

Article

Karyotypes of 10 Anuran Species from the Qinghai–Tibetan Plateau

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Abstract: The Qinghai–Tibet Plateau (QTP) is the highest and largest mountain plateau in the world, which has become a focus area of amphibian biodiversity research and conservation, depending on its large number of endemic and threatened species. Among the 58 families of Anura, only a few species of four families (Megophryidae, Bufonidae, Dicroglossidae, Ranidae) are distributed in QTP. Revealing the genetic diversity of these species is crucial for research on their environmental adaptability and biodiversity conservation. Chromosome rearrangements are a critical source of genetic variation, which is recognized as a driver of speciation, providing the genetic material for differentiation and environmental adaptation of amphibians. Here, we identified the karyotypes of 10 species of the above families from the QTP. The karyotypes of these species were obtained from new sites that were not previously reported. Among them, the karyotypes of *D. himalayanus* and tetraploid *B. zamdaensis* were reported for the first time. In particular, the ploidy of *B. zamdaensis* from Zanda, China, was found to be distinctly different from the ploidy from Spiti River, India. This indicates that they have presented species differentiation and supports the multiple and complicated polyploidization events in the *Bufo* toads. Furthermore, the different locations of the secondary constriction between the Weixi and Zhongdian populations of *O. xiangchengensis* support that there is a karyotypic variation between the two subspecies (*O. xiangchengensis xiangchengensis* vs. *O. xiangchengensis deqinicus*). A series of chromosomal variations may have facilitated the rapid evolution of amphibians in the QTP, and our study will provide support for further research on amphibian genetic diversity and biodiversity conservation.

Keywords: Qinghai–Tibet Plateau; amphibians; karyotype; chromosome



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1. Introduction

The Qinghai–Tibet Plateau (QTP) is the highest and largest mountain plateau in the world, with a mean elevation of over 4500 m above sea level and a surface area of 2.3 million km² [1]. It is known as ‘the roof of the world’ and has been classified as a biodiversity hotspot [2]. Its landscape of river gorges and steep mountains has led to dramatic ecological stratification and environmental heterogeneity in a relatively small area [3], resulting in one of the most diversified fauna and flora in the world [4,5].

QTP has become a focus area of amphibian biodiversity research and conservation, given its large number of endemic and threatened species [6]. Only a few species of four families (Megophryidae, Bufonidae, Dicroglossidae, Ranidae) among the 58 families of Anura are distributed in QTP [6,7], attracting growing scientific attention. To explore the influence of a high-elevation environment on amphibian species and how they adapt to it, numerous aspects of amphibians in QTP have been studied, including phylogeography [8,9], diversity [10], ecological niche [11], functional phenotype [12], and symbiotic

microbiome [13]. Previous studies have suggested that high-elevation environment adaptation of amphibians may benefit from their unique genetic diversity.

Systematic data on native taxa are critical to understanding environmental adaptation and biodiversity, which is crucial for biodiversity conservation in QTP. Cytogenetic data provide some of the most fundamental information about the genome [14] and have been used as clues to study phylogenies and geographical clines [15]. Karyotypic variations, including polyploidization, inversion, and translocation, have been found to drive speciation, adaptive divergence, and alternate reproductive strategies [16–19]. Research on amphibian cytotaxonomy has a history of more than 140 years [20]. To date, over 8600 amphibian species have been described [7], and about 1900 of them have been reported to possess karyotypes [14], indicating an urgent need for more cytogenetic data. Previous studies on genomes and karyotypes have suggested that amphibians exhibit high levels of diversity in chromosome numbers and genome sizes [14,21], which likely reflects their unique evolutionary history [22]. Therefore, the identification of karyotypes in amphibians from QTP is important for the diversity and conservation of amphibians in this region.

In this study, we investigated the karyotypes of 10 anuran species in the QTP and explored variations in chromosome number and structure. These data will contribute to revealing the genetic diversity of amphibians in the QTP. Furthermore, our karyotypic data will provide support for research on environmental adaptation and conservation of amphibians.

2. Materials and Methods

2.1. Specimens

We collected 25 individuals of 10 amphibian species from Yunnan, Xinjiang, and Xizang of China in 2019 and 2022 (Table 1). The taxonomic identity of each individual was confirmed by morphological and molecular data. After having taken samples for the karyotype test, specimens were fixed in 75% ethanol and deposited at the Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS), Chengdu, China.

2.2. Chromosome Preparation and Measurement

Mitotic metaphase cells were obtained from bone marrow using the procedures described by Schmid [23], and each individual produced four slides. Chromosomes were stained with 10% Giemsa for 30 min and photographed using an Optec B302 microscope equipped with a Sony (Tokyo City, Japan) ICX285A CCD camera. Chromosomal measurements were conducted using ImageView (Chongqing Optec Instrument Co., Ltd., Chongqing City, China) software and analyzed based on Levan's criterion [24]. Ten mitotic metaphase cells were randomly selected for measurement from each species. The character terminology and abbreviations used in this study are provided below:

p—short arm; q—long arm; SC—secondary constriction; cen—centromeric; inter—interstitial; per—peripheral; AR—arm ratio; CI—centromeric index (%); RL—relative length (%); LC—location of centromere; M—metacentric chromosome: $1.00 < AR \leq 1.70$, $37.50 < CI \leq 50.00$; SM, submetacentric chromosome: $1.70 < AR \leq 3.00$, $25.00 < CI \leq 37.50$; ST—subtelocentric chromosome: $3.00 < AR \leq 7.00$, $12.50 < CI \leq 25.00$; T—telocentric chromosome, $7.00 < AR$, $0 < CI \leq 12.50$.

Table 1. Information of samples used in karyotype identification.

Species	Locality	Longitude (°E)	Latitude (°N)	Gender	Voucher Number
<i>Oreolalax xiangchengensis</i>	Weixi Lisu Autonomous County, Yunnan, China	98.94837582	27.68311651	♀	CIB5334220116
<i>Oreolalax xiangchengensis</i>	Weixi Lisu Autonomous County, Yunnan, China	98.94837582	27.68311651	♀	CIB5334220118
<i>Scutiger boulengeri</i>	Dingjie County, Xizang, China	87.081458	28.592544	♀	CIBXJ2021121
<i>Scutiger boulengeri</i>	Dingqing County, Xizang, China	95.416961	31.053237	♂	CIBXJ2021124
<i>Scutiger boulengeri</i>	Dingqing County, Xizang, China	95.416961	31.053237	♂	CIBXJ2021125
<i>Scutiger boulengeri</i>	Zhongba County, Xizang, China	84.047854	29.775521	♂	CIBXJ2021133
<i>Scutiger boulengeri</i>	Zhongba County, Xizang, China	84.047854	29.775521	♀	CIBXJ2021134
<i>Bufo gargarizans</i>	Lijiang Naxi autonomous county	99.712708	27.06504	♂	CIByN201909214
<i>Duttaphrynus himalayanus</i>	Dingjie County, Xizang, China	87.081458	28.592544	♀	CIBXJ2021130
<i>Duttaphrynus himalayanus</i>	Dingjie County, Xizang, China	87.081458	28.592544	♂	CIBXJ2021131
<i>Duttaphrynus himalayanus</i>	Dingjie County, Xizang, China	87.081458	28.592544	♂	CIBXJ2021132
<i>Bufotes taxkorensi</i>	Taxkorgan Tajik Autonomous County, Xinjiang, China	75.21498056	37.83938056	♂	CIBXJ2021119
<i>Bufotes taxkorensi</i>	Taxkorgan Tajik Autonomous County, Xinjiang, China	75.21498056	37.83938056	♂	CIBXJ2021126
<i>Bufotes taxkorensi</i>	Taxkorgan Tajik Autonomous County, Xinjiang, China	75.21498056	37.83938056	♂	CIBXJ2021127
<i>Bufotes zandaensis</i>	Zanda County, Xizang, China	79.984408	31.534458	♂	CIBXJ2021120
<i>Bufotes zandaensis</i>	Zanda County, Xizang, China	79.984408	31.534458	♂	CIBXJ2021135
<i>Bufotes zandaensis</i>	Zanda County, Xizang, China	79.984408	31.534458	♂	CIBXJ2021136
<i>Maculopaa chayuensis</i>	Derung-Nu Autonomous County, Yunnan, China	98.59665692	27.76807508	♂	CIByN201909280
<i>Maculopaa chayuensis</i>	Derung-Nu Autonomous County, Yunnan, China	98.59665692	27.76807508	♀	CIByN201909282
<i>Gynandropaa yunnanensis</i>	Binchuan County, Yunnan, China	100.331225	25.91264	♂	CIB5334220131
<i>Nanorana parkeri</i>	Dingjie County, Xizang, China	87.081458	28.592544	♀	CIBXJ2021122
<i>Nanorana parkeri</i>	Dingjie County, Xizang, China	87.081458	28.592544	♂	CIBXJ2021123
<i>Nanorana parkeri</i>	Nanmulin County, Xizang, China	89.1059	29.34904722	♂	CIBXJ2021128
<i>Rana chaochiaoensis</i>	Weixi Lisu Autonomous County, Yunnan, China	99.42701547	27.58048148	♂	CIB5334220132
<i>Rana chaochiaoensis</i>	Weixi Lisu Autonomous County, Yunnan, China	99.42701547	27.58048148	♀	CIB5334220104

3. Results

3.1. Karyotype of Megophryidae

The karyotype of *Oreolalax xiangchengensis* consists of 26 chromosomes, including 6 pairs of large chromosomes and 7 pairs of small chromosomes (Figures 1A and 2A). The SC is located on the 6q^{inter}, and the AR ranges from 1.03~1.71. Among these chromosomes, all except chromosome No. 3 are metacentric, and chromosome No. 3 is submetacentric (2SM + 24M).

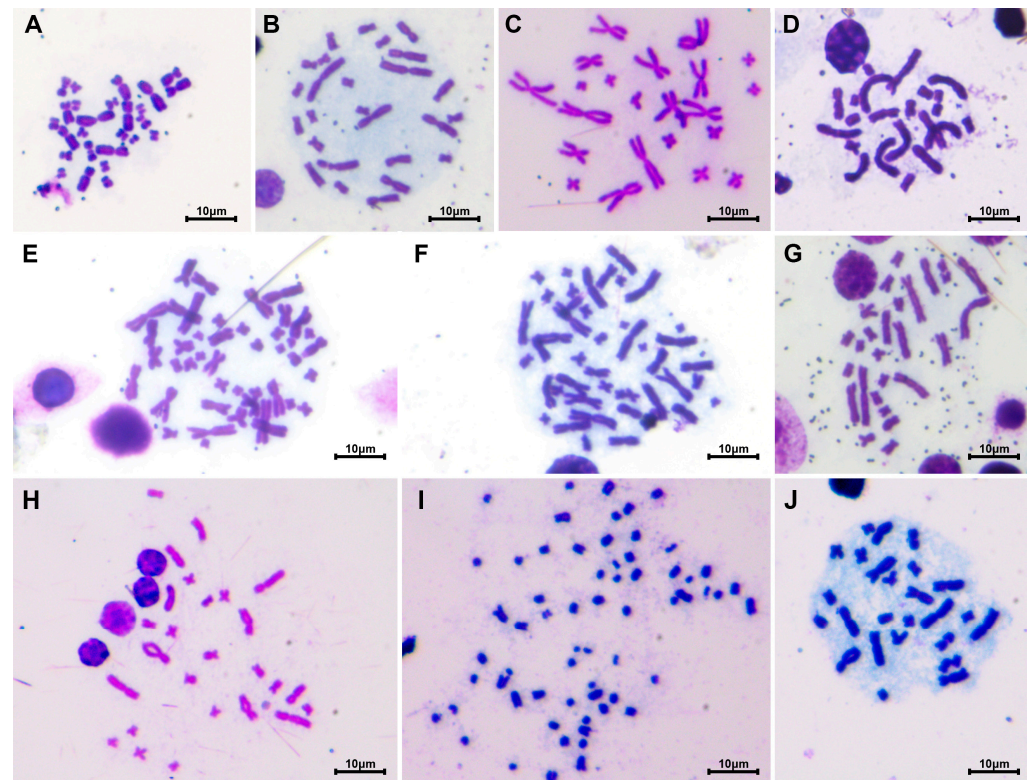


Figure 1. Karyotype of ten amphibians. (A) *Oreolalax xiangchengensis*; (B) *Scutigera boulengeri*; (C) *Bufo gargarizans*; (D) *Duttaphrynus himalayanus*; (E) *Bufotes taxkorensis*; (F) *Bufotes zamdaensis*; (G) *Nanorana parkeri*; (H) *Maculopaa chayuensis*; (I) *Gynandropaa yunnanensis*; and (J) *Rana chaochiaoensis*.

The karyotype of *Scutigera boulengeri* comprises 26 chromosomes, including 6 pairs of large chromosomes and 7 pairs of small chromosomes (Figures 1B and 2B). Among these chromosomes, No. 5, 7, and 8 are submetacentric, while the others are metacentric (6SM + 20M). The secondary constriction (SC) is located on the 2p^{inter}.

3.2. Karyotype of Bufonidae

The karyotype of *Bufo gargarizans* comprises 22 chromosomes, including 5 pairs of large chromosomes and 8 pairs of small chromosomes (Figures 1C and 2C). All chromosomes are metacentric, and the AR ranges from 1.06 to 1.63. The SC is located on 6q^{ter}.

The karyotype of *Duttaphrynus himalayanus* consists of 22 chromosomes, including 6 pairs of large chromosomes and 5 pairs of small (Figures 1D and 2D). All the chromosomes are metacentric (22M), and the AR ranges from 1.05 to 1.52. Chromosome No. 4 has the largest AR, and no obvious SC was observed.

Both *Bufotes taxkorensis* and *Bufotes zamdaensis* have a chromosome number of 44, and the SCs are located on the 11q^{ter} (Figures 1E,F and 2E,F). The karyotype of *B. zamdaensis* includes one pair ST (No. 21) and two pairs of SM (No. 9, 13); the remaining chromosomes are metacentric (2ST + 4SM + 38M). While *B. taxkorensis* has four pairs of SM (No. 7, 8, 13, 14), and the remaining chromosomes are M (8SM + 36M).

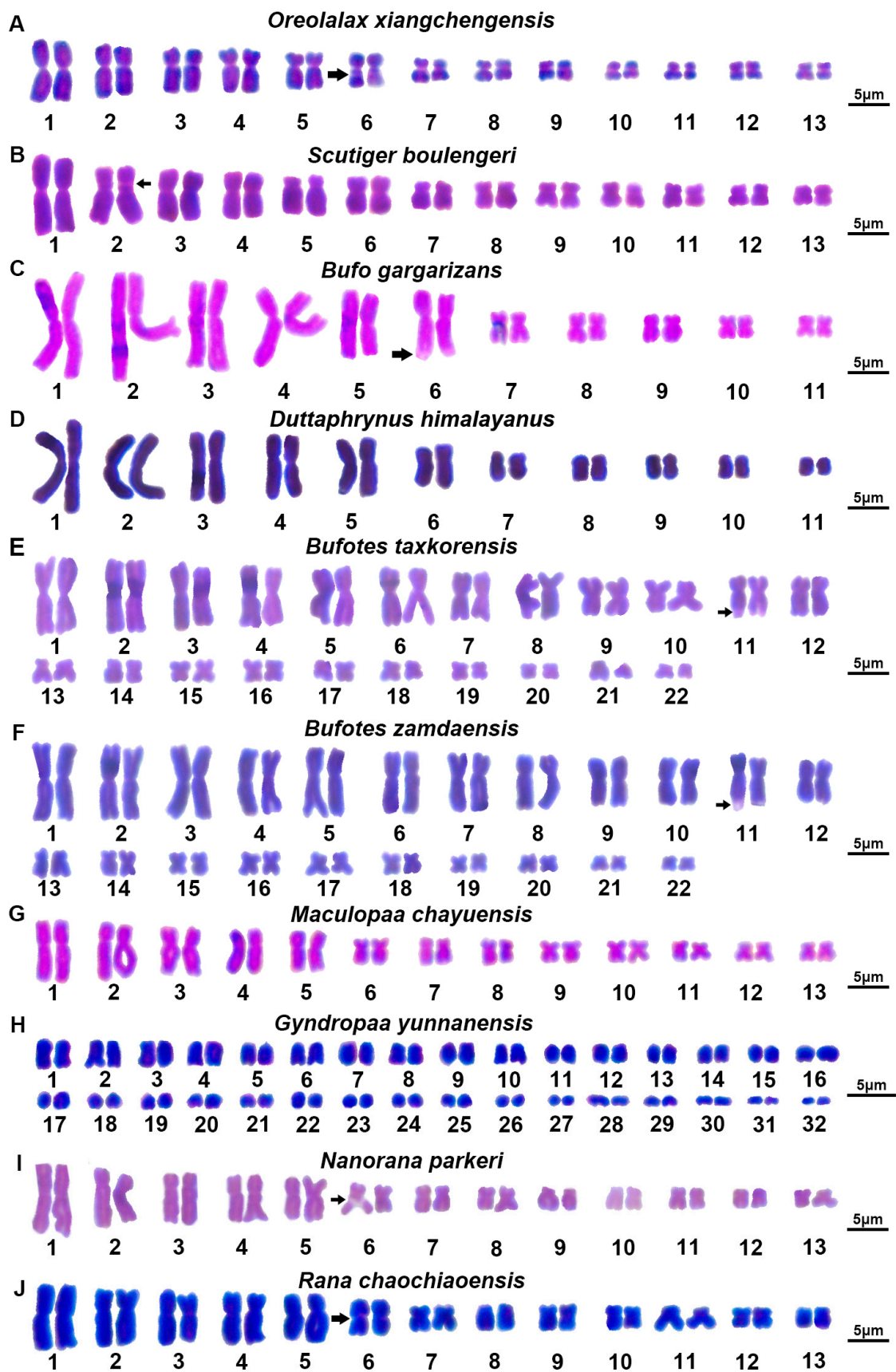


Figure 2. Paired chromosome of 10 amphibians. The pairs of chromosomes were arranged by relative length, and the secondary constrictions were marked by a black arrow.

3.3. Karyotype of *Dicroglossidae*

The karyotype of *Maculopaa chayuensis* consists of 26 chromosomes, consisting of 5 pairs of large chromosomes and 8 pairs of small (Figures 1H and 2G). The AR of chromosomes ranges from 1.23 to 1.99, and five pairs of them are SM, while the other pairs are M (10SM + 16M). No obvious SC were observed.

The karyotype of *Gynandropaa yunnanensis* comprises 64 chromosomes, all of which are telocentric chromosome (64T) (Figures 1I and 2H). The RL of 32 pairs of chromosomes ranges from 1.32~5.57. Since all chromosomes only have a long arm and no short arm, there are no AR or CI values in this karyotype (Table 2). No obvious SC was observed in this karyotype.

The karyotype of *Nanorana parkeri* consists of 26 chromosomes, including 5 pairs of large chromosomes and 8 pairs of small chromosomes (Figures 1G and 2I). The SC is located on the 6q^{ter}. The AR ranges from 1.20 to 2.56, and 4 pairs of chromosomes are SM, while other pairs are M (8SM + 18M).

3.4. Karyotype of *Ranidae*

Rana chaochiaoensis has a chromosomes number of $2n = 26$, including five large chromosomes and eight small chromosomes (Figures 1J and 2J). The SC is on the 6q^{per}. The AR of chromosomes changes from 1.16~3.33. One pair of chromosomes is T (No. 8), 4 pairs are SM (No. 2, 4, 11, 13), and the other pairs are all M (2T + 8SM + 16M).

Table 2. The measuring data of karyotype to 10 amphibians.

Chr.	Index (Mean ± SD)	<i>Oreolalax xiangchengensis</i>	<i>Scutiger boulenger</i>	<i>Bufo gargarizans</i>	<i>Duttaphrynus himalayanus</i>	<i>Bufores taxkorensi</i>	<i>Bufores zandaensis</i>	<i>Maculopaa chayuensis</i>	<i>Gynandropaa yunnanensis</i>	<i>Nanorana parkeri</i>	<i>Rana chaochiaoensis</i>
1	AR	1.15 ± 0.04	1.23 ± 0.06	1.22 ± 0.06	1.29 ± 0.07	1.18 ± 0.07	1.19 ± 0.05	1.45 ± 0.05	--	1.20 ± 0.05	1.16 ± 0.06
	CI	46.48 ± 0.84	44.79 ± 0.90	45.08 ± 0.87	43.58 ± 0.87	45.79 ± 1.02	45.57 ± 0.95	40.88 ± 0.85	--	45.47 ± 0.84	46.34 ± 1.02
	RL	15.00 ± 0.59	15.92 ± 0.62	16.06 ± 0.57	17.44 ± 0.76	8.23 ± 0.34	8.77 ± 0.33	14.09 ± 0.54	5.57 ± 0.23	15.27 ± 0.57	13.55 ± 0.53
	LC	M	M	M	M	M	M	M	T	M	M
2	AR	1.49 ± 0.07	1.36 ± 0.06	1.26 ± 0.05	1.06 ± 0.05	1.16 ± 0.05	1.26 ± 0.05	1.99 ± 0.09	--	2.32 ± 0.10	1.95 ± 0.09
	CI	40.21 ± 0.78	42.45 ± 0.84	44.23 ± 0.82	48.55 ± 0.97	46.31 ± 1.01	44.21 ± 0.88	33.44 ± 0.67	--	30.09 ± 0.59	33.95 ± 0.70
	RL	12.28 ± 0.48	11.92 ± 0.47	15.86 ± 0.68	14.47 ± 0.55	7.84 ± 0.30	8.61 ± 0.33	12.86 ± 0.50	4.99 ± 0.21	12.21 ± 0.47	11.72 ± 0.47
	LC	M	M	M	M	M	M	SM	T	SM	SM
3	AR	1.71 ± 0.06	1.38 ± 0.05	1.52 ± 0.06	1.48 ± 0.07	1.11 ± 0.06	1.20 ± 0.06	1.81 ± 0.07	--	2.35 ± 0.11	1.36 ± 0.05
	CI	36.85 ± 0.70	42.10 ± 0.83	39.75 ± 0.74	40.32 ± 0.79	47.29 ± 1.00	45.55 ± 0.92	35.59 ± 0.68	--	29.83 ± 0.55	42.37 ± 0.85
	RL	10.71 ± 0.40	10.39 ± 0.38	14.04 ± 0.57	13.59 ± 0.54	7.77 ± 0.31	7.41 ± 0.29	10.91 ± 0.44	4.98 ± 0.19	11.37 ± 0.47	10.93 ± 0.42
	LC	SM	M	M	M	M	M	SM	T	SM	M
4	AR	1.61 ± 0.08	1.29 ± 0.07	1.63 ± 0.10	1.52 ± 0.07	1.16 ± 0.07	1.07 ± 0.04	1.97 ± 0.09	--	1.50 ± 0.10	1.73 ± 0.07
	CI	38.35 ± 0.70	43.68 ± 0.83	37.98 ± 0.78	39.71 ± 0.77	46.32 ± 0.85	48.20 ± 0.94	33.65 ± 0.69	--	40.03 ± 0.81	36.63 ± 0.69
	RL	10.59 ± 0.42	9.76 ± 0.37	11.98 ± 0.48	11.92 ± 0.48	7.21 ± 0.29	7.15 ± 0.28	10.31 ± 0.40	4.40 ± 0.17	10.32 ± 0.40	10.73 ± 0.46
	LC	M	M	M	M	M	M	SM	T	M	SM
5	AR	1.59 ± 0.08	2.37 ± 0.13	1.07 ± 0.06	1.28 ± 0.07	1.49 ± 0.08	1.30 ± 0.07	1.33 ± 0.06	--	1.35 ± 0.07	1.53 ± 0.09
	CI	38.56 ± 0.78	29.70 ± 0.60	48.39 ± 0.97	43.88 ± 0.86	40.14 ± 0.90	43.41 ± 0.87	42.85 ± 0.87	--	42.56 ± 0.83	39.52 ± 0.80
	RL	8.84 ± 0.34	8.08 ± 0.34	10.18 ± 0.42	11.15 ± 0.46	7.21 ± 0.30	6.85 ± 0.27	9.39 ± 0.37	4.14 ± 0.17	9.69 ± 0.38	10.12 ± 0.39
	LC	M	SM	M	M	M	M	M	T	M	M
6	AR	1.30 ± 0.06	1.29 ± 0.07	1.54 ± 0.06	1.43 ± 0.08	1.56 ± 0.05	1.33 ± 0.07	1.91 ± 0.10	--	1.76 ± 0.09	1.21 ± 0.05
	CI	43.54 ± 0.89	43.76 ± 0.75	39.39 ± 0.82	41.23 ± 0.80	39.11 ± 0.75	42.84 ± 0.85	34.38 ± 0.69	--	36.25 ± 0.75	45.22 ± 0.98
	RL	8.60 ± 0.35	7.98 ± 0.30	10.10 ± 0.43	8.04 ± 0.31	6.76 ± 0.25	6.73 ± 0.26	6.11 ± 0.24	3.93 ± 0.16	6.47 ± 0.28	7.66 ± 0.32
	LC	M	M	M	M	M	M	SM	T	SM	M
7	AR	1.17 ± 0.05	1.76 ± 0.08	1.34 ± 0.07	1.27 ± 0.05	1.76 ± 0.08	1.10 ± 0.07	1.58 ± 0.09	--	1.57 ± 0.08	1.42 ± 0.06
	CI	46.19 ± 0.85	36.19 ± 0.72	42.66 ± 0.87	44.09 ± 0.97	36.24 ± 0.75	47.67 ± 0.90	38.75 ± 0.80	--	38.85 ± 0.81	41.40 ± 0.78
	RL	5.73 ± 0.23	5.94 ± 0.25	5.15 ± 0.20	5.42 ± 0.19	6.10 ± 0.25	5.59 ± 0.23	6.31 ± 0.27	4.30 ± 0.17	6.20 ± 0.26	5.63 ± 0.23
	LC	M	SM	M	M	SM	M	M	T	M	M
8	AR	1.33 ± 0.06	1.84 ± 0.09	1.11 ± 0.05	1.23 ± 0.05	1.89 ± 0.12	1.89 ± 0.08	1.52 ± 0.07	--	1.23 ± 0.06	3.33 ± 0.16
	CI	42.88 ± 0.81	35.22 ± 0.69	47.41 ± 1.01	44.84 ± 0.83	34.63 ± 0.71	34.66 ± 0.64	39.63 ± 0.72	--	44.81 ± 0.97	23.09 ± 0.46
	RL	5.14 ± 0.19	5.76 ± 0.25	4.62 ± 0.18	5.07 ± 0.19	5.97 ± 0.25	5.49 ± 0.22	5.83 ± 0.23	3.92 ± 0.15	5.24 ± 0.22	5.59 ± 0.25
	LC	M	SM	M	M	SM	SM	M	T	M	ST
9	AR	1.40 ± 0.06	1.39 ± 0.06	1.50 ± 0.08	1.16 ± 0.05	1.50 ± 0.07	1.48 ± 0.04	1.42 ± 0.06	--	2.56 ± 0.16	1.55 ± 0.06
	CI	41.61 ± 0.80	41.75 ± 0.91	40.01 ± 0.80	46.40 ± 0.93	40.00 ± 0.78	40.40 ± 0.89	41.35 ± 0.79	--	28.07 ± 0.55	39.15 ± 0.75
	RL	4.94 ± 0.21	5.48 ± 0.22	4.52 ± 0.18	4.79 ± 0.19	5.55 ± 0.23	5.33 ± 0.21	5.08 ± 0.22	4.19 ± 0.18	5.16 ± 0.20	5.46 ± 0.22
	LC	M	M	M	M	M	M	M	T	SM	M
10	AR	1.03 ± 0.04	1.30 ± 0.08	1.13 ± 0.06	1.05 ± 0.06	1.14 ± 0.05	1.67 ± 0.08	1.27 ± 0.07	--	1.38 ± 0.07	1.38 ± 0.07
	CI	49.23 ± 1.05	43.52 ± 0.87	46.99 ± 0.95	48.78 ± 0.97	46.66 ± 0.88	37.48 ± 0.81	43.99 ± 0.92	--	42.05 ± 0.84	42.09 ± 0.79
	RL	4.79 ± 0.16	5.37 ± 0.24	3.86 ± 0.16	4.60 ± 0.21	5.20 ± 0.21	5.27 ± 0.20	5.08 ± 0.19	3.61 ± 0.14	4.93 ± 0.20	4.93 ± 0.21
	LC	M	M	M	M	M	M	M	T	M	M
11	AR	1.04 ± 0.04	1.31 ± 0.08	1.06 ± 0.06	1.07 ± 0.06	1.06 ± 0.04	1.28 ± 0.04	1.50 ± 0.08	--	1.45 ± 0.05	2.23 ± 0.11
	CI	49.13 ± 0.96	43.24 ± 0.87	48.64 ± 0.89	48.35 ± 1.01	48.43 ± 1.00	43.78 ± 0.88	40.05 ± 0.76	--	40.82 ± 0.81	31.00 ± 0.66
	RL	4.64 ± 0.18	4.68 ± 0.19	3.62 ± 0.13	3.50 ± 0.12	5.06 ± 0.20	4.43 ± 0.17	4.74 ± 0.19	3.45 ± 0.14	4.89 ± 0.21	4.87 ± 0.20
	LC	M	M	M	M	M	M	M	T	M	SM
12	AR	1.18 ± 0.07	1.15 ± 0.05			1.27 ± 0.06	1.42 ± 0.08	1.23 ± 0.05	--	1.33 ± 0.06	1.30 ± 0.06
	CI	45.86 ± 0.86	46.44 ± 0.99			44.14 ± 1.01	41.32 ± 0.79	44.92 ± 0.89	--	42.85 ± 0.82	43.40 ± 0.90
	RL	4.49 ± 0.17	4.49 ± 0.17			4.61 ± 0.18	4.11 ± 0.18	4.69 ± 0.18	3.55 ± 0.15	4.33 ± 0.17	4.35 ± 0.18
	LC	M	M			M	M	M	T	M	M

Table 2. Cont.

Chr.	Index (Mean ± SD)	<i>Oreolalax xiangchengensis</i>	<i>Scutiger boulenger</i>	<i>Bufo gargarizans</i>	<i>Duttaphrynus himalayanus</i>	<i>Bufores taxkorensi</i>	<i>Bufores zamdaensis</i>	<i>Maculopaa chayueni</i>	<i>Gynandropaa yunnanensis</i>	<i>Nanorana parkeri</i>	<i>Rana chaochiaoensis</i>
13	AR	1.23 ± 0.05	1.09 ± 0.06			1.74 ± 0.07	1.90 ± 0.11	1.96 ± 0.12	--	1.37 ± 0.07	1.96 ± 0.10
	CI	44.84 ± 1.03	47.75 ± 0.99			36.54 ± 0.75	34.48 ± 0.70	33.80 ± 0.63	--	42.15 ± 0.83	33.78 ± 0.73
	RL	4.26 ± 0.17	4.23 ± 0.18			2.89 ± 0.13	3.27 ± 0.13	4.60 ± 0.19	3.66 ± 0.14	3.92 ± 0.17	4.47 ± 0.17
	LC	M	M			SM	SM	SM	T	M	SM
14	AR					1.80 ± 0.07	1.37 ± 0.08		--		
	CI					35.73 ± 0.71	42.26 ± 0.79		--		
	RL					2.92 ± 0.11	2.89 ± 0.11		3.02 ± 0.13		
	LC					SM	M		T		
15	AR					1.14 ± 0.05	1.30 ± 0.06		--		
	CI					46.72 ± 0.87	43.45 ± 0.89		--		
	RL					2.52 ± 0.11	2.73 ± 0.11		3.02 ± 0.12		
	LC					M	M		T		
16	AR					1.20 ± 0.06	1.03 ± 0.04		--		
	CI					45.54 ± 0.91	49.22 ± 10.00		--		
	RL					2.46 ± 0.11	2.63 ± 0.10		2.70 ± 0.10		
	LC					M	M		T		
17	AR					1.17 ± 0.06	1.18 ± 0.07		--		
	CI					46.04 ± 0.93	45.79 ± 1.03		--		
	RL					2.36 ± 0.10	2.45 ± 0.09		3.08 ± 0.13		
	LC					M	M		T		
18	AR					1.61 ± 0.09	1.22 ± 0.06		--		
	CI					38.30 ± 0.79	45.02 ± 0.93		--		
	RL					2.22 ± 0.09	2.39 ± 0.10		2.70 ± 0.10		
	LC					M	M		T		
19	AR					1.10 ± 0.06	1.27 ± 0.07		--		
	CI					47.67 ± 1.02	44.08 ± 0.82		--		
	RL					2.00 ± 0.08	2.18 ± 0.08		2.76 ± 0.11		
	LC					M	M		T		
20	AR					1.04 ± 0.05	1.16 ± 0.06		--		
	CI					49.09 ± 0.99	46.30 ± 0.90		--		
	RL					1.86 ± 0.08	2.00 ± 0.08		2.60 ± 0.10		
	LC					M	M		T		
21	AR					1.43 ± 0.06	3.76 ± 0.18		--		
	CI					41.13 ± 0.83	20.99 ± 0.42		--		
	RL					1.67 ± 0.07	1.94 ± 0.08		2.44 ± 0.10		
	LC					M	ST		T		
22	AR					1.23 ± 0.05	1.51 ± 0.06		--		
	CI					44.92 ± 0.96	39.86 ± 0.78		--		
	RL					1.60 ± 0.06	1.79 ± 0.07		2.81 ± 0.10		
	LC					M	M		T		
23	AR								--		
	CI								--		
	RL								2.60 ± 0.11		
	LC								T		
24	AR								--		
	CI								--		
	RL								2.60 ± 0.10		
	LC								T		

Table 2. Cont.

Chr.	Index (Mean \pm SD)	<i>Oreolalax xiangchengensis</i>	<i>Scutigera boulengeri</i>	<i>Bufo gargarizans</i>	<i>Duttaphrynus himalayanus</i>	<i>Bufo taxkorensis</i>	<i>Bufo zandaensis</i>	<i>Maculopaa chayueni</i>	<i>Gynandropaa yunnanensis</i>	<i>Nanorana parkeri</i>	<i>Rana chaochiaoensis</i>
25	AR								--		
	CI								--		
	RL								2.54 \pm 0.09		
	LC								T		
26	AR								--		
	CI								--		
	RL								2.23 \pm 0.09		
	LC								T		
27	AR								--		
	CI								--		
	RL								1.91 \pm 0.08		
	LC								T		
28	AR								--		
	CI								--		
	RL								1.91 \pm 0.07		
	LC								T		
29	AR								--		
	CI								--		
	RL								1.91 \pm 0.08		
	LC								T		
30	AR								--		
	CI								--		
	RL								1.75 \pm 0.07		
	LC								T		
31	AR								--		
	CI								--		
	RL								1.43 \pm 0.06		
	LC								T		
32	AR								--		
	CI								--		
	RL								1.32 \pm 0.05		
	LC								T		

4. Discussion

4.1. Karyotype Comparison

4.1.1. Karyotype of Megophryidae

The karyotype of *O. xiangchengensis* population from Zhongdian County (ZD), Yunnan Province, has been reported by Li [25]. They have 26 chromosomes, including 3 pairs of SM (No. 3, 4, 5) and 10 pairs of M, with one pair of SC on the 6q^{per}. On the other hand, the karyotype of the population from Weixi County (WX), Yunnan Province, only has one pair of SM (No. 3), and the SC is on the 6q^{inter}. This suggests that there may be a chromosomal inversion or changes in the NORs (nucleolar organizing regions) locations occurring between different geographic populations of the *O. xiangchengensis*.

In a previous study, the karyotype of *S. boulengeri* from Kangding City, Sichuan Province, was reported as $2n = 26$, with No. 7 chromosomes being SM, No. 5 being ST, and others being M, with the SC located on the 2p^{inter} [26]. In this study, the chromosomal number and SC location of the population from Zanda County, Xizang Province, were found to be consistent with the Kangding population. However, the karyotype of the Zanda population, which includes 3 pairs of SM, 10 pairs of M and no ST, suggests that there are variations in AR and LC between these two populations of *S. boulengeri*.

4.1.2. Karyotype of Bufonidae

The karyotype of *D. himalayanus* population from Derung-Nu Autonomous County, Yunnan Province, has been identified in [22], while the specimens from this area have later been identified as *D. cyphosus* [6]. Therefore, in this study, the karyotype of *D. himalayanus* from Dingjie County, Xizang Province, is the first report of this species, which consisted of 22 metacentric chromosomes (22M).

B. gargarizans is widely distributed in China, and its karyotypes in several areas (Heilongjiang, Beijing, Shanghai, Sichuan, Fujian) have been reported [22]. In most previous studies, the karyotype of *B. gargarizans* had a chromosome number of 22, and the SC was located on the 6q^{ter}, which was consistent with our result.

The *B. viridis* complex comprises diploid ($2n = 22$), triploid ($3n = 33$) and tetraploid ($4n = 44$) bisexually reproducing taxa [27,28]. Among them, *B. pewzowi* is an allotetraploid with karyotype $4n = 44$ [28,29]. The same chromosome number as *B. taxkorensis* and *B. zamdaensis* indicates that they are both allopolyploids. The *B. pewzowi* has four pairs of SM (No. 7, 8, 13, 14) that is identical to *B. taxkorensis*, while different from *B. zamdaensis* (2ST + 4SM + 38M). The SCs in *B. taxkorensis* and *B. zamdaensis* are located on 11q^{ter}, while in *B. pewzow*, they are located on 12q^{ter} [29]. As they are all allopolyploids, it indicates that the SCs are located on only one pair of chromosomes in the No.6 tetrad, and therefore the positions of the SCs between these three tetraploid species are the same.

4.1.3. Karyotype of Dicroglossidae

The karyotype of *M. chayuensis* population in Lushui County, Yunnan Province, was previously reported to be $2n = 26$, and it has 5 pairs of SM (No. 2, 3, 4, 6, 8) [30]. In this study, *M. chayuensis* from Derung-Nu Autonomous County, Yunnan, China, also has 26 chromosomes, including 5 pairs of SM (No. 2, 3, 4, 6, 13). However, among them, 4 pairs (No. 2, 3, 4, 6) are the same as the Lushui population, and 2 pairs (No. 8, 13) have different centromere positions.

All species in the genus *Gynandropaa* have 64 telocentric chromosomes (64T), and the SC location varies between different species or geographic populations [22]. In this study, no obvious SCs have been observed. Previous studies have reported that *G. yunnanensis* from Jingdong County, Jinping County, Tengchong City in Yunnan Province have SCs on 4q^{per}, 2q^{inter} and 15q^{inter}, respectively [22,30,31]. Two different SC locations have been reported for *G. phrynooides* (Yimen County: 18q^{inter}, Qujing City: 20q^{inter}) [22,32]. *G. sichuanensis* from Zhaojue County, Sichuan Province, has an SC on 32q^{ter} [33]. Due to the similar chromosome morphology in *G. sichuanensis*, the differences in SC positions could be attributed to SC location polymorphism, or potential differences in measurement and

ordering processes. Therefore, more cytogenetic techniques are needed to be conducted in different *G. sichuanensis* populations.

The karyotype of *N. parkeri* from Dingjie and Nanmulin Counties, Xizang Province, was found to be $2n = 26$, with SC on the $6q^{ter}$, which is consistent with the karyotype of the population from Lasa City, Xizang Province [26]. However, the locations of centromeres are different on some chromosomes. In this study, the karyotype of *N. parkeri* has 4 pairs of SM (No. 2, 3, 6, 9), while a previous study reported 6 pairs of SM (No. 2, 3, 4, 6, 8, 9) Sin Lasa population. It is difficult to determine whether these chromosomal differences arise from karyotypic variations or are simply caused by a problem in establishing chromosomal homologies. Therefore, further research is needed using more samples and additional techniques.

4.1.4. Karyotype of Ranidae

The karyotype of the *R. chaochiaoensis* populations from Kunming City, Zhongdian County, Yunnan Province, and Yanyuan City, Sichuan Province, have been reported previously [22,34,35]. All the results indicated that *R. chaochiaoensis* has a chromosome number of 26, and the SC is located at $6q^{per}$, which coincides with the findings of this study. Among different geographic populations, the No. 8 chromosomes are consistently ST, while the number of SM varies. The Yanyuan population has 5 pairs of SM (No. 2, 3, 7, 9, 13); the Kunming population has 3 pairs (No. 3, 9, 13); the Zhongdian population has 4 pairs; and the Weixi population has 4 pairs (No. 2, 4, 11, 13). These results suggest that the main characteristics of karyotypes among different populations of *R. chaochiaoensis* were constant, while the AR and SC positions show rich variations.

4.2. Karyotypic Differences and Phylogenetic Differentiation

The chromosome number of Megophryidae species ranges from 22 to 30 [14]. Among them, all species belonging to the genus *Oreolalax* and *Scutigera* have the same chromosome number of 26 [14,22], but they can be easily distinguished by the disparate locations of SCs. The SCs of species in the genus *Oreolalax* are located entirely on the No. 6 chromosomes ($6q^{per}$, $6q^{ter}$ or $6q^{inter}$), while those in the genus *Scutigera* are located entirely on the No. 2 chromosome ($2p^{per}$) [22]. The difference in the location of SCs between *Oreolalax* and *Scutigera* species may cause by chromosomal structural variations, and it serves as an important characteristic for distinguishing the karyotypes of these two genera. These findings imply that the chromosomal rearrangement may be causally related to the divergence of *Oreolalax* and *Scutigera*.

In 1990, Yang described the *O. xiangchengensis* from Deqin County as a subspecies of *O. xiangchengensis deqinicus* [36] based on the differences in webbing morphology compared to *O. xiangchengensis xiangchengensis* [36,37]. Zhongdian County is close to Xiangcheng County, while Weixi County is close to Deqin County. There are significant differences in karyotype between these two populations (Table 3), specifically in the location of the SC, which is located in the $6q^{per}$ (Zhongdian County) and $6q^{inter}$ (Weixi County), respectively. This suggests the presence of an inversion or changes in the NOR location on the long arm of the No. 6 chromosome among different subspecies of *O. xiangchengensis*. Furthermore, despite the species' high diversity and significant morphological differences in the genus *Oreolalax*, genetic distances based on mitochondrial DNA are relatively closer [38]. Therefore, the chromosomal variation may be one of the facilitating factors in the speciation within the genus *Oreolalax*.

In 1998, Fei et al. described *B. zamdaensis* as a valid species based on its morphological differences from *B. taxkorensis* and *B. pewzowi* [39]. Karyotypic data indicated that *B. zamdaensis* is not only diverse from *B. taxkorensis* and *B. pewzowi* in morphology but also in karyotype (Table 3). However, the karyotype of *B. zamdaensis* from Spiti River, India, has previously been reported as triploid ($3n = 33$) [33]. The different ploidy levels in the *B. viridis* complex are the result of multiple whole genome duplication (WGD) events, for which a relatively well-supported hypothesis for their formation has been proposed [28]. Prior to

this report, tetraploid population of *B. zamdaensis* has not been identified in the karyotype. The individuals in this study were collected from the type locality of this species, and there is geographical isolation between these tetraploid populations and the previously reported triploid populations [40]. The discovery of tetraploid population in *B. zamdaensis* provides new evidence supporting the occurrence of multiple WGD events in the *B. viridis* complex.

Table 3. Comparison of the karyotypes of 10 Anuran species.

Species	Chr. Number	Karyotype Composition	SM	ST	T	SC	Location	Ref.
<i>Oreolalax xiangchengensis</i>	26	24M + 2SM	3			6q ^{inter}	Weixi Lisu Autonomous County, Yunnan, China	--
	26	20M + 6SM	3, 4, 5			6q ^{per}	Zhongdian County, Yunnan Province, China	[25]
<i>Scutigera boulengeri</i>	26	20M + 6SM	5, 7, 8			2p ^{inter}	Dingjie County, Xizang, China	--
	26	20M + 6SM	5, 7, 8			2p ^{inter}	Dingqing, County, Xizang, China	--
	26	20M + 6SM	5, 7, 8			2p ^{inter}	Zhongba County, Xizang, China	--
	26	22M + 2SM + 2ST	7	5		2p ^{inter}	Kangding City, Sichuan Province, China	[26]
<i>Bufo gargarizans</i>	22	22M				6q ^{inter}	Lijiang Naxi autonomous county, China	--
	22	18M + 4SM	4, 9			6q ^{inter}	Heilongjiang Province, China	[22]
	22	18M + 4SM	4, 9			6q ^{inter}	Beijing City, China	[22]
	22	18M + 4SM	4, 9			6q ^{inter}	Shanghai City, China	[22]
	22	18M + 4SM	4, 9			6q ^{inter}	Sichuan Province, China	[22]
	22	18M + 4SM	4, 9			6q ^{inter}	Fujian Province, China	[22]
<i>Duttaphrynus himalayanus</i>	22	22M				/	Dingjie County, Xizang, China	--
<i>Bufo taxkorensis</i>	44	38M + 2ST + 4SM	9, 13	21		11q ^{ter}	Taxkorgan Tajik Autonomous County, Xinjiang, China	--
<i>Bufo zamdaensis</i>	44	36M + 8SM	7, 8, 13, 14			11q ^{ter}	Zanda County, Xizang, China	--
	33	/	/	/	/	/	Spiti River, India	[40]
<i>Bufo pewzowi</i>	44	36M + 8SM	7, 8, 13, 14			12q ^{ter}	Hotan Prefecture, Xizang Province, China	[29]
<i>Maculopaa chayuensis</i>	26	16M + 10SM	2–4, 6, 13			6p ^{per}	Derung-Nu Autonomous County, Yunnan Province, China	--
	26	16M + 10SM	2–4, 6, 8			6p ^{per}	Lushui County, Yunnan Province, China	[30]
<i>Gynandropaa yunnanensis</i>	64	64T			1–32	/	Binchuan County, Yunnan, China	--
	64	64T			1–32	4q ^{per}	Jingdong County, Yunnan Province, China	[31]
	64	64T			1–32	2q ^{inter}	Jinping County, Yunnan Province, China	[22]
	64	64T			1–32	15q ^{inter}	Tengchong City, Yunnan Province, China	[30]
<i>Gynandropaa phrynoides</i>	64	64T			1–32	18q ^{inter}	Yimen County, Yunnan Province, China	[22]
	64	64T			1–32	20q ^{inter}	Qujing County, Yunnan Province, China	[32]
<i>Gynandropaa sichuanensis</i>	64	64T				32q ^{ter}	Zhaojue County, Sichuan Province, China	[33]
<i>Nanorana parkeri</i>	26	18M + 8SM	2, 3, 6, 9			6q ^{ter}	Dingjie and Nanmulin Counties, Xizang Province, China	--
	26	16M + 10SM	2–4, 6, 8, 9			6q ^{ter}	Lasa City, Xizang Province, China	[26]
<i>Rana chaochiaoensis</i>	26	16M + 8SM + 2ST	2, 4, 11, 13	8		6q ^{per}	Weixi Lisu Autonomous County, Yunnan, China	--
	26	18M + 6SM + 2ST	3, 9, 13	8		6q ^{per}	Kunming City, Yunnan Province, China	[34]
	26	16M + 8SM + 2ST	2, 3, 9, 13	8		6q ^{per}	Zhongdian County, Yunnan Province, China	[22]
	26	14M + 10SM + 2ST	2, 3, 7, 9, 13	8		6q ^{per}	Yanyuan City, Sichuan Province, China	[35]

5. Conclusions

In conclusion, we identified the karyotypes of 10 amphibian species from the QTP. The karyotypes of these species were obtained from new sites that were not previously reported. Among them, the karyotypes of *D. himalayanus* and tetraploid *B. zamdaensis* were reported for the first time. The different ploidies of *B. zamdaensis* populations from Zanda, China ($4n = 44$) and the Spiti River, India ($3n = 33$) imply species differentiation and support the occurrence of multiple and complicated polyploidization events in the Bufo toads. Furthermore, there are differences in the secondary constriction locations between the two subspecies of *O. xiangchengensis* (*O. x. xiangchengensis* and *O. x. deqinicus*). This study will provide further support for research on amphibian genetic diversity and biodiversity conservation.

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