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Chromosome study of *Rasbora trilineata* and *Rasbora borapetensis* (Cyprinidae, Cypriniformes): Reveal by conventional staining technique

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Abstract - The present study aims to construct the karyotype and idiogram of two *Rasbora*, namely *Rasbora trilineata* and *R. borapetensis*. Specimens were collected from Salween, Song Kharm, Mae Khlong basin and Sirindhorn Peat Swamp Forest, Thailand. Fish chromosome preparation was prepared directly from renal cells. Conventional staining technique was applied to stain the chromosomes with Giemsa's solution. The results showed that two species the number of diploid chromosomes is 2*n*=50. The fundamental number (NF) of *R. trilineata* is 100, while *R. borapetensis* is 98. The types of chromosomes of *R. trilineata* were 12 metacentric, 24 submetacentric and 14 acrocentric chromosomes, and *R. borapetensis* eight metacentric 18 submetacentric, 20 acrocentric and four telocentric chromosomes. The karyotype formula are as follows: *R. trilineata* 2*n* (50) = 12m+24sm+14a, *R. borapetensis* 2*n* (50) = 8m+18sm+20a+4t.

Keywords: Karyotype, *Rasbora trilineata*, *Rasbora borapetensis*

1. Introduction

Genus *Rasbora*, Bleeker, 1859 is a small to moderate-sized genus in the family Cyprinidae that lives throughout a vast geographical range within Asia, including the Indian subcontinent, southern China, and Southeast Asia, with currently 77 species, *Rasbora* constitutes the most species-rich genus in the cyprinid subfamily Danioninae (Eschmeyer, 2013; Froese & Pauly, 2013). Taxonomically, *Rasbora* is known as catch all group due to considered insufficient unique diagnostic characteristics per species and morphological difficulty in characterization (Muchlisin *et al.*, 2012). *Rasbora trilineata*, commonly known as the scissortail *Rasbora* is native to Cambodia, Indonesia, Lao People's Democratic Republic, Malaysia, Thailand, and Viet Nam. This species is popular in the aquarium trade, because distinctive feature of the tail is forked, and bears bands of yellow and black, ending with a band of white at the tip.

R. trilineata has nine dorsal soft rays and eight anal soft rays. A short blackish line is present on each side along the base of the anal fin. The line on both sides uniting behind anal fin and extending to the base of the caudal fin along the lower edge of the caudal peduncle (Taki, 1974). The narrow dark mid-lateral stripe on side, black stripe along middle of back (Kottelat, 2001). This species has 13 predorsal scale rows and no red in caudal fin (Rainboth, 1996). Species widespread in lakes, swamps, slow flowing areas of rivers, usually in open areas (Kottelat, 1998; Vidthayanon, 2002). It seems to prefer habitats in forest (Kottelat & Widjanarti, 2005). A common resident of surface waters in streams, canals, ditches and occasionally of reservoirs in lowland areas (Rainboth, 1996). Inhabits medium to large rivers, flooded fields and brooks of the middle Mekong (Taki, 1978).

Rasbora borapetensis is a beautiful streamlined fish and popular aquarium fish throughout the world. In Thailand, this is a popular aquarium fish, exported in large numbers, the main source. Wild caught fish are increasingly rare in the trade as they are being produced commercially in several countries. This species has 9 dorsal soft rays and 8 anal soft rays. It has an incomplete lateral line, reaching at most to the anal fin origin, a dark brown mid-lateral stripe from gill opening to somewhat in front of caudal fin base, not widening posteriorly and above it a second, pale stripe (Kottelat, 1998; Kottelat, 2001). *R. borapetensis* has no black pigment on its fins (Rainboth, 1996). The distributions in Thailand are found in Mekong, Chao Phraya and Meklong basins (Kottelat, 1998). Collected in the Mekong basin at Nam Man about 2 km upstream of Amphoe Dan Sai in Loei Province (Kottelat, 1990) also from Khon Kaen, Chachoengsao, Sakon Nakhon, Nakhon Sawan, Nakhon Ratchasima, Narathiwat, Phrae, Chanthaburi, Phitsanulok, Prachin Buri and Surat Thani (Monkolprasit *et al.*, 1997).

Nowadays, several methods namely, conventional staining, C-banding, Ag-NOR banding and FISH (fluorescence *in situ* hybridization) have been used by ichthyologists for the gathering of cytogenetic information of fish (Sola *et al.*, 2000; Kavaco *et al.*, 2005), yet each of these methods provides a different aspect of the karyotype characteristics. They prove to be useful chromosome pattern in fish studies, particularly as cytotaxonomically markers in systematic and evolutionary studies of closely related fish taxa (Ren *et al.*, 1996;

Gornung *et al.*, 2001; Galetti *et al.*, 2006; Wang *et al.*, 2010). Up to the present, cytogenetic data in genus *Rasbora* have been performed in fifteen species (Table 1).

Table 1. Cytogenetic publications of the genus *Rasbora*.

Species	2*n*	NF1	NF2	Karyotype formula	References
Rasbora agilis	*50*	*100*	*100*	*24m+26sm*	*Donsakul et al. (2009)*
R. aurotaenia	*50*	*92*	*90*	*14m+26sm+2a+8t*	*Seetapan and Moeikum (2004)*
	50	*98*	*74*	*8m+16sm+24a+2t*	*Thongnetr et al. (2021)*
R. borapetensis	*50*	*88*	*88*	*24m+14sm+12t*	*Donsakul et al. (2005)*
R. buchanani	*50*	*100*	*96*	*30m+18sm+2a*	*Manna and Khuda-Bukhsh (1977)*
R. caudimaculata	*50*	*98*	*96*	*20m+26sm+2a+2t*	*Donsakul and Magtoon (2002)*
R. daniconius	*50*	*80*	*74*	*18m+6sm+6a+20t*	*Khuda-Bukhsh et al. (1979)*
	50	*92*	*90*	*32m+8sm+2a+8t*	*Donsakul et al. (2005)*
R. dorsicellata	*50*	*92*	*92*	*18m+24sm+8t*	*Donsakul et al. (2009)*
R. einthovenii	*50*	*94*	*86*	*6m+30sm+8a+6t*	*Donsakul et al. (2005)*
	50	*100*	*84*	*16m+18sm+16a*	*Yeesaem et al. (2019)*
R. heteromorpha	*48*	-	-	-	*Post (1965)*
	48	*74*	*72*	*14m+10sm+2a+22t*	*Donsakul et al. (2005)*
R. myersi	*50*	*90*	*84*	*20m+14sm+6a+10t*	*Donsakul and Magtoon (2002)*
R. paviei	*50*	*100*	*84*	*10m+24sm+16a*	*Donsakul and Magtoon (2002)*
R. paviana	*50*	*98*	*74*	*8m+16sm+24a+2t*	*Thongnetr et al. (2021)*
R. rebrodorsalis	*50*	*88*	*86*	*26m+10sm+2a+12t*	*Donsakul and Magtoon (2002)*
	50	*82*	*82*	*16m+16sm+18t*	*Donsakul et al. (2009)*
R. sumatrana	*50*	*94*	*92*	*26m+16sm+2a+6t*	*Donsakul and Magtoon (1995)*
R. trilineata	*48*	-	-	-	*Post (1965)*
	50	*94*	*92*	*26m+16sm+2a+6t*	*Donsakul et al. (2005)*
	50	*100*	*86*	*12m+24sm+14a*	*This study*
R. borapetensis	*50*	*98*	*90*	*8m+18sm+20a+4t*	*This study*

Remarks: 2*n*=diploid chromosome, NF1=fundamental number m, sm, a =2, t=1, NF2=fundamental number m, sm=2, a, t=1, m=metacentric, sm=submetacentric, st=subtelocentric, a = acrocentric and t=telocentric chromosome.

Most of these species have 2*n*=50 chromosome consisting of mono-and bi-arm only chromosomes and there was no observation of strange size chromosomes related to sex.

This cytogenetic study, a report on karyotype analysis of two *Rasbora*, including *R. trilineata* and *R. borapetensis*. This study is the first chromosome record of *R. borapetensis*. In the future, basic knowledge and cytogenetics of two species could be applied to several studies, especially for their cytotaxonomy.

2. Materials and methods

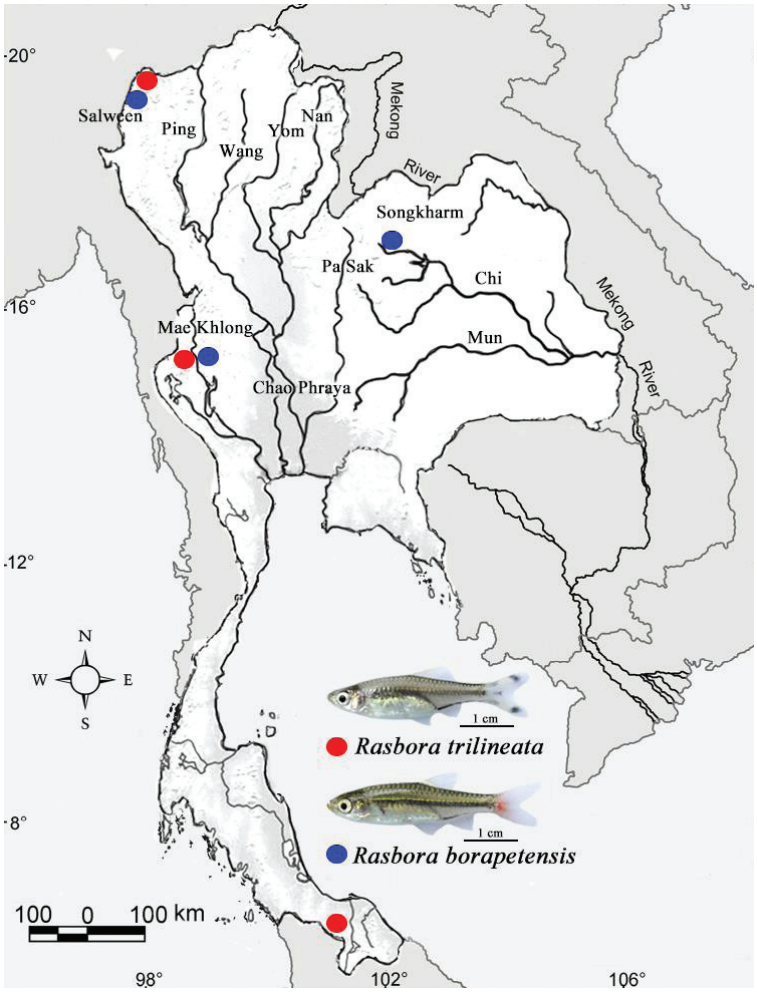

Figure 1. Collection sites of sampling in Thailand examined in the present study (Map of the basin Thailand)

Rasbora trilineata and *R. borapetensis* specimens used in this study were collected from Salween, Song Kharm, Mae Khlong basin and Sirindhorn Peat Swamp Forest, Thailand (Figure 1). The fishes were alive when transported to the laboratory and were kept for 72 hours prior to processing. The preparation of chromosomes was accomplished after Phimphan *et al.* (2021), with slight adaptations as follows. The 0.05% colchicine (1 mL to 100 g body weight) was injected into the fish's abdominal cavity and left for 1 hour. Chromosomes were prepared from kidney cells by air-drying technique. Kidney tissues were cut into small pieces, then mixed with hypotonic solution (0.075 M KCl) and incubated for 30 min. Cells were fixed in fresh cool fixative (3 absolute methanol: 1 glacial acetic acid) gradually added up to 7 ml before centrifuging again at 1,500 rpm for 8 min, whereupon the supernatant was discarded. Fixation was repeated until the supernatant was clear and the pellet was

mixed with 1 ml fixative. The mixture was dropped onto a clean and cold slide by a micropipette, and then the air-dry technique was applied. Conventional staining was done using 10% Giemsa's solution for 10 min (Phimphan *et al.*, 2021). Chromosomes photographs of 100 metaphase spreads were taken from each species by conventional Giemsa's staining and the best 20 were used for karyological examination. The length of the short arm chromosome (Ls) and the long arm chromosome (Ll) were measured and the length of the total arm chromosome (LT, LT=Ls+Ll) was calculated. The relative length (RL), the centromeric index (CI), and standard deviation (SD) of RL and CI were estimated (Turpin & Lejeune, 1965). The CI (q/p+q) between 0.50-0.59, 0.60-0.69, 0.70-0.89, and 0.90-0.99 were described as metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively. All data were used in karyotyping and idiograming.

3. Results and discussion

The *R. trilineata* and *R. borapetensis* species herein showed the same diploid number (2*n*) of 50 chromosomes. The fundamental number (NF) of *R. trilineata* was 100, while that of *R. borapetensis* was 98. The diploid number of *R. trilineata* were in concordance with previous cytogenetics data (Donsakul *et al.*, 2005), and similar to another species in *Rasbora* (Donsakul *et al.*, 2009; Seetapan & Moeikum, 2004; Aiumsumang *et al.*, 2021; Donsakul *et al.*, 2005; Manna and Khuda-Bukhsh, 1977; Donsakul and Magtoon, 2002; Khuda-Bukhsh *et al.*, 1979; Yeesaem *et al.*, 2019; Post, 1965; Donsakul and Magtoon, 1995). These species have the chromosome diploid number of 2*n*=50, which is the apparent modal diploid number of the *Rasbora*. Accordingly, it can be concluded that chromosome number in this genus is conserved. However, it differs from some species of previous report in the *R. heteromorpha* (Post, 1965; Donsakul *et al.*, 2005) and *R. trilineata* (Post, 1965) which was 2*n*=48.

Table 2. Karyomorphological details of *R. trilineata* from 20 metaphases chromosome, 2*n* (diploid)=50.

Chromosome pairs	Ls (µm)	Ll (µm)	LT (µm)	CI±SD	RL±SD	Chromosome size	Chromosome type
1	1.196	2.496	3.692	0.576±0.025	0.046±0.000	Large	metacentric
2	1.728	1.851	3.579	0.532±0.037	0.043±0.001	Large	metacentric
3	1.582	1.879	3.461	0.541±0.030	0.043±0.000	Large	metacentric
4	1.295	2.855	3.250	0.595±0.003	0.051±0.002	Large	metacentric
5	1.414	1.774	3.188	0.556±0.007	0.040±0.000	Medium	metacentric
6	1.302	1.694	2.996	0.565±0.022	0.037±0.001	Medium	metacentric
7	1.035	3.356	4.391	0.664±0.009	0.055±0.003	Large	submetacentric
8	0.871	2.907	3.779	0.669±0.030	0.047±0.001	Large	submetacentric
9	0.887	2.746	3.632	0.656±0.010	0.045±0.001	Large	submetacentric
10	1.222	2.311	3.533	0.654±0.002	0.044±0.000	Large	submetacentric
11	0.818	2.481	3.299	0.652±0.013	0.041±0.002	Large	submetacentric

Table 2. Karyomorphological details of *R. trilineata* from 20 metaphases chromosome, 2n (diploid)=50. (cont.)

Chromosome pairs	Ls (µm)	Ll (µm)	LT (µm)	CI±SD	RL±SD	Chromosome size	Chromosome type
12	1.150	2.089	3.239	0.645±0.006	0.040±0.001	Large	submetacentric
13	0.845	2.367	3.212	0.637±0.014	0.040±0.002	Medium	submetacentric
14	0.784	2.227	3.010	0.640±0.004	0.037±0.001	Medium	submetacentric
15	1.049	1.940	2.990	0.649±0.013	0.037±0.000	Medium	submetacentric
16	0.988	1.645	2.634	0.625±0.012	0.033±0.001	Medium	submetacentric
17	0.963	1.603	2.566	0.625±0.044	0.032±0.001	Medium	submetacentric
18	0.852	2.433	3.284	0.741±0.006	0.041±0.000	Medium	submetacentric
19	0.775	2.286	3.061	0.747±0.016	0.038±0.001	Medium	acrocentric
20	0.708	2.202	2.910	0.757±0.007	0.036±0.000	Medium	acrocentric
21	0.685	2.143	2.828	0.758±0.013	0.035±0.001	Medium	acrocentric
22	0.788	2.002	2.789	0.718±0.010	0.035±0.001	Medium	acrocentric
23	0.684	1.924	2.608	0.738±0.004	0.032±0.000	Medium	acrocentric
24	0.709	1.756	2.465	0.712±0.004	0.031±0.000	Medium	acrocentric
25	0.655	1.706	2.361	0.723±0.017	0.029±0.001	Medium	acrocentric

Remarks: Ls=short arm chromosome, Ll=length of long arm chromosome, LT=length of total chromosomes, RL=relative length, CI=centromeric index, S.D.=standard deviation.

Table 3. Karyomorphological details of *R. borapetensis* from 20 metaphases chromosome, 2*n* (diploid)=50.

Chromosome pairs	Ls (µm)	Ll (µm)	LT (µm)	CI±SD	RL±SD	Chromosome size	Chromosome type
1	1.828	1.871	3.699	0.537±0.020	0.043±0.001	Large	metacentric
2	1.776	1.899	3.675	0.544±0.030	0.043±0.000	Large	metacentric
3	1.614	1.794	3.408	0.556±0.007	0.040±0.000	Medium	metacentric
4	1.502	1.714	3.216	0.565±0.021	0.037±0.001	Medium	metacentric
5	1.235	3.376	4.611	0.764±0.009	0.055±0.003	Large	submetacentric
6	1.495	2.975	4.470	0.695±0.003	0.053±0.002	Large	submetacentric
7	1.071	2.927	3.998	0.769±0.030	0.047±0.001	Large	submetacentric
8	1.396	2.516	3.912	0.676±0.025	0.046±0.000	Medium	submetacentric
9	1.422	2.331	3.753	0.654±0.002	0.044±0.000	Medium	submetacentric
10	1.350	2.109	3.459	0.645±0.006	0.040±0.001	Medium	submetacentric
11	1.249	1.960	3.209	0.649±0.013	0.037±0.000	Medium	submetacentric
12	1.188	1.665	2.853	0.625±0.012	0.033±0.001	Medium	submetacentric
13	1.163	1.623	2.786	0.625±0.044	0.032±0.001	Medium	submetacentric
14	1.087	2.766	3.853	0.756±0.010	0.045±0.001	Large	acrocentric
15	1.018	2.501	3.519	0.752±0.013	0.041±0.002	Large	acrocentric
16	1.052	2.453	3.505	0.741±0.006	0.041±0.000	Large	acrocentric

Table 3. Karyomorphological details of *R. borapetensis* from 20 metaphases chromosome, 2n (diploid)=50. (cont.)

Chromosome pairs	Ls (µm)	Ll (µm)	LT (µm)	CI±SD	RL±SD	Chromosome size	Chromosome type
17	1.045	2.387	3.432	0.737±0.014	0.040±0.002	Large	acrocentric
18	0.975	2.306	3.281	0.747±0.016	0.038±0.001	Medium	acrocentric
19	0.984	2.247	3.231	0.740±0.004	0.037±0.001	Medium	acrocentric
20	0.908	2.222	3.130	0.757±0.007	0.036±0.000	Medium	acrocentric
21	0.885	2.163	3.048	0.758±0.013	0.035±0.001	Medium	acrocentric
22	0.988	2.022	3.010	0.718±0.010	0.035±0.001	Medium	acrocentric
23	0.884	1.944	2.828	0.738±0.004	0.032±0.000	Medium	acrocentric
24	0.000	1.776	1.776	1.000±0.004	0.031±0.000	Medium	telocentric
25	0.000	1.726	1.726	1.000±0.017	0.029±0.001	Medium	telocentric

Remarks: Ls=short arm chromosome, Ll=length of long arm chromosome, LT=length of total chromosomes, RL=relative length, CI=centromeric index, S.D.=standard deviation.

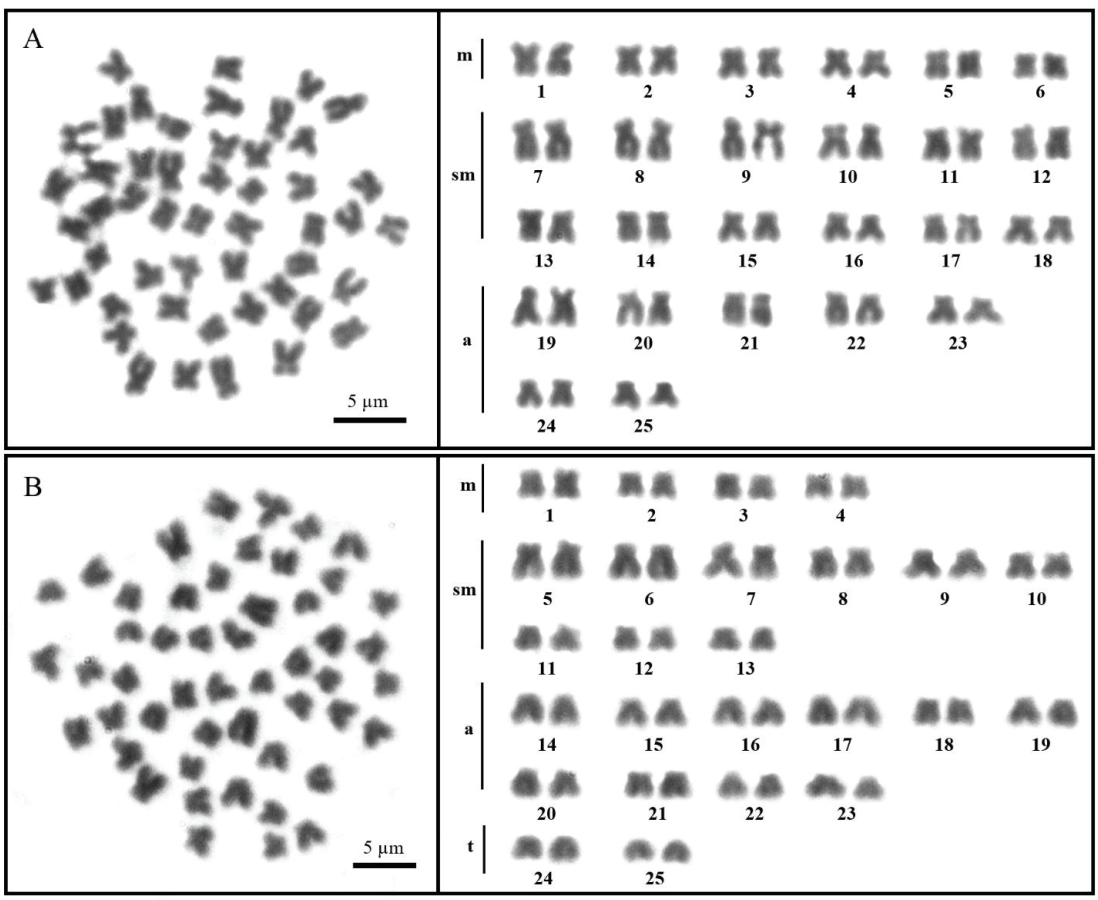

Figure 2. Metaphase chromosome and karyotype showing of the *R. trilineata* (A) and *R. borapetensis* (B), 2n (diploid)=50, by conventional staining technique.

The karyotype of *R. trilineata* was composed of 12 metacentric, 24 submetacentric and 14 acrocentric chromosomes. The mean values calculated from twenty mitotic metaphases showed the centromeric index of chromosome complements ranging from 0.532±0.037 to 0.723±0.017. The karyotype formula of *R. trilineata* could be deduced as: 2*n* (50) = 12m+24sm+14a. The type chromosomes of *R. trilineata* herein differ from those previously reported (Donsakul *et al.*, 2005). These differences may be due to different criteria used for the chromosome classification and/or inter-populational variation in this species. Meanwhile, *R. borapetensis* showed eight metacentric 18 submetacentric, 20 acrocentric and four 4 telocentric chromosomes. The present investigation in this fish species revealed that the mean value of centromeric index ranged from 0.537±0.020 to 1.000±0.017. The suggested karyotype of this species was 2*n* (50) = 8m+18sm+20a+4t. (Table 2, 3) (Figure 2). The number and type chromosome, this is the first report of *R. borapetensis*. The NFs of the genus *Rasbora* range from 74 to 100 and karyotypes are composed of both mono-and bi-arms chromosomes. Nirchio *et al.* (2002) proposed that species with high NF is advanced state or apomorphic character whereas one with low NF is a primitive state or plesiomorphic character. Thus, the *Rasbora* seems to be advanced karyotype.

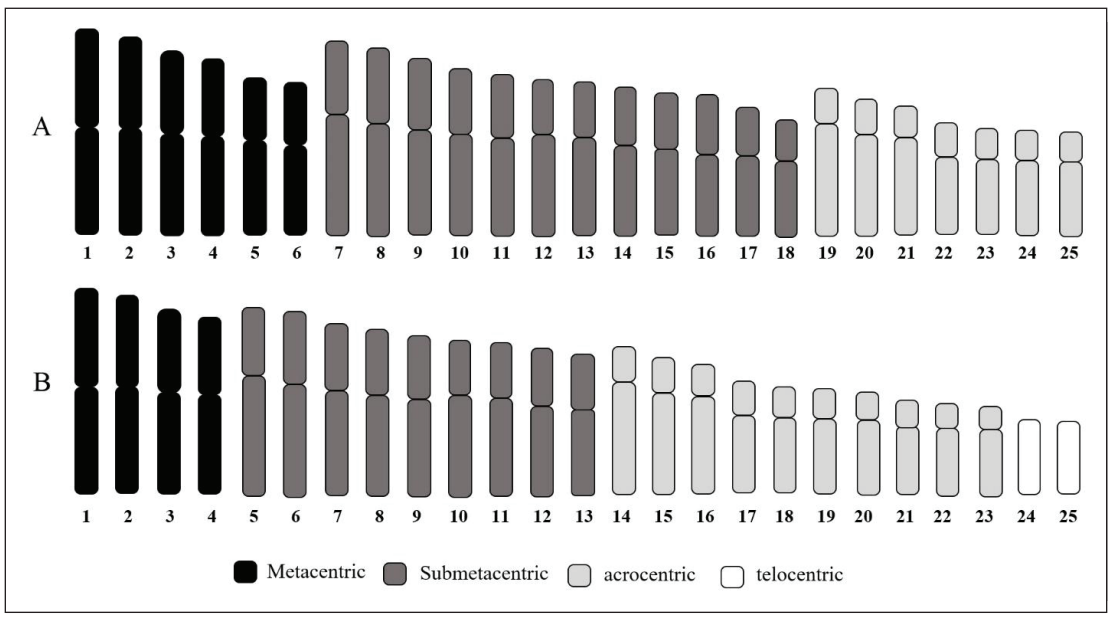

Figure 3. diogram showing lengths and shape of chromosomes of the *R. trilineata* (A) and *R. borapetensis* (B), 2n (diploid)=50, by conventional staining technique.

The ideogram (Figure 3) shows a continuous length gradation of chromosomes. The size differences between the largest and smallest chromosomes show approximately two-fold. The data of the chromosome measurement on mitotic metaphase cells (from all specimens) are shown in Table 2. Our results add new knowledge that can be used for karyological comparative analyses in *Rasbora* species, on the basis of the classical banding basis of the approach within this taxon.

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