The First Chromosomal Analysis of Ghost Stag Beetle, *Odontolabis siva* (Coleoptera, Scarabaeoidea, Lucanidae)

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บทคัดย่อ

การศึกษาแคริโอไทป์ครั้งแรกของด้วงคีมซิว่า (*Odontolabis siva*) เตรียมโครโมโซมจากอัณฑะของด้วงเพศผู้จำนวน 10 ตัว ด้วยเทคนิคการบดขยี้เซลล์ ผลการศึกษาพบว่า ด้วงคีมซิว่าเพศผู้มีจำนวนโครโมโซมดิพลอยด์ (2*n*) เท่ากับ 29 แท่ง มีจำนวนโครโมโซมพื้นฐานเท่ากับ 55 ในเพศผู้ ประกอบด้วยโครโมโซมชนิดเมทาเซนทริกขนาดใหญ่ 10 แท่ง ซับเมทาเซนทริกขนาดใหญ่ 14 แท่ง อะโครเซนทริกขนาดใหญ่ 2 แท่ง และเทโลเซนทริกขนาดเล็ก 2 แท่ง มีการกำหนดเพศระบบ XO โดยโครโมโซมเอ็กซ์เป็นชนิดเทโลเซนทริกขนาดเล็กมากที่สุด การศึกษาการแบ่งเซลล์ไมโอซิส พบว่า ในระยะเมทาเฟส 2 มีจำนวนโครโมโซมแฮพลอยด์ (*n*) เท่ากับ14 แท่ง (14+O) และ 15 แท่ง(14+X) ด้วงคีมซิว่าเพศผู้มีสูตรแคริโอไทป์ (*2n*=29) = L^{m}_{10}+ L^{sm}_{14}+ L^{a}_{2}+ S^{t}_{2}+ โครโมโซมเพศ (XO)

คำสำคัญ: โครโมโซม, แคริโอไทป์, ด้วงคีมซิว่า

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Abstract

This research is the first karyotype study of ghost stag beetles (*Odontolabis siva*). Chromosome preparation was collected from testes of 10 male's beetles by squash technique. The results showed the diploid number of chromosome was 2*n*= 29, the fundamental number (NF) was 55 in males. Karyotype was present as 10 large metacentric, 14 large submetacentric, 2 large acrocentric and 2 small telocentric chromosomes. Sex chromosome system is XO system which the X-chromosome is the smallest telocentric chromosome. Consequently, the *O. siva* chromosome number on metaphase II of meiotic cell division was both *n* (haploid) = 14 (14+O) and 15 (*n* = 14+X). The karyotype formula of male *O. siva* was as follows: *2n* (diploid) 29 = L^{m}_{10}+ L^{sm}_{14}+ L^{a}_{2}+ S^{t}_{2}+ sex-chromosome (XO).

Keywords: Chromosome, Karyotype, Ghost stag beetle, *Odontolabis siva*

1. Introduction

Ghost stag beetle (*Odontolabis siva*) belongs to order Coleoptera, superfamily Scarabaeoidea, family Lucanidae. Their body was entirely black. Male was bears head with a sharp post-ocular spine, protonum with an additional front tooth in large male which absent in small male (Figure 1). Female head was short without a post- ocular spine, protonum with lateral and hind teeth similar to the small male. In additions, they were distributed in Tibet, Bhutan, China, Laos, Malaysia, Myanmar, North East of India, Vietnam and Thailand (Ek-Amnuay, 2008).

There are no currently published studies on cytogenetics of *Odontolabis* species. The karyological picture of the superfamily Scarabaeoidea emerges from few contributions mostly reporting on haploid (*n*) and/or diploid (2*n*) chromosome numbers (Salamanna, 1965, 1972; Vidal, & Giacomozzi, 1977; Yadav, Pillai, & Karamjeet, 1979; Yadav, Burra, & Dange, 1989) and conventional staining technique (Vidal and Giacomozzi, 1979; Colomba, Monteresino, Vitturi, & Zunino, 1996) . Furthermore, it was only a few publications on cytogenetics of Lucanidae species

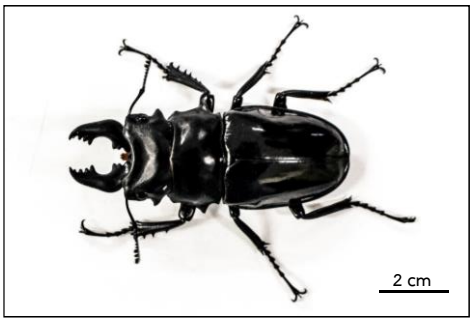

Figure 1 General characteristics of the ghost stag beetle, *Odontolabis siva* (Coleoptera, Lucanidae).

The previous studies reported that diploid number (*2n*) of Lucanid species (Table 1) were 2*n* = 6-32 and sex chromosome systems were XY system (Virkki, 1966, 1967; Abe, Kudoh, & Saitoh, 1976; Abe, Ichikawa, Kudoh, & Saitoh; 1992a, 1992b; Kudoh, Abe, Kondoh, Satoh, & Saitoh, 1970; Colomba, Vitturi, & Zunino, 2000; Abe, & Kudoh, 2005; Abe, & Terayama, 2009; Dutrillaux, & Dutrillaux, 2012).

The present study is aimed to analyze karyotypes and ideogram of *O. siva* by the conventional staining techniques from mitotic and meiotic cell divisions. The results obtained can provide increasing cytogenetic information for future studies on taxonomy and evolutionary relationships of these beetles. Moreover, it provides useful basic information for the conservation and breeding practices, and studies of chromosome evolution of this beetle.

Table 1

Review of beetle cytogenetic publications in the family Lucanidae.

Species	2*n*	NF	Karyotype	Sex chro.	Reference
Aegus laevicolis subnitidus	26	-	-	X(a), Y(dot)	Abe and Terayama. (2009)
Aesalus a. asiaticus	20	-	-	XY	Abe *et al*. (1992b)
Ceruchus l.lignarius	20	39	18m+2sm	X(a), Y	Kudoh *et al*. (1970)
	20	39	18m+2sm	X(a), Y	Abe and Terayama. (2009)
Dorcus antaeus	16	32	10m+4sm	X(m), Y(sm)	Abe *et al*. (2006)
D. b. bisignatus	6	11	4m	X(m), Y	Abe *et al*. (2006)
D. curvidens binodulosus	18	35	6m+6sm+4a	X(sm), Y	Abe *et al*. (2006)
D. metacostatus	14	28	8m+4sm	X(m), Y(a)	Abe and Kudoh (2005)
D. m. montivagus	12	24	10m	X(m), Y(sm)	Abe *et al*. (2006)
D. parallelopipedus	18	-	-	XY	Virkki (1959)
	18	36	8m/sm+8st	X(sm), Y(m)	Colomba *et al*. (2000)
D. r. rectus	18	35	10m+6sm	X(sm), Y	Abe *et al*. (1976)
D. r. miekoae	18	35	10m+6sm	X(sm), Y	Abe *et al*. (2006)

Species	2*n*	NF	Karyotype	Sex chro.	Reference
D. rubrofemoratus	10		8m	X(sm), Y	Abe *et al*. (1976)
D. s. striatipennis	14	26	12m	X(dot), Y	Abe *et al*. (1976)
	14	26	12m	X(dot), Y	Abe *et al*. (2006)
D. titanus	14	-	-	XY	Abe and Kudoh (2005)
D. t. castanicolor	12	23	6m+4sm	X(sm), Y(dot)	Abe and Terayama. (2009)
D. t. daitoensis	12	23	6m+4sm	X(sm), Y(dot)	Abe *et al*. (2006)
D. t. elegans	12	23	6m+4sm	X(sm), Y(dot)	Abe *et al*. (2006)
D. t. pilifer	12	23	6m+4sm	X(sm), Y(dot)	Abe *et al*. (2006)
D. t. platymelus	12	23	6m+4sm	X(sm), Y(dot)	Abe *et al*. (2006)
D. t. sika	12	23	6m+4sm	X(sm), Y(dot)	Abe *et al*. (2006)
D. t. tatsutai	12	23	6m+4sm	X(sm), Y(dot)	Abe and Terayama. (2009)
D. t. Taurus	10	19	6m+2sm	X(m), Y	Abe and Kudoh (2005)
D. t. titanus	12	23	6m+4sm	X(sm), Y(dot)	Abe *et al*. (2006)
Figgulus boninensis	20	40	20m	-	Abe *et al*. (1992a)
F. binodulus	20	40	20m	-	Abe *et al*. (1992a)
F. punctatus	26	51	4m+20a	X(a), Y(dot)	Abe *et al*. (1992a)
Lucanus cervus	22	-	-	XY	Dutrillaux and Dutrillaux (2012)
L. maculifemoratus	26	-	-	XY	Abe *et al*. (1992b)
Nicagus japonicas	20	19	12m+6sm	X(sm), Y(dot)	Abe and Terayama. (2009)
Nigidius lewisi	32	63	30m	X(sm), Y(dot)	Abe and Terayama. (2009)
Odontolabis siva	**29**	**55**	**10m+14sm+2a+2t**	**male X(t), O**	**Present study**
Platycerus d.delicatulus	20	39	16m+2a	X(a), Y(dot)	Abe *et al*. (1992b), Abe and Terayama. (2009)
Prismognathus a. angularis	26	-	-	XY	Abe *et al*. (1992b)
P. dauricus	26	51	2m/sm+18sm+4a	X(a), Y(dot)	Abe and Terayama. (2009)
Prosopocoilus i. inchinatus	20	-	-	XY	Abe *et al*. (1976)
P. hachijoensis	20	-	-	XY	Abe *et al*. (1992b)
Sinodendron rugosum	18	-	-	XY	Virkki (1966, 1967)

Notes: 2*n* = diploid chromosome number, NF = fundamental number, chro. = chromosome,
m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric, t = telocentric,
X = X-chromosome, Y = Y-chromosome, O = not Y-chromosome and - not available.

2. Materials and Methods

The 10 males of *O. Siva* were collected from Chiang Mai Province, Thailand. The chromosome preparation technique was applied form Supiwong, Tanomtong, Jumrusthanasan, Khakhong, Neeratanaphan, & Sanoamuang (2013). The Individual of specimen was injected with 0.01% colchicine into abdominal cavity and left for 1 hour. After that, testis was collected from abdominal cavity and incised to single cells in hypotonic solution (0.075 M KCl) by using surgical scissors and incubated in KCl for 30 minute. Then, the mixture was centrifuged at 3,000 rpm for 10 minute, supernatant was discarded and cell suspensions were fixed by fixative solution (methanol: glacial acetic acid, 3:1). Next, the mixture was centrifuged and the fixation was repeatedly done until the supernatant was clear. After wards, the cell suspensions were dropped on clear slides and slides were staining with 20% Giemsa's dye in Gurr's buffer for 20 minute and rinsed with tap water. Chromosome counting was performed on metaphase cell under light microscopes.

Standardized karyotypes and idiogram of this beetle were constructed. Chromosome checking was performed on mitotic and meiotic metaphase cells under a light microscope. The frequencies of chromosome number per cell were counted. The maximum frequency of chromosome number per cell is the diploid ($2n$) and haploid (n) chromosome number of this beetle. Ten cells of each male with clearly observable and well spread chromosome were selected for karyotyping. The length of short arm chromosome (Ls) and long arm chromosome (Ll) were measured and calculated to the length of total arm chromosome (LT, LT = Ls+Ll). The relative length (RL), the Centromeric Index (CI) and S of RL and CI were calculated (Table 2).

The CI (q/p+q) between 0.50 - 0.59, 0.60 - 0.69, 0.70 - 0.89 and 0.90 - 0.99 are representing the metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively (Chaiyasut, 1989). The fundamental number (number of chromosome arm, NF) is obtained by assigning a value of two to metacentric, sub- metacentric and acrocentric chromosomes and one to telocentric chromosome. All parameters were used in karyotyping. The idiogram was constructed using a model drawing of karyotype and accomplished by a computer program.

Table 2

Mean of length of short arm chromosome (Ls), length 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 10 cell of male ghost stag beetles (*Odontolabis siva*), 2*n*=29.

Chro. pair	Ls	Ll	LT	RL±SD	CI±SD	Size	Type
1	0.997	1.272	2.269	0.077±0.009	0.560±0.021	Large	Metacentric
2	0.885	1.098	1.982	0.067±0.007	0.555±0.025	Large	Metacentric
3	0.859	1.045	1.904	0.064±0.005	0.550±0.026	Large	Metacentric
4	0.842	1.012	1.855	0.062±0.005	0.549±0.023	Large	Metacentric
5	0.773	0.949	1.722	0.058±0.004	0.551±0.031	Large	Metacentric
6	1.136	1.782	2.917	0.098±0.006	0.611±0.038	Large	Submetacentric
7	1.042	1.645	2.687	0.090±0.006	0.610±0.028	Large	Submetacentric
8	0.898	1.509	2.407	0.081±0.006	0.623±0.023	Large	Submetacentric
9	0.918	1.438	2.357	0.079±0.005	0.609±0.035	Large	Submetacentric
10	0.898	1.416	2.313	0.078±0.006	0.614±0.039	Large	Submetacentric
11	0.829	1.291	2.119	0.071±0.006	0.607±0.033	Large	Submetacentric
12	0.790	1.159	1.949	0.064±0.009	0.603±0.031	Large	Submetacentric
13	0.632	1.468	2.101	0.070±0.009	0.702±0.034	Large	Acrocentric
14	0.000	0.809	0.809	0.027±0.007	1.000±0.000	Small	Telocentric
X	0.000	0.340	0.340	0.012±0.011	1.000±0.000	Small	Telocentric

Remarks: L=large chromosome (LT>1.629), S=small chromosome (LT<1.459) and chro. = chromosome.

3. Results and Discussion

The diploid chromosome number (*2n*) of male *O. siva* was 29 with funda-mental number (NF) was 55, and the presence of 10 large metacentric, 14 large sub-metacentric, 2 large acrocentric and 2 small telocentric chromosomes. Sex chromosome system was XO system with X being the smallest telocentric chromosome. Consequently, the *O. Siva* chromosome number on metaphase II of meiotic cell division was both *n* = 14 (*n* = 14+ O) and *n* = 15 (*n* = 14+ X). The karyotype formula of male *O. siva* is as follows: *2n* (diploid) 29 = L^{m}_{10}+ L^{sm}_{14}+ L^{a}_{2}+ S^{t}_{2}+sex-chromosome (XO) or 10m + 14sm + 2a +2t+ XO (Figure 2, 3, & 4).

In addition, this result showed the diploid chromosome number of *O. Siva* is

different from other species in the same family that the diploid numbers (*2n*) of Lucanidae published species were 6-32. In the other hand, the NF of male *O. Siva* was 55 disagree with other Lucanids studies that reported fundamental number as 11-39 (Virkki, 1966, 1967; Abe *et al*., 1976, 1992a, 1992b; Kodoh *et al*., 1970; Colomba *et al*., 2000; Abe and Kudoh, 2005; Abe, & Terayama, 2009; Dutrillaux, & Dutrillaux, 2012).

Nevertheless, this study is the first data of the XO sex chromosome system in the family Lucanidae. It's surprising that the sex chromosome system of *O. siva* was XO while all of previous studies presented only XY system.

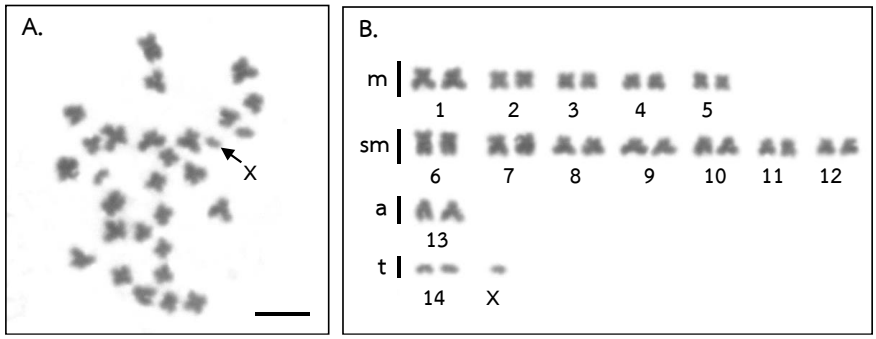

Figure 2 The mitotic cell division of metaphase chromosome plate (A.) and karyotype (B.) of male ghost stag beetle (*Odontolabis siva*), 2*n*= 29 by conventional staining technique. Scale bar indicate 5 micrometers. The sex-chromosome system is XO in which the X chromosome classified as the smallest telocentric chromosome.

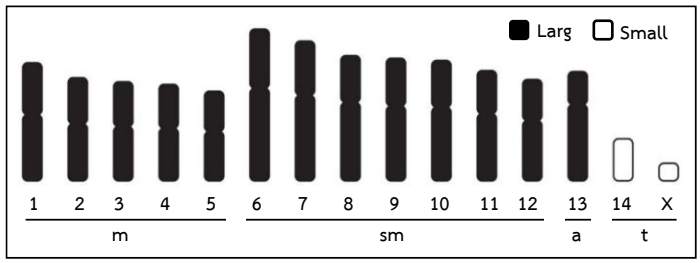

Figure 3 Standardized idiogram showing lengths and shapes of chromosomes of the ghost stag beetles (*Odontolabis siva*), 2*n*=29 by conventional staining technique.

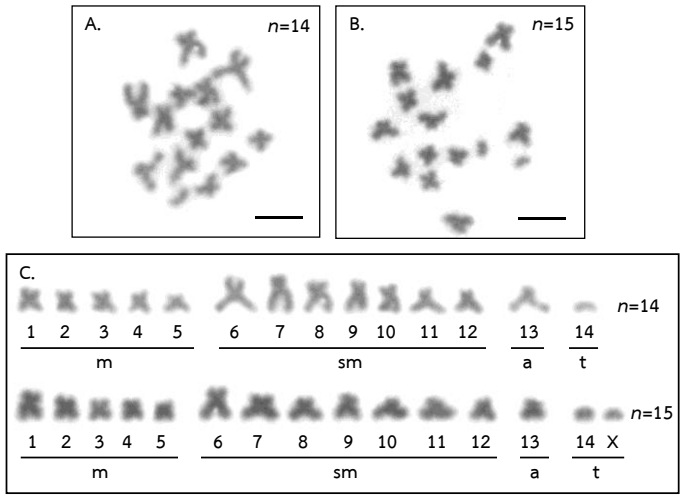

Figure 4 The meiotic cell division of metaphase II chromosome plates (A. and B.) and karyotype (C.) of male ghost stag beetle (*Odontolabis siva*), *n*= 14 and *n*= 15by conventional staining technique. Scale bars indicate 5micrometers.

In present study on meiotic cell division of *O. siva* showed the synapsis of homologous chromosome during metaphase I, comprising 14 bivalents and X chromosome (Figure 5 H.), and 15 haploid chromosomes (*n* = 15) at metaphase II (Figure 5 I.). Beside, we found that *O. Siva* had the clarity of the observable leptotene (initiation of chromosome shrinking, Figure 5 C.), zygotene (homologous chromosomes were initiated to synapsis, Figure 5 D.), pachytene (completion of chromosome synapsis, Figure 5 E.), diplotene (chiasma and crossing over, Figure 5 F.), diakinesis (each of chromosome was separated, Figure 5 G.) in prophase I of meiosis I.

The family Lucanidae has long been deliberated as one of the most primitive groups of living Scarabaeoids (Kim, & Farrell, 2015). Established on fossil verification of *Paralucanus mesozo*icus (Paralucanidae), proposed family Lucanidae to have varied from its common ancestor with family Passalidae during the late Jurassic, for which followed by the phylogenetic system of Browne, & Scholtz (1998, 1999). They estimated the stem group lucanids to have arisen during the middle Jurassic around 167 MYA. The two lucanids included in their analyses belong to the genera *Lucanus* and *Nigidius*, respectively, whose divergence age is expected to be around

94 MYA in their analyses. The two genera do not represent the earliest split within family Lucanidae and hence.

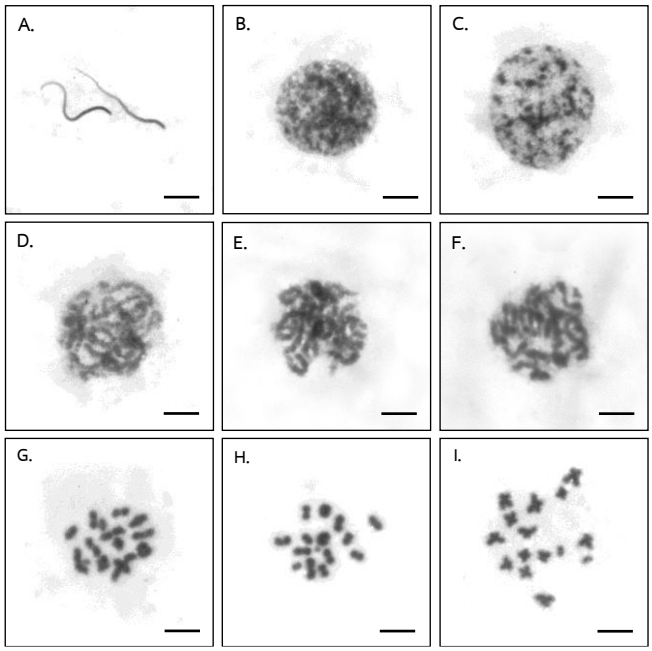

Figure 5 Meiotic cell division of male ghost stag beetle (*Odontolabis siva*), 2*n* = 29 on mature sperm (A), interphase (B), leptotene (C), zygotene (D), pachytene (E), diplotene (F), diakinesis (G), metaphase I (H), and metaphase II (I). Scale bars indicate 5 micrometers.

Within the Lucanidae, *Aesalus* constituted the earliest branching clade, a molecular phylogenies point the genus *Aesalus* as a monophyletic lineage that represents the ancestral clade inside this family indicated 2*n* = 2 (Abe *et al.*, 2009). A second clade includes genera *Ceruchus*, *Nicagus*, *Sinodendro*n and *Platycerus*, presents the standard karyotype formed about 2*n* = 20 except genus *Sinodendro*n show 2*n*= 18 (Virkki 1966, 1967; Kudoh *et al.*, 1970; Abe *et al.*, 1992b; Abe and Terayama, 2009) and the least one from the other clades, more recently derived, encompasses *Nigidus*, *Prismognathus*, *Lucanus*, *Odontolabis*, *Dorcus* and *Aegus* demonstrated the diploid number of 6-32 (Abe *et al.*, 1992b; Abe and Terayama,

2009; Dutrillaux and Dutrillaux, 2012; present study) (Figure 6).

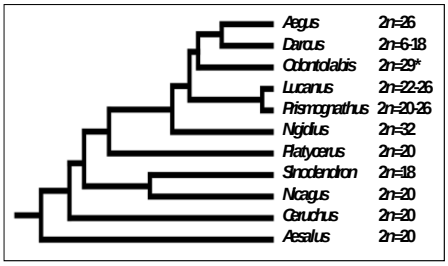

Figure 6 Phylogenetic relationship of the family Lucanidae, adapted from Kim and Farrell (2015).
Note: * = present study

In summarizing, the present study delivers the first detailed karyological information on the genus *Odontolabis* which linked to future cytogenetic data on other genera belonging to the same family will provide a set of hypothetically informative characters suitable to understand the phylogenetic situation of Lucanidae within Scarabaeoidea, which according to recent information (Colomba *et al*., 2000) is still unclear.

Acknowledgements

This work was financially supported in part by a full scholarship form the Science Achievement Scholarship of Thailand and Toxic Substances in Livestock and Aquatic Animals Research Group, Khon Kaen University. And I would like to thanks department of biology faculty of science Khon Kaen University and cytogenetics research groups.

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