



## A Review on the Molecular Study of the Species of Family Sciuridae (Rodentia: Mammalia)

*Archana Bahuguna and Ashutosh Singh*

*Northern Regional Centre, Zoological Survey of India,  
218-Kaulagarh Road, Dehradun, (Uttarakhand), India*

*(Received 05 June, 2013, Accepted 15 July, 2013)*

**ABSTRACT:** There are 250 species of the squirrels residing globally with rich biodiversity in tropical countries. Very little information is available about their status, distribution and phylogeny. An extensive deforestation and poaching are the major threats to the species of family Sciuridae. Molecular study is one of the very effective tools for identification of the species hence in their conservation. The present review describes the utility of this tool in phylogeny, the population study and in status survey done to a limited extend in their range of distribution by using Cyt b, Cyt Oxidase, c-myc and RAPD techniques by various workers.

**Key words:** Conservation, molecular study, phylogeny, Sciuridae

### INTRODUCTION

Squirrels, the small active, bushy-tailed mammals acquired the name from the Greek for “shade-tailed”. Squirrels are commonly known as “Shekhri”. The squirrel family Sciuridae is one of the largest families of mammals. There are 250 species of squirrels and are divided into two groups—those that live in trees and those that live on the ground. It contains an abundant and diverse group of species that have been the subject of numerous and important studies on behaviour, ecology, reproductive biology and morphology. Sciurids are among the best understood mammals in this regards (Steppan *et al.* 2003). Moreover the Sciuridae are found throughout the world (absent only from southern South America, Madagascar and Australia), and their diverse habitats, from dense forest to open deserts, make them very useful for study of mammalian adaptation to temperature extremes and resource scarcity and of development of behaviors in different environments. However the utility of this body of knowledge is compromised by an incomplete understanding of their phylogenetic relationships.

Until recently (Mercer & Roth, 2003), no comprehensive phylogeny of the family has been published, although a number of morphological systematic studies have been done (Black, 1972; Moore, 1959; Thorington *et al.* 2002).

Koprowski & Nandini in 2008 reported that the tropics particularly the forests of south and Southeast Asia are hotspots of squirrel diversity, but

this region generates the fewest scientific publications on squirrels. Moreover they reported that the most endangered squirrels occur in tropical countries with high deforestation rates and flying squirrels are more at risk than tree squirrels. They recommended that due to high diversity, extinction risk and lack of knowledge of sciurid in the tropics, increased efforts should be done to know their status and biology.

According to Herrer *et al.* (2003), the squirrel family Sciuridae is one of the largest and most widely dispersed families of mammals. In spite of the widespread distribution and conspicuousness of this group, phylogenetic relationships remain poorly understood. They used DNA sequence data from the mitochondrial cytochrome b gene of 114 species in 21 genera to infer phylogenetic relationships among sciurids based on maximum parsimony and Bayesian phylogenetic methods. Although they evaluated more complex alternative models of nucleotide substitution to reconstruct Bayesian phylogenies none provided a better fit to the data than the GTR+G+I model. They used the reconstructed phylogenies to evaluate the current taxonomy. At essentially all levels of relationships they found the phylogeny of squirrels to be in substantial conflict with the current taxonomy. At the highest level, the flying squirrels do not represent a basal divergence and the current division of Sciuridae into two subfamilies is therefore not phylogenetically informative.

At the tribal level, the Neotropical pygmy squirrel *Sciurillus*, represents a basal divergence and is not closely related to the other members of the tribe Sciurini. At the genus level, the sciurine genus *Sciurus* is paraphyletic with respect to the dwarf squirrels (*Microsciurus*) and the Holarctic ground squirrels (*Spermophilus*) are paraphyletic with respect to antelope squirrels (*Ammospermophilus*), prairie dogs (*Cynomys*), and marmots (*Marmota*). Finally, several species of chipmunks and Holarctic ground squirrels do not appear monophyletic, indicating a need for reevaluation of alpha taxonomy.

Steppan *et al.* in 2003 said that although the family Sciuridae is large and well known, phylogenetic analyses are scarce. They reported on a comprehensive molecular phylogeny for the family. Two nuclear genes (c-myc and RAG1) comprising approximately 4500 bp of data (most in exons) were applied for the first time to rodent phylogenetics. Parsimony, likelihood and Bayesian analyses of the separate gene regions and combined data revealed five major lineages and refuted the conventional elevation of the flying squirrels (Pteromyinae) to subfamily status. Instead flying squirrels are derived from one of the tree squirrel lineages. C-mycindels corroborate the sequence based topologies. The common ancestor of extant squirrels appears to have been arboreal, confirming the fossil evidence. The results also revealed an unexpected clade of mostly terrestrial squirrels with African and Holarctic centers of diversity. They presented the revised classification of squirrels and demonstrated the phylogenetic utility of relatively slowly evolving nuclear exonic data even for relatively recent clades.

In order to investigate phylogenetic relationships of the family Sciuridae living in Japan, they sequenced partial regions (379 bases) of mitochondrial 12S rRNA genes in six species of Japanese and other Asian squirrels. Phylogenetic trees constructed by sequence data indicated that two genera of flying squirrels (*Petaurista* and *Pteromys*) were clustered in a group distinct from non-flying squirrels, suggesting a possible monophyletic relationship of these flying squirrels. The evolutionary distance between the Japanese squirrel (*Sciuruslis*) from Honshu Island and the Eurasian red squirrel (*Sciurus vulgaris*) from Hokkaido island was comparable to intraspecific distances of the remaining species examined (Oshida *et al.* 1996)

In a study by Piaggio (2001), there are currently 25 recognized species of the chipmunk genus *Tamias*. In this study they sequenced the complete mitochondrial cytochrome *b* (cyt *b*) gene of 23 *Tamias* species. They analyzed the cyt *b* sequence and then analyzed a combined data set of cyt *b* along with a previous data set of cytochrome oxidase subunit II (COII) sequence. Maximum-likelihood was used to further test the fit of models of evolution to the cyt *b* data. Other sciurid cyt *b* sequence was added to examine the evolution of *Tamias* in the context of other sciurids. Relationships among *Tamias* species are discussed, particularly the possibility of a current sorting event among taxa of the South western United States and the extreme divergences among the three subgenera (*Neotamias*, *Eutamias*, and *Tamias*).

Lee *et al.* in 2008 said that, twenty-five chipmunk species occur in the world, of which only the Siberian chipmunk, *Tamiasibiricus*, inhabits Asia. To investigate mitochondrial cytochrome *b* sequence variations and population structure of the Siberian chipmunk in northeastern Asia, they examined mitochondrial cytochrome *b* sequences (1140 bp) from 3 countries. Analyses of 41 individuals from South Korea and 33 individuals from Russia and northeast China resulted in 37 haplotypes and 27 haplotypes, respectively. There were no shared haplotypes between South Korea and Russia northeast China. Phylogenetic trees and network analysis showed 2 major maternal lineages for haplotypes, referred to as the S and R lineages. Haplotype grouping in each cluster was nearly coincident with its geographic affinity. In particular, 3 distinct groups were found that mostly clustered in the northern, central and southern parts of South Korea. Nucleotide diversity of the S lineage was twice that of lineage R. The divergence between S and R lineages was estimated to be 2.98-0.98 Myr. During the iceage, there may have been at least two refuges in South Korea and Russia - northeast China. The sequence variation between the S and R lineages was 11.3%, which is indicative of specific recognition in rodents. These results suggest that *Tamias sibiricus* from South Korea could be considered a separate species. However, additional information, such as details of distribution, nuclear genes data or morphology, is required to strengthen this hypothesis.

In 2004, Oshida *et al.*, studied phylogenetic relationship among five species of the genus *Petaurista* (*P. alborufus*, *P. elegans*, *P. leucogenys*,

*P. petaurista* and *P. philippensis*) was investigated using the complete cytochrome b gene sequence (1140 bp). Phylogenetic tree indicated (1) *P. alborufus castaneus* from southern China was closely related to *P. petaurista albiventer* from Pakistan, (2) *P. alborufuslena* from Taiwan, *P. petaurista melanotus* from China and Laos, and *P. philippensis grandis* from Taiwan were grouped and (3) *P. leucogenys* which is endemic to Japan, distinctly separated from other species. Their result critically refuse the present classification in *Petaurista* and suggest that it is reasonable to regard *P. alborufuslena* as a distinct species from *P. alborufus castaneus* and *P. petaurista albiventer* as a distinct species from *P. petaurista melanotus*.

Selonen *et al.* in 2005 studied microsatellite loci in the Siberian flying squirrel (*Pteromys volanus*). It is an arboreal rodent that inhabits spruce-dominated forest. The species has declined in Finland, probably due to modern forestry. They studied genetic variation in the flying squirrel (n = 159) between eight sampling sites in Finland using seven microsatellite loci. Heterozygosity levels were similar in the flying squirrel as found for other Sciurid species. There were slight heterozygote excesses over all loci. Isolation by distance was clear and genetic differentiation between sampling sites was high. Radio telemetry studies by them revealed that the dispersal abilities of flying squirrels were good. But the high differentiation between sampling sites indicated that the actual gene flow over large distance was low, thus they emphasized the importance of landscape-level-management in the conservation of flying squirrel.

According to Yu *et al.*, (2006) with modified DNA extraction and purification protocols, the complete cytochrome b gene sequence (1140 bp) were determined from degraded museum specimens. Molecular analysis and morphological examination of cranial characteristics of the giant flying squirrels of *Petaurista philippensis* complex (*P. grandis*, *P. hainana*, and *P. yunanensis*) and other *Petaurista* species yielded new insights into long-standing controversies in the *Petaurista* systematics. Patterns of genetic variations and morphological differences observed in this study indicate that *P. hainana*, *P. albiventer*, and *P. yunanensis* can be recognized as distinct species, and *P. grandis* and *P. petaurista* are conspecific populations. Phylogenetic relationships reconstructed by using parsimony, likelihood, and

Bayesian methods reveal that, with *P. leucogenys* as the basal branch, all *Petaurista* groups formed two distinct clades. *Petaurista philippensis*, *P. hainana*, *P. yunanensis*, and *P. albiventer* are clustered in the same clade, while *P. grandis* shows a close relationship to *P. petaurista*. Deduced divergence times based on Bayesian analysis and the transversional substitution at the third codon suggests that the retreating of glaciers and upheavals or movements of tectonic plates in the Pliocene–Pleistocene were the major factors responsible for the present geographical distributions of *Petaurista* groups.

Phylogeographic relationship among five *Callosciurus* species (*C. caniceps*, *C. erythraeus*, *C. finlaysoni* and *C. prevostii*) from Southeast Asia were inferred from partial mitochondrial cytochrome b gene sequences (1,800 bases pairs). Maximum parsimony (MP), neighbor-joining (NJ) and maximum likelihood (ML) trees showed very similar branching patterns although the phylogenetic position of *C. caniceps* in NJ trees showed very similar branching patterns, although the phylogenetic position of *C. caniceps* in NJ tree was different from those in MP and ML trees. The five *Callosciurus* species were clustered in two lineages : one cluster consisting of *C. caniceps*, *C. erythraeus* and *C. finlaysoni* (79% bootstrap value in MP tree and 57% support value in ML tree) and the other cluster consisting of *C. nigrovittatus* and *C. prevostii* (100% bootstrap values in MP and NJ trees and 100% support value). Based on morphological and zoogeographical data, *Callosciurus* was previously categorized into two groups: the mainland unit and Sundal unit. The study done by Oshida *et al.* in 2001 supports this hypothesis. Phylogenetic relationship and divergences estimated from molecular data suggested that early divergences between two lineages might have occurred during Miocene and that subsequent divergence in each lineage might have taken place during the Pliocene (Oshida *et al.* 2001).

Yu *et al.* investigated in 2004 that the genetic diversity between the populations of woolly flying squirrels (*Eupetaurus*) from the eastern and western extremes of the Himalayas. They analyzed the partial mitochondrial cytochrome b gene sequences (390-810 bp) that were determined from the museum specimens using maximum parsimony (MP) and maximum likelihood (ML) methods.

The molecular data revealed that the two specimens that were collected in northwestern Yunnan (China) were members of the genus *Eupetaurus*. Reconstructed phylogenetic relationships showed that the populations of *Eupetaurus* in the eastern and western extremes of the Himalayas are two distinct species with significant genetic differences (12%) and diverged about 10.8 million years ago. *Eupetaurus* is significantly different from *Petaurista* and *Pteromys*. Considering the divergence time of the two *Eupetaurus* groups, the glaciations and the uplift of the Himalayas and Qinghai-Tibet plateau during the Pliocene-Pleistocene period might be the major factors affecting the present distribution of *Eupetaurus* along the Himalayas.

Oshida *et al.* (2000) elucidated the phylogenetic relationships among four species belonging to the genus *Petaurista* (*P. alborufusc astaneus*, *P. alborufuslena*, *P. leucogenys leucogenys*, *P. leucogenys nikkonis*, *P. petaurista melanotus*, and *P. philippensis grandis*), they investigated the partial sequences (1,068 bp) of the mitochondrial cytochrome *b* gene for these giant flying squirrels. Phylogenetic trees (NJ, MP, and ML trees) constructed from cytochrome *b* sequences indicated that *P. leucogenys* was grouped independently with other species, and that *P. philippensis* was most closely related to *P. petaurista* with 99–100% bootstrap values. In addition, two subspecies of *P. alborufus* did not form a single clade: *P. alborufus castaneus* from China was most distantly related to the other species, whereas *P. alborufus lena* from Taiwan was closely related to *P. petaurista* and *P. philippensis* with 82–90% bootstrap values. This result suggests that it is reasonable to regard *P. alborufus lena* as a distinct species from *P. alborufus castaneus*.

In a study done by Yu *et al.* in 2008 the analyses of the mitochondrial cytochrome *b* gene (1140 bp) showed that *Dremomys lokriah*, *D. pernyi*, *D. pyrrhomerus*, *D. rufigenis* and *D. gularis* all are separate species. *Dremomys pyrrhomerus* showed 8.5% sequence variation from *D. rufigenis*, and the level of estimated sequence divergence observed among *D. gularis*, *D. lokriah* and *D. pernyi* was > 11%. With *Tamiops* and *Callosciurus* as the outgroup taxa, in both maximum likelihood and Bayesian analyses, the five *Dremomys* species formed one strongly supported monophyletic group and *D. pyrrhomerus* is closely related to *D. rufigenis*.

The derived divergence times and fossil record suggested that the present geographical distributions of *Dremomys* owe much to the uplifting of the Himalayas and the successive glacial and interglacial in the Pliocene–Pleistocene.

In India 27 species of squirrels have been reported. Most of them are data deficient and under threat due to poaching and habitat destruction. Among the species present in India *Ratufa indica* (Erxleben), *Ratufa bicolor* (Sparrman) are under Schedule II, *Ratufa macroura* (Pennant), *Petinomysfus cocapillus* (Jerdon) are under Schedule I and *Funambulus palmarum* (Linn.) is under Schedule IV of IWPA. And all of them including *Petaurista petaurista* (Pallas), *Petaurista philippensis* (Elliot) are data deficient. *Ratufa indica* is also listed under Appendix II of CITES, as VU (Nationally), DD (Globally) under CAMP; *Ratufa macroura* listed as EN under RDB, EN (Nationally), DD (Globally) under CAMP and under Appendix II (CITES). *Ratufa bicolor* is listed under Appendix II (CITES), VU (Nationally) and DD (Globally) under CAMP. The Travancore Flying Squirrel (*Petinomysfus cocapillus*) is a flying squirrel found in South India and Sri Lanka. Travancore flying squirrels were thought to be extinct but rediscovered in 1989 after a gap of 100 years. Gundia is one of the only three important locations in Karnataka where the Travancore Flying Squirrel (*Petinomysfus cocapillus*) is recorded according to scientific studies by wildlife biologists of Mysore University (Ananthakrishnan, 2006; Rajamani *et al.*, 2008).

There are very few studies in India regarding their status and ecology. They are also killed for their fur (Bahuguna, 2007; Bahuguna, 2008). In India, some of the studies have been done for their identification through primary guard hair (Bahuguna, 2007; Bahuguna, 2008; Bahuguna *et al.*, 2010a; Bahuguna, 2010b) and these studies are useful for providing scientific proof in the court as well as for ecological study. But molecular study is required for providing the scientific proof for the court as well as for ecological studies (for their status, prey-predator relationship).

According to Spiridonova *et al.* (2005), populations of two ground squirrel species, *Spermophilus major* and *S. erythrogegens*, from the inter fluvial area of the Tobol and Ishim rivers, where their ranges overlap, have been examined using RAPD-PCR.

They have identified 253 loci, which included taxon-specific markers for *S. major* and *S. erythrogegens* as well as markers for geographic populations. Estimation of genetic diversity and construction of phylogenetic relationships were performed using software programs POPGENE, TEPGA, and TREECON. In all, based on morphological traits, animals from the Tobol-Ishim interfluvium were assigned to the two parental morphotypes and showed similar levels of genetic variability. However, the total polymorphism level proved to be higher in ground squirrels with the *major* morphotype than in animals with the *erythrogegens* morphotype. Nevertheless, the number of rare alleles was high in both cases, constituting about 70% of the total number. Inter population differentiation was considerably higher in *S. major* = 0.50 than in *S. erythrogegens* = 0.41. The genetic differentiation between local samples from the Tobol-Ishim interfluvial area was lower than that between the parental species. A significant part of the genetic diversity of the species examined and animals from the zone of overlapping ranges was accounted for by intra population variability. Animals from the northern and southern parts of the Tobol-Ishim interfluvium were characterized by the core traits of *S. major* and *S. erythrogegens*, respectively, falling into two distinct clusters in the UPGMA and NJ reconstructions. In addition to three hybrid individuals, identified by the bioacoustic method, three hybrid animals were distinguished using RAPD analysis. These animals earlier were thought to be “pure” species and formed their own clusters in phylogenetic reconstructions. Thus, the RAPD-PCR results directly showed the existence of stable hybridization (20% genetic hybrids) between *S. major* and *S. erythrogegens* in the Tobol-Ishim interfluvial area, which is more extensive than inferred previously from morphological and bioacoustic data.

Ermakov *et al.* in 2002 studied four species of ground squirrel—yellow (*Spermophilus fulvus*), russet (*S. major*), small (*S. pygmaeus*), and spotted (*S. suslicus*)—occur in the Volga region. Between *S. major* and *S. pygmaeus*, *S. major* and *S. fulvus*, and *S. major* and *S. suslicus*, sporadic hybridization was reported. Using sequencing and restriction

analysis, they have examined the mtDNA C region in 13 yellow, 60 russet, 61 small, 45 spotted ground squirrels, and 9 phenotypic hybrids between these species. It was shown that 43% of *S. major* individuals had “alien” mitotypes typical of *S. fulvus* and *S. pygmaeus*. Alien mitotypes occurred both within and outside sympatric zones. No alien mitotypes were found in 119 animals of the other three species, which suggests that only one parental species (*S. major*) predominantly participates in backcrosses. Phenotypic hybrids *S. fulvus* × *S. major* and *S. major* × *S. pygmaeus* were reliably identified using RAPD-PCR of nuclear DNA. However, they could find no significant traces of hybridization in *S. major* with alien mitotypes. Analysis of p53 pseudo genes of *S. major* and *S. fulvus* that were for the first time described produced similar results: 59 out of 60 individuals of *S. major* (including *S. major* with *S. fulvus* mitotypes) had only the pseudo gene variant specific for *S. major*. This situation is possible even at low hybridization frequencies (less than 1% according to field observations and 1.4 to 2.7% according to nuclear DNA analysis) if dispersal of *S. major* from the sympatric zones mainly involved animals that obtained alien mtDNA via backcrossing. The prevalence of animals with alien mitotypes in some *S. major* populations can be explained by the founder effect. Further studies based on large samples are required for clarifying the discrepancies between mitochondrial and nuclear DNA data, as discussed by Ermakov *et al.* in 2002.

Individuals of many species cache food to gain direct benefits from consuming their own caches, but individuals of a few species also gain indirect benefits by sharing caches with kin. They investigated whether gray squirrels cache primarily to gain direct benefits or if they also gain indirect benefits by sharing caches with kin. If squirrels share caches with kin, then genetically related squirrels should live near one another and cache near one another to facilitate cache sharing. In contrast, if squirrels cache primarily for direct benefits, then they should clump their caches near the center of their ranges to facilitate cache defense. This study was conducted with 140 squirrels in a 10 ha forest.

DNA was extracted from blood samples taken from squirrels, and genetic similarity scores from randomly amplified polymorphic DNA (RAPD) loci were used to measure relatedness. Squirrels were given piles of pecans for caching at six sites and observed from a blind. They recorded the direction squirrels took nuts from piles and at one site determined the location of caches. For male–female comparisons, related squirrels lived significantly closer to one another than unrelated squirrels, but this was not the case for female–female and male–male comparisons. The genetic similarity of neighboring squirrels did not influence the location of caches or the direction that squirrels took nuts from piles. Squirrels clumped their own caches and moved nuts toward their own home range centers. These results suggest that gray squirrels cache primarily to gain direct benefits rather than indirect benefits (Spritzer and Brazeau, 2003).

Tsvirka *et al.* (2008) reported that genetic diversity in the four east Palearctic ground squirrel species of the genus *Spermophilus* — *S. undulatus*, *S. parryi* (subgenus *Urocitellus*), *S. dauricus*, and *S. relictus* (subgenus *Citellus*)—was investigated using RAPD PCR with ten random primers. Siberian chipmunk, *Tamias sibiricus*, was used as an out-group. Molecular markers for different taxonomic ranks were identified, including those for the genera *Spermophilus* and *Tamias*, subgenera *Urocitellus* and *Citellus*, as well as for each of the four species, *S. undulatus*, *S. parryi*, *S. dauricus*, and *S. relictus*.

For the ground squirrel species and subgenera, genetic differentiation indices were calculated. In addition, for these groups the NJ phylogenetic reconstructions and UPGMA dendrograms of genetic similarity of the individuals and combined populations were constructed. Comparative molecular genetic analysis revealed a high genetic differentiation between *S. undulatus*, *S. dauricus*, *S. relictus*, and *S. parryi*, along with a low level of genetic differentiation of the subgenera *Citellus* and *Urocitellus*, distinguished in accordance with the existing taxonomic systems of the genus *Spermophilus*.

Analysis of *Spermophilus pallidicauda* Satunin, 1903 from three localities in Mongolia using cytogenetic characteristics (chromosome number and morphology, as well as the number and localization of nucleolus organizer regions) revealed the presence of a first-generation ( $F_1$ ) hybrid animal ( $2n = 36$ ), of the pale tailed *S. pallidicauda* ( $2n =$

34) and alashanic *S. alaschanicus* ( $2n = 38$ ) ground squirrels in the contact zone of their ranges. Analysis of nuclear DNA from ten ground squirrels (from a set of karyologically examined animals) by RAPD-PCR with eight oligonucleotide primers (OPA10, OPA12, OPC02, OPC05, OPC08, OPC09, OPC12, and OPD05) revealed four hybrids in two sites. The position of the hybrids in phylogenetic reconstructions made for the subgenera *Citellus* and *Colobotis* species varied depending on the method used. In the UPGMA dendrogram of genetic similarity hybrids formed their own subcluster with high bootstrap index (949) within the cluster of *Citellus* species. In the NJ phylogenetic tree, hybrids also clustered with high bootstrap index (886). But in this case they were located between the *Colobotis* and *Citellus* species clusters. The mtDNA haplotypes of the three hybrids examined were highly similar to the *Colobotis* ground squirrels, albeit in phylogenetic reconstructions they were placed between *Colobotis* and *Citellus*. The sites of the hybrid animals identification were located more than 200 km apart. Hence, the contact zone between the *S. pallidicauda* and *S. alaschanicus* can encompass a large territory in Mongolia (Tsvirka *et al.*, 2006)

Antolin *et al.* in 2001 studied that Piute ground squirrels (*Spermophilus mollis*) are distributed continuously in habitat dominated by native shrubs and perennial grasses in the Snake River Birds of Prey National Conservation Area in Idaho, U.S.A. This habitat is being fragmented and replaced by exotic annual plants, changing it to a wild fire-dominated system that provides poor habitat for ground squirrels. To assess potential effects of this fragmentation on ground squirrel populations, they combined an estimate of effective population size ( $N_e$ ) based upon a demographic study with a population genetic analysis. The study area included three subpopulations separated from each other by 8.13 km. The ratio of effective population size to census number ( $N_e/N$ ) was 0.57. Combining  $N_e/N$  with dispersal distances from a radio-tracking study, they calculated that neighborhood size was 62.2 ha, which included between 204 and 480 individuals. The population genetic analysis (based on randomly amplified polymorphic DNA (RAPD) and microsatellite markers) showed relatively low levels of genetic differentiation between subpopulations and no inbreeding within subpopulations ( $f = 0.0003$ ).

Table 1: Conservation status, population trend and the native countries of some squirrels species of Asia.

Species	Conservation Status	Population Trend	Native Countries									
			India	Bhutan	Pakistan	Bangladesh	Nepal	Myanmar	Thailand	China	Malaysia	Sri Lanka
<i>Ratufa bicolor</i>	Near Threatened	Decreasing	✓	✓		✓	✓	✓		✓	✓	
<i>Ratufa indica</i>	Least Concern	Decreasing	✓									
<i>Ratufa macroura</i>	Near Threatened	Decreasing	✓									✓
<i>Ratufa affinis</i>	Near Threatened	Decreasing							✓		✓	
<i>Sciurus pyrrhinus</i>	Data Deficient	Unknown										
<i>Callosciurus quinquestriatus</i>	Near Threatened	Decreasing					✓			✓		
<i>Funambulus palmarum</i>	Least Concern	Increasing	✓									✓
<i>Funambulus pennantii</i>	Least Concern	Unknown	✓		✓		✓					
<i>Funambulus sublineatus</i>	Vulnerable	Decreasing	✓									✓
<i>Tamiops mcclellandii</i>	Least Concern	Decreasing	✓	✓			✓	✓	✓	✓	✓	
<i>Tamiops swinhoei</i>	Least Concern	Stable						✓		✓		
<i>Marmota himalayana</i>	Least Concern	Unknown	✓		✓		✓			✓		
<i>Eoglaucomys fimbriatus</i>	Least Concern	Unknown	✓		✓							
<i>Hylopetes alboniger</i>	Least Concern	Decreasing	✓	✓		✓	✓	✓	✓	✓		
<i>Hylopetes phayrei</i>	Least Concern	Stable						✓	✓	✓		
<i>Petinomysfus cocapillus</i>	Near Threatened	Decreasing	✓									✓
<i>Belomys pearsonii</i>	Data Deficient	Unknown	✓	✓			✓	✓	✓	✓		
<i>Eupetaurus cinereus</i>	Endangered	Unknown			✓							
<i>Petaurista elegans</i>	Least Concern	Stable	✓	✓			✓	✓	✓	✓	✓	
<i>Petaurista magnificus</i>	Least Concern	Decreasing	✓	✓			✓			✓		
<i>Petaurista philippensis</i>	Least Concern	Decreasing	✓					✓	✓	✓		✓
<i>Petaurista petaurista</i>	Least Concern	Decreasing	✓				✓	✓	✓	✓	✓	
<i>Dremomys lokriah</i>	Least Concern	Decreasing	✓			✓	✓	✓		✓		
<i>Dremomys pernyi</i>	Least Concern	Stable	✓					✓		✓		

These estimates of population subdivision translate into an effective migration rate ( $N_e m$ ) of 2.3-3.3 per year, which represents a high level of gene flow. Invasion by exotics will reduce the overall productivity of the habitat, and will lead to isolation among subpopulations if favorable habitat patches become isolated.

The red squirrel (*Sciurus vulgaris* L.) is widespread in Lithuania; it lives and nests in deciduous and coniferous forests. Genetic diversity of 20 Lithuanian red squirrels from 11 locations in Lithuania was examined using a random amplified polymorphic DNA (RAPD) method. Ten random primers were used for amplification. Reproducible amplification patterns were obtained using six primers. In total, sixty-three amplification patterns were generated during analyses. Fifty-five (87.3%) of them were polymorphic. Visual examination of electrophoresis gels and analysis of banding patterns showed that the studied red squirrels are genetically quite different. Clustering analysis did not indicate any differentiation among study sites, a correlation between genetic and geographical distances was not detected either. These findings proved that the Lithuanian population of red squirrels is heterogenic. RAPD analysis revealed high-level genetic diversity within Lithuanian red squirrels (Paulauskas *et al.*, 2006). Trizio *et al.* (2005) investigated the genetic variability of the red squirrels using mitochondrial DNA mt(DNA) and microsatellite. Both mitochondrial and nuclear DNA data indicated the significant correlation between genetic and geographical distance. Twenty six haplotype were detected. The main aim of the study was to investigate genetic diversity of the red squirrels from different area of Lithuania using Random amplification polymorphic DNA, RAPD methods and the results of this analysis showed that the high level diversity of RAPD marker in Lithuanian red squirrels.

## CONCLUSION

The molecular study *i.e.* cyt b gene, cyt oxidase. C myc, RAPD analysis, done by many workers globally indicate that such studies are useful in phylogeny, resolving taxonomic problems, differentiation of hybrid population and in providing information about the behavior of the species of family sciuridae and their status survey.

However, very few molecular studies have been done in tropical countries, with high deforestation particularly in the South and Southeast Asia, where the most endangered squirrels occurs. There is a need to do molecular study in case of species of squirrels residing Asian countries.

## ACKNOWLEDGEMENT

We are grateful to the Director, Zoological Survey of India, Kolkata Dr. K. Venkatraman for encouraging to write this review. Thanks are also due to Additional Director, Northern Regional Centre, Zoological survey of India Mr P.I. Bhitia for providing all facilities to conduct the study.

## REFERENCES

- Ananthkrishnan G. (2006). Squirrels in focus, The Hindu, retrieved 6/13/2007 Two flying squirrel species.
- Antolin, M.F., Horne, B.V., Berger, M.D. Jr., Holloway, A.K., Roach, J.L. & Weeks, R.D. Jr. (2001). Effective population size and genetic structure of a Piute ground squirrel (*Spermophilus mollis*) population, *Canadian Journal of Zoology*, **79**(1), 26-34.
- Bahuguna, A. (2007). Trichotaxonomy of Red giant flying squirrels, *Petaurista petaurista* (Pallas) and Northern palm squirrels, *Funambulus pennantii* (Wroughton) (Mammalia: Rodentia: Sciuridae). *Annals of forestry*, **15** (2), 58-69.
- Bahuguna, A. (2008). Identification of Indian species of *Callosciurus* Gray, through dorsal guard hair) (Mammalia: Rodentia: Sciuridae), *Biosystematica*, **1**(2), 25-32.
- Bahuguna, A., Shajpal, V., Goyal, S.P., Mukherjee, S.K. & Thakur, V. (2010a). Forensic manual, Species Identification from Guard hair of selected Indian Mammals. Pub: Wildlife Institute of India, Defra UK, TRAFFIC WWF pp: 400.
- Bahuguna, A. (2010 b). Trichotaxonomy of Sciuridae, Viverridae, Mustelidae, Tragulidae and Herpestidae: under publication by Director ZSI, pp 311, Plates 49C.
- Black, C.C. (1972). Holarctic evolution and dispersal of squirrels (Rodentia: Sciuridae). *Evol. Biol.* **6**, 305-322.



- Ermakov, O.A., Surin, V.L., Titov, S.V., Tagiev, A.F., Luk'yanenko, A.V. & Formozov, N.A. (2002). A Molecular Genetic Study of Hybridization in Four Species of Ground Squirrels (Spermophilus: Rodentia, Sciuridae), *Russian Journal of Genetics*, **38**(7), 796-809.
- Herron, M.D., Castoe, T.A. & Parkinson, C.L. (2003). Sciruid phylogeny and the paraphyly of Holarctic ground squirrels (Spermophilus). *Molecular phylogenetics and evolution*, **31**, 1015-1030.
- Koprowski, J.L. & Nandini, R. (2008). Global hotspot and knowledge gaps for tree and flying squirrels, *Current Science*, **95**(7), 857-865.
- Lee, M.Y., Lissovsky, A.A., Park, S.K., Obolenskaya, E.V., Dokuchaev, E.N., Zhang, Y.P., Yu, L., Kim, Y.J., Voloshina, I., Myslenkov, A., Choi, T.Y., Min, M.S. & Lee, H. (2008). Mitochondrial Cytochrome b Sequence Variations and Population Structure of Siberian Chipmunk (Tamiassibiricus) in Northeastern Asia and Population Substructure in South Korea, *Molecules and Cells*, **26**, 566-575.
- Mercer, J. & Roth, V.L. (2003). The effects of Cenozoic global change on squirrel phylogeny. *Science*, **299**, 1568-1572.
- Moore, J.C. (1959). Relationship among the living squirrels of the Sciuridae. *Bull. Am. Mus. Nat. Hist.*, **118**, 157-206.
- Oshida, T., Masuda, R. & Yoshida, M.C. (1996). Phylogenetic Relationships among Japanese Species of the Family Sciuridae (Mammalia, Rodentia), Inferred from Nucleotide Sequences of Mitochondrial 12S Ribosomal RNA Genes, *Zoological Science*, **13**(4), 615-620.
- Oshida, T. & Masuda, R. (2000). Phylogeny and Zoogeography of Six Squirrel Species of the Genus Sciurus (Mammalia, Rodentia), Inferred from Cytochrome b Gene Sequences, *Zoological Science*, **17**(3), 405-409.
- Oshida, T., Lin, L.K., Masuda, R. & Yoshida, C.M. (2000). Phylogenetic Relationships among Asian species of *Petaurista* (Rodentia, Sciuridae), Inferred from Mitochondrial Cytochrome b Gene Sequences, *Zoological Science*, **17**(1), 123-128.
- Oshida, T., Shafique, C.M., Barkati, S., Fujita, J., Lin, L.K. & Masuda, R. (2004). A preliminary study on molecular phylogeny of giant flying squirrels, genus *Petaurista* (Rodentia, sciuridae) based on mitochondrial cytochrome b gene sequences, *Russian Journal of Theriology*, **3**(1), 15-24.
- Oshida, T., Yasuda, M., Endo, H., Hussien, N.A. & Masuda, R. (2001). Molecular phylogeny of five squirrel species of the genus *Callosciurus* (Mammalia: Rodentia) inferred from cytochrome b gene sequence. *Mammalia*, **65**(4), 473-482.
- Paulaskas, A., Radzijeuskaja, J. & Namavieute, E.V. (2006). Genetic diversity of red squirrel (*Sciurus vulgaris* L.) in Lithuania. *Acta Zoologica Lituanica*, **16**(2), 124-129.
- Piaggio, A. & Spicer, G.S. (2001). Mitochondrial Cytochrome b and Cytochrome Oxidase II Gene Sequences, *Molphylogenetics* vol, **20**(3), 335-350.
- Rajamani, N., Molur, S. & Nameer, P.O. (2008). *Petinomys fuscocapillus*. In: ICUN 2008. IUCN Red List of Threatened Species.
- Selonen, V., Painter, J.N. & Hanski, I.K. (2005). Microsatellite variation in the Siberian flying squirrel in Finland. *Ann. Zool. Fennici* **42**, 505-511.
- Spiridonova, L.N., Chelomina, G.N., Starikov, V.P., Korablev, V.P., Zvirka, M.N. & Lyapunova, E.A. (2005). RAPD-PCR Analysis of Ground Squirrels from the Tobol-Ishim Interfluvium: Evidence for Interspecific Hybridization between Ground Squirrel Species *Spermophilus major* and *S. erythrogastrus*, *Russian Journal of Genetics*, **41**(9), 991-1001.
- Spritzer, M.D. & Brazeau, D. (2003). Direct vs. Indirect Benefits of Caching by Gray Squirrels (*Sciurus carolinensis*), *Ethology*, **109**(7), 559-575.
- Stephan, S.J., Storz, B.L. & Hoffmann, R.S. (2003). Nuclear DNA phylogeny of the squirrels (Mammalia: Rodentia) and the evolution of arboreality from c-myc and RAG1, *Molecular Phylogenetic and Evolution*, **30**, 703-719.

- Thronigton, R.W.J., Hoffmann, R.S. (2002). In press Family Sciuridae in: Wilson, D.E., Reeder, D.M. (Eds), *Mammals Species of the World*. Smithsonian Institute Press. Washington, D.C.
- Trizio, I., Crestanello, B., Galbusera, P., Wauters, L.A., Tori, G. & Matthysen, E. (2005). Geographical distance and physical barrier shape the genetic structure of Eurassian red squirrels (*Sciurus vulgaris*). *Mol Ecol*, **14**, 469-481.
- Tsvirka, M.V., Chelomina, G.N. & Korablev, V.P. (2006). Genetic evidence of hybridization between paletailed *Spermophiluspallidicauda* Satunin, 1903 and alashanic *S. alaschanicus* Büchner, 1888 ground squirrels in Mongolia, *Russian Journal of Genetics*, **42**(4), 421-428.
- Tsvirka, M.V., Spiridonova, L.N. & Korablev, V.P. (2008) Molecular genetic relationships among east Palaerctic ground squirrels of the genus *Spermophilus* (Sciuridae, Rodentia), *Russian Journal of Genetics*, **44**(8), 966-974.
- Yu, F., Yu, F., McGuire, P.M., Kilpatrick, C.M., Pang, J., Wang, Y., Lu, S. & Woods, C.A. (2004). Molecular phylogeny and biogeography of woolly flying squirrel (Rodentia: Sciuridae), inferred from mitochondrial cytochrome b gene sequences, *Molecular Phylogenetics and Evolution*, **33**, 735-744.
- Yu, F., Yu, F., Pang, J., Kilpatrick, C.W., McGuire, P.M., Wang, Y., Lu, S. & Woods, C.A. (2006). Phylogeny and biogeography of the *Petaurista Philippensis* complex (Rodentia: Sciuridae), inter and intra-specific relationship inferred from molecular and morphometric analysis, *Molecular Phylogenetics and Evolution* **38**(3), 755-766.
- Yu, F., Li, S., Yang, S., Wang, Y., Jiang, X., McGuire, P.M., Feng, Q. & Yang, J. (2008). Molecular phylogeny of five species of *Dremomys* (Rodentia: Sciuridae), inferred from cytochrome b gene sequences, *Zoological Scripta*, **37**(4), 349-354.