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Chromosomal description of three *Dixonius* (Squamata, Gekkonidae) from Thailand

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Abstract. Chromosomal characteristics and karyological analysis of three *Dixonius*, including *D. hangseesom*, *D. siamensis* and *D. melanostictus*, from Thailand were studied. Chromosome preparations were conducted by squash technique from bone marrow and testis. Conventional Giemsa's staining and Ag-NOR banding techniques were applied to stain the chromosome. The results showed that the diploid chromosomes are 2n=40, for *D. hangseesom* and *D. siamensis*; and 2n=42, for *D. melanostictus*. The fundamental number (NF) is 42 in *D. hangseesom* and *D. siamensis* and 44 in *D. melanostictus*. The types of chromosomes were 2 metacentrics and 38 telocentrics for *D. hangseesom* and *D. siamensis*, while the karyotype of *D. melanostictus* comprised 2 acrocentrics and 40 telocentrics. In the *D. hangseesom* and *D. siamensis*, NORs are located to the near centromere on long arm of the telocentric chromosome pair 13. Although, the NORs of *D. melanostictus* are situated on the subtelomeric region of telocentric chromosome pair 8. There are no sex differences in karyotypes between males and females of these three geckos species. We found that during metaphase I and metaphase II on male meiosis of the *D. hangseesom* and *D. siamensis*, the homologous chromosomes showed synapsis of 20 bivalents and 20 haploid chromosomes (n=20). Moreover, metaphase I and metaphase II of the male *D. melanostictus* showed synapsis of 21 bivalents and 21 haploid chromosomes (n=21). Their karyotype formulas is as follows: *D. hangseesom* (2n=40): $L^m_2 + L^t_2 + M^t_4 + S^t_{32}$, *D. siamensis* (2n=40): $L^m_2 + L^t_6 + M^t_4 + S^t_{28}$, and *D. melanostictus* (2n=42): $L^a_2 + L^t_{12} + M^t_4 + S^t_{24}$.

Keywords: *Dixonius*, chromosome, karyotype, Nucleolar Organizer Region (NOR).

INTRODUCTION

Dixonius is a genus of Asian geckos or commonly known as leaf-toed geckos that belong to the class Reptilia, order Squamata, and family Gekkonidae. The *Dixonius* was first chosen to accommodate Southeast Asian leaf-toed geckos previously to the polyphyletic and nearly cosmopolitan *Phyllodactylus* (Bauer et al. 1997). The *D. siamensis* is the type species of the genus *Dixonius*, which receive name from the first zoologist that note *siamensis* (Dixon 1964). In Thailand, total 7 species of *Dixonius* were found including *D. dulayaphitakorum, D. hangseesom*, *D. kaweesaki, D. mekongensis, D. melanostictus*, *D. pawangkhananti and D. siamensis* (Sumontha et al. 2017; Pauwels et al. 2020, 2021).

Only about 10% of gekkonid species have been karyotyped and were studied with classical cytogenetic methods, including routine staining, as well as R, NOR and C banding (Moritz 1984; Shibaike et al. 2009). The series from 2n=28 to 46 of the diploid chromosomes is characteristic of the gekkonid lizard's karyotype (Gorman 1973; King 1987; Schmid et al. 1994). The typical karyotype consists of a gradual series of mono-armed chromosome (sometimes with a few bi-armed chromosome), and there is no distinction between macro- and microchromosomes are present, the centromere is often subterminal (Gorman 1973). Karyotype evolution within the group is accompanied by Robertsonian fusions, fissions and pericentric inversions (Gorman 1973; King 1987). Examples of the gekkonid's chromosome study in Thailand that were reported: *Gekko gecko*, 2n=38=12bi-armed+26mono-armed (Patawang et al. 2014); *Hemidactylus frenatus*, 2n=40=34bi-armed+6mono-armed; *H. platyurus*, 2n=46=2bi-armed+44mono-armed (Patawang and Tanomtong 2015); *Cyrtodactylus kunyai*, 2n=40=12bi-armed+28mono-armed; *C. interdigitalis*, 2n=42=10bi-armed+32mono-armed (Thongnetr et al. 2019); *C. jarujini*, 2n=40=28bi-armed+12mono-armed; and *C. doisuthep*, 2n=34=28bi-armed+6mono-armed (Thongnetr et al. 2021) etc.

In Thailand, there is only one previous report on *Dixonius* species chromosome. Ota et al. (2001) demonstrated that *D. siamensis*' karyotype by conventional staining technique is as 2n=42, male and female specimen from Mae Yom National Park, Phrae Province, Thailand; and 2n=40, male from Phu Wua Wild Life Reserve Area, Nongkhai Province, Thailand. The present study of the karyological analysis of *D. hangseesom*, *D. siamensis* and *D. melanostictus*, provides the first report on the Ag-NOR banding technique, chromosome size, chromosome type, karyotype formula, and standardized idiogram which are compared to earlier reports.

MATERIALS AND METHODS

Sample collection

Both male and female of three *Dixonius* species (Figure 1) were collected from three sites in Thailand, *D. hangseesom* from Kanchanaburi Province; *D. siamensis* from Chiang Mai Province; and *D. melanostictus* from Saraburi Province. The geckos were transferred to the laboratory and kept under standard conditions for one day prior to the experimentation.

Chromosome preparation

Chromosomes were directly prepared *in vivo* (Patawang et al. 2014) by injecting phytohemagglutinin (PHA) solution into its abdominal muscle. After ten hours, colchicine is injected to animal intramuscular and its abdominal cavity and left for 8-10 hours. Bone marrow (in male and female) and testis (in male) are cut

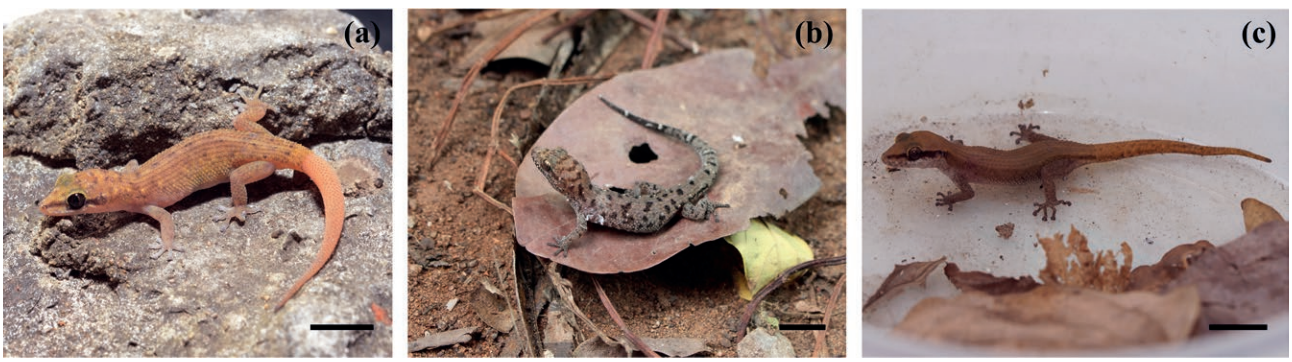

Figure 1. General characteristic of the *D. hangseesom* (a), *D. siamensis* (b) and *D. melanostictus* (c) from Thailand. Scale bars indicate 1 centimeter.

into small pieces then mix squash with 0.075M potassium chloride (KCl). After discarding all large cell pieces, 15 ml of cell sediment is transferred to a centrifuge tube and incubated for 30-40 minutes. Cells were fixed in fresh cool Canoy fixative (3 absolute methyl alcohol: 1 glacial acetic acid) gradually added up to 8 ml before centrifuging again at 3,000 rpm for 10 minutes, 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 micropipette followed by the air-drying.

Chromosome staining

Conventional staining (Gosden 1994)

The mixture is dropped onto a clean and cold slide by micropipette followed by the air-dry technique. The slide is conventionally stained with 20% Giemsa's solution for 30 minutes.

Ag-NOR banding (Howell and Black 1980)

The two drops of each 50% silver nitrate and 2% gelatin were added on slides, respectively. Then it was sealed with cover glasses and incubated at 60 °C for 5-10 minute. There after that it was soaked in distilled water until cover glasses are separated.

Chromosome checking

Ten clearly observable cells with well spread chromosomes of each male and female were selected and photographed. The length of short arm chromosome (Ls) and long arm chromosome (Ll) were measured and the length of 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 and Lejeune 1965; Chaiyasut 1989). The CI (q/[p+q]) between 0.500-0.599, 0.600-0.699, 0.700-0.899, and 0.900-1.000 were described as metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively. The fundamental number (NF, number of chromosome arms) was obtained by assigning a value of 2 to the metacentric, submetacentric and acrocentric chromosomes and 1 to the telocentric chromosome. All data were used in karyotyping and idiograming.

RESULTS AND DISCUSSION

Diploid number and chromosome characteristics

Karyomorphology of the *D. hangseesom* and *D. siamensis* revealed that the diploid chromosome number (2n) is 40 and the *D. melanostictus* showed 2n=42. The karyotypes of male and female *D. hangseesom* composed 2 large metacentrics, 2 large telocentrics, 4 medium telocentrics and 32 small telocentrics (Table 1 and Figures 2a-b, 4a). For the karyotypes of male and female *D. siamensis* comprised 2 large metacentrics, 6 large telocentrics, 4 medium telocentrics and 28 small telocentrics (Table 2 and Figures 2c-d, 4b). Though, both sexs of *D. melanostictus*'s karyotypes consisted 2 large acrocentrics, 12 large telocentrics, 4 medium telocentric and 24 small telocentrics (Table 3 and Figures 2e-f, 4c). All three *Dixonius* species exhibit no sex differences in karyotypes between males and females (Figures 2a-f). The chromosomal characteristic of *D. hangseesom* and *D. siamensis* showed more the closed relationship than *D. melanostictus*. Karyotypes of the *D. hangseesom* and *D. siamensis* revealed the same of diploid number and chromosome type, which has only different chromosome size. Their karyotype formulas is as follows:

$2n=40=L^m_2 + L^t_2 + M^t_4 + S^t_{32}$ (*D. hangseesom*)
$2n=40=L^m_2 + L^t_6 + M^t_4 + S^t_{28}$ (*D. siamensis*)
$2n=42=L^a_2 + L^t_{12} + M^t_4 + S^t_{24}$ (*D. melanostictus*).

The chromosome character of *D. hangseesom* (2n=40) and *D. melanostictus* (2n=42) from this study is the first report of both species. However, the diploid result of *D. siamensis* (2n=40) in this report both showed difference and accordance with *D. siamensis* in the reported of Ota et al. (2001). It is possible that reported by Ota et al. (2001) may have studied more than one with the complex species. However, overall of these karyotypes of *D. hangseesom*, *D. siamensis* and *D. melanostictus* resemble to other gekkonid, which comprised many gradient mono-armed (telocentric) and few bi-armed chromosomes (meta-, submeta- or acrocentric). Proximity of chromosome number and karyotype feature within genus *Dixonius* represents a close evolutionary line in the group.

Nucleolar organizer regions and haploid number

This study is the first report of nucleolar organizer regions in *D. hangseesom*, *D. siamensis* and *D. melanostictus*. In both sexs of the *D. hangseesom* and *D. siamensis*, we found the clearly observable NORs on the region

Table 1. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), length of total chromosomes (LT), centromeric index (CI), relative length (RL) and standard deviation (SD) of CI and RL from 20 metaphases of male and female yellow-tailed leaf-toed gecko (*Dixonius hangseesom*) 2n=40.

Chr pair	Ls	Ll	LT	CI±SD	RL±SD	Chr size	Chr type
1	6.156	6.718	12.874	0.522±0.024	0.143±0.002	Large	Metacentric
2	0.000	10.305	10.305	1.000±0.000	0.114±0.002	Large	Telocentric
3	0.000	7.204	7.204	1.000±0.000	0.080±0.001	Medium	Telocentric
4	0.000	6.470	6.470	1.000±0.000	0.072±0.003	Medium	Telocentric
5	0.000	5.445	5.445	1.000±0.000	0.060±0.002	Small	Telocentric
6	0.000	4.924	4.924	1.000±0.000	0.055±0.003	Small	Telocentric
7	0.000	4.434	4.434	1.000±0.000	0.049±0.003	Small	Telocentric
8	0.000	4.037	4.037	1.000±0.000	0.045±0.003	Small	Telocentric
9	0.000	3.661	3.661	1.000±0.000	0.040±0.001	Small	Telocentric
10	0.000	3.484	3.484	1.000±0.000	0.039±0.002	Small	Telocentric
11	0.000	3.297	3.297	1.000±0.000	0.037±0.002	Small	Telocentric
12	0.000	3.036	3.050	1.000±0.000	0.034±0.001	Small	Telocentric
13*	0.000	3.050	3.036	1.000±0.000	0.034±0.002	Small	Telocentric
14	0.000	3.007	3.007	1.000±0.000	0.033±0.001	Small	Telocentric
15	0.000	2.724	2.724	1.000±0.000	0.030±0.003	Small	Telocentric
16	0.000	2.661	2.671	1.000±0.000	0.030±0.003	Small	Telocentric
17	0.000	2.671	2.661	1.000±0.000	0.029±0.003	Small	Telocentric
18	0.000	2.434	2.434	1.000±0.000	0.027±0.001	Small	Telocentric
19	0.000	2.273	2.273	1.000±0.000	0.025±0.001	Small	Telocentric
20	0.000	2.239	2.239	1.000±0.000	0.025±0.002	Small	Telocentric

Abbreviations: Chr, chromosome; *, NORs bearing chromosomes.

adjacent to subcentromeric of the telocentric chromosome pair 13th (Figures 3a-d, 4a-b). Whereas, the NORs of male and female *D. melanostictus* are situated on the subtelomeric region of telocentric chromosome pair 8th (Figures 3e-f, 4c). The NORs characteristic of *D. hangseesom* and *D. siamensis* showed more the closed relationship than *D. melanostictus*. Position of nucleolar organizer regions of the *D. hangseesom* and *D. siamensis* revealed the same located, subcentromeric of telocentric chromosome pair 13th. Compared with other geckos, most showed two NORs appearing near terminal region (centromere or telomere) of small bi-armed or small mono-armed chromosome. An example of the previous reports of the geckos' NOR localization included in the genus *Cyrtodactylus* (Thongnetr et al. 2019, 2021), *Gehyra* (King 1983), *Gekko* (Chen et al. 1986; Shibaike et al. 2009; Patawang et al. 2014), *Hemidactylus* (Patawang and Tanomtong 2015), *and Lepidodactylus* (Trifonov et al. 2015). These previous studies showed the NOR appearing near terminal region of one homologous small chromosome.

The metaphase I (meiosis I, reductional division) was found which can be defined as the 20 bivalents for *D. hangseesom* (Figure 5a) and *D. siamensis* (Figure 5c), and 21 bivalents for *D. melanostictus* (Figure 5e). No metaphase I cells with partially paired bivalents, which are speculated to be male heteromorphic sex chromosomes in these three *Dixonius* species. Moreover, haploid chromosome of n=20 in *D. hangseesom* (Figure 5b), n=20 in *D. siamensis* (Figure 5d) and n=21 in *D. melanostictus* (Figure 5f) were found at metaphase II (meiosis II, equational division) of spermatid cells. For these results, behavior and number of chromosomes in metaphase I and metaphase II confirmed of each other's accuracy and also verified the accuracy of diploid chromosome in somatic cells.

An overview of Dixonius chromosomal feature and their chromosome evolution

Gekkonid chromosome that has been reported in the past, most species show the gradient karyotype, which comprising of many mono-armed chromosomes and few bi-armed chromosomes. Present results of *D. hangseesom*, *D. siamensis* and *D. melanostictus* agree with chromosomal evolution line hypothesis within the gekkonid group. The karyotype of these three *Dixonius* showed the gradient of major telocentric, while just comprised 2 bi-armed chromosomes. These features conform to the

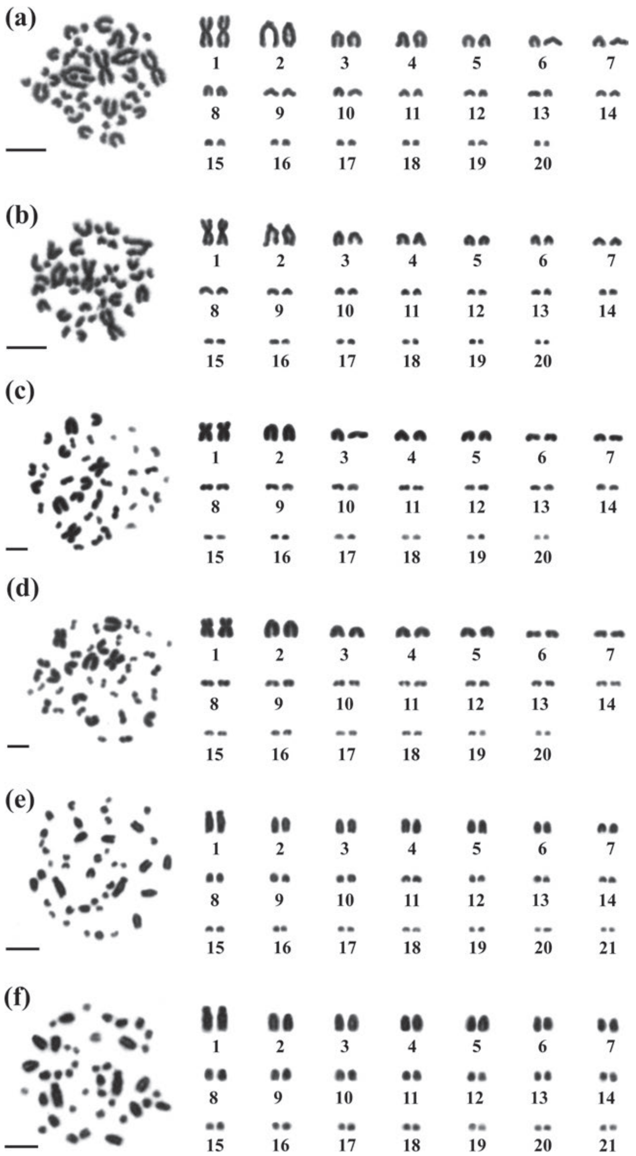

Figure 2. Metaphase chromosome plates and karyotypes using conventional Giemsa's staining of male (a) and female (b) *D. hangseesom* (2n=40); male (c) and female (d) *D. siamensis* (2n=40); and male (e) and female (f) *D. melanostictus* (2n=42). Scale bars indicate 10 micrometers.

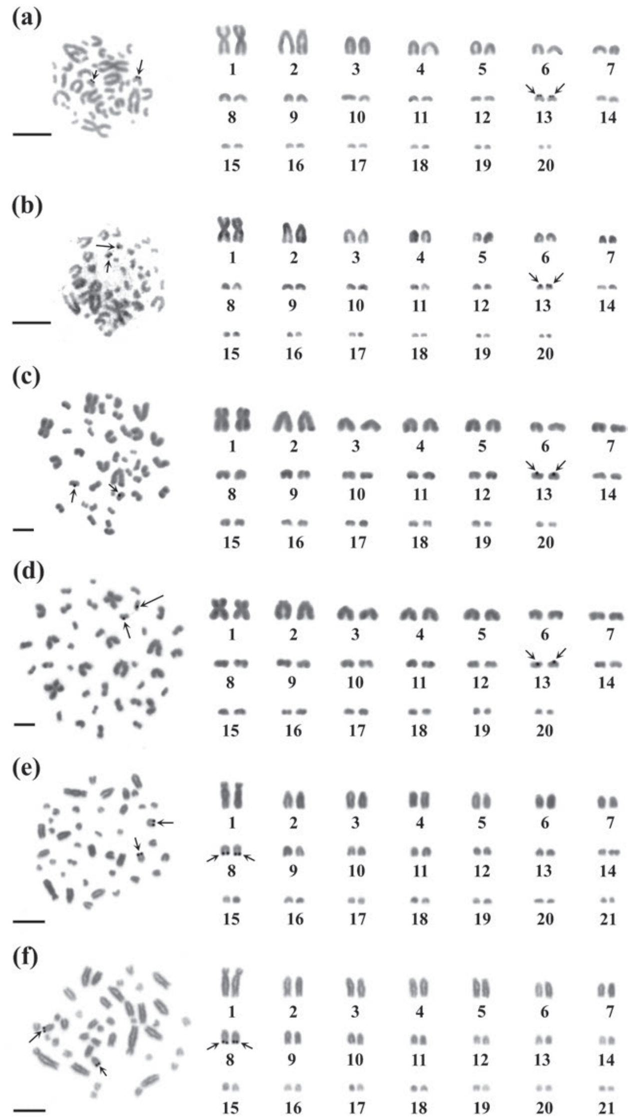

Figure 3. Metaphase chromosome plates and karyotypes using Ag-NOR staining of male (a) and female (b) *D. hangseesom* (2n=40); male (c) and female (d) *D. siamensis* (2n=40); and male (e) and female (f) *D. melanostictus* (2n=42). Arrows indicate nucleolar organizer regions (NORs) and scale bars indicate 10 micrometers.

hypothesis of rearrangement from ancestral karyotype by Robertsonian fusions, fissions or pericentric inversions (Gorman 1973; King 1987). In this study, from all chromosome characters by conventional Giemsa's staining and Ag-NOR banding techniques, we suggest that the chromosome of *Dixonius* showed a high genetic conservation and there were only a few changes at the chromosome structure level. However, the chromosome of *D. hangseesom* and *D. siamensis* showed more the closed evolutionary relationship than *D. melanostictus*.

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Table 2. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), length of total chromosomes (LT), centromeric index (CI), relative length (RL) and standard deviation (SD) of CI and RL from 20 metaphases of male and female Siamese leaf-toed gecko (*Dixonius siamensis*), 2n=40.

Chr pair	Ls	Ll	LT	CI±SD	RL±SD	Chr size	Chr type
1	5.925	6.102	12.027	0.507±0.032	0.125±0.002	Large	Metacentric
2	0.000	10.928	10.928	1.000±0.000	0.113±0.001	Large	Telocentric
3	0.000	8.012	8.012	1.000±0.000	0.083±0.002	Large	Telocentric
4	0.000	7.072	7.072	1.000±0.000	0.073±0.003	Large	Telocentric
5	0.000	6.821	6.821	1.000±0.000	0.071±0.003	Medium	Telocentric
6	0.000	6.121	6.121	1.000±0.000	0.064±0.002	Medium	Telocentric
7	0.000	5.814	5.814	1.000±0.000	0.060±0.002	Small	Telocentric
8	0.000	5.214	5.214	1.000±0.000	0.054±0.001	Small	Telocentric
9	0.000	4.012	4.012	1.000±0.000	0.042±0.002	Small	Telocentric
10	0.000	3.628	3.628	1.000±0.000	0.038±0.003	Small	Telocentric
11	0.000	3.421	3.421	1.000±0.000	0.036±0.002	Small	Telocentric
12	0.000	3.214	3.214	1.000±0.000	0.033±0.001	Small	Telocentric
13*	0.000	3.042	3.042	1.000±0.000	0.032±0.001	Small	Telocentric
14	0.000	2.988	2.988	1.000±0.000	0.031±0.002	Small	Telocentric
15	0.000	2.756	2.756	1.000±0.000	0.029±0.003	Small	Telocentric
16	0.000	2.524	2.524	1.000±0.000	0.026±0.003	Small	Telocentric
17	0.000	2.326	2.326	1.000±0.000	0.024±0.001	Small	Telocentric
18	0.000	2.214	2.214	1.000±0.000	0.023±0.001	Small	Telocentric
19	0.000	2.112	2.112	1.000±0.000	0.022±0.002	Small	Telocentric
20	0.000	2.042	2.042	1.000±0.000	0.021±0.002	Small	Telocentric

Abbreviations: Chr, chromosome; *, NORs bearing chromosomes.

Table 3. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), length of total chromosomes (LT), centromeric index (CI), relative length (RL) and standard deviation (SD) of CI and RL from 20 metaphases of male and female dark-sides ground gecko (*Dixonius melanostictus*), 2n=42.

Chr pair	Ls	Ll	LT	CI±SD	RL±SD	Chr size	Chr type
1	2.019	5.945	7.964	0.746±0.029	0.101±0.002	Large	Acrocentric
2	0.000	7.363	7.363	1.000±0.000	0.094±0.002	Large	Telocentric
3	0.000	7.241	7.241	1.000±0.000	0.092±0.001	Large	Telocentric
4	0.000	7.026	7.026	1.000±0.000	0.089±0.003	Large	Telocentric
5	0.000	6.736	6.736	1.000±0.000	0.086±0.003	Large	Telocentric
6	0.000	6.159	6.159	1.000±0.000	0.078±0.002	Large	Telocentric
7	0.000	4.739	4.739	1.000±0.000	0.060±0.002	Large	Telocentric
8*	0.000	4.506	4.506	1.000±0.000	0.057±0.001	Medium	Telocentric
9	0.000	4.102	4.102	1.000±0.000	0.052±0.003	Medium	Telocentric
10	0.000	3.824	3.824	1.000±0.000	0.049±0.002	Small	Telocentric
11	0.000	3.256	3.256	1.000±0.000	0.041±0.003	Small	Telocentric
12	0.000	2.109	2.109	1.000±0.000	0.027±0.003	Small	Telocentric
13	0.000	2.047	2.047	1.000±0.000	0.026±0.001	Small	Telocentric
14	0.000	2.024	2.024	1.000±0.000	0.026±0.001	Small	Telocentric
15	0.000	1.812	1.812	1.000±0.000	0.023±0.001	Small	Telocentric
16	0.000	1.556	1.556	1.000±0.000	0.020±0.002	Small	Telocentric
17	0.000	1.508	1.508	1.000±0.000	0.019±0.003	Small	Telocentric
18	0.000	1.375	1.375	1.000±0.000	0.017±0.002	Small	Telocentric
19	0.000	1.130	1.130	1.000±0.000	0.014±0.002	Small	Telocentric
20	0.000	1.062	1.062	1.000±0.000	0.014±0.002	Small	Telocentric
21	0.000	1.057	1.057	1.000±0.000	0.013±0.001	Small	Telocentric

Abbreviations: Chr, chromosome; *, NORs bearing chromosome.

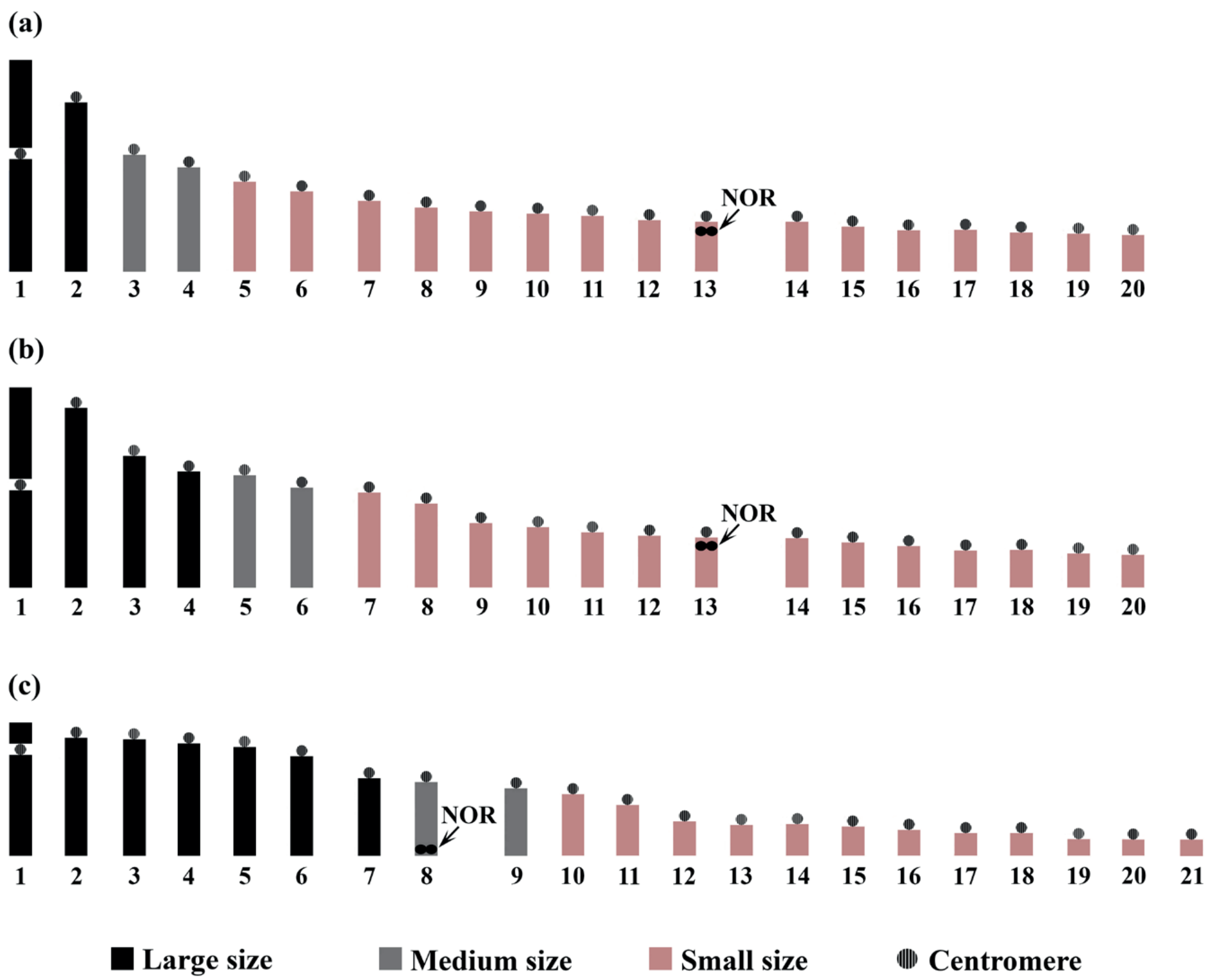

Figure 4. Standardized idiogram of *D. hangseesom* (a), *D. siamensis* (b) and *D. melanostictus* (c). Arrows indicate nucleolar organizer regions (NORs).

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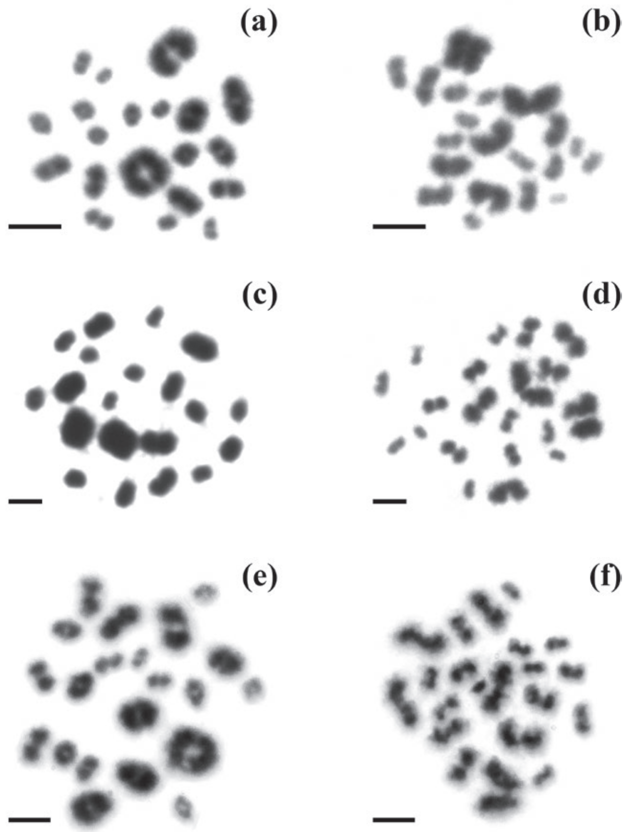

Figure 5. Respective bivalent on metaphase I and haploid chromosome on metaphase II of male *D. hangseesom* (20 bivalents, a; 20 haploid chromosomes, b), male *D. siamensis* (20 bivalents, c; 20 haploid chromosomes, d) and male *D. melanostictus* (21 bivalents, e; 21 haploid chromosomes, f). Scale bars indicate 5 micrometers.

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