

# Pelagic-benthic resource polymorphism in *Schizopygopsis thermalis* Herzenstein 1891 (Pisces, Cyprinidae) in a headwater lake in the Salween River system on the Tibetan Plateau

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## Abstract

Resource polymorphism is a ubiquitous phenomenon in vertebrates and may represent a critical intermediate stage in speciation. Freshwater lakes in high-altitude areas represent a natural system for understanding resource polymorphism in fishes in diverse lacustrine environments and a few co-distributed species. We report resource polymorphism in a cyprinid fish, *Schizopygopsis thermalis*, in Lake Amdo Tsonak Co, a headwater lake in the upper Salween River system. Two morphs, planktivorous and benthivorous, were identified according to geometric morphological and traditional linear traits. The planktivorous morph exhibits a longer head and lower jaw, larger asymptotic standard length ( $L_{\infty}$ ), lower growth rate ( $k$ ) and higher growth performance index ( $\phi$ ) than the benthivorous morph. With respect to descriptive traits, the planktivorous morph possesses a terminal mouth and a highly developed mucus cavity in the cheek and chin, while the benthivorous morph is characterized by an inferiorly positioned mouth with a sharpened horny edge on the lower jaw. Our results indicate that distinct pelagic-benthic resources and low interspecific competition in the lake drove the initial differentiation of the two morphs and that partial spatial reproductive isolation might maintain and reinforce the differences between them.

## INTRODUCTION

Resource polymorphism is a phenomenon whereby a single species exhibits two or more morphs (morphotypes) showing differences in morphology, behaviour, coloration, or life history characteristics (e.g., growth characters) (Smith & Skúlason, 1996). These differences are considered to be an adaptation to habitat heterogeneity through the differentiation of feeding biology and habitat utilization. Resource polymorphism is not uncommon in freshwater fish species; examples include *Gasterosteus* (McPhail, 1992), *Coregonus* (Ostbye et al., 2005; Ostbye et al., 2006) (Ostbye et al. 2005; Ostbye et al. 2006), and *Salvelinus* species (Jonsson & Jonsson, 2001; Savvaitova, 1995). For example, two trophic morphs of *Gasterosteus aculeatus* coexist in several postglacial lakes, where one morph utilizes the littoral area and feeds on benthic invertebrates while the other occupies the open-water area and feeds on zooplankton (Schluter & Mcphail, 1992). The morphological adaptations of such morphs appear to be similar among cases; that is, the planktivorous morph often has longer and denser gill rakers, an elongated head and a longer lower jaw, while the benthivorous morph is characterized by shorter and sparser gill rakers, a blunt, round head and a shorter lower jaw (Fraser, Adams, & Huntingford, 1998; Jonsson & Jonsson, 2001; Smith & Skúlason, 1996). In addition to the typical benthivorous-planktivorous pairs, three or more morphs occur in some lake systems. For instance, five and four sympatric morphs have been found in *Coregonus lavaretus* in Fennoscandia and *Salvelinus alpinus* in

Thingvallavatn, respectively (Hudson, Vonlanthen, & Seehausen, 2010; Jonsson & Jonsson, 2001; Praebel et al., 2013).

Phenotypic plasticity, a process by which individuals express alternate phenotypes in response to different environments, is viewed as a mechanism of resource polymorphism (Seehausen et al., 2014; Skúlason et al., 2019). Praebel et al. (2013) found that European whitefish (*C. lavaretus*) formed three sympatric morphs that differed in gill raker counts due to rapid adaptive radiation into the littoral, pelagic and profundal lacustrine environments in three northern Fennoscandian lakes. The differences in size, diet, and jaw features between lean and huronius morphs of lake charr (*Salvelinus namaycush*) are typical examples of adaptation to shallow- and deep-water environmental conditions (Chavarie et al., 2016). The morphs of the African barb (*Labeobarbus gananensis* complex), which differ in mouth morphology, gill rakers, diet and gut length, display a typical example of adaptation involving distinct feeding strategies (Levin et al., 2018). Muir et al. (2016) evaluated four morphs of *S. namaycush* in many small post-glacial lakes throughout the Holarctic. Although there were differences in the number of morphs among lakes, the observed morphological variation resulted from adaptation to the separation of food resources (piscivorous and invertivorous feeding strategies), which was driven by lacustrine environmental diversity.

Cyprinids, the largest family of vertebrates, also exhibit resource polymorphism, with multiple morphs occurring in a single freshwater system. In the Genale River (Ethiopian highlands, East Africa), a barb (*L. gananensis*) complex was found to be composed of six forms, five of which were related to mouth morphology, which represents a typical form of adaptive radiation in response to different resources (Levin et al., 2018). Schizothoracins, the largest and most diverse group of the Qinghai-Tibetan Plateau ichthyofauna (Chen & Cao, 2000), also show morphological diversity within species or even sympatric speciation within a single lake. For instance, four morphs of *Schizopygopsis stoliczkai* were described in Lake Yashilkul, Pamir (Savvaitova et al., 1987). Furthermore, Zhao et al. (2009) demonstrated sympatric speciation between *Gymnocypris eckloni eckloni* and *G. e. scoliotomus* in Lake Sunmucu, a small glacial lake on the Tibetan Plateau.

Two morphs were found in *Schizopygopsis thermalis* Herzenstein 1891 (Cyprinidae: Schizothoracinae) in Lake Amdo Tsonak Co during our team field investigations in 2014-2018. The two morphs correspond to a resource axis in the lake: one form (planktivorous) predominately feeds on plankton and inhabits pelagic lake habitats, and the other form (benthivorous) mainly feeds on benthos and dwells in the benthic zone and a tributary (Nagchu River) of the lake. The former morph possesses a normal lower jaw, a terminal mouth, and moderately or highly dense gill rakers, while the latter morph is characterized by a shortened lower jaw, an inferior mouth with a sharpened horny edge on the lower jaw and short and sparse gill rakers.

However, the two morphs of *S. thermalis* have not been verified via morphological analysis. In addition, their biological characteristics (e.g., growth, feeding habit, and reproductive traits) are still unclear. To address these gaps in knowledge and better understand the ecological mechanisms of polymorphism in *S. thermalis*, the specific objectives of this study were to (1) characterize the morphological variation of the two significantly distinct morphs coexisting in Lake Amdo Tsonak Co and quantitatively analyse their morphological characters by a combination of morphometric and traditional linear measures; (2) define the biological characters (e.g., growth, feeding habit, and reproductive traits) of the two morphs of *S. thermalis*; and (3) elucidate the potential ecological mechanism of resource polymorphism in *S. thermalis*.

## MATERIAL AND METHODS

### 2.1 Study area

Lake Amdo Tsonak Co (31.55-32.08°N, 91.25-91.33°E) is a resource-poor and freshwater headwater lake of the Salween River system on the Tibetan Plateau, with a surface area of 182 km<sup>2</sup>, an elevation of 4,587 m above sea level, and a maximum depth of over 20 m. The annual mean air temperature is -3-0 °C, the annual total rainfall is 350-420 mm, the mean conductivity is 581.5 µs/cm (534.2-563.6 µs/cm), the mean salinity is 0.29, oxygen concentration is 6.53 mg/L (range: 5.63-7.21 mg/L), the mean phosphorus concentration is 0.028 mg/L, and the mean nitrogen concentration is 0.59 mg/L. The water chemistry values were measured and assessed by our colleagues.



## 2.2 Field sampling

Fishes were collected from Lake Amdo Tsonak Co and its tributary (Nagchu River) (Figure 1) in May and September 2017 and April and July 2018 with gill nets (mesh size: 30 mm), cast nets and hand nets. We labelled and photographed the left lateral side of each captured fish in the field. The standard length (SL, 0.1 mm), total length (TL, 0.1 mm), total weight ( $W$ ), sex, and stage of maturity of each specimen were recorded in the field. Only adult individuals were considered in the subsequent analyses due to the less obvious characteristics of the juveniles. We also measured gill raker number and length and pharyngeal tooth row number and recorded the presence or absence of parasites and the degree of mucus cavity development. In addition, we also measured the width of the horny edge of the lower jaw of some samples with the method shown in Figure 2 and described their lower jaw traits.

## 2.3 Morphological analysis

With respect to the analysis of both morphometric and linear traits, 154 specimens were analysed in this study. The left lateral side of the specimens was photographed using a Canon PowerShot G12 camera under natural light conditions with a ruler for scale. To obtain meaningful landmarks (homologous points), specimens were placed with the fins straightened and mouth closed in an orthogonal position. Specifically, the camera was fixed on a tripod with the lens parallel to the surface of the samples. Data on body shape and linear measurements for each fish were collected using digital landmarks on the photographs. A total of 24 landmarks were marked on each photograph (Figure 3) with tpsUtil and tpsDig2 (Rohlf, 2010). Body shapes were estimated by extracting the individual centroid size in MorphoJ 1.06 (Klingenberg, 2008). Simultaneously, new coordinates ( $xy$ ) for each fish were extracted using generalized Procrustes superimposition for subsequent analysis (Markevich, Esin, & Anisimova, 2018). To increase the accuracy of body shape estimates, some linear head traits (e.g., snout length, upper jaw length, lower jaw length, head length, head height and distance between the anterior termini of the upper and lower jaws) were measured. Allometry is common in fish, indicating that morphology and body size are typically related (Zelditch, Swiderski, & Sheets, 2004). Thus, a multivariate regression of body shape (Procrustes coordinates) on centroid size was used to correct for allometric effects, and regression residuals were used in geometric morphometric analyses (Elmer et al., 2014). Principal component analysis (PCA) was conducted via MorphoJ 1.06 to assess body shape variation (a geometric morphometric trait) among individuals without *a priori* grouping and to capture the maximum amount of variation with the smallest number of variables. The abbreviations and standardization of the linear head traits were as follows: HeadL = head length, SnoutL = snout length, Upper2 = upper jaw length, LowerL = lower jaw length and JawD = the distance between the anterior termini of the upper and lower jaws, which were normalized by  $SL$ , and HeadH = head height and UpperL1 = upper jaw length, which were standardized by head length and lower jaw length, respectively. Morphs were initially identified with unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis using Past 3.2.6 (<http://folk.uio.no/ohammer/past/>) based on 7 linear traits and scores from the first two principal components (PCs) of body shape from geometric morphometric analysis (Chavarie, Howland, & Tonn, 2013). To quantify the importance of each variable for the ordination axes and thus to summarize the variation in the morphs identified with the cluster analysis, PCA of body shape PCs and linear traits was then conducted. This procedure was performed with the ggbiplot package in R (version 0.55; Vu, 2011). Discriminant function analysis (DFA) and posterior jackknife cross-validation were performed with SPSS 22.0 on morphs defined by cluster analysis to determine whether the morphs were significantly different. The efficacy of the DFA was evaluated with Wilks'  $\lambda$ , which varies between 0 and 1, with zero indicating perfect identification. Finally, we performed multivariate analysis of variance (MANOVA) with Tukey's honestly significant difference (HSD) post hoc comparisons (SPSS 22.0) on body shape PCs and linear traits of samples between morphs to test the validity of the discriminant analysis results. Morphological variation between the sexes was estimated by implementing t-tests of body shape PCs and linear traits. To visualize body shape differences between morphs, we reconstructed body shapes using landmark coordinates based on DFA of only geometric morphometric data (Procrustes coordinates) (Elmer et al., 2014; Jakubavičiūtė, De Blick, Dainys, Ložys, & Olsson, 2018). For countable variables (e.g., gill raker number and pharyngeal tooth row number) and the measurable variable (e.g., gill raker length, which was standardized

by SL), the Kolmogorov-Smirnov test and Levene's test were performed. Analysis of variance (ANOVA) was conducted for variables that were normally distributed with variance homogeneity, and nonparametric tests were implemented for variables (after logx transformation) that showed a non-normal distribution or variance heterogeneity. To test whether the presence or absence of parasites affected body shape, analysis of covariance (ANCOVA) was performed using parasites as a covariate, morph as a factor and linear traits and PCs as dependent variables.

## 2.4 Diet analysis

To evaluate the feeding habits of the fish, we inspected the gut contents (since cyprinid fish do not have an obvious stomach, we chose the anterior intestine: the first bend from the pharynx to the intestine, where the food had not yet been digested) of 25 individuals of one morph (planktivorous) and 30 individuals of the other morph (benthivorous) and dissected them under a dissecting stereomicroscope before other gelatinous substances were excluded, such as gastric juices. Prey items were identified at the phylum or genus level. Then, we divided all prey items into six categories: zooplankton, small fishes (including fishes and their remains), hydrophilic insects, periphytic algae, zoobenthos and others (hydrophyte debris, organic debris and small grains of sand). First, the diet compositions were estimated by occurrence rate and wet weight percentage.

$$F \% = (N_i / N_{total}) \times 100\%$$

$F \%$  is the percentage of occurrence of prey  $i$ ,  $N_i$  is the frequency of occurrence of prey  $i$ , and  $N_{total}$  represents the total number of gut samples with food.

$$W \% = (W_c / W_{total}) \times 100\%$$

$W \%$  is the wet weight percentage of prey category  $c$  (one of the six food categories),  $W_c$  represents the wet weight of prey category  $c$ , and  $W_{total}$  represents the total weight of all prey items in each sample. Schoener's index ( $D_{xy}$ ) (Schoener, 1970) of proportional diet overlap was calculated and used to evaluate the difference in food composition between the two morphs.

$$C_{xy} = 1 - 0.5\{|P_{xc} - P_{yc}|\}$$

$C_{xy}$  represents the diet overlap index between the two forms ( $x$  and  $y$ ).  $P_{xc}$  and  $P_{yc}$  represent the shared food category of form  $x$  and  $y$  ( $W \%$ ), respectively. Values range between 0 (no diet overlap) and 1 (complete diet overlap), and values greater than 0.6 generally indicate biologically significant overlap (Wallace, 1981). Finally, the nonparametric Kruskal-Wallis test (Zar, 1999) was implemented to estimate the food composition ( $W \%$ ) difference between the two morphs.

## 2.5 Growth analysis

In total, 140 specimens (lapillus otoliths) were used to study the growth characteristics of the two morphs (planktivorous,  $N = 66$ ; benthivorous,  $N = 74$ ). Preparation of otolith sections and age determination were performed by an experienced worker. Each otolith was interpreted three times, and otoliths without at least 2 identical interpretations were excluded from the analysis. Photographs of all otolith sections were captured using MicroPublisher (5.0 RTV) under a light microscope (BH2; Olympus Optical, Tokyo, Japan). Otolith radius and ring diameter were measured with Autook (Image Analyser 2.0).

The relationship of  $SL$  with otolith radius was described by Frase-Lee regression, and the back-calculated  $SL$  of all ages was obtained using the modified Frase-Lee function (Johnson & Noltie, 1997):

$$\log_e L_i = a + (\log_e L_c - a)(\log_e O_i / \log_e O_c)$$

where  $L_c$  is the  $SL$  of the specimens,  $a$  is the intercept of the  $SL$ -otolith radius regression,  $O_c$  is the radius of the otolith at capture,  $O_i$  is the radius of the otolith at age  $i$  and  $L_i$  is the back-calculated  $SL$  at age  $i$ .

Back-calculated  $SL$  was used to fit a von Bertalanffy growth function (VBGF) (von Bertalanffy, 1938) and obtain the growth parameters of each morph:

$$L_t = L_{[\varphi]} (1 - \exp^{-k \times (t - t_0)})$$

where  $L_t$  is the back-calculated  $SL$  at age  $t$ ,  $L_{[\varphi]}$  is the asymptotic  $SL$ ,  $k$  is the growth coefficient,  $t$  is age, and  $t_0$  is the theoretical age at zero length.

To compare growth parameters, the growth performance index ( $\varphi$ ) was calculated according to the equation of Munro & Pauly (1983):

$$\varphi = \log_{10} K + 2 \log_{10} L_{[\varphi]}$$

where  $K$  is the growth coefficient and  $L_{[\varphi]}$  is the asymptotic  $SL$ .

Because of the nonlinear formulation of the VBGFs, a general linear model could not be used for ANCOVA. Instead, an analysis of residuals of the sum of squares (ARSS) was performed to compare the VBGFs between sexes and morphs (Chen, Jackson, & Harvey, 1992), and the degree of fit was denoted by the correlation coefficient and coefficient of determination ( $R^2$ ).

## 2.6 Genetic analysis

In the laboratory, fin tissue was collected from each specimen and preserved in 95% ethanol, and genetic samples were taken from the planktivorous ( $N = 36$ ) and benthivorous ( $N = 33$ ) morphs. Total genomic DNA was isolated using a standard phenol-chloroform method (Sambrook, Fritsch, & Maniatis, 1989). Two mitochondrial gene sequences, the cytochrome *b* gene (Cyt *b*, 1,140 bp) and the control region (D-loop, 839 bp), were amplified for all individuals using PCR. Detailed primer information is given in Table S1. PCR was performed in 37  $\mu$ L reactions containing 1  $\mu$ L of DNA, 0.375  $\mu$ L of dNTP mix, 0.3 units of Taq polymerase (TaKaRa), 3.8  $\mu$ L of 10 $\times$  reaction buffer, and 0.5  $\mu$ L of each primer. All reactions were performed under the following thermal cycler conditions: denaturation at 94  $^{\circ}$ C for 3 min, followed by 30 cycles of 95  $^{\circ}$ C for 30 s, annealing at 55  $^{\circ}$ C for 50 s and primer extension at 72  $^{\circ}$ C for 90 s for Cyt *b* and 45 s for D-loop and a final extension at 72  $^{\circ}$ C for 10 min (He & Chen, 2006; Liang et al., 2017). After visualization of the fragments using a 1% agarose gel, the PCR products were sequenced on an ABI 3730 capillary sequencer by Sangon Biotech Company (Shanghai, China).

The chromatograms were visually checked in BioEdit 7.0 (Hall, 1999), and the sequences were aligned with ClustalX (Larkin et al., 2007). Five species in the genus *Schizopygopsis* (*S. younghusbandi*, *S. pylzovi*, *S. stoliczkai*, *S. bangongensis*, and *S. malacanthus*) and the closely related species *Herzensteinia microcephalus* were analysed together. Two species, *G. eckloni* and *G. przewalskii*, were designated as outgroups. Phylogenetic relationships were constructed under maximum likelihood (ML) and Bayesian inference (BI) in CIPRES (Miller, Pfeiffer, & Schwartz, 2010) using a concatenated sequence of Cyt *b* and the D-loop, respectively (Zhao et al., 2009). Analysis of molecular variance (AMOVA, Excoffier, Smouse, & Quattro, 1992) was performed using Arlequin 3.5 (Excoffier & Lischer, 2010) to estimate the genetic structure of and differentiation among populations (corresponding to the confirmed morphs).

## RESULTS

### 3.1 Morphological analysis

Across all samples, two morphs were identified with UPGMA cluster analysis on the basis of body shape (Figure 4), referred to here as morph 1 (planktivorous,  $N = 74$ ) and morph 2 (benthivorous,  $N = 80$ ). DFA showed that the two morphs differed significantly in terms of body shape (Wilks'  $\lambda = 0.13$ ,  $N = 154$ ,  $p < 0.001$ ). A posteriori jackknife cross-validation showed high success, with 98.6 and 98.8% of the samples being correctly allocated to the planktivorous and benthivorous morphs, respectively. According to the MANOVA results shown in Table 1, seven linear traits and two body shape PCs were effective variables for the discriminant analysis. Based on the PCA and reconstructed body shape results (Figure 5), the samples of the planktivorous morph had a more terminal mouth, a longer, more robust head shape and a longer upper jaw, lower jaw and snout length than those of the benthivorous morph. In addition, among all the samples, morphological differences between the sexes were not detected (t-test,  $df = 152$ , all  $p > 0.05$ , males:females = 1:1.8). With regard to parasites, only cestodes and *Caenorhabditis elegans* were identified in a small number

of samples (planktivorous,  $N = 3$ ; benthivorous,  $N = 9$ ). Parasitism also did not affect the morphology of the specimens (ANCOVA;  $p = 1.00, > 0.05$ ).

With respect to descriptive traits, the two morphs (planktivorous and benthivorous) significantly differed in head characters. Planktivorous individuals possessed a terminal mouth with a slightly horny edge (width of horny edge:  $< 0.02$  cm) or lacked a horny edge on the lower jaw and had a highly developed mucus cavity in the cheek and chin. The benthivorous individuals were characterized by an inferior or sub-inferior mouth with a sharpened horny edge on the lower jaw (width of horny edge:  $0.14-0.42$  cm) and the lack of a mucus cavity or only a small mucus cavity in the cheek and chin.

Obvious differences and similarities between morphs were observed in some specialized organs related to ingestion. Although pharyngeal teeth in one or two rows were observed in both morphs (Figure 6), the percentage of individuals with a single row of pharyngeal teeth was larger in the benthivorous morph (30.0%) than in the planktivorous morph (10.8%). The numbers and lengths of gill rakers were similar between morphs (t-test; all  $p > 0.05$ ). A summary of basic information regarding the specimens of the two morphs captured in the field is given in Table S2.

### 3.2 Diets

Resource segregation was clear in the two morphs of *S. thermalis*. The planktivorous and benthivorous morphs showed low dietary overlap ( $C_{xy} = 0.42$ ). By wet weight, zooplankton composed a larger portion (43.9%) (Figure 7) of the gut contents in the planktivorous morph than in the benthivorous morph. Most of the zooplankton belonged to Rotifera (37.67%) and Copepoda (5.99%), both of which also had a high occurrence rate (100%) but constituted a small portion (0.02%) of the gut contents of the benthivorous morph. Small fishes (34.98%) and hydrophilic insects (17.08%) composed the second largest portion of prey in the gut of the planktivorous morph, which contained a larger portion of these prey items than did the benthivorous morph (29.40% and 8.64%, respectively). However, the benthivorous morph ingested a larger proportion of periphytic algae (47.93%) and zoobenthos (12.78%), based on the gut contents, than did the planktivorous morph, and Bacillariophyta (43.66%) and Chironomida larvae (9.56%) constituted the largest portion of the periphytic algae and zoobenthos, respectively. The nonparametric Kruskal-Wallis test showed that the morphs differed significantly in the wet percentage of food composition of periphytic algae ( $\chi^2$ : 40.30,  $p < 0.001$ ), zooplankton ( $\chi^2$ : 46.28,  $p < 0.001$ ), zoobenthos ( $\chi^2$ : 43.34,  $p < 0.001$ ), hydrophilic insects ( $\chi^2$ : 25.58,  $p < 0.001$ ), small fishes ( $\chi^2$ : 15.91,  $p < 0.001$ ) and others ( $\chi^2$ : 5.29,  $p < 0.05$ ). The detailed dietary data are shown in Table S3.

### 3.3 Growth

Back-calculation of otolith length was performed for males, females, and both sexes of each morph. We did not find a significant difference in the relationship of  $SL$  with otolith radius (ANCOVA after log transformation) between males and females in the planktivorous morph ( $N = 66$ , males:females: 1:1.87,  $F = 0.382$ ,  $p = 0.539 > 0.05$ ) or benthivorous morph ( $N = 74$ , males:females: 1:1.96,  $F = 3.297$ ,  $p = 0.073 > 0.05$ ). Therefore, the back-calculated  $SL$  of all ages was obtained using a modified Frase-Lee function for both sexes (males and females combined) of each morph, as follows:

planktivorous morph:

$$\log_e L_i = 5.0789 + (\log_e L_c - 5.0789)(\log_e O_i / \log_e O_c) \quad (R^2 = 0.9241)$$

benthivorous morph:

$$\log_e L_i = 5.1594 + (\log_e L_c - 5.1594)(\log_e O_i / \log_e O_c) \quad (R^2 = 0.8436)$$

The back-calculated  $SL$  of the samples (Table S4) was used to fit a VBGF for females, males and both sexes of each morph (Figure 8).

For the samples of the planktivorous morph, we found that females exhibited a larger asymptotic  $SL$  ( $L_{[\infty]} = 408.23$ ), lower growth rate ( $k = 0.104$ ) and higher growth performance index ( $\phi = 4.24$ ) than males ( $L_{[\infty]} =$

372.22,  $k = 0.12$  and  $\varphi = 4.22$ ). However, for the benthivorous morph, the results differed. Males presented a larger asymptotic SL ( $L_{[\varphi]} = 371.88$ ), higher growth rate ( $k = 0.139$ ) and higher growth performance index ( $\varphi = 4.29$ ) than did females ( $L_{[\varphi]} = 371.23, k = 0.12$  and  $\varphi = 4.22$ ). According to the VBGF test performed via ARSS, we found a significant difference between males and females for each morph (planktivorous morph:  $F_{0.01(65,64)} < 4.45, p < 0.01$ ; benthivorous morph:  $F_{0.01(73,72)} < 681.74, p < 0.01$ ).

Females of the planktivorous morph showed a larger asymptotic SL ( $L_{[\varphi]} = 408.23$ ), lower growth rate ( $k = 0.104$ ) and higher growth performance index ( $\varphi = 4.24$ ) than those of the benthivorous morph ( $L_{[\varphi]} = 371.23, k = 0.12$  and  $\varphi = 4.22$ ). Males of the planktivorous morph exhibited a larger asymptotic SL ( $L_{[\varphi]} = 372.22$ ), lower growth rate ( $k = 0.12$ ) and lower growth performance index ( $\varphi = 4.22$ ) than those of the benthivorous morph ( $L_{[\varphi]} = 371.88, k = 0.139$  and  $\varphi = 4.23$ ). A significant difference was observed for females ( $F_{0.01(91,90)} < 115.86, p < 0.01$ ) and males ( $F_{0.01(47,46)} < 47.82, p < 0.01$ ) of the two morphs. In the samples of both sexes, individuals of the planktivorous morph exhibited a larger asymptotic SL ( $L_{[\varphi]} = 405.14$ ), lower growth rate ( $k = 0.102$ ) and higher growth performance index ( $\varphi = 4.22$ ) than individuals of the benthivorous morph ( $L_{[\varphi]} = 374.22, k = 0.116$  and  $\varphi = 4.21$ ). The significantly different VBGFs between morphs were supported by the ARSS results ( $F_{0.05(139,138)} = 1.64, p < 0.05$ ). The detailed results are shown in Table 2 and Table 3.

### 3.4 Phylogenetic analyses

Phylogenetic analyses of the concatenated sequence data (Cyt b + D-Loop) based on ML and BI analysis produced similar topologies (Figure 9). With respect to outgroups and other closely related taxa, all analyses showed that the individuals of the two morphs were genetically mixed and did not support the monophyly of the forms (bootstrap support (BS) = 100; Bayesian posterior probability (PP) = 1.00). In addition, AMOVA revealed that most of the genetic variation (97.01%) was found within morphs, while little genetic variation (2.99%) was present between the two morphs. These results indicate that the two morphs of *S. thermalis* are significantly genetically differentiated ( $F_{st} = 0.0299, p < 0.05$ ).

## DISCUSSION

To utilize the available separated food resources in environments, species often display morphology with a certain degree of polymorphism (Jonsson & Jonsson, 2001; Smith & Skulason, 1996). Resource polymorphism is a ubiquitous phenomenon in vertebrates and may represent a critical intermediate stage in speciation. In our study, we demonstrated the existence of two forms (planktivorous and benthivorous) of *S. thermalis*. Both morphological and ecological analyses showed that the two morphs of *S. thermalis* coexist in Lake Amdo Tsonak Co, which is likely caused by their different feeding habits. The two morphs of *S. thermalis* might also represent an important intermediate stage in ecological speciation.

### 4.1 Morphological analysis

Our results showed that head shape significantly differed between the planktivorous and benthivorous morphs. The planktivorous morph had a significantly longer snout and upper and lower jaw, exhibited a longer and more robust head shape and was limited to the lake. In contrast, the benthivorous morph possessed a shorter snout and upper and lower jaw, displayed a blunt head shape, and was distributed in both the lake and its tributaries. Differences in head morphometry are predominant between morphs. For example, planktivorous morphs often have robust heads and long lower jaws, while benthivorous morphs are characterized by blunt round heads and short lower jaws. These findings are similar to those for European whitefish (*C. lavaretus*) in Lake Ruskebukta and Lake Skrukkebukta and *Thymallus nigrescens* in Lake Hovsgol (Amundsen et al., 2004; Olson et al., 2019).

The other differences in morphological features between the two morphs are related to the horny edges on the lower jaw and the mucus cavity. The terminal mouth of the planktivorous morph, which is characterized by a slight horny edge or the absence of a sharpened horny edge on the lower jaw, is beneficial for capturing zooplankton, small fishes and hydrophilic insects, and the highly developed mucus cavity in the cheek and chin may aid in swimming for long periods. In contrast, the benthivorous morph possessed an inferior or

sub-inferior mouth with a sharpened horny edge on the lower jaw, facilitating the scraping of periphytic algae adhered to rocks (Liang, 2017; Ji, 2008).

Furthermore, the number of pharyngeal tooth rows was also found to significantly differ between the two morphs, which may be associated with their feeding functions. Eastman (1971, 1977) indicated that pharyngeal teeth are important feeding organs used by fish to crush food and are present in all cyprinids (and other families). Our results showed that a larger proportion of benthivores than of planktivores had one row of such teeth. The difference in the number of pharyngeal tooth rows between morphs could result from functional responses to increased crushing force during mastication as the “tooth-food-chewing pad complex” is working (Smits, Witte, & Povel, 1996; Uzar, Andrzejewski, & Kozak, 2019).

In contrast to the morphs of European whitefish and three-spined sticklebacks (Praebel et al., 2013; Schluter & Mcphail, 1992), our morphs showed a high degree of similarity in the number and length of gill rakers, which might indicate that the morphological differentiation in gill raker number and length between the two morphs is in the initial stage.

## 4.2 Growth features

Slow growth is one of the characteristics of plateau fishes (Chen et al., 2008; Duan, 2015; Zhu & Chen, 2009). The samples of *S. thermalis* were captured in Lake Amdo Tsonak Co, which is located at an altitude of 4,587 m on the Tibetan Plateau, and the average growth performance index was approximately 4.23. Hence, the growth rate of this species is also slow. This slow growth is related to both life history characteristics and the unique water chemistry caused by the very high altitude. In our study, the results of the ARSS test revealed significant differences in growth features between females and males in both the planktivorous morph and the benthivorous morph. There are two possible explanations for these differences. One is the widely divergent ratios of males to females (planktivorous morph: males:females = 1:1.87; benthivorous morph: males:females = 1:1.96). The other is the use of different age groups to fit the growth equations. For the planktivorous morph, the age of females ranged from 6-27 yr, while the age of males ranged from 7-22 yr. For the benthivorous morph, the age of females ranged from 6-30 yr, while the age of males ranged from 6-18 yr.

Different growth characteristics between morphs normally relate to feeding biology (Hindar & Jonsson, 1982). In this paper, disparate growth characteristics between morphs were shown via the ARSS test in both females and males. Overall, in comparison to the benthivorous morph, the planktivorous morph had a higher growth performance index ( $\phi$ ) and a larger asymptotic value ( $L_{\infty}$ ) at a lower growth coefficient ( $k$ ). The distinct growth characteristics of the two morphs could be explained by dietary differences. Although both morphs represent typical omnivorous fish, their diet compositions are significantly dissimilar (He & Chen, 2006). The planktivorous morph predominately feeds on zooplankton and small fishes in the pelagic area, thus feeding partially on animals, while the benthivorous morph mainly feeds on periphytic algae and zoobenthos in benthic zones and the river, thus preferring a plant-based diet. Jonsson & Jonsson (2001) reported that the somatic growth rate and maximum size of fish generally depended on the quality and quantity of food. Therefore, planktivorous individuals with partially animal-based diets obtain more energy than benthivorous individuals with primarily plant-based diets. That is, the individuals of planktivorous fish have access to more higher-quality food than do those of benthivorous fish, which may facilitate nutrition acquisition during growth (Jonsson & Jonsson 1997, 1998). Our results are similar to those on the growth features of lake charr (*S. namaycush*) in Rush Lake (Chavarie et al., 2016) and *S. younghusbandi* in Yarlung Zangbo River (Duan, 2015). In addition, Swanson et al. (2003) demonstrated that intraspecific competition was reduced in a sympatric population by a decrease in dietary overlap. Therefore, in our study, dietary divergence in *S. thermalis* facilitates resource partitioning and helps maintain the population-level stability of different growth patterns.

## 4.3 Phylogenetic analyses

Resource polymorphism in fishes, such as salmonid species or cyprinid species, may be the result of phenotypic plasticity or trait heritability (Klemetsen, 2010; Seehausen et al., 2014; Skúlason et al., 2019). In the African barb (*L. gananensis*), mouth polymorphism is attributed to phenotypic plasticity (Levin et al., 2018), while in the European whitefish (*C. lavaretus*), which exhibits differences in gill raker count, the trophic niches of littoral, pelagic, and profundal morphs are heritable (Praebel et al., 2013). In our study, phylogenetic analyses and AMOVA results did not support the two morphs being two different species. However, the genetic differentiation of the two morphs was significant ( $F_{st} = 0.0299$ ,  $p < 0.05$ ), which may explain the significant phenotypic differences between morphs. Compared with *G. e. eckloni* and *G. e. scoliotomus*, which are sympatric species of schizothoracins in Lake Sunmucu, a small glacial lake on the Tibetan Plateau (Zhao et al., 2009), the *S. thermalis* in our study exhibited a low level of genetic differentiation. Our findings are similar to those of Chavarie et al. (2016), who described lean and huronicus morphs (*S. namaycush*) in Rush Lake.

#### 4.4 Ecological mechanisms of resource polymorphism in *S. thermalis*

Multiple factors could have resulted in the resource polymorphism observed in *S. thermalis*. First, Lake Amdo Tsonak Co is a high-altitude headwater lake in the Salween River system on the Tibetan Plateau that shows minimal interspecific competition due to scarce species (only one highly specialized schizothoracin species and three species of *Triplophysa*) and a lack of predatory fish. In addition, Lake Amdo Tsonak Co has a discrete environment with more than two distinguishable habitats that have separated food resources. For example, the lake contains plentiful zooplankton in the pelagic area, abundant periphytic algae and zoobenthos in the benthic zone, and a large amount of aquatic plants on its shoals. Thus, the availability of vacant niches (separated food resources) and intense intraspecific competition might be fundamental prerequisites driving the differentiation in morphology. Ecological differences may establish barriers to gene flow, and reproductive isolation may occur when barriers are sufficient to prevent recent gene flow. During the field investigation in Lake Amdo Tsonak Co, we observed two different types of spawning: river spawning and lacustrine spawning. Because planktivores inhabit only the lake and benthivores are distributed in the lake and its outlet and tributaries, we speculate that the planktivores spawn only on shoals within the lake, which are rich in aquatic plants, while the benthivores may spawn both on lake shoals and in watercourses. This difference in spawning ground indicates a strong possibility for the existence of partial spatial isolation. Hence, the morphological differentiation of *S. thermalis* might be further maintained via this partial spatial isolation.

#### CONCLUSION

Two morphs, planktivorous and benthivorous, related to resource polymorphism were confirmed in *S. thermalis* in Lake Amdo Tsonak Co. The morphs differed in morphological characteristics (such as mouth position, jaw features, mucus cavity, and pharyngeal teeth), feeding habit and growth features. Although phenotypic plasticity representing the mechanism of resource polymorphism in *S. thermalis* cannot be excluded, the individuals of the two morphs exhibited a significant level of genetic differentiation. The pelagic-benthic resources and scarce species in the lake might have driven the initial morphological differentiation, and the dietary dissimilarity in the two morphs might be ultimately maintained and reinforced by partial spatial isolation in the spawning grounds in the freshwater environment. Resource polymorphism might be a common phenomenon in Tibetan Plateau freshwater lakes, which sheds light on intraspecific morphological polymorphism and speciation. However, our sampling was insufficient along the lake-depth gradient (e.g., littoral, pelagic and profundal) due to inconvenient traffic and extreme environmental conditions. Thus, the detailed distribution of the two morphs within lakes needs to be further investigated. It is also not clear whether the feeding difference between the two morphs developed a long time ago or only recently; hence, carbon and nitrogen stable isotope analysis, a more reliable method that could indicate individual long-term food resource use, should be conducted to explore the dietary differences between the two morphs in future studies. In addition, the putatively recent origin of the two morphs is also unclear; thus, more sensitive genetic markers, such as y-satellite or single nucleotide polymorphism (SNP) markers, should be used to assess the level of more recent divergence between the two morphs.

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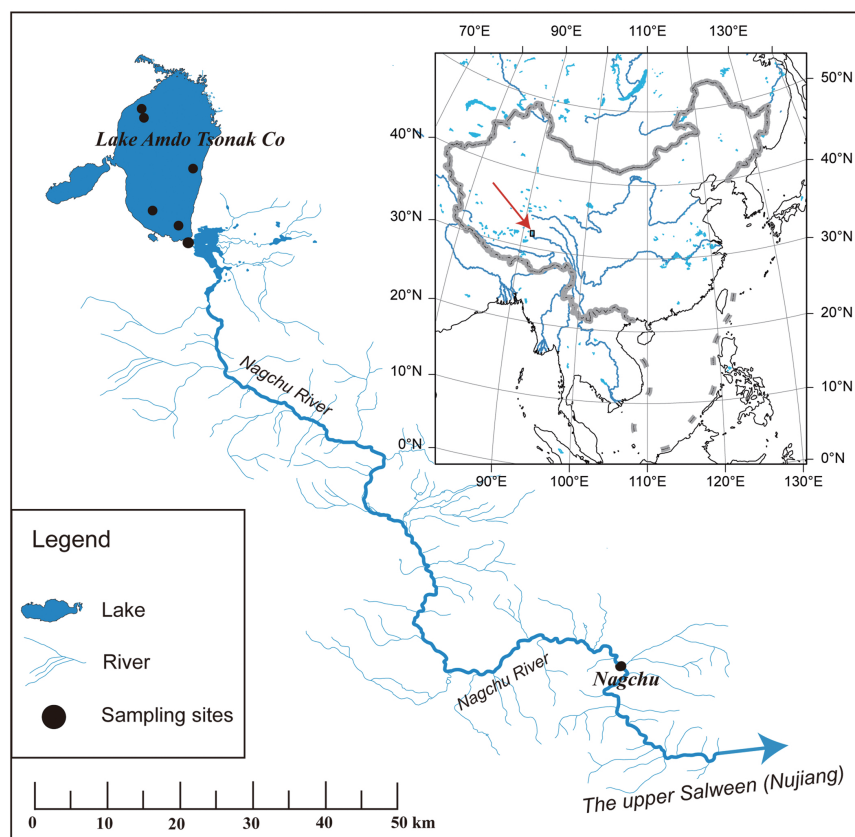
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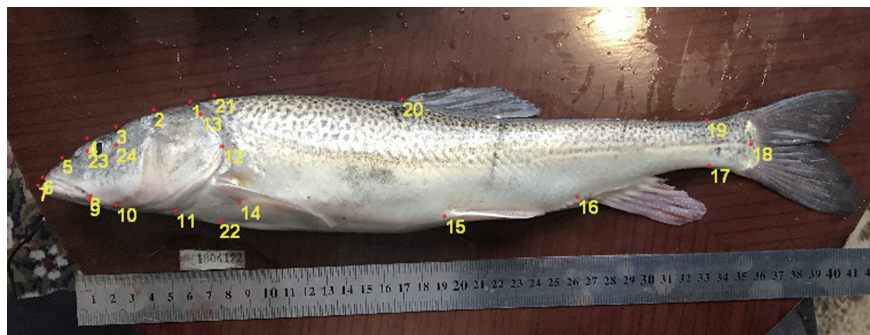
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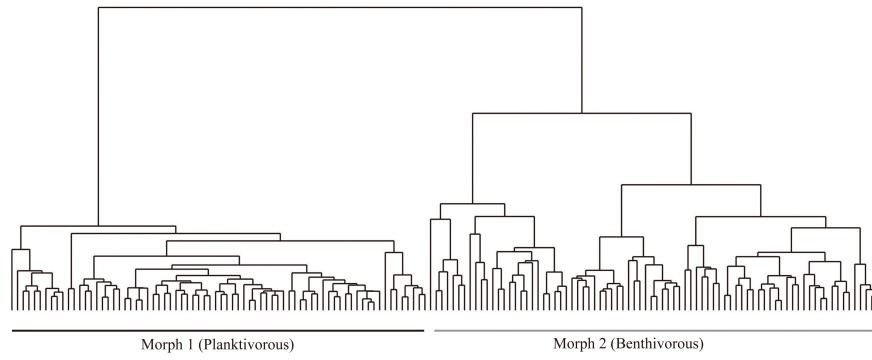
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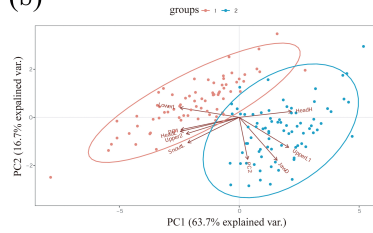
(a) planktivorous morph



benthivorous morph



(b)



(c)

