J Vector Borne Dis 46, March 2009, pp. 52–56

Karyosystematic and morphometric characterization of the rodents as reservoir hosts of zoonotic cutaneous leishmaniasis in an endemic focus of Isfahan Province, Iran

L. Shirani Bidabadi[a], M.A. Nilforoushzadeh[a,b], A.A. Akhavan[c], H. Abdoli[e], A.H. Siadat[a], F. Jaffary[a,d], S.H. Hejazi[a], N. Shareghi[e], M. Ghanei[e], M. Arandian[e] & S.H. Moradi[a]

[a]*Skin Diseases and Leishmaniasis Research Center (Sedigheh Tahereh), Isfahan University of Medical Sciences, Isfahan;* [b]*Research Center of Training of Leprosy and Skin Diseases, Department of Dermatology, University of Medical Sciences , Tehran;* [c]*Department of Medical Entomology and Vector Control, School of Public Health and Institute of Public Health Research,Tehran University of Medical Sciences, Tehran;* [d]*Department of Pharmacology and Toxicology, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan;* [e]*Research Center of Training of Isfahan, School of Public Health and Institute of Public Health Research,Tehran University of Medical Sciences,Tehran, Iran*

Abstract

Background & objectives: Rodents belonging to Gerbillinae subfamily are the main reservoir hosts of zoonotic cutaneous leishmaniasis (ZCL) in Iran. Regarding the important role of these rodents in the maintenance of *Leishmania major* in the nature, their identification with morphometric, cytogenetic and molecular methods seems to be essential. The karyotype study of these species, captured from a new focus of zoonotic cutaneous leishmaniasis located in the south of Isfahan Province was carried out in 2007.

Methods: Twenty specimens containing seventeen *Meriones persicus* and three *Nesokia indica* were captured from Mobarakeh rural district south of Isfahan. Giemsa-stained karyotypes of these two species were prepared from bone marrow chromosome preparations. Systematic important characters of the body and cranium (incisors, molars, occipitonasal, condylobasal, zygomatic, tympanic bullae, etc.) of these rodents were studied. Cranium size was measured using a Vernier calipers.

Results: Specimens of *M. persicus* and *N. indica* had 2n = 42. The karyotype study of these species included metacentric, sub-metacentric and acrocentric chromosomes. Morphological studies were completely matched with the reported characters of these species and further confirmed the diagnoses.

Interpretation & conclusion: Based on the results of this study, *M. persicus* and *N. indica* are two completely differentiated rodents species that were collected from a new focus and they can also be differentiated morphologically.

Key words Karyosystematic – *Meriones persicus* – *Nesokia indica* – reservoir host – zoonotic cutaneous leishmaniasis

Introduction

Rodents belonging to Gerbillinae subfamily are the main reservoir hosts of zoonotic cutaneous leishmaniasis (ZCL) in Iran. Regarding the importance of these rodents in the maintenance of the *Leishmania major* in the nature, their identification using morphometric, cytogenetic and molecular methods could be considered as a preliminary step in the control of CL. Karyotypes of Iranian rodents are poorly known[1–4]. Furthermore, karyotypes of certain species show geographical variation in the fundamental number of chromosomes as well as the diploid number of chromosomes as in the Genera Nannospalax

and Meriones[5–7]. The present study focused on the karyotypes of rodent species from Dehsorkh rural district, Mobarakeh county, a new foci of ZCL, south of Isfahan Province.

Material & Methods

During a field survey in south of Isfahan Province, Mobarakeh rural district, Iran in 2007, 20 rodents were collected (Fig. 1). Measurements of four external characters (total length, tail length, ear length and hindfoot length in mm) and weight (g) were taken from these specimens, which were also karyotyped in accordance with the conventional bone marrow method. Systematic important characters of the body and cranium (incisors, molars, occipitonasal, condylobasal, zygomatic, tympanic bullae, length of nasal, diastema, anterior palatine foramina, tympanic bullae; width of tympanic bullae, upper cheek teeth, lower cheek teeth, rostrum, mandible and height of skull) of rodents were studied. A total of 20 slides were prepared for each specimen, and well-spread metaphase cells were analyzed.

Cranium size was measured with Calipers. The diploid number of chromosomes (2n) were determined by examining photographs of the slides. The skin, skulls and karyotype preparations were deposited in Parasitology and Mycology Department, School of Medicine, Isfahan University of Medical Sciences, Iran.

Fig. 1: Colony of rodents reservoir of ZCL from Dehsorkh foci around Isfahan, Iran

Results

The karyotyped specimens were identified as *M. persicus* (17 specimens) and *N. indica* (3 specimens). The results of external and cranial of five adult specimens of *M. persicus* and three adult specimens of *N. indica* from Dehsorkh, Mobarakeh County, Isfahan Province are summarized in Tables 1 and 2, respectively. Their measurements were comparable with the reported sizes of these species.

Karyotype study: Karyotype study revealed diploid chromosome number of 2n = 42 in 17 specimens of *M. persicus* from south of Isfahan, Mobarakeh rural district (Figs. 2 and 3). The autosomal set included 18 pairs meta and submetacentric and three pairs acrocentric chromosomes. Both sex chromosomes were submetacentric with smaller Y-chromosome compared to X-chromosome.

Three karyotyped specimens of *N. indica* showed the diploid chromosome number of 2n = 42. Their autosomal set included 17 pairs meta and submetacentric

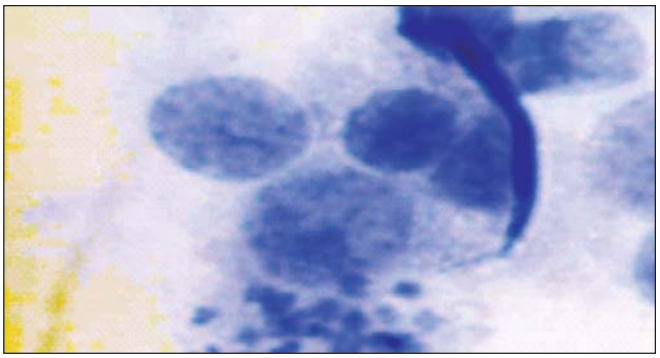

Fig. 2: Chromosome set of *M. persicus*

Fig. 3: Female karyotype of *M. persicus* from Dehsorkh foci around Isfahan, Iran

Table 1. The external and cranial measurements of five adult specimens of *M. persicus* from Dehsorkh, Mobarakeh County, Isfahan Province

Characteristics (mm)	Specimen numbers				
	F1	M2	F3	F4	F5
Total length	325	378	295	295	215
Head and body length	200	210	135	145	135
Tail length	125	168	160	150	80
Hind foot Length	40	42	43	39	42
Ear length	20	18	19	20	20
Weight (g)	300	100	100	100	75
Zygomatic width	21/3	22/1	21/6	18/5	21/7
Least interorbital width	5/8	8/7	8/4	7/3	8/3
Condylobasal length	42/7	38/1	41/1	40/2	39/4
Occipitonasal length	45/7	42/1	43/3	43/5	44/7
Cranial width	18/9	11/2	13/2	18/2	17/7
Length of nasal	17/3	16/5	16/9	12/7	19
Length of diastema	11/9	11/7	12/9	11/9	11/8
Length of anterior palatine foramina	7/2	8/9	8/2	9/9	7/5
Length of tympanic bullae	11/1	11/9	11/2	11/8	11/1
Width of tympanic bullae	9/3	8/3	10/6	9/8	7/7
Upper cheek teeth	6/9	5/7	6/3	6/7	6/8
Lower cheek teeth	5/6	6	5/4	5/7	5/5
Height of skull	15/7	14/5	14/4	15/7	15/1
Width of rostrum	4/4	4/3	5/8	4/4	4/5
Mandible	20/8	13/5	22/1	21/4	21/6

F—Female; M—Male.

and four pairs of acrocentric chromosomes (Figs. 4 and 5).

Discussion

The objective of the current study was to determine the closely related rodent species through karyotypic study, determination of chromosomal variabilities and chromosomal abnormalities along with morphologic identification of them that will help to identify the species specificity as reservoir host of cutaneous leishmaniasis in an endemic area to control the disease in future.

Rodents belonging to Gerbillinae subfamily are the main reservoir hosts of ZCL in Iran. The results of current study showed *M. persicus* and *N. indica* were two completely differentiated rodent species that were collected from a new focus and they could also be differentiated morphologically.

External characteristics of *M. persicus* and *N. indica* collected in a new focus were comparable with Etemad rodent identification key characters[8]. The external and cranial measurements of adult specimens of the collected specimens were also matched with above. Our karyotype finding of *M. persicus*

Table 2. The external and cranial measurements of three adult specimens of *N. indica* from Dehsorkh, Mobarakeh County, Isfahan Province

Characteristics (mm)	Specimen numbers		
	1F	2M	3M
Total length	320	325	310
Head and body length	210	200	210
Tail length	110	125	100
Hind foot length	37	40	35
Ear length	19	20	18
Weight (g)	300	125	391
Zygomatic width	29/4	28/7	29/5
Least interorbital width	9/6	11/8	10/2
Condylobasal length	43/7	42/7	41/7
Occipitonasal length	45/9	44/7	46
Cranial width	17/6	17/9	17/8
Length of nasal	20/8	18/9	19/2
Length of diastema	15/6	14/9	14/7
Length of anterior palatine foramina	8/5	7/6	9
Length of tympanic bullae	8/6	8/6	8/6
Width of tympanic bullae	6/9	6/4	6/5
Upper cheek teeth	9/9	11/7	10/2
Lower cheek teeth	8/7	8/7	8/7
Height of skull	13/3	15/1	14
Width of rostrum	8/9	7/4	7/6
Mandible	29/3	29/3	29/3

F—Female; M—Male.

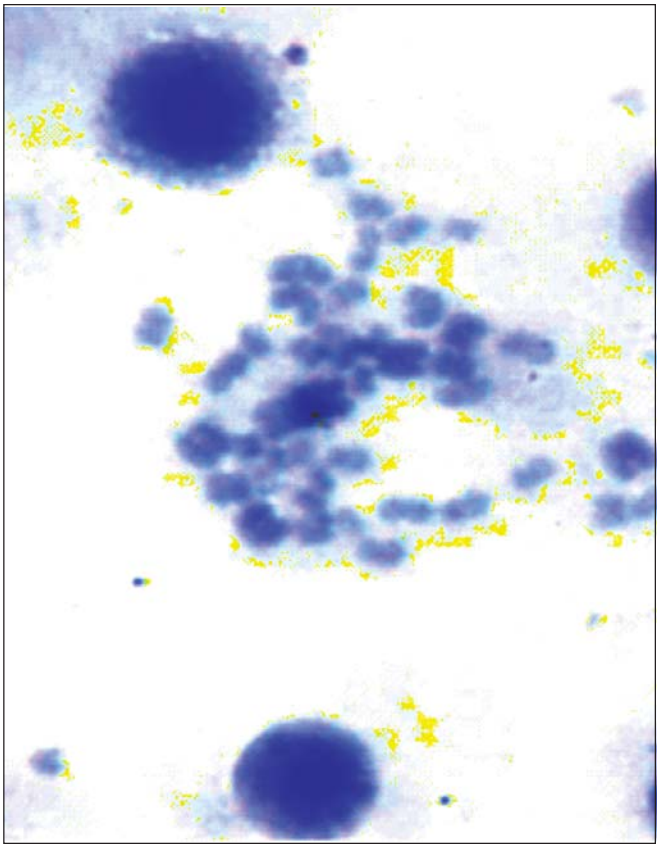

Fig. 4: Chromosome set of *N. indica*

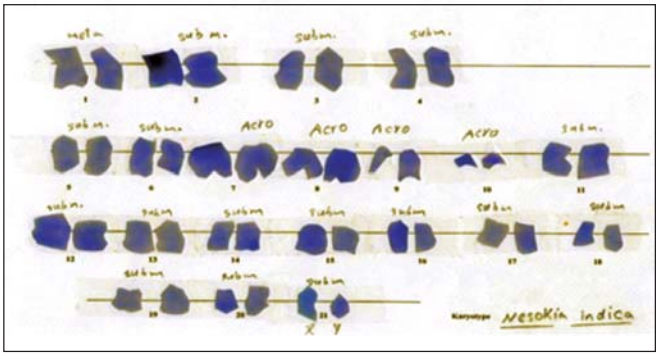

Fig. 5: Male karyotype of *N. indica* from Dehsorkh foci in and around Isfahan, Iran.

was consistent with those given by Matthey[9], Nuri *et al*[10],Vorontsov & Korobitsina[11], Benazzou *et al*[3], and Nuri and Ercument[12], but different from that given by Matthey[13] who reported that the diploid and the fundamental number of 42 and 74, respectively. These findings support the proposed stable diploid number of chromosomes in *M. persicus* populations.

Biometrical comparisons of *M. persicus* could not be made because of the insufficient specimen numbers in both the present study and in other published papers, but the tail length given by Vinogradov and Argyropulo[14] is longer than that in Iranian specimens. Furthermore, the karyotype finding of *N. indica* was consistent with those given by Matthey[9].

In conclusion, this is the first report of *M. persicus* and *N. indica* as two potential reservoirs of ZCL in some endemic areas of Isfahan, Iran with confirmed morphological and karyotyped identification. Further

studies on leishmaniasis infection of these rodents are warranted.

Acknowledgement

We are grateful to the staff of Public Health Center of Mobarakeh area, Isfahan, Iran.

References

1. Matthey R. Researches Sur Les chromosomes des Muridae. *Caryologia pisa* 1954; *6:* 1–44.
2. Nadler CF, Lay DM. Chromosomes of some species of Meriones (Mammalia: Rodentia). *Z Sagetierkunde* 1967; *32:* 285–91.
3. Benazzou T, Viegas-Pequignot E, Petter F, Dutrillax B. Phylogenie chromosomique dequatre especes de Meriones (Rongeur, Gerbillidae). *Ann Genet* 1982; *25:* 19–24.
4. Akhavan AA, Yaghoobi-Ershadi MR, Shirani-Bidabadi L. Karyosystemic and morphometric characterization of *Rhombomys opimus* and *Meriones libycus*, the main reservoir hosts of zoonotic cutaneous leishmaniasis in the endemic foci of Isfahan Provinces, Iran. 12th Iranian Congress on Infectious Diseases and Tropical Medicine 2004.
5. Gharkheloo M, Kivanc E. A study on the morphology, karyology and distribution of *Ellobius fisher*, 1814 (Mammalia : Rodentia) in Iran. *Turk J Zool* 2003; *27:* 281–92.
6. Nevo E, Fillippucci MG, Redi C, Korol A, Beiles A, Chromosomal speciation and adaptive radiation of mole rats in Asia minor correlated with increased ecological stress. *Proc Natl Acad Sci* 1994; *91*(17): 8160–4.
7. Colak E , Yigit N, Sozen M, Ozkurt S. A study on taxonomic status of *Microtus subterraneus* (de selys and Longchamps, 1836) and *Microtus majori* Thomas 1906 (Mammalia: Rodentia) in Turkey. *Tr J Zoology* 1998; *22:* 119–29.
8. Etemad E, Iranian Mammale. Rodents and their identification key 1978, I edn. Tehran: National Association of Natural Resources Conservation and Human Resource Environment publication 1978; p. 286.
9. Matthey R. Les Chromosomes des Muridae, Revision critique et materiaux pour server a listore de levolution chromosomique chez les rongeurs, Rev. *Suisse Zool* 1953; *60:* 225–83.
10. Nuri Y, Ercument C, Reyhan V. A Study on the distribution, morphology and karyology of *Tatera indica* (Hardwicke 1807) (Mammalia: Rodentia) in Turkey. *Turk J Zool* 2001; *25:* 67–70.
11. Vorontsov NN, Korobitsina KV. Materials on a comparative karyology of Gerbillinae. *T Zoologiya* 1970; *12:* 152–7.
12. Nuri Y, Ercument C. A study of the taxonomy and karyology of *Meriones persicus* (Blanford 1875) (Mammalia: Rodentia) in Turkey. *Tr J Zoology* 1999; *23:* 269–74.
13. Matthey R. Cytologie et taxonomie du genre Meriones, Illiger (Rodentia-Muridue-Gerbillinae). *Saugetierkdl Mitt* 1957; *5:* 145–50.
14. Vinogradov BS, Argyropulo AL. Fauna of the USSR mammals key to the rodents. Moscow: Leningrad Publication 1941.

Corresponding author: Leila Shirani Bidabadi, Skin Diseases and Leishmaniasis Research Center (Sedigheh Tahereh), Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: L_shirani@mui.ac.ir

Received: 14 August 2008 *Accepted in revised form:* 4 November 2008