

Morphometrical, Biometrical and Karyological Analyses on Subspecies of *Meriones tristrami* (Thomas, 1892) in Iran

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Abstract

In this study, three subspecies of *Meriones tristrami blackleri*, *Meriones tristrami lycaon* and *Meriones tristrami intraponticus* which were recorded from Iran were evaluated by means of morphological (n = 45). According to the morphological evaluations, *M.t. blackleri* with marked white tail tip appears to be more diverged one among these subspecies. The statistical analyses were carried out with PCA and DFA methods and the biometric differences were determined the difference among the subspecies. Thus, of the characteristics measured, tail length, T x 100/HB, hind foot length, interorbital width, occipitonasal length, mastoid width and palatal length, were found to be distinguishing characteristics among subspecies. According to UPGMA cluster, *M.t. blackleri* and *M.t. intraponticus* are closer each other, and *M.t. lycaon* is connected to this group and is the most diverged subspecies. All the subspecies have 72 number of chromosome, and number of fundamental arm (NF) is 76 in *blackleri*, 82 in *lycaon*, 84 in *intraponticus*.

Key Words: Biometry, karyology, chromosome, Iran

INTRODUCTION

The first record of *Meriones* species from Iran was defined as *Meriones persicus* in the vicinity of Isfahan [1]. This record was followed by *Meriones blackleri lycaon* [2] from Ahvaz and *Meriones blackleri intraponticus* [3] from Qazvin, the sub-species of this species. According to museum specimens, Neuhauser [3] defined *M. tristrami* population, which was distributed in certain sections of Iran, as *Meriones blackleri bogdanovi* sub-species. Ellerman [4] considered *Meriones tristrami* as a sub-species of *Meriones shawii* and accepted *Meriones blackleri* as a valid taxa. However, later studies considered *M. blackleri* as the synonymous of *M. tristrami* and *M. tristrami* was evaluated at species level and accepted *bogdanovi*, *intraponticus*, *blackleri*, *lycaon* as a sub-species of *M. tristrami* [5,6,3,7,8]. Lay [9] analyzed *Meriones tristrami* species collected from some regions of Iran and morphological properties of the species. The analysis showed that three different geographical variations of *M. tristrami* can be found in Iran. *M.t. lycaon*, whose tail is longer than head-body length collected from Isfahan, Ahvaz and Qazvin; *M.t. bodenheimeri*, whose tail tip is always smaller than head-body length which is collected from Hamadan and Kurdistan and the samples whose tail lengths are greater or smaller than head-body length and whose tail tip has or hasn't got a white feather collected from Sistan and Baluchestan were identified as *M.t. ackleri*. In addition, two different karyotypes were determined in the specimens which are NFa = 80 and NFa = 76. Misonne [10] carried out a comprehensive study on the taxonomic status of *Meriones tristrami* in Iran. They investigated taxonomic species and

identification characteristics of *Meriones tristrami blackleri*, *M.t. lycaon*, *M.t. intraponticus*, *M.t. bodenheimeri*, *M.t. bogdanovi* sub-species based on the specimens collected from Iran. According to morphologic and biometric comparisons, southeastern and eastern species were found to be closer to *M.t. bodenheimeri* and *bogdanovi* than *M.t. lycaon*. White tail type of *M.t. blackleri*; back color and baculum shape of *lycaon*; white incisors of *intraponticus* and short hind feet and kulak length of *bodenheimeri*, were determined as the characteristics that distinguish the taxa from each other. In addition, number of diploid chromosomes (2n) of *M. tristrami* is 72. However, *blackleri* has basic chromosome number of (NF) 76 and *lycaon*, *intraponticus*, *bodenheimeri*, have basic chromosome number of 82. Recently developed methods in molecular systematics are widely used in animal systematics to distinguish species and to form phylogenetic relationships. However, there are few molecular systematic studies on 6 sub-species of *Meriones* genus and there is no study on the specimens collected from Iran. In Iran, 6 sub-species of *Meriones tristrami* were identified generally based on their morphological properties. No detailed statistical analyses or evaluations within the scope of molecular systematic were conducted and sub-species statuses were not supported by statistical and molecular methods. In the present study, morphological properties of *Meriones tristrami*, *blackleri*, *lycaon* and *intraponticus* topotypes collected from different regions were compared for the first time using advanced statistical methods and alloenzyme variances were identified for the first time. Thanks to this identification, validity of sub-species and possible distinguishing characteristics between the sub-species were determined.

MATERIAL AND METHOD

This study used 55 of the specimens collected in field studies in 2005-2006. Live collection traps were used for collection of specimens. The traps were established in higher locations like animal shelter, store, garden and roofs particularly in rural settlement areas. Mostly bread and peanut butter mixture was used as bait in traps. Habitat properties and outer morphology of the locality where the specimens were collected were recorded. Standard 4 dimensions (total height, tail length, hind foot and ear lengths mm) and weight (gr) of all specimens were measured according to Harrison and Bates [11]. The specimens which were caught dead were measured and alive and field specimens were embalmed in laboratory as museum samples. Alive specimens were taken to laboratory by transport cages. The specimens were kept in animal care room for a certain time and karyotypes were prepared using Ford and Hamerton [12] Colchicine Hypotonic Citrate technique based on their morphologies to avoid misidentification and to determine karyological properties. Of the prepared karyotype preparates, approximately 20 metaphase plates of each specimen were analyzed with x100 immersion objective and number of chromosomes (2n), number of basic chromosome arms (NF) and number of autosomal arms (NFa) values of the specimen were identified. The metaphase plates which looked good were photographed and karyotic properties were determined. Main blood tissue and liver, kidney, heart and blood serums were collected from the specimens to use in electrophoretic studies and were kept at -70 c deep freezer.

FINDINGS

External Morphological Characteristics

Dorsal fur color of *Meriones tristrami* shows a wide variation from matt dark brown to light yellowish brown or reddish yellow. All individuals have a white or grayish circle around their eyes. There is a pure white stain at the back of the ears. Long hairs at the tip of rostrum are generally black although rarely white hairs are also observed. Dorsal and ventral color of tail is very similar; however dorsal color of tail can be slightly darker in some specimens. General color of the tail is lighter than dorsal fur color. Hind foot soles are hairy; hair color get whiter from dorsal to hind foot. Forefoot sole has five pads; three in front and two at the back. Hind foot soles have four pads all of which are in the front. Dorsal fur color completely differs from ventral fur color in sides. Ventral fur is pure White. There is a callous transverse 1-2 cm hairless region in abdominal section in ventral. Females have a total of 7-8 nipples. *M.t.blackleri* sub-species are distinguished from other sub-species with 1 cm or longer white feather at tail tip. Body size of the specimens of these sub-species are generally smaller than others. Tail tip white feather is also found in specimens of *M.t.intraponticus* sub-species. However, the white feather is quite smaller than *blacklei*. Although dorsal fur color of *M.t.lycaon* specimens are not much distinct, it is darker reddish in color.

Skull Characteristics

Collected *Meriones tristrami* specimens were first divided into 3 categories according to type localities. Certain statistical procedures were performed based on 38 measurement characteristics collected from the body and skull. Among the analyzed three sub-species, the extent of distinguishing skull characteristics was explained in the following tables and figures.

Analysis of biometric data

Firstly, 4 exterior dimensions (total height, tail, hind foot and ear lengths) were measured of all specimens. A index was formed using body and tail lengths ($T \times 100 / HB$) B index was obtained by multiplying tail length by 100 and dividing it into body length. Other 33 measurement characteristics belong only to skull. Mean lengths and standard deviations of measured characteristics in specimens of 3 different sub-species are presented in Table 1, 2 and 3. Furthermore, mean and standard deviations of 75 specimens belonging to 3 populations are presented in Table 4.

Karyological Characteristics

Karyotype of M.t.lycaon

Ideogram of a female individual which has the best karyotype plate for *M.t.lycaon* was made (Figure 4.1). Diploid chromosome number was found to be (2n)72, number of basic chromosome arm number was found to be (NF) 82, number of autosomal chromosome arm number was found to be (Nfa)78. The karyotype had 6 submetacentric, 2 telocentric and 60 acrocentric chromosomes. Chromosome X is quite large and in submetacentric structure.

Karyotype of M.t.intraponticus

One male individual among 12 alive *M.t.intraponticus* sub-species specimens was selected for karyotype. Karyotype plate and ideogram based on only one individual are presented in Figure 4.2. number of diploid chromosomes was found to be 72(2n=72); number of basic chromosome arms was found to be 84(NF=84) and number of autosomal chromosome arms was found to be (Nfa=80) 80. The karyotype contained 2 metaentric, 10 submetacentric, 62 acrocentric chromosomes. Chromosome X is the largest chromosome in the plate and is metacentric. Chromosome Y is also metacentric and much smaller than chromosome X.

Karyotype of M.t. blackleri:

Of 20 alive specimens, 2 were used for karyotic study. We obtained similar results with the study of Misonnex [13]. Number of diploid chromosomes was found to be (2n) 72, number of basic chromosome arms was found to be (NF) 76 and number of autosomal chromosome arms (Nfa) was found to be 72. The karyotype contained 2 metasentric, 2 submetacentric chromosomes and 68 acrocentric chromosomes. Chromosome X and Y are submetacentric. Chromosome X is the morphologically largest chromosome in the karyotype while chromosome Y is medium sized (Figure 4.3).

Table 1. External and cranial average measurements of *M.t.lycaon* (\pm SD = Standard Deviation)

(Group 1 = Isfahan - Shiraz samples).

Characters (mm)	Average	\pm SD
Total length	275,9 400	24,98 297
Tail length	135,12 00	11,4 1179
Hind foot length	36,6 600	1,42657
Ear length	21,1 200	1,64671
T x 100 / HB	97,00 88	6,64 702
Zygomatic breadth	22,1211	1,58976
Interorbital constriction	6,5 428	0,41297
Condilobasal length	26,01 68	2,3 9419
Condilonasal length	37,9 880	2,53826
Occipitonasal length	39,1225	2,42989
Basilar length	33,1628	2,55 243
Nasal length	15,9 769	1,55312
Nasal breadth	3,9987	0,32253
Frontal sutur length	12,0121	0,81807
Perietal sutur length	7,6519	0,56091
Face area length	21,9985	1,77 457
Brain area length	17,0015	1,05504
Mastoid breadth	11,8 976	0,83903
Bullal braincase length	15,0 460	0,48588
Without bullal, braincase length	12,22 20	0,43058
Occipital breadth	17,0056	0,67431
Braincase breadth	16,9 989	0,55664
Diestama length	11,0 750	0,94440
Length of palate	17,98 40	1,31 415
Foreman incisiva length	7,1221	0,70 543
Timpanik bulla height	9,0123	0,79095
Timpanik bulla length	12,01 60	0,77997
Timpanik bulla breadth	8,9889	0,45 360
Mandibul length	20,9 660	1,48222
Mandibul height	10,1122	0,75545
Upper molar alveol length	6,0 625	0,329 11
Lower molar alveol length	5,9 832	0,459 41
Upper M1 length crown	3,0 014	0,18445
Upper M2 length crown	1,3772	0,07888
Upper M3 length crown	,6188	0,08722
Lower M1 length crown	2,5 264	0,13849
Lower M2 length crown	1,4452	0,13249
Lower M3 length crown	,5738	0,17777

Table 2. External and cranial average measurements of *M.t.blackleri* (\pm SD = Standard Deviation) (Group 2 = Ahvaz samples).

Characters (mm)	Average	\pm SD
Total length	291,4055	24,44871
Tail length	152,1 000	9,01848
Hind foot length	35,5980	1,175 60
Ear length	22,4 200	1,55888
T x 100 / HB	111,3418	12,31648
Zygomatic breadth	21,111 4	1,03642
Interorbital constriction	5,99 84	0,31644
Condilobasal length	34,6 784	1,74045
Condilonasal uzunluk	36,9587	1,72988
Occipitonasal length	37,6 755	1,50766
Basilar length	32,132 4	1,67861
Nasal length	15,5 724	0,82162
Nasal breadth	4,2452	0,25018
Frontal sutur length	11,2254	0,55488
Parietal sutur length	7,4476	0,460 25
Face area length	21,7 980	1,01546
Brain area length	16,2920	0,87328
Mastoid breadth	11,7932	0,50880
Bullal braincase length	14,1 766	0,32 634
Without bullal, braincase length	12,01 84	0,47156
Occipital breadth	16,012 6	0,39573
Braincase breadth	16,79 76	0,41369
Diestama length	9,7552	0,71122
Length of palate	17,4998	0,91864
Foreman incisiva length	6,4715	0,49527
Timpanik bulla height	8,3158	0,56670
Timpanik bulla length	11,0000	0,37077
Timpanik bulla breadth	9,0 132	0,30 711
Mandibul length	19,6740	1,06126
Mandibul height	9,5558	0,45464
Upper molar alveol length	5,5312	0,21555
Lower molar alveol length	5,3678	0,25821
Upper M1 length crown	2,3015	0,17728
Upper M2 length crown	1,2799	0,07237
Upper M3 length crown	,5771	0,07289
Lower M1 length crown	2,2112	0,11689
Lower M2 length crown	1,3189	0,08095
Lower M3 length crown	,5424	0,08113

Table 3. External and cranial average measurements of *M.t.intraponticus* (\pm SD = Standard Deviation) (Group 3 = Hamadan - Qazvin samples).

Characters (mm)	Average	\pm SD
Total length	299,7 000	18,64012
Tail length	152,1277	11,01510
Hind foot length	37,1 700	1,86227
Ear length	21,8089	1,68335
T x 100 / HB	110,4798	7,91050
Zygomatic breadth	21,1 628	1,44097
Interorbital constriction	6,6780	0,32993
Condilobasal length	35,5442	2,25899
Condilonasal uzunluk	38,1658	2,50513
Occipitonasal length	39,0 604	2,37667
Basilar length	33,00 22	2,300 49
Nasal length	15,5912	1,38241
Nasal breadth	4,4885	0,19955
Frontal sutur length	11,6272	0,56873
Parietal sutur length	8,0 260	0,42672
Face area length	21,8888	1,52256
Brain area length	17,5876	5,93244
Mastoid breadth	13,0 076	0,79395
Mastoid breadth	14,1865	0,44082
Without bullal, braincase length	11,3922	0,43541
Occipital breadth	16,2037	0,68990
Braincase breadth	17,0698	0,58598
Diestama length	10,2855	0,89597
Length of palate	18,3324	1,12111
Foreman incisiva length	6,2978	0,63022
Timpanik bulla height	8,6316	0,42817
Timpanik bulla length	11,01 24	0,49618
Timpanik bulla breadth	9,101 2	0,34755
Mandibul length	20,4234	1,61894
Mandibul height	9,2886	1,42777
Upper molar alveol length	5,8214	0,32936
Lower molar alveol length	5,7906	0,33796
Upper M1 length crown	2,3534	0,17760
Upper M2 length crown	1,3186	0,05480
Upper M3 length crown	,5523	0,05927
Lower M1 length crown	2,3208	0,12084
Lower M2 length crown	1,3238	0,04147
Lower M3 length crown	,4890	0,09029

Table 4. External and cranial average measurements of *M.tristrami* (\pm SD = Standad Deviation)

Characters (mm)	Average	\pm SD
Total length	286,5 133	23,5107
Total length	146,1688	13,07051
Hind foot length	36,4 800	1,56578
Ear length	21,5134	1,71645
T x 100 / HB	106,7485	11,28902
Zygomatic breadth	21,0 795	1,40175
Interorbital constriction	6,4567	0,45069
Condilobasal length	35,2 311	2,15280
Condilonasal uzunluk	38,12 04	2,34471
Occipitonasal length	39,4806	2,24495
Basilar length	33,11 85	2,17000
Nasal length	15,6755	1,30340
Nasal breadth	4,6 543	0,27750
Frontal sutur length	11,3898	0,67183
Parietal sutur length	7,6398	0,50793
Face area length	20,0 871	1,48789
Brain area length	17,0 393	3,51029
Mastoid breadth	12,3598	0,85442
Mastoid breadth	14,2216	0,43015
Without bullal, braincase length	11,5459	0,47602
Occipital breadth	16,2542	0,64544
Braincase breadth	17,11 72	0,54006
Diestama length	10,2487	0,92912
Length of palate	18,1 422	1,17602
Foreman incisiva length	6,5128	0,61505
Timpanik bulla height	8,5980	0,64599
Timpanik bulla length	11,0142	0,68212
Timpanik bulla breadth	9,9 598	0,40 181
Mandibul length	20,3555	1,52316
Mandibul height	9,4942	0,96575
Upper molar alveol length	5,7680	0,34774
Lower molar alveol length	5,7835	0,49035
Upper M1 length crown	2,3795	0,20338
Upper M2 length crown	1,3086	0,07128
Upper M3 length crown	,5822	0,07865
Lower M1 length crown	2,2829	0,13658
Lower M2 length crown	1,3599	0,10601
Lower M3 length crown	,5411	0,12911

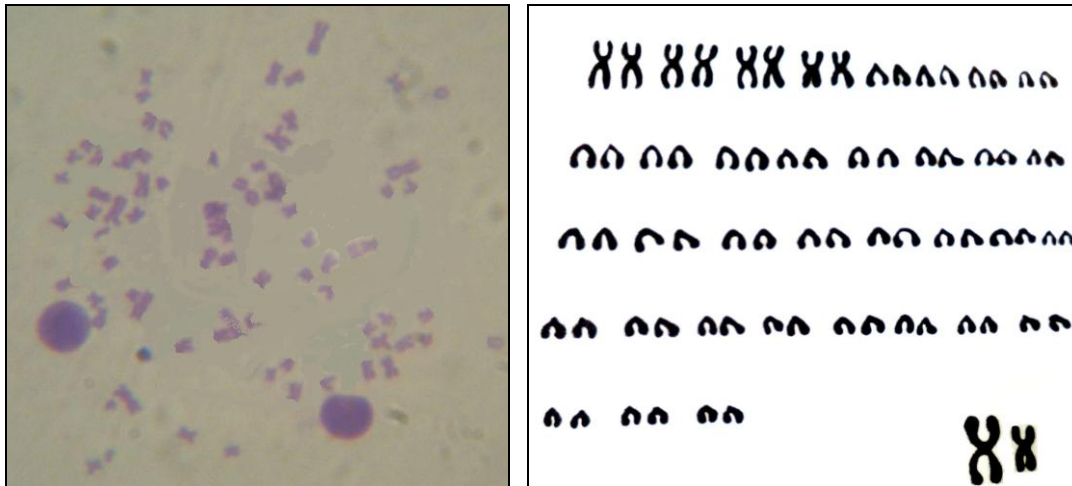


Figure 4.1. Ideogram and karyotype plate of *M.t.lycaon*, un idiogram ($2n = 72$, $NF = 82$, $Nfa=78$)

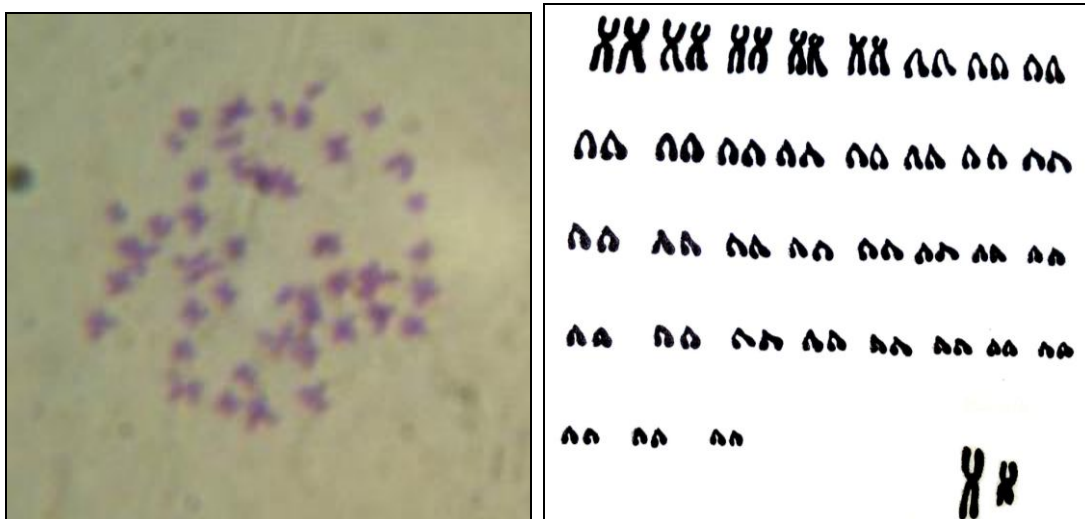


Figure 4.2. Ideogram and karyotype plate of *M.t.intraponticus* ($2n = 72$, $NF = 84$, $Nfa=80$)

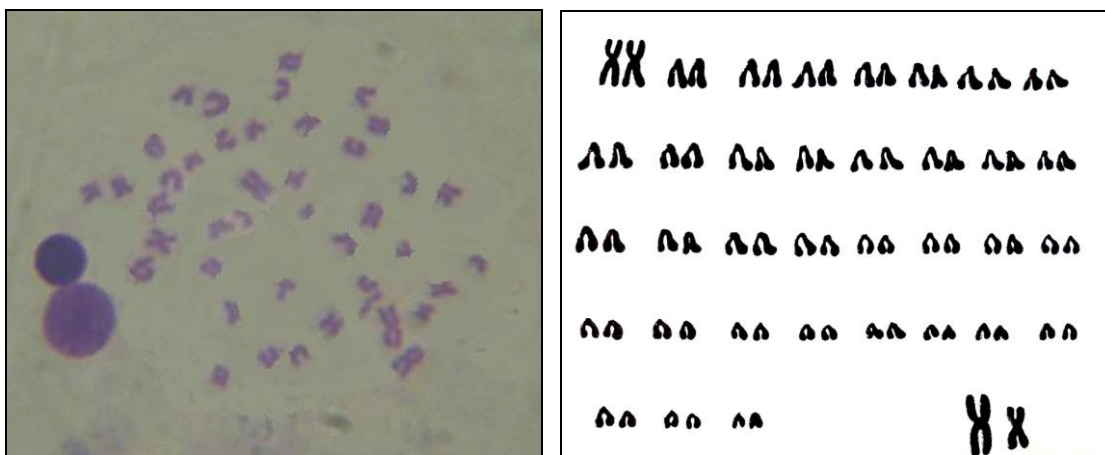


Figure 4.3. Idiogram and karyotype plate of *M.t.blackleri* ($2n = 72$, $NF=76$, $Nfa=72$)

DISCUSSION

Morphologic Properties

Thomas[14] defined *M.tristrami* nominative subspecies as pale brownish yellow dorsal, partially black fur color; the hairs are whiter in the tips; abdominal and hind feet hairs are white; palms and hid feet soles are hairless; inner section of tail has a black feathers; but is fragile and narrow; parietal section is not widened; upper incisors have one canal and molars are hyposodont. Following the definition of Thomas[14], Harrison[11], Osborn and Helmy[15] and Harrison and Bates[7] defined *M.tristrami* almost in the same manner. In addition to these definitions, Lehmann[16] reported that there was a characteristic callus in median line of abdominal fur in adult male specimens. Neuhauser [3] reported that *M.t.intraponticus* had dark brownish red dorsal fur color; longer tail, head-body length and that there was a white section in tail in all of the specimens he analyzed. Color and abdominal callus of the analyzed specimens are similar with the definitions above. In *M.t.intraponticus*, tail feather whiteness is shorter than that in *blackleri*. Furthermore, a small white stain was detected in the middle of frontal region of *M.t.blackleri* specimens we collected. This stain is present in 60% of the specimens. We found 1 cm callus in our adult male specimens. Color and abdominal callus of the analyzed specimens are similar with the definitions above.

Biometrik characteristics

In t-test statistical analysis of biometric data, Yiğit et al[17] indicated measurement characteristics that distinguished *lycaon*, *blackleri* and *intraponticus* sub-species. According to that the proportion of tail to head-body length was (CD= 0.92, 82%) mastoid width (CD= 0.81, 79%), siestama lengths (CD=0.73,76%) characteristics distinguished *lycaon* and *blackleri* sub-species on the other hand, *M.t.intraponticus* is distinguished from *lycaon* with tail length (CD= 0.85, 83%) and from *blackleri* with hid feet length (CD=1,24,%89), occipital length (CD=0.73,76%), plate length (CD=0.80,%77) and diastema (CD=0.81,79%). In our study, according to Qazvin HSD results, *lycaon* sub-species is distinguished from *blackleri* sub-species with tail length, hind feet length, ear length, T x 100/HB, interorbital narrowing, occipitonasal length, mastoid width, occipital width, diastema length, palate length, tympanic bulla height, length and width, mandibular length, upper and lower molar and alveolar lengths, upper M1 and M2 crown length, lower M1 and M2 crown lengths. Similarly, it was found that *lycaon* was distinguished from *intraponticus* with tail length, T x 100/HB, mastoid width, foramen incisive length, tympanic bulla length, lower molar alveolar length and upper M1 and M3 crown length and lower M2 crown lengths. It was found that hind feet length, interorbital narrowing, occipitonasal length, parietal suture length, mastoid width, brain capsule length without , palate length, tympanic bulla length, upper and lower molar alveolar length and lower M1 crown length were the important characteristics to distinguish *blackleri* and *intraponticus* sub-species. All of these 3 sub-species are distinguished from each other with certain characteristics. According to this test, the farthest populations seem to be *lycaon* and *blackleri*.

Karyological Properties

In karyologic studies, Matthey[5] determined Iraq specimens of *M.tristrami* as $2n = 72$ and $NF = 74$ and Matthey[5] determined chromosomes X and Y as metacentric. In addition Matthey [5] reported that sex chromosomes of all Gerbillinae family was metacentric. However, other studies reported that in *Meriones tristrami*, which belonged to this family, sex chromosomes were submetacentric. Yiğit et al.[17] carried out a karyologic study on four sub-species of *Meriones tristrami* (*lycaon*, *blackleri*, *intraponticus*, *bodenheimeri*) distributed in Turkey and published karyotype of *blackleri* and *bodenheimeri* sub-species for the first time. In that study, although Yiğit *et al* [17] reported number of basic chromosome arms of *intraponticus* sub-species as (FN) 82, the present study reported FN of this sub-species as 82. Furthermore, Yiğit *et al* [17] identified sex chromosome of *blackleri* specimens as metacentric; however in the present study we found sex chromosomes as submetacentric. Lidicker,w.z [18] identified the specimens collected from Iran Zencan as $2n=72$ and $NF= 80$ and reported that sex chromosomes were submetacentric. In the same study, Yiğit found karyotype of *M.tristrami* specimens collected from Dogubeyazit as $2n= 72$ $NF= 84$. Wilson [19]carried out a karyological study on the specimens generally collected from Central Anatolia and pointed out that there were two different chromosomal forms. He collected *M.t. intraponticus* and *lycaon* specimens from type locations and found as $2n= 72$, $NF= 84$. In addition he reported that there was another chromosomal form with FN 80 and that this form was found in rather low numbers. In our study, FN of specimens of *lycaon* sub-species was found as 82 and no chromosomal form with $FN=80$ was found. Misonnex [13] and Kadhim et al., [20] reported the specimens collected from Hamadan as $2n = 72$, $NF = 82$.

CONCLUSION

In conclusion, considering morphological differences between *M.tristrami* sub-species, it can be stated that sub-species shows variations in terms of dorsal color and tail tip feather and that *M.t.blackleri* and *lycaon*, were well distinguished in terms of these criteria. Furthermore, shorter tail length of *lycaon* than head-body length distinguishes this sub-species from two other sub-species. Analysis results of biometric data found characteristics which significantly distinguished all three analyzed sub-species. UPGMA tree and conical distinguishing graph data also revealed that the sub-species were distinguished from each other. *blackleri* and *intraponticus* populations which are close are completely different from *lycaon* population and all of them are at sub-species level in biometrical terms. In karyological terms, number of basic chromosome arms of analyzed sub-species was the same (72). However, in all sub-species, the difference in number of arms is striking. This characteristic can be considered as a taxonomic criterion if geographic limits are determined.

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