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CHROMOSOMAL AND CYTO-TAXONOMICAL STUDY OF HIMALAYAN MOUSE HARE (PIKAS, *OCHOTONA;* LAGOMORPHA)

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ABSTRACT
In present study, The metaphase chromosome of Himalayan mouse hare (*Ochotona roylei*) was prepared using standard blood cell culture, colchicine-citrate-flam drying, gimsa staining method and Classified on the basis of their arm ratio. Chromosomes counted in thirty one well spread metaphase plates, the diploid chromosome number was determined as 2N=62 for Himalayan mouse hare (*Ochotona roylei;* Lagomorpha) from Tungnath Garhwal Himalaya and Chromosome formula established as "6m + 9sm + 6st + 9t + X-sm + Y-t". On the basis of chromosome data accumulated up to 2011 mouse hare (*Ochotona* family) classified into five groups. The lowest 5th group having chromosome 2N=38, the 4th group having chromosome number 2N=40 to 42. The 3rd group belonging to the Qinghai Tibet Plateau group mouse hare and having the chromosome number 2N=46 to 54, the second group is having chromosome number 2N=60 to 62 from the surrounding Himalayan and also include the present study on *Ochotona roylei* which having 2N=62 from the Kedarnath wild life century Indian Himalaya. The highest number of chromosome 2N=68 has been reported from central Asia and North America and included in first group of classification.

KEYWORDS: Pika, *Ochotona roylei*, Himalayan mouse hare, Lagomorpha, Kedarnath wild life Sanctuary, chromosome, arm ratio.

INTRODUCTION

Mouse hare (family Ochotonidae) is small mammal; with comparatively short ears, small limbs and a visibly hairy small brush like structure in the origin of tail (Bahuguna and Upadhyay, 2009). The taxonomy of Himalayan mouse Hare is poorly understood and on the basis of little evidence, a good number of nominal species and subspecies have been described.

TABLE 1. Chromosomal Data of Lagomorpha Family Ochotonidae

S.No.	Group	Species	Chromosome Number (2N)	Reference
1.	1st Group	*Ochotona pusilla*	68	Vorontsov and Ivanitskaya, 1973.
2.		*Ochotona collaris*	68	Rausch and Ritter, 1973.
3.		*Ochotona princeps*	68	Adams, 1971; Stock, 1976.
4.	2nd The Surrounding	*Ochotona roylei*	62	Capanna, *et al*., 1991.
5.	Himalaya Group	*Ochotona macrotis*	62	Vorontsov and Ivanitskaya, 1973.
6.		*Ochotona rutila*	62	Vorontsov and Ivanitskaya, 1973.
7.		*Ochotona rufescens*	62, 60	Vorontsov and Ivanitskaya, 1973; Puget and Berland, 2008.
8.	3rd Qinghai Tibet	*Ochotona forresti*	54	Ye *et al*., 2011.
9.	Plateau Group	*Ochotona daurica*	50	Vorontsov and Ivanitskaya, 1973; Ivanitskaya, 1978.
10.		*Ochotona curzoniae*	46	Tan and Bai, 1987.
11.	4th Northern Paleartic	*Ochotona alpine*	42	Vorontsov and Ivanitskaya, 1973.
12.	Group	*Ochotona hyperborean*	40	Hayta and Shimba, 1969; Vorontsov and Ivanitskaya, 1973.
13.	5th Group	*Ochotona argentata*	38	Erbajeva and Ma, 2006; Howell, 1929.
14.		*Ochotona pricei*	38	Vorontsov and Ivanitskaya, 1973; Ivanitskaya, 1978.
15.		*Ochotona pallasi*	38	Erbajeva and Ma, 2006; Howell, 1929.

The order Lagomorpha comprises of two families, Leporidae (hares and rabbits) and Ochotonidae (Mouse-hares). According to different systematic and taxonomical studies, 30 living species of *Ochotona* are known (Mayers, *et al*., 2008; Bahuguna and Upadhyay, 2009). The classifications published on species level by different

authors (Allen, 1938; Ognev, 1940; Ellerman and Morrison-Scott, 1951 and 1966; IUCN, 1990) are based on the same peculiar characteristics i.e. skull morphology, dentition and coat color of body. Over all the systematic context is uncertain, while the morphological differentiation is inadequate to support the phyletic relationships among species, it is crucial to look for other, non-morphological, methods (i.e. the comparison of chromosome structure) allowing for the identification of different lineages within the genus. Karyotypic relationships among species within a genus offer a suitable tool for classification (Capanna *et al*., 1991). Accordingly, chromosomal data accumulating on pikas (*Ochotona*) up to 2010, currently the chromosome number of fifteen out the thirty living species of pikas are known (Nadler *et al*., 1969; Hayata and Shimba, 1970; Adams, 1971; Hsu and Benirschke, 1971; Wurster *et al*., 1971; Rausch and Ritter, 1973; Vorontsov and Ivanitskaya, 1973; Stock, 1976; Kimura *et al*., 1983; Capanna *et al*., 1991; Puget and Berland, 2008; Ye *et al*., 2011). About cyto-taxonomy, Capanna *et al*., (1991) proposed classification of *Ochotona* in different karyological groups. The updated with recent data were shown in table 1. Till date, research work is lacking from this area especially on chromosomal and cytogenetic aspects on Pikas from Kedarnath wild life sanctuary, Uttarakhand India. In the absence of research resources it is too conjectural to assume the taxonomical status of species. Hence an effort has been made to study the chromosomal and taxonomical status of the Himalayan mouse hare (*Ochotona roylei*, Lagomorpha).

SAMPLE COLLECTION AND METHODOLOGY

The Himalayan mouse hare were collected from Tungnath Himalaya situated at Kedarnath wild life Sanctuary, within the ambit of 30°26′N 78°54′E to 30°43’N 78°90’E and 30°45′N 79°36′E to 30°75’N 79°60’E, Geographically situated in the district Chamoli and Rudraprayag, Uttarakhand by using trapping method (Bonvicino *et al*., 2005). The blood samples were collected from adult male and female animal by using 5 ml sterile dispo-van syringes from fore lags in 4 ml tube containing 68 units USP Sodium Heparin (Ref No. 367871) and transported in departmental laboratories at body temperature. After blood sample collection mouse hare were release in same geographical areas.

The blood samples were placed into 15 ml glass centrifuge tube containing 8 ml of RPMI-1640 culture media with L-Glutamine (10.3 ml sterile Glutamine solution per litre of media added prior to use) and add 50µl of 0.05 % colchicine. The tubes were puts into the incubator pre-set at temperature 27^0C to incubate for 75 minutes then centrifuge at 1200 rpm for 10 minutes. Decant the supernatant and add 8 ml hypotonic solution (0.56 % KCl) to the cell pellet. Keep the cells into the solution for 25 minutes for swelling and then hypotonic action was stopped by adding 1.0 ml of freshly prepared chilled Carneys fixative (methanol + acetic acid in 3:1 ratio) slowly and mixed gently with pasture pipette. To get cell pellets, centrifuge cell suspension at 1200 rpm for 10 minutes. Then remove supernatant and add slowly 8 ml freshly prepared chilled fixative and keep the cells in cooled refrigerator for overnight for fixation. Repeat centrifugation three times to obtain clear transparent suspension, then take small quantity of cell suspension in a pasture pipette and drop it from a height of 1.5 feet on the grease free pre cleaned slide. Allow to air flame dry and keep slide for ageing for 2 days. Then Stain with 5% Giemsa stain with phosphate buffer (pH 6.8) for 20 minutes than washed with double distilled water. Kept it for air drying and observe metaphase in bright field microscope to obtain the quality of staining. For preparation of karyotype, photograph were taken and cut the individual chromosomes. The group of the chromosome were prepared as followed by Levan *et al*., (1964). Details about the Centromere position, arm ratio and symbol were given in table 2.

TABLE 2: Classification of chromosome as per Levan, *et al*.,1964

Centro-mere position	Arm ratio (L/S)	Chromosome type	Symbol
Medium	1.0- 1.7	Metacentric	m
Sub-medium	1.71 - 2.99	Sub-metacentric	sm
Sub-terminal	3.01 - 7.00	Sub-telocentric	st
Terminal	Above 7.00	Acrocentric	t

RESULTS

On the basis of chromosomes counted in thirty one well spread metaphase plates, the diploid chromosome number was determined as 62 (2N=62) for Himalayan mouse hare (*Ochotona roylei;* Lagomorpha) present in Tungnath, Garhwal, Himalaya, Uttarakhand India. The well spread autosomes consist of six pair metacentric (6-m), nine pair sub-metacentric (9-sm), six pair sub-telocentric (6-st) and 9 pair telocentric (9-t) with well determined sex chromosome X sub metacentric (sm) and Y telocentric (t). Chromosome formula in this study for Himalayan mouse hare (*Ochotona roylei*) is established as “6-m + 9-sm + 6-st + 9-t + X-sm + Y-t”. In Present study (Fig. 1) chromosome number 1 to 6 shows metacentric chromosome, 7 to 15 shows sub-metacentric chromosome, 16 to 21 shows sub-telocentric chromosome, 22 to 30 shows telocentric chromosome and X metacentric and Y telocentric shows sex chromosome of male Himalayan mouse hare (*Ochotona roylei*).

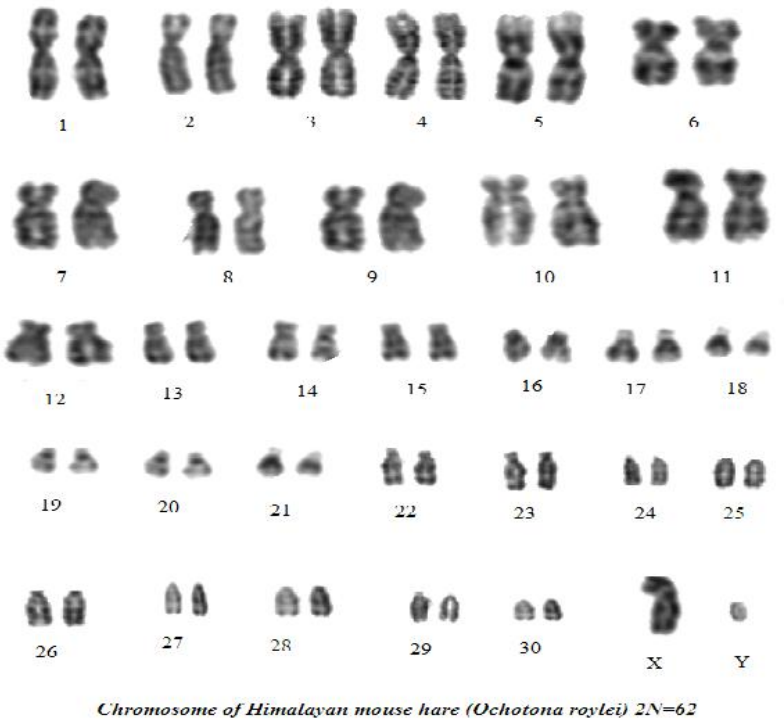

FIGURE 1: G-banding pattern photograph of male mouse hare (*Ochotona roylei*) chromosome

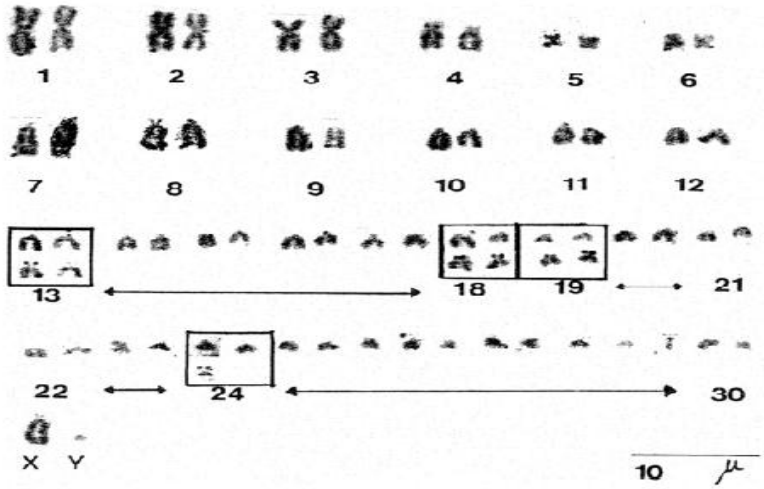

FIGURE 2: Chromosome photograph of *Ochotona roylei* (2n-62) done by Capanna, *et al*., (1991).

DISCUSSION:

In present study diploid number chromosome of Himalayan mouse hare is 2N=62 from Kedarnath wild life sanctuary, Uttarakhand India. Present findings also supported by Capanna *et al*., (1991), reported similar number of chromosome for *Ochotona roylei* from other alpine zone. Similar number of chromosome (2N=62) reported by several authors in different species of *Ochotona* i.e. *Ochotona rutile, Ochotona macrotis* and *Ochotona rufescens* (Vorontsov and Ivanitskaya, 1973; Capanna *et al*., 1991; Puget and Berland, 2008). In present study larger autosomes contain 6 pair of meta-centric chromosome and 9 pair of sub-metacentric chromosome. Capanna *et al*., (1991) recorded three pair of larger meta-centric chromosome and one larger sub-metacentric chromosome (Fig. 2). In present studied chromosome banding pattern also agree with Hayata and Shimba, (1969) and recorded similar banding pattern (Fig. 3) and reported larger 4 pair of metacentric, 3 pair sub-metacentric and three pair sub-telocentric chromosome in Japanese mouse hare (*Ochotona hyperboea*). In present study size is recorded as decreasing from metacentric to telocentric chromosome. Present findings are also supported by Capanna *et al*., (1991) and reported

decreasing size from metacentric to telocentric chromosome in same species. Vorontsov and Ivanitskaya, (1969) reported similar observation in other Lagomorpha. In present study sex chromosomes are well determined as larger X chromosome sub-metacentric (sm) and small Y chromosome telocentric (t). Present observation supported the findings of Capanna *et al*., (1991) in *Ochotona roylei* from Northern Indian Himalaya. Very similar observations were also recorded by Vorontsov and Ivanitskaya, (1969), Hayata and Shimba, (1969) in other *Ochotona spp.* (Lagomorpha) from different alpine zones.

On the basis of cyto-taxonomical and karyological data the *ochotona* family may be classified into five groups based on the chromosome number. The lowest chromosome 2N=38 was recorded by Howell (1829); Vorontsov and Ivanitskaya (1973); Ivanitskaya (1978); Erbajeva and Ma (2006) etc. from northern Palaearctic zone and place in 5th group. The 4th group also from the same geographical area reported by Hayata and Shimba (1969); Vorontsov and Ivanitskaya (1973) which having the chromosome number 2N=40 to 42. The 3rd group reported from Qinghai Tibet Plateau having the chromosome number 2N=46 to 54 reported by Vorontsov and Ivanitskaya (1973); Ivanitskaya (1978); Tan and Bai (1987); Ye *et al*. (2011) etc. The second group is having chromosome number 2N=60 to 62 from the surrounding Himalayan areas (Nadler *et al*., 1969; Vorontsov and Ivanitskaya, 1973; Capanna *et al*., 1991; Puget and Berland, 2008). The second group also include the present study on *Ochotona roylei* which having 2N=62 from the Kedarnath wild life sanctuary, Uttarakhand India. The highest number of chromosome 2N=68 has been reported from central Asia and north America put into first group as reported by Stock (1976); Adams, (1971); Vorontsov and Ivanitskaya (1973); Rausch and Ritter (1973).

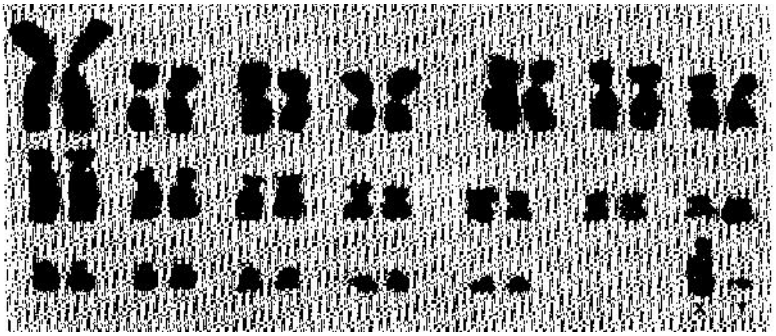

FIGURE 3: Chromosome photograph of Ochotona hyperborean (2n-40) done by Hayata and Shimba, (1969).

In first group, chromosome number 2N=68 included three species (*Ochotona pusilla*, *Ochotona princeps, Ochotona collaris*) belonging to different mountainous geographical areas and place point divide in subgroups. According to Niu *et al*. (2004) the same diploid number of chromosomes and the similar archaic feature in teeth morphology, the North American pikas (*Ochotona princeps* and *Ochotona collaris*) are considered to be much closer to *Ochotona pusilla*. There is a hypothesis that the ancient *pusilla*-like taxon probable ancestral of *Ochotona pusilla*, *Ochotona princeps* and *Ochotona collaris* migrated from Asia to the North America at the end of late Pliocene and the beginning of Pleistocene, and became distributed widely to south-eastern America (Erbajeva, 1994 and 1998). However, cyto-taxonomical data support this hypothesis and placed in same group. The 2nd cyto-taxonomic group, based on the chromosome number 2N=62 is having four species (*Ochotona roylei, Ochotona macrotis, Ochotona rutila and Ochotona rufescens*) included in same group as per bio-geographic requirements (Capanna, *et al*., 1991) and the morphological similarities among the animals (IUCN, 1990). According to Capanna *et al*. (1991), the four species are continuously distributed, along high mountain ranges, from North Iran to North Burma in similar climatic and geographical condition in different alpine zones. Morphological study done by Angelici and Corti (1990) also supported present gapping and suggested a closer affinity between *Ochotona roylei, Ochotona macrotis, Ochotona rutile and Ochotona rufescens*. Karyological data (2N=62) also agreed with the opinion of Angelici and Corti (1990) and included in same group.

Third cyto-taxonomical grouping included three species (*Ochotona daurica, Ochotona forresti and Ochotona curzoniae*) reported to same geographical areas from the Qinghai Tibet Plateau. The chromosome number of the group indicates variation from 2N=46 to 54, but due to biogeographically demand, and species placed in same group. According to Capanna *et al*. (1991), the distribution range of *Ochotona daurica* shoes adequate from the area of the second group species. This biogeographic condition further agrees with its cyto-taxonomic peculiarity to place in third group. Our study also supports the hypothesis that the differentiation of genus *Ochotona* in the Palearctic region was closely related to the gradual uplifting of the Qinghai-Tibet Plateau as given the opinion by Yu *et al*. (1992; 1996; 1997 and 2000). The species of the surrounding Qinghai-Tibet Plateau group and the Huanghe group are just distributed in the Qinghai-Tibet Plateau and adjacent mountains. Obviously, differentiations of the three groups are closely related to the uplifting of the Qinghai-Tibet Plateau and subsequent climatic changes (Niu *et al*., 2004). The species within the surrounding Qinghai-Tibet Plateau group are typical plateau and high altitude adapted species, which have undergone a rapid radiation since the early Pleistocene (about 2.2 Million years ago). The molecular data also indicates that radiation of this group may be multi-diverse and shows also in form of variation in chromosome number.

Fourth group consist two species *Ochotona alpina* and *Ochotona hyperborea* having chromosome number variation from 2N=40 to 42. According to IUCN (1990) *Ochotona alpine* and *Ochotona hyperborea* both are distributed in different type of geographical and environmental condition. According to Huntley and Webb (1989), environmental changes can often produce strong selective pressures that can result in rapid morphological diversification. Geographical condition causes mutation in molecular data also affected the number of chromosome. Niu *et al*. (2004) proposed *Ochotona alpina* and *Ochotona hyperborea* in same group due to molecular affinity in mt-DNA sequences. According to Niu *et al*. (2004), Northern group consist of five species, and can be divided into two subgroups, North Palearctic group and Nearctic species and placed *Ochotona alpina* and *Ochotona hyperborea* in North Palearctic group. Present comparative cytogenetic analysis also revealed the chromosome divergence in fourth group with low chromosome number: 2N=40 - 42 (Vorontsov and Ivanitskaya, 1973) in comparision to Nearctic species group with high diploid chromosome number 2N=68 (Hsu and Benirschke, 1971; Rausch and Ritter, 1973).

Fifth group consists three species having same chromosome number 2N=38. Geographic study indicates that separation between these species (IUCN, 1990) and the morphological similarities within species probably due to convergent evolution, apparently because the morphological characters have tracked the environment and resulted in adaptive modification in structure that increase the probability of survival (Yu *et al*., 1997). The convergent event may also have happened between the groups.

CONCLUSION

In conclusion, the diploid chromosome number was determined as 2n=62 for himalayan mouse hare (*ochotona roylei;* lagomorpha) from Tungnath Garhwal Himalaya and chromosome formula established as "6m + 9sm + 6st + 9t + x-sm + y-t". On the basis of chromosome number data accumulated up to 2011, *ochotona* family classified into five groups. The lowest 5th group having chromosome 2n=38, the 4th group having chromosome number 2n=40 to 42. The 3rd group belonging to the qinghai tibet plateau group mouse hare and having the chromosome number 2n=46 to 54, the second group is having chromosome number 2n=60 to 62 from the surrounding Himalayan and also include the present study on *ochotona roylei* which having 2n=62 from the Kedarnath wild life century Indian Himalaya. The highest number of chromosome 2n=68 has been reported from central Asia and north America and included in first group of classification.

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