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Original Article

The first chromosome characterization of the family Tragulidae (Artiodactyla) in Thailand by conventional staining

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Abstract

Karyotypes were studied from the family Tragulidae of Thailand, representing a single genus with two species namely; lesser Malay mouse-deer (*Tragulus javanicus*) and larger Malay mouse-deer (*Tragulus napu*). Blood samples were taken from the two species kept in Khoa Kheow Open Zoo, Chonburi province and Songkhla Zoo, Songkhla province, Thailand. After standard whole blood lymphocyte culture in presence of colchicine, the metaphase spreads were performed on microscopic slides and air-dried. Conventional Giemsa's staining was applied to visualize chromosomes. The karyotype of lesser Malay mouse deer showed that diploid chromosome number was 2*n*=32 and fundamental numbers (NF) were 64 in both female and male. The autosomes consist of 6 large metacentric, 6 large submetacentric, 14 medium metacentric, 2 submetacentric and 2 small metacentric chromosomes. The X chromosome was a large submetacentric chromosome while the Y chromosome was a small metacentric chromosome. For our result, the first karyotypic study of *T. napu*, the larger Malay mouse-deer, the karyotype shows that diploid chromosome number was 2*n*=32, and NF were 64 in both female and male. The autosomes consist of 6 large metacentric, 6 large submetacentric, 12 medium metacentric, 2 medium submetacentric, 2 medium acrocentric and 2 small submetacentric chromosomes. The X chromosome was a large submetacentric chromosome while the Y chromosome was the smallest metacentric chromosome.

Keywords: karyotype, Tragulidae, lesser Malay mouse-deer (*Tragulus javanicus*), larger Malay mouse-deer (*Tragulus napu*)

1. Introduction

Mouse-deer or chevrotains (Tragulidae, Artiodactyla) are small ruminants. They have primitive features with no horn or antler. Both sexes possess enlarged upper canine. The family Tragulidae can be divided into 3 genera and 9 species including chevrotains (genus *Hyemoschus* and *Moschiola*) 3 species and mouse-deer or genus *Tragulus* 6 species. Mouse-deer can found only in the tropical forests of Africa, India, Sri Lanka, and South-east Asia. Two species in family Tragulidae found in Thailand are the lesser Malay mouse-deer and the large Malay mouse-deer (Lekagul and McNeely, 1977, 1988). Smit-Van (1889) investigated on skin skull and characters of Tragulidae with keys to species in which found clearly distinct characters exist between the lesser Malay mouse-deer and the large Malay mouse-deer.

According to existing previous report, the study on

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lesser Malay mouse-deer cytogenetic (Yong, 1973; Shi and Chen, 1989; Gallagher *et al*., 1996), there is no cytogenetic report on large Malay mouse-deer. Thus, this study is the first karyotypic description for the large Malay mouse-deer.

2. Materials and Methods

Blood samples from the jugular vein were collected using aseptic technique from 2 females and 2 males lesser Malay mouse-deer, 3 females and 3 males larger Malay mouse-deer, which were kept in Khoa Kheow Open Zoo (KKOZ), Chonburi province and Songkhla Zoo (SZ), Songkhla province, Thailand. The samples were kept in 10 milliliters vacuum tubes containing heparin to prevent blood clotting and then cooled on ice until arriving at the laboratory.

2.1 Cell culture

The lymphocytes were cultured using whole blood microculture technique adapted from Campiranon (2003). The RPMI 1640 medium was prepared with 2% phytohemagglutinin (PHA) as a mitogen and kept in blood culture bottles of 5 ml each. The 0.5 milliliters of blood sample was dropped into medium and well mixed. The culture bottle was loosely capped, incubated at 37C under 5% of carbon-dioxide environment and regularly shaken every morning and evening. When reaching harvest time at the 72nd hour of incubation, colchicine was introduced and well mixed followed by further incubation for 30 minutes.

2.2 Cell harvest

The blood sample mixture was centrifuged at 1,200 rpm (85 xg) for 10 minutes and the supernatant was discarded. 10 ml of hypotonic solution (0.075 M KCl) was applied to the pellet and the mixture was incubated for 30 minutes. After centrifugation again and discarding supernatant, cells were fixed by fresh cool fixative (1 methanol : 3 glacial acetic acid) gradually added up to 8 ml before centrifuging and the supernatant discarded. The 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 using micropipette followed by the air-dry technique.

2.3 Chromosomal checks karyotyping and idiograming

Chromosomal checks were performed on mitotic metaphase cells under light microscope. Twenty cells of females and males lesser Malay mouse deer and larger Malay mouse deer with clearly observable and widely-spread chromosomes were selected and photographed. Metaphase chromosomes were used in karyotyping and idiograming following Nash and O'Brien (1987) and Wada *et al*. (1991).

3. Results and Discussion

After cells were cultured at 37^{o}C for 72 hours and conventionally stainined, we found that lesser Malay mouse-deer has 2*n*=32 and fundamental number (NF) were 64 in both of male and female (Figures 1, 2 and 3). This result is in agreement with Yong (1973), Shi and Chen (1989) and Gallagher *et al*. (1996). For larger Malay mouse-deer, 2*n*= 32 and fundamental number (NF) was 64, too (Figures 4, 5 and 6). Moreover, we also concluded that lesser and larger Malay mouse-deer has the same chromosome number and fundamental number.

Lesser Malay mouse-deer has 6 large metacentric, 6 large submetacentric, 14 medium metacentric, 2 submetacentric and 2 small metacentric autosomes (Figures 1, 2 and 3). This is similar to Yong (1973), Shi and Chen (1989) and Gallagher *et al*. (1996), were reported the autosomes of lesser Malay mouse-deer as metacentric and submetacentric chromosomes. The character of the autosomes was complete Robertsonian fusion (centric fusion). For larger Malay mouse-deer, the autosomes were 6 large metacentric, 6 large

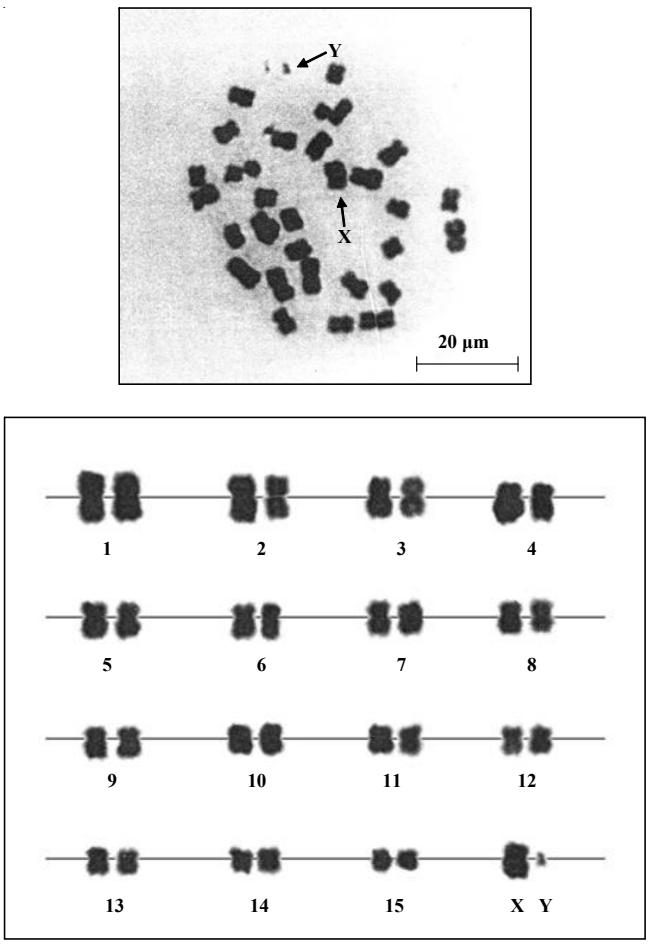

Figure 1. Metaphase and karyotype of male lesser Malay mouse-deer (*Tragulus javanicus*) 2n=32 by conventional staining, arrow indicate sex-chromosomes

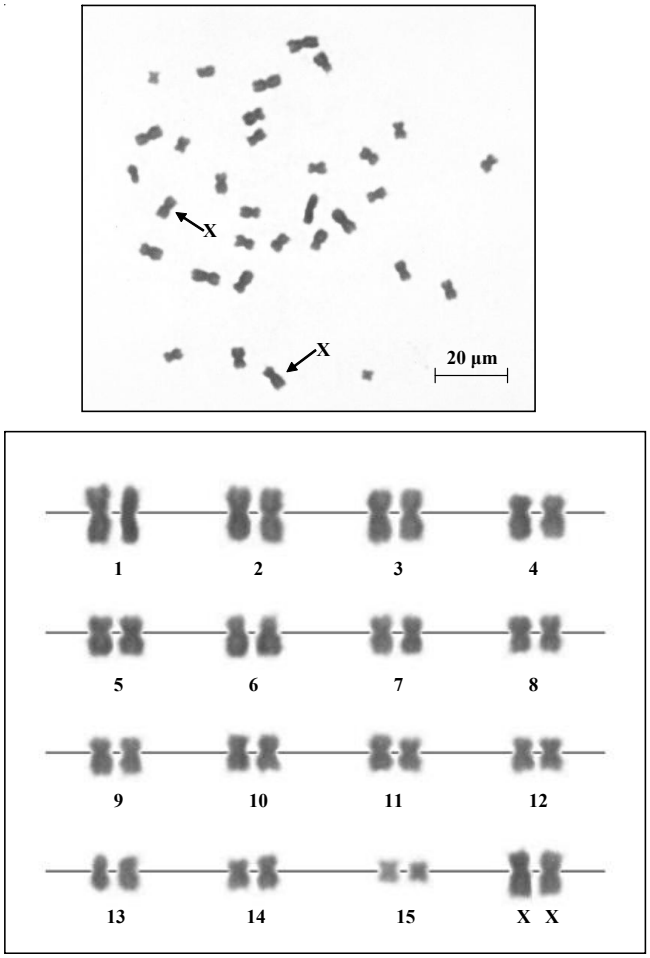

Figure 2. Metaphase and karyotype of female lesser Malay mouse-deer (*Tragulus javanicus*) 2n=32 by conventional staining, arrow indicate sex-chromosomes

submetacentric, 12 medium metacentric, 2 medium submetacentric, 2 medium acrocentric and 2 small submetacentric chromosomes (Figures 4, 5 and 6). The autosomes of lesser Malay mouse-deer and larger Malay mouse-deer have much similarity. Most of all of both species have the same autosomes except in chromosome pair 8 in which appear acrocentric and metacentric chromosome in lesser Malay mouse deer and larger Malay mouse-deer, respectively. The change of chromosome pair 8 between acrocentric and metacentric may have resulted from pericentric inversion.

The X chromosome of lesser Malay mouse deer was a large submetacentric chromosome and the Y chromosome was a small metacentric chromosome (Figures 1, 2 and 3) confirming the reports of Yong (1973), Shi and Chen (1989) and Gallagher *et al*. (1996). For larger Malay mouse-deer, the X chromosome was a large submetacentric chromosome and the Y chromosome was the smallest metacentric chromosome (Figures 4, 5 and 6). These results revealed that both of Malay mouse-deer have the same size and type of X chromosome while the size of their Y chromosome is quite difference. Lesser Malay mouse-deer has a small Y chromo-

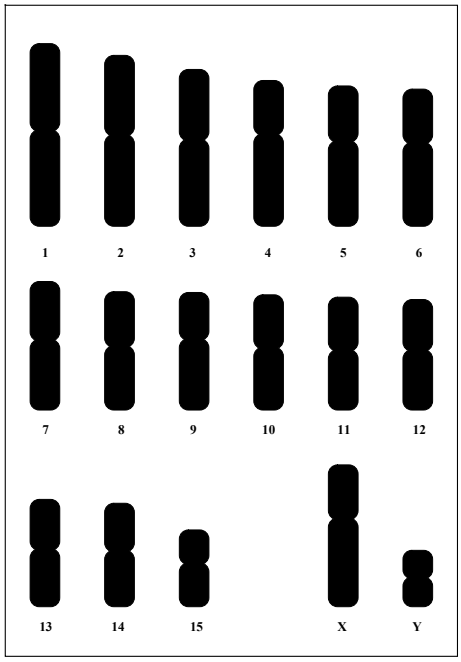

Figure 3. Idiogram of lesser Malay mouse-deer (*Tragulus javanicus*) 2n=32 by conventional staining

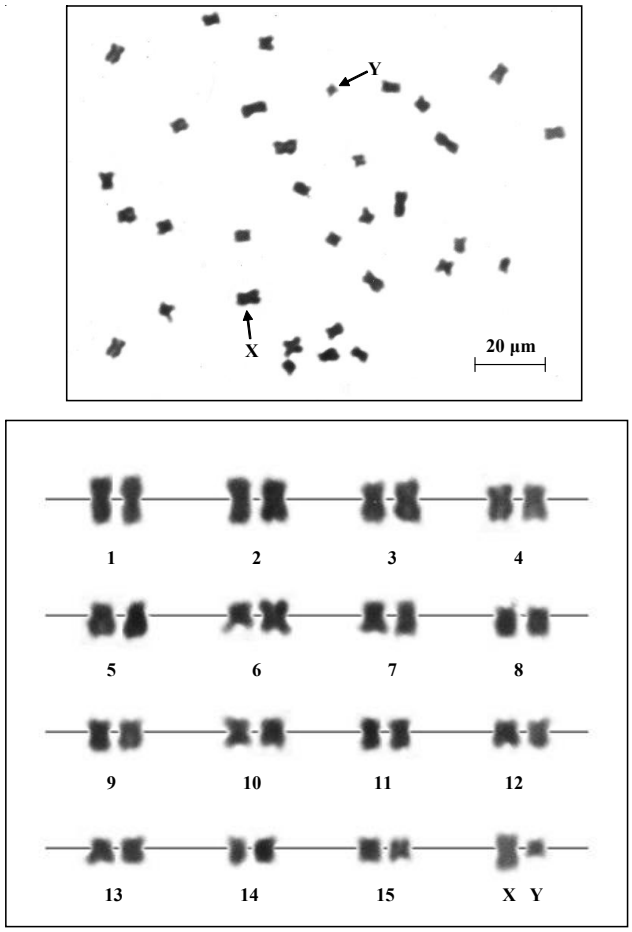

Figure 4. Metaphase and karyotype of male larger Malay mouse-deer (*Tragulus napu*) 2n=32 by conventional staining, arrow indicate sex-chromosomes

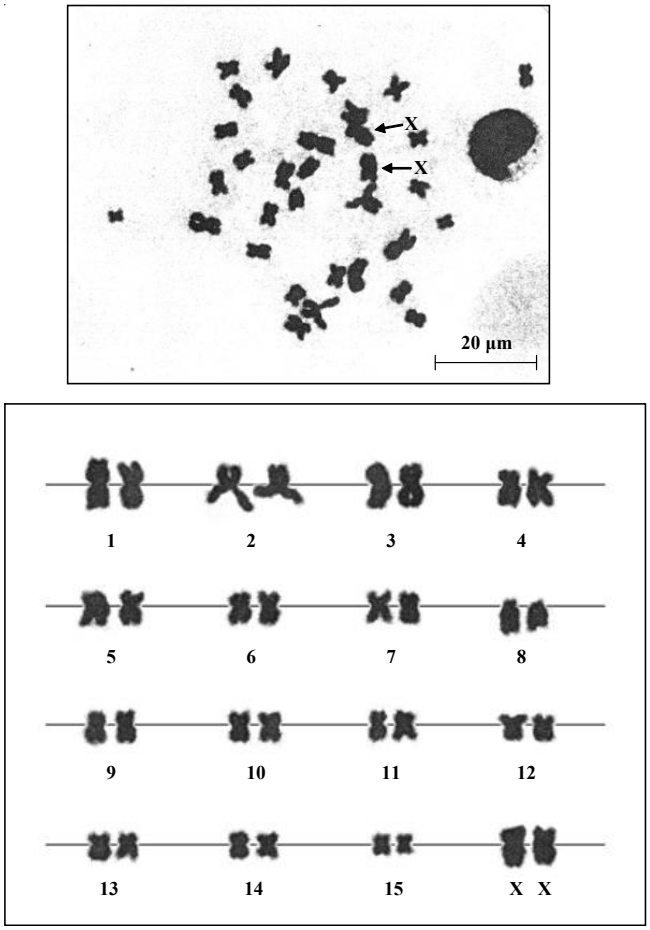

Figure 5. Metaphase and karyotype of female larger Malay mouse-deer (*Tragulus napu*) 2n=32 by conventional staining, arrow indicate sex-chromosomes

some but larger Malay mouse-deer has an even smaller than those of Y chromosome (Figures 1 and 4).

The 20 metaphase cells of males and females lesser and larger Malay mouse-deer were used the chromosome measuring at 3,800 fold size. The average length of short arm (Ls), long arm (Ll) and the length of the chromosome (LT) were determined. The relative length (RL), centromeric index (CI) and SD of RL and CI were calculated. The size and type of each chromosome were determined (Tables 1 and 2).

The karyotype formula for the lesser Malay mouse-deer is as follows:

$2n\ (32) = L_6^{m}+L_6^{sm}+M_{14}^{m}+M_2^{sm}+S_2^{m}$ + sex chromosomes

The karyotype formula for the larger Malay mouse-deer is as follows:

$2n\ (32) = L_6^{m}+L_6^{sm}+M_{12}^{m}+M_2^{sm}+M_2^{a}+S_2^{m}$+sex chromosomes

The present study demonstrated that larger Malay mouse-deer and lesser Malay mouse-deer have much similarity in karyotype. We found that there is only one difference point of both karyotypes at chromosome 8, with the presence of acrocentric and metacentric in the larger Malay mouse-deer and lesser Malay mouse-deer, respectively. According to the report of Slate *et al*. (2002) and Huang *et al*. (2005), Pecoran ancestor (an ancestor of order Artiodactyla) has *2n*=58. The types of autosomes were 27 acrocentric and 1 submetacentric chromosomes, with the X and Y-chromosome being submetacentric and acrocentric chromosome, respectively. The changing of larger Malay mouse-deer and lesser Malay mouse-deer's karyotype could be assumed as following. Larger Malay mouse-deer chromosomes has changed from *2n*=58 to *2n*=32. Its 2 autosomes were resemble to an ancestor; 1) 1 metacentric or submetacentric and 2) acrocentric chromosomes, while 13 of 26 acrocentric chromosomes has become metacentric or submetacentric as found as larger Malay mouse-deer that resulted from the complete Robertsonian translocation (centric fusion). Most of all of both species have the same autosomes except in chromosome pair 8 in which appear acrocentric and metacentric chromosome in lesser Malay mouse deer and larger Malay mouse-deer, respectively. The changing of chromosome pair 8 between acrocentric in larger Malay mouse-deer and metacentric in lesser Malay mouse-deer may result from pericentric inversion. The changing of sex chromosome in both species from pecoran ancestor, the submetacentric X-chromosomes in both species are similar to their ancestor while the metacentric Y-chromosome are derived from heterochromatin addition or pericentric inversion (Figure 7).

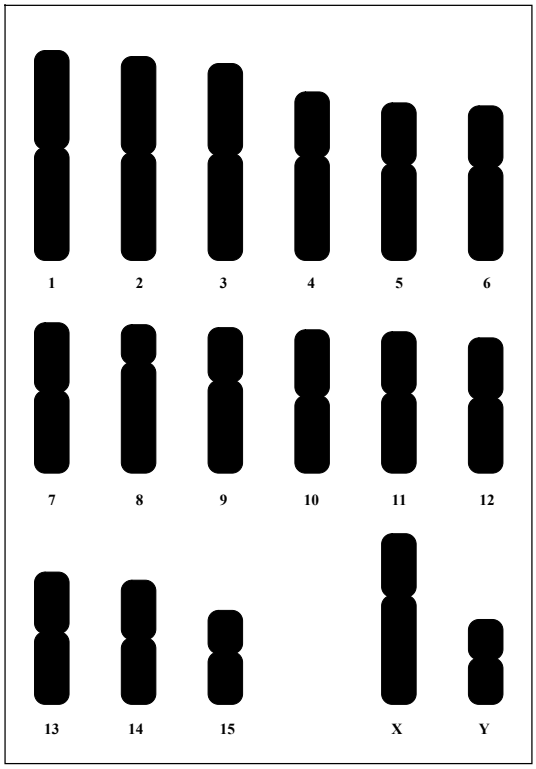

Figure 6. Idiogram of larger Malay mouse-deer (*Tragulus napu*) 2n=32 by conventional staining

The changing of family Tragulidae's karyotype may result from Robertsonian fusion and fission and may also

Table 1. Mean of length short arm chromosome (Ls), length long arm chromosome (Ll), length total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL and CI of metaphase chromosome from 20 cells in male and female lesser Malay mouse deer (*Tragulus javanicus*) 2n (diploid) = 32

chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	size of chromosome	type of chromosome
1	1.10	1.22	2.32	0.086±0.004	0.525±0.017	L	m
2	1.01	1.16	2.17	0.081±0.002	0.534±0.015	L	m
3	0.90	1.10	2.01	0.075±0.003	0.550±0.023	L	m
4	0.69	1.17	1.86	0.069±0.002	0.630±0.021	L	sm
5	0.71	1.08	1.78	0.066±0.001	0.604±0.027	L	sm
6	0.69	1.06	1.75	0.065±0.002	0.607±0.031	L	sm
7	0.75	0.89	1.64	0.061±0.001	0.542±0.026	M	m
8	0.70	0.81	1.51	0.056±0.003	0.537±0.028	M	m
9	0.60	0.90	1.50	0.056±0.002	0.602±0.029	M	sm
10	0.68	0.79	1.47	0.055±0.001	0.538±0.030	M	m
11	0.68	0.76	1.43	0.053±0.003	0.529±0.021	M	m
12	0.65	0.76	1.40	0.052±0.003	0.540±0.015	M	m
13	0.64	0.73	1.36	0.051±0.001	0.532±0.013	M	m
14	0.61	0.71	1.32	0.049±0.002	0.538±0.014	M	m
15	0.44	0.55	0.99	0.037±0.001	0.557±0.025	S	m
X	0.69	1.12	1.81	0.067±0.003	0.617±0.011	L	sm
Y	0.35	0.38	0.73	0.027±0.001	0.521±0.010	S	m

Table 2. Mean of length short arm chromosome (Ls), length long arm chromosome (Ll), length total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL and CI of metaphase chromosome from 20 cells in male and female larger Malay mouse deer (*Tragulus napu*) 2n (diploid) = 32

chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	size of chromosome	type of chromosome
1	0.97	1.11	2.08	0.082±0.001	0.534±0.018	L	m
2	0.96	1.06	2.02	0.080±0.001	0.526±0.016	L	m
3	0.90	1.05	1.95	0.077±0.002	0.538±0.023	L	m
4	0.64	1.03	1.67	0.066±0.001	0.617±0.030	L	sm
5	0.61	0.95	1.56	0.062±0.005	0.608±0.012	L	sm
6	0.60	0.93	1.53	0.060±0.001	0.606±0.024	L	sm
7	0.68	0.81	1.48	0.058±0.004	0.545±0.010	M	m
8	0.38	1.09	1.47	0.058±0.001	0.744±0.011	M	a
9	0.53	0.91	1.43	0.056±0.001	0.634±0.028	M	sm
10	0.66	0.76	1.42	0.056±0.002	0.537±0.019	M	m
11	0.61	0.79	1.40	0.055±0.002	0.563±0.035	M	m
12	0.60	0.74	1.34	0.053±0.001	0.551±0.026	M	m
13	0.60	0.71	1.30	0.051±0.003	0.543±0.018	M	m
14	0.59	0.65	1.24	0.049±0.002	0.527±0.006	M	m
15	0.43	0.51	0.94	0.037±0.001	0.547±0.014	S	m
X	0.62	1.07	1.68	0.066±0.004	0.634±0.015	L	sm
Y	0.40	0.45	0.85	0.034±0.001	0.529±0.007	S	m

cooperate with inversion, translocation, duplication and deletion. Cytogenetics knowledge obtained from G-banding, NOR-banding, High-resolution technique and FISH (fluorescence *in situ* hybridization) could provide a clearer answer regarding the chromosome evolution. At present, there is little cytogenetic knowledge of the family Tragulidae. There

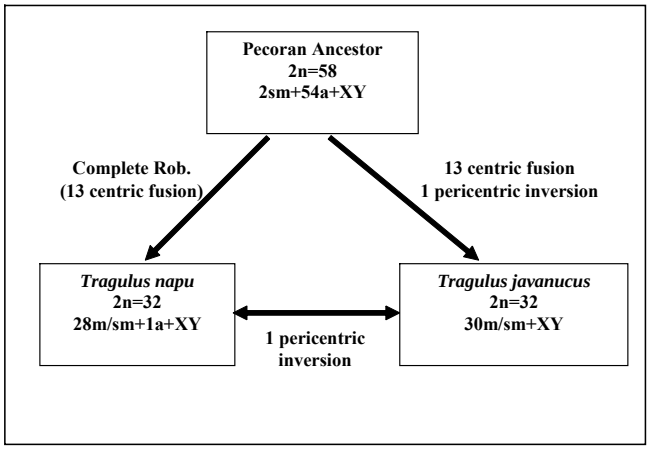

Figure 7. The chromosome evolution of family Tragulidae in Thailand including larger Malay mouse-deer (*Tragulus napu*) and lesser Malay mouse-deer (*T. javanicus*) from Pecoran ancestor (the ancestor of order Artiodactyla)

are just 1 genus and 2 species' karyotypes available for this family (3 genus 9 species), it is difficult to make an overview comparison of chromosome evolution of the animals in this family.

4. Conclusion

The karyotype of lesser Malay mouse-deer (*T. javanicus*) shows that the diploid chromosome number was 2*n*=32, and NF were 64 in both female and male individuals. The autosomes consist of 6 large metacentric, 6 large submetacentric, 14 medium metacentric, 2 submetacentric and 2 small metacentric chromosomes. The X chromosome is a large submetacentric chromosome while Y chromosome is a small metacentric chromosome. This research is the first karyotypic study of larger Malay mouse-deer (*T. napu*). The karyotype shows that diploid chromosome number was 2*n*= 32 and NF were 64 in both female and male. The autosomes consist of 6 large metacentric, 6 large submetacentric, 12 medium metacentric, 2 medium submetacentric, 2 medium acrocentric and 2 small submetacentric chromosomes. The X chromosome is a large submetacentric chromosome while the Y chromosome is the smallest metacentric chromosome. Most of both species have the same autosomes except in chromosome pair 8 in which appear acrocentric and metacentric chromosome in lesser Malay mouse deer and larger Malay mouse-deer, respectively. The changing of chromosome pair 8 between acrocentric in larger Malay mouse-deer and metacentric in lesser Malay mouse-deer may result from pericentric inversion.

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