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First cytogenetic study of the Somphong's rasbora (*Trigonostigma somphongsi*) (Perciformes, Cyprinidae), a critically endangered species in Thailand

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Abstract. The first chromosome study of *Trigonostigma somphongsi* (Meinken,1958) from Thailand. Specimens were collected from Pak Phli District, Nakhornnayok Province, central Thailand. The mitotic chromosomes were directly prepared from kidney tissues of two males and two females. Conventional staining and Ag-NOR banding techniques were applied to stain the chromosomes. The results shown that diploid chromosome number of *T. somphongsi* was 2*n*=50, the fundamental numbers (NF) were 92 in both male and female. The types chromosomes consisted of 10 metacentric, 20 submetacentric, 12 acrocentric and 8 telocentric chromosomes. The result also exhibited that the interstitial nucleolar organizer regions (NORs) were clearly observed at the short arm of chromosome pair 2. The karyotype formula could be deduced as: 2*n* (diploid) 50=10m+20sm+12a+8t.

Keywords: karyotype, chromosome, *Trigonostigma somphongsi.*

INTRODUCTION

The *Trigonostigma somphongsi* is a critically endangered species in natural habitat of Thailand. It has been discovered in a deep water rice field, floodplain of Bangpakong Basin, Nakhornnayok Province, central Thailand. (Petsut et al. 2014). The *Trigonostigma* is a genus of small cyprinid fish found in Southeast Asia. There are currently five recognized species in this genus including *T. heteromorpha*, *T. hengeli*, *T. somphongsi*, *T. espei* and *T. truncate* (Tan, 2020). The *T. somphongsi* was found in the rice field and in a

Figure 1. The characteristic of *T. somphongsi*. Scale bar indicate 0.2 cm.

densely vegetated ditch, which was flooded by a nearby river. It differs from the rest of the genus in its dark pattern which occurs as a strait horizontal line that extends from the base of the caudal fin and ends just after it passed the posterior part of the dorsal fin, instead of showing the wedge-like marking of other species in the genus (Petsut et al, 2014) (Fig. 1). Although the Somphong's rasbora is important for fish biodiversity of Thailand, there were quite scarce data about cytogenetics in these fishes especially banding analysis in fish chromosomes.

Up to date, information about karyotypes in genus *Trigonostigma* are rare and usually based on conventional staining technique. Only *T. heteromorpha* has been published, and the result showed that the diploid chromosome number (2*n*) was 50 (Khuda 1979). The cytogenetic studies using conventional staining technique provided valuable information on the great karyotype diversity shown by these animals. Analyses of cytogenetic markers, included the number and karyotype formula, number and location of nucleolar organizer regions (NORs). The present study is the first report on the chromosomal characteristics of *T. somphongsi* determined using conventional staining and Ag-NOR banding techniques. *T. somphongsi* is a critically endangered species in natural habitats of Thailand. The results enhance the level of cytogenetic information available and enable future comprehensive studies to be conducted on taxonomy and evolutionary relationships. Moreover, the data provide useful basic information for conservation and on breeding practices as well as an analysis of the chromosomal evolution of this species of *Trigonostigma*.

MATERIALS AND METHODS

Sample collection

Two males and two female of *T. somphongsi* were collected from agricultural land, Pak Phli District, Nakhornnayok Province, central Thailand, and were grown starting from May 2023 (permission from an ethical committee ID U1-04498-2559). All specimens were maintained in aerated, flowing seawater acquaria until the analysis.

Chromosome preparation

Chromosomes preparations were obtained from kidney by cell suspension technique. Briefly, the kidney was cut into small pieces and then mixed with 0.075 M potassium chloride (KCl). After discarding all large piece tissues, cell sediments were transferred to a centrifuge tube and incubated for 30 minutes, then centrifuged at 1,500 rpm for 5 minutes. The KCl was discarded from the supernatant after centrifugation at 1,500 rpm for 5 minutes. Cells were fixed in fresh cool fixative (3 methanol:1 glacial acetic acid) and gradually made up to 8 ml before centrifuging again at 1,500 rpm for 5 minutes, whereupon the supernatant was discarded. Fixation was repeated until the supernatant was clear. The mixture was dropped onto a clean and cold slide by micropipette followed by air-drying technique.

Chromosome staining

Conventional staining of the chromosomes in the air-dried slides was done using 10% Giemsa solution for 10 minute (Rooney 2001). Ag-NOR banding was carried out following method of Howell and Black (1980) by adding 4 drops of 50% silver nitrate and 2% gelatin on slides. The slides were then sealed with cover glasses and incubated at 60°C for 5 minutes. After that the slides were soaked in distilled water until the cover glasses were separated.

Chromosome checking

Twenty clearly observable cells with well spread chromosomes of each male and female were selected and photographed under Olympus Bx63 microscope. Metaphase figures were analyzed according to the chromosome classification of Turpin and Lejeune (1965). 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. 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 chromo-

somes, respectively. The fundamental number (NF, number of chromosome arms) was obtained by assigning a value of 2 to metacentric, submetacentric and acrocentric chromosomes and 1 to telocentric chromosome. All parameters were used in karyotyping and idiogram.

RESULTS AND DISCUSSION

This is the first report on cytogenetic characterization using conventional staining and Ag-NOR banding techniques for *T. somphongsi*. The results indicated diploid chromosome number (2*n*) were found 50 in all studies samples as show in Fig. 2. This result is coincident with *T. heteromorpha* reports by Khuda 1979, and similar to another species in *Rasbora* (Post 1965; Manna and Khuda-Bukhsh 1977; Khuda-Bukhsh et al. 1979; Donsakul and Magtoon 1995; Donsakul and Magtoon 2002; Seetapan and Moeikum 2004; Donsakul et al. 2005; Donsakul et al. 2009; Yeesaem et al. 2019; Aiumsumang et al. 2021; 2022). From the previous report, most of cyprinid species have 2*n*=50, chromosome consisting of both mono- and bi-arm chromosomes. There is no observation of strange size chromosomes related to sex, which is in accordance to the author of this genus (Khuda 1979). The types chromosomes of *T. somphongsi* were 10 metacentric, 20 submetacentric, 12 acrocentric and 8 telocentric chromosomes. The mean values calculated from twenty mitotic metaphases showed the centromeric index of chromosome complements ranging from 0.569±0.001 to 1.000±0.000 (Table 1).

To our results, NORs could be observed in one pair of chromosomes in both male and female of *T. somphongsi*. The result demonstrated that the chromosome marker shows in the chromosome pair 2, which is metacentric chromosome (Fig. 3). An important characteristic of Nucleolar Organizer Regions (NORs) in fish is related to that it has inter- and intra-species polymorphism. NORs characters can be a cytogenetic marker for cytotaxonomic studies and also have been used for studying of phylogenetic relationships among the Cyprinid fishes (Amemyia and Gold 1988; Galetti Jr 1998; Almeida-Toledo et al. 2000). The important karyotype feature of *T. somphongsi* is the symmetrical karyotype, which were found in four types of chromosomes (metacentric, submetacentric, acrocentric, and telocentric chromosomes). Figure 4 show the idiograms from conventional staining and Ag-NOR banding techniques. The karyotype formula could be deduced as: 2*n* (diploid) 50=10m+20sm+12a+8t. The study on fish chromosomes is the basic knowledge which can be applied for the several fields such as classification, evolution, heredity, systematic (Gold et al. 1990; Ueda et al. 2001; Barat et al. 2002; Barat and Sahoo 2007), breeding, rapid production of inbred lines and cytotaxonomy (Kirpichnikov 1981). Furthermore, cytogenetic studies on fish have also been used as biological indicator to determine the ecological toxicology (Klinkhardt, 1993) and cytogenetic techniques have been widely applied to improve farmed stocks in many aquaculture species in the World (Beardmore et al. 2001; Desprez et al. 2003).

Here, we have that the karyotype of *T. somphongsi* is 2*n*=50 and might represent a derived character, probably also shared by all members of the *Trigonostigma* clade. Besides, our study is the first cytogenetic karyotype data to describe in detail the karyotypic features of the Som-

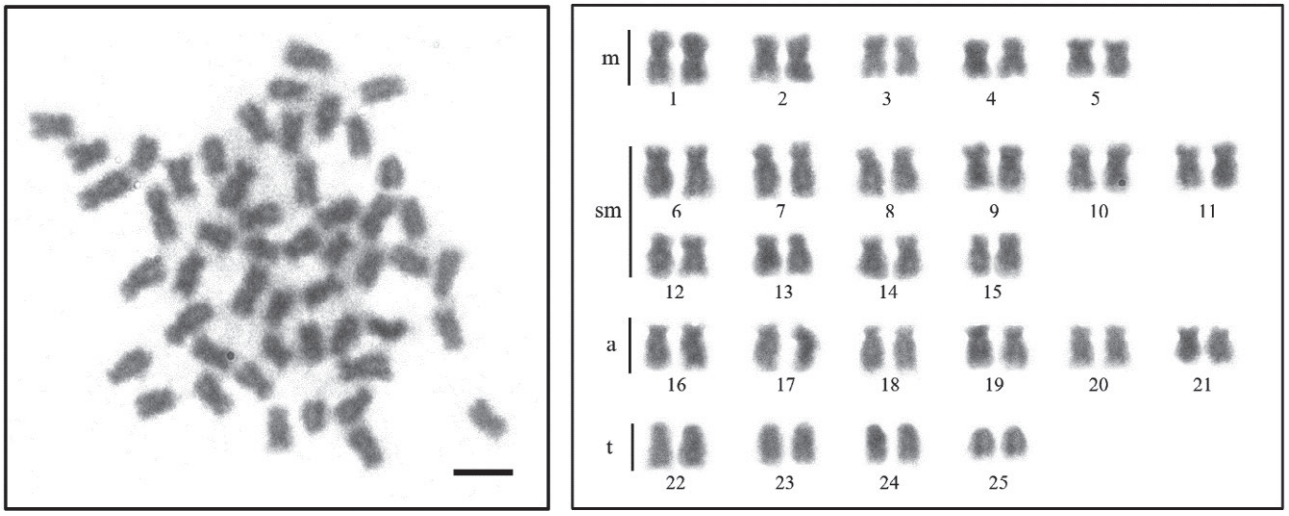

Figure 2. Metaphase chromosome plates and karyotypes of the *T. somphongsi* by conventional staining. Species share the karyotype composed of 50 chromosomes. Scale bar indicate 5 μm.

Table 1. Karyomorphological details of *T. somphongsi* from 20 metaphases chromosome, 2*n* (diploid)=50.

Chro. pair	Ls	Ll	LT	RL ± SD	CI ± SD	Chro. size	Chro. type
1	2.651	2.965	5.616	0.047±0.002	0.569±0.001	Large	metacentric
2	2.547	2.882	5.429	0.045±0.002	0.574±0.001	Large	metacentric
3	2.433	2.716	5.149	0.043±0.003	0.571±0.002	Large	metacentric
4	2.337	2.709	5.046	0.040±0.001	0.583±0.002	Medium	metacentric
5	2.927	2.609	5.536	0.039±0.001	0.583±0.002	Medium	metacentric
6	2.800	3.982	6.782	0.045±0.002	0.688±0.004	Large	submetacentric
7	2.692	3.237	5.929	0.044±0.001	0.680±0.001	Large	submetacentric
8	2.764	3.753	6.517	0.042±0.004	0.682±0.002	Large	submetacentric
9	2.985	3.706	6.691	0.040±0.004	0.674±0.002	Large	submetacentric
10	2.597	3.646	6.243	0.037±0.005	0.694±0.003	Medium	submetacentric
11	2.564	3.480	6.044	0.035±0.003	0.692±0.005	Medium	submetacentric
12	2.488	2.842	5.330	0.030±0.003	0.653±0.001	Medium	submetacentric
13	2.488	2.842	5.330	0.030±0.003	0.653±0.001	Medium	submetacentric
14	2.293	4.470	6.763	0.029±0.008	0.669±0.002	Medium	submetacentric
15	2.121	3.942	6.063	0.025±0.003	0.664±0.003	Medium	submetacentric
16	0.549	3.998	4.547	0.041±0.001	0.898±0.004	Large	acrocentric
17	0.404	3.565	3.969	0.038±0.001	0.894±0.004	Large	acrocentric
18	0.354	3.445	3.799	0.038±0.001	0.871±0.004	Large	acrocentric
19	0.344	3.014	3.358	0.037±0.001	0.874±0.004	Medium	acrocentric
20	0.175	2.985	3.160	0.035±0.001	0.872±0.004	Medium	acrocentric
21	0.168	2.014	2.182	0.031±0.001	0.890±0.004	Medium	acrocentric
22	0.000	4.624	4.624	0.029±0.001	1.000±0.000	Large	telocentric
23	0.000	3.615	3.615	0.027±0.001	1.000±0.000	Large	telocentric
24	0.000	3.550	3.550	0.026±0.001	1.000±0.000	Medium	telocentric
25	0.000	2.989	2.989	0.026±0.001	1.000±0.000	Medium	telocentric

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

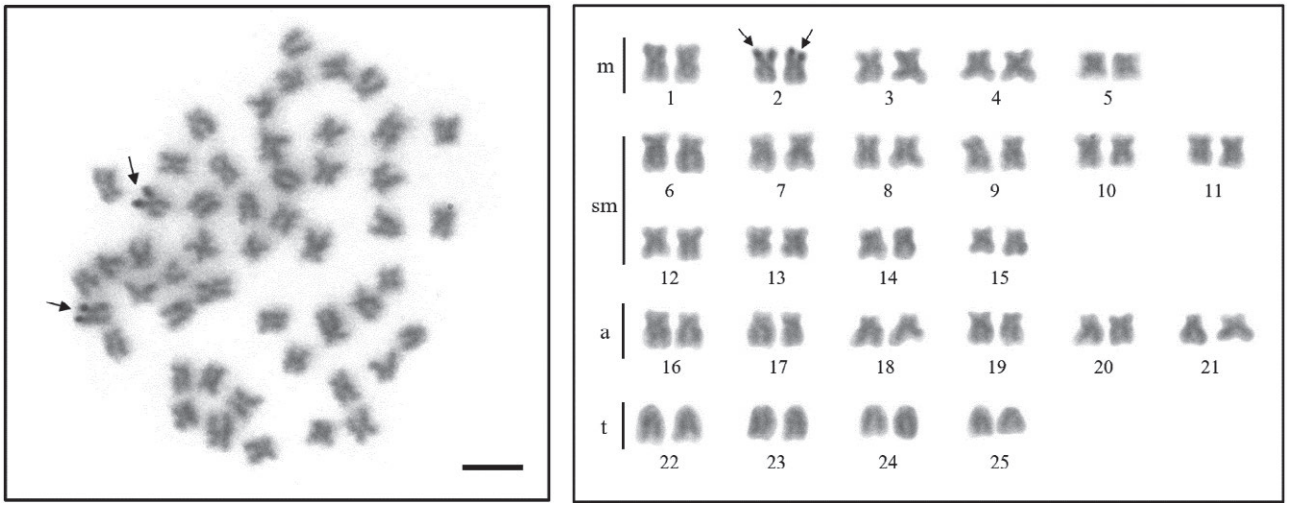

Figure 3. Metaphase chromosome plates and karyotypes of the *T. somphongsi*. The arrows indicate NOR banding by Ag-NOR staining technique. Scale bar indicate 5 μm.

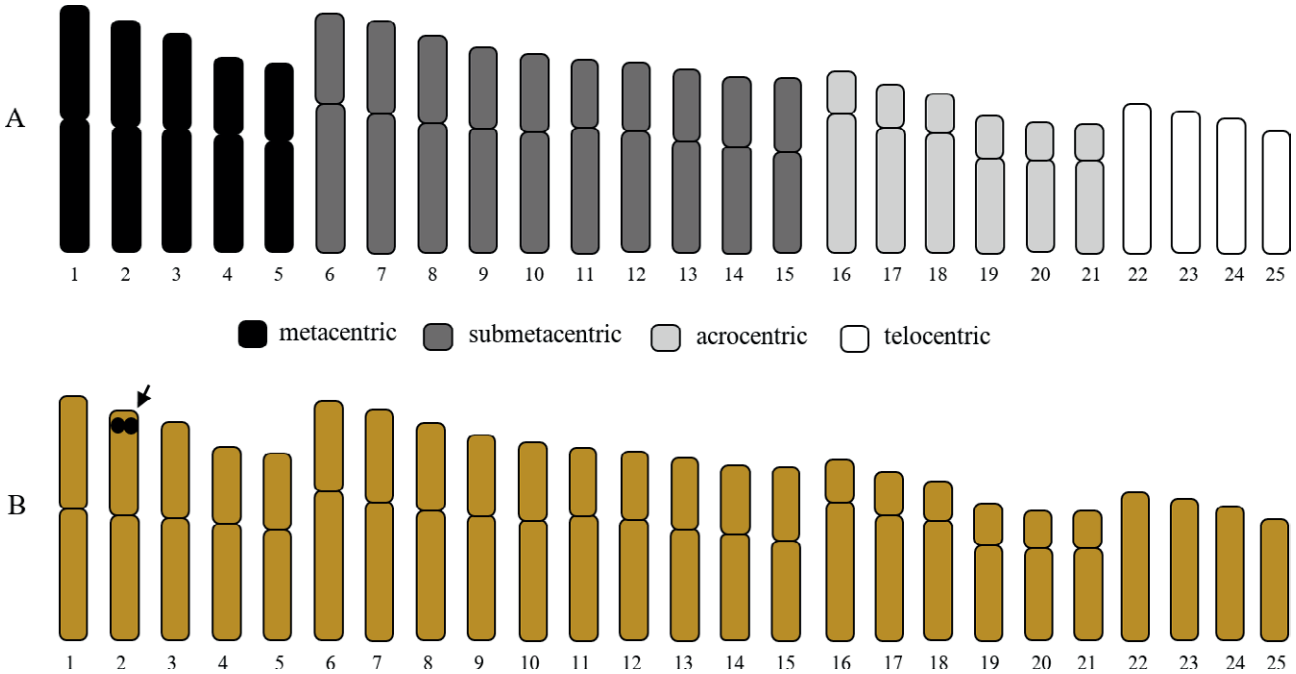

Figure 4. Idiogram showing lengths and shape of chromosomes of the *T. somphongsi*, *n* (haploid)=50, by conventional staining (A) and Ag-NOR staining technique (B). The arrows indicate NOR banding.

phong's rasbora species. The data can be a support for the investigation of chromosomal cytotaxonomy evolutionary history of relationships, conservation and on breeding practices within *Trigonostigma*.

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