UNIVERSITI PUTRA MALAYSIA

COMPARATIVE KARYOTYPES OF FALLOW DEER AND SPOTTED DEER

NUR RASHIDAH RAHMAT

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FACULTY OF VETERINARY MEDICINE

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COMPARATIVE KARYOTYPES OF FALLOW DEER AND SPOTTED DEER

NUR RASHIDAH RAHMAT

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It is hereby certified that we have read this paper entitled "Comparative Karyotypes of Fallow Deer and Spotted Deer" by Nur Rashidah Rahmat and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999-Final Year Project.

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Dedicated to……..

My parents,

Brothers and Sisters,

Nieces and Nephews,

and All my Beloved Friends.

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An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Final Year Project.

COMPARATIVE KARYOTYPES BETWEEN FALLOW DEER AND SPOTTED DEER

by

Nur Rashidah Rahmat

2016

Supervisor: Dr. Mohd Shahrom Salisi

Co-supervisor: A.P. Dr. Rosnina Hj Yusoff

This study was conducted to characterize the chromosomes of two deer species raised in Malaysia, namely *Dama dama* (fallow deer) and *Axis axis* (spotted deer) using conventional procedures for construction of karyotypes. The medium RPMI-1640, supplemented with pokeweed mitogen, fetal calf serum, penicillin-streptomycin and amphotericin B was used for lymphocytes culture in order to produce a good quantity and quality of metaphase spreads for chromosomes analysis. The fallow deer and spotted deer had diploid number (2n) of 68 and 66 respectively. The conventional karyotype showed that fallow deer has 33 pairs of autosomes (32 pairs of acrocentrics and 1 pair of metacentric) while spotted deer has 32 pairs of autosomes (31 pairs of acrocentrics and 1

pair of metacentric). The X chromosome is a longest acrocentric while the Y chromosome is a smallest submetacentric for both species. The fundamental number of fallow deer is 71 for male and 70 for female while for spotted deer the fundamental number is 69 for male and 68 for female. Thus, fallow deer and spotted deer have the different diploid number (2n) and fundamental number but similar in chromosome morphology.

Keywords: *Dama dama*, *Axis axis*, lymphocyte culture, karyotype, fundamental number

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir.

KARIOTIP BANDINGAN UNTUK RUSA PARANG DAN RUSA BINTIK

oleh

Nur Rashidah Rahmat

2016

Penyelia: Dr. Mohd Shahrom Salisi

Penyelia Bersama: PM Dr. Rosnina Hj Yusoff.

Kajian ini telah dijalankan untuk menciri kromosom rusa parang (*Dama dama*) dan rusa bintik (*Axis axis*) yang terdapat di Malaysia, mengguna prosedur pembentukan kariotip konvensional. Medium kultur RPMI 1640 (80%) yang ditambah dengan mitogen pokeweed, serum anak lembu fetus, penicilin-streptomisin, dan amfoterisin B digunakan dalam kultur limfosit untuk menghasilkan lepa metafasa baik kualiti dan kuantitinya untuk analisis kromosom. Rusa parang dan rusa bintik masing-masing mempunyai nombor diploid kromosom (2n) 68 dan 66. Kariotip konvensional menunjukkan rusa parang mempunyai 33 pasang autosom (32 pasang acrosentrik, 1 pasang metasentrik),

manakala rusa bintik mempunyai 32 pasang autosom (31 pasang acrosentrik, 1 pasang metasentrik). Kromosom X untuk kedua-dua spesies rusa ini adalah panjang akrosentrik sambil kromosom Y kecil submetasentrik. Nombor fundamental bagi rusa parang jantan adalah 71 dan bagi betina 70. Untuk rusa bintik jantan, nombor fundamental adalah 69 dan 68 untuk betina. Kesimpulannya, rusa parang dan rusa bintik berbeza dalam nombor diploid (2n) dan fundamental tetapi sama morfologi kromosomnya.

Kata kunci: *Dama dama*, *Axis axis*, kultur limfosit, kariotip, nombor fundamental

1.0 INTRODUCTION

Deer are any of the 43 species of hoofed ruminants in the family, Cervidae and order, Artiodactyla. They are notable for having two large and two small hooves on each foot and also for having antlers in the males of most species and in the females of one species, which is the reindeer (*Rangifer tarandus*). Deer are native to all continents except Australia and Antarctica, and many species have been widely introduced beyond their original habitats as game animals (Hernandez-Fernandez and Vrba, 2005).

The fallow deer (*Dama dama*) and spotted deer (*Axis axis*) are classified under the family, Cervidae and subfamily, cervinae. This subfamily comprises of nine genera; *Axis*, *Cervus*, *Dama*, *Elaphodus*, *Elaphurus*, *Muntiacus*, *Przewalskium*, *Rucervus* and *Rusa*. The genus, *Dama* comprises the fallow deer (*Dama dama*) and Persian fallow deer (*Dama mesopotamica*). Spotted deer (*Axis* axis), calamian deer (*Axis calamianensis*), bawean deer (*Axis kuhlii*) and hog deer (*Axis porcinus*) are categorized under the genus, *Axis* (Hernandez-Fernandez and Vrba, 2005).

Dama dama and *Axis axis* are classified as least concern species in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. There are no major threats to this fallow deer in Europe. In their native range, hunting and habitat conversion for agriculture caused massive decline in the past (Wemmer, 1998). In addition, the tiny remaining population in the native range in Turkey is at risk from inbreeding and hunting. As for spotted deer, they are hunted for food and sports and it is

also unclear on the numbers of captive spotted deer (The IUCN Red List of Threatened Species, 2008).

The domestication and farming of deer in Malaysia have become a growing interest due to demand for velvet, skin and meat (Habiba, 2005). In 1990, 45 females and 5 males *Cervus timorensis* imported from Mauritius, were placed at the Infoternak Farm in Sg. Siput, Perak. Apart from this, there are also other deer species imported into the country for other purposes including for display in zoos and animal parks. The introduction of various deer species had resulted in the introduction of new germplasm and the risk of combination of the germplasm. Therefore, there is a need to evaluate the genetic background of the various deer species before they are indiscriminately diluted or altered (Habiba, 2005).

In the earlier research on genetic characterization of deer species raised in Malaysia, only three deer species, namely *Cervus timorensis* (rusa), *Cervus unicolor* (sambar) and *Cervus Nippon* (sika) had been karyotyped (Habiba, 2005). Thus, the present study was conducted to determine the diploid number (2n), nombre fundamental (NF), to construct the karyotypes of males and females of these two deer species and to describe the chromosome morphology of *Dama dama* (fallow deer) and *Axis axis* (spotted deer).

Hypothesis:

H_o: Fallow deer and spotted deer have same diploid number (2n), fundamental number (NF) and chromosome morphology.

H_a: Fallow deer and spotted deer have different diploid number (2n), fundamental number (NF) and chromosome morphology.

2.0 LITERATURE REVIEW

2.1 Fallow Deer

Fallow deer have a natural range in southern European regions, Asia Minor (south-western part of Asia), along the Mediterranean Sea and possibly in northern Africa. They have been widely introduced to 38 countries in North and South America, the Leeward Islands, Europe, South Africa, Australia, New Zealand and Fiji (Feldhamer*et. al.,* 1998; Nowak 1999). They inhabit many regions in the world with climates ranging from cool-humid to warm-dry. The preferred habitat usually is a combination of vegetation type with old, deciduous, broad-leaf forests of varying densities interspersed with grassy areas. They are also found in mixed forests, broad-leaf forests, subalpine vegetation, woodlands, low mountains, scrublands and topical or subtropical grassland (Feldhamer*et. al.,* 1998; Grizmek 1990).

They stand 0.9 to 1 m tall at the shoulder and has a body length between 1.3 and 1.75 m. (Walker, 1964). They have multi-point antlers (palmate) that distinguish them from all other deer. The antlers are usually shed annually in April and the new ones are regrown and free of velvet (velvet covers the growing bone and cartilage that develops into antlers) by August, until the fifth or sixth year. Females are generally without antlers (Feldhamer*et. al.,* 1998; Grizmek 1990).

They have the most variable pelage coloration (white, black, menil and common) of any species of deer. Typically, the pelage is darker on the dorsal surface of the body and lighter on the ventral surface, chest and lower legs. White peleage is cream coloured

at birth, becoming paler as they mature and adults are almost pure white in winter. Black peleage is have no spots at any time of the year and do not have the light area surrounding the tail. Common peleage is a deep chestnut with white spots, which in winter turns to a dark brown and the spots fade. Menil peleage is similar to the common peleage except that it has a greater number of spots and the area around the tail is bordered by brown, and the spots are still visible on the winter coat (Feldhamer*et. al.,* 1998; Grizmek 1990; Nowak 1999).

2.2 Spotted Deer

Spotted deer are historically found in India and Ceylon. They have been introduced to Texas and Hawaii in the United States of America. They occupy the grasslands and very rarely move into areas of dense jungle that may occur adjacent to them in their native lands. Therefore, short grasslands are important areas for them due to a lack of cover for predators such as tigers (Moe and Wegge, 1994). They stand 0.6 to 1 m tall at the shoulder and has a body length of about 1.5 m. Their body color is reddish with white spots on the belly, inner legs and underneath their short tail. The males tend to be darker and have black facial markings. Characteristic white spots occur in both sexes and run longitudinally in rows throughout the duration of the animal's life (Ables, 1977).

2.3 Chromosomes

Chromosomes are the hereditary material consisting of a nucleic acid and histones (a type of protein), that support its structure. In 1943, Avery and Chase first reported that the nucleic acid (deoxyribonucleic acid, DNA or ribonucleic acid, RNA) is the hereditary material. Each chromosome has a constriction point called the centromere (kinetochore or primary constriction), which divides the chromosome into two sections or "arms." The short arm of the chromosome is labeled as 'p' arm while the long arm is labeled the 'q' arm. The location of the centromere on each chromosome gives the chromosome its characteristic shape and can be used to help in describing the location of specific genes (Darbeshwar, 2009).

2.4 Karyotype

Karyotype refers to chromosome size, morphology and number of a chromosome complement of an individual (Battaglia, 1952). Chromosomes vary in size, location of the centromere and the presence or absence of satellites, attached to the proximal portion of chromosome arm through a thin stalk structure called secondary constriction. When the centromere is at the tip of the chromosome, it is classified as telocentric chromosome and such chromosome has got only one arm. When the centromere is near the end of the chromosome, it is classified as acrocentric chromosome and such chromosome has one very short arm. When the centromere lies in an approximately central position of the chromosome, it is classified as metacentric chromosome and such chromosome both arms

are equal length. Meanwhile in submetacentric chromosome, the centromere is nearer to one arm than the other arm resulting in the two arms being unequal: one short arm and one long arm. Certain chromosomes have secondary constriction which forms the nucleolus during interphase, thus secondary constriction is also called nucleolus organizer region (Darbeshwar, 2009).

2.5 Lymphocyte culture

According to Moorhead *et. al.,* 1960, a combination of cytological and leukocyte culture techniques have a convenient, reliable approach for chromosome studies of humans as they have several advantages; i) relative ease of obtaining blood and only small volume required, ii) adequate mitotic yield from the short-term culture of leukocytes and iii) high numbers of "exact count" quality metaphase spreads, permitting a critical analysis of chromosome morphology. According to Janossy and Greaves, 1971, they demonstrated that phytohaemagglutinin (PHA) selectively activates thymus-derived (T) cells and pokeweed mitogen (PWM) activates 'bursa-equivalent'-derived (B) cells. Therefore, in culturing of lymphocyte, these mitogen will stimulate lymphocytes to enter mitosis when they are being incubated for 72 hours. A mitotic inhibitor is added to the culture 1-2 hours before harvesting the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated (Moorhead *et. al.,* 1960).

3.0 MATERIALS AND METHODS:

The animals used in this study comprised of 2 males and 2 females from each species, fallow deer and spotted deer. The fallow deer were belonged to University Agriculture Park, Universiti Putra Malaysia and spotted deer were belonged to Livestock Animal Centre, Lenggong, Perak.

3.1 Animal restraining

Both deer species were kept in the dark room in the morning of blood collection day. The fallow deer were then physically restrained in the dark room by 4 person in which 1 person secured the head and neck, 1 person hold the fore limbs, 1 person hold the abdominal region and 1 person hold the hind limbs. Meanwhile, the spotted deer was brought to the crush and a rope was used to tie up the limbs. They were also sedated with sedative drug, xylazine at dose of 3 to 5 mg/kg.

3.2 Blood collection

The blood collection site was clipped by using a shaver. Then, the area was swabbed with 80% alcohol. Blood samples were collected in heparinized blood tubes by using 18 G venoject needle from the jugular vein of the animals.

The blood samples were agitated by hand and gently to allow anti-coagulant reagent (heparin) to act on the blood. Then, the blood tubes were kept in a cold box in which the ice pads were arranged surrounding (left and right sides, top and bottom) of the tube rack without any direct contact of the ice pads to the blood tube to prevent the blood

from being frozen. These blood tubes were transported immediately to cytogenetic laboratory to be processed. However, the blood samples of spotted deer from Livestock Animal Centre, Lenggong took about 4 hours of journey to arrive to the laboratory.

3.3 Chromosomes preparation

3.3.1 Blood culture

The basic short-term lymphocyte culture technique by Moorhead *et. al.,* 1960 was adopted with some modifications. Blood samples were centrifuged at 1800 rpm for 10 minutes to obtain the buffy coat containing leucocytes. The buffy coat is then aspirated and transferred into culture flasks containing culture medium. The culture medium comprised of 8.0 ml RPMI-1640 medium (Gibco®, Grand Island, New York), 2.0 ml fetal bovine serum (Gibco®, Grand Island, New York), 1000 µg penicillin G-streptomycin (Fischer Scientific Co., Fair Lawn, New Jersey), 1000 µg amphotericin B (Fugizone®, Gibco®, Grand Island, New York) and 1 µg pokeweed mitogen (Sigma-Aldrich Co., St. L ouis, Missouri, United States).

The mixture was agitated gently for uniform mixture and cultured at 37ºC for 72 hours. During the incubation, the culture were agitated gently twice a day (morning and late evening) to prevent leucocytes adhering to the wall of the culture flask.

3.3.2 Harvesting the culture

One hour before harvesting, 0.1 ml of colcemid solution (KaryoMAX® COLCEMID® Solution, Gibco®, Grand Island, New York) was added into the culture

flask and agitated gently. Just before harvesting, the culture flasks were agitated gently and the suspension was transferred into a 15 ml conical centrifuge tube. The suspension was spun for 8 minutes at 1800 rpm. The supernatant was decanted leaving behind only 2.0 ml of the medium and the cell button. 6.0 ml of pre-warmed 0.075 M potassium chloride solution was added into each tube, uniformly mixed with the cell and incubated at 37ºC for another 20 minutes. The tubes were centrifuged at 1800 rpm for 8 minutes.

The supernatant were decanted leaving behind only the cell button. The cell button was broken up with a Pasteur pipette and 6.0 ml of Carnoy's fixative (1 part of glacial acetic acid and 3 parts of methanol). The tubes were centrifuged at 1800 rpm for 8 minutes and the supernatant were decanted. This procedure was repeated 3 times and then the culture was kept at 4ºC for overnight.

3.4 Preparation of slides

Glass slides with frosted ends were cleaned and immersed in a mixture of 1 part of ethanol and 1 part of diethyl ether, wiped with a Kimwipes and left them dried by air dry. Two drops of cell suspension were placed on a precleaned glass slide, the second drop was placed adjacent to the first drop to avoid overlapping of the metaphase spreads. The slide was put on the warm air to let it dried and then it was stained with 10% Giemsa solution for 2 ½ minutes.

3.5 Construction of karyotypes

Slides were examined under a light microscope (Motic BA400) and good metaphase spreads were photographed using image analyzer (Moticam Pro 285A). The diploid number of chromosomes were calculated manually from the printed image and were tabulated. The karyotypes were constructed from chromosomes cut of the printed image and arranged according to International System for Cytogenetic Nomenclature of Domestic Animals (ISCNDA, 1989) in which the chromosomes were arranged from longest pairs to shortest pairs of autosomes and followed by sex chromosomes.

4.0 RESULTS

4.1 Chromosomes number

The numerical distribution chromosomes is based on a combination series of lymphocytes cultures in pokeweed mitogen. The numerical distribution will be converted into percentage distribution in order to determine the diploid number. The diploid number was determined based on those diploid number that have percentage distribution of 50% and above. The remaining metaphase spreads that had less or more than diploid number are presumably resulting from preparation artifact.

The numerical distribution chromosomes of fallow deer was tabulated in Table 1.0. In this distribution, males and females had 71 and 59 metaphase spreads respectively with 68 chromosomes per metaphase spread. This made up of 65.1% and 53.2% of percentage distribution chromosomes as presented in Figure 1.0. Therefore, the diploid number of fallow deer was 68.

Meanwhile, the numerical distribution chromosomes of spotted deer was tabulated in Table 2.0. In this distribution, males and females had 99 and 88 metaphase spreads with 66 chromosomes per metaphase spread. This made up of 69.7% and 67.7% of percentage distribution chromosomes as presented in Figure 2.0. Therefore, the diploid number of spotted deer was 66.

Table 1.0: The numerical distribution chromosomes of fallow deer

Diploid chromosomes number	Male	Female
	No. of metaphase spreads	No. of metaphase spreads
60	4	9
61	4	6
62	7	5
63	3	3
64	2	5
65	5	8
66	7	12
67	4	3
68	71	59
69	2	1
Total	**109**	**111**

Figure 1.0: Percentage distribution chromosomes of fallow deer

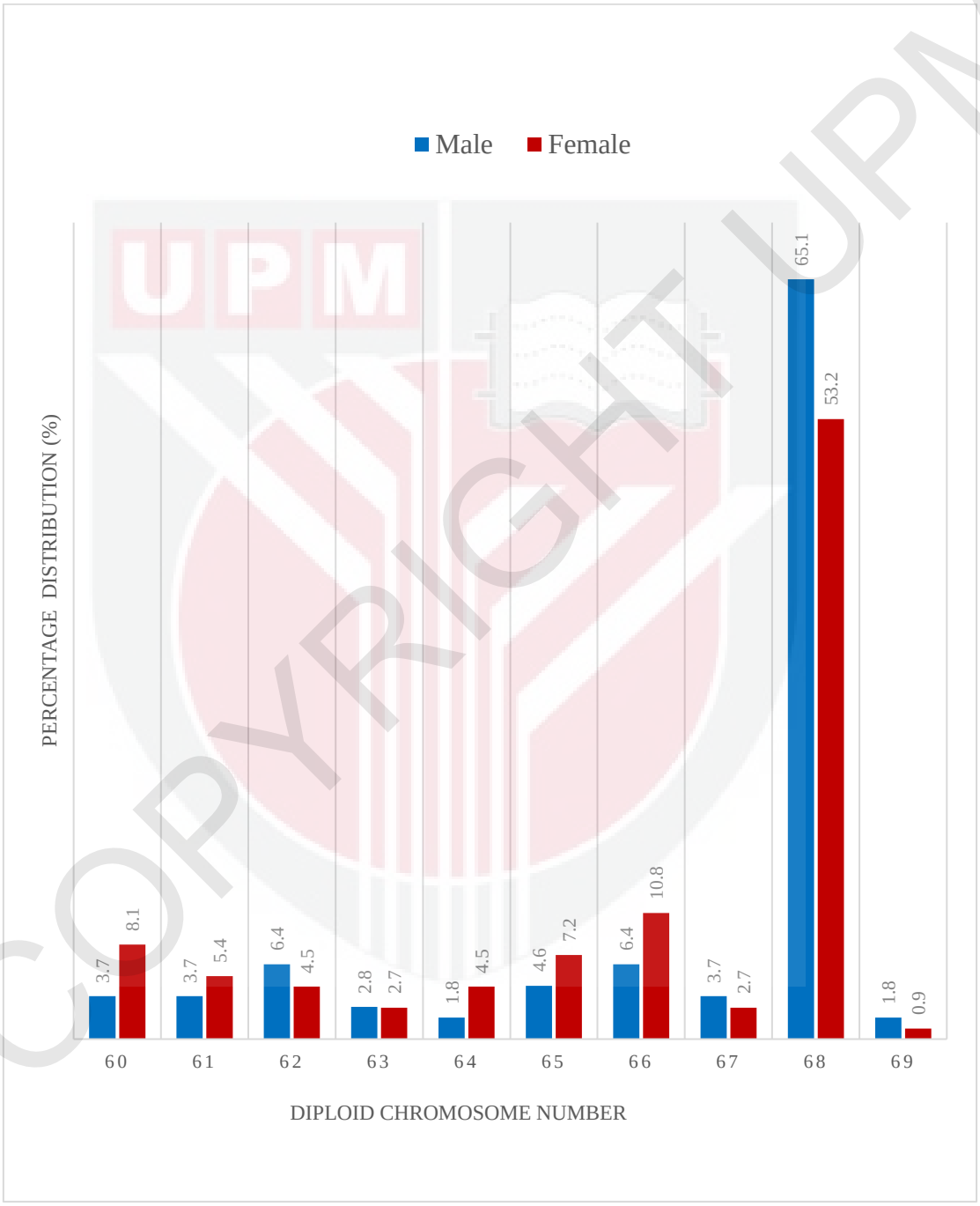
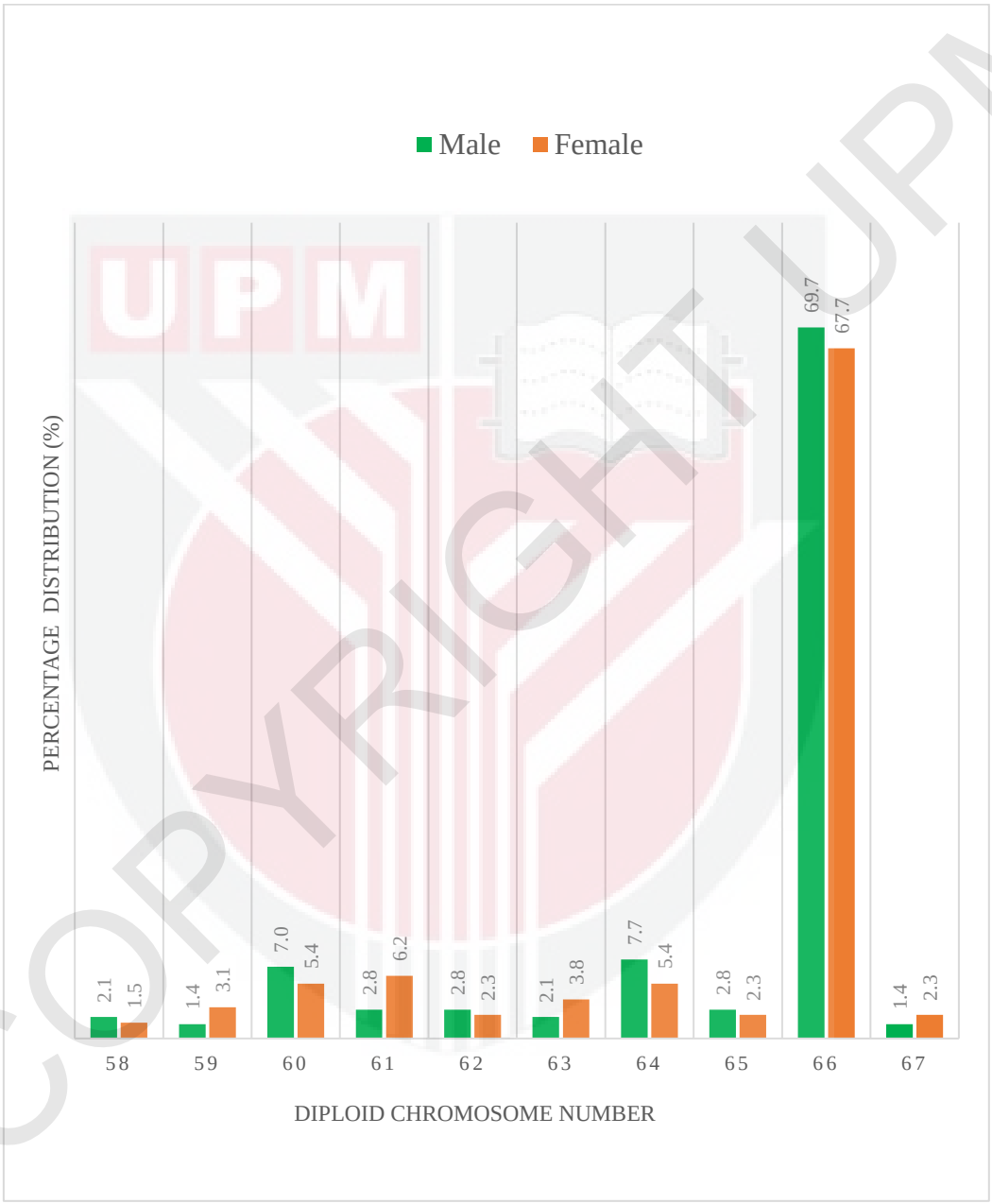


Table 2.0: The numerical distribution chromosomes of spotted deer

Diploid chromosomes number	**Male**	**Female**
	No. of metaphase spreads	**No. of metaphase spreads**
58	3	2
59	2	4
60	10	7
61	4	8
62	4	3
63	3	5
64	11	7
65	4	3
66	99	88
67	2	3
Total	**142**	**130**

Figure 2.0: Percentage distribution chromosomes of spotted deer



4.2 Karyotypes

In Figure 3.0, the conventional karyotype of male fallow deer was constructed and it comprised of 33 pairs of autosomes (32 pairs of acrocentrics, 1 pair of long metacentric) and a pair of sex chromosome. The X chromosome as a longest acrocentric while the Y chromosome as a smallest submetacentric. In Figure 4.0, the conventional karyotype of female fallow deer was constructed in which the autosomes were similar as male. Meanwhile, the sex chromosomes were XX. The morphological characteristics of chromosomes and fundamental number of fallow deer were depicted in Table 3.0.

In Figure 5.0, the conventional karyotype of male spotted deer was constructed and it comprised of 32 pairs of autosomes (31 pairs of acrocentrics, 1 pair of long metacentric) and a pair of sex chromosome. The X chromosome as a longest acrocentric while the Y chromosome as a smallest submetacentric. In Figure 6.0, the conventional karyotype of female spotted deer was constructed in which the autosomes were similar as male. Meanwhile, the sex chromosomes were XX. The morphological characteristics of chromosomes and fundamental number of spotted deer were depicted in Table 4.0.

Figure 3.0: Conventional karyotype of male fallow deer

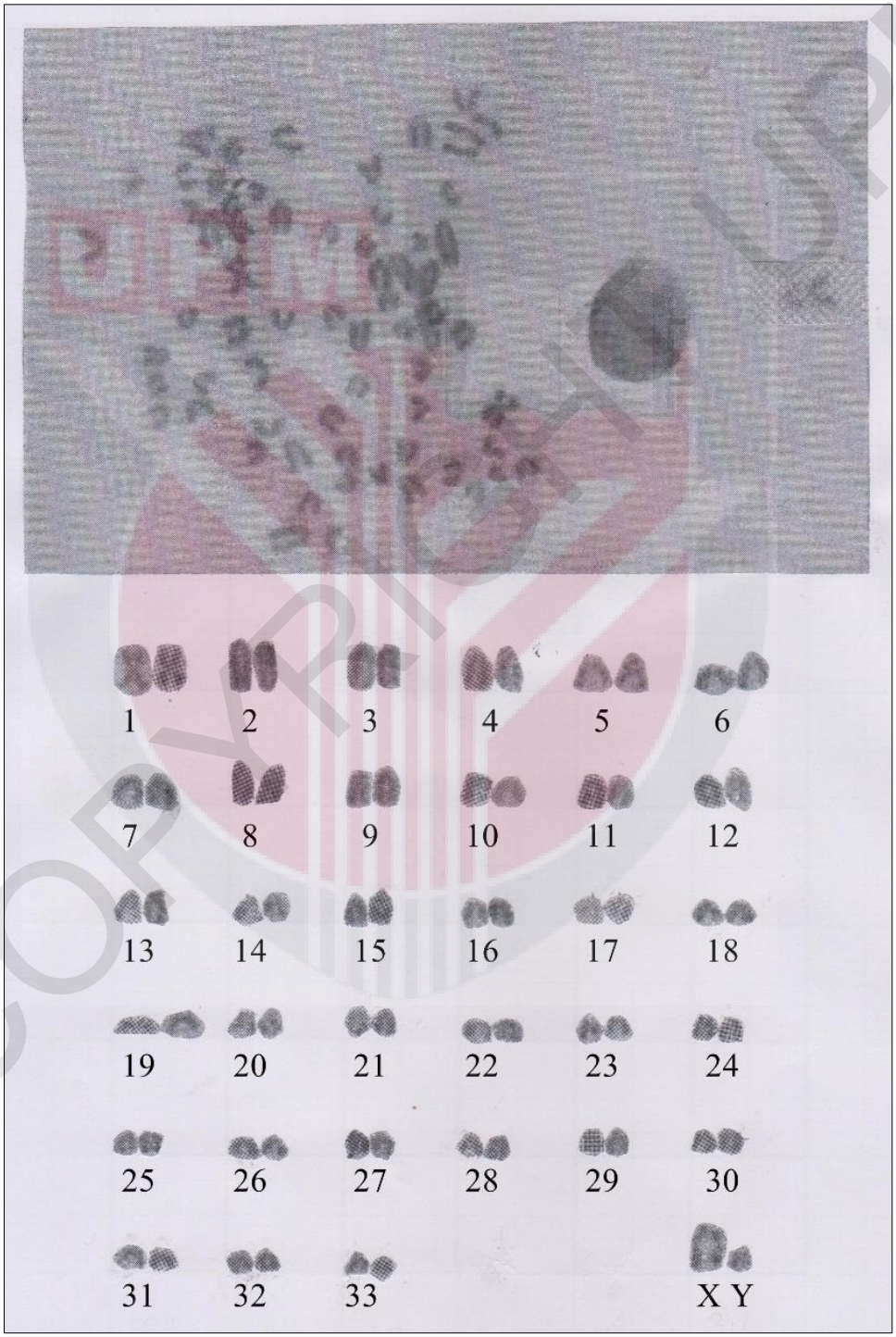


Figure 4.0: Conventional karyotype of female fallow deer.

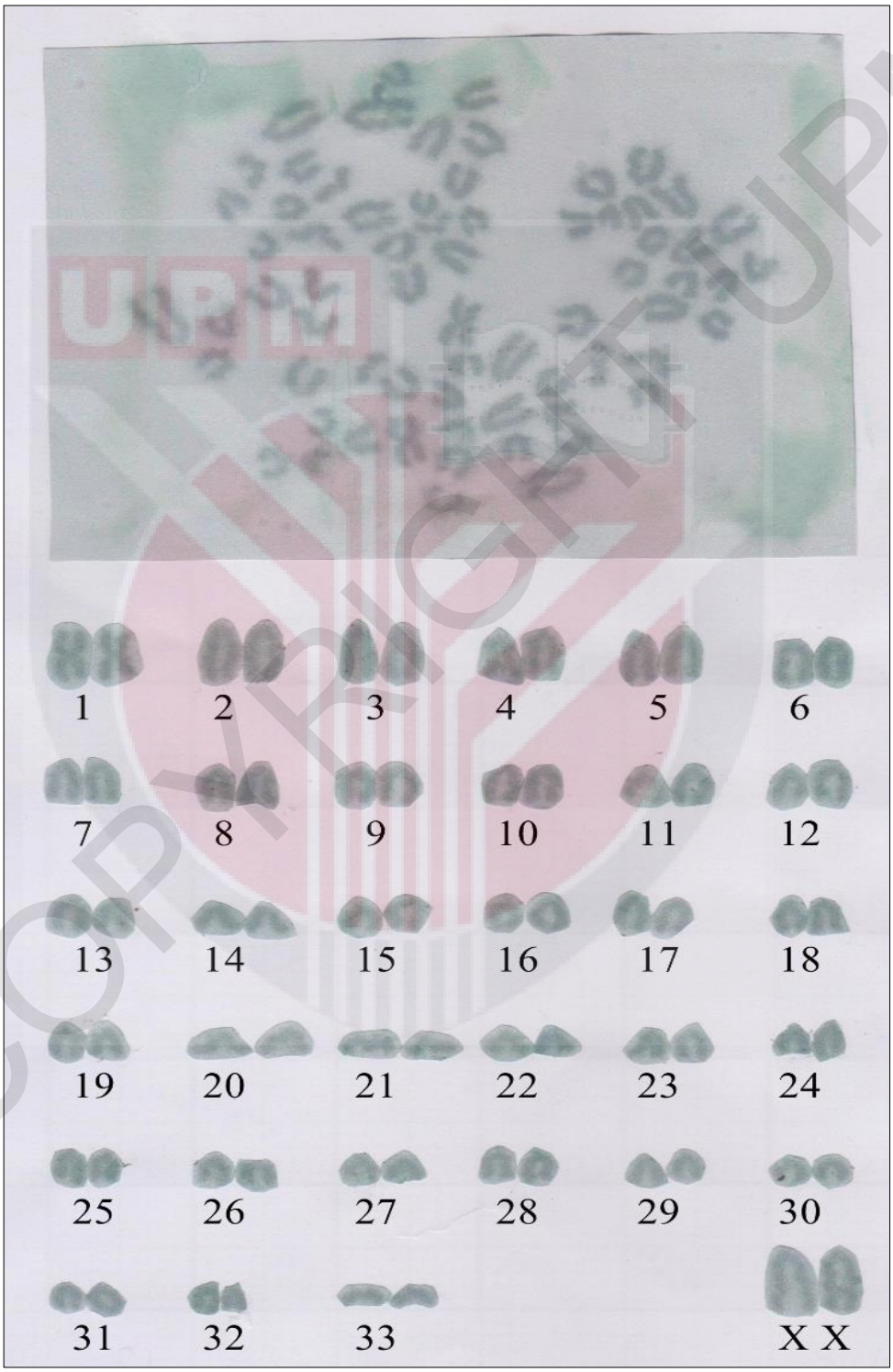


Table 3.0: Morphological characteristics of chromosome of fallow deer

	Autosomes		Sex Chromosome		Total Number	Fundamental Number
	M	AC	X	Y		
Male	2	64	AC	SM	68	71
Female	2	64	AC	-	68	70

M : Metacentric

SM : Submetacentric

AC : Acrocentric

Figure 5.0: Conventional karyotype of male spotted deer

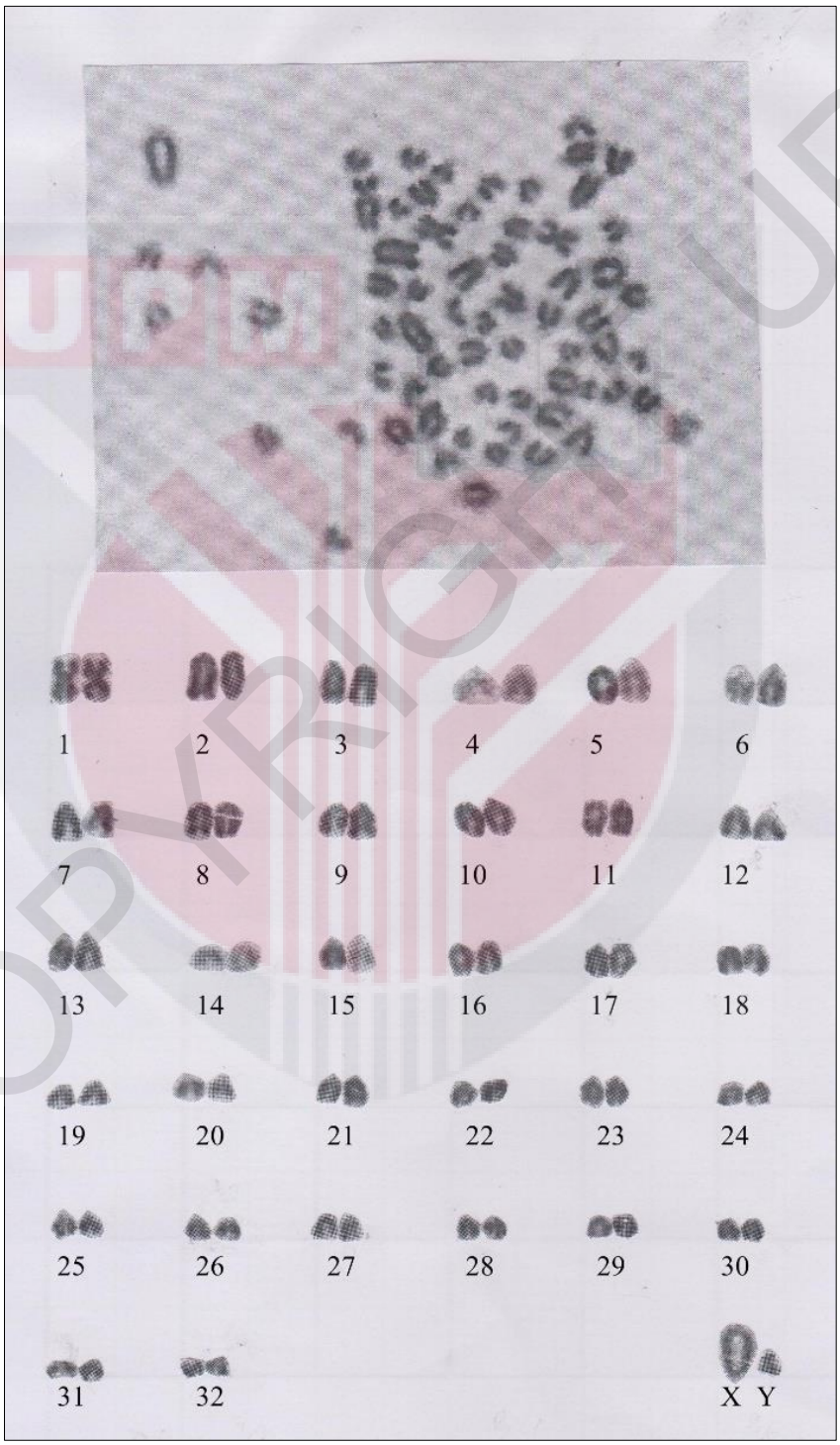


Figure 6.0: Conventional karyotype of female spotted deer

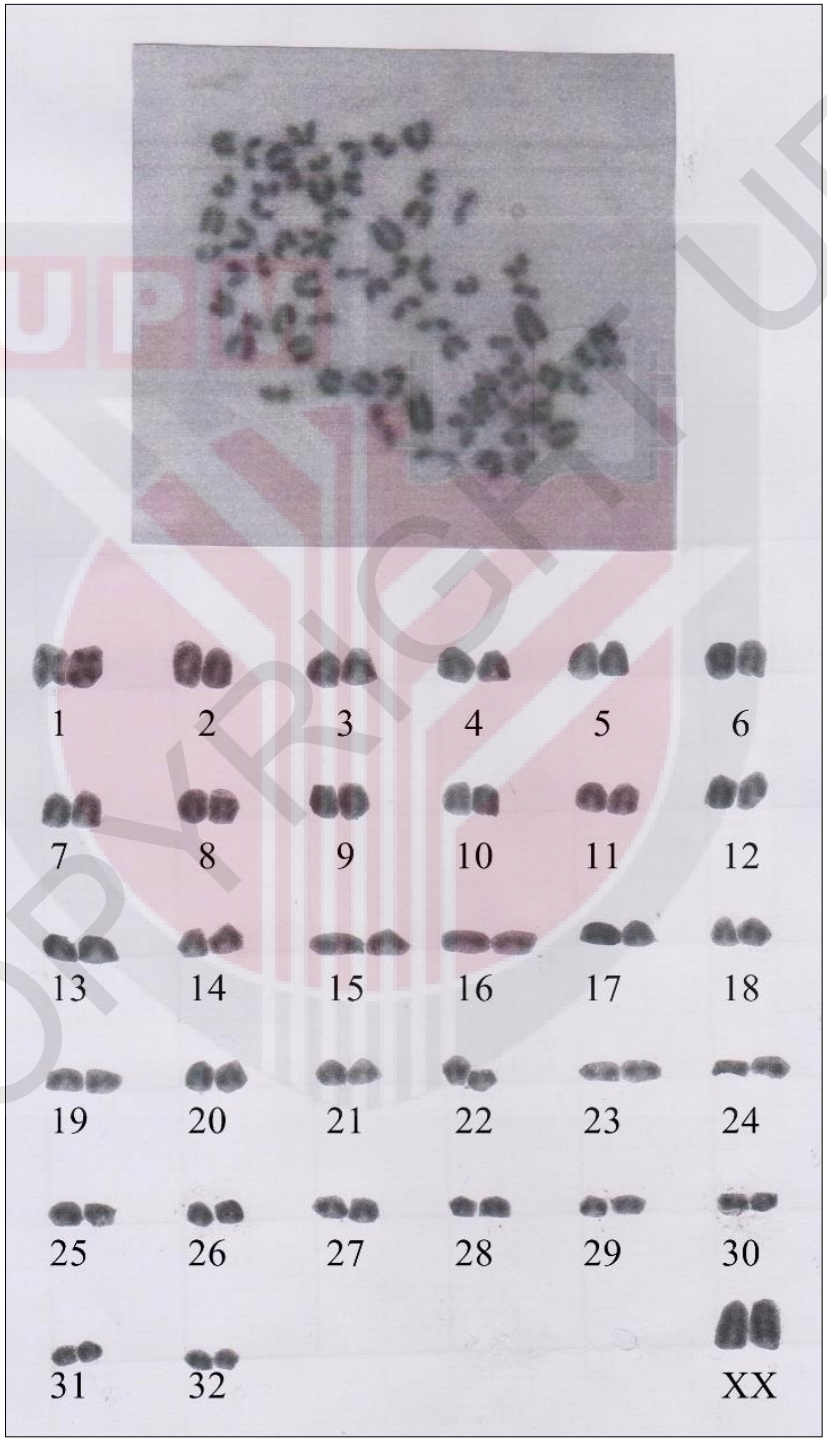


Table 4.0: Morphological characteristics of chromosome of spotted deer

	Autosomes		Sex Chromosome		Total Number	Fundamental Number
	M	AC	X	Y		
Male	2	62	AC	SM	66	69
Female	2	62	AC	-	66	68

M : Metacentric

SM : Submetacentric

AC : Acrocentric

5.0 DISCUSSION

From our study, the diploid number of 68 in fallow deer with 33 pairs of autosome (1 pair of metacentric and 32 pairs of acrocentric) and a pair of sex chromosomes (X chromosome as longest acrocentric and Y chromosome as smallest submetacentric) was in agreement with the study done by Kozubska-Sobocińska *et. al.*, 2013; Markov, 1997; Gripenberg and Wessman, 1993; Gurtavsson and Sundt, 1968. The fundamental number of chromosomes were based on the number of arms displayed in the chromosome morphology. In this study it was found that the fallow deer had fundamental number of 71 in male and 70 in female.

The diploid number of 66 in spotted deer with 32 pairs of autosome (1 pair of metacentric and 31 pairs of acrocentric) and a pair of sex chromosomes (X chromosome as longest acrocentric and Y chromosome as smallest submetacentric) was in agreement with the study done by Shanthi *et. al.*, 2008; Robinson and Elder, 1993; Hsu and Benirschke, 1975. In this study it was found that the spotted deer had fundamental number of 69 in male and 68 in female.

Since these findings were in agreement with other scholars, so there is less risk of combination of the germplasm of this two species. However, there is a need to further confirm with biochemical polymorphisms and randomly amplified polymorphic DNA (RAPD) markers techniques like the study done by Habiba Ali, 2005 on the other species of deer.

Besides that, this study also examined the species of different genus from subfamily, Cervinae namely *Dama dama* (fallow deer) and *Axis axis* (spotted deer). Meanwhile in previous study by Habiba Ali, 2005, they did a study of 'Genetic Characterization of Deer Raised in Malaysia' that involved deer species from the same genus, *Cervus* namely *Cervus timorensis* (rusa), *Cervus unicolor* (sambar) and *Cervus nippon* (sika).

From those studies, all of these deer species had different diploid number in which fallow deer, spotted deer, rusa, sambar and sika displayed a total of 68, 66, 60, 62 and 66 chromosomes. They were also differ in terms of autosomes number and morphology. Fallow deer had 33 pairs of autosome (1 pair of metacentric and 32 pairs of acrocentric). Spotted deer had 32 pairs of autosome (1 pair of metacentric and 31 pairs of acrocentric). Rusa had 29 pairs of autosome (5 pairs of metacentric/submetacentric and 24 pairs of acrocentric). Sambar had 30 pairs of autosome (4 pairs of metacentric/submetacentric and 26 pairs of acrocentric). Sika had 32 pairs of autosome (2 pairs of metacentric/submetacentric and 30 pairs of acrocentric autosomes).

The morphology of sex chromosome of fallow deer and spotted deer were similar in which X chromosome was characterized as the largest acrocentric, while Y chromosome was a smallest submetacentric. Meanwhile, the morphology of sex chromosome of rusa, sambar and sika were similar in which X chromosome was characterized as the largest acrocentric, while the Y chromosome was a small acrocentric.

There were also difference on the fundamental number of fallow deer and spotted deer. Fallow deer had fundamental number of 70 in male and 71 in female while spotted deer had 69 in male and 68 in female. However, the fundamental number of rusa, sambar and sika were similar, 70 in both sexes.

Although these five species of deer belong to the same family, Cervidae and subfamily Cervinae, their chromosome constitution are different and even if they were belong to the same genus *Cervus*, such as rusa, sambar and sika, their chromosome constitution are also different. This shows through years of evolution and geographical isolation, the genus *Cervus* have arises into different species with different features for identification.

6.0 CONCLUSIONS

The fallow deer and spotted deer have different diploid number and fundamental number but similar in chromosome morphology that consisted of 1 pair of metacentric in the autosome and in the sex chromosome, X chromosome as largest acrocentric while Y chromosome as smallest submetacentric in the karyotypes.

7.0 RECOMMENDATIONS

Banding techniques such as Giemsa (G)- banding and Centromere (C)- banding can be conducted to further confirm the karyotypes of fallow deer and spotted deer. A further cytogenetic study can also be done on another deer species raised in Malaysia such as *Cervus elaphus* (wapiti) and *Cervus canadensis* (elk).

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