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Cytogenetics of Black-bearded Tomb bat (*Taphozous melanopogon*) by Conventional staining, Ag-NOR staining and Fluorescence in situ hybridization Techniques

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ABSTRACT

Black-bearded Tomb bat is classified as the genus of *Taphozous* and has a very similar morphology. The knowledge can provide cytogenetic information potentially useful to support on taxonomic classification. Five males and six females were collected from Mahasarakam province and Khon Kaen university, Khon Kaen province. The 20% Giemsa's and 50% silver nitrate solution, respectively were applied to stain the chromosomes. Chromosomes were prepared from bone marrow tissue. The chromosomes harvesting was investigated by hypotonic-fixation-air drying technique. Giemsa's staining, Ag-NOR staining and fluorescence in situ hybridization (FISH) staining techniques, as well as microsatellites $(GC)_{15}$ and $(CGG)_{10}$ as probes were applied. The karyotype, idiogram, position of NORs and microsatellites were performed in the present study. The results show that the diploid chromosomes number was 2n = 42 and the fundamental number (NF) were 74 chromosomes in both female and male of *T. melanopogon*. The autosomes consisting of 8 large metacentric, 8 medium metacentric, 2 medium submetacentric, 2 medium acrocentric, 2 small metacentric, 4 small

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submetacentric, 4 small acrocentric and 10 small telocentric chromosomes. The sex determination is XY, X chromosome is medium metacentric chromosomes and Y chromosome is small submetacentric chromosomes. NOR sites appeared to telomere of the short arm of the chromosome pairs 15 small acrocentric type and chromosome pairs 20 small telocentric type. The pattern of both microsatellite $(GC)_{15}$ and $(CGG)_{10}$ repeats were distributed throughout the genome as well as to all chromosomes. Karyotype formula described as 2n (42) = $L^{m}{}_{8}$ + $M^{m}{}_{8}$ + $M^{sm}{}_{2}$ + $M^{a}{}_{2}$ + $S^{m}{}_{2}$ + $S^{sm}{}_{4}$+ $S^{a}{}_{4}$ + $S^{t}{}_{10}$ + Sex Chromosome.

INTRODUCTION

Black-bearded Tomb bat is a common bat in Thailand. Belongs to the family Emballonuridae. There are 14 species of taphozous. it can be found in 4 species in Thailand, black-bearded tomb bat (*Tasphozous melanopogon*), long-winged tomb bat (*T. longimanus*), large-winged bat (*T. theobaldi*), naked-rumped pouched bat (*T. saccolaimus*) (1). Studies have shown that bats in this genus have the number of diploid chromosomes is between 42 and 44 rods (2n = 42 - 44) (2). It is estimated that the black-bearded tomb bat has similar numbers of diploids to another bat of the same genus. Karyological analysis were studied of the lesser Asiatic house bat (*Scotophilus kuhlii*) from Thailand. The results showed that the fundamental number (NF) was 52 in both male and female and the diploid chromosome number of *S. kuhlii* was 2n=36 (3). Chromosome analysis and morphometrics of the intermediate roundleaf bat (*Hipposideros larvatus*) from Northeast Thailand were studied. The results showed that the diploid chromosome number of *H. larvatus* was 2n=32, and the fundamental number as 66 in both males and females (4). The karyotype was studied of *M. horsfieldii* was 2n= 44 from Thailand. The results showed that the diploid chromosome number of *S. kuhlii* was 2n= 36, and the fundamental number was 52 in both male and female. (5). The number of diploids was 42 rods (2n = 42). The karyotype were studied of *Taphozous nudiventris*, which the source of the sample is in the region of Turkey. (6). The Karyology was studied of Ten Vespertilionid Bats (Chiroptera: Vespertilionidae) from Taiwan. The result showed three *Myotis* species (*M. formosus watasei*, *M. latriostris*, and *M. taiwanensis*) have the standard Myotis karyotype of 2n = 44 with FN = 50 (7). Cytogenetics is the study of genetics through cytological methods and genetics. structure, number, function, behavior and variation of chromosomes. These properties that affect the expression of the gene (8). Studying cytogenetics brings benefits in areas such as in medicine, it is used to diagnose genetic diseases.

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The evolution of organisms from the cellular change. This is because the study and comparison of karyotypes are important in the study of phylogenetic closeness and Taxonomy to classify certain organisms (9). Karyotype were studied of three Lonchophylla species (Chiroptera, Phyllostomidae) from Southeastern Brazil. All three species showed the same diploid number 2n = 28 and an autosomal fundamental number FNa= 50 (9). Conventional staining technique has been used to determine chromosome number and karyotype composition. Structure, number, type, size, and morphology of a nucleolar organizer region (NOR) may be specific to populations, species, and subspecies. NOR staining is frequently used to compare variations, as well as to identify and explain specifications (10). Molecular cytogenetic experiments have demonstrated that NORs are the chromosomal site of gene coding for 5.8S, 18S, and 28S rRNA, in humans and several mammalian species. NORs can be used as markers for evolutionary chromosome studies (10). Recently, molecular cytogenetic studies using fluorescence in situ hybridization (FISH) for mapping repetitive DNA sequences have provided important contributions to the characterization of biodiversity and the evolution of divergent fish groups (11). However, conventional cytogenetic and fluorescence in situ hybridization technique is the conduction of DNA probes binds to target DNA on Black-bearded Tomb bat have not yet been performed. Black-bearded Tomb bat is classified as the genus of Taphozous and has a very similar morphology. Accordingly, the present study is the cytogenetic report on *Tasphozous melanopogon* of Thailand and accomplished with both classical and molecular cytogenetics. It is therefore the source for the study of the cytogenetics of the Black-bearded Tomb bat (*T. melanopogon*) by Giemsa's staining, Ag-NOR staining and fluorescence in situ hybridization techniques, which results from a study of genetics. The cellular level of the Black-bearded tomb bat will give us more detailed insight into the chromosome level as a supporting material for further identification of bat species.

MATERIALS AND METHODS

Sample collection

Five males and six females of Black-bearded Tomb Bat were collected from Mahasarakam province and Khon Kaen university, Khon Kaen province.

Chromosome preparation, Giemsa' s staining and AgNORs banding technique

Metaphase chromosomes were directly prepared in vivo as following (8) and (9). Subsequently, chromosomes were stained with 20% Giemsa solution and 50 % silver nitrate for Ag-NOR banding (12).

Chromosome checking

Chromosome counting was performed on mitotic metaphase cells under light microscope. Twenty cells each of male and female with clearly observable and well-spread chromosomes were selected and photographed. The length of short arm (Ls) and the length of

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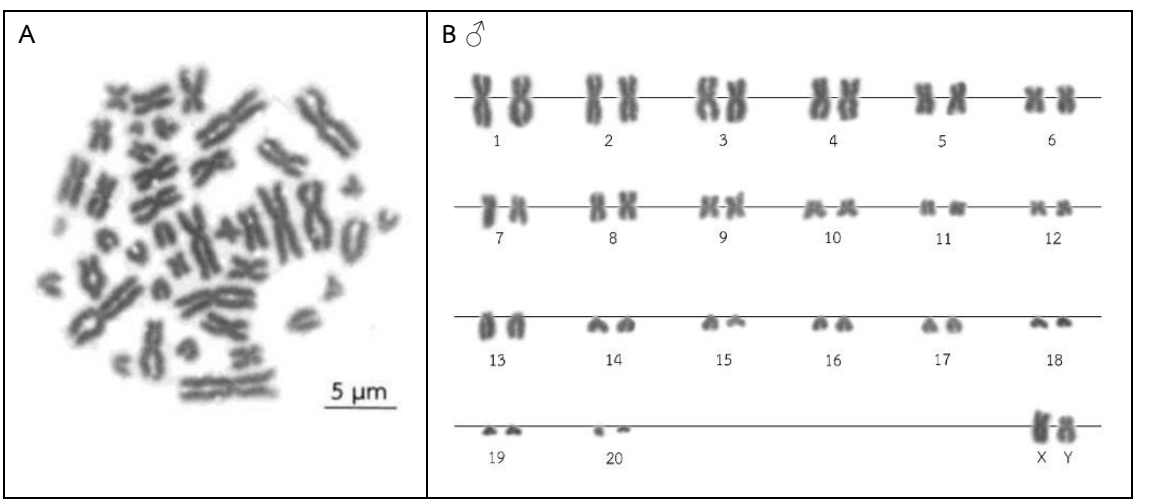
long arm of chromosome (Ll) were measured to calculate the length of total arm chromosome (LT, LT = Ls + Ll). The relative length (RL), the centromeric index (CI) and standard deviation (SD) of RL, CI were also computed to classify the types and size of chromosomes according to (13). All parameters were used in karyotyping and ideogram according to (12), and (13). 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 (m), submetacentric (sm), acrocentric (a), and telocentric (t) chromosomes, respectively. The fundamental number (NF) was obtained by assigning a value of 2 to the m, sm and a chromosome and 1 to the t chromosome. All data were used in karyotyping and diagramming (14)

Fluorescence in situ hybridization (FISH) with $d(GC)_{15}$, and $d(CGG)_{10}$ probes

FISH was performed on metaphase chromosome spreads with specific probes (15). Both rDNA probes were directly labeled with the Nick-translation Labeling Kit (Jena Bioscience, Jena, Germany), using the fluorescent labels Atto488 (18S rDNA) and Atto550 (5S rDNA), according to the manufacturer's manual (16). The usage of microsatellites $d(GC)_{15}$, and $d(CGG)_{10}$ probes described by (12) was followed with slight modifications. Sequences were directly labeled with Cy3 at 5′ terminals during synthesis by Sigma (St. Louis, MO, USA). FISH was performed on mitotic chromosome spreads (17) under highly stringent conditions, as previously reported (18).



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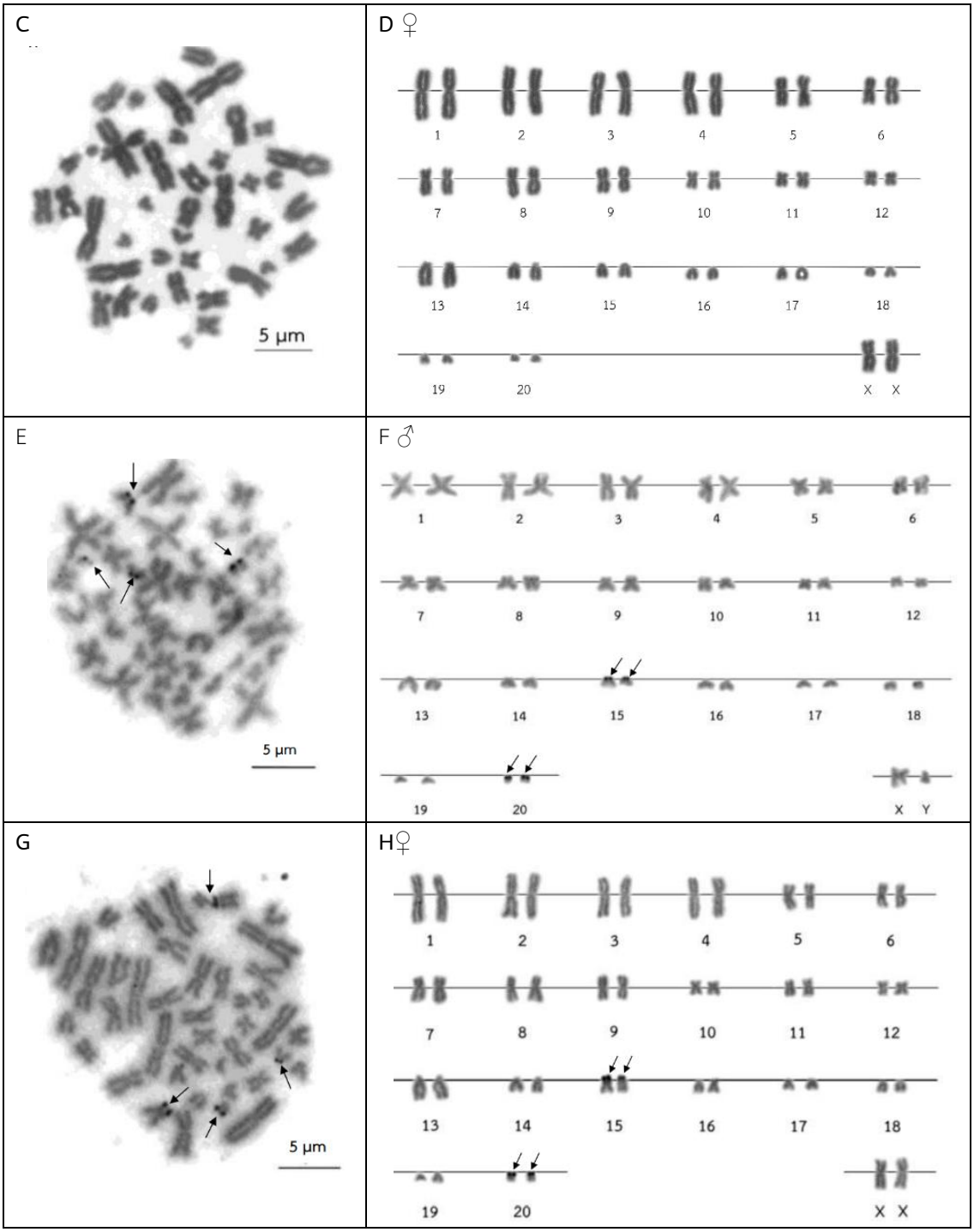

Figure 1 The metaphase and karyotypes chromosomes of the Black- bearded Tomb Bat (*T. melanopogon*) 2n= 42, male (A- B and E- F) and female (C- D and G- H) with conventional chromosome staining and Ag-NOR staining banding technique (E-H). Bars indicate 5 µm.

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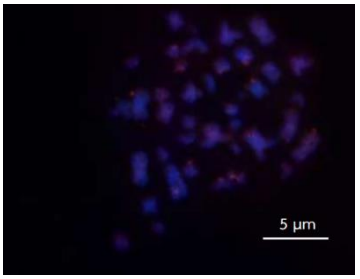
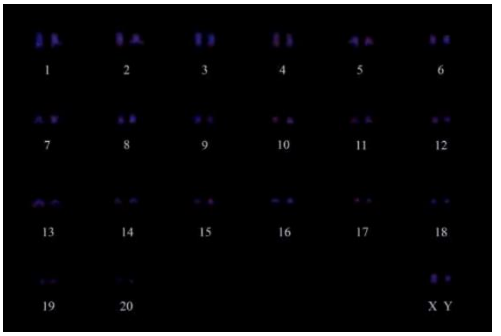
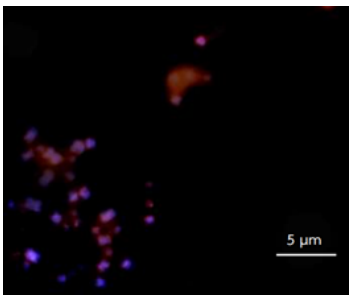
A



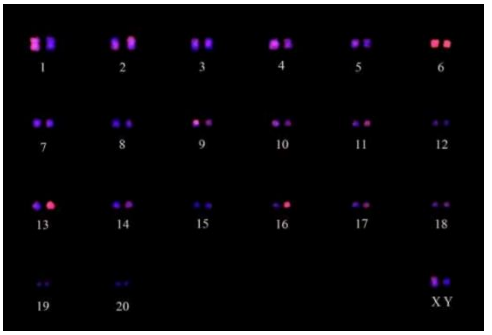
C d(GC)$_{15}$♂



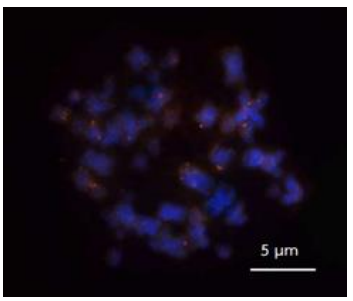
B



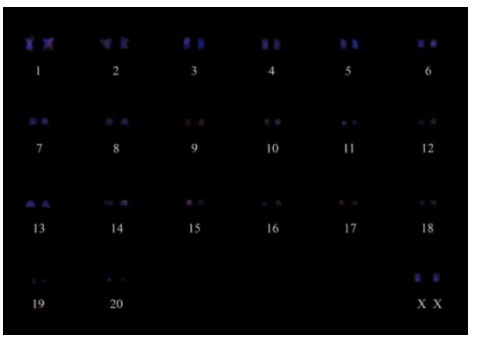
D d(CGG)$_{10}$♂



E



G d(GC)$_{15}$♀



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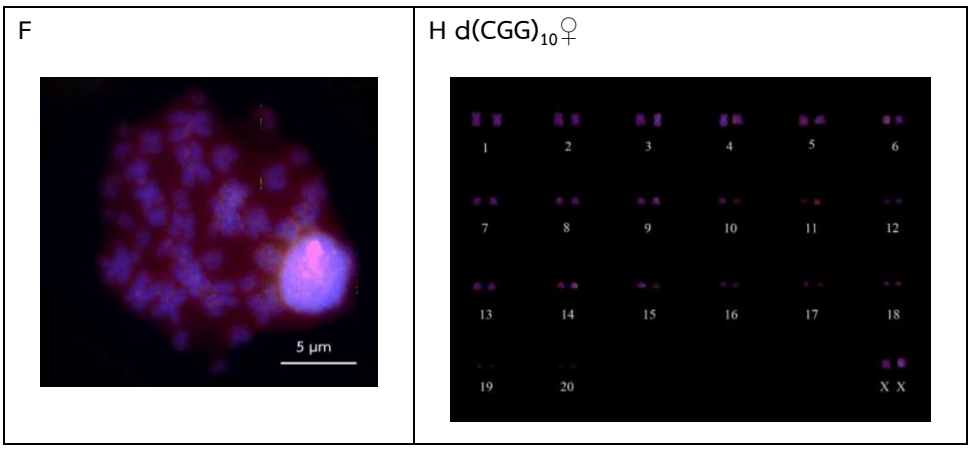
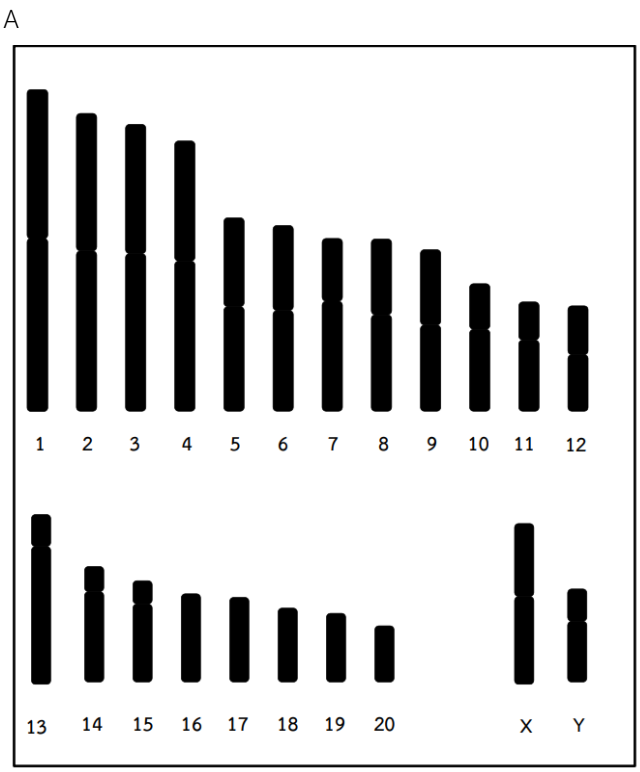


Figure 2 Metaphase chromosome and karyotypes of male (A-D) and female (E-H), Black-bearded Tomb Bat (*T. melanopogon*), 2n=42. Fluorescence in situ hybridization (FISH) with d(CA)$_{15}$ probe of male (C) and female (G), FISH with d(CGG)$_{10}$ of male (D) and female (H). Bars indicate 5 µm. The arrows indicate probe signals.



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B

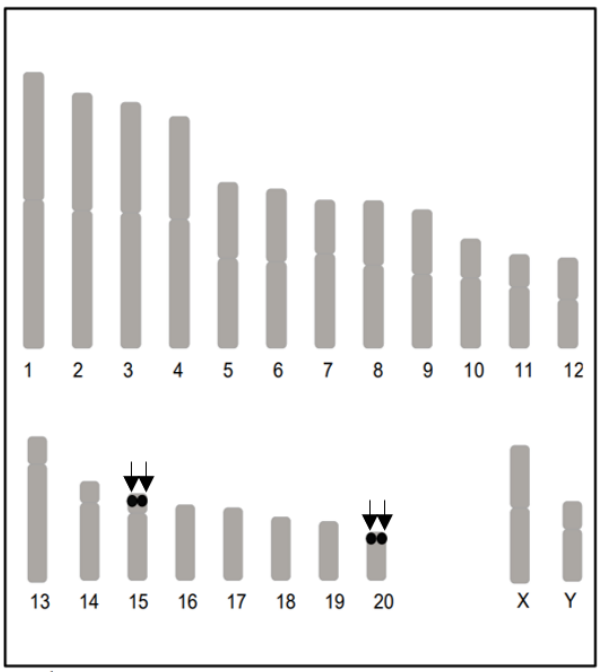

Figure 3 Standard idiograms of the Black-bearded Tomb Bat (*T. melanopogon*, 2n = 42) from conventional chromosome staining (A) and Ag–NOR staining banding (B) techniques.

Table 1 Cytogenetics review in the genus Taphozous

Species	2n	NF	Karyotype	Sex system	Locality	Reference
Taphozous melanopogon	42	64	18m + 6sm + 16t	X(m)/Y(a)	India	Ray-Chaudihuri et al. (1971)
T. longimanus	42	64	18m + 8sm + 16t	X(sm)/Y(a)	India	Ray-Chaudihuri et al. (1971)
T. theobaldi	42	64	24msm + 16t	X(m)/Y(sm)	Thailand	Harada et al. (1982)
T. melanopogon	42	62	16m + 6sm + 2a + 14t	X(m)/Y(sm)	Thailand	Narumon et al. (2012)
T. nudiventris	42	64	18m + 6sm + 16t	X(m)/Y(a)	Turkey	Asan et al. (2007)
T. nudiventris	42	66	10m + 12sm + 4a + 14t	X(sm)/Y(t)	Egypt	Attia et al. (2007)
T. nudiventris	42	64	18m + 6sm+ 16t	X(m)/Y(a)	Turkey	Asan and Albatrak (2007)

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Species	2n	NF	Karyotype	Sex system	Locality	Reference
T. melanopogon	42	64	24msm + 16t	X(sm)/Y	Philippines	Rickart et al. (1999)
T. nudiventris	42	64	24msm + 16t	X(msm)/ Y(a)	Turkey	Arsalan and Zima (2014)

Remarks: 2n = diploid chromosome number NF = base chromosome number m = metacentric chromosome sm = submetacentric chromosome a = acrocentric chromosome and t = telocentric chromosome

Table 2 Means of the short arm length (Ls), long arm length (Ll) and total arm length of chromosomes (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI of 20 metaphase cells of the male and female (Black-bearded Tomb Bat), 2n=42

Chro. Pair	LS	LL	LT	RL±SD	CI±SD	Chro. Size	Chro. Type
1	2.920	3.387	6.307	0.0953±0.004	0.537±0.021	Large	Metacentric
2	2.699	3.144	5.843	0.088±0.004	0.537±0.019	Large	Metacentric
3	2.536	3.092	5.628	0.085±0.005	0.548±0.025	Large	Metacentric
4	2.358	2.945	5.303	0.08±0.005	0.554±0.027	Large	Metacentric
5	1.744	2.052	3.796	0.057±0.003	0.540±0.026	Medium	Metacentric
6	1.670	1.975	3.645	0.055±0.002	10.542±0.017	medium	metacentric
7	1.231	2.158	3.390	0.051±0.004	0.638±0.030	medium	submetacentric
8	1.487	1.892	3.379	0.05±0.003	0.558±0.029	medium	metacentric
9	1.479	1.691	3.170	0.048±0.003	0.534±0.019	medium	metacentric
10	0.896	1.606	2.502	0.038±0.004	0.643±0.026	small	submetacentric
11	0.748	1.397	2.145	0.032±0.002	00.653±0.036	small	submetacentric
12	0.958	1.109	2.067	0.031±0.003	0.535±0.024	small	metacentric
13	0.628	2.706	3.333	0.05±0.003	0.811±0.020	medium	acrocentric
14	0.494	1.774	2.268	0.034±0.003	0.782±0.036	small	acrocentric
15	0.456	1.532	1.988	0.03±0.002	0.770±0.036	small	acrocentric
16	0.000	1.732	1.732	0.026±0.002	1.000±0.000	small	telocentric
17	0.000	1.662	1.662	0.025±0.002	1.000±0.000	small	telocentric
18	0.000	1.451	1.451	0.021±0.002	1.000±0.000	small	telocentric
19	0.000	1.349	1.349	0.02±0.002	1.000±0.000	small	telocentric

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Chro. Pair	LS	LL	LT	RL±SD	CI±SD	Chro. Size	Chro. Type
20	0.000	1.104	1.104	0.0167±0.002	1.000±0.000	small	telocentric
X	1.432	1.724	3.156	0.046±0.016	0.543±0.018	medium	metacentric
Y	0.634	1.193	1.826	0.03±0.012	0.651±0.019	small	submetacentric

Remarks: Chro.: Chromosome

RESULTS AND DISCUSSION

Karyotype, diploid number of chromosomes (2n) and fundamental chromosome number (NF) of the Taphozous melanopogon

The results show that the diploid chromosomes number was 2n = 42 and the fundamental number (NF) were 74 chromosomes in both female and male of T. melanopogon in both male and female. Karyotype formula described as. 2n (42) = $L^{m}{}_{8}$ + $M^{m}{}_{8}$ + $M^{sm}{}_{2}$ + $M^{a}{}_{2}$ + $S^{m}{}_{2}$ + $S^{sm}{}_{4}$+ $S^{a}{}_{4}$ + $S^{t}{}_{10}$ + Sex chromosome.

Chromosome type and size of *Taphozous melanopogon*

The karyotype is composed of 8 large metacentric, 8 medium metacentric, 2 medium submetacentric, 2 medium acrocentric, 2 small metacentric, 4 small submetacentric, 4 small acrocentric and 10 small telocentric chromosomes. The sex determination is XY, X chromosome is medium metacentric chromosomes and Y chromosome is small submetacentric chromosomes (Table 2) (Figure 1).

Chromosome marker of *Taphozous melanopogon*

The determination of a chromosome marker for this species was firstly obtained by Ag-NOR staining. The nucleolar organizer regions (NORs) were determination of a chromosome marker for this species was firstly obtained by Ag-NOR staining. The nucleolar organizer regions were appeared to telomere of the short arm of the chromosome pairs 15 small acrocentric type and chromosome pairs 20 small telocentric type. (Figure 3).

Patterns of microsatellite d(GC)$_{15}$ and d(CGG)$_{10}$ repeats in Taphozous melanopogon

The mapping of microsatellite repeats on the chromosomes of *T. melanopogon* showed that $(GC)_{15}$ and $(CGG)_{10}$ signals were observed on all chromosome 1, 6 and 13 pairs. (Figure 2. A-H).

Idiograms of Taphozous melanopogon chromosomes

All previous results were summarized, and idiograms presenting shapes, sizes and probe signals on the chromosomes of *T. melanopogon* are shown in Figure 3.

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CONCLUSION

The result of black-bearded tomb bat indicated that the karyotype formula described as: 2n (42) = L^{m}_{8} + M^{m}_{8} + M^{sm}_{2} + M^{a}_{2} + S^{m}_{2} + S^{sm}_{4}+ S^{a}_{4} + S^{t}_{10} + Sex chromosome. Diploid number was 2n = 42 chromosomes and fundamental number was 74 (NF = 74) for male and female of *T. melanopogon*. which is consistent with the number of diploid somatic chromosomes and previous studies from (19) and (20). And the results of the study showed that the type of sex chromosome was consistent with the previously study of (21). The number of diploids being studied is found in black-bearded tomb bat, for example in the study of *T. longimanus* chromosomes (22), *T. theobaldi*. (23) and *T. nudiventris* (24). With the corresponding number of diploid chromosomes. It shows that the black-bearded tomb bat does not have a large variety of chromosomes within the genus. While the number of fundamental chromosomes is between 62 and 66, they are not highly variable. Number, type and size of *T. melanopogon* chromosomes, 8 large metacentric, 8 medium metacentric, 2 medium submetacentric, 2 medium acrocentric, 2 small metacentric, 4 small submetacentric, 4 small acrocentric and 10 small telocentric chromosomes. The sex determination is XY, X chromosome is medium metacentric chromosomes and Y chromosome is small submetacentric chromosomes. (Table 1). Which is consistent but there are a number of chromosome types acrocentric more It is also different from previous studies that did not detect the acrocentric chromosome (24). Chromosome marker of *T. melanopogon*, the determination of a chromosome marker for this species was firstly obtained by Ag-NOR staining. The nucleolar organizer regions (NORs) sites appeared to telomere of the short arm of the chromosome pairs 15 small acrocentric type and chromosome pairs 20 small telocentric type. The pattern of microsatellite $(GC)_{15}$ and $(CGG)_{10}$ signals were observed on all chromosome 1, 6 and 13 pairs.

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REFERRENCES

1. Thongchai N, Kasidis R, Sakarin S, Art-ong P. Biodiversity of balts and small mammals in the secondary forest, RSPG area. Research Results and Research Progress Report; 2015.
2. Sotero- Caio CG, Baker RJ, Volleth M. Chromosomal evolution in Chiroptera. Genes. 2017;8(10):1-25.
3. Praween S, Alongklod T, Sumpars K, Krit P, La-orsri S. Karyological analysis and morphometrics of the Lesser Asiatic House Bat, *Scotophilus kuhlii* (Chiroptera, Vespertilionidae). Cytologia. 2012;77(3):401-11.
4. Praween S, Alongklod T, Sumpars K, Sarawut K, Krit P, La-orsri S. Karyological analysis and

ISSN: 2773-9376 (Print), 2773-9473 (Online)

morphometrics of Horsfield's Bat, Myotis horsfieldii (Chiroptera, Vespertilionidae). Cytologia. 2013;78(1):15-24.

5. Sitthisak J, Praween S, Alongklod T, Sumpars K, Narumon Pr, Sarawut K. Chromosome analysis and morphometric of Intermediate Roundleaf Bat, *Hipposideros larvatus* (Chiroptera, Hipposideridae) by conventional, GTG-banding and Ag-NOR banding techniques. Cytologia. 2014;79(4):445-56.

6. Asan N, Albayrak I. Some taxonomic features of *Taphozous nudiventris* Cretzschmar, 1830 vel 1831 from Turkey (Chiroptera: Emballonuridae). Turk J Zool. 2007;12(31):165-70.

7. Liang-Kong L, Masaharu M, Masashi H. Karyology of Ten Vespertilionid Bats (Chiroptera: Vespertilionidae) from Taiwan. Zool Stud. 2002;41(4):347-54.

8. Schulz- Schaeffe J. Cytogenetics: Plants, Animals, Humans. New York: Springer-Verlag New York Inc.; 1980.

9. Almeida B, Novaes RLM, Aguieiras M, Souza RF, Esbérard CEL, Geise L. Karyotype of three Lonchophylla species (Chiroptera, Phyllostomidae) from Southeastern Brazil. Comp Cytogenet. 2016;10(1):109-15.

10. Nuntiya M, Weerayuth S, Alongklod T. Chromosomal analysis and Nors polymorphism of bagarius suchus (Siluriformes: Sisoridae) by conventional banding and fish techniques. J Nat Appl Sci. 2016;2(1):10-9.

11. Gálvez F, Symonová R, Kovařík A. Evolutionary trends in animal ribosomal DNA loci: Introduction to a new online database. Chromosoma. 2018;127:141-50.

12. Supiwong W, Tanomtong A, Pinthong K, Kaewmad P, Poungnak P, Jangsuwan N. The first chromosomal characteristics of nucleolar organizer regions and karyological analysis of pink anemonefish, Amphiprion perideraion (Perciformes, Amphiprioninae). Cytologia. 2015;80(3):271-8.

13. Kasiroek W, Indananda C, Luangoon N, Pinthong K, Supiwong W, Tanomtong A. First chromosome analysis of the Humpback cardinalfish, Fibramia lateralis (Perciformes, Apogonidae). Cytologia. 2017;82(1):9-15.

14. Tanomtong A, Supiwong W, Jearranaiprepame P, Khakhong S, Kongpironchuen C, Getlekha N. A new natural autotetraploid and chromosomal characteristics of dwarf snakehead fish, Channa gachua (Perciformes, Channidae) in Thailand. Cytologia. 2014;79(1):15-27.

15 Turpin R, Lejeune J. Les chromosomes humans. Paris: Gauthier-Pillars; 1965.

16. Nash WG, OíBrien SJ. A comparative chromo-some banding analysis of Ursidae and their relation- ship to other carnivores. Cytogenet Cell Genet. 1987;45:206-12.

17. Wada MY, Lim Y, Wurster- Hill DH. Bandedkaryotype of wild- caught male Korean raccoon dog, Nyctereutes procyonoides koreensis. Genomics. 1991;34:302-6.

18. Rodrigues DS, Rivera M, Lourenço LB. Molecular organization and chromosomal localization of 5S rDNA in Amazonian Engystomops (Anura, Leiuperidae). BMC Genet. 2012;13(1):1-13.

19. Koubínová D, Sreepada K, Koubek P, Zima J. Karyotypic variation in rhinolophid and hipposiderid bats (Chiroptera: Rhinolophidae, Hipposideridae). Acta Chiropt. 2010;12:393–400.

20. Seim I, Fang X, Xiong Z, Lobanov AV, Huang Z, Ma S, et al. Genome analysis reveals insights into physiology and longevity of the Brandt's bat Myotis brandtii. Nat Commun. 2013;4:1–8

21. Rickart AE, Mercier AJ, Heaney RL. Cytogeography of philippine bat (Mammalia: Chrioptera). Biological society of Washington. 1999;112(3):453-69.

22. Asan N, Albayrak I. Some taxonomic features of taphozous nudiventris cretzschmar, 1830 vel 1831 from Turkey (Chiroptera: Emballonuridae). Turk J Zool. 2007;12(31):165-70.

23. Harada M, Minezawa M, Takada S, Yenbutra S, Nunpakdee P, Ohtani S. Karyological analysis of 12 species of bats from Thailand. Caryologia. 1982;352:269-78.

24. Sotero- Caio CG, Baker RJ, Volleth M. Chromosomal Evolution in Chiroptera. Genes. 2017;8(10):1-25.

25. Ray-Chaudhuri SP, Pathak S, Sharma T. Karyotypes of five Indian species of microchiroptera. Caryologia. 1971;24(2): 239-45.

26. Arsalan A, Zima J. Karyotypes of the mammals of Turkey and neighbouring regions: A review. Folia Zool. 2014;63(1):1–62.