



## รายงานพันธุศาสตร์ระดับเซลล์ครั้งแรกของงูสายม่านพระอินทร์ (*Dendrelaphis pictus*, Colubrinae) ด้วยการย้อมสีโครโมโซมแบบจิมซ่า

### First Cytogenetic Report of Painted Bronzeback (*Dendrelaphis pictus*, Colubrinae) by Giemsa's Staining

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#### บทคัดย่อ

การศึกษาครั้งนี้เป็นการศึกษาพันธุศาสตร์ระดับเซลล์ครั้งแรกของงูสายม่านพระอินทร์ (*Dendrelaphis pictus*) ใช้ตัวอย่างงูเพศผู้ 10 ตัว และเพศเมีย 10 ตัว จากจังหวัดมหาสารคาม ฉีดโคลชิซิน 0.03% เข้าไปในตัวอย่างสัตว์เป็นเวลา 18 ชั่วโมง เตรียมโครโมโซมจากม้ามและลำไส้ด้วยวิธีบดขยี้เซลล์ และเทคนิคไฮโปโทนิค-ฟิกเซชัน-แอร์รรายอิ่ง ย้อมสีโครโมโซมแบบธรรมดาด้วยสีจิมซ่า 20% ผลการศึกษาพบว่างูสายม่านพระอินทร์มีโครโมโซมดิพลอยด์เท่ากับ 36 แห่ง ประกอบด้วยโครโมโซมชุดใหญ่ 16 แห่ง และโครโมโซมชุดเล็ก 20 แห่ง โครโมโซมชุดใหญ่ประกอบด้วยโครโมโซมชนิดเมทาเซนทริก 2 แห่ง ซับเมทาเซนทริก 10 แห่ง เทโลเซนทริก 2 แห่ง โครโมโซมเพศเป็นระบบ ZZ/ZW โครโมโซมแซตเป็นชนิดซับเมทาเซนทริก และโครโมโซมดับเบิลยูเป็นชนิดเทโลเซนทริก พบรอยคอดที่สองบนแขนข้างยาวของโครโมโซมชนิดซับเมทาเซนทริกคู่ที่ 2 โครโมโซมชุดเล็กทั้ง 10 คู่มีขนาดเล็กเป็นจุด ไม่สามารถจำแนกชนิดได้ งูสายม่านพระอินทร์มีสูตรแคโรไทป์ดังนี้

$$2n = 36 = L^m_2 + L^{sm}_4 + M^{sm}_2 + S^{sm}_4 + S^t_2 + ZZ/ZW + 20 \text{ โครโมโซมชุดเล็ก}$$

## ABSTRACT

This study is the first cytogenetic study of painted bronzeback (*Dendrelaphis pictus*). Ten male and ten female snakes from Maha Sarakham province were treated by 0.03% colchicine for 18 hours. The chromosomes prepared from spleen and intestine using squash technique. Then, Hypotonic-fixation-air-drying technique was applied. The chromosomes were stained with 20% Giemsa's solution. The karyotype showed the  $2n=36$  comprises 16 macrochromosomes and 20 microchromosomes. The macrochromosomes comprises 2 metacentric, 10 submetacentric and 2 telocentric chromosomes. The sex chromosome system is ZZ/ZW. The Z-chromosome is submetacentric and W-chromosome is telocentric chromosome. The secondary constrictions were found on the long arm of submetacentric chromosome pair 2. The ten pairs of microchromosomes are dot-like chromosome which cannot classify the type of chromosomes. The painted bronzeback has karyotypic formula as follows:

$$2n = 36 = L^m_2 + L^{sm}_4 + M^{sm}_2 + S^{sm}_4 + S^t_2 + ZZ/ZW + 20 \text{ microchromosomes}$$

**คำสำคัญ:** งูสายม่านพระอินทร์ (*Dendrelaphis pictus*) พันธุศาสตร์ระดับเซลล์ แคริโอไทป์

**Keywords:** Painted Bronzeback (*Dendrelaphis pictus*), Cytogenetics, Karyotype

## INTRODUCTION

Painted bronzeback (*Dendrelaphis pictus*) is a species of snake in the family Colubridae, subfamily Colubrinae. *Dendrelaphis* is the largest genus, with 45 species. This genus has 6 species in Thailand including *D. caudolineatus*, *D. pictus*, *D. kopsteini*, *D. striatus*, *D. ngansaoensis* and *D. cyanochlosis*. The subfamily Ahaetuliinae comprises five genera containing 63 species i. e. *Ahaetulla*, *Chrysopelea*, *Dryophiops*, *Proahaetulla* and *Dendrelaphis*. (Pyron et al., 2013; Chan-ard et al., 2015).

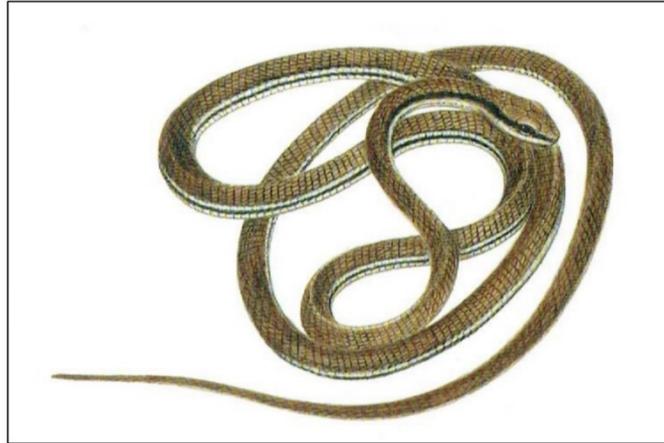
The painted bronzeback (*Dendrelaphis pictus*) is small to medium snake (up to 125 centimeters). The coloration is bronze-brown dorsal, yellow ventrolateral stripe edge with black along flank, forehead brown with black postocular stripe. The neck can display blue or greenish-blue patch when excited. This snake is widespread throughout in South Asia, Southeast Asia and south China. The status of this snake was not evaluate (NE) by IUCN (International Union for Conservation of Nature) (Das, 2010; Chan-ard et al., 2015).

Up to the present, there is no report on cytogenetics of painted bronzeback. There are only two cytogenetic reports of *Dendrelaphis* including *D. ahaetulla* by Sharma and Nakhasi (1980) and *D. puntulata* by Mengden (1982). The diploid number of previous reports was  $2n=36$ . In the present study, we exhibit the standardization of karyotype and idiogram. This report describes the first chromosome staining by conventional Giemsa's staining in *D. pictus*. The obtained results can provide more cytogenetic information for future studies on taxonomy and evolutionary relationships of the genus and family.

## MATERIALS AND METHODS

The study of standardized karyotype and idiogram by conventional Giemsa's staining of painted bronzeback (*D. pictus*) divide into four steps including the snake chromosome preparation, chromosome counting, chromosome measuring and chromosome analysis.

1. Snake chromosome preparation: The 10 males and 10 females of *D. pictus* (Figure 1) were collected from natural area from Muang district, Maha Sarakham, Thailand. The samples were checked by identification key, Das (2010) and Chan-ard (2015).



**Figure 1.** External morphology of painted bronzeback (*Dendrelaphis pictus*) (Modified from Chan-ard et al. 2015)

2. Chromosome counting: Many pictures of the chromosomes in metaphase cells were taken for counting the number of chromosomes. The twenty metaphase cells of each male and female were measured for the length of short arm (Ls) and long arm (Ll) chromosomes.

3. Chromosome measuring: The Ls and Ll were measured and calculated for the length of total arm chromosomes (LT,  $LT = Ls + Ll$ ). Relative length (RL), centromeric index (CI) and standard deviation (SD) were also calculated. The CI was calculated for the classification of the shape of chromosomes according to Chaiyasut (1989) and Tanomtong et al. (2019). All parameters were used in standardized karyotyping and idiogramming.

4. Chromosome analysis: Some species of reptiles and birds, chromosomes are clearly divided into two sets. The larger set is called macrochromosomes, which is larger than 1.0 micron. The smaller set is called the microchromosomes, which is smaller than 1.0 micron. In karyotype formula, the size and type of

Chromosome preparation was conducted by the colchicine-hypotonic-fixation-air drying technique, from spleen and intestine tissues. The chromosomes were stained with 20% Giemsa staining solution for 30 minutes according to Verma and Babu (1995).

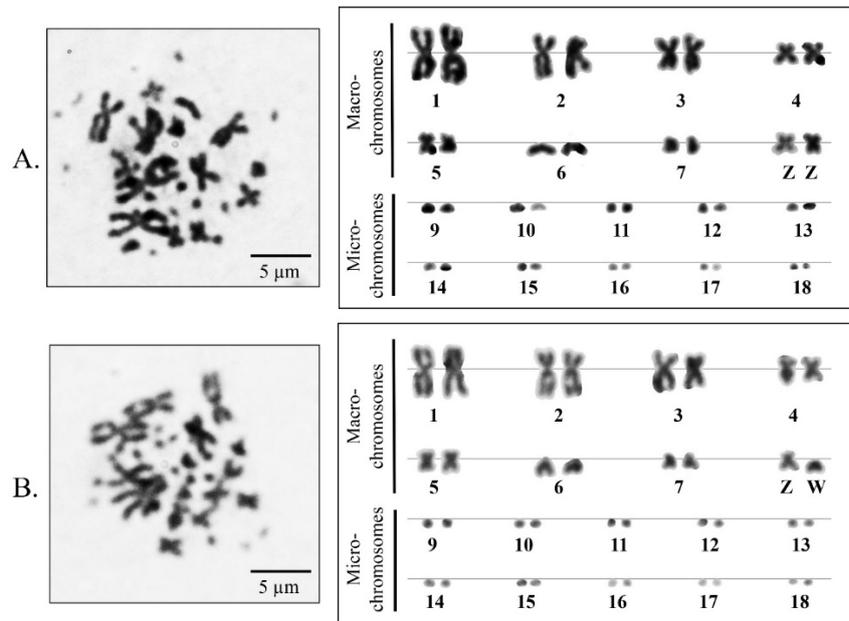
chromosome were classified, which the symbol “L, M and S” represented large, medium and small chromosomes, respectively. The symbol “m, sm, a, and t” represented metacentric, submetacentric, acrocentric and telocentric type of chromosomes, respectively. The fundamental number (NF) was obtained by assigning the value of two for metacentric, submetacentric and acrocentric chromosomes and one for the telocentric chromosome.

## RESULTS

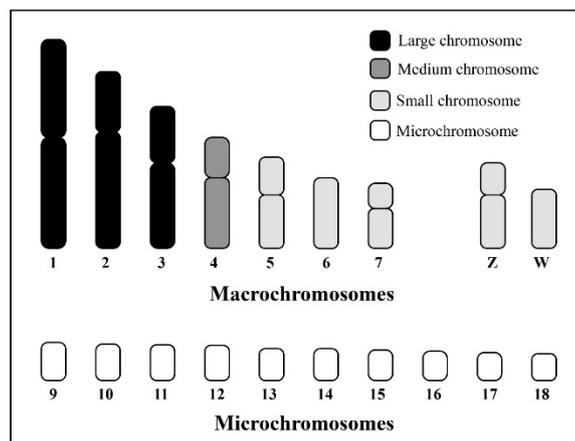
The karyotype of painted bronzeback (*Dendrelaphis pictus*) showed that  $2n = 36$  including 16 macrochromosomes and 20 microchromosomes (Figure 2). The macrochromosomes consist of one pair of large metacentric, two pairs of large submetacentric, one pair of medium submetacentric, two pairs of small submetacentric and one pair of small telocentric chromosomes. The macrochromosome can be classified into three types of large, medium and small. The size of large chromosome was ranging from 3.57 to

5.25 micron, the medium chromosome was ranging from 2.31 to 3.56 micron and the small chromosome was lower than 2.31 micron. For the microchromosome, it is the dot-like chromosomes which its size is lower than one micron, thus that it cannot be classified for

the type of chromosomes. Twenty metaphase cells of each male and female were measured for Ls, Ll, Lt, Ci, Rl, Sd, chromosome sizes and types were shown in Table 1. The standardized conventional idiograms of painted bronzeback shown in Figure 3.



**Figure 2.** Metaphase plates and standardized karyotypes of male, ZZ (A.) and female, ZW (B.) of painted bronzeback (*Dendrelaphis pictus*);  $2n=36$  by using Giemsa's staining. Scale bars indicate 5 µm.



**Figure 3.** Standardized idiogram of painted bronzeback (*Dendrelaphis pictus*);  $2n=36$  by using Giemsa's staining.

Sex determination of painted bronzeback is ZZ/ZW system. The Z-chromosome is small submetacentric and W- chromosome is small telocentric chromosome. Secondary constrictions were found on

interstitial long arm of submetacentric chromosome pair 2. The painted bronzeback (*Dendrelaphis pictus*) has karyotypic formular as follows:

$$2n = 36 = L^m_2 + L^{sm}_4 + M^{sm}_2 + S^{sm}_4 + S^t_2 + ZZ/ZW + 20 \text{ microchromosomes}$$

**Table 1.** Mean length (micron) 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 males and females of painted bronzeback (*Dendrelaphis pictus*),  $2n=36$ .

Chromosome pair	Ls (μm)	Ll (μm)	LT (μm)	CI±SD	RL±SD	Size	Type
1	2.46	2.80	5.26	0.532±0.029	0.154±0.020	Large	Metacentric
2	1.61	2.85	4.46	0.639±0.009	0.130±0.012	Large	Submetacentric
3	1.41	2.16	3.57	0.606±0.036	0.104±0.010	Large	Submetacentric
4	1.03	1.78	2.81	0.635±0.024	0.082±0.009	Medium	Submetacentric
5	0.92	1.38	2.30	0.601±0.015	0.067±0.008	Small	Submetacentric
6	0.00	1.77	1.77	1.000±0.000	0.052±0.009	Small	Telocentric
7	0.61	1.02	1.63	0.628±0.030	0.048±0.006	Small	Submetacentric
Z	0.78	1.39	2.17	0.640±0.018	0.063±0.011	Small	Submetacentric
W	0.00	1.46	1.46	1.000±0.000	0.043±0.007	Small	Telocentric
9	0.00	0.95	0.95	-	0.028±0.004		microchromosome
10	0.00	0.93	0.93	-	0.027±0.003		microchromosome
11	0.00	0.88	0.88	-	0.026±0.002		microchromosome
12	0.00	0.86	0.86	-	0.025±0.003		microchromosome
13	0.00	0.81	0.81	-	0.024±0.002		microchromosome
14	0.00	0.80	0.80	-	0.023±0.002		microchromosome
15	0.00	0.77	0.77	-	0.023±0.003		microchromosome
16	0.00	0.75	0.75	-	0.022±0.002		microchromosome
17	0.00	0.72	0.72	-	0.021±0.001		microchromosome
18	0.00	0.62	0.62	-	0.020±0.001		microchromosome

## DISCUSSION

This study is the first cytogenetic study of painted bronzeback (*Dendrelaphis pictus*). The karyotype showed 16 macrochromosomes and 20 microchromosomes. The macrochromosomes comprised of 14 bi-arm and 2 uni-arm chromosomes. There are only two cytogenetic reports of *Dendrelaphis*, namely *D. ahaetulla* and *D. puntulata* which exhibited the diploid number ( $2n$ ) of 36. The *D. ahaetulla* showed 16 bi-arm macrochromosomes, 20 microchromosomes and no sex chromosomes. The *D. puntulata* showed 16 macrochromosome, 20 microchromosomes and ZZ/ZW sex chromosome system (Sharma and Nakhasi 1980; Mengden 1982).

In Thailand, there were only 3 species in 3 families has been karyotype reports, Typhlopidae, Homalopsidae and Colubridae. Common blind snake (*Ramphotyphlops braminus*, Typhlopidae) was found triploid number,  $3n=42$  with consisting of 24 bi-arm macrochromosomes and 18 microchromosomes. Puff-Faced Water Snake (*Homalopsis buccata*, Colubridae) has showed polymorphism of diploid number. The population from Sakon Nakhon Province has  $2n=32$  while the population from Khon Kaen Province has  $2n=36$ . For Colubridae family, chequered keelback (*Xenochrophis piscator*) has  $2n=30$  which included of 8 bi-arm, 2 uni-arm macrochromosomes and 20

microchromosomes (Luangpirom et al., 1999; Pinthong et al., 2013; Patawang and Tanomtong, 2014).

The chromosome complement of snakes is relatively conserved. The putative ancestor has  $2n=36$  consisting of 16 macrochromosomes, 20 microchromosomes and isomorphic sex-chromosomes. The primitive snake families have quite similar to the ancestral karyotype, such as, Leptotyphlopidae, Typhlopidae, Aniliidae, Tropidophiidae, Cyndrophidae, Xenopeltidae, Loxocemidae, Pythonidae, Boidae and Bolyeriidae. The advance snakes has heteromorphic ZZ/ZW sex-chromosomes, such as, Acrochordidae, Xenodermidae, Preatidae, Viperidae, Homalopsidae, Lamphrophiidae, Elapidae and Colubridae. Most snakes have  $2n=36$  karyotypes, consisting of 16 macrochromosomes, 20 microchromosomes, ZZ/ZW sex chromosome system. The lowest diploid number in snake is  $2n=20$  (16 macro- and 8 microchromosomes)

which was found in the genus *Hydronastes* (Colubridae) and *Achalinus* (Xenopeltidae). The genus *Apostolepis*, *Sordallina* and *Pseudoboa* in family Colubridae have the highest diploid number of 50 (14 macro- and 36 microchromosomes). The mechanism of snake chromosome evolution is the fusion and fission.

In Thailand, there are only three snake species in three families which are: common blind snake (*Ramphotyphlops braminus*), puff-faced water snake (*Homalopsis buccata*), Chequered keelback (*Xenochrophis piscator*) have been cytogenetic reported. The common blind snake showed triploid number of 42, the puff-faced water snake showed polymorphism of diploid number of 32 and 36, the chequered keelback showed untypical diploid number of 30 (Table 2). Thus, Thailand still needs more snake cytogenetic information in order to understanding evolution process, taxonomic classification, systematics and breeding plan.

**Table 2.** Review of cytogenetic reports of snakes in Thailand (Reptilia; Squamata)

Species	2n	NF	Macro.		Micro.	Sex	Locality	Reference
			Bi.	Uni.				
<b>Family Typhlopidae</b>								
Common blind snake ( <i>Ramphotyphlops braminus</i> )	42 (3n)	-	24	0	18	-	Maha Sarakham	Patawang and Tanomtong (2014)
<b>Family Homalopsidae</b>								
Puff-faced water snake ( <i>Homalopsis buccata</i> )	32	-	10	4	16	-	Sakon Nakhon	Luangpirom et al. (1999)
	36	52	14	0	20	ZZ/ZW	Khon Kaen	Pinthong et al. (2013)
<b>Family Colubridae</b>								
Chequered keelback ( <i>Xenochrophis piscator</i> )	30	-	8	2	20	-	Sakon Nakhon	Luangpirom et al. (1999)
Painted bronzeback ( <i>Dendrelaphis pictus</i> )	36	28/27	12	2	20	ZZ/ZW	Maha Sarakham	This study

**Remark:** 2n=diploid number, 3n=triploid number, NF=Fundamental number, Macro.=macrochromosome, Bi.=bi-arm chromosome, Uni.=uni-arm chromosome, Micro.=microchromosome, Sex=Sex chromosome system

## CONCLUSION

The karyotype of painted bronzeback (*Dendrelaphis pictus*) showed that  $2n = 36$  including 16 macrochromosomes and 20 microchromosomes. The macrochromosomes consist of one pair of large metacentric, two pairs of large submetacentric, one pair of medium submetacentric, two pairs of small

submetacentric, one pair of small telocentric chromosomes. Sex determination of painted bronzeback is ZZ/ZW system. The Z-chromosome is small submetacentric and W-chromosome is small telocentric chromosome. Secondary constrictions were found on interstitial long arm of submetacentric chromosome pair 2. The painted bronzeback (*Dendrelaphis pictus*) has karyotypic formula as follows:

$$2n = 36 = L^m_2 + L^{sm}_4 + M^{sm}_2 + S^{sm}_4 + S^t_2 + ZZ/ZW + 20 \text{ microchromosomes}$$

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