

# Karyological Analysis of the Indo-Chinese Water Dragon, *Physignathus cocincinus* (Squamata, Agamidae) from Thailand

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**Summary** Karyological analysis of the Indo-Chinese water dragon (*Physignathus cocincinus*) from Northeast Thailand was studied. Dragon lizard chromosome preparations were conducted by a squash technique from bone marrow and testis. Conventional staining and Ag-NOR banding techniques were applied to stain the chromosome with Giemsa's solution. The results showed that the number of diploid chromosome was  $2n=26$ , while the fundamental number (NF) were 48 in both males and females. The types of chromosome were five metacentric pairs and one submetacentric pair of macrochromosomes, and 12 pairs of apparent microchromosomes. No irregularly sized chromosomes related to sex were observed. Nucleolar organizer regions (NORs) are located at the secondary constriction of the long arm near telomere in macrochromosome pair 2. We found that during metaphase I the homologous chromosomes showed synapsis, which can be defined as 18 bivalents and 18 haploid chromosomes at metaphase II as in diploid species. The karyotype formula is as follows:  $2n (36)=L_4^m+L_2^{sm}+M_4^m+S_2^m+24$  microchromosomes.

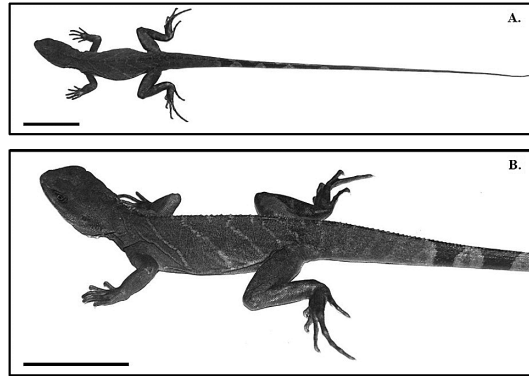
**Key words** Indo-Chinese water dragon, *Physignathus cocincinus*, Karyotype, Ag-NOR banding.

Agamid lizards of the family Agamidae comprise about 380 species in Africa, Asia, Australia and some in Southern Europe. Many species are commonly called dragon lizards (Cogger and Zweifel 1998, Uetz 2010). The Indo-Chinese water dragon belongs to the kingdom Animalia, phylum Chordata, class Reptilia, order Squamata, suborder Lacertilia, infraorder Iguania, family Agamidae, subfamily Agaminae, genus *Physignathus* and species *Physignathus cocincinus* (Cuvier 1829). The general characteristics of *P. cocincinus* are: long snout, moderate body in juveniles and robust and compressed in adults, small dorsal scales and uniform, femoral pores in males (eight pairs), a bright green dorsum changeable to brownish-green, a white venter with bright blue patches, and a snout-vent length of 250 mm in adults (Das 2010).

Karyotypically, agamids are a reasonably well studied group with karyotypes available for about 30% of species described worldwide. Cytogenetic studies can give useful information for classification of agamid lizards. However, recent cytogenetic surveys indicated that it is difficult to

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**Fig. 1.** General characteristics of the Indo-Chinese water dragon, *Physignathus cocincinus* (Agamidae, Squamata) from Northeast Thailand, (A) dorsal view and (B) lateral view (scale bars indicate 5 cm).

resolve the phylogenetic relationships among agamids due to the scarcity of phylogenetically informative characters in chromosome morphology (Honda *et al.* 2000).

There is only one previous cytogenetics study on *P. cocincinus*. De Smet (1981) demonstrated by conventional staining technique that the Indo-Chinese water dragon's karyotype was  $2n$  (diploid)=36, with a fundamental number (NF, number of chromosome arms) of 48. The chromosomes were composed of 12 bi-arm macrochromosomes and 24 microchromosomes. Here, we examine the karyotypes and idiogram of *P. cocincinus* from mitotic and meiotic cell divisions, including the first application of the Ag-NOR chromosome banding technique in *P. cocincinus*.

#### Materials and methods

Samples of the dragon lizard, *P. cocincinus*, were obtained from Surin Province, Northeast Thailand (Fig. 1). Three males and three females were transferred to the laboratory and were kept under standard conditions for 7 d prior to the experiments. Chromosome preparations were conducted by a squash technique from bone marrow and testis. The chromosomes were stained with 10% Giemsa's for 30 min and identified for NORs by the Ag-NOR banding technique (Howell and Black 1980). The length of the short arm (Ls) and long arm (Ll) chromosomes were measured and the total arm length of the chromosomes were determined (LT,  $LT=Ls+Ll$ ), as well as the relative length (RL) and centromeric index (CI). CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiogramming.

#### Results and discussion

All specimens possessed a standard karyotype of  $2n=36$ , with five metacentric pairs and one submetacentric pair of macrochromosomes, and 12 pairs of apparent microchromosomes. The sizes of the macrochromosomes were 4 large metacentric, 2 large submetacentric, 4 medium metacentric and 2 small metacentric (Fig. 2). This is similar from the previous study by De Smet (1981) who found  $2n=36$ , and the types of chromosomes were 12 macrochromosomes and 24 microchromosomes for *P. cocincinus*.

In the family Agamidae, two karyomorphs are typical: (1)  $2n=30, 32, 34$  or  $36$  chromosomes, including six pairs of metacentric and submetacentric macrochromosomes and 11, 12, 13 or 14 pairs of microchromosomes; and (2)  $2n=44, 46$  or  $48$  chromosomes, all telocentric macrochromosomes (11 or 12 pairs) and 11, 12 or 13 pairs of microchromosomes (Tables 1 and 2).

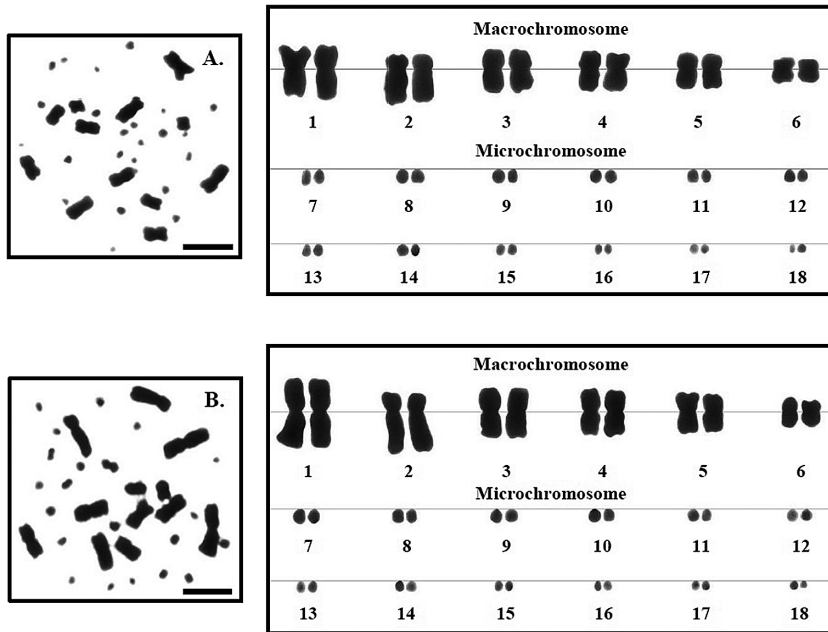


Fig. 2. Metaphase chromosome plates and karyotypes of male (A) and female (B) Indo-Chinese water dragon (*Physignathus cocincinus*)  $2n=36$  by a conventional staining technique (scale bars indicate  $5\mu\text{m}$ ).

One of these karyomorphs is considered to be derived from the other through a series of Robertsonian rearrangements (centric fusion) of macrochromosomes, sometimes accompanied with addition or deletion of one microchromosome pair (Bickham 1984, Diong *et al.* 2000).

The karyotypes of the sex chromosome of males and females are not different in contrast to the karyotypes of other agamid lizards (Diong *et al.* 2000, Ota *et al.* 2002, Srikulnath *et al.* 2009). Morphologically differentiated sex chromosomes have been described in only two species of agamid lizards, *Phrynocephalus vlangalii* and *Pogona vitticeps*. For these species, the sex chromosomes are the largest macrochromosome pair and show female heterogamety (ZZ/ZW) in *Phrynocephalus vlangalii* (Zeng *et al.* 1997), and a small microchromosome pair (ZZ/ZW micro-sex chromosomes) in *Pogona vitticeps* (Ezaz *et al.* 2005).

The results of our cytogenetic study of *P. cocincinus* using the Ag-NOR banding technique are as follows. The objective of this technique is to detect NORs which represent the location of genes that have functions in ribosome synthesis (18S and 28S ribosomal RNA) (Sharma *et al.* 2002). The region adjacent to the long arm telomeric region of chromosome pair 2 showed clearly observable NORs (Fig. 3). Moreover, the results gained from meiotic cell division on interphase (Fig. 6C) and pachytene (Fig. 6F) showed the two positions of NORs. In contrast, the head of mature spermatozoa (Fig. 6A) showed only one position of NOR. The previous studies using the Ag-NOR banding technique in the family Agamidae showed that one pair of secondary constriction/NOR in all genera is present on the large macrochromosome pair. These include the NOR which occur on the largest macrochromosome in genera *Agama* and *Leiolepis*, and pair 2 of macrochromosome in genera *Acanthocercus* and *Calotes*, and pair 9 of macrochromosome in *Prynocephalus* (Hall 1970, Solleder and Schmid 1988, Zeng *et al.* 1997).

The present study on meiotic cell division of *P. cocincinus* found that during metaphase I (Figs. 5 and 6H) the homologous chromosomes showed synapsis, which can be defined as the 18 bivalent (6 ring bivalents of macrochromosomes and 12 bivalents of microchromosomes), and 18

**Table 1.** Review of lizard cytogenetic publications in family Agamidae (Reptilia, Squamata).

Genera	TSN	SSN	2n	Mac.	Mi.	References
<i>Acanthocercus</i>	8	1	34–36	10–12 (8–10bi, 2t)	22–26	Gorman and Shochat (1972), Solleder and Schmid (1988)
<i>Acanthosaura</i>	5	1	32	12 (bi)	20	Ota <i>et al.</i> (2002)
<i>Agama</i>	33	4	42–50	20–28 (2–6bi, 18–24t)	20–24	De Smet (1981), Solleder and Schmid (1988)
<i>Amphibolurus</i>	3	2	32 *48 (3n)	12 (bi)	20	Hall (1970), Witten (1978), Witten (1983)
<i>Bronchocela</i>	9	1	48	28 (t)	20	Hall (1970)
<i>Calotes</i>	24	4	32–34	12 (bi)	20–22	Hall (1970), Singh <i>et al.</i> (1970), Sharma and Nakhasi (1980), De Smet (1981), Solleder and Schmid (1988), Kritpetcharat <i>et al.</i> (1999)
<i>Ceratophora</i>	5	1	34	12 (bi)	22	Hall (1970)
<i>Chlamydosaurus</i>	1	1	32	12 (bi)	20	Witten (1983)
<i>Cophotis</i>	2	1	28	12 (bi)	16	Hall (1970)
<i>Ctenophorus</i>	24	10	32	12 (bi)	20	Hall (1970), King (1981), Witten (1983)
<i>Diporiphora</i>	15	4	32	12 (bi)	20	Hall (1970), Witten (1983)
<i>Draco</i>	28	1	34	12 (bi)	22	Hall (1970), Kritpetcharat <i>et al.</i> (1999)
<i>Gonocephalus</i>	16	3	32–42	12–22 (bi)	20	Ota <i>et al.</i> (1992), Diong <i>et al.</i> (2000)
<i>Hypsilurus</i>	19	1	36	12 (bi)	24	Witten (1983)
<i>Japalura</i>	24	5	34–46	10–26 (0–10bi, 0–24t)	22–26	Nakamura (1935), Makino and Momma (1949), Ota (1988), Ota (1989)
<i>Laudakia</i>	20	10	34–36	12 (bi)	22–24	Hall (1970), Gorman and Shochat (1972), Arronet (1973), Sokolovsky (1975), De Smet (1981), Kupriyanova (1984)
<i>Leirolepis</i>	7	5	34–36 *54 (3n)	12 (bi) *18(3n)	22–24 *36 (3n)	Kupriyanova (1984), Solleder and Schmid (1988), Darevsky and Kupriyanova (1993), Aranyavalai <i>et al.</i> (2004), Srikulnath <i>et al.</i> (2009)
<i>Lophognathus</i>	4	2	32, 40	12 (bi), 20	20	Hall (1970), Witten (1983)
<i>Lyriocephalus</i>	1	1	30	12 (bi)	18	Moody and Hutterer (1978)
<i>Phrynocephalus</i>	44	31	44–48	20–24 (0–22bi, 8–24t)	22–26	Arronet (1973), Sokolovsky (1974), Manilo <i>et al.</i> (1991), Manilo and Golubev (1993), Manilo and Golubev (1994), Zeng <i>et al.</i> (1997), Manilo (2000)
<i>Physignathus</i>	2	2	36	12 (bi)	24	Hall (1970), De Smet (1981)
<i>Pogona</i>	7	2	32	12 (bi)	20	Witten (1983), Ezaz <i>et al.</i> (2005)
<i>Psammophilus</i>	2	1	32	12 (bi)	20	Krishna and Aswathanarayana (1979)
<i>Pseudotrapelus</i>	1	1	44	22 (0–10bi, 12–22t)	22	Gorman and Shochat (1972)
<i>Ptycolaemus</i>	2	1	34	12 (bi)	22	Sharma and Nakhasi (1980)
<i>Rankinia</i>	1	1	32	12 (bi)	20	Witten (1983)
<i>Sitana</i>	4	1	44–46	22–24 (t)	22	Dutt (1968), Makino and Asana (1948)
<i>Trapelus</i>	14	8	44–46	22–24 (t)	22–24	Hall (1970), Gorman and Shochat (1972), Sokolovsky (1975), Solleder and Schmid (1988)
<i>Tympanocryptis</i>	7	1	32	12 (bi)	20	Witten (1983)
<i>Uromastix</i>	17	3	34–36	12 (bi)	22–24	Gorman and Shochat (1972), Makino and Asana (1948)

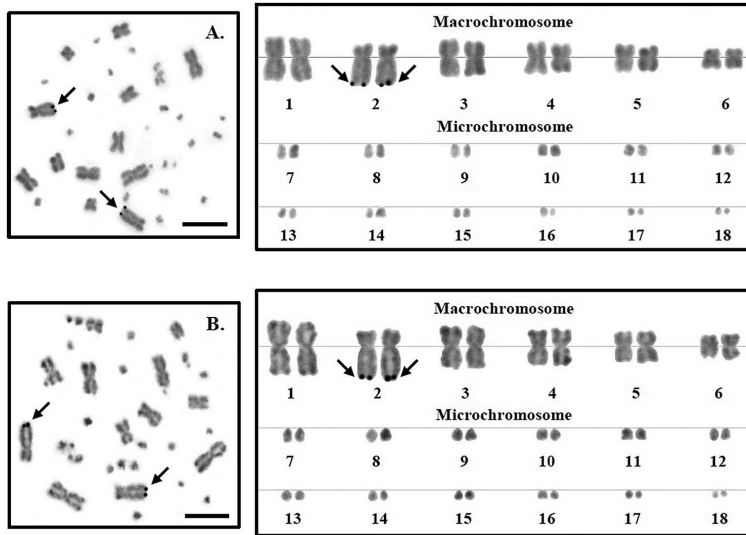
\* TSN from Roskov *et al.* (2013).

Remarks: TSN=total species number, SSN=studied species number, 2n=diploid chromosome number, 3n=triploid chromosome number, Mac.=macrochromosome, Mi.=microchromosome, bi=bi-arms chromosome, and t=telocentric chromosome.

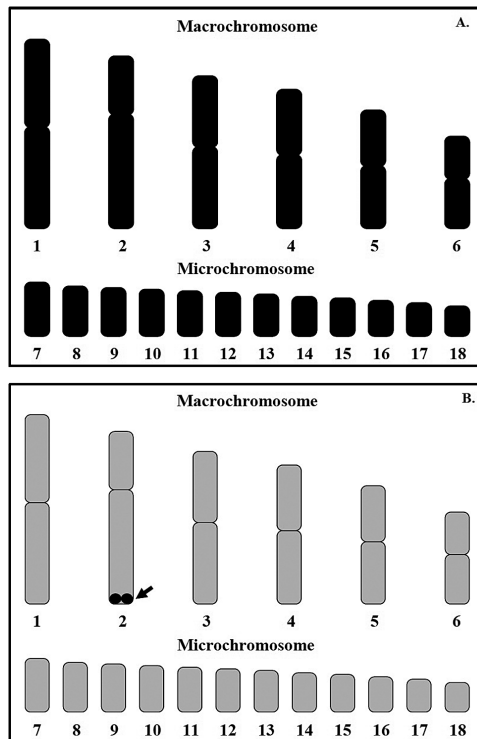
**Table 2.** Review of water dragon lizard cytogenetic publications in the genus *Physignathus* (Squamata, Agamidae)

Species	2n	NF	Karyotype formulas	NOR	Locations	References
<i>P. lesuerii</i>	36	48	12bi-arms+24mi.	—	—	Hall (1970)
<i>P. cocincinus</i>	36	48	12bi-arms+24mi.	—	—	De Smet (1981)
	36	48	10m+2sm+24mi.	p (L) 2	Thailand	Present study

Remarks: 2n=diploid chromosome number, NF=fundamental number (number of chromosome arm), m=metacentric, sm=submetacentric, mi=microchromosome, p=chromosome pair, L=long arms, and —=not available.



**Fig. 3.** Metaphase chromosome plates and karyotypes of male (A) and female (B) Indo-Chinese water dragon (*Physignathus cocincinus*)  $2n=36$  by Ag-NOR banding technique. Nucleolar organizer regions/NORs appear to the telomere on long arm of the submetacentric chromosome pair 2 (arrows). Scale bars indicate  $5\mu\text{m}$ .



**Fig. 4.** Standardized idiogram of Indo-Chinese water dragon (*Physignathus cocincinus*)  $2n=36$  by conventional staining (A) and Ag-NOR banding techniques (B). Arrow indicates nucleolar organizer region/NOR.

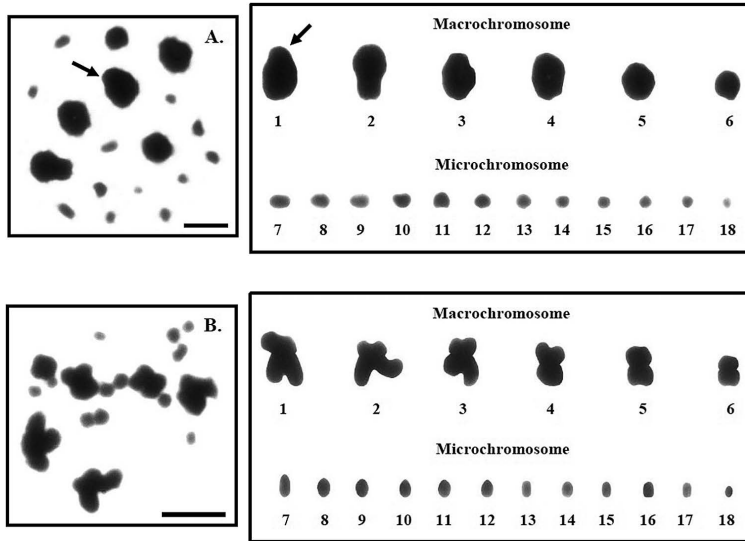


Fig. 5. The meiotic cell division of metaphase I chromosome (A) and metaphase II chromosome (B) of Indo-Chinese water dragon (*Physignathus cocincinus*) by conventional staining technique. Scale bars indicate 5  $\mu$ m.

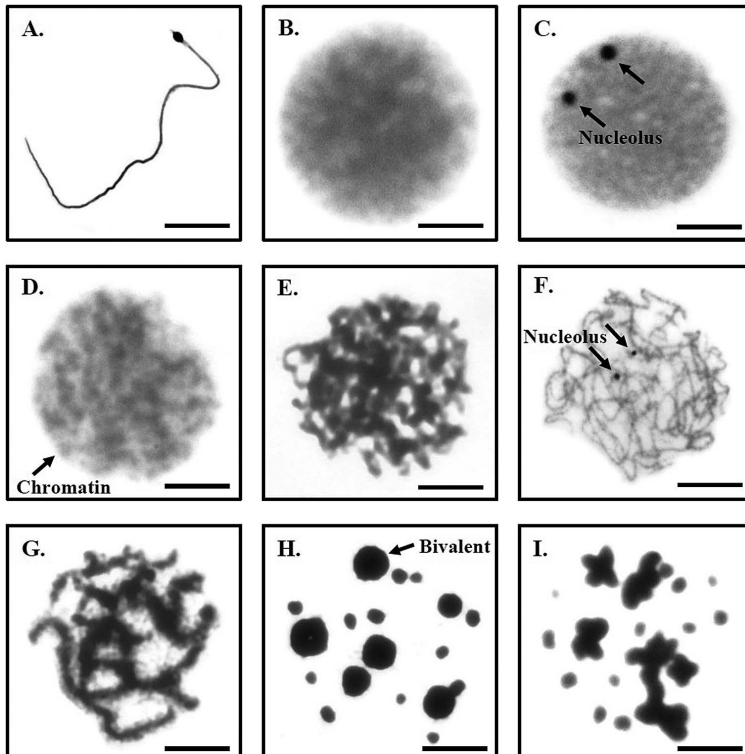


Fig. 6. Meiotic cell division of the Indo-Chinese water dragon (*Physignathus cocincinus*)  $2n=36$  on mature sperm (A), interphase (B and C), leptotene (D), pachytene (E and F), diplotene (G), metaphase I (H), and metaphase II (I). Scale bars indicate 10  $\mu$ m

**Table 3.** Mean length (L) of the short arm chromosome (Ls), long arm chromosome (Ll), and total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from metaphase chromosomes in 20 cells of male and female Indo-Chinese water dragon (*Physignathus cocincinus*),  $2n=36$ .

Chromosome pairs	Ls	Ll	LT	CI±SD	RL±SD	Types	Sizes
1	1.039	1.199	2.238	0.536±0.009	0.139±0.011	m	L
2*	0.683	1.352	2.035	0.664±0.025	0.126±0.010	sm	L
3	0.847	0.959	1.806	0.531±0.007	0.112±0.006	m	L
4	0.772	0.865	1.637	0.528±0.013	0.102±0.004	m	M
5	0.654	0.740	1.394	0.531±0.018	0.086±0.003	m	M
6	0.499	0.582	1.081	0.538±0.012	0.067±0.003	m	S
7	—	—	0.636	—	0.039±0.005	microchromosome	
8	—	—	0.589	—	0.037±0.004	microchromosome	
9	—	—	0.569	—	0.035±0.003	microchromosome	
10	—	—	0.551	—	0.034±0.003	microchromosome	
11	—	—	0.531	—	0.033±0.003	microchromosome	
12	—	—	0.507	—	0.032±0.003	microchromosome	
13	—	—	0.487	—	0.030±0.002	microchromosome	
14	—	—	0.463	—	0.029±0.002	microchromosome	
15	—	—	0.444	—	0.028±0.002	microchromosome	
16	—	—	0.416	—	0.026±0.002	microchromosome	
17	—	—	0.386	—	0.024±0.002	microchromosome	
18	—	—	0.352	—	0.022±0.002	microchromosome	

Remark: \*=Nucleolar organizer region/NOR, m=metacentric, sm=submetacentric, L=large, M=medium, and S=small.

haploid chromosomes at metaphase II (Figs. 5 and 6I) as in diploid species. The largest metacentric chromosome pair 1 is the largest ring bivalent. No metaphase I cells with partially paired bivalents that are speculated to be heteromorphic sex chromosomes, and no metaphase II cells with condensed chromosomes that are speculated to be the Y or W chromosomes were detected. In prophase I (meiosis I), we found that *P. cocincinus* had the distinctness of the observable leptotene (initiation of chromosome shrinking, Fig. 6D); pachytene (completion of chromosome synapsis, Figs. 6E and F) and diplotene (chiasma and crossing over, Fig. 6G).

The important chromosome marker of *P. cocincinus* is the symmetrical karyotype that was found in only metacentric and submetacentric macrochromosomes. The largest and smallest chromosomes show size differences (approximately eight fold). The chromosome marker is macrochromosome pair 1 which is the largest metacentric chromosome. Data of the chromosomal checks on mitotic metaphase cells are shown in Table 3. An idiogram by conventional staining and Ag-NOR banding techniques is shown in Fig. 4. The idiogram shows gradually decreasing length of macrochromosomes and microchromosomes. The karyotype formula of *P. cocincinus* is as follows:  $2n(36)=L_4^m+L_2^{sm}+M_4^m+S_2^m+24$  microchromosomes.

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