

# Karyological characteristics of the Koh Tao caecilian, *Ichthyophis kohtaoensis* (Amphibia: Gymnophiona: Ichthyophiidae) by conventional staining and Ag-NOR banding techniques

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**Abstract** The karyotypic analysis of the Koh Tao caecilian, *Ichthyophis kohtaoensis* Taylor, on 1960 from Surin Province, Thailand was obtained from the present study. Mitotic chromosome was prepared directly from the small intestine of specimens after in vivo colchicine treatment. The metaphase spreads were performed on microscopic slides and air-dried. Conventional staining, and Ag-NOR banding techniques were applied to stain the chromosome with Giemsa's solution. Results showed that the number of diploid chromosome was  $2n = 42$ , while the fundamental number (NF) was 64 in both males and females. The type of chromosomes included 6 large metacentric, 2 large submetacentric, 12 small metacentric, 2 small submetacentric, and 20 small telocentric chromosomes. We found that nucleolar organizer regions/NORs (the representative of chromosome marker) were located on the small telocentric chromosome pair 19. No cytologically distinguishable sex chromosome was observed. The karyotype formula is as follows:  $2n (42) = L_6^m + L_2^{sm} + S_{12}^m + S_2^{sm} + S_{20}^t$ .

**Keywords** *Ichthyophis kohtaoensis* · Karyotype · Chromosome

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## 1 Introduction

Gymnophiona comprise one of the three living orders of Amphibia. Approximately 160 nominate species of caecilians, belonging to six families, are known to date. The family Ichthyophiidae Taylor, 1968, is considered one of the most primitive caecilians and includes three genera, *Uraeotyphlus* Peter, 1879, *Ichthyophis* Fitzinger, 1826, and *Caudacaecilia* Taylor, 1968 (Wilkinson et al. 2011). The genus *Ichthyophis*, composed of about 50 species, has the widest distribution range among the three genera, and found in most regions of South Asia and Southeast Asia (Nishikawa et al. 2012). The Koh Tao caecilian (*Ichthyophis kohtaoensis*) is an amphibian in the Ichthyophiidae family found in Cambodia, Laos, Myanmar, Thailand, and Vietnam. Its natural habitats are subtropical or tropical moist lowland forests, subtropical or tropical moist wet forests, rivers, intermittent rivers, swamps, freshwater marshes, intermittent freshwater marshes, plantations, rural gardens, urban areas, heavily degraded former forests, irrigated land, and seasonally flooded agricultural land. The scientific name refers to the Koh Tao Island in the Gulf of Siam, where the type specimen was collected (van Dijk et al. 2004).

Karyological analyses in caecilians thus far has differentiated species based on mitotic metaphase chromosomal morphology while sporadic reports have based the species differentiation based on meiotic metaphase chromosomal morphology. Thus, the basic diploid number of the order Gymnophiona is in the range of 20–42 (Venu 2014b). The primitive caecilian in the family Ichthyophiidae includes the genera *Ichthyophis* and *Caudacaecilia*, consisting of  $2n = 42$  with chromosomal constitutions characterized by asymmetrical karyotypes (metacentric, submetacentric, acrocentric, and telocentric chromosomes). In contrast, the

higher caecilian family Caecilian includes a larger chromosome numbers, with diploid numbers ranging from 20 to 38 chromosomes of diverse size and shape (Venu 2014c).

Previous cytogenetic publications of caecilians in the family Ichthyophiidae were conducted by Seshachar (1936, 1937a, b, 1939), Elayidom et al. (1963), Wake and Case (1975), Seto and Nussbaum (1976), Nussbaum and Treisman (1981), Wen and Pang (1990), Venkatachalaiah and Venu (2002), Matsui et al. (2006), Venu et al. (2011), Venu (2013, 2014a, d), and Venu and Venkatachalaiah (2013) (Table 1). Our present report provides a karyotypic description of *I. kohtaoensis*, based on conventional staining and Ag-NOR banding techniques. This obtained knowledge would be useful as support cytogenetic information for further study on taxonomy and evolutionary relationships.

## 2 Materials and methods

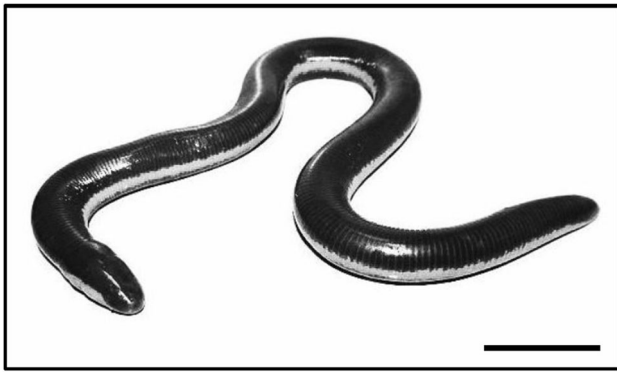
Specimens of both sexes of *I. kohtaoensis* were collected from June to September 2013 in Surin Province, Thailand, by digging into the soil adjacent to small streams (Fig. 1). Metaphase chromosomes were obtained from intestinal epithelia using methods modified from Wake et al. (1980). Conventional staining was done using 20 % Giemsa's solution for 30 min. Ag-NOR banding [5] was performed by adding 2 drops of 50 % silver nitrate and 2 % gelatin on slides (Howell and Black 1980). The lengths of short arm (Ls) and long arm (Ll) chromosomes were measured to calculate the length of total arm chromosome (LT,  $LT = Ls + Ll$ ). Relative length (RL) and centromeric index (CI) was estimated. CI was also computed to classify

**Table 1** Review of Asiatic tailed caecilian cytogenetic reports in the family Ichthyophiidae (genera; *Caudacaecilia*, *Ichthyophis*, and *Uraeotyphlus*)

Species	2n	NF	Karyotype	Ag-NOR	Locality	References
<i>Caudacaecilia asplenia</i>	42	62	20biarm + 22uniarm	–	Malaysia	Matsui et al. (2006)
<i>Ichthyophis bannanicus</i>	42	62	14m + 6sm + 22t	–	China	Wen and Pang (1990)
	42	60	10m + 8sm + 24t	–	India	Venu (2014d)
	44	66	10m + 10sm + 24t	–	India	Venu (2014d)
<i>I. beddomei</i>	42	60	10m + 8sm + 8a + 16t	–	India	Venkatachalaiah and Venu (2002)
	42	58	10m + 6sm + 26t	–	India	Venu (2013)
	42	–	12mac + 30micro	–	–	Seshachar (1936, 1937a, b)
<i>I. glutinosus</i>	48	–	28mac + 20micro	–	–	Wake and Case (1975)
	42	58	16m + 26t	–	Asia	Nussbaum and Treisman (1981)
	42	58	16m + 26t	–	China	Wen and Pang (1990)
<i>I. kodaguensis</i>	42	60	10m + 8sm + 24t	–	India	Venu (2013)
<i>I. kohtaoensis</i>	42	64	16m + 6sm + 20t	–	Asia	Nussbaum and Treisman (1981)
	42	64	18m + 4sm + 20t	2(t)	Thailand	Present study
<i>I. malabarensis</i>	36	60	24m/sm + 12t	–	India	Venkatachalaiah and Venu (2002)
<i>I. orthopicatus</i>	42	62	14m + 4sm + 2a + 22t	–	Sri Lanka	Seto and Nussbaum (1976)
<i>I. peninsularis</i>	42	58	6m + 10sm + 26t	–	India	Venu (2014d)
<i>I. tricolor</i>	42	64	Cytotype A: 20m + 2sm + 20t	–	India	Venu (2014a)
	42	62	Cytotype B: 20m + 2sm + 20t	–	India	Venu (2014a)
	44	64	Cytotype C: 10m + 8sm + 2a + 24t	–	India	Venu (2014a)
<i>Uraeotyphlus gansi</i>	42	58	14m + 2sm + 26t	2(-)	India	Venu et al. (2011)
<i>U. interruptus</i>	36	58	10m + 12sm + 14t	–	India	Venu and Venkatachalaiah (2013)
<i>U. menoni</i>	36	58	10m + 12sm + 14t	–	India	Venu and Venkatachalaiah (2013)
	36	–	–	–	India	Elayidom et al. (1963)
<i>U. narayani</i>	36	58	10m + 12sm + 14t	–	India	Venu and Venkatachalaiah (2013)
	36	–	–	–	India	Seshachar (1939)
	36	–	16mac + 20micro	–	India	Elayidom et al. (1963)
<i>U. oxyurus</i>	36	58	10m + 12sm + 14t	2 (sm)	India	Venu and Venkatachalaiah (2013)

2n diploid chromosome number, NF fundamental number (number of chromosome arm), m metacentric, sm submetacentric, a acrocentric, t telocentric chromosome, NOR nucleolar organizer region, mac macrochromosome, micro microchromosome

– Not available



**Fig. 1** General characteristic of Koh Tao caecilian (*L. kohtaoensis* Taylor, 1960), scale bar indicates 2 cm

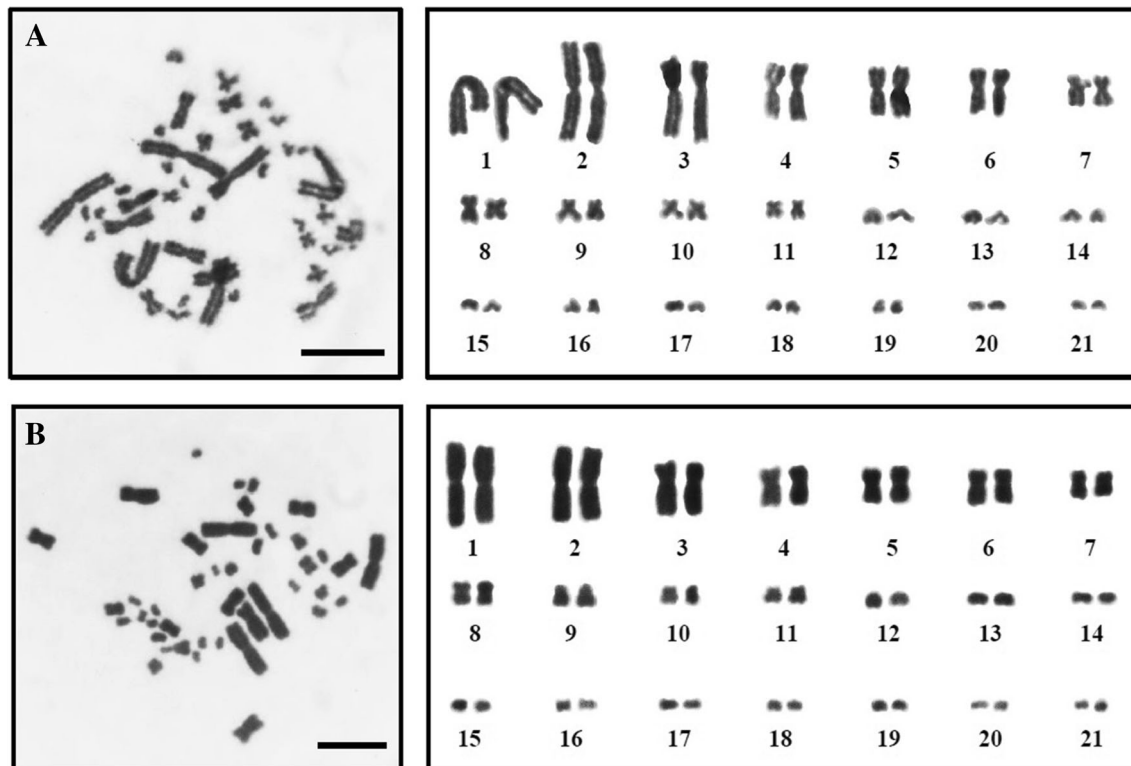
the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiogramming.

### 3 Results and discussion

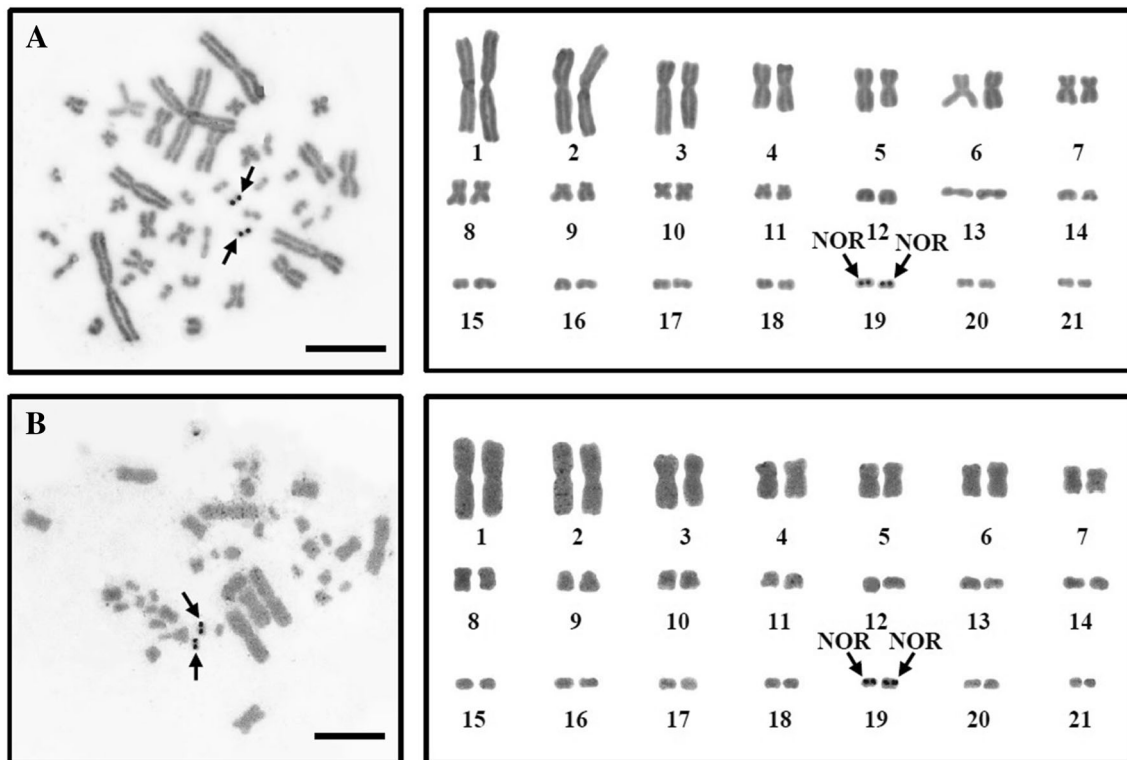
The mitotic karyotypes of both sexes have a diploid number ( $2n$ ) of 42. Twenty-one pairs of the homologous chromosomes in the somatic metaphase sets were found (Fig. 2). The fundamental number (NF) was 64 in both

males and females. This is in accordance with the previous study by Nussbaum and Treisman (1981). The types of chromosomes identified were 18 metacentric, 4 submetacentric, and 20 telocentric chromosomes. This is inconsistent to the report of Nussbaum and Treisman (1981), identified 16 metacentric, 6 submetacentric, and 20 telocentric chromosomes in the *I. kohtaoensis*. Earlier cytogenetic investigation reported conventionally stained metaphase chromosomes of nine species of the genus *Ichthyophis* including *I. bannanicus* (Wen and Pang 1990), *I. beddomei* (Venkatachalaiah and Venu 2002; Venu 2013), *I. glutinosus* (Nussbaum and Treisman 1981; Seshachar 1936, 1937a, b), *I. kodaguensis* (Venu 2013), *I. kohtaoensis* (Nussbaum and Treisman 1981), *I. malabarensis* (Venkatachalaiah and Venu 2002), *I. orthoplicatus* (Seto and Nussbaum 1976), *I. peninsularis* (Venu 2014d), and *I. tricolor* (Venu 2014a). Format cytogenetic analyses of ichthyophiid caecilians based on conventional chromosome staining methods have indicated closer relationships prevailed within the family Ichthyophiidae. These studies revealed that the genus *Ichthyophis* is a karyologically conserved taxa in terms of chromosome number ( $2n = 36$  to 48) and chromosome arm morphology (NF = 58 to 66).

In the present report, the karyotype of *I. kohtaoensis* shows 21 pairs of chromosomes. This karyotype presents



**Fig. 2** Metaphase chromosome plates and karyotypes of male (a) and female (b) Koh Tao caecilian (*L. kohtaoensis*)  $2n = 42$  by conventional staining technique, scale bars indicate 5 micrometers



**Fig. 3** Metaphase chromosome plates and karyotypes of male (a) and female (b) Koh Tao caecilian (*Ichthyophis kohtaoensis*)  $2n = 42$  by Ag-NOR banding technique, the arrows indicate nucleolar organizer regions/NORs (scale bar 5  $\mu$ m)

all necessary cytogenetic characteristic to be considered as the species with primitive karyotype perhaps similar to the presumptive ancestral karyotype of the genus *Ichthyophis* which is one among the striped Ichthyophiidae caecilians studies. Chromosomal variations either at the intra or interspecific level are the result of chromosome rearrangements within the set of chromosomes of populations or of species limiting to the extent of genetic changes. There are two possible chromosomal rearrangements: Robertsonian change (centric fusion and fission) and non-Robertsonian type (pericentric and paracentric inversions, tandem fusion and reciprocal translocations), which bring about the change in number and morphology during the course of assessment of chromosome lineages. It seems more likely that the Robertsonian processes, mainly centric fusions, have dominated their way in tracing out the evolution of amphibian karyotype, more explicitly in caecilians (Venu and Venkatachalaiah 2005).

No heteromorphic pairs of chromosomes were observed in both sexes in the metaphase complement, which is similar to the results from Nussbaum and Treisman (1981) and the other caecilians in the family Ichthyophiidae (Venu et al. 2011; Venu and Venkatachalaiah 2013; Venu 2013, 2014a). It is possible that the caecilian's sex chromosomes are dependent on an initiation of differentiation. Therefore,

chromosomes containing sex-determination gene cannot be found by cytogenetic analyses.

The present study accomplished using Ag-NOR staining technique, is the first cytogenetic report of *I. kohtaoensis*. The objective of this technique is to call attention to the nucleolar organizer regions/NORs, which represent the location of genes that function in ribosome synthesis (18S and 28S ribosomal RNA) (Sharma et al. 2002). The NORs were clearly observable on the small telocentric chromosome pair 19 (Fig. 3). It is quite consistent to the reports of Venu and Venkatachalaiah (2013), who reported that *Uraeotyphlus oxyurus* showed 1 pair of NORs-bearing on submetacentric chromosomes. Normally, most Asiatic tailed caecilians have only one pair of NORs on their chromosomes.

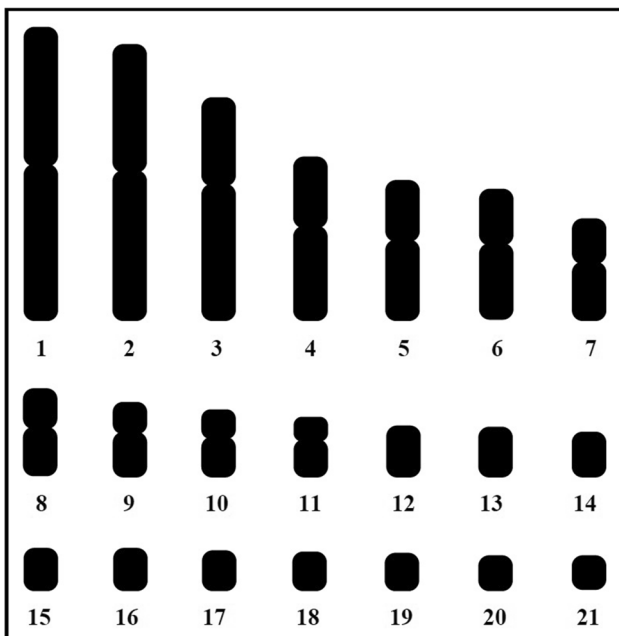
The *I. kohtaoensis* demonstrated that the chromosome marker is the chromosome pair 1, which is the largest metacentric chromosome. The largest chromosome is four times larger than the smallest chromosome. The chromosome length of 20 cells (males and females) in mitotic metaphase was measured (in centimeters). The mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), total length of arm chromosome (LT), relative length (RL), centromeric index (CI), size, and type of chromosome are presented in Table 2. Figures 4 and 5

**Table 2** Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosomes (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of

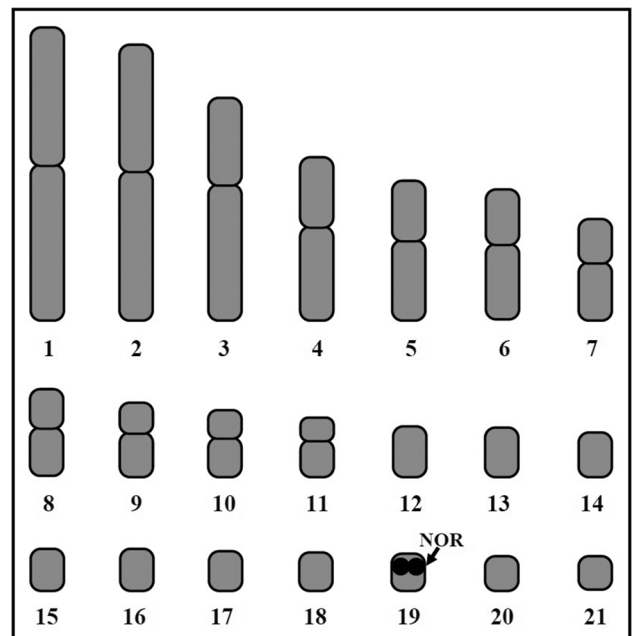
CI, RL from 20 metaphases of male and female Koh Tao caecilian (*L. kohtaoensis*)  $2n$  (diploid) = 42

Chromosome pair	Ls	Ll	LT	CI $\pm$ SD	RL $\pm$ SD	Chromosome size	Chromosome type
1	14.951	16.843	31.795	0.529 $\pm$ 0.017	0.145 $\pm$ 0.010	Large	Metacentric
2	13.754	16.204	29.958	0.539 $\pm$ 0.016	0.136 $\pm$ 0.010	Large	Metacentric
3	9.543	14.633	24.170	0.612 $\pm$ 0.041	0.108 $\pm$ 0.012	Large	Submetacentric
4	7.616	10.126	17.743	0.569 $\pm$ 0.026	0.080 $\pm$ 0.006	Large	Metacentric
5	6.493	8.714	15.207	0.573 $\pm$ 0.025	0.069 $\pm$ 0.004	Small	Metacentric
6	6.048	8.174	14.222	0.574 $\pm$ 0.031	0.064 $\pm$ 0.004	Small	Metacentric
7	4.725	6.310	11.034	0.569 $\pm$ 0.026	0.050 $\pm$ 0.004	Small	Metacentric
8	4.212	5.223	9.435	0.556 $\pm$ 0.022	0.043 $\pm$ 0.002	Small	Metacentric
9	3.309	4.814	8.123	0.596 $\pm$ 0.049	0.037 $\pm$ 0.003	Small	Metacentric
10	3.154	4.253	7.407	0.574 $\pm$ 0.040	0.034 $\pm$ 0.004	Small	Metacentric
11	2.530	3.982	6.512	0.616 $\pm$ 0.038	0.030 $\pm$ 0.003	Small	Submetacentric
12	0.000	5.537	5.537	1.000 $\pm$ 0.000	0.025 $\pm$ 0.003	Small	Telocentric
13	0.000	5.352	5.352	1.000 $\pm$ 0.000	0.025 $\pm$ 0.004	Small	Telocentric
14	0.000	4.860	4.860	1.000 $\pm$ 0.000	0.022 $\pm$ 0.003	Small	Telocentric
15	0.000	4.523	4.523	1.000 $\pm$ 0.000	0.021 $\pm$ 0.003	Small	Telocentric
16	0.000	4.216	4.516	1.000 $\pm$ 0.000	0.021 $\pm$ 0.003	Small	Telocentric
17	0.000	4.215	4.215	1.000 $\pm$ 0.000	0.019 $\pm$ 0.002	Small	Telocentric
18	0.000	4.130	4.130	1.000 $\pm$ 0.000	0.019 $\pm$ 0.003	Small	Telocentric
19 <sup>a</sup>	0.000	3.963	3.963	1.000 $\pm$ 0.000	0.018 $\pm$ 0.003	Small	Telocentric
20	0.000	3.961	3.961	1.000 $\pm$ 0.000	0.017 $\pm$ 0.003	Small	Telocentric
21	0.000	3.952	3.952	1.000 $\pm$ 0.000	0.016 $\pm$ 0.003	Small	Telocentric

<sup>a</sup> NORs bearing chromosomes



**Fig. 4** Ideogram showing lengths and shape of chromosomes of the Koh Tao caecilian (*L.kohtaoensis*),  $2n = 42$  by conventional staining



**Fig. 5** Ideogram of chromosomes of the Koh Tao caecilian (*L.kohtaoensis*)  $2n = 42$  by Ag-NOR banding technique, the arrow indicates NOR-bearing telocentric chromosome pair 19

show the ideograms from conventional staining and Ag-NOR banding techniques. The ideogram shows the gradually decreasing length of the chromosomes. The karyotype formula was deduced as follows:  $2n(42) = L_6^m + L_2^{sm} + S_{12}^m + S_2^{sm} + S_{20}^t$ .

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