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RESEARCH PAPER

Karyological Analysis of Two Species in the Subfamily Schizothoracinae (Cypriniformes: Cyprinidae) from China, with Notes on Karyotype Evolution in Schizothoracinae

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Abstract

The karyotypes of *Schizothorax kozlovi* and *S. lissolabiatus* in the Subfamily Schizothoracinae, both of which are endemic to China, were investigated for the first time using the methods of kidney cell culture *in vivo*, and the numbers, sizes and morphology of the chromosomes in the two species were expatiated. The karyotype formula of *S. kozlovi* could be stated as 2n=92 = 18M+14SM+16ST+44T, FN=124, and that of *S. lissolabiatus* might be described as 2n=148=44M+36SM+20ST +48T, FN=228. *S. kozlovi* was probably a tetraploid species while *S. lissolabiatus* should be a hexaploid one, both these two species should belong to the primitive group in Schizothoracinae.

Almost all species karyologically investigated were polyploid including tetraploid and hexaploid in Schizothoracinae. The karyotypes in the subfamily might be originated from the primitive karyotype in Cyprinidae through approaches of centromere breakage, polyploidization and polyploidization with centromere breakage, Robertsonian translocation or chromosome deletion, under the pressure of rigorously natural selection in the Qinghai-Tibet Plateau. Also, the karyotypes evolved with the increase of number proportion of the metacentric (submetacentric) chromosomes in somatic cell of the species in Schizothoracinae.

Keywords: *Schizothorax kozlovi, Schizothorax lissolabiatus*, Schizothoracinae, karyotype, chromosome.

Introduction

Since the 1960s, karyological studies in teleost fishes have made valued contributions in the fields related to genetics, taxonomy and environmental toxicology (Cucchi & Baruffaldi, 1990). Karyological analysis could provide basic information on the number, size and morphology of chromosomes (Khan, Bhise, & Lakra, 2000; Tan, Qin, Chen, Chen, & Li, 2004) and increase the knowledge of evolutionary mechanisms and genetic question in the species investigated (Tripathy & Das, 1988; Macgregor, 1993). Also, the study of fish karyotype may supply necessary and important information for the use of chromosome manipulation techniques of fishes, including induction of fish polyploid, gynogenesis, androgenesis and inter or intra-species hybridization to produce new and good inbred lines in aquaculture. The study of fish chromosomes has being an active area of research in recent decades.

The Family Cyprinidae (Teleostei: Cypriniformes) is one of the richest and most important family of fish, and its members are distributed throughout the world (Al-Sabti, 1991). A total of about 1500 species have been recorded in the family worldwide (Gül, Colak, Sezgin, & Kaloğlu, 2004). The Subfamily Schizothoracinae, which is characterized by the large anal scales along two sides of anal hole and anal fin on the ventral side of body, belongs to Cyprinidae and lived in the area and countries around the Qinghai-Tibet Plateau (Chen & Cao, 2000), including China, India, Nepal, Bangladesh, Burma, Kazakhstan, Kyrgyzstan, Tajikistan, Pakistan, Afghanistan, Turkey, Iran, etc. Schizothoracinae comprises approximately 12 genera and more than 100 species, of which 76 species or subspecies from 11 genera are distributed in China (Mirza,1991; Chen & Cao, 2000). Up to now, there are 34 species or subspecies in 8 genera in the subfamily which have been karyologically investigated (Table 1).

Systematically, *Schizothorax kozlovi* and *S. lissolabiatus* belong to Cypriniformes, Cyprinidae and Schizothoracinae and are endemic to China (Chen & Cao, 2000). *Schizothorax kozlovi*, which possesses 2 pairs of long barbels, a smooth lower-lip consisting of 3 lobes, a low jaw without sharp horn and a thorax with minute scales, a strong last simple ray of dorsal

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Table 1. The karyotypes of 36 species or subspecies karyologically investigated in Schizothoracinae

Species	2n	Karyotype formula	Number proportion of st-t chromosomes	FN	FN/2n	References
Chuanchia labiosa	92	32m+26sm+18st+16t	36.957	150	1.630	Yu, Zhou, Li, Li, & Zhou,1989
Diptychus dybowskii lansdelli	98	54m-sm+4st+40a	44.898	152	1.551	Mazik, Toktosunov, & Ráb, 1989
D. gymnogaster microcephalus	100	70m-sm+6st+24st-a	30.000	170	1.700	Mazik *et al*., 1989
D. micromaculatus	98	62m-sm+6st+30st-a	36.735	160	1.633	Mazik *et al*., 1989
D. sewerzowi	98	80m-sm+14st-a+4a	18.367	178	1.816	Mazik *et al*., 1989
Diptychus sp.	98	28m+32sm+38st-t	38.776	158	1.612	Zan, Liu, & Song, 1985
Gymnocypris chui	92	36m+16sm+12st+28a	43.478	144	1.565	Zhang, Tang, Feng, Liu, & Zhao, 2016
G. dobula	66	32m+10sm+4st+20t	36.364	108	1.636	Wu, Kang, Men, & Wu, 1999
G. eckloni eckloni	94	26m+28sm+22st+18t	42.553	148	1.574	Yu *et al*., 1989
G. przewalskii	92	18m+18sm+16st+40t	60.870	128	1.391	Qi, 2004
G. scleracanthus	92	26m+12sm+18st+36a	58.696	130	1.413	Zhang *et al*., 2016
G. waddelli	94	24m+14sm+22st+34t	59.574	132	1.404	Wu *et al*., 1999
Gymnodiptychus dybowskii	98	28m+30sm+12st+28t	40.816	156	1.592	Kong, Hu, & Wang, 2011
Oxygymnocypris stewartii	92	26m+30sm+22st+14t	39.130	148	1.609	Yu *et al*., 1989
Platypharodon extremus	90	24m+30sm+20st+16t	40.000	144	1.600	Yu *et al*., 1989
Schizopygopsis pylzovi	92	32m+26sm+20st+14t	36.957	150	1.630	Yu *et al*., 1989
S. younghusbandi	90	26m+28sm+20st+16t	40.000	144	1.600	Yu *et al*., 1989
younghusbandi	94	22m+8sm+46st+18t	68.085	124	1.319	Wu *et al*., 1999
S. younghusbandi himalayensis	88	40m+16sm+12st+20t	36.364	144	1.636	Wu, Wu, & Lei, 1996
Schizothorax curvifrons	94	26m+20sm+20st+28t	51.064	140	1.489	Ganai, Yousuf, Dar, Tripathi, & Wani, 2011
S. davidi	98	20m+34sm+24st+20t	44.898	152	1.551	Li, Li, Gui, & Zhou, 1987
S. esocinus	98	30m+22sm+10st+36t	46.939	150	1.531	Ganai *et al*., 2011
S. grahami	148	52m+30sm+66st-t	44.595	230	1.554	Zan *et al*., 1985
S. kumaonensis	98	18 m + 10sm + 70a	71.429	126	1.286	Lakara, John, & Barat, 1997
S. labiatus	98	24m+20sm+2st+52t	55.102	142	1.449	Ganai *et al*., 2011
S. niger	98	24m+32sm+22st+20t	42.857	154	1.571	Ganai *et al*., 2011
S. o'connori	92	30m+26sm+20st+16t	39.130	148	1.609	Yu *et al*., 1989
	106	24m+26sm+30st+26t	52.830	156	1.472	Wu *et al*., 1999
S. plagiostomus	96	24m+18sm+54t	56.250	138	1.438	Ganai *et al*., 2011
S. prenanti	148	28m+40sm+36st+44t	54.054	216	1.459	Li *et al*., 1987
S. progastus	98	16m+20sm+12st+50a	63.265	134	1.367	Rishi, Singh, & Kaul, 1983
S. richardsonii	98	16 m + 40sm + 42a	42.857	154	1.571	Lakara *et al*., 1997
Schizothorax sp.	148	50m+28sm+70st-t	47.297	226	1.527	Zan *et al*., 1985
S. taliensis	148	48m+30sm+70st-t	47.297	226	1.527	Zan *et al*., 1985
S. waltoni	92	26m+28sm+22st+16t	41.304	146	1.587	Yu *et al*., 1989
	112	26m+24sm+28st+34t	55.357	162	1.446	Wu *et al*., 1999
S. zarudnyi	96	18m + 28sm + 50st	52.083	142	1.479	Kalbassi, Hosseini, & Tahergorabi, 2008
S. kozlovi	92	18m+14sm+16st+44t	65.217	124	1.348	this research
S. lissolabiatus	148	44m+36sm+20st+48t	45.946	228	1.541	this research

fin and a pair of ventral fins the origins of which are behind the origins of dorsal fins, is distributed in the Yalong River, Jinsha River and Wujiang River, Yangtze River system in China, while *S. lissolabiatus* that has 2 pairs of barbels, 3 rows of pharyngeal teeth, a smooth lower-lip comprising 2 lobes, a low jaw with sharp horn and naked thorax and fore-abdomen occurs in the upper Pearl River, the Yuanjiang River, the Lancang River and the Nujiang River in the Southwest China (Wu, 1989; Chen & Cao, 2000) (Figure 1). Although morphological and taxonomic characteristics of *Schizothorax kozlovi* and *S. lissolabiatus* have been stated (Chen & Cao, 2000), no information of karyotype is available on the two species at present.

This study aims to investigate the karyotypes and provide detailed information on the number, size and morphology of diploidy chromosomes in *Schizothorax kozlovi* and *S. lissolabiatus*. The study is also intended to increase the knowledge available for chromosome manipulation, aquaculture, selective breeding, genetic improvement of these two species and improve the understanding of the mechanism of karyotype evolution and speciation in Schizothoracinae.

Materials and Methods

Twelve individuals in each of *Schizothorax kozlovi* and *S. lissolabiatus* were collected respectively from the upper Wujiang River, the Yangtze River system, China in November, 2008 and the upper Beipan River, the Pearl River system, China in April, 2009 for karyotype analysis. The specimens of *Schizothorax kozlovi* were 122 – 153 mm in standard length and 25.2 - 48.9 g in weight, while the individuals of *S. lissolabiatus* were 162 – 228 mm in standard length and 59.3 - 213.3 g in weight. The fish individuals of both species were kept respectively to live in 400 L aquarium at 18 °C before analysis. All the chemicals used in this study were purchased from SIGMA.

To stimulate and increase cell mitotic divisions, the fish individuals of *Schizothorax kozlovi* and *S. lissolabiatus* received a celiac injection of phytohaemagglutinin at the base of pectoral fin at a final dose of 10 μg/g body weight in 0.2 mL distilled water solution respectively after they were maintained in the aquarium for 24 hours. Twenty-four hours after the phytohaemagglutinin injection, the specimens of the two species were injected into the abdominal cavity with colchicine from the base of pectoral fin at a final dose of 5 μg/g body weight respectively to form mitotic metaphase cells (Esmaeili *et al*. 2009). The fish individuals were kept to live in 150 mg/L tricaine for 5 minutes for anaesthesia and then completely exsanguinated by cutting its gill lamella on both sides of head with an ophthalmic scissors and their anterior kidneys removed 4 hours after the colchicine injection.

The tissue from the anterior kidney of each individual in *Schizothorax kozlovi* and *S. lissolabiatus* was collected respectively and sliced into small pieces and minced completely in a culture dish with a pincet. The visible biomembrane was discarded from the tissue. Then, the tissue was immersed in the cold 0.075 M KCl solution for hypotonic treatment for 45 minutes in a 10-mL centrifuge tube. The hypotonized tissue was centrifuged at 1 000 r/min for 10 minutes. The supernatant was discarded and the cold fresh Carnoy’s fixative (3:1 v/v methanol and glacial acetic acid) was added and mixed completely with the tissue in the centrifuge tube. Thirty minutes later, the mixture was centrifuged at 1 000 r/min for 10 minutes. Then, another three replacements of fixative with centrifugation were repeated before spreading. After fixation, the mixture was dropped onto the glass slides which were kept at 4 °C in a refrigerator for 20 minutes in advance, from a height of about 50 cm and air-dried at room temperature. The slides were stained with 3% Giemsa buffered solution (pH 7.2) for 30 minutes, then gently washed with distilled water and air-dried.

Mitotic metaphases were observed using an Olympus CH20 microscope with an oil immersion lens at 10×100 magnification. The chromosomes at the metaphase stage of somatic cells were photographed with a digital image capture system (DM200, Beijing Groupca Technology Co., Ltd, Beijing, China). Karyotyping was conducted

Figure 1. *Schizothorax kozlovi* (a) and *S. lissolabiatus* (b) (Photographed by Yinggui Dai). *S. kozlovi* possesses a low jaw without sharp horn while *S. lissolabiatus* has a low jaw with sharp horn.

according to 5 best mitotic metaphases images in each of *Schizothorax kozlovi* and *S. lissolabiatus*. The numbers of chromosomes in the somatic cells were counted, and chromosome morphometric data were determined using ADOBE PHOTOSHOP 6.0 photographic software with a micrometre scale. The average lengths of the short and long arms, the whole chromosome and the centromeric index (CI, the ratio of the short arm length to the total length of chromosome), arm ratio (the ratio of the long arm length to the short arm length of chromosome) and relative length (the percentage of absolute length of each chromosome pair in the sum of absolute lengths of total chromosome pairs in a somatic cell) were then calculated for each chromosome pair in the two species.

The chromosomes in each spread were paired and counted using the criteria of maximum resemblance based on the total length and the CI (Tan, Qin, Chen, Chen, & Li, 2004). The chromosome pairs were classified and counted following the criteria used by Levan, Fredga, and Sandberg (1964) into metacentric (M), submetacentric (SM), subtelocentric (ST) and telocentric (T), with CI ranges of 0.375 - 0.500, 0.250 - 0.375, 0.125 - 0.250 and 0 - 0.125 respectively. In this resarch, metacentric and submetacentric chromosomes were considered to have two arms, and telocentric and subtelocentric chromosomes to have only one. The fundamental number (FN) of chromosome arms was then calculated by summation of arm numbers of all types of chromosomes in the karyotype of the somatic cell in *Schizothorax kozlovi* and *S. lissolabiatus*. Finally, the karyotype of each of these two species was constructed respectively by placing the chromosome pairs into the mentioned classes on the basis of centromic position and arranging the homologous pairs in decreasing length order within each group. The ideogram was arranged using EXCEL 2007 (Microsoft) to provide the common feature of the chromosomes for each of the two species.

Results

Karyotype of *Schizothorax kozlovi*

One hundred mitotic metaphases (more than 5 per fish individual) from 12 individuals of *S. kozlovi* were available for the kayotype analysis of this species. The count of chromosomes varied from less than 88 to more than 100 per metaphase, with a mode of 92 representing 64.00% of the metaphases (Figure 2). No heteromorphic sex chromosome was identified in the species. The diploid chromosomes (2n = 92) of *S. kozlovi* comprised 9 pairs of metacentric (M), 7 pairs of submetacentric (SM), and 8 pairs of subtelocentric (ST) and 22 pairs of telocentric chromosomes (T) (Table 2). The average length of the chromosomes ranged from 6.000 to 12.144 μm and the haploid chromosome length of the species was 422.592 ± 35.142 μm on the basis of measurements of 5 best mitotic metaphases.

Therefore, the karyotype formula for *S. kozlovi* could be summarized as 2n = 92 = 18M + 14SM + 16ST + 44T, and the arm number was FN = 124. An example of the metaphase spreads and the karyotype of this species were shown in Figure 3, and its ideogram was shown in Figure 4.

Karyotype of *Schizothorax lissolabiatus*

A total of 96 metaphase spreads (more than 5 per fish individual) from 12 individuals of *S. lissolabiatus* in this research showed that the count of chromosomes ranged from 135 to148 per metaphase and the modal chromosome number was 2n = 148, which represented 59.38% of the cells examined (Figure 5). No heteromorphic sex chromosome was distinguished in this species. There were 22 pairs of metacentric chromosomes (M), 18 pairs of submetacentric chromosomes (SM), 10 pairs of subtelocentric chromosomes (ST) and 24 pairs of telocentric chromosomes (T) identified and measured

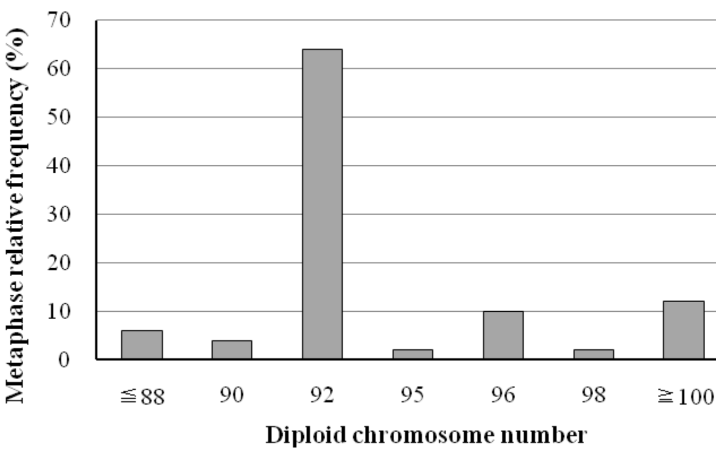


Figure 2. Frequency distribution of chromosome numbers recorded in 100 diploid metaphases of *Schizothorax kozlovi*.

Table 2. Numeral characteristics of the karyotype of *Schizothorax kozlovi* showing the mean values of measurements from 5 best mitotic metaphases

Chromosome pair no.	Long arm length (µm)	Short arm length (µm)	Total length (µm)	Relative length (%)	Centromeric index (CI)	Arm ratio	Classification
1	6.912±1.263	5.232±1.131	12.144±2.380	2.869±0.468	0.429±0.015	1.331±0.083	M
2	6.528±1.404	5.568±1.240	12.096±2.610	2.842±0.387	0.460±0.017	1.174±0.084	M
3	7.536±1.793	4.512±1.065	12.048±2.857	2.837±0.550	0.375±0.004	1.670±0.027	M
4	6.672±0.855	5.376±0.939	12.048±1.734	2.850±0.325	0.445±0.023	1.253±0.119	M
5	6.960±1.601	4.272±0.951	11.232±2.548	2.639±0.432	0.381±0.007	1.628±0.045	M
6	6.768±1.938	4.416±1.183	11.184±3.095	2.625±0.553	0.395±0.016	1.533±0.105	M
7	6.576±1.711	4.080±1.018	10.656±2.724	2.504±0.487	0.383±0.008	1.611±0.052	M
8	5.952±0.821	4.224±0.402	10.176±1.207	2.410±0.241	0.416±0.014	1.405±0.080	M
9	5.952±0.462	4.080±0.339	10.032±0.747	2.383±0.223	0.407±0.015	1.462±0.092	M
10	8.160±1.583	3.600±0.537	11.760±2.022	2.776±0.343	0.308±0.029	2.271±0.307	SM
11	8.208±1.351	3.456±0.626	11.664±1.777	2.769±0.448	0.297±0.032	2.403±0.360	SM
12	7.776±0.998	3.840±0.563	11.616±1.466	2.750±0.294	0.330±0.021	2.038±0.201	SM
13	7.440±0.975	3.936±0.498	11.376±1.426	2.707±0.406	0.346±0.016	1.893±0.137	SM
14	6.960±1.731	3.504±0.691	10.464±2.404	2.461±0.410	0.337±0.018	1.973±0.159	SM
15	7.440±0.975	3.024±0.553	10.464±1.364	2.476±0.271	0.288±0.031	2.502±0.369	SM
16	7.056±1.552	3.312±0.547	10.368±2.074	2.442±0.356	0.321±0.018	2.120±0.177	SM
17	8.208±1.274	2.544±0.402	10.752±1.649	2.552±0.415	0.237±0.011	3.232±0.204	ST
18	8.208±1.228	2.256±0.364	10.464±1.456	2.478±0.308	0.216±0.025	3.672±0.550	ST
19	7.728±1.614	2.160±0.537	9.888±2.046	2.334±0.420	0.218±0.026	3.642±0.573	ST
20	8.064±1.158	1.776±0.274	9.840±1.325	2.336±0.333	0.181±0.021	4.575±0.586	ST
21	7.968±0.547	1.872±0.313	9.840±0.814	2.339±0.253	0.189±0.018	4.313±0.468	ST
22	8.160±1.188	1.632±0.201	9.792±1.351	2.313±0.209	0.167±0.013	5.005±0.490	ST
23	7.344±0.578	1.392±0.107	8.736±0.626	2.080±0.240	0.160±0.012	5.293±0.468	ST
24	6.816±1.095	1.824±0.498	8.640±1.537	2.058±0.423	0.209±0.027	3.841±0.606	ST
25	9.264±0.468	0	9.264±0.468	2.197±0.088	0	∞	T
26	9.024±0.602	0	9.024±0.602	2.138±0.070	0	∞	T
27	8.832±0.573	0	8.832±0.573	2.092±0.037	0	∞	T
28	8.640±0.537	0	8.640±0.537	2.047±0.052	0	∞	T
29	8.400±0.720	0	8.400±0.720	1.988±0.043	0	∞	T
30	8.208±0.547	0	8.208±0.547	1.945±0.051	0	∞	T
31	8.064±0.553	0	8.064±0.553	1.911±0.079	0	∞	T
32	7.920±0.588	0	7.920±0.588	1.876±0.065	0	∞	T
33	7.824±0.498	0	7.824±0.498	1.854±0.065	0	∞	T
34	7.632±0.573	0	7.632±0.573	1.807±0.018	0	∞	T
35	7.536±0.498	0	7.536±0.498	1.785±0.047	0	∞	T
36	7.344±0.626	0	7.344±0.626	1.739±0.064	0	∞	T
37	7.152±0.573	0	7.152±0.573	1.693±0.043	0	∞	T
38	7.056±0.648	0	7.056±0.648	1.670±0.064	0	∞	T
39	7.056±0.274	0	7.056±0.274	1.677±0.127	0	∞	T
40	6.768±0.547	0	6.768±0.547	1.603±0.063	0	∞	T
41	6.768±0.313	0	6.768±0.313	1.609±0.137	0	∞	T
42	6.672±0.520	0	6.672±0.520	1.581±0.087	0	∞	T
43	6.576±0.553	0	6.576±0.553	1.558±0.086	0	∞	T
44	6.384±0.553	0	6.384±0.553	1.512±0.089	0	∞	T
45	6.192±0.462	0	6.192±0.462	1.467±0.065	0	∞	T
46	6.000±0.339	0	6.000±0.339	1.424±0.091	0	∞	T
Total			422.592 ± 35.142				

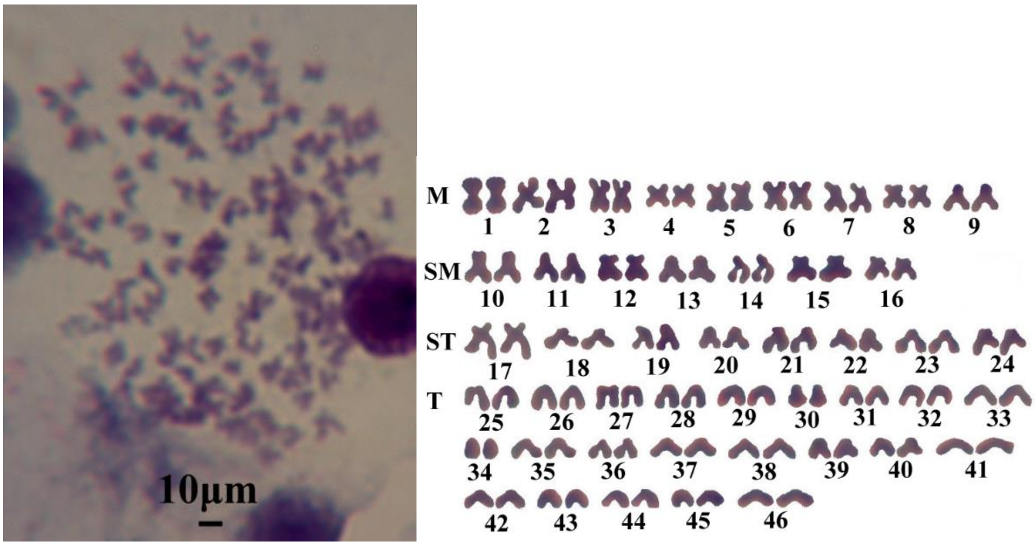
in the diploid somatic cell (2n=148) of *S. lissolabiatus* according to 5 clear mitotic metaphases (Table 3). The size of the chromosomes in the species varied from 7.200 to 14.496 µm, and the total length of haploid chromosomes in the species was 810.576 ± 59.978 µm. The karyotype and ideogram for *S. lissolabiatus* were shown in Figure 6 and Figure 7 respectively.

The karyotype formula for *S. lissolabiatus* might be described as 2n=148=44M+36SM+20ST+48T, with the fundamental chromosome arm number being FN=228.

Discussion

Notes on Karyotype Evolution in Schizothoracinae

According to the 36 species or subspecies karyologically investigated in Schizothoracinae, the chromosome numbers of them were 2n=66-148



(a) (b)

Figure 3. (a) Metaphase chromosomes of *Schizothorax kozlovi*. (b) Karyotype of *Schizothorax kozlovi* (2n = 92).

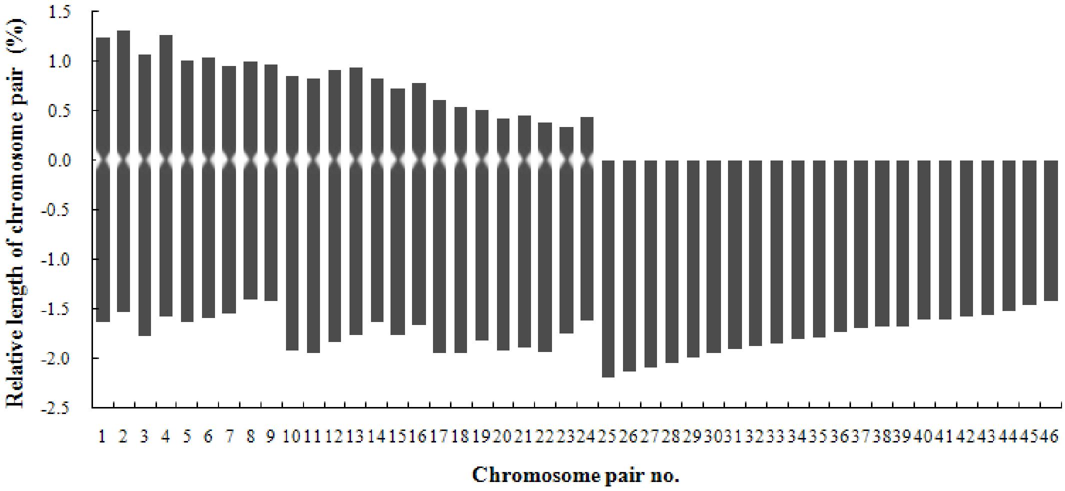


Figure 4. Ideogram of *Schizothorax kozlovi* (n=46). The plus values represent the relative lengths of short arms of chromosome pairs, and the minus values those of long arms of chromosome pairs.

respectively (Table1). On the basis of karological analysis of 141 species of cyprinid, Arai (1982) considered that karyotype of 2n=50 chromosomes was the primitive type in Cyprinidae. Also, Zhou (1984) thought that the fundamental number of karyotype in the species in Cyprinidae was 2n = 50 or 48. In fact, many species in Cyprinidae are polyploid fishes which have several chromosome series (2n>50). For example, the species *Carassius auratus* (2n=100), *Cyprinus carpio* (2n=100), *Tor putitora* (2n=100), *T. khudree* (2n=100) and *T. tor* (2n=100) in Cyprinidae are all tetraploid (Khuda–Bukhsh, 1982). It was believed that the fishes living in the area with the protean climate and cold winter might be intended to become polyploid during their evolution (Khuda-Bukhsh & Nayak, 1982; Rishi, Singh, & Kaul, 1983) and the polyploidy could enhance the survival of fishes under natural selection pressure (Oellerman & Skeleton, 1990). By comparison of number, arm number of chromosome and content of DNA in cell nucleus, Zan, Liu, and Song (1985) concluded that *Diptychus sp.* was a tetraploid species with the chromosome number of karyotype 2n=98, but species *Schizothorax graham* and *S. taliensis* were hexaploid with 2n=148 in Schizothoracinae. Of the 36 species or subspecies karyologically investigated in Schizothoracinae (Table 1), *Gymnocypris dobula* was probably a diploid species with 2n=66, 5 species should be hexaploid (2n=148), and the remaining 30 species or subspecies might be all tetraploid, of which the 2n chromosome number of karyotype was approximately 100. As a result, almost all species karyologically studied in Schizothoracinae were polyploid including tetraploid and hexaploid, which might evolve from the primitive karyotype in Cyprinidae under the pressure of rigorously natural

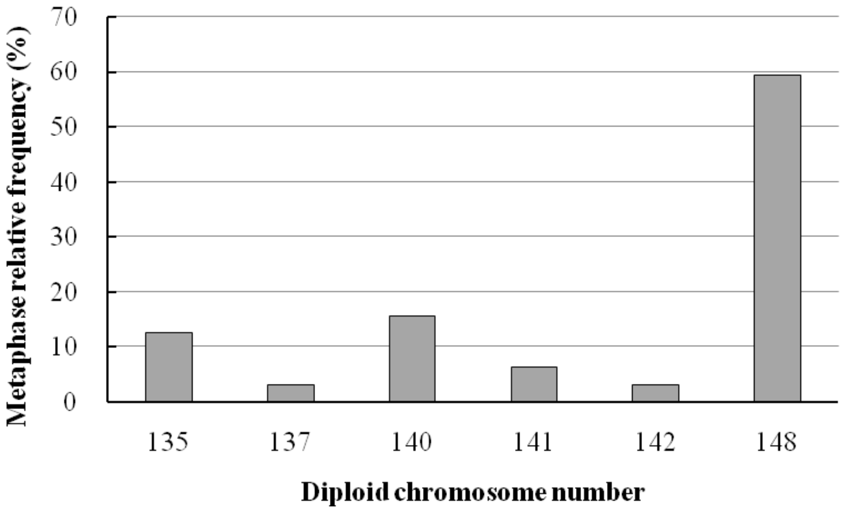


Figure 5. Frequency distribution of chromosome numbers recorded in 96 diploid metaphases of *Schizothorax lissolabiatus*.

selection in the Qinghai-Tibet Plateau, and the tetraploid species was dominant in the subfamily. Also, there were 9, 12 species karyologically investigated that respectively had karyotypes of 2n = 92, 98 chromosomes in Schizothoracinae (Table 1), which showed that tetraploids with 2n = 92, 98 chromosomes were two important patterns of karyotypes in the subfamily.

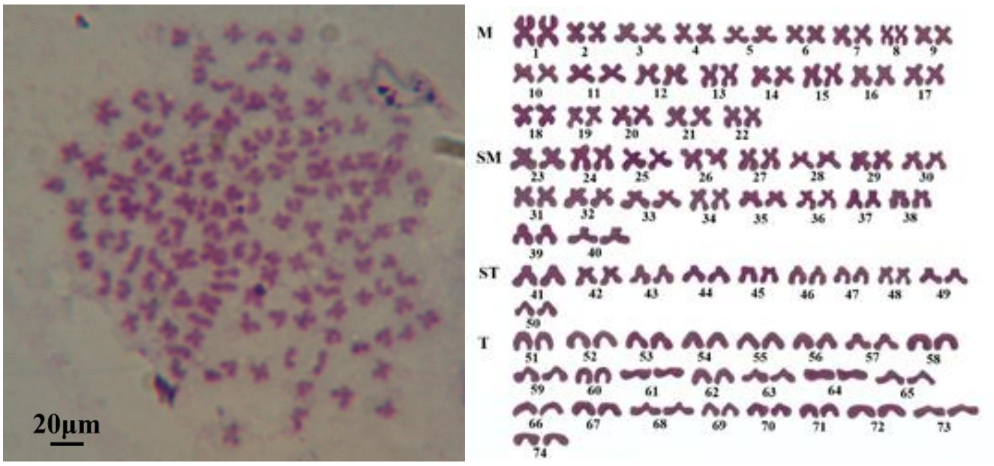
It was summarized that the chromosome number of the karyotypes in Cyprinidae evolved mainly through 5 approaches (Arai, 1982): ① centromere breakage**,** 2n = 50 or 48 → 78, ② aneuploid increase and centromere breakage, 2n=50 → 52, ③ polyploidization, 2n=50 → 100 or 98, ④ Robertsonian translocation, 2n=50 → 48 or 2n=48 → 44, ⑤ chromosome deletion, 2n=48 → 46. The 36 species karyologically studied in Schizothoracinae each had the karyotype of 2n = 66, 88, 90, 92, 94, 96, 98, 100, 106, 112 or 148 chromosomes (Table 1), it might be guessed that the karyotypes of species in Schizothoracinae should be originated from the primitive karyotype 2n = 50 or 48 chromosomes in Cyprinidae respectively through the procedures of centromere breakage**,** polyploidization and polyploidization with centromere breakage, Robertsonian translocation or chromosome deletion (Table 4).

Schizothoracinae comprised three groups based on morphological data: the primitive group, the specialized group and the highly specialized group (Cao, Chen, Wu, & Zhu, 1981). The primitive group which was composed of the genera *Aspiorhynchus*, *Schizocypris* and *Schizothorax* represented the primitive grade during evolution, the specialized group including the genera *Diptychus*, *Gymnodiptychus* and *Ptychobarbus* and the highly specialized group that consisted of the genera *Chuanchia*, *Gymnocypris*, *Herzensteinia*, *Oxygymnocypris*, *Platypharodon* and *Schizopygopsis* belonged to the evolutionary one in the subfamily. It was reported that to a wide extent, animal species showed a decreasing trend in number of telocentric (subtelocentric) chromosomes in their karyotypes during their evolution (Li, 1986). All uniarmed karyotypes were considered to be ancestral in fish species (Ohno, 1974). On basis of studies on the karyotypes of more than 800 fishes, Kojima Yoshio (1990) considered that fishes with less FN (fundamental chromosome arm number of karyotype) had a relatively lower evolutionary grade. The index of FN / 2n was useful to analyze karyotype evolution of fish species, and the cyprinid showed an increase trend of FN / 2n value in evolution (Yu, Li, & Zhou, 1990). According to the species karyologically investgated in Schizothoracinae (Table 1), most of the species in *Schizothorax* had higher number proportion of the telocentric (subtelocentric) chromosomes and less FN in their karyotypes than those in the remaining genera, and the value of FN / 2n in almost all of the species in *Schizothorax* was lower than 1.60. However, most of the species in the genera except for *Schizothorax* had higher number proportion of the metacentric (submetacentric) chromosomes and more FN in their somatic cell than those in *Schizothorax* and the FN/2n value of them was close to or higher than 1.60. Accordingly, the increase of number proportion of the metacentric (submetacentric) chromosomes in somatic cell of the species should be another considerable pattern of karyotype evolution in Schizothoracinae.

Dobzhansky (1936) and Muller (1940, 1942) considered that during evolution, the independent evolution within isolated populations could result in the genetic incompatibilities between populations which bring on speciation, and no population had to pass through a low-fitness transitional state, which is called the Dobzhansky–Muller Model. According to the species karyologically investigated in

Table 3. Numeral characteristics of the karyotype of *Schizothorax lissolabiatus* showing the mean values of measurements from 5 clear mitotic metaphases

Chromosome pair no.	Long arm length (μm)	Short arm length (μm)	Total length (μm)	Relative length (%)	Centromeric index (CI)	Arm ratio	Classification
1	7.584±1.570	6.912±1.414	14.496±2.947	1.810±0.455	0.477±0.015	1.098±0.068	M
2	8.304±1.542	6.048±1.038	14.352±2.548	1.763±0.211	0.422±0.017	1.372±0.092	M
3	8.496±1.485	5.712±1.193	14.208±2.615	1.759±0.343	0.401±0.021	1.501±0.123	M
4	7.440±1.420	6.336±0.939	13.776±2.331	1.698±0.248	0.462±0.018	1.169±0.085	M
5	8.064±1.833	5.472±0.995	13.536±2.812	1.667±0.312	0.407±0.019	1.464±0.111	M
6	8.160±0.740	5.376±0.807	13.536±1.495	1.679±0.237	0.396±0.019	1.530±0.116	M
7	7.536±0.923	5.856±0.402	13.392±1.308	1.662±0.229	0.438±0.015	1.284±0.080	M
8	7.680±1.637	5.568±1.434	13.248±3.042	1.648±0.436	0.419±0.016	1.392±0.087	M
9	7.152±1.168	6.000±1.212	13.152±2.317	1.618±0.224	0.455±0.024	1.202±0.117	M
10	8.064±0.602	5.040±0.635	13.104±1.207	1.622±0.176	0.384±0.015	1.609±0.095	M
11	7.728±1.414	5.328±0.687	13.056±2.090	1.610±0.232	0.410±0.018	1.443±0.103	M
12	7.920±1.336	4.896±0.892	12.816±2.204	1.579±0.236	0.382±0.012	1.623±0.081	M
13	7.056±1.041	5.664±0.923	12.720±1.912	1.564±0.146	0.445±0.019	1.251±0.095	M
14	7.680±0.814	4.896±0.274	12.576±1.027	1.556±0.143	0.390±0.020	1.567±0.122	M
15	7.440±0.945	5.040±0.537	12.480±1.410	1.537±0.073	0.404±0.020	1.477±0.115	M
16	7.584±1.231	4.848±0.981	12.432±2.156	1.533±0.233	0.389±0.020	1.578±0.122	M
17	7.536±1.385	4.848±0.838	12.384±2.177	1.520±0.174	0.392±0.019	1.556±0.119	M
18	6.816±1.343	5.136±0.908	11.952±2.228	1.473±0.252	0.430±0.015	1.325±0.081	M
19	7.296±0.553	4.464±0.526	11.760±1.060	1.457±0.167	0.379±0.012	1.641±0.079	M
20	6.960±1.292	4.608±0.821	11.568±2.060	1.420±0.159	0.399±0.021	1.514±0.124	M
21	6.624±0.908	4.704±0.602	11.328±1.474	1.401±0.185	0.415±0.016	1.410±0.089	M
22	6.816±0.923	4.272±0.573	11.088±1.454	1.367±0.148	0.385±0.016	1.599±0.099	M
23	8.976±1.343	3.984±0.824	12.960±2.092	1.594±0.180	0.306±0.025	2.287±0.257	SM
24	9.072±2.317	3.792±1.052	12.864±3.315	1.573±0.306	0.294±0.027	2.420±0.300	SM
25	8.400±1.430	4.416±0.732	12.816±2.137	1.575±0.164	0.345±0.012	1.904±0.097	SM
26	8.304±1.343	4.176±0.892	12.480±2.200	1.543±0.277	0.333±0.018	2.010±0.156	SM
27	7.872±1.038	4.176±0.770	12.048±1.799	1.494±0.264	0.345±0.012	1.898±0.099	SM
28	7.872±1.540	4.080±0.849	11.952±2.372	1.489±0.369	0.341±0.012	1.936±0.103	SM
29	8.160±1.410	3.648±0.821	11.808±2.163	1.450±0.170	0.307±0.026	2.270±0.262	SM
30	8.592±1.726	3.216±0.732	11.808±2.450	1.448±0.223	0.271±0.010	2.689±0.140	SM
31	7.632±1.878	4.080±0.882	11.712±2.754	1.432±0.227	0.350±0.011	1.862±0.088	SM
32	8.448±0.644	2.928±0.201	11.376±0.842	1.406±0.096	0.257±0.003	2.884±0.049	SM
33	8.112±1.156	3.120±0.563	11.232±1.700	1.395±0.263	0.277±0.012	2.615±0.163	SM
34	7.872±1.414	3.120±0.679	10.992±2.060	1.350±0.168	0.283±0.016	2.539±0.202	SM
35	7.440±1.032	3.504±0.468	10.944±1.436	1.349±0.137	0.321±0.020	2.129±0.190	SM
36	7.440±2.232	3.360±0.814	10.800±2.998	1.340±0.404	0.314±0.024	2.204±0.244	SM
37	7.920±0.416	2.784±0.215	10.704±0.626	1.326±0.125	0.260±0.006	2.849±0.082	SM
38	7.824±0.691	2.832±0.313	10.656±0.998	1.315±0.084	0.265±0.006	2.769±0.093	SM
39	7.440±0.930	3.168±0.547	10.608±1.362	1.305±0.090	0.298±0.029	2.381±0.322	SM
40	7.248±1.444	3.024±0.751	10.272±2.109	1.272±0.274	0.293±0.027	2.435±0.309	SM
41	9.168±0.920	1.584±0.131	10.752±0.981	1.326±0.054	0.148±0.012	5.805±0.563	ST
42	8.784±1.524	1.872±0.520	10.656±1.976	1.316±0.234	0.174±0.025	4.851±0.820	ST
43	8.496±1.332	1.728±0.394	10.224±1.624	1.258±0.145	0.169±0.020	5.000±0.707	ST
44	8.208±1.038	1.824±0.436	10.032±1.393	1.240±0.169	0.181±0.024	4.621±0.775	ST
45	8.448±2.074	1.536±0.274	9.984±2.331	1.221±0.198	0.156±0.013	5.462±0.573	ST
46	7.776±1.242	2.208±0.313	9.984±1.466	1.235±0.189	0.222±0.020	3.533±0.435	ST
47	7.776±0.274	2.112±0.263	9.888±0.462	1.224±0.096	0.213±0.019	3.726±0.449	ST
48	7.632±1.118	1.920±0.379	9.552±1.404	1.183±0.189	0.201±0.025	4.047±0.619	ST
49	7.296±0.875	1.968±0.394	9.264±1.231	1.144±0.139	0.211±0.020	3.772±0.467	ST
50	7.440±0.379	1.776±0.215	9.216±0.274	1.142±0.095	0.193±0.025	4.261±0.758	ST
51	11.328±0.966	0	11.328±0.966	1.397±0.049	0	∞	T
52	10.992±1.168	0	10.992±1.168	1.354±0.066	0	∞	T
53	10.464±1.343	0	10.464±1.343	1.287±0.077	0	∞	T
54	10.320±1.379	0	10.320±1.379	1.269±0.080	0	∞	T
55	10.080±1.004	0	10.080±1.004	1.242±0.050	0	∞	T
56	9.888±0.966	0	9.888±0.966	1.219±0.053	0	∞	T
57	9.792±0.936	0	9.792±0.936	1.207±0.055	0	∞	T
58	9.744±0.908	0	9.744±0.908	1.201±0.044	0	∞	T
59	9.600±1.018	0	9.600±1.018	1.183±0.058	0	∞	T
60	9.456±1.095	0	9.456±1.095	1.165±0.068	0	∞	T
61	9.312±0.951	0	9.312±0.951	1.147±0.043	0	∞	T
62	9.168±0.920	0	9.168±0.920	1.130±0.049	0	∞	T
63	9.024±0.998	0	9.024±0.998	1.111±0.051	0	∞	T
64	8.928±0.995	0	8.928±0.995	1.099±0.047	0	∞	T
65	8.880±0.898	0	8.880±0.898	1.094±0.039	0	∞	T
66	8.784±0.969	0	8.784±0.969	1.082±0.053	0	∞	T
67	8.640±0.882	0	8.640±0.882	1.065±0.047	0	∞	T
68	8.496±0.954	0	8.496±0.954	1.046±0.047	0	∞	T
69	8.400±1.018	0	8.400±1.018	1.034±0.056	0	∞	T
70	8.160±0.612	0	8.160±0.612	1.007±0.027	0	∞	T
71	8.016±0.670	0	8.016±0.670	0.989±0.026	0	∞	T
72	7.872±0.520	0	7.872±0.520	0.972±0.030	0	∞	T
73	7.488±0.520	0	7.488±0.520	0.924±0.021	0	∞	T
74	7.200±0.416	0	7.200±0.416	0.890±0.036	0	∞	T
Total			810.576 ± 59.978				



(a) (b)

Figure 6. (a) Metaphase chromosomes of *Schizothorax lissolabiatus*. (b) Karyotype of *Schizothorax lissolabiatus* (2n = 148).

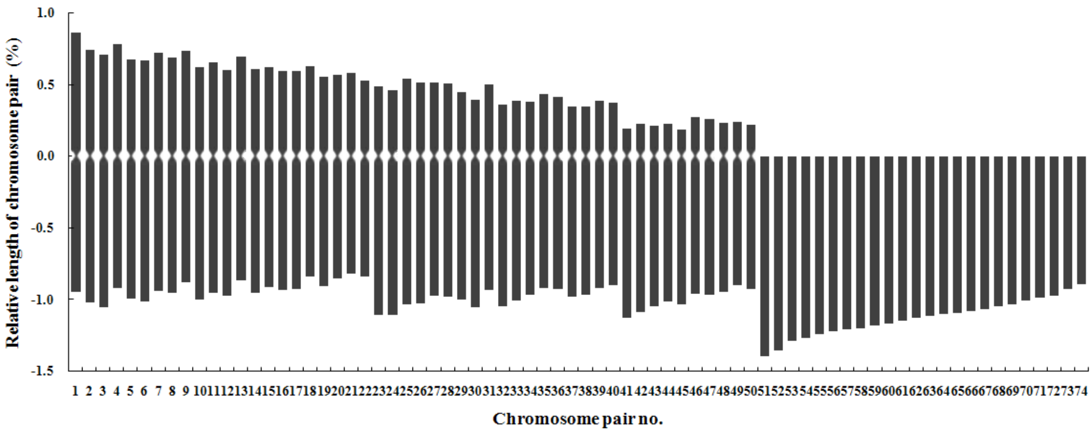


Figure 7. Ideogram of *Schizothorax lissolabiatus* (n = 74). The plus values represent the relative lengths of short arms of chromosome pairs, and the minus values those of long arms of chromosome pairs.

Schizothoracinae, the 36 species or subspecies respectively had 2n=66–148 chromosomes and FN=124–230 in their karyotypes in the family (Table1), which showed the the genetic incompatibility resulted from the variance of chromosome number and FN value in cells of the insulated populations in Schizothoracinae probably was one of important reasons that decided the speciations of these species which were originated from the ancestral species that had 2n=50 or 48 uniarmed chromosomes in its karyotype through the independent evolution of its segregated offspring populations in the diverse and complicated environments around the Qinghai-Tibet Plateau.

Analysis of Karyotypes of *Schizothorax kozlovi* and *S. lissolabiatus*

According to Zan *et al*. (1985), *Schizothorax kozlovi* had the karyotype of 2n=92 chromosomes and therefore was probably a tetraploid species, while *S. lissolabiatus* had 2n=148 chromosomes in its karyotype and should be a hexaploid one in this research. Therefore, karyotypes of the species *Schizothorax kozlovi* and *S. lissolabiatus* might be originated from the primitive one in Cyprinidae respectively through polyploidization with chromosome deletion and polyploidization with Robertsonian translocation during their evolution under the changeful and cold climate in the Yunnan-Guizhou Plateau in the Southwest China.

Both *Schizothorax kozlovi* and *S. lissolabiatus* had higher number proportion of the telocentric (subtelocentric) chromosomes and lower FN / 2n value in their karyotypes than most species in the genera except for *Schizothorax* (Table 1), which hinted that the two species should belong to the primitive group in Schizothoracinae.

So far, a total of 18 species in *Schizothorax* have been karologically investigated (Table 1). The chromosome number of their karyotypes was 2n=92 - 148, the range of FN / 2n value of them was 1.286 - 1.609. *Schizothorax kozlovi* had less chromosomes and lower FN / 2n value of karyotype than most of the

Table 4. Evolutionary approaches of karyotypes of species in Schizothoracinae

Chromosome no.of karyotype (2n)	Ploidy	Evolutionary approach
66	Diploid	Centromere breakage
88, 94, 96	Tetraploid	Polyploidization and Robertsonian translocation
90	Tetraploid	Polyploidization, Robertsonian translocation and chromosome deletion
92, 96	Tetraploid	Polyploidization and chromosome deletion
98, 100	Tetraploid	Polyploidization,
106, 112	Tetraploid	Polyploidization and centromere breakage
148	Hexaploid	Polyploidization and Robertsonian translocation

Note: The Evolutionary approaches were identified according to Arai (1982).

remaining species and therefore might be situated at the low evolutionary grade while *S. lissolabiatus* should represent the high one because it had much more chromosomes and higher value of FN/2n in its karyotype than most other species in *Schizothorax* (Table 1).

The chromosome set manipulation techniques of fishes had made significant contributions to aquaculture and aquaculture genetic researches (Chai, Li, Lu, & Clarke, 2009). For example, the gynogenetic and triploid populations of brown trout were cultured in Europe, especially in France (Hulata, 2001). The species *Schizothorax kozlovi* and *S. lissolabiatus* are valuable food fishes for the local people and potential breed for aquaculture in China. This study provides the first information on the karyotypes of *Schizothorax kozlovi* and *S. lissolabiatus* which will improve the understanding of chromosomal manipulations to facilitate the aquaculture of these two species in the future.

The polyploidy had larger size, a longer life, faster growth and greater ecological adaptability than diploidy in Cyprinidae (Uyeno & Smith, 1972), provided redundant gene loci (Becak, Becak, & Ohno, 1966) and are the basis of the characteristic life-history traits of species (Oellermann & Skelton, 1990), so the polyploid fishes might develop some desirable characteristics for domestication in aquaculture. *Schizothorax kozlovi*, *S. lissolabiatus* were respectively tetraploid, hexaploid species, therefore it is promising to breed excellent strains of these two species for aquaculture.

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