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A New Report on Karyological Analysis of the Jewel-Beetle, *Sternocera ruficornis* (Coleoptera, Buprestidae) from Thailand

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Abstract-The first karyological analysis of the *Sternocera ruficornis* (Coleoptera, Buprestidae) was achieved by using the squash technique collected from the male gonad. Furthermore, the chromosomes were stained by conventional staining and the Ag-NOR banding techniques. The results showed the diploid number of chromosomes was 2*n*=26, comprising 4 metacentric, 6 submetacentric, 6 acrocentric and 8 telocentric chromosomes. The sex chromosome system was XY system with X and Y chromosomes being metacentric and acrocentric chromosomes, respectively. Heteromorphism (1a1b) nucleolar organizer regions (NORs) are clearly observed near the centromere of chromosome pair one and the subcentromeric region of chromosome pair nine. The karyotype formula demonstrated as follows: 2*n* (26) =Lm2+Sm2+Ssm6+Sa6+St8+XY.

Keywords: Jewel-beetles, *Sternocera ruficornis*, karyotype, Ag-NOR banding

1. Introduction

The family Buprestidae (Coleoptera) is one of the largest group of Polyphagan beetles comprising approximately 15,500 species in 775 genera. In addition, almost 100 fossil species have been described (Bellamy 2008a-d, 2009). The jewel-beetles (*Sternocera ruficornis*) are classified into the order Coleoptera, family Buprestidae, subfamily Julodinae, genus *Sternocera*. In Thailand, there are two species of buprestid belonging to the genus Sternocera. They are red-legged *Sternocera ruficornis* and green legged *Sternocera aequisignata* which can be which can be found throughout Thailand (Ohmomo & Akiyama, 1972). The characteristics of *S. ruficornis* are a rounded metallic green body, reddish legs, and an average body length of between 30-45 mm (Figure 1). There are widely overspread in Laos, Vietnam, and Thailand, especially in the northeast of Thailand (Ek-Amnuay, 2008).

Jewel-Beetles are edible, then, their wings are made into jewelry and other marketable objects. Moreover, their numbers have declined because they are quite popular among insect collectors. (Pinkaew, 2001)

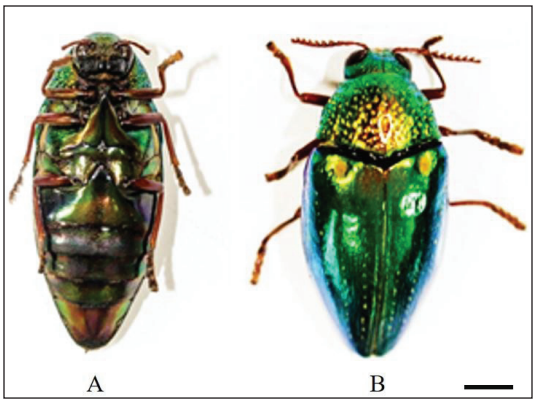

Figure 1. General characteristics of the Jewel-Beetles, *Sternocera ruficornis*, (A) ventral view, and (B) dorsal view (scale bar indicate 1 cm).

Karyotype studies of jewels-beetles have been published for 92 species (34 from Armenia) in 22 genera, 14 tribes and 5 subfamilies (Julodinae, Polycestinae, Chrysochroinae, Buprestinae and Agrilinae) (Smith & Virkki 1978 ; 1991 ; Karagyan & Kuznetsova 2000 ; Karagyan 2001 ; Karagyan *et al*., 2004 ; Karagyan & Lachowska 2007 ; Moura *et al*., 2008 ; Karagyan *et al*., 2012). Recently, there are only three cytogenetics studies on genus *Sternocera* as *Sternocera* sp., *S. laevigata* and *S. nitidicollis* (Karagyan & Kuznetsova, 2000), demonstrated by conventional staining technique suggesting that 2n=26 with XY sex chromosome system (Table 1). Our work aimed to analyze the chromosomal information of *S. ruficornis* by conventional staining and Ag-NOR staining techniques. Furthermore, the cytogenetic data of *S. ruficornis* will be applied for studies of breeding, conservation and chromosomal evolution among related beetle species.

Table 1. Cytogenetic data review for genus *Stenocera*

species	2*n*	NORs	Karyotype	references
Stenocera sp	26	-	24 autosomes+XY	Dasgupta (1977)
Sternocera laevigata	26	-	24 autosomes+XY	Karagyan and Kuznetsova (2000)
Sternocera nitidicollis	26	-	24 autosomes+XY	Karagyan and Kuznetsova (2000)
Sternocera ruficornis	26	2 pair	24 autosomes+XY	This study

2. Materials and Methods

2.1 Chromosome Preparation

The specimens were collected from the Kalasin province, northeastern Thailand and identified according to the categories of Ek-Amnuay (2008). Chromosomes were prepared directly in vivo. Firstly, colchicine was injected into the abdominal cavity of each insect and left for 1 hour. Gonad cells were collected by cutting tissue into small pieces, mixed and incubated with 0.075 M KCl for 30-45 minutes. The fresh and cool fixative (3 methanol: 1 glacial acetic acid) terminated KCl activities then centrifuged at 3,000-3,200 rpm for 10 minutes. The supernatants were separated and discarded. Fixative solutions were added and centrifuged again to wash the cell sediments for 2-3 times. The obtained cell sediments were kept at -20°C for chromosome studies.

2.2 Conventional Staining and Ag-NOR Banding Technique

The cell suspension was dropped on to completely clean slides and fixed with a fixative solution. The slides were infused in a mixture of 20% Giemsa's for 15 minutes and rinsed with distilled water (Tanomtong *et al*., 2014).

Ag-NOR banding was performed by applying two drops of 2% gelatin on the slides, followed by four drops of 50% silver nitrate, respectively. The slides were sealed with cover glasses and incubated at 60 °C for 5-10 minutes, then soaked in distilled water until cover glasses are separated. The slides were then kept for examination with a light microscope (Chaiyasan *et al*., 2018).

2.3 Data Analysis

Twenty clearly observable cells with well spread chromosomes were selected and photographed. 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 (Chaiyasut, 1989). 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 0.90–1.00 are described as metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively.

Table 2. Mean of length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from metaphase chromosome in 20 cells of male Jewel-Beetles (*Sternocera ruficornis*, 2*n*=26)

Chro. pairs	Ls (µm)	Ll (µm)	LT (µm)	RL±SD	CI±SD	Size	Type
1a	6.021	6.761	12.782	0.269±0.006	0.530±0.024	Large	Metacentric
1b	5.476	6.237	11.713	0.247±0.008	0.533±0.031	Large	Metacentric
2	1.246	1.708	2.955	0.061±0.007	0.581±0.046	Small	Metacentric
3	1.152	1.744	2.896	0.061±0.003	0.604±0.024	Small	Submetacentric
4	1.106	1.694	2.799	0.058±0.004	0.605±0.039	Small	Submetacentric
5	0.823	1.823	2.646	0.055±0.002	0.687±0.061	Small	Submetacentric
6	0.726	1.835	2.561	0.054±0.002	0.717±0.053	Small	Acrocentric
7	0.548	1.867	2.415	0.051±0.003	0.772±0.046	Small	Acrocentric
8	0.522	1.711	2.234	0.047±0.004	0.773±0.149	Small	Acrocentric
9	0.000	1.973	1.973	0.041±0.002	1.000±0.000	Small	Telocentric
10	0.000	1.864	1.864	0.039±0.003	1.000±0.001	Small	Telocentric
11	0.000	1.844	1.844	0.039±0.004	1.000±0.002	Small	Telocentric
12	0.000	1.671	1.671	0.035±0.004	1.000±0.003	Small	Telocentric
X	2.706	1.671	5.813	0.122±0.004	0.534±0.028	Small	Metacentric
Y	0.615	3.075	3.689	0.077±0.003	0.836±0.081	Small	Acrocentric

* Large chromosome (LT>6.141), Small chromosome (LT<6.132),
** Chro. = Chromosome

3. Results

3.1 Diploid Chromosome Number (2n) and Karyotype of *Sternocera Ruficornis*

The diploid chromosome number of *S. ruficornis* was 2*n*=26 (24+XY) for male and 2*n*=26 (24+XX) for female. The karyotype composed of 2 large metacentric, 2 small metacentric, 6 small submetacentric, 6 small acrocentric, 8 small telocentric (Table 2). In addition, the male karyotype showed an exclusive small acrocentric chromosome identified as the Y chromosome. Hence, it occurs as the XX/XY sex-determination system (Figure 2 and 3).

3.2 Chromosome marker of *Sternocera ruficornis*

The determination of chromosome marker for this species was firstly obtained by using the Ag-NOR banding technique. The nucleolar organizer regions (NORs) were observed on the centromere of chromosome one and subcentromeric region of chromosome nine (Figure 3 and 4).

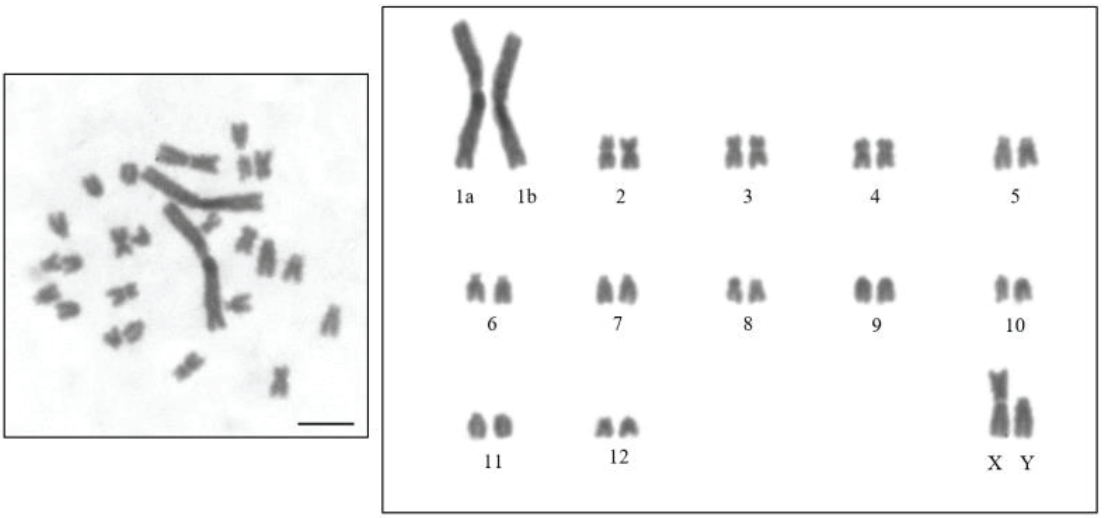

Figure 2. Metaphase chromosome plate and karyotypes of male Jewel-Beetles (*Sternocera ruficornis*) 2*n*=26 by a conventional staining technique (scale bar indicate 5 µm)

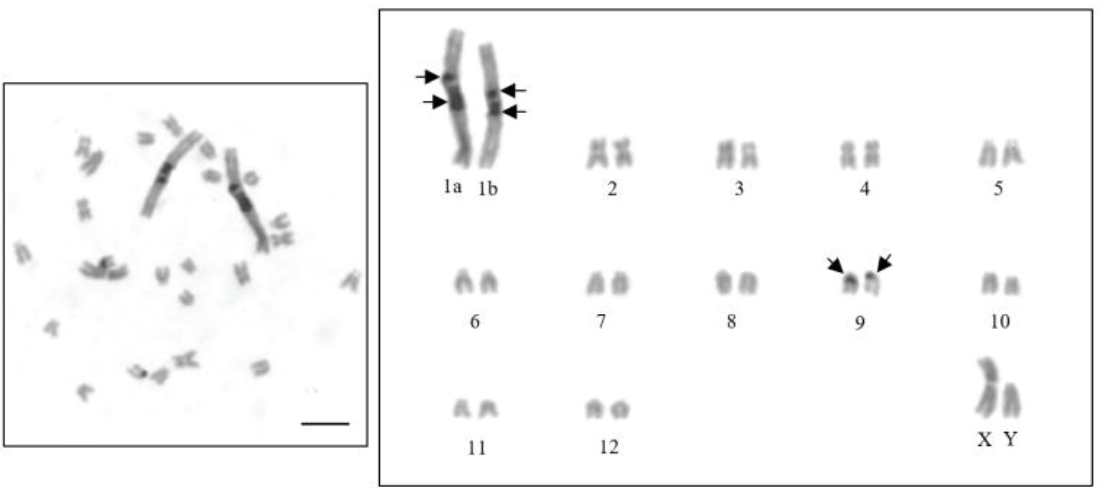

Figure 3. Metaphase chromosome plate and karyotypes of male Jewel-Beetles (*Sternocera ruficornis*) 2*n*=26 by Ag-NOR banding technique.
Nucleolar Organizer Regions/NORs near centromere of chromosome pair one.
A subcentromeric region of chromosome pair nine (arrows) and scale bar indicates 5 µm.

4. Discussions and Conclusion

All of the specimens showed the diploid number (2*n*) of *Sternocera ruficornis* was 26, similarality with chromosome number (2*n*) of *Sternocera laevigata*, *S. nitidicollis* and most published species in subfamily Julodinae (Karagyan & Kuznetsova, 2000). In a comparative karyotypic analysis of males and females in *S. ruficornis*, we found morphologically differentiated sex chromosomes of the type XX/XY, the X chromosome represented by a small sized metacentric and the Y chromosome by a small sized acrocentric. This result consistent with the previous study about

the sex chromosome system in Buprestidae suggested that most species were XY system (Karagyan & Kuznetsova, 2000).

The Ag-NOR banding technique showing that the Nucleolar Organizer Regions (NORs) band appeared at 2 sites near the centromere of chromosome one and subcentromeric region of chromosome nine. This is the first report on polymorphism of NORs in *S. ruficornis*. As commonly understood, in Coleoptera NORs may be located in some autosomal pair and/or sex chromosomes (Almeida *et al*., 2000; Moura *et al*., 2003; Bione *et al*., 2005; Holecová *et al*., 2008). The most common pattern in Coleoptera is the location of the nucleolus organizer region in one autosomal pair (Virkki 1983; Virkki *et al*., 1984; Vitturi *et al*., 1990; Colomba *et al*., 2000; Moura *et al*., 2003; Almeida *et al*., 2006; Schneider *et al*., 2007). The results showed heteromorphism in chromosome pair one with a different size of NORs. The purpose of this technique is to detect NORs, the location where genes encoding ribosomal RNA were located.

The idiograms of *S. ruficornis* showed size polymorphism of chromosome 1 due to size differentiations of NOR. The heteromorphic size of this region was detected between homologous chromosomes. Thus, this variation in NOR size in *S. ruficornis* was due to a variable number of rDNA genes. This could be explained by mechanisms such as unequal crossing-over, transpositions, or other rearrangements including deletions and/or duplications, involve segments of homologous chromosomes, thus, frequently attributed to mechanisms related to structural modifications of NORs.

The present study is the first report on size heteromorphism (1a1b) of the Ag-NOR in the Buprestidae species. The result has shown that the size heteromorphism of NOR observed represents a true polymorphism, since it involves variations in the copy number of genes in homologous chromosomes.

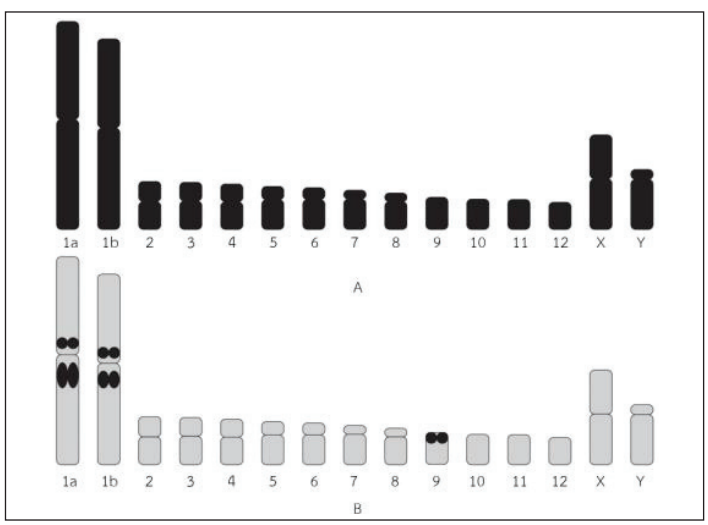

Figure 4. Idiograms showing lengths and shapes of chromosomes of Jewel-Beetles (*Sternocera ruficornis*, 2*n*=26) by (A) conventional staining technique and (B) Ag-NOR banding technique.

5. Acknowledgement

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