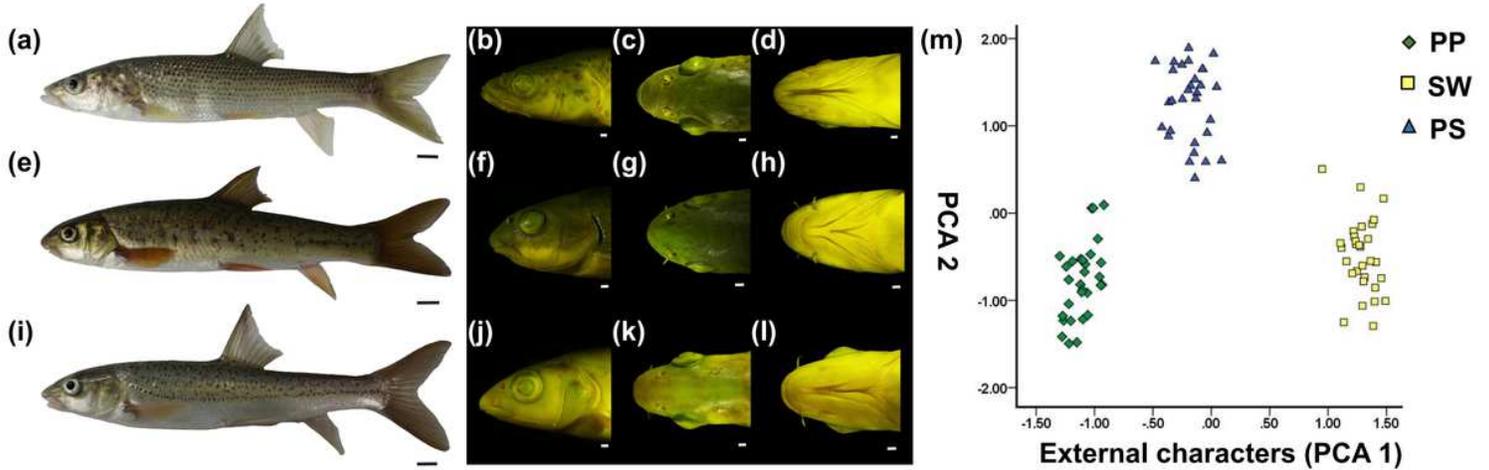
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661 **Fig. 8**



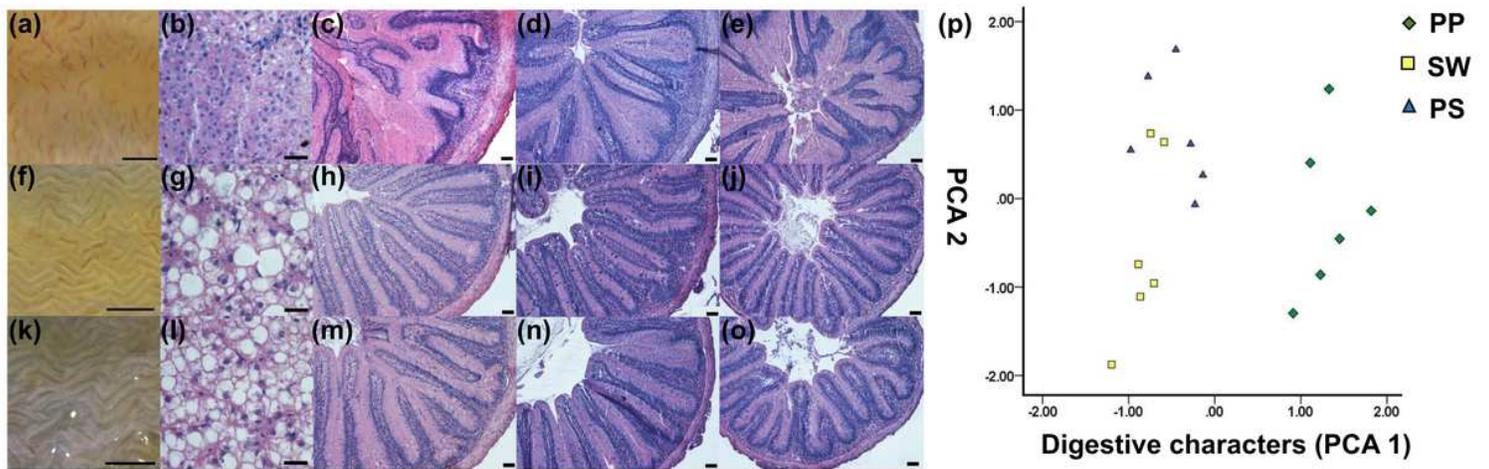
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# Figures



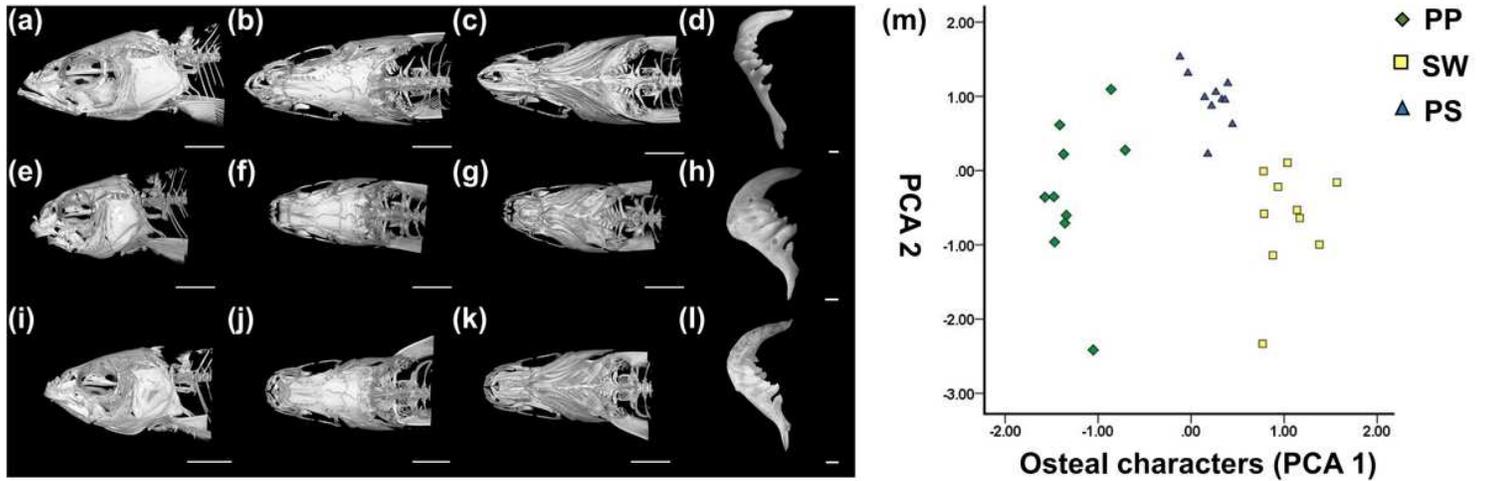
**Figure 1**

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**Figure 2**

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**Figure 3**

Osteal characters comparison. (a-c) The MicroCT image of head characters of PP. (d) The pharyngeal bone of PP. (e-g) The MicroCT image of head characters of SW. (h) The pharyngeal bone of SW. (i-k) The MicroCT image of side head of PS. (l) The pharyngeal bone of SP. (m) The PCA of osteal characters. The scale of MicroCT images is 6 mm, and the scale of pharyngeal bones is 1 mm.



**Figure 4**

Comparison of foraging habit. (a) Small fish (*S. taeniatus*). (b) Tubificidae worms. (c) Small shrimp (*N. denticulate*). (d) Periphytic algae (*Spirogyra*). (e) Small fish debris. (f) The FL of different foods among PP, SW and PS. The scale of all figures is 1 mm. The different superscripts (a, b) above the boxes differ significantly at  $P < 0.05$  based on Tukey test. The boxes give the first and third quartiles, the thick lines give the medians and whiskers indicate means  $\pm$  SD.

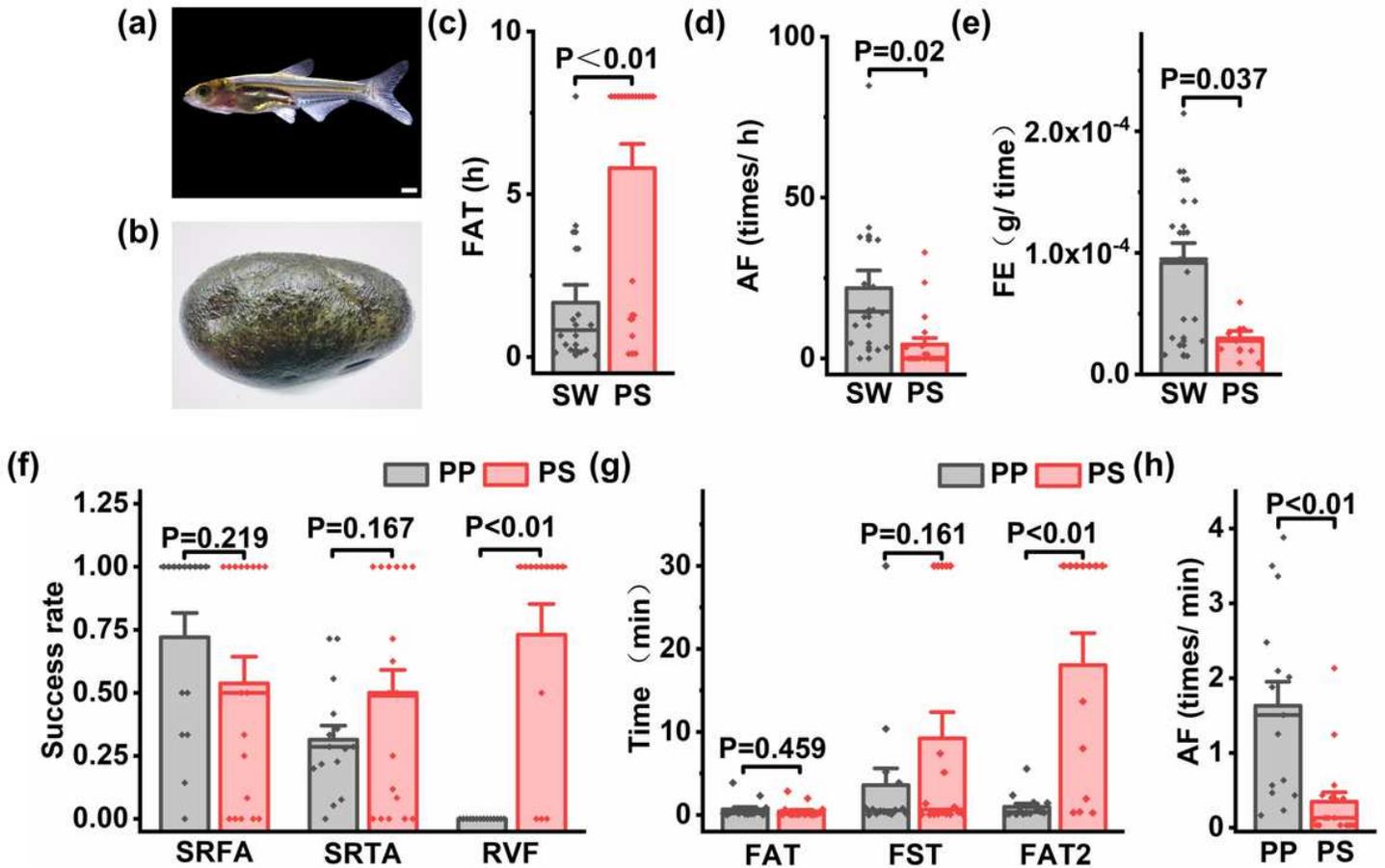
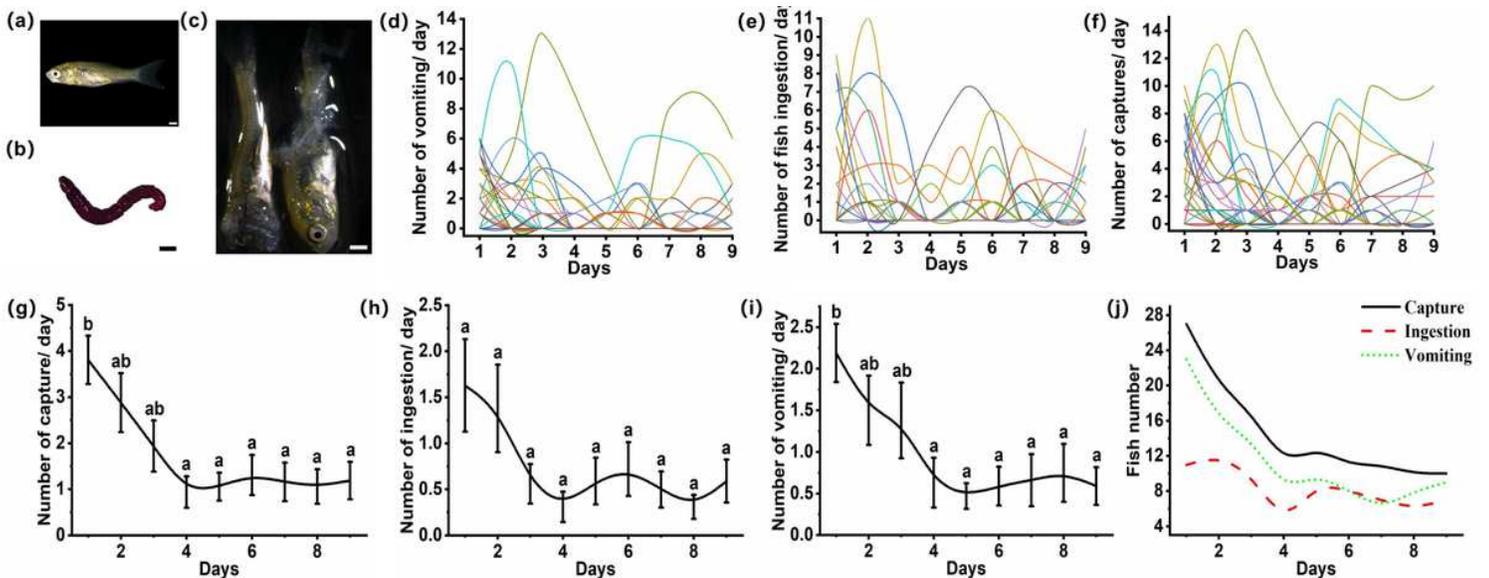


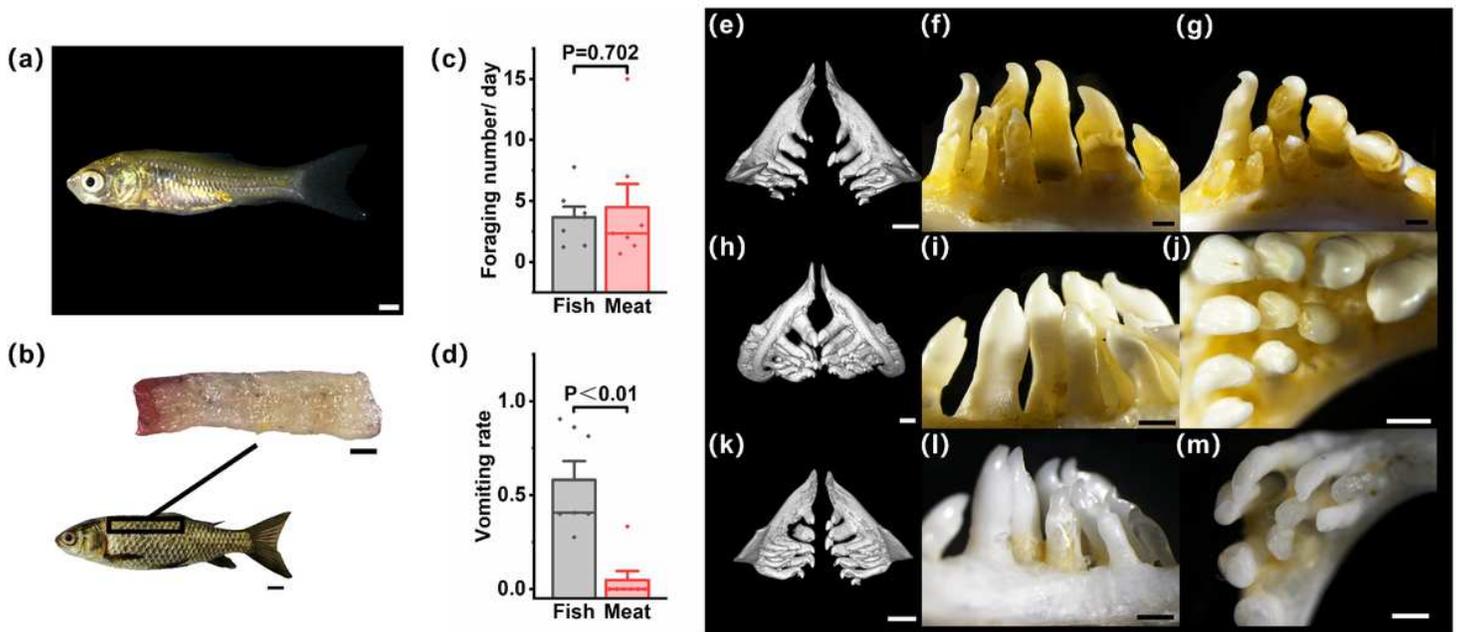
Figure 5

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## Figure 6

The changes of the related indicators of foraging fish in hybrid fish with time. (a) Small fish (*C. auratus*). (b) Blood worm (Chironomidae larvae). (c) Little fish debris. (d) The trends of vomiting of every SP with time. (e) The trends of ingestion of every SP with time. (f) The trends of captures of every SP with time. (g) The mean trend of captures of SP with time. (h) The mean trend of ingestion of SP with time. (i) The mean trend of vomiting of SP with time. (j) The trends in the number of SP involved in capture, ingestion and vomiting. In (d), (e) and (f), each line represents an individual. The scale of all figures is 1 mm. The different superscripts (a, b) above the lines differ significantly at  $P < 0.05$  based on Tukey test, and whiskers indicate mean  $\pm$  SE.



## Figure 7

Mechanism of hybrid fish vomiting small fish. (a) Small fish (*C. auratus*). (b) A small piece muscle in the back of *C. auratus*. (c) The ANDF for small fish or meat by these SP with a persistent vomiting-fish behavior. (d) Compare the VR of SP between foraging small fish and meat. (e) The MicroCT image of pharyngeal bones of PP. (f-g) The detail image of pharyngeal bones of PP. (h) The MicroCT image of pharyngeal bones of SW. (i-j) The detail image of reverse pharyngeal bones of SW. (k) The MicroCT image of pharyngeal bones of SP. (l-m) The detail image of pharyngeal bones of SP. (m) The scale in (a) is 1mm, in (b), the meat is 1mm and the fish is 10mm, in (e), (h) and (k) is 2mm, in (f), (g), (i), (j), (l) and (m) is 0.5mm. The numbers above these columns give the P-value based on Tukey test, the height give the mean, the thick lines give the medians, and whiskers indicate mean  $\pm$  SE.

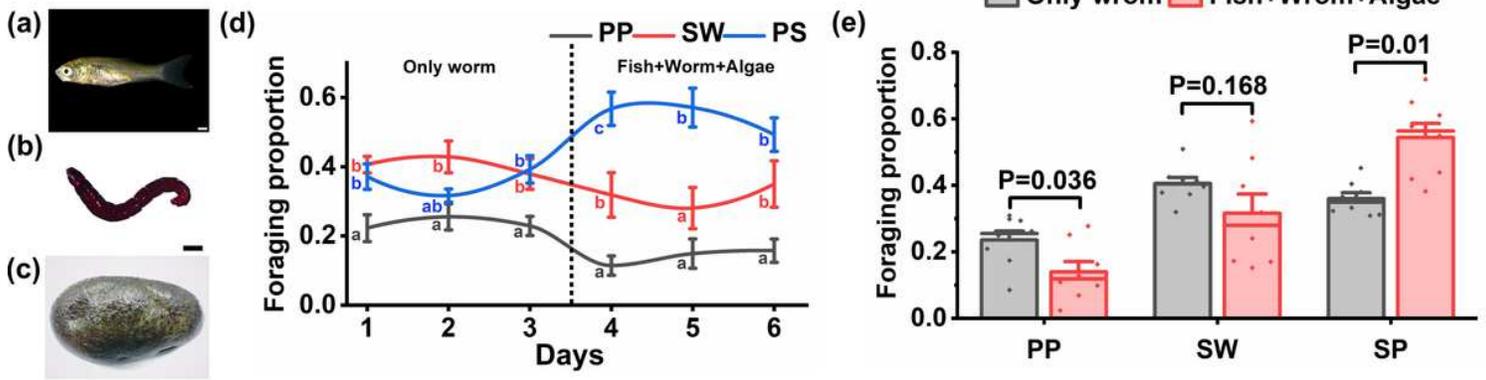


Figure 8

New ecological niche formation of hybrid fish. (a) Small fish (*C. auratus*). (b) Blood worm (Chironomidae larvae). (c) Rock with periphytic algae (*Spirogyra*). (d) The trends of daily FP of PP, SW and SP in an environment with only worms or fishes, worms and periphytic algae. (e) Comparison of total FP of PP, SW and PS between an environment with only worms or with fishes, worms and periphytic algae. The scale of all figures is 1 mm. The different superscripts (a, b) above the boxes or lines differ significantly at  $P < 0.05$  based on Tukey test. The numbers above the columns give the P-value based on Tukey test. The heights of boxes give the mean, the thick lines give the medians and whiskers indicate mean  $\pm$  SE.

## Supplementary Files

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