A DOCTORAL DISSERTATION

Taxonomic Study of Muridae and Phylogenetic Relationship of *Mus* and *Rattus* in Nepal Inferred to Mitochondrial DNA *Cytochrome B* (*CytB*) Gene

Faculty of Science Education

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미토콘드리아 DNA *Cytochrome B* (*CytB*) 유전자에 근거한 네팔에 서식하는 *Mus*속과 *Rattus*속의 계통 유연관계 및 쥐과(Muridae)의 분류학적 연구

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프라딥 아디카리

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Taxonomic Study of Muridae and Phylogenetic Relationship of *Mus* and *Rattus* in Nepal Inferred to Mitochondrial DNA *Cytochrome B* (*CytB*) Gene

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Abstract

In the current study, taxonomy of murids occurring in three locations Lumbini, Pokhara, and Kathmandu of Nepal have been studied using both morphological and molecular analyses. Morphological identification of murids was carried out by assessing the external morphology including fur color, footpad, tail, ear, external genitalia, and pairs of mammary glands in females and measuring the external body. Altogether, five species namely *Bandicota bengalensis*, *Mus booduga*, *Niviventer fulvescens*, *Rattus pyctoris*, and *Tatera indica* were identified using morphological analysis. However, four species *M. musculus*, *R. nitidus*, *R. rattus* and *R. tanezumi* could not identify through morphological analysis. The morphological traits were compared between two species *R. rattus* and *R. tanezumi* but there were no consistently discernible coat color differences to distinguish them. Also, there was no significant difference in morphometric measurement (Student *t*-test, n=52, *df*=50, $p>0.05$). These two species were distinguished with *R. nitidus* and *R. pyctoris* by tail length and tail color, respectively. Similarly, the morphology of *M. musculus* was compared between collection from different locations Lumbini and Pokhara. They were distinguished by coat color but there was no significant difference in morphometric measurement (Student *t*-test, n=23, *df*=21, $p>0.05$).

Molecular identification was carried out using mitochondrial DNA (mtDNA) *Cytochrome B* (*CytB*) gene sequences and successfully identified eight taxa (*B. bengalensis*, *M. booduga*, *M. musculus*, *N. fulvescens*, *R. nitidus*, *R. rattus*, *R. pyctoris*, and *R. tanezumi*) in species level and one taxon (*Mus* sp.) at genus level. The molecular data generated in this study was also used to distinguish intraspecific and inter specific variations. *CytB* haplotypes were determined in all taxa and found altogether forty-four unique haplotypes in 114 *CytB* sequences of murids. The *M. booduga* (2), *M. musculus* (6), *Mus*

sp. (2), *R. nitidus* (4), and *R. rattus* (26), occupied multiple haplotypes but *B. bengalensis*, *N. fulvescens*, *R. pyctoris* and *R. tanezumi* occupied a single haplotype in this study. Genetic distances were computed among conspecific haplotypes of *R. rattus*, *M. musculus*, *R. nitidus*, and *M. booduga*, which were ranged 0.001-0.017, 0.001-0.016, 0.001-0.008 and 0.001-004, respectively. Genetic distances were also computed between nine identified murids and found highest genetic distance between *B. bengalensis* and *M. musculus* (0.278) and lowest genetic distance between *R. rattus* and *R. tanezumi* (0.048). Phylogenetic studies of two genera *Mus* and *Rattus* was carried using *CytB* gene sequences. Phylogenetic tree (neighbor joining tree, NJ tree) was constructed based on the genetic distance between the haplotypes of intrageneric species. Three *Mus* taxa *M. musculus*, *M. booduga* and *Mus* sp. were found to be clustered in two species groups (*M. musculus* species group and *M. booduga* species group) of subgenus *Mus*. Former one was in *M. musculus* species group and later two were in *M. booduga* species group. The phylogenetic analysis of *M. musculus* revealed that the haplotypes sequences of mtDNA *CytB* gene distinguished into two distinct clades on a NJ tree representing two subspecies, *M. m. bactrianus* and *M. m. castaneus* in Pokhara and Lumbini, respectively. The divergence time estimation between these two subspecies showed they were diverged approximately, 0.68 million years before present (MYBP). Phylogenetic analysis suggested two population of *M. booduga* abundant in Nepal and India are occurring in two different lineages. Although the *Mus* sp. could not identify at species level but phylogenetic analysis revealed it has close genetic relation with *M. nitidulus* recorded in Myanmar. Phylogenetic relationship was studied on four species of *Rattus* identified in this study. The *R. rattus* abundant in Nepal has a close genetic relation with *R. rattus* found in Pakistan and have been clustered together in a group at the phylogenetic tree. Genetically, these two populations are close with South Indian population of *R. rattus*, which were

estimated to be diverged about 2.097–2.344 MYBP. The results of phylogenetic study also revealed two subpopulations of *R. rattus* in Nepal, which were estimated to be separated approximately between 0.529 and 0.592 MYBP. Phylogenetic analysis showed two different subgroups (A and B); subgroup A contains the sequences of *R. tanezumi* only found in Nepal, and another subgroup B contains those from South and East Asian countries including Bangladesh, Laos, Vietnam, and South Korea. The genetic distance between these two subgroups was found higher than 0.02, which suggested being different lineages of *R. tanezumi*. The genetic distance between the haplotypes of *R. nitidus* abundant in Nepal, India, Laos, and Vietnam were ranged between 0.001 and 0.009 and were clustered together in a group at the phylogenetic tree, indicating close genetic relation among the different populations. The *R. pyctoris* have a single haplotype and have no other distinct genetic population so its phylogenetic relation studied with respect to haplotypes of other species. Genetically, it has close relation with *R. tanezumi*, which were diverge about 5.192–5.806 MYBP.

This study provided the morphological and molecular dataset of murids found in Nepal. The molecular datasets generated in this study provided new records of *M. m. bactrianus* and *R. tanezumi* for Nepal. Though this study was carried out in selected areas of Nepal, the findings suggested that integrative studies of morphological and molecular analyses are required for correct identification and understanding the evolutionary phenomenon in murids. Further, extensive survey and collection of specimen from different localities across Nepal are required for determining the taxonomic status of murids and their phylogenetic relationship.

I. INTRODUCTION

1. General introduction

The Rodentia is the largest mammalian order due to the extraordinary proliferation of rats and mice comprising about 42% of all mammalian diversity (Musser and Carleton, 2005). The Muridae is the single most specious and ubiquitous rodential family comprised of approximately 300 genera and 1,300 species over the world (Aplin *et al.*, 2003a; Musser and Carleton, 2005). In South Asia, it comprises 24 genera and 71 species (Srinivasulu and Srinivasulu, 2012) and in Nepal, it occupied 40.84% of the total species found in the South Asia (Baral and Shah, 2008; Jnawali *et al.*, 2011; Thapa, 2014). It is estimated that origin of rodents was approximately 61.7–62.4 million years before present (MYBP) and murids were about 23 MYBP (Wu *et al.*, 2012). After that, intergeneric and interspecific diversification occurred in the different interval of time (Wu *et al.*, 2012). For instance, divergence time of two genera *Mus* and *Rattus* were estimated about 8–12.3 MYBP (Jacobs and Flynn, 2005; Wu *et al.*, 2012).

Geographically, murids are distributed in all the continents and oceanic islands, except Antarctica (Aplin *et al.*, 2003a; Musser and Carleton, 2005; Robins *et al.*, 2010; Pimsai *et al.*, 2014). They are adapting to a wide range of environments from humid tropical forests to the hottest and driest desert and tundra region in different forms of lifestyles, like fossorial, arboreal, scansorial and semiaquatic (Kingdon, 1997; Nowak, 1999; Pimsai *et al.*, 2014). They are inhabiting in various types of habitats, for instance, grassland, shrubland, forest, agriculture land, and human settlements (Clausnitzer and Kityo, 2001; Aplin *et al.*, 2003a; Pimsai *et al.*, 2014).

In Nepal, murids are distributed from lowland *terai* region (65 m above sea

level, ASL) to a height of 4,100 m ASL in different altitudes and habitats (Abe, 1982; Baral and Shah, 2008; Jnawali *et al.*, 2011). Murids species like *Rattus rattus*, *R. nitidus*, *R. pyctoris*, *Mus musculus*, *M. booduga*, and *Niviventer fulvescens*, were recorded up to 4,100 m ASL in and around the human settlements, agriculture land, bushland and deciduous broadleaf forest (Ellerman, 1961; Abe, 1982; Baral and Shah, 2008). Some species have recorded only at a certain range of altitudes from eastern, central, and western, Nepal. The *Apodemus gurkha*, is an endemic species of Nepal has been recorded between 2000-3,600 m ASL in Gorkha (Hinton, 1924; Jnawali *et al.*, 2011), Ghrorepani (Mekada *et al.*, 2001), and Tukuche (Abe, 1982). The *N. fulvescens*, *N. eha*, *R. pyctoris*, and *M. cervicolor* have been recorded 2,100-3,200 m ASL, 2,600-3,700 m ASL, 1,200-4,500 m ASL and 200-3,200 m ASL, respectively (Abe, 1982; Newton *et al.*, 1990; Baral and Shah, 2008). Similarly, some species like *Nesokia indica*, *Dacnomys millardi*, *Golunda ellioti*, *Bandicota indica* were found below 1,200 m ASL near to the human settlement and agriculture land (Abe, 1982; Baral and Shah, 2008). All the murids recorded in Nepal are considered as the least concerned species except *Apodemus gurkha*, which is an endangered species of Nepal according to the national redlist of mammal category (Jnawali *et al.*, 2011).

Murids have ecological, economic, biomedical, social, and culture value (Bryda, 2013; Sunyer *et al.*, 2013). They are considered keystone species in ecological perspective are playing a key role in pruning or eliminating vegetation, spreading of seeds and pollens, nutrient cycling, and an indicator of habitat change (Munoz and Bonal, 2011; Sunyer *et al.*, 2013). In the terrestrial ecosystem, they are acting as the consumers of plants and being the prey of the reptiles, birds, and other mammals. They are laboratory animals, widely used in biomedical research and drug test (Bryda, 2013). Globally, some murids are the major pest in agriculture. In Asia, rats and mice, causing 5-10% pre-harvest lost in rice farming (Singleton, 2003). Mostly, three taxa *Rattus* species, *Bandicota* species, and *M. musculus* are well-recognized pest species (Singleton,

2003). In addition, they are the transmitter of the pathogen, spreading of diseases such as Hantaviruses, plague, and rat typhus (Gratz, 1994; Mills, 1999).

Murids exhibit a short reproductive cycle, omnivorous in diet, adaptive in various environments, high dispersal capacity, and commensal with humans (Nowak, 1999 Aplin *et al.*, 2003a; Ruscoe and Murphy, 2005). These characteristics contribute to their extraordinary potential for invasion and have now well established in the world. Four species (*R. rattus*, *R. exulans*, *R. norvegicus* and *M. musculus*) are worst invasive species spreading worldwide (Long, 2003). These invasive species have severe effects on the human health (Meerburg *et al.*, 2009), agriculture system (Singleton, 2003), and native organisms (Harper and Bunbury, 2015). To control these invasive species rodenticides, physical trapping, and biological controls are some commonly used techniques (Singleton, 2003). Recently, Ecologically Based Rodent Management concept based on the fertility control has been emerged in many developed and developing countries (Chambers *et al.*, 1999; Belmain *et al.*, 2007, Sharma *et al.*, 2015).

2. Morphological study of murids

1) Morphological characteristics of murids

The murids are characterized by the extremely enlarged and V-shaped infraorbital foramen, modified into a wider upper portion for muscle transmission and a narrower lower portion for nerve transmission (Ellerman, 1961). Usually, the anterior root of zygomatic arch flattened in the form of the zygomatic plate is tilted upward to a greater or lesser degree for muscle attachment (Ellerman, 1961; Agrawal, 2000). It has two pairs of rootless and continuously growing incisors, lack of canine and premolar teeth (Miller and Gidley, 1918; Ellerman,

1961). The dental formula consist 1,0,0,3/1,0,0,3, and molar teeth are cuspidate, laminate, prismatic, and cusps arranged in 2-3 longitudinal rows (Agrawal, 2000).

The murids have slender bodies, pointed snouts, tilted zygomatic plate in an upward direction, brawny jaw muscle and fused tibia and fibula (Miller and Gidley, 1918; Ellerman, 1961; Nowak, 1999; Agrawal, 2000; Aplin *et al*., 2003a). They have strong limbs, scaled tail sensitive whiskers and pinna, 2-8 pairs of mammary glands, and different coat colors (Ellerman, 1961; Abe, 1982; Aplin *et al*., 2003a, Pimsai *et al*., 2014). Usually, the tail of the murids is longer than head body except for few species such as *R. norvegicus* and *B. bengalensis* (Aplin *et al*., 2003a). The oestrous cycle, gestation length, litter size, and post-partum oestrus varied in different species. Usually, *Rattus* species have a short oestrous cycle of 4-7 days with a post-partum oestrus, short gestation length 21-24 days, and average litter size 4-8 (Breed, 1978, Aplin *et al*., 2003a). However, most of the non-*Rattus* species have reduced rate of reproductive potential with longer oestrous cycles about 6-10 days and longer gestation lengths about 27-38 days (Breed, 1978; Aplin *et al*., 2003a; Thitipramote *et al*., 2009).

2) Morphological identification of murids

Characterization and comparison of the macromorphological traits including cranial analysis are the globally applied techniques in murids taxonomy (Ellerman 1961; Abe, 1971, 1982; Martens and Niethammer, 1972; Niethammer and Martens 1975; Newton *et al*., 1990; Agrawal, 2000; Aplin, *et al*., 2003a; Geffen *et al*., 2011; Yazdi and Adriaens, 2013; Darvish *et al*., 2014; Pimsai *et al*., 2014). Assessment of external morphology included coat color, footpad, tail and ear morphology and mammary pairs in females and measurement of body dimension in sexually matured specimens are the basic and fundamental procedures in species identification (Ellerman, 1961; Newton *et al*., 1990;

Agrawal *et al.*, 2000; Aplin *et al.*, 2003a; Pimsai *et al.*, 2014). Similarly, the internal morphological analysis included skull and skeletal analysis (Ellerman, 1961; Abe, 1971, 1982; Agrawal *et al.*, 2000; Pimsai *et al.*, 2014), and examination of reproductive anatomy (Geffen *et al.*, 2011) are also applied in murids identification. Morphological analysis has some qualitative merits such as it is applicable on the museum specimens preserved from long time and extinct species found in the fossilized form (Hillis, 1987). It is most economical, convenient, and applicable even in the field. However, it has some downfalls too. It could not give correct identification result to the sibling species (Jiggins, 1998) such as *R. rattus* and *R. tanezumi* and phenotypic plasticity (Price *et al.*, 2003).

3. Taxonomic studies of murids in Nepal

In Nepal, the taxonomic studies of murids using morphological analysis have been started since third decades of 19th century. Hodgson (1832) first time published a classified catalogue of the mammals of Nepal including rats and mice but his first substantive description on murids was published in 1845 describing 11 species of murids (Hodgson, 1845). Hinton (1922) described the taxonomy of *R. nitidus*, *R. rattoides* and four subspecies of *R. rattus*. Hinton and Fry (1923) published a checklist of mammalian species, of which 16 species were murids. They claimed that *M. brunneusculus* identified by Hodgson (1845) was the subspecies of *R. rattus*, which is closely related to *R. r. sikkimenesis* (*R. andamanensis*). Thomas (1924) described a new species of a field mouse, *A. gurkha* from Laprak, Gorkha, which is an endemic mammal of Nepal. Biswas and Khajuria (1955) reported two new subspecies *R. r. khumbuensis* and *M. m. pygmaeus* from the eastern part of Nepal. Martens and Neithmmer (1972) reported another new species *A. sylvaticus wardi* (*A. pallipes*) together with *A.*

gurkha and well explained about the distribution pattern of these species. Chesmore (1970) recorded *B. bengalensis* and *M. booduga* from Birganj, Nepal. Abe (1971, 1977, 1982) carried out the vertical survey in central and western, Nepal from *terai* region to Langtang National Park and Pokhara to Tukuche, respectively and described the morphology of murids based on the external morphology and cranial analysis. Martens and Niethammer (1972) recorded a new species *Apodemus sylvaticus wardii* for Nepal. Mitchell (1975) published a checklist of mammalian species including 11 species of rodents. Marshall (1977) published an erudite monograph on Asian species of the genus *Mus*, which included the analysis results of the *M. cervicolor*, *M. cookii*, and *M. musculus* collected in Royal Chitwan National Park, Hetwada, and Kathmandu. Ingles *et al.* (1980) reported a new record of *Diomys crumpi* from the estern *terai* of Nepal. Since 1990 to 2014, various faunal surveys carried out in Nepal and described the habit, habitat, and geographical distribution of murids (Newton *et al.*, 1990; Mekeda *et al.*, 2001; Adhikari, 2001; Nembang, 2003; Dahal, 2011; Adhikari, 2014).

Nepalese zoologists have produced few accounts of mammalian species including murids, but noteworthy publications include Shrestha (1997), Majupuria and Kumar (1998), Baral and Shah (2008), Jnawali *et al.* (2011), Thapa, (2014). Earlier morphological studies on murids were well succeeding to describe the taxonomy and ecology of various species in Nepal. However, there was no consistency to deal with taxonomic status and nomenclature of many taxa such as *M. platythrix*, *A. flavicollis*, *A. sylvaticus*, *M. saxicola*, *R. tanezumi*, *B. maxima*, *M. dubius*, *M. m. homorus*, and *M. m. urbanus*, in Nepal (Pearch, 2011; Thapa, 2014). Due to the similar morphology, rapid radiation, and high intraspecific and interspecific biodiversity in murids, their correct identification has been difficult (Robins *et al.*, 2007; Aplin *et al.*, 2011; Rowe *et al.*, 2011). Therefore, there could be high chances of misidentification and wrong interpretation on systematic depending on the morphological analysis only.

4. Phylogenetic studies of murids

The phylogenetic study determines the evolutionary relationship and trend of development among the different group of organisms. The phylogenetic relation is usually represented as branching, tree-like diagrams that represent an estimated pedigree of the inherited relationships among molecules 'gene tree' and organisms (Brinkman, 2001). Phylogenetic study helps to understand the pattern of gene and genome evolution, a relation between the different group of taxa, identifying species and clades for future studies as well as valuable to the other non-evolutionary disciplines like physiology, genomics, immunology, and oncology (Steppan *et al.*, 2004). It has been studying using both morphological characters and molecular sequencing data (Brown, 2002). Morphological phylogeny is based upon the similarities and differences in physical characteristics, but molecular phylogeny is based on the mitochondrial or nuclear DNA gene sequences (Hillis, 1987).

There are some qualitative features in mtDNA such as maternal inheritance, no recombination, significant high sequence variations among closely related species, and rapid evolution rate about 5-10 times faster than nuclear DNA (Brown *et al.*, 1979; Irwin *et al.*, 1991, Gissi *et al.*, 2000; San Mauro *et al.*, 2006). It is used extensively as a genetic marker particularly made it amenable to identify species, to evaluate phylogenetic relationships among the different populations, and to estimate tentative divergence time from their common ancestor based on its sequence variability (Brown *et al.*, 1979; De Mandal *et al.*, 2014). Therefore, intraspecific and interspecific variations, population structures of diverse taxa, have been studied using mtDNA (Page and Holmes, 1998; Page *et al.*, 2010; Suzuki *et al.*, 2013; Robins *et al.*, 2014). It has been reported that mtDNA sequences evolve most rapidly in rodents compared to large mammals (Nabholz *et al.*, 2008), and therefore, mtDNA sequences are widely used in rodent taxonomy and phylogenetics.

In mtDNA, the *CytB* gene has both slow and fast evolving regions occupying both conserved and variable sites, which makes it possible to examine deep divergences and more recent ones (Irwin *et al.*, 1991). The *CytB* gene has been using as a powerful genetic marker in the taxonomic study and phylogenetic relationship among closely related taxa within genera and family levels (Patwardhan *et al.*, 2014). In addition, it eagerly allows for establishing positional homology with unequivocal alignments and the studies shown that it is suitable for studying evolutionary activities that take place within the past 20 million years before present (MYBP) (Irwin *et al.*, 1991). Thus, mtDNA *CytB* gene sequences are usually used to determine phylogenetic relationship between different taxa including *Mus* and *Rattus*.

1) Phylogenetic study of the *Mus* species

The genus *Mus* is a moderately specious murine rodent comprising 41 well-recognized species believed to native taxa of Eurasia and African continents, but it is distributed globally except Antarctica (Musser and Carleton, 2005; Suzuki and Aplin, 2012). It is divided into four subgenera namely *Mus*, *Pyromys*, *Nannomys* and *Coelomys* having discrete morphological, biochemical and chromosomal traits (Marshall, 1977; Lundrigan *et al.*, 2002). Marshal (1977) first time distinguished into four groups based on the craniodental criteria but Lundrigan *et al.*, (2002) confirmed to each subgenus as phylogenetic entities.

The subgenus *Mus* is possibly originated somewhere in the South-central Asia and Indian sub-continent (Suzuki and Aplin, 2012). Analysis of phylogenetic relationship within the Eurasian subgenus *Mus* consistently indicates three clusters of species (Suzuki, *et al.*, 2004; Veyrunes *et al.*, 2005). Suzuki *et al.* (2004) named to these three evolutionary lineages as *M. musculus* species group, *M. cervicolor* species group and *M. booduga* species group for Palearctic, South-east Asian, and Indian species, respectively. It comprised 12 species in three different species groups. The *M. musculus* species group

included *M. musculus*, *M. spretus*, *M. spicilegus*, *M. macedonicus*, and *M. cypriacus*, *M. cervicolor* species group included *M. cervicolor*, *M. caroli*, and *M. cookie*, and *M. booduga* species group included *M. booduga*, *M. terricolor* , *M. famulus* and one Southeast Asian species *M. fragilicauda* (Suzuki *et al.*, 2004). In Nepal, five species of subgenus *Mus* (*M. booduga*, *M. terricolor*, *M. cervicolor*, *M. cookie*, and *M. musculus*) and two species of subgenus *Pyromys* recorded yet (Baral and Shah, 2008; Thapa, 2014).

Within the *M. musculus* species group the *M. musculus* is a most abundant taxa in Eurasia supposed to have originated in the Northern part of the Indian Subcontinent (Boursot *et al.*, 1993; Din *et al.*, 1996), but currently it has been spreading all over the world's continents and islands except Antarctica (Musser and Carleton, 2005). Now, it is recognized that *M. musculus* species consists of genetically diverse and differentiated species having at least six different subspecies (*M. m. castaneus*, *M. m. musculus*, *M. m. domesticus*, *M. m. bactrianus*, *M. m. gentilulus* and *M. m. isaticus*) have been described throughout the world (Prager *et al.*, 1998; Terashima *et al.*, 2006; Searle *et al.*, 2009; Suzuki *et al.*, 2013; Hardouin *et al.*, 2015; Hamid *et al.*, 2017). It has 2n=40 karyotypes (Malovi *et al.*, 2015). The *M. booduga* and *M. terricolor* are two indigenous species of Indian subcontinent recorded in Nepal, India, and Bangladesh (Suzuki *et al.*, 2004). The karyotypes of these two sibling species have recorded 2n=40 (Sharma, 1996). Similarly, three sister taxa *M. cervicolor*, *M. caroli* and *M. cookii* are abundant in South-east Asian countries also found in India and Nepal (Marshall, 1977).

The rate of mtDNA evolution with respect on nuclear DNA in *Mus* species have relatively low compares to average rate recorded in mammalian species. According to She *et al.* (1990), it evolves two to six times faster than nuclear DNA. The rate of mtDNA sequence divergence varied with species to species, but they have simultaneous evolution. Chatterjee *et al.* (1994) commented that *M. booduga* and *M. terricolor* group might have evolved simultaneously with other groups but little later than *cervicolor*, *caroli*, and *cookii* group.

DNA-based phylogenetic studies were carried on two *Mus* species (*M. booduga* and *M. musculus*) found in Nepal. Suzuki *et al.* (2004) studied on *M. booduga* using *CytB* gene and found both populations occurred in Nepal and India have a close genetic relation with *M. fragilicauda* found in Laos. However, the analysis of Shimada *et al.* (2009) using *Melanocortin 1 receptor* (*MC1R*) gene showed it has a close genetic relation with *M. terricolor*. In contrast to Prager *et al.* (1998), Tereshima *et al.* (2006) and Suzuki *et al.* (2013) determined a unique group of *M. musculus* found in Nepal as comparing to six different subspecies (*M. m. castaneus*, *M. m. musculus*, *M. m. domesticus*, *M. m. bactrianus*, *M. m. gentilulus* and *M. m. isaticus*) recorded in Eurasia. Despite the *M. booduga* and *M. musculus*, the phylogeny of other *Mus* species found in Nepal has not studied yet. However, several studies on *Mus* phylogeny found in the world using various types of genetic markers (Lundrigan *et al.*, 2002; Suzuki *et al.*, 2004, 2008, 2013, Chevret *et al.*, 2005; Terashima *et al.*, 2006; Rudra *et al.*, 2016; Hamid *et al.*, 2017).

2) Phylogenetic study of the *Rattus* species

The genus *Rattus* is the most specious mammalian taxa comprising about 66 described species (Musser and Carleton, 2005). It is believed to have originated in the mainland of Asia (Watt and Baverstock, 1994; Chaimanee and Jaeger, 2000) but currently worldwide in distribution (Musser and Carleton, 2005). Robins (2007, 2010) studied phylogenetic and identified two major groups of *Rattus* namely Asian group, including the rats from mainland and islands of Southeast Asia and the Australo-Papuan group including the rats from Australia and New Guinea based on the studies on mtDNA. In Asian group, three species of *Rattus* namely *R. rattus*, *R. norvegicus* and *R. exulans* are globally distributed through the transportation by humans (Matisoo-Smith and Robins, 2004) but the rats from Australo-Papuan group are restricted in the Australia

and New Guinea except *R. praetor* (Robins *et al.*, 2014).

In Nepal, five species of *Rattus* namely *R. rattus*, *R. nitidus*, *R. pyctoris*, *R. norvegicus* and *R. sikkimensis* (*R. andamanensis*) have been recorded (Hodgson, 1845; Hinton, 1922; Ellerman, 1961; Mitchel, 1975; Abe, 1982; Baral and Shah, 2008; Jnawali *et al.*, 2011; Thapa, 2014). However, the *R. tanezumi*, have not recorded before this study. In fact, it is a morphologically non-distinguishing species with a sister taxon, *R. rattus* (Aplin *et al.*, 2003a; Musser and Carleton, 2005). These cryptic species can be differentiated using either cytogenetic or molecular technique. In karyotype studies, they can be differentiated based on different numbers (*R. rattus*, 2n=38 and *R. tanezumi*, 2n=42) of chromosomes (Yosida *et al.*, 1974; Baverstock *et al.*, 1983; Chingangbam *et al.*, 2014a). Meanwhile in molecular studies, the differentiation can be carried out by analysis of intraspecific genetic divergence using nucleotide sequences including the mtDNA *CytB* gene sequence (Brown and Simson, 1981; Aplin *et al.*, 2011). Thus, Aplin *et al.* (2003a, 2011) and Yasuda *et al.*, (2014) have described to them under *R. rattus* complex (RrC).

The genus *Rattus* is a relatively least understood taxon, which has complex taxonomy (Aplin *et al.*, 2003a). Several species of *Rattus* are morphologically non-distinguishing so that there is a high level of misidentification. Thus, the molecular technique should be employed for correct identification. However, still, it could not get sufficient attention from geneticists and taxonomists (Yosida, 1980; Aplin *et al.*, 2003a; Musser and Carleton, 2005). Therefore, attention should be focused not only on taxonomy but also on phylogenetic relations within and between the species in order to distinguish species and determine their taxonomic status.

In murids, mtDNA is often used to resolve the phylogenetic relationships (Martin *et al.*, 2000; Pages *et al.*, 2010; Yasuda *et al.*, 2014; Chingangbam *et al.*, 2015). More recently, the *CytB* marker has been employing to establish evolutionary relationship and estimation of tentative divergence time from common ancestors in *Rattus* (Robins *et al.*, 2007; 2008, 2010, 2014; Mostert,

2009; Tollenaere *et al*., 2010; Aplin *et al*., 2011; Page *et al*., 2011). Despite the morphological study of *Rattus* abundant in Nepal, its molecular taxonomy, and evolutionary standpoint poorly understood. Aplin *et al*. (2011) studied the phylogenetic position of *R. rattus* found in Nepal regarding the global population of *R. rattus* and found a distinct lineage (lineage III) of *R. rattus* together with the *R. rattus* population abundant in Pakistan. However, phylogenetic studies have not carried on the other *Rattus* taxa (*R. nitidus*, *R. norvegicus*, *R. pyctoris*, *R. tanezumi*, and *R. andamanensis*) present in Nepal yet.

5. Research purposes

In Nepal, taxonomic and systematics studies of murids have been carrying out since 1832, but several controversies are existed in their identification and systematics. In addition, there was no uniformity on the nomenclature of many species. Thus, this study aimed characterization and comparison of external morphology and molecular technique in identification of different species of murids abundant in Lumbini, Pokhara, and Kathmandu of Nepal. This study also aimed to determine the phylogenetic relationship of *Mus* species and *Rattus* species recorded in the study to understand their evolutionary phenomena.

II. MATERIALS AND METHODS

1. Study area

This study was carried out in three locations Lumbini (83.25°-84.36° E, 27.28°-27.95° N), Pokhara (28.03°-28.30° N 83.50°-84.10° E) and Kathmandu (27.58°-27.80° N 85.20°-85.38° E) of Nepal (Fig. 1). Three districts Kapilbastu, Rupandehi, and Palpa are included in the Lumbini, one district Kaski is included in Pokhara, and two districts Kathmandu and Lalitpur are included in Kathmandu. The study sites in Lumbini, Pokhara, and Kathmandu were ranged between 90-2,000 m ASL, 500-2,300 m ASL and 1,250-2,700 m ASL, respectively. Eighteen species of murids included *R. rattus*, *R. pyctoris*, *R. nitidus*, *M. musculus*, *M. booduga*, *B. bengalensis*, *N. fulvescens*, *A. sylvaticus* are reported in Lumbini, Pokhara and Kathamandu (Baral and Shah, 2008; Jnawali *et al*., 2011).

2. Specimens collection

The live traps, Sherman live traps and traditional mouse catching trap baited with the sausage, peanuts, and cookies were set in the grid of the randomly selected plots. The distance between any two traps was maintained in five meters. All the species were captured using the live traps except *Tatera indica*. The outer opening holes made by the rats were followed through the digging of the field and captured to them nearly two-meter depth inside.

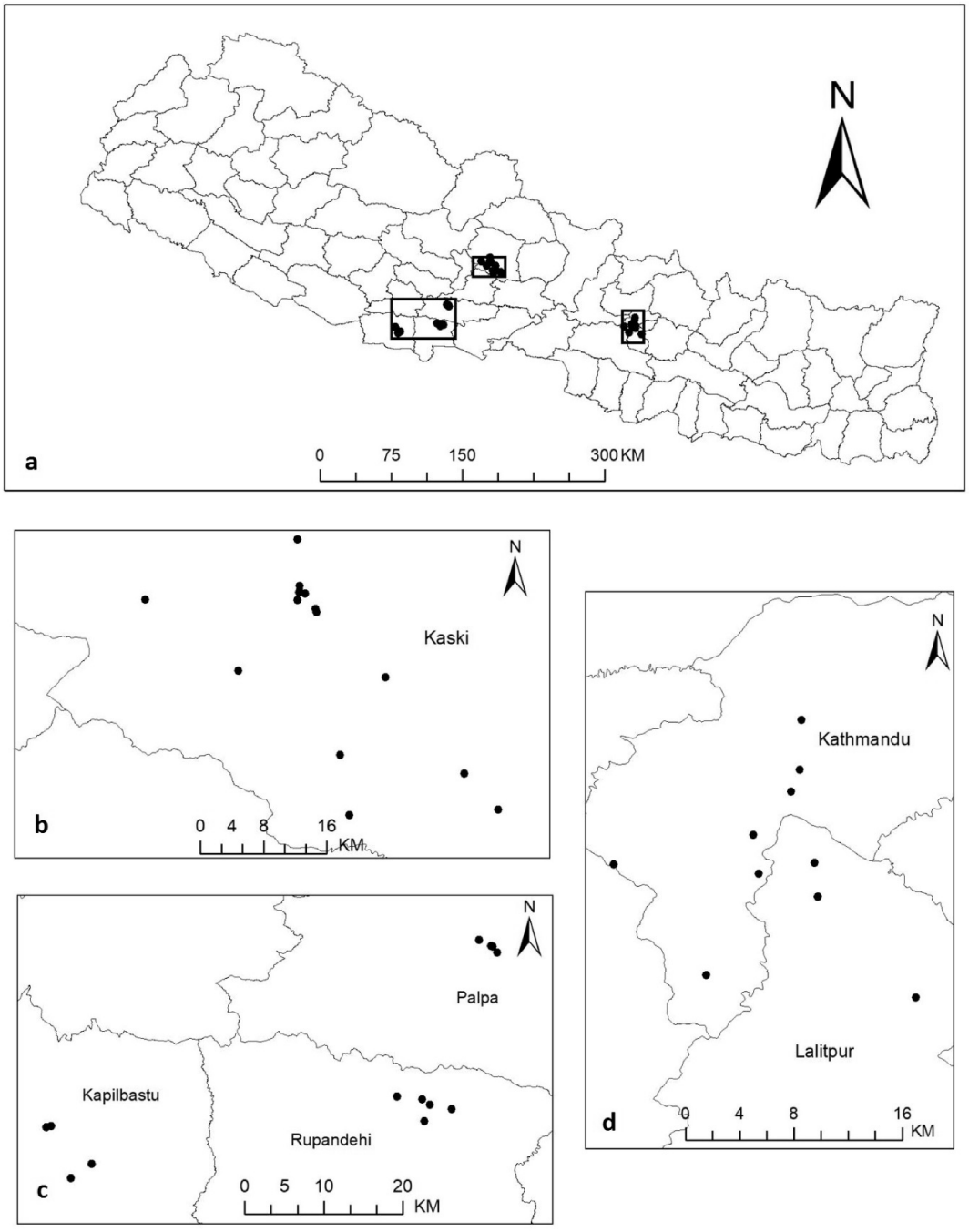


Fig. 1. Map of Nepal and showing the specimen collection locations (a). Dots indicate the collection sites of murids in three locations Pokhara (b), Lumbini (c), and Kathmandu (d). Detail information of collection sites of mice have been included in Table 1.

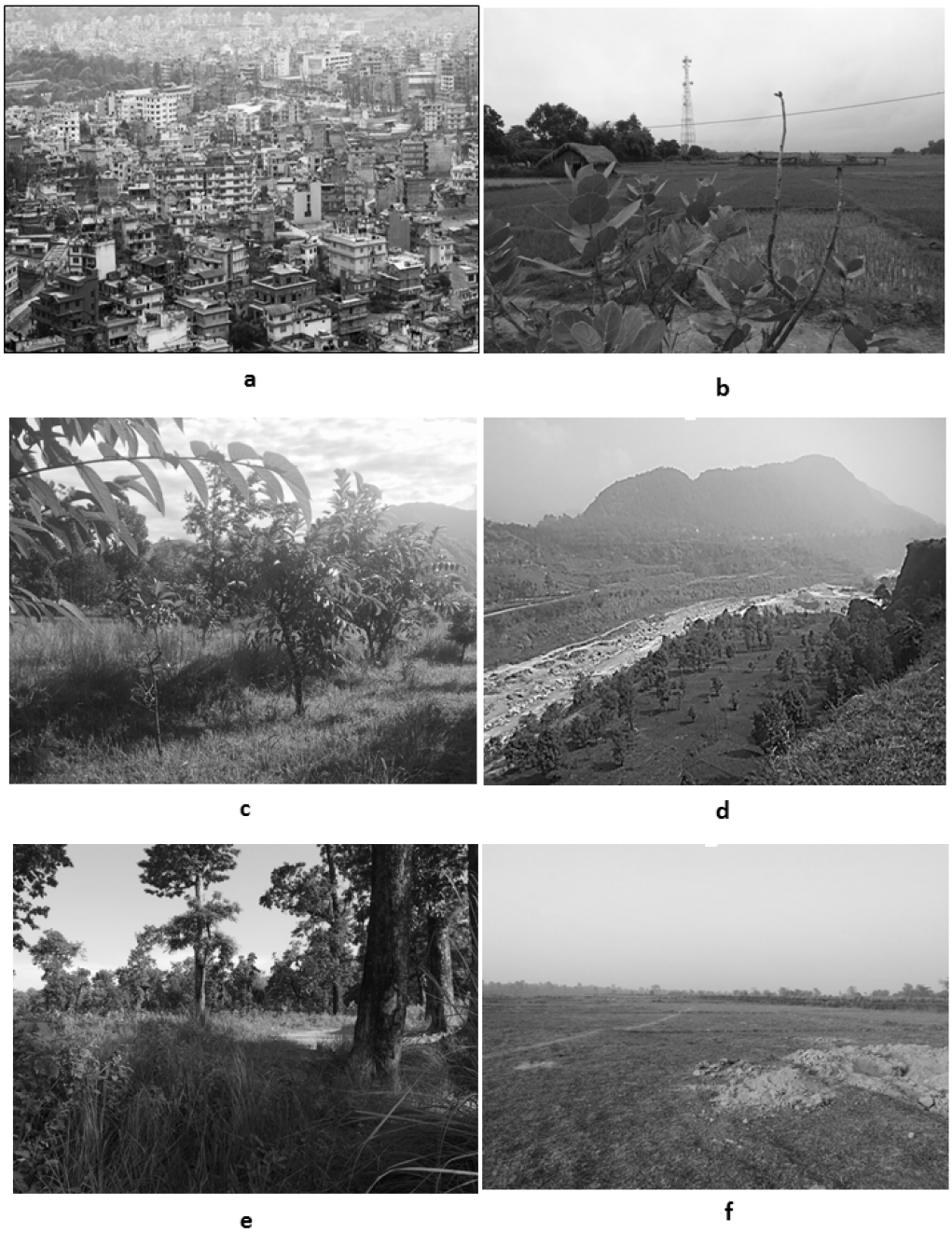


Fig. 2. Representative photos of specimen collection sites in different habitats. Human settlement (a), agriculture land (b), shrubland (c) grassland (d), forest (e), and barren land (f).

Specimen sampling was done in six types of habitats namely, human settlement, agriculture land, grassland, shrubland, forest and shrubland and barren land (Fig. 2). Specimens were collected during the years 2014 to 2016.

3. External morphological measurement and identification

External morphological characters of adult rats and mice included coat color, footpad, tail and ear morphology were examined in the field. Morphological measurement included head-body length (HBL), tail length (TL), hindfoot length (HFL), and ear length (EL) was carried out using a digital caliper (CD-15, Mitutoyo, Japan) to the nearest 0.01 mm (Fig. 3). Similarly, body weight (BW) of each was measured by digital weight machine (MW11300, Cas, Korea). All the collected specimens were identified based on the morphological characteristics and following to the earlier reports (Ellerman, 1961; Agrawal, 2000; Aplin *et al*., 2003a; Baral and Shah, 2008). Tail tip of each mouse and rat was cut off and kept in the sterilized tube for molecular analysis. Some representative specimens (NPL001-NPL040) were preserved in 80% alcohol and remaining others was released in nature.

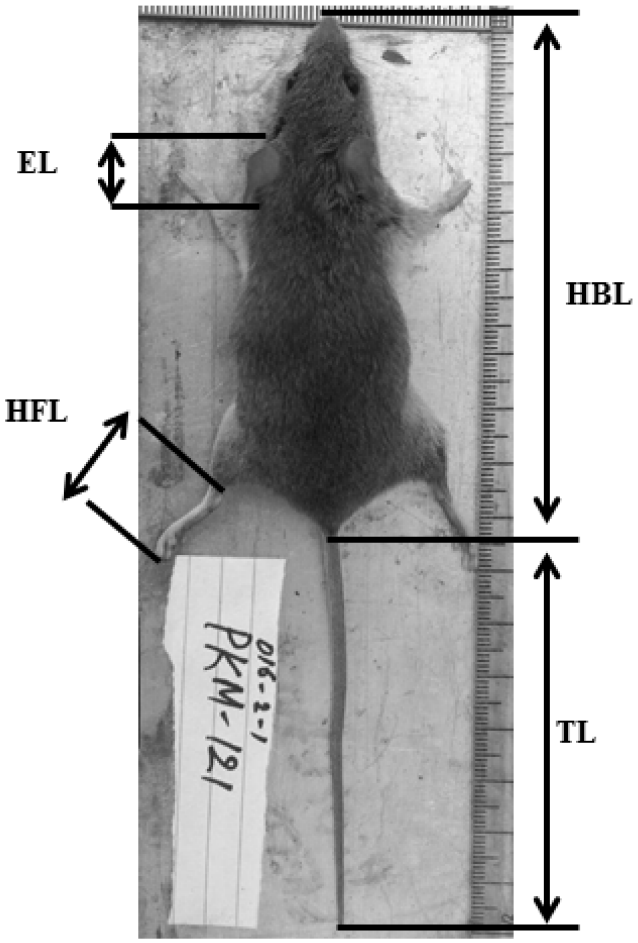


Fig. 3. Measurement of external morphological traits. HBL, head body length, TL, tail length, HFL, hindfoot length, EL, ear length.

4. Statistical analysis

Mean value with the standard deviation (SD) of all species was determined in each external morphological measurement of the adult individuals. The independent sample *t*-test was used to compare the means of external morphological characters between male and female of each species *M. musculu*, *R. rattus*, and *T. indica*. Mean of external morphological characters *R. rattus* and *R. tanezumi* was compared using independent sample *t*-test. Similarly, external morphological characters of *M. musculus* found in Lumbini and Pokhara were also compared through independent sample *t*-test. All the statistical tests were carried out using the IBM SPSS 20.0 (IBM Corp. Armonk, USA).

5. DNA extraction and Polymerase chain reaction (PCR)

Genomic DNA was extracted from the tissue sample of each rat and mouse using Wizard Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer's instructions. The final concentration of total DNA was maintained about 50 ng/μl. MtDNA *CytB* gene was amplified using universal primers L14724 (CGA AGC TTG ATA TGA AAA ACC ATC GTT) and H15915 (AAC TGC AGT CAT CTC CGG TTT ACA AGA C) designed by Irwin *et al.* (1991). Polymerase chain reactions (PCR) were performed in a total volume 20 μl, 10× PCR buffer, 10 mM dNTP, 10 pmol each primer, 2.0 units of *Taq* DNA polymerase (Genet Bio, Daejeon, South Korea), and 1 μl genomic DNA (50 ng/μl) were mixed and reaction mixtures were kept in Master cycler (Eppendorf, Hamburg, Germany). The thermal cycling parameters for the PCR of *CytB* were 95ºC for 2 min and 40 cycles of 95ºC for 30 sec, 48ºC for 1 min and 72ºC for 1 min, followed by final extension of 72ºC for 5 min. PCR products

were separated using 1.5% agarose gel electrophoresis and temporarily stored at 4°C.

6. DNA sequencing and molecular identification

The purified PCR product was directly analyzed by a DNA sequencing ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster, CA). Similarity searches for all DNA sequences were conducted using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) nucleotide database to identify the potential species present, which were then compared morphologically. All *CytB* sequences determined in this study were deposited in the NCBI database.The accession numbers of each deposited sequence of different murids have been tabulated in Table 6.

7. Phylogenetic analysis

Multiple sequence alignments were generated using the mtDNA *CytB* sequences of the murids taxa identified in this study and the reference sequences of murids available in the NCBI database, which were executed by using the CLUSTAL W program (Larkin *et al*., 2007) with the default setting. All the sequences were trimmed and determined the *CytB* haplotypes of each species using DnaSP v5 program (Librado and Rozas, 2009). MEGA 7.0 software (Kumar *et al*., 2016) was used in all phylogenetic analyses. Pairwise genetic distances were calculated between two haplotypes determined in this study and reference sequences taken from NCBI database. Genetic distances were computed between two groups, subgroups, species, and subspecies determined during

molecular identification and phylogenetic studies of the *Mus* species and *Rattus* species. Similarly, mean genetic distances within the genera, groups, and subgroups as well as overall mean distances of each analysis were computed. The evolutionary relationships were inferred using a neighbor-joining (NJ) tree based on *CytB* haplotype sequences. Tentative divergence times for all branching points in the topology of the NJ tree were calculated based on the genetic distance and fossil-based calibration interval of *Mus* and *Rattus* divergence 11-12.3 MYBP (Jacobs and Flynn, 2005). In each analysis, the Tamura-Nei model (Tamura and Nei, 1993) with Gamma distribution was used as the best-fitted nucleotide substitution model, and reliability of nodes was assessed by bootstrap analysis (Felsenstein, 1985) using 1,000 bootstrap replications.

III. Results and Discussion

1. Morphological identification of murids in Nepal

1) Specimen collection and identification

Altogether, 169 individuals of five genera and nine species (*Bandicota bengalensis*, *Mus booduga*, *M. musculus*, *Niviventer fulvescens*, *Rattus nitidus R. pyctoris*, *R. rattus*, *R. tanezumi and Tatera indica*) of murids were collected and identified using both morphological and molecular analysis (Table 1). The specimen collection was highest in Lumbini (49.11%), following to the Pokhara (34.91%), and Kathmandu (15.97%). Six species were collected in the Lumbini, five in Pokhara and five in Kathmandu. The specimen size was highest in the genus *Rattus* (67.45%) following to the *Mus* (18.93%), *Tatera* (10.05%), *Bandicota* (2.95%), and *Niviventer* (1.44%). Similarly, the highest proportion of specimen was collected from the human settlement (65.08%) following to the barren land (10.65%), agriculture land (9.46%), grassland (7.69%), and forest and shrubland (7.1%). The *R. rattus* was found to be occurring in all types of habitats, but *M. musculus* and *B. bengalensis* were found only in and around the human settlement. Easy access to food and space for nesting might be the reason for the habitation of these species in human settlement. Among the collected specimens, 102 were males, and 67 were females (Fig. 4), and 105 individuals were adults, 21 individuals were subadults, and 23 individuals were young.

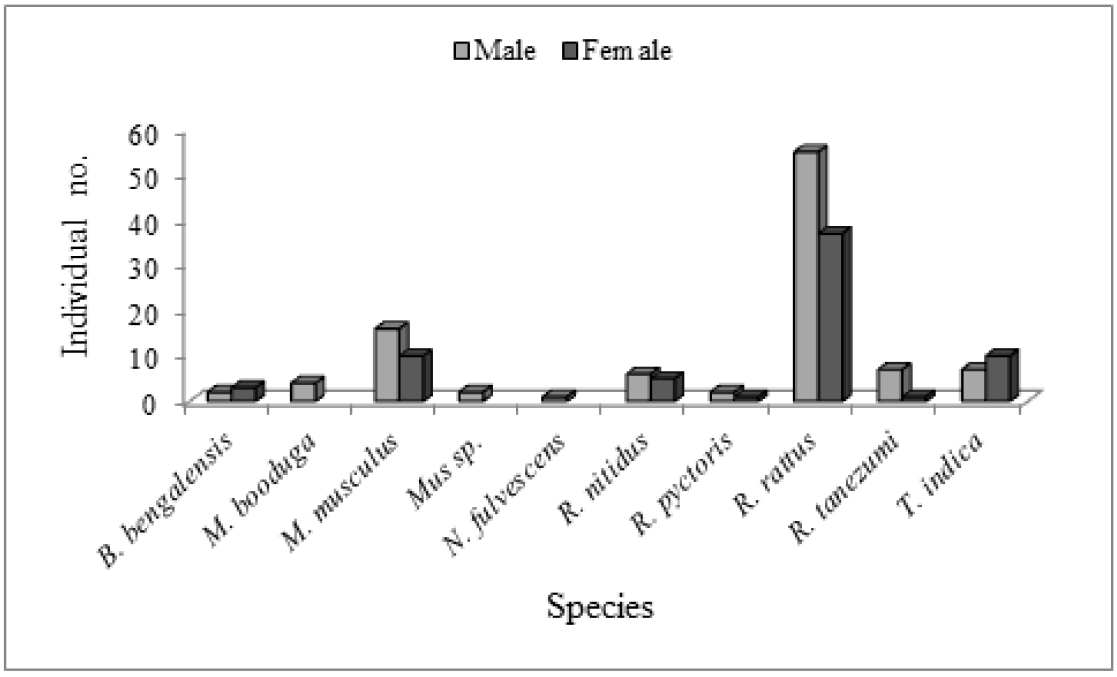


Fig. 4. Sex composition of individuals of different species of murids collected

Table 1. Sampling sites of murids in Nepal.

Location	District	Sampling site	Coordinate	Sample size and species	Habitat
Lumbini	Kapilbastu	Buddhabatiaka	27.65° N, 83.04° E	25 (Mm 17, Rr 6, Rt 2)	Human settlement
		Buddhabatika	27.66° N, 83.04° E	4 (Rr 3, Rt 1)	Forest and shrubland
		Segrahawa	27.60° N, 83.06° E	17 (Ti 17)	Barren land
		Jagadispur	27.61°N, 83.09°E	3 (Rr 3)	Agriculture land
	Rupandehi	Butwal (Badelpokhari)	27.69° N, 83.43° E	3 (Mm 1, Rr 2)	Human settlement
		Devdaha (Charange)	27.67° N, 83.50° E	9 (Mm 1, Rr 4, Rt 4)	Human settlement
		Butwal (Tilottama Campus)	27.66° N, 83.47° E	2 (Bb 1, Rn 1)	Human settlement
		Butwal (Sukhanagar)	27.69° N, 83.46° E	8 (Mm 1, Rr 7)	Human settlement
		Butwal (Ramnagar)	27.68° N, 83.47° E	7 (Rr 7)	Humansettlement
	Palpa	Tansen (Tribhuvan Campus)	27.87° N, 83.53° E	1 (Rr 1)	Barren land
		Tansen (Tundikhel)	27.86° N, 83.54° E	2 (Mm 2)	Human settlement
		Tansen (Buspark)	27.86° N, 83.54°E	1 (Rr 1)	Human settlement
		Bartung	27.85° N, 83.55° E	1 (Rr 1)	Human settlement
Pokhara	Kaski	Hemja (Lampata)	28.28° N, 83.94° E	3 (Mb 1, Rr 2)	Grassland
		Hemja (Babiotar)	28.27° N, 83.95° E	5 (Rr 4, Rn 1)	Agriculture land
		Lamachaur	28.27°N, 83.95° E	1 (Rr 1)	Forest and shrubland
		Nagdanda	28.28° N, 83.85° E	2 (Rr 2)	Agriculture land
		Nirmalpokhari	28.15° N, 83.97° E	2 (Rr 2)	Human settlement

Bb, *B. bengalensis*; Mb, *M. booduga*; Mm, *M. musculus*; Msp, *Mus* sp.; Nf, N. *fulvescens*; Rn, *R. nitidus*; Rp, *R. pyctoris*; Rr, *R. rattus*,; Rt, *R. tanezumi*; Ti, *T. indica*.

Table 1. Continued

Location	District	Sampling site	Coordinate	Sample size and species	Habitat
Pokhara	Kaski	Lekhanath (Budhibazar)	28.18° N, 84.03° E	2 (Rr 2)	Human settlement
		Lekhanath (Talchok)	28.16° N, 84.05° E	2 (Rr 2)	Agriculture land
		Purunchaur (Adhikaritol)	28.29° N, 83.94° E	14 (Rr 10, Mb 1, Mm 2, Rn 1)	Human settlement
		Purunchaur (Takuro)	28.28° N, 83.94° E	4 (Nf 1, Rr 3)	Forest and shrubland
		Purunchaur (Manidada)	28.17° N, 83.56° E	6 (Rr 6)	Human settlement
		Purunchaur (Joginegade)	28.28° N, 83.94° E	5 (Rr 5)	Grassland
		Purunchaur (Chimire)	28.28° N, 83.94° E	7 (Mb 2, Mm 2, Rr 3)	Human settlement
		Ghachok	28.31° N, 83.94° E	1 (Rr 1)	Human settlement
		Pokhara (Bagar)	28.23° N, 83.99° E	1 (Rr 1)	Human settlement
		Raniban, Pokhara	28.19° N, 83.96° E	1 (Rr 1)	Forest and shrubland
		Pame, Thulakhet	28.24° N, 83.90° E	3 (Rr 3)	Human settlement
Kathmandu	Kathmandu	Dakshinkali	27.60° N, 85.26° E	3 (Rr 3)	Human settlement
		Kirtipur (Chobhar)	27.66° N, 85.29° E	4 (Rp 3, Rn 1)	Grassland
		Kirtipur (Tribhuvan University)	27.68° N, 85.28° E	4 (Msp 2, Rr 2)	Agriculture land
		Chandragiri forest	27.66° N, 85.21° E	2 (Rr 1, Rn 1)	Forest and shrubland
		Lainchaur	27.71° N, 85.31° E	4 (Bb 4)	Human settlement
		Manmaiju	27.74° N, 85.31° E	2 (Rr 2)	Human settlement
		Indrachok	27.70° N, 85.30° E	1 (Rr 1)	Human settlement
	Lalitpur	Godawari park	27.59° N, 85.37° E	1 (Rn 1)	Forest and shrubland
		Khumaltar	27.64° N, 85.32° E	3 (Rn 3)	Human settlement
		Lagankhel	27.66° N, 85.32° E	3 (Rn 3)	Human settlement

Five species (*B. bengalensis*, *M. booduga*, *N. fulvescens*, *R. pyctoris*, and *T. indica*) were identified by morphological analysis and four taxa (*M. musculus*, *R. nitidus*, *R. rattus*, and *R. tanezumi*) were identified through molecular analysis. One taxon from the genus *Mus* could not identify at species level through the molecular analysis and named as *Mus* sp. Details of molecular analysis have been provided in next sections molecular identification and phylogenetic analysis. The *Mus* and *Rattus* genera have high species diversity, a high degree of similarity between the intrageneric species and almost exist in similar habitat so could not distinguish easily using only morphological analysis (Aplin *et al*., 2003a; Musser and Carleton, 2005).

2) Morphological characterization and comparison

Morphological characterization was carried out in all murids identified through morphological analysis and molecular analysis. It was based on the examination of external morphology like coat and tail color, body shape and size, and measurement of external body parts. The morphometric data of each taxon are tabulated in Table 2.

(1) *Bandicota bengalensis*

The *B. benagalensis* was the single species collected from the genus *Bandicota*. Its coat color was dark brown with uniformly distributed black spine guard hairs on the back and gray and yellowish gray hairs on the belly. Hair size was shorter on the belly compare to back. Hairs on the both limbs were black. The photos of lateral and ventral views of *B. bengalensis* have been provided in Fig. 5. The tail was naked and uniformly black on the both surfaces. The ear was slightly pinkish, short and thinly haired and eight pairs of mammae on the female. The mean body weight was 146.81±9.39 g, and HBL ranged 170–181 mm, which was 50 mm longer than TL (Table 2).

Coat color and other morphological characteristics are similar to the previous studies carried out in Nepal and India (Ellerman, 1961; Agrawal, 2000; Aplin *et al*., 2003a; Baral and Shah, 2008). The short tail is the characteristics feature of the *B. bengalensis*, usually, have three-quarters (~80%) of the head body (Ellerman, 1961; Agrawal, 2000). Out of the three *Bandicota* species recorded in Nepal, it is the smallest rat, which can distinguish easily due to its body size dimension (Aplin *et al*., 2003a). Numerous mamame (12-19), short tail and dull under parts may create confusion in morphological identification with *R. norvegicus* however; it is entirely distinct from any *Rattus* (Ellerman, 1961).

(2) *Mus booduga*

The *M. booduga* was small field mouse having coat color grayish brown to light gray on the back and whitish or white on the belly with some patches of brown or gray hairs. Both limbs were white, and the tail was naked and bicolored (dark above and pale below). The photos of dorsal and ventral views of *M. booduga* have been provided in Fig. 5. The eyes were large, ears were rounded, and the muzzle was pointed compares to other *Mus* species. Its mean body weight was 11.27±3.81 g and HBL was ranged 82.03-85.6 mm, which was 2-6 mm longer than TL (Table 2).

Newton *et al*. (1990) reported TL was longer than HBL in *M. booduga*, but this study found all the individuals had shorter TL than HBL as like to Chesmore (1970), Marshal (1977), Agrawal *et al*. (2000), Aplin *et al*. (2003a), and Baral and Shah (2008).

(3) *Mus musculus*

The *M. musculus* have been collected from two locations (Lumbini and Pokhara) have different coat color. The mice collected in Lumbini were varied from brown-gray to brown-black on the back and brownish-yellow or tawny on the belly. However, in Pokhara they were light brown with an intermix of black hairs on the back and uniformly light-gray hairs on the belly.

Table 2. Measurement and comparison of external morphology of murids

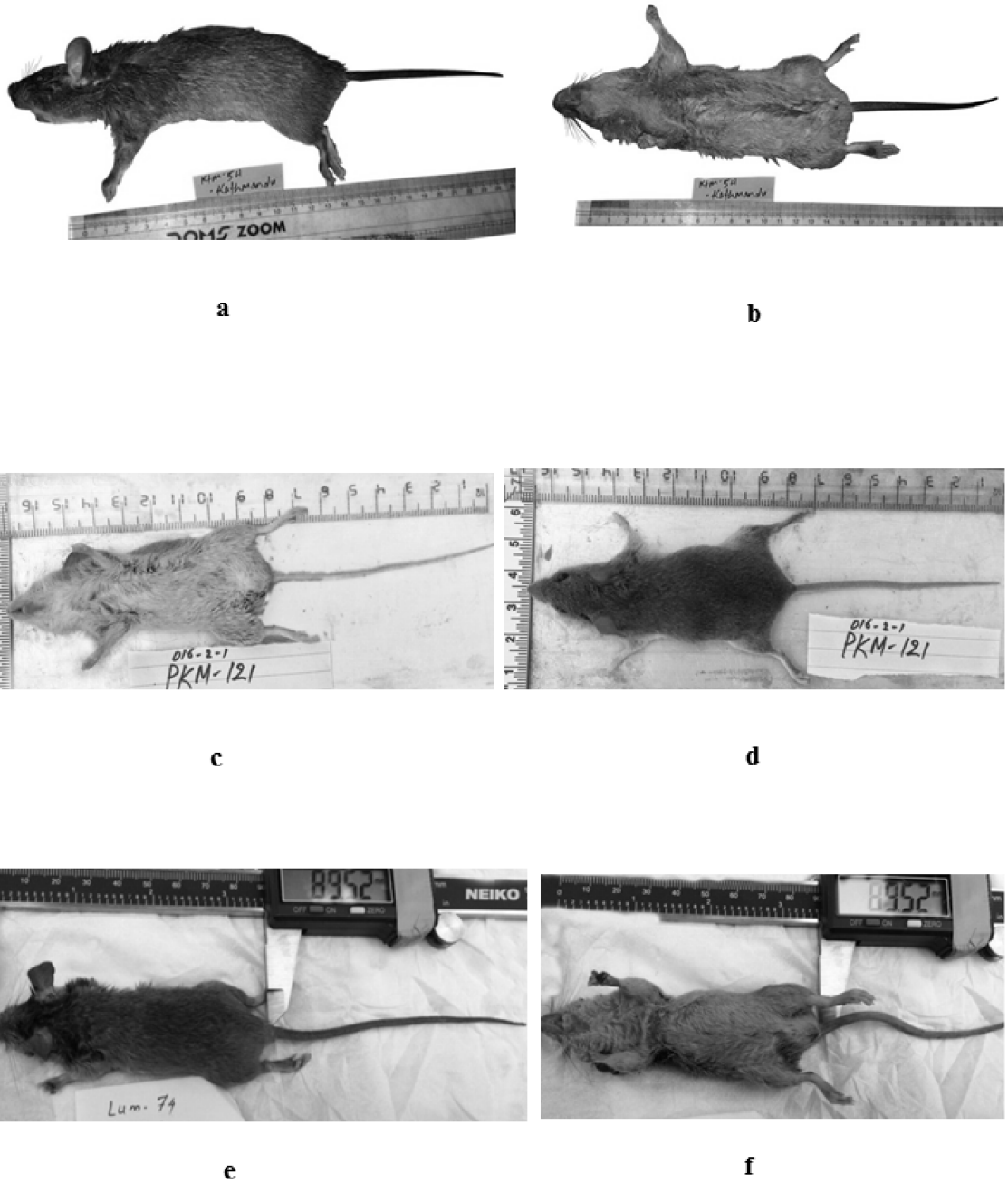
Species name	Category	Character[1] (Mean±SD)					Reference
		BW (g)	HBL (mm)	TL (mm)	HFL (mm)	EL (mm)	
B. bengalensis (n=5)	Mean±SD	146.81±9.39	175.6±4.82	125.20±7.25	31.64±2.24	19.92±1.51	This study
	Range	131.19-156.4	170-181	115-131	27.97-33.96	17.64-21.38	
		-	170-210	155-157	35-37	22-24	Ellerman (1961)
		to 310	75-254	44-177	19-41	-	Aplin *et al.* (2003a)
M. booduga (n=4)	Mean±SD	11.27±3.81	80.24±7.05	79.87±2.21	17.07±0.47	13.26±0.82	This study
	Range	8.4-16.9	82.03-85.6	77.94-83	16.52-17.66	12.17-13.39	
		-	52-85	51-72	13-16	-	Agrawal (2000)
	Mean±SD	7.9±2.63	58.2±11.47	62.2±4.79	14±1.08	11.6±1.11	Newton *et al.* (1990)
		to 14	to 80	to 70	to 17	to 12.5	Aplin *et al.* (2003a)
M. musculus (n=23)	Mean±SD	13.82±4.21	75.08±9.31	84.45±10.41	16.64±1.64	12.39±1.43	This study
	Range	8.2-22.4	61-86	75-110	14.9-23.29	10.34-13.97	
		-	60-87	75	18-19	-	Ellerman (1961)
		to 26	26-95	45-117	13-20	-	Aplin *et al.* (2003a)
		-	52-100	60-90	-	-	Baral and Shah (2008)
Mus sp. (n =2)	Mean±SD	9.90±2.40	70.7±4.94	62.5±8.34	16.13±0.84	13.42±0.50	This study
	Range	8.2-11.6	67.2-74.2	56.6-68.4	15.53-16.73	13.06-13.78	
N. fulvescens (n=1)		46.4	115	170	26.5	19.38	This study
	Range	-	114-135	178-212	27-30	20-22	Ellerman (1961)
		60-76	129-158	167-210	27-32.5	19.5-23	Abe (1971)

[1], Abbreviations of each character were given in the Materials and Methods.

Table 2. Continued

Species name	Category	Character[1] (Mean±SD)					Reference
		BW (g)	HBL (mm)	TL (mm)	HFL (mm)	EL (mm)	
R. pyctoris (n=3)	Mean±SD	111.79±29.16	173±11.78	183.00±12.12	30.51±0.66	23.45±0.58	This study
	Range	92-145.29	163-186	170-194	29.96-31.25	23.04-24.12	
	Range	-	149-170	184-225	33-36	25-27	Ellerman (1961)
		100-200	168-215	167-213	31-38	19-25	Aplin *et al.* (2003a)
R. rattus (n=47)	Mean±SD	105.63±28.99	164.56±15.92	186.31±18.34	30.92±2.72	22.28±1.91	This study
	Range	70.3-167.5	115-190	120-212	25.35-34.4	20.69-26.41	
		-	140-203	178-233	31-35	22-25	Ellerman (1961)
		-	151-176	172-210	29-32.5	21.5-24.5	Pages *et al.* (2011)
R. tanezumi (n=5)	Mean±SD	89.50±18.77	155.00±10.00	194.00±14.50	31.24±1.34	21.59±1.07	This study
	Range	70.3-110.9	145-165	174-210	29.05-32.6	20.32-22.24	
	Mean±SD	118.00±33.71	166.55±14.42	182.75±13.54	33.30±0.60	13.86±1.08	Kim *et al.* (2013)
	Range	80-200	111-182	145-185	31-34	19-24	Chingangbam *et al.* (2014b)
R. nitidus (n=4)	Mean±SD	116.55±31.63	169.50±8.54	167.00±11.74	32.85±2.30	23.56±1.75	This study
	Range	90.1-162.4	160-180	150-177	30.17-35.7	21.32-25.41	
	Range	-	167±12	177±17	35±2	22±1.2	Agrawal (2000)
T. indica (n=12)	Mean±SD	127.36±24.31	165.58±13.18	187.58±18	38.21±0.88	23.70±2.64	This study
	Range	83.3-181.4	147-190	175-210	37.03-39.73	20.63-28.83	
		-	130-195	150-226	31-42	-	Agrawal (2000)
		-	143-188	205	-	-	Baral and Shah (2008)

[1], Abbreviations of each character were given in the Materials and Methods.



a b c d e f

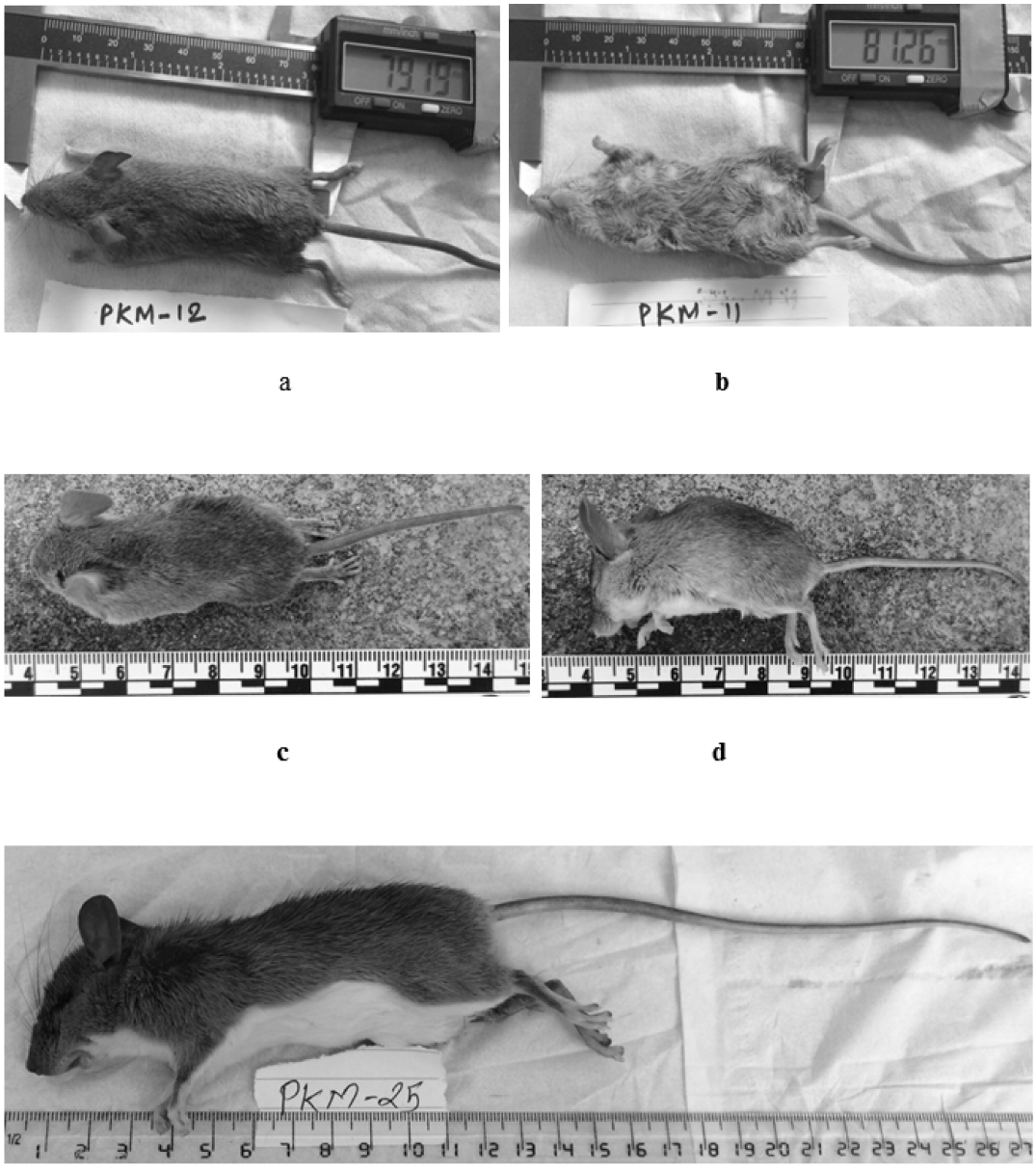
Fig. 5. Photos of *B. bengalensis* (a, lateral view, b, ventral view), *M. booduga* (c, ventral view, d, dorsal view), and *M. musculus* collected in Lumbini (e, dorsal view, f, ventral view).

All the mice have white fingers and white toes, but the tail was unicolor (Lumbini) and bicolor (Pokhara). The nose was blunt, ears were brown or similar to body color, and mammae on females were five pairs. The photos of dorsal and ventral views of *M. musculus* collected in Lumbini and Pokhara have been shown in Fig. 5 and Fig. 6, respectively. The mean body weight was 13.82±4.21 g, and HBL ranged 61–86 mm, which were 5–15 mm shorter than TL (Table 2).

Similar morphometric variations were found in the earlier studies (Ellerman, 1961; Marshal, 1977; Aplin *et al*., 2003a; Baral and Shah, 2008; Menon, 2014). The morphometric comparisons between the mice collected in Lumbini and Pokhara (Table 3) as well as two different sexes (Table 4) revealed there was no significant difference between collection sites and have no sexual dimorphism (Student *t*-test, n=23, df=21, $p>0.05$). These results indicate that body size of the *M. musculus* has no sharp variation with sex and geographical location. However, it is a polytypic species (Musser and Carleton, 2005) having a wide variation in coat color among different subspecies. Earlier studies carried in Nepal have been reported different coat colors in different subspecies of *M. musculus* namely, *M. m. castaneus*, *M. m. dubius*, *M. m. homourus*, *M. m. urbanus* (Hodgson, 1845; Mitchell, 1975; Marshall, 1977).

(4) *Mus* sp.

The *Mus* sp. has the light brown with intermixes of black and white hairs present on the back, but the belly was total, white. Both limbs were white, and the tail was bicolored. The photos of dorsal and lateral views of *Mus* sp. have been provided in Fig. 6. The mean body weight was 9.90±2.40 g and of HBL ranged 61–86 mm, which were 6–10 mm longer than TL (Table 2). The sample size was not sufficient to compare morphology, but this study found shortest tail and largest ear in *Mus* sp. comparing to other *Mus* species recorded in Nepal.



a b

c d

e

Fig. 6. Photos of *M. musculus* collected in Pokhara (a, dorsal view, b, ventral view), *Mus* sp. (c, dorsal view, d, lateral view), and *N. fulvescens* (e, lateral view).

Table 3. Comparison of morphological traits of *M. musculus* found in Pokhara and Lumbini

Character[1]	Mean±SD		*p*-value	Significance[2]
	Pokhara (N=4)	Lumbini (N=19)		
BW (g)	13.10±5.89	13.97±3.97	0.715	NS
HBL (mm)	79.10±2.77	74.24±10.01	0.354	NS
TL (mm)	83.01±3.87	84.75±11.37	0.769	NS
HFL (mm)	16.15±1.21	16.75±1.73	0.518	NS
FFL (mm)	18.32±0.74	19.20±2.25	0.453	NS
EL (mm)	12.13±0.72	12.44±1.55	0.701	NS

[1], abbreviations of each character were given in the materials and methods section.

[2], NS indicates not significant at p=0.05 level.

The external morphological characters determined in this taxon do not match with the previously recorded specimens of Nepal. It could be new species. Therefore, further specimen collection and morphological analysis including cranial analysis are required to confirm its taxonomy.

(5) *Niviventer fulescens*

The *N. fulvescens* synonymized, as *R. fluvescens* or *M. fulvescens* was the single species collected from the genus *Niviventer*, having chestnut-brown and black spine guard hairs uniformly distributed on the back and pure white hairs on the belly. Both limbs were brown black, but toes and fingers were white.

Agrawal (2000) has reported its spiny hairs in summer and smooth hairs in winter, but this study found spiny hairs in winter season too. The tail was found naked and bicolored, dark above and yellowish white below. According to Agrawal (2000), both unicolored and bicolored tail present in *N. fulvescens*. The photos of the lateral view of *N. fulvescens* has been provided in Fig. 6. Abe (1971) have collected *N. fulvescens* from the Northern part of Pokhara and found bright reddish brown rats at high altitude (-2,000 m), indicated that slightly color variation occurred in the same species present in different locations and habitat. The tail was extremely longer (170 mm) than the head body (115 mm). Usually, TL exceeds 140% of the head body (Ellerman, 1961; Agrawal, 2000), which may indicate its arboreal habit.

A single individual was captured during this study so the data could not represent well to the morphometric characters of the species. However, the morphology of *N. fulvescens* was similar to the earlier studies in Nepal (Ellerman, 1961; Abe, 1971, 1982; Newton *et al.*, 1990; Barala and Shah, 2008) and India (Agrawal, 2000). Tail morphology and body color are the key distinguishing features of the *N. fulvescens* among the congeneric speceis found in Nepal.

Table 4. Comparison of external morphology between male and female of murids

Species name	Characters[1]	Male			Female			p-value	Significance[2]
		N	Range	Mean±SD	N	Range	Mean±SD		
M. musculus	BW (g)	13	9.6-22.4	12.71±4.66	10	8.2-19.5	15.26±3.21	0.155	NS
	HBL (mm)	13	61-78.81	71.95±10.67	10	69-86	79.16±5.24	0.064	NS
	TL (mm)	13	75-110	84.41±12.45	10	82.27-97	84.49±7.61	0.985	NS
	HFL (mm)	13	15.53-17.26	17.01±1.97	10	14.9-17.13	16.16±0.99	0.228	NS
	EL (mm)	13	10.34-16.15	12.47±1.65	10	11.36-15	12.28±1.15	0.755	NS
R. rattus	BW (g)	26	70.3-167.5	98.52±28.32	21	77.6-177.1	114.44±27.99	0.061	NS
	HBL (mm)	26	135-190	160.71±15.98	21	135-190	169.33±14.86	0.064	NS
	TL (mm)	26	151-210	181.69±18.13	21	160-212	192.04±17.32	0.053	NS
	HFL (mm)	26	25.82-34.4	31.22±2.47	21	23.52-32.59	30.54±3.02	0.398	NS
	EL (mm)	26	20.02-26.41	22.40±1.46	21	20.07-25.6	22.13±2.38	0.634	NS
T. indica	BW (g)	6	130-181.4	141.78±19.72	6	90.8-129.1	112.95±20.32	0.032	*
	HBL (mm)	6	165-190	176.16±9.49	6	147-160	155±4.85	0.001	*
	TL (mm)	6	190-207	202.83±7.08	6	160-185	172.33±10.23	1.31×10^{-4}	*
	HFL (mm)	6	37.52-39.73	38.84±0.77	6	37.03-38.2	37.59±0.43	0.006	*
	EL (mm)	6	21.24-28.83	24.28±2.52	6	21.6-28.69	23.11±2.86	0.469	NS

[1], abbreviations of each character were given in the Materials and Methods section.

[2], NS indicates not significant at p=0.05 level, *, Significant difference in sexual dimorphism at p=0.05 level.

(6) *Rattus nitidus*

The coat color of the *R. nitidus* was dark to light brown on the back with a distinct dark patch on the lower back extended up to tail. Similarly, silvery white to dull gray hairs uniformly present on the belly. The tail was weakly bicolored (dark above and paler below), the ear was large and lightly haired, and both limbs were pure white. The photos of dorsal and ventral views of *R. nitidus* have been provided in Fig. 7. The mean BW was 116.55±31.63 g, and HBL ranged 160–180 mm (Table 2). The TL was 93.75–98.33% of the HB. Based on the fur color and relative length of tail comparing to HBL it was classified into two subspecies in earlier studies.

Hinton (1919) differentiated *R. n. obsoletus* from other subspecies *R. n. nitidus* based on the fur color at the undersurface of the body, at which grey with rusty tinge in *R. n. obsoletus* and silvery in *R. n. nitidus*. Similary, Ellerman (1961) reported two subspecies could distinguish by tail length, which is shorter in *obsoletus* (99% of HB) and longer in *nitidus* (107% of HB). Agrawal (2000) was not totally agreed with Hinton (1919) and Ellerman (1961) because he found TL in *R. n. nitidus* 87–131% of the head body and 80–107% in *R. n. obsoletus* and concluded both subspecies were the synonym and overlapping their characteristics. The ranges of morphometric values determined in this study were similar to Agrawal (2000). Ellerman (1961) mentioned the type localities for *R. n. nitidus* is Nepal and *R. n. obsoletus* is west Myanmar. In contrast to Ellerman (1961), this study determined shorter tail length than the head body in all individuals. Thus, the further study required to distinguish it in subspecies level.

(7) *Rattus pyctoris*

The *R. pyctoris* synonymized as *M. rattoid*, *R. rattoid*, *R. turkestanicus* have soft and gray hairs with an intermix of black hairs on the back but whitish gray hairs on the belly. The tail was soft, smooth, and strongly bicolored (dark above and silvery white below).

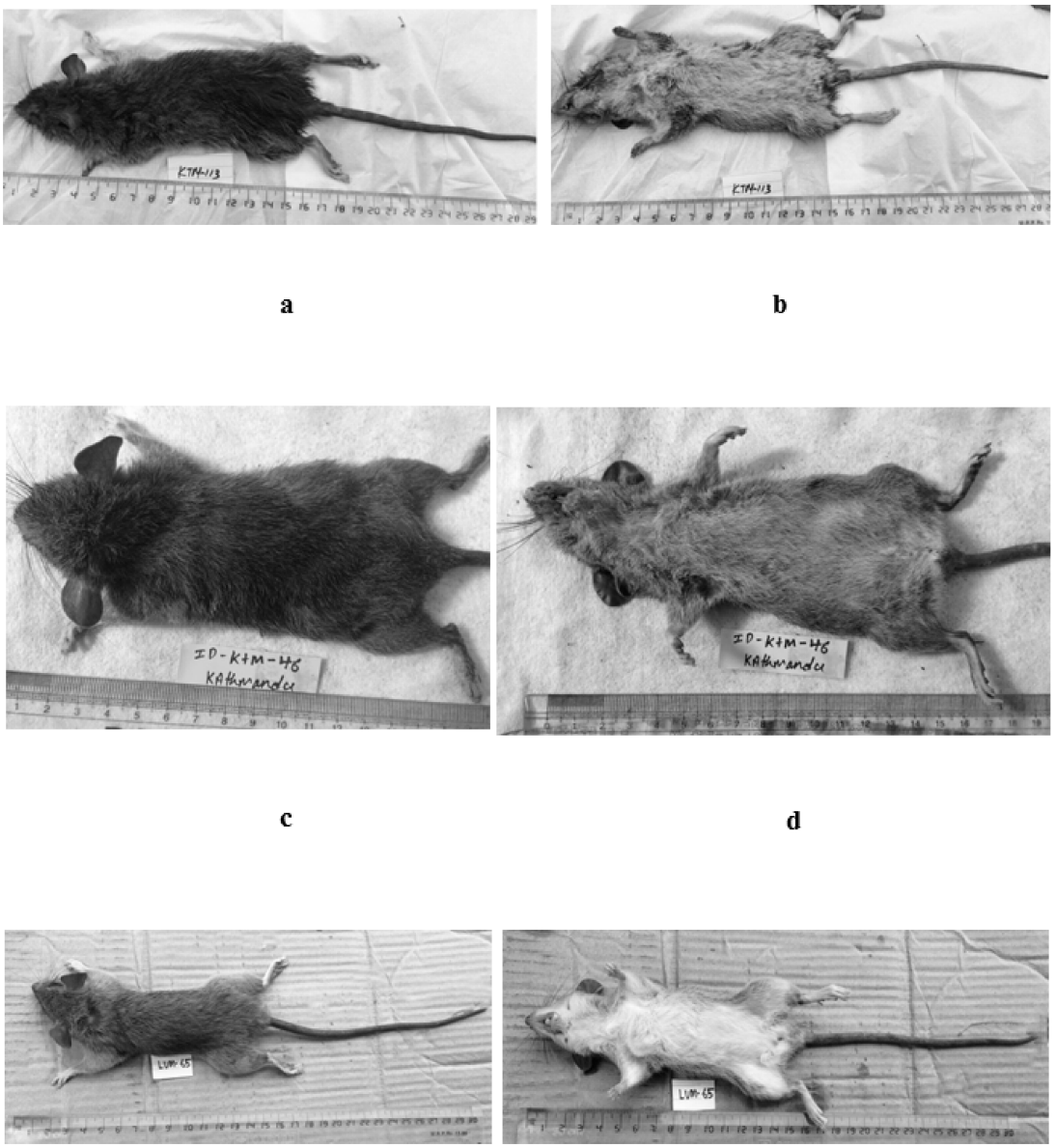


Fig. 7. Photos of *R. nitidus* (a, dorsal view, b, ventral view), *R. pyctoris* (c, dorsal view, d, ventral view), and *R. rattus* (e, dorsal view, f, ventral view).

The photos of dorsal and ventral views of *R. pyctoris* have been provided in Fig. 7. The mean BW was 111.79±29.16 g, and HBL ranged 163-186 mm, which was 10-15 mm shorter than TL (Table 2). Toes and fingers were white, and snout was short and broad. Mammae on the female were six pairs but Ellerman (1961) have found five pairs of mammae in India. Ellerman (1961) and Abe (1972) reported similar coat color and external morphology of *R. pyctoris* from Kathmandu and northern part of Pokhara valley, respectively. However, Hodgson (1845) described to this species, as *M. rattoid* but he did not mention its tail color. Morphometric comparison between male and female revealed that males (HBL, 186 mm) were relatively bigger in size than female (HBL, 170 mm). Although sample size was not sufficient for the comparison of morphometric data with earlier studies, however, the range of morphometric variation was similar to the previous studies conducted in Nepal (Hodgson, 1845; Hinton, 1922; Ellerman, 1961; Abe, 1971, 1982; Baral and Shah, 2008), India (Ellerman, 1961; Agrawal, 2000) and Turkestan (Aplin *et al.*, 2003a). Tail morphology is the main distinguishing feature of *R. pyctoris* among the genus *Rattus*.

(8) *Rattus rattus* and *Rattus tanezumi*

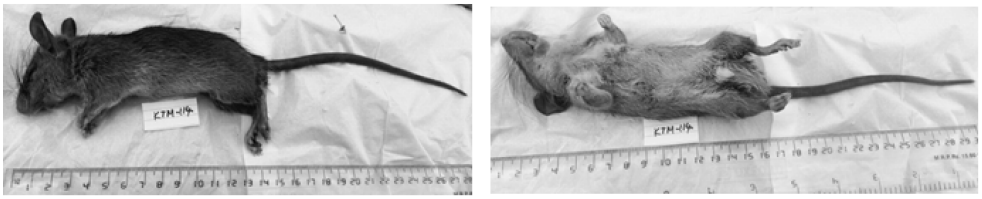
The *R. rattus* and *R. tanezumi* could not distinguish through the gross morphological analysis. These two species have not consistently discernible coat color difference within and between the species as like to Mostert (2009). Both species have spiny, brownish, grayish to reddish hairs with flat black spine, projecting on the back. Similarly, belly was varied from uniform grayish, brownish to whitish with or without chest patches.

The color variation was noticed within the same collection sites of the Lumbini, Pokhara, and Kathmandu. In both species, ears were thinly haired, the tail was elongated, scaly, dark, and longer than head-body, limbs were black or gray on the upper surfaces but white on the fingers and toes, and 5-6 pairs of mammae on the female. The photos of dorsal and ventral views

of *R. rattus* collected in Lumbini and Kathmandu have been provided in Fig. 7. Similarly, the lateral and ventral views of *R. tanezumi* have been shown in Fig. 8.

Morphometric measurement and comparison are the key points for distinguishing many species of *Rattus* (Aplin *et al.*, 2003a). In adult individuals, average TL was longer in *R. tanezumi* (194.00±14.50 mm) than that of *R. rattus* (186.31±18.34 mm), but the average value of HBL and BW was lower in *R. tanezumi* than those of *R. rattus* (Table 2). However, statistically, there was no significant difference between their morphological characters (Student *t*-test, n=52, *df*=50, $p>0.05$) (Table 5). Due to the morphologically non-distinguishable characters, both species have been regarded as the part of *R. rattus* species complex (Aplin *et al.*, 2003a; Musser and Carleton, 2005; Robins *et al.*, 2007; Aplin *et al.*, 2011; Chingangbam *et al.*, 2015). In *R. rattus*, females were found relatively bigger dimension than male (Table 4) but statistically, there was no significant sexual dimorphism (Student *t*-test, n=47, *df*=45, $p>0.05$). In *R. tanezumi*, the sample size was low so did not compare the morphometric values between male and female. The range of morphometric variation was wide in *R. rattus* (Table 2) but almost similar with earlier studies carried out in Nepal (Hinton, 1922; Ellerman, 1961; Abe, 1971; Baral and Shah, 2008), India (Ellerman, 1961; Aplin *et al.*, 2003a; Pages *et al.*, 2011), and Bangladesh (Aplin *et al.*, 2003a).

However, the *R. tanezumi* has not been recorded in Nepal before this study. Therefore, its morphometric comparison carried out with the earlier studies in India (Chingangbam, 2014b) and South Korea (Kim *et al.*, 2013), which showed similar morphometric values except for TL. TL was relatively longer in the *R. tanezumi* found in Nepal. These comparisons indicate that body size of both species has largely varied.



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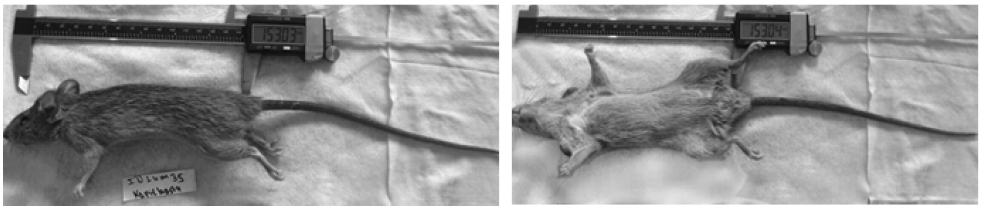

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Fig. 8. Photos of *R. rattus* (a, lateral view, b, ventral view), *R. tanezumi* (c, lateral view, d, ventral view), and *T. indica* (e, dorsal view, f, ventral view).

Table 5. Comparison of morphological characters between *R. tanezumi* and *R. rattus*

Character[1]	Mean±SD		*p*-value	Significance[2]
	R. tanezumi (n=5)	*R. rattus* (n=47)		
BW (g)	89.50±18.77	105.63±28.99	0.231	NS[b]
HBL (mm)	155.00±10.00	164.56±15.92	0.197	NS
TL (mm)	194.00±14.50	186.31±18.34	0.37	NS
HFL (mm)	31.24±1.34	30.92±2.72	0.799	NS
EL (mm)	21.59±1.07	22.28±1.91	0.434	NS

[1], abbreviations of each character were given in the materials and methods section.

[2], NS indicates not significant at p=0.05 level.

The broad range of size variation is considered to be occurred by the environment gradients such as habitats and geography, and biological factors such as age and sex, (Faleh *et al.*, 2012; Pergams *et al.*, 2015). In addition, several morphological variations including body color in *R. rattus*, it has been classified into many subspecies such as *R. r. arboreus*, *R. r. rufescens*, *R. r. brunneaus*, *R. r. brunneusculus*, *R. r. gangutrainaus* and *R. r. khumbuensis* (Hodgson, 1845; Hinton, 1922; Hinton and Fry, 1923; Biswas and Khajuria, 1955). Musser and Carleton (2005) synonymize to *R. r. brunneus and R. r. brunneusculus* as *R. tanezumi* but Hinton and Fry (1923) described to them as the subspecies of *R. rattus*. Moreover, Ellerman (1961) and Abe (1982) have followed to the Hinton and Fry (1923) for the taxonomy of *Rattus* species. The recent cytogenetic study also suggested that *R. r. brunneusculus* (*M. brunneusculus*) is different species than *R. tanezumi* due to having different karyotypes (Chingangbam, 2014a,b). Thus, morphological analysis is not sufficient for describing the correct taxonomy of *R. rattus* and *R. tanezumi*.

(9) *Tatera indica*

The *T. indica* was the single species collected from the genus *Tatera*, have rusty brown and black on the back and white belly. The tail was long, soft-hairy, distinctly bicolored, and dark blackish brown with grayish sides as well as prominent black tuft on the tip. The photos of dorsal and ventral surfaces of *T. indica* have been provided in Fig. 8.

Ears were long and naked, moderate, rounded, thinly clad. Eyes were large and hind feet were long (38.21±0.88 mm), well developed, and white. Mammae on females were four pairs. Agrawal (2000) and Menon (2014) have been described similar color pattern and morphology of *T. indica* in Indian continent. The mean body weight was 127.36±24.31 g, and HBL ranged 147-190 mm, which were 10-35 mm lower than TL (Table 2). Normally, its TL exceeds 100-140% of HB (Ellerman, 1961; Agrawal, 2000). The

morphometric comparison revealed that male was the significantly bigger dimension (Table 4) than female (Student *t*-test, n=12, *df*=10, $p<0.05$). The ranges of morphometric variation were similar to the earlier reports on *T. indica* in Nepal and India (Agrawal, 2000; Baral and Shah, 2008; Menon, 2014).

As like to other mammalian species characterization and comparison of morphological characters such as coat color, external body parts including ears, tail, limbs, digits, and fingers, and measurement of body dimension are the usually applied diagnostic features of murids (Agrawal *et al.*, 2000; Aplin *et al.*, 2003a). Coat color is the phenotypic characteristics of mammals used in the thermoregulation, communication, and camouflage adaptation to prevent from the predator (Rios and Alvarez-Castaneda, 2012). On taxonomic and systematic studies of mammals, coat color has been considering as a distinguishing feature for species identification because mammalian hairs show considerable intra and interspecific variation (Caro, 2005, 2009). However, it can vary with genetic factors (Kambe *et al.*, 2012), biological (age and sex) factors (Rios and Alvarez-Castaneda, 2012), and environmental (season and habitat) factors (Rios and Alvarez-Castaneda, 2012). Some advanced devices such as spectroradiometers can quantify little variation of integument color in many species (Sandoval Salinas, 2017) for their identification, but it may not be accessed easily everywhere. Thus, examination of coat color is a basic phenomenon but it could not be sufficient for species identification. It could be applicable to the taxa, which have distinct coat color, few species number, and have no phenotypic plasticity.

Based on the morphological characteristics some species were found to be distinguished easily with other closely related species. The *N. fulvescens* can distinguish with *N. niviventer* and *N. eha* by distinct coat color (chestnut-brown back and pure white belly) and extremely long tail, >140% of HB (Ellerman, 1961; Agrawal, 2000; Baral and Shah, 2008). The *B.*

bengalensis can distinguish with *B. indica* and *B. maxima* by body weight and body dimension (Baral and Shah, 2008; Jnawali *et al*., 2011). *M. booduga* can distinguish with *M. musculus* and other species *Mus* by its tail color and tail length (Ellerman, 1961). *R. pyctoris* can distinguish with other species of *Rattus* by tail color and tail surface (Ellerman, 1961; Baral and Shah, 2008; Jnawali *et al*., 2011). *T. indica* can distinguish from other species by its long and naked ears and naked soles (Jnawali *et al*., 2011).

The high degrees of similarities between the morphological data generated in this study and earlier report revealed that all the identification might be correct. Altogether, ten murids including five genera and nine species were identified and specified their morphological characters. The *Mus* sp. could not identify at the species level because the morphological and molecular analyses performed in this study could not sufficient for describing their taxonomic position thus, further morphological and molecular studies required to confirm their taxonomic position. Similarly, two cryptic species *R. rattus* and *R. tanezumi* have not consistently discernible coat color and other morphological difference. Therefore, they were distinguished by only molecular analysis. However, previous studies carried out in Nepal were based only in morphological analysis so there could be possibility of misidentification in these taxa. This study suggested that an approach integrating morphological and molecular analyses could be appropriate for the accurate and effective identification of cryptic species like *R. tanezumi* and *R. rattus*.

All the species described in this section were also, identified by the molecular analysis. Thus, the possibility of misidentification was low and the morphological characterization considered to be used in the taxonomic key. Careful analysis of external morphology and comparing with relevant reports can distinguish the *B. bengalensis*, *M. booduga*, *N. fulvescens*, *R. pyctoris*, and *T. indica* from closely related species, which have been discussed briefly in this section. Although, some species have low sample size and were not

sufficient to analyze statistically, this study filled the research gap remained in taxonomic study of murids since long time. The morphological traits and colored photographs of each taxon provided in this study will facilitate in morphological identification in future. Extensive survey and detail studies of external morphology, cranial, and molecular analyses are required to understand the taxonomic status murids occurred in Nepal.

2. Molecular identification of murids

1) BLAST results

Altogether, 113 individuals of murids collected from 39 different sites of Lumbini, Kathmandu, and Pokhara, Nepal were successfully amplified and sequenced (Table 1). However, 17 DNA samples of *T. indica* could not amplify due to either low quality of DNA or amplification failure. All nucleotide sequences were subjected to the BLAST and determined the most identical putative species, which were compared with the results of morphological identification. Altogether eight taxa (*B. bengalensis*, *M. musculus*, *M. booduga*, *N. fulvescens*, *R. nitidus*, *R. pyctoris*, *R. rattus*, and *R. tanezumi*) were identified at species level having query cover 100% and identity was over 95% except *N. fulvescens* (Table 6). Interestingly, *N. fulvescens* have low identity (94%) in BLAST result, but it was perfectly matched with morphological identification based on its distinct external morphology, which were described in the earlier reports (Ellerman, 1961; Abe, 1982; Baral and Shah, 2008). Two sequences of Mus taxa have identity 95.45% with *M. nitidulus* (Shimada *et al.*, 2016).

Table 6. Identification of species using nucleotide BLAST analysis

Expected species name	Haplotype	N	Accession number[a]	Putative species	Accession number[b]	Identity %	Reference
R. rattus	RraNPL003	1	KY985274	*R. rattus* III	JN675599	99.53	Aplin *et al.*, 2011
R. rattus	RraNPL004	3	KY002796	*R. rattus* III	JN675599	99.07	Aplin *et al.*, 2011
R. rattus	RraNPL008	6	KY985275	*R. rattus* III	JN675599	99.07	Aplin *et al.*, 2011
R. rattus	RraNPL009	8	KY985276	*R. rattus* III	JN675599	99.18	Aplin *et al.*, 2011
R. rattus	RraNPL013	2	KY985277	*R. rattus* III	JN675599	99.3	Aplin *et al.*, 2011
R. rattus	RraNPL023	1	KY985278	*R. rattus* III	JN675599	99.88	Aplin *et al.*, 2011
R. rattus	RraNPL025	1	KY985279	*R. rattus* III	JN675599	99.3	Aplin *et al.*, 2011
R. rattus	RraNPL026	1	KY985280	*R. rattus* III	JN675599	99.07	Aplin *et al.*, 2011
R. rattus	RraNPL039	1	KY985281	*R. rattus* III	JN675599	99.3	Aplin *et al.*, 2011
R. rattus	RraNPL040	14	KY002799	*R. rattus* III	JN675599	99.42	Aplin *et al.*, 2011
R. rattus	RraNPL042	1	KY002801	*R. rattus* III	JN675599	99.3	Aplin *et al.*, 2011
R. rattus	RraNPL051	3	KY002802	*R. rattus* III	JN675599	99.3	Aplin *et al.*, 2011
R. rattus	RraNPL052	1	KY985282	*R. rattus* III	JN675599	99.88	Aplin *et al.*, 2011
R. rattus	RraNPL081	1	KY002808	*R. rattus* III	JN6755601	99.3	Aplin *et al.*, 2011
R. rattus	RraNPL088	1	KY985283	*R. rattus* III	JN675599	98.84	Aplin *et al.*, 2011
R. rattus	RraNPL096	1	KY985284	*R. rattus* III	JN675599	99.65	Aplin *et al.*, 2011
R. rattus	RraNPL099	1	KY002812	*R. rattus* III	JN675599	99.88	Aplin *et al.*, 2011
R. rattus	RraNPL100	2	KY002813	*R. rattus* III	JN675599	99.65	Aplin *et al.*, 2011
R. rattus	RraNPL134	3	KY985288	*R. rattus* III	JN675599	99.3	Aplin *et al.*, 2011
R. rattus	RraNPL140	1	KY985289	*R. rattus* III	JN675599	98.72	Aplin *et al.*, 2011
R. rattus	RraNPL144	2	KY985290	*R. rattus* III	JN675599	99.3	Aplin *et al.*, 2011
R. rattus	RraNPL146	1	KY985291	*R. rattus* III	JN675599	98.84	Aplin *et al.*, 2011
R. rattus	RraNPL158	1	KY985292	*R. rattus* III	JN6755601	99.19	Aplin *et al.*, 2011
R. rattus	RraNPL192	2	KY985285	*R. rattus* III	JN675599	99.76	Aplin *et al.*, 2011
R. rattus	RraNPL199	1	KY985286	*R. rattus* III	JN675599	99.07	Aplin *et al.*, 2011
R. rattus	RraNPL202	1	KY985287	*R. rattus* III	JN675599	98.72	Aplin *et al.*, 2011
R. tanezumi	RtaNPL073	7	KY002823	*R. tanezumi*	JX534065	100	Pages *et al.*, 2013
R. pyctoris	RpyNPL053	3	KY587428	*R. pyctoris*	JN675512	100	Aplin *et al.*, 2011
R. nitidus	RniNPL002	2	KY985270	*R. nitidus*	AB973109	99.53	Suzuki and Chingangbam, 2015*
R. nitidus	RniNPL017	1	KY985271	*R. nitidus*	AB973109	99.76	Suzuki and Chingangbam, 2015*

N, number of *CytB* sequences; [a], accession number of the haplotypes determined in this study; [b], accession number of the most identical sequence.

Table 6. Continued

Expected Species name	Haplotype	N	Accession number [a]	Putative species	Accession number [b]	Identity %	Reference
R. nitidus	RniNPL018	6	KY985272	*R. nitidus*	AB973109	99.88	Suzuki and Chingangbam, 2015*
R. nitidus	RniNPL028	1	KY985273	*R. nitidus*	AB973109	99.65	Suzuki and Chingangbam, 2015*
M. musculus	MmuNPL062	3	KY418170	*M. m. bactrianus*	KT376775	99.65	Hamid *et al*., 2017
M. musculus	MmuNPL063	1	KY418171	*M. m. bactrianus*	KT376775	99.53	Hamid *et al*., 2017
M. musculus	MmuNPL077	11	KY418172	*M. musculus*	AB820897	99.88	Suzuki *et al*., 2013
M. musculus	MmuNPL078	1	KY418173	*M. musculus*	AB820897	99.76	Suzuki *et al*., 2013
M. musculus	MmuNPL084	5	KY418174	*M. musculus*	AB973115	99.88	Suzuki and Chingangbam, 2015*
M. musculus	MmuNPL093	1	KY418175	*M. musculus*	AB973115	99.76	Suzuki and Chingangbam, 2015*
M. booduga	MboNPL057	2	KY587423	*M. booduga*	AB125761	99.65	Suzuki *et al*., 2004
M. booduga	MboNPL204	2	KY587425	*M. booduga*	AB125761	99.88	Suzuki *et al*., 2004
Mus sp.	MspNPL048	1	-	*M. nitidulus*	AB262423	95.41	Shimada *et al*., 2016*
Mus sp.	MspNPL049	1	-	*M. nitidulus*	AB262423	95.17	Shimada *et al*., 2016*
B. bengalensis	BbeNPL107	4	KY587421	*B. bengalensis*	JN675474	99.3	Aplin *et al*., 2011
N. fulvescens	NfuNPL195	1	KY587417	*N. fulvescens*	KY068720	94	Zhang *et al*., 2016

*, Unpublished reference; N, number of *CytB* sequences; a, accession number of the haplotypes determined in this study; b, accession number of the most identical sequence.

However, the *M. nitidulus* has been reported as an endemic mouse of Myanmar (Shimada *et al*., 2007). Only two sequences were determined from this species, and the morphology could not match with other *Mus* taxa recorded in Nepal. Therefore, it is hard to confirm them at the species level. Out of the 22 sequences of *M. musculus*, four were distinguished into subspecies level on BLAST results, which were over 99.50% identical with *M. m. bactrianus*. Details on subspecies of *M. musculus* have been provided in the phylogenetic study of *M. musculus* section.

2) Haplotype distribution

Altogether, forty-four unique haplotypes were found in 114 CytB sequences of murids. The *M. booduga* have two haplotypes in four sequences, *M. musculus* have six haplotypes in 22 sequences, Mus sp. have two haplotypes in two sequences, *R. nitidus* have four haplotypes in 10 sequences, and *R. rattus* have 26 haplotypes in 61 sequences (Table 7) indicate intraspecific variations in those species. However, four species *B. bengalensis*, *N. fulvescens*, *R. pyctoris* and *R. tanezumi* have a single haplotype in four, one, three and seven sequences, respectively indicate no intraspecific variation in the collection of this study.

All the haplotypes sequences determined in this study were submitted to NCBI database, which accession numbers have been tabulated in Table 6. The *R. rattus* and *M. musculus* have found relatively higher number of haplotypes compare to other species, which indicate high genetic diversity on those species. Both of those are globally distributing and remarkably adapting species in different environmental conditions (Musser and Carleton, 2005). Current molecular studies revealed the *R. rattus* and *M. musculus* are polytypic species having at least three lineages in *R. rattus* and seven lineages in *M. musculus* (Prager *et al*., 1998; Aplin *et al*., 2011; Suzuki *et al*.,

2013; Hamid *et al.*, 2017). Molecular phylogeny and lineages of *R. rattus* and *M. musculus* have been discussed in details in next two sections (Phylogenetic study of *Rattus* and phylogenetic study of *Mus*). As like to Mostert (2009), two *Rattus* taxa (*R. rattus* and *R. tanezumi*) were not distinguished at different species level from the morphological analysis but from the molecular analysis, these two were distinguished clearly into two species. Because of the complex taxonomy, and morphologically indistinguishable at species level, Aplin *et al.* (2003b, 2011) and Robins *et al.* (2007) describe to them as *Rattus rattus* complex.

3) Phylogenetic analysis

The pair wise genetic distance between the conspecific haplotypes of *R. rattus*, *M. musculus*, *R. nitidus*, and *M.* booduga were ranged 0.001-0.017, 0.001-0.016, 0.001-0.008 and 0.001-004, respectively (Table 7). Genetic distance was computed between nine murids identified in this study and two reference taxa *R. norvegicus* and *M. nitidulus*. The genetic distance between each species has been summarized in Table 8. Highest genetic distance was found between *B. bengalensis* and *M. musculus* (0.278) and lowest genetic distance between *R. rattus* and *R. tanezumi* (0.048). The mean genetic distance within the genus *Rattus*, *Mus*, *Bandicota* and *Niviventer* was found 0.067, 0.108, 0.058 and 0.068, respectively and overall mean distance was determined 0.153. As like to Chaimamee and Jaeger (2000), *Bandicota* was found genetically closest with genus *Rattus* following to the *Niviventer* and *Mus*. Genetic distances were found to be increased with higher taxonomic level from intra-species, interspecies, and inter genera, which support the significant change in genetic divergence at the species boundaries (Lakra *et al.*, 2011; Li *et al.*, 2015).

Table 7. Haplotypes determination in murids collected in Nepal

Species name	N	H	Location
B. bengalensis	4	1	Kathmandu
M. booduga	4	2	Pokhara
M. musculus	22	6	Lumbini, Pokhara
Mus sp.	2	2	Kathmandu
N. fulvescens	1	1	Pokhara
R. nitidus	10	4	Lumbini, Pokhara, Kathmandu
R. pyctoris	3	1	Kathmandu
R. rattus	61	26	Lumbini, Pokhara, Kathmandu
R. tanezumi	7	1	Lumbini

N, number of *CytB* sequences, H, number of haplotypes.

Table 8. Pairwise genetic distance between the haplotypes of murids

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. RraIIINEP001																														
2. RraIIIPAK002	0.011																													
3. RraNPL003	0.006	0.015																												
4. RraNPL004	0.004	0.012	0.002																											
5. RraNPL008	0.010	0.013	0.010	0.007																										
6. RraNPL009	0.008	0.012	0.008	0.006	0.006																									
7. RraNPL013	0.007	0.011	0.007	0.005	0.005	0.004																								
8. RraNPL023	0.002	0.013	0.004	0.004	0.010	0.008	0.007																							
9. RraNP025	0.007	0.013	0.007	0.005	0.007	0.001	0.005	0.007																						
10. RraNPL026	0.008	0.012	0.008	0.006	0.006	0.000	0.004	0.008	0.001																					
11. RraNPL039	0.008	0.012	0.008	0.006	0.006	0.000	0.004	0.008	0.001	0.000																				
12. RraNPL040	0.006	0.010	0.006	0.004	0.004	0.002	0.001	0.006	0.004	0.002	0.002																			
13. RraNPL042	0.007	0.008	0.007	0.005	0.005	0.004	0.002	0.007	0.005	0.004	0.004	0.001																		
14. RraNPL051	0.007	0.011	0.007	0.005	0.005	0.004	0.002	0.007	0.005	0.004	0.004	0.001	0.002																	
15. RraNPL052	0.002	0.013	0.006	0.004	0.010	0.008	0.007	0.002	0.007	0.008	0.008	0.006	0.007	0.007																
16. RraNPL081	0.008	0.007	0.012	0.010	0.011	0.010	0.008	0.011	0.011	0.010	0.010	0.007	0.008	0.008	0.011															
17. RraNPL088	0.012	0.016	0.012	0.010	0.010	0.008	0.007	0.012	0.010	0.008	0.008	0.006	0.007	0.005	0.012	0.013														
18. RraNPL096	0.005	0.016	0.005	0.005	0.010	0.008	0.007	0.002	0.007	0.008	0.008	0.006	0.007	0.007	0.005	0.013	0.012													
19. RraNPL099	0.002	0.013	0.006	0.004	0.010	0.008	0.007	0.002	0.007	0.008	0.008	0.006	0.007	0.005	0.002	0.011	0.010	0.005												
20. RraNPL100	0.005	0.011	0.006	0.004	0.007	0.006	0.005	0.005	0.005	0.006	0.006	0.004	0.005	0.005	0.005	0.008	0.010	0.007	0.005											
21. RraNP134	0.007	0.011	0.007	0.005	0.005	0.004	0.002	0.007	0.005	0.004	0.004	0.001	0.002	0.002	0.007	0.008	0.007	0.007	0.007	0.005										
22. RraNPL140	0.012	0.016	0.012	0.010	0.010	0.008	0.007	0.012	0.010	0.008	0.008	0.006	0.007	0.007	0.012	0.013	0.012	0.012	0.012	0.010	0.007									
23. RraNPL144	0.007	0.011	0.007	0.005	0.005	0.004	0.002	0.007	0.005	0.004	0.004	0.001	0.002	0.002	0.007	0.008	0.007	0.007	0.007	0.005	0.002	0.005								
24. RraNPL146	0.008	0.012	0.008	0.006	0.006	0.005	0.004	0.008	0.006	0.005	0.005	0.002	0.004	0.004	0.008	0.010	0.008	0.008	0.008	0.006	0.004	0.008	0.004							
25. RraNPL158	0.012	0.008	0.013	0.013	0.015	0.013	0.012	0.015	0.015	0.013	0.013	0.011	0.012	0.012	0.015	0.006	0.017	0.017	0.015	0.012	0.012	0.017	0.012	0.013						
26. RraNPL192	0.004	0.015	0.005	0.005	0.011	0.010	0.008	0.001	0.008	0.010	0.010	0.007	0.008	0.008	0.004	0.012	0.013	0.001	0.004	0.006	0.008	0.013	0.008	0.010	0.016					
27. RraNPL199	0.008	0.012	0.008	0.006	0.006	0.000	0.004	0.008	0.001	0.000	0.000	0.002	0.004	0.004	0.008	0.010	0.008	0.008	0.008	0.006	0.004	0.008	0.004	0.005	0.013	0.010				
28. RraNPL202	0.013	0.017	0.013	0.011	0.004	0.010	0.008	0.013	0.011	0.010	0.010	0.007	0.008	0.008	0.013	0.015	0.013	0.013	0.013	0.011	0.008	0.013	0.008	0.010	0.018	0.015	0.010			
29. RtaLAO001	0.048	0.044	0.047	0.047	0.051	0.050	0.048	0.048	0.048	0.050	0.050	0.047	0.046	0.046	0.046	0.041	0.048	0.048	0.046	0.046	0.049	0.051	0.049	0.047	0.046	0.047	0.050	0.056		
30. RtaKOR002	0.047	0.043	0.046	0.046	0.050	0.048	0.047	0.047	0.047	0.048	0.048	0.046	0.044	0.044	0.044	0.040	0.047	0.047	0.044	0.044	0.047	0.050	0.047	0.047	0.044	0.046	0.048	0.054	0.005	
31. RtaNPL073	0.051	0.050	0.050	0.050	0.054	0.053	0.051	0.051	0.051	0.053	0.053	0.050	0.048	0.049	0.051	0.044	0.054	0.051	0.049	0.046	0.048	0.054	0.051	0.053	0.049	0.050	0.053	0.058	0.030	0.029
32. RpyNPL001	0.107	0.109	0.106	0.106	0.115	0.110	0.111	0.107	0.108	0.110	0.110	0.110	0.108	0.108	0.108	0.103	0.114	0.107	0.104	0.108	0.112	0.119	0.112	0.111	0.108	0.105	0.110	0.120	0.109	0.109
33. RpyNPL053	0.107	0.109	0.106	0.106	0.115	0.110	0.111	0.107	0.108	0.110	0.110	0.110	0.108	0.108	0.108	0.103	0.114	0.107	0.104	0.108	0.112	0.119	0.112	0.111	0.108	0.105	0.110	0.120	0.109	0.109
34. RniIND001	0.148	0.156	0.155	0.152	0.156	0.152	0.154	0.151	0.150	0.152	0.152	0.152	0.155	0.151	0.152	0.146	0.156	0.155	0.148	0.151	0.155	0.163	0.155	0.150	0.155	0.153	0.152	0.157	0.161	0.169
35. RniVNM003	0.154	0.153	0.159	0.156	0.155	0.151	0.152	0.154	0.154	0.151	0.151	0.151	0.149	0.150	0.155	0.148	0.155	0.158	0.151	0.154	0.153	0.161	0.153	0.149	0.157	0.157	0.151	0.155	0.159	0.167
36. RniLAO002	0.151	0.160	0.159	0.156	0.160	0.156	0.157	0.154	0.154	0.156	0.156	0.156	0.158	0.154	0.155	0.149	0.162	0.158	0.151	0.154	0.158	0.166	0.158	0.154	0.159	0.157	0.156	0.160	0.164	0.173
37. RniNPL002	0.151	0.159	0.154	0.152	0.156	0.153	0.154	0.150	0.150	0.153	0.153	0.152	0.155	0.151	0.152	0.149	0.156	0.154	0.148	0.151	0.155	0.163	0.155	0.150	0.158	0.152	0.153	0.157	0.162	0.170
38. RniNPL017	0.151	0.159	0.157	0.155	0.159	0.156	0.157	0.153	0.153	0.156	0.156	0.156	0.158	0.154	0.155	0.149	0.159	0.157	0.151	0.154	0.158	0.166	0.158	0.153	0.158	0.155	0.156	0.160	0.164	0.172
39. RniNPL018	0.150	0.158	0.155	0.154	0.158	0.154	0.155	0.151	0.152	0.154	0.154	0.154	0.156	0.152	0.153	0.148	0.158	0.155	0.150	0.152	0.156	0.164	0.156	0.152	0.157	0.153	0.154	0.158	0.162	0.170
40. RniNPL028	0.150	0.158	0.152	0.151	0.155	0.151	0.152	0.148	0.149	0.151	0.151	0.151	0.154	0.150	0.151	0.148	0.155	0.152	0.147	0.150	0.154	0.161	0.154	0.149	0.157	0.151	0.151	0.155	0.161	0.169
41. RnoCHN001	0.154	0.154	0.155	0.152	0.156	0.152	0.154	0.154	0.149	0.152	0.152	0.152	0.150	0.151	0.155	0.149	0.157	0.158	0.151	0.151	0.155	0.163	0.155	0.150	0.158	0.156	0.152	0.162	0.159	0.162
42. MmuNPL062	0.215	0.230	0.215	0.215	0.225	0.219	0.219	0.214	0.217	0.219	0.219	0.221	0.219	0.219	0.211	0.224	0.224	0.214	0.212	0.218	0.219	0.232	0.224	0.221	0.231	0.214	0.219	0.232	0.223	0.225
43. MmuNPL063	0.217	0.232	0.217	0.217	0.227	0.221	0.221	0.215	0.218	0.221	0.221	0.223	0.220	0.221	0.213	0.226	0.226	0.216	0.214	0.220	0.220	0.234	0.225	0.223	0.233	0.215	0.221	0.233	0.224	0.227
44. MmuNPL077	0.213	0.227	0.213	0.213	0.225	0.216	0.217	0.211	0.214	0.216	0.216	0.218	0.216	0.217	0.209	0.221	0.221	0.212	0.209	0.215	0.216	0.230	0.221	0.218	0.228	0.211	0.216	0.231	0.220	0.222
45. MmuNPL078	0.215	0.229	0.215	0.215	0.227	0.218	0.218	0.213	0.216	0.218	0.218	0.220	0.218	0.218	0.210	0.223	0.223	0.213	0.211	0.217	0.218	0.231	0.223	0.220	0.230	0.213	0.218	0.233	0.222	0.224
46. MmuNPL084	0.215	0.230	0.215	0.215	0.228	0.219	0.219	0.214	0.217	0.219	0.219	0.221	0.219	0.219	0.211	0.224	0.224	0.214	0.212	0.218	0.219	0.232	0.224	0.221	0.231	0.214	0.219	0.234	0.218	0.220
47. MmuNPL093	0.215	0.230	0.215	0.215	0.227	0.219	0.219	0.213	0.216	0.219	0.219	0.220	0.218	0.219	0.211	0.223	0.224	0.214	0.212	0.217	0.218	0.232	0.223	0.220	0.230	0.213	0.219	0.234	0.225	0.227
48. MmbIRN001	0.209	0.224	0.209	0.209	0.222	0.213	0.213	0.208	0.211	0.213	0.213	0.215	0.212	0.213	0.205	0.218	0.218	0.208	0.206	0.212	0.212	0.226	0.217	0.215	0.225	0.208	0.213	0.228	0.222	0.224
49. MmcCHN002	0.215	0.226	0.211	0.211	0.224	0.215	0.215	0.213	0.212	0.215	0.215	0.217	0.214	0.215	0.211	0.220	0.220	0.214	0.211	0.214	0.214	0.228	0.219	0.217	0.227	0.213	0.215	0.230	0.218	0.221
50. MmNPL002	0.224	0.239	0.224	0.224	0.237	0.228	0.232	0.223	0.226	0.228	0.228	0.230	0.228	0.229	0.220	0.233	0.234	0.223	0.221	0.227	0.228	0.242	0.233	0.228	0.240	0.223	0.228	0.244	0.241	0.244
51. MmcIND004	0.217	0.232	0.217	0.217	0.230	0.221	0.221	0.216	0.218	0.221	0.221	0.223	0.220	0.221	0.213	0.226	0.226	0.216	0.214	0.220	0.220	0.234	0.225	0.223	0.233	0.216	0.221	0.236	0.225	0.227
52. MboNPL057	0.233	0.245	0.230	0.230	0.234	0.238	0.234	0.231	0.235	0.238	0.238	0.236	0.239	0.234	0.234	0.244	0.240	0.232	0.230	0.232	0.239	0.248	0.239	0.234	0.246	0.231	0.238	0.235	0.246	0.249
53. MboNPL204	0.227	0.239	0.224	0.224	0.229	0.232	0.229	0.226	0.229	0.232	0.232	0.230	0.233	0.229	0.228	0.239	0.234	0.226	0.224	0.227	0.233	0.242	0.233	0.228	0.240	0.226	0.232	0.229	0.246	0.249
54. MboNPL001	0.227	0.239	0.224	0.224	0.229	0.232	0.229	0.226	0.229	0.232	0.232	0.230	0.233	0.229	0.228	0.239	0.234	0.226	0.224	0.227	0.233	0.242	0.233	0.229	0.240	0.226	0.232	0.229	0.246	0.249
55. MspNPL048	0.216	0.222	0.214	0.212	0.220	0.220	0.217	0.214	0.217	0.220	0.220	0.218	0.221	0.220	0.211	0.216	0.225	0.215	0.216	0.215	0.216	0.230	0.221	0.217	0.224	0.214	0.220	0.226	0.232	0.241
56. MspNPL049	0.220	0.226	0.218	0.217	0.224	0.223	0.221	0.218	0.220	0.223	0.223	0.223	0.225	0.224	0.216	0.220	0.229	0.219	0.220	0.219	0.220	0.234	0.225	0.221	0.229	0.218	0.223	0.230	0.236	0.246
57. MniMMR001	0.233	0.239	0.231	0.229	0.243	0.238	0.234	0.231	0.235	0.238	0.238	0.236	0.233	0.234	0.234	0.244	0.240	0.232	0.229	0.232	0.233	0.248	0.239	0.234	0.248	0.231	0.238	0.250	0.235	0.245
58. BbeNPL107	0.176	0.169	0.178	0.175	0.171	0.175	0.176	0.176	0.172	0.175	0.175	0.175	0.172	0.173	0.172	0.167	0.181	0.176	0.173	0.173	0.172	0.181	0.172	0.172	0.175	0.174	0.175	0.171	0.169	0.169
59. BbeLKA001	0.165	0.162	0.162	0.163	0.165	0.168	0.170	0.163	0.166	0.168	0.168	0.168	0.166	0.166	0.161	0.160	0.174	0.163	0.162	0.166	0.166	0.169	0.166	0.168	0.165	0.161	0.168	0.166	0.155	0.155
60. NfuNPL195	0.220	0.229	0.220	0.219	0.229	0.225	0.225	0.219	0.222	0.225	0.225	0.225	0.222	0.223	0.222	0.221	0.232	0.223	0.217	0.217	0.228	0.237	0.228	0.222	0.226	0.222	0.225	0.226	0.223	0.232
61. NfuCHN001	0.212	0.218	0.212	0.210	0.215	0.211	0.211	0.210	0.214	0.211	0.211	0.211	0.208	0.209	0.213	0.210	0.214	0.215	0.209	0.215	0.213	0.223	0.213	0.208	0.221	0.213	0.211	0.218	0.215	0.221

Table 8. Continued

Haplotype	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
31. RtaNPL073																															
32. RpyNPL001	0.110																														
33. RpyNPL053	0.110	0.000																													
34. RniIND001	0.162	0.139	0.139																												
35. RniVNM003	0.161	0.139	0.139	0.007																											
36. RniLAO002	0.166	0.141	0.141	0.004	0.011																										
37. RniNPL002	0.164	0.139	0.139	0.005	0.010	0.008																									
38. RniNPL017	0.166	0.142	0.142	0.002	0.010	0.006	0.005																								
39. RniNPL018	0.164	0.140	0.140	0.001	0.008	0.005	0.004	0.001																							
40. RniNPL028	0.162	0.137	0.137	0.004	0.008	0.007	0.001	0.004	0.002																						
41. RnoCHN001	0.161	0.151	0.151	0.067	0.064	0.066	0.067	0.070	0.069	0.066																					
42.MmuNPL062	0.230	0.265	0.265	0.223	0.221	0.226	0.224	0.225	0.224	0.222	0.205																				
43. MmuNPL063	0.231	0.267	0.267	0.225	0.223	0.228	0.225	0.227	0.225	0.224	0.206	0.001																			
44. MmuNPL077	0.232	0.264	0.264	0.232	0.230	0.235	0.232	0.234	0.232	0.231	0.213	0.015	0.016																		
45. MmuNPL078	0.234	0.266	0.266	0.233	0.232	0.237	0.234	0.236	0.234	0.232	0.215	0.016	0.015	0.001																	
46. MmuNPL084	0.230	0.271	0.271	0.232	0.230	0.236	0.233	0.235	0.233	0.231	0.213	0.013	0.015	0.004	0.005																
47. MmuNPL093	0.237	0.270	0.270	0.231	0.230	0.235	0.232	0.234	0.232	0.230	0.213	0.015	0.016	0.005	0.006	0.004															
48. MmbIRN001	0.223	0.264	0.264	0.226	0.224	0.229	0.226	0.228	0.226	0.225	0.199	0.007	0.008	0.015	0.016	0.013	0.015														
49. MmcCHN002	0.231	0.266	0.266	0.231	0.229	0.235	0.232	0.234	0.232	0.230	0.209	0.013	0.015	0.004	0.005	0.002	0.004	0.013													
50. MmNPL002	0.242	0.256	0.256	0.223	0.221	0.226	0.221	0.223	0.221	0.219	0.222	0.036	0.037	0.036	0.037	0.035	0.034	0.033	0.034												
51. MmclIND004	0.232	0.264	0.264	0.229	0.227	0.232	0.229	0.231	0.229	0.228	0.210	0.006	0.007	0.016	0.017	0.015	0.016	0.008	0.015	0.037											
52. MboNPL057	0.260	0.258	0.258	0.254	0.258	0.258	0.253	0.257	0.255	0.253	0.267	0.188	0.190	0.183	0.185	0.183	0.179	0.186	0.179	0.194	0.190										
53. MboNPL204	0.260	0.258	0.258	0.254	0.258	0.258	0.253	0.257	0.255	0.253	0.261	0.194	0.195	0.189	0.190	0.188	0.185	0.192	0.184	0.199	0.195	0.002									
54. MboNPL001	0.260	0.258	0.258	0.254	0.258	0.258	0.253	0.257	0.255	0.253	0.261	0.194	0.195	0.189	0.190	0.188	0.185	0.192	0.184	0.199	0.196	0.004	0.001								
55. MspNPL048	0.224	0.251	0.251	0.216	0.214	0.210	0.213	0.216	0.214	0.213	0.225	0.176	0.178	0.179	0.180	0.178	0.175	0.172	0.174	0.178	0.180	0.124	0.124	0.127							
56. MspNPL049	0.228	0.256	0.256	0.220	0.219	0.214	0.217	0.220	0.218	0.217	0.228	0.179	0.180	0.181	0.183	0.181	0.175	0.174	0.177	0.175	0.183	0.128	0.128	0.130	0.005						
57. MniMMR001	0.232	0.255	0.255	0.221	0.214	0.214	0.218	0.221	0.219	0.218	0.218	0.186	0.187	0.180	0.182	0.175	0.176	0.179	0.176	0.177	0.185	0.129	0.129	0.127	0.054	0.057					
58. BbeNPL107	0.175	0.173	0.173	0.193	0.189	0.191	0.195	0.197	0.195	0.193	0.196	0.277	0.279	0.265	0.267	0.269	0.264	0.279	0.265	0.273	0.279	0.290	0.284	0.281	0.273	0.275	0.271				
59. BbeLKA001	0.155	0.152	0.152	0.195	0.185	0.193	0.195	0.197	0.195	0.194	0.211	0.292	0.294	0.277	0.279	0.278	0.280	0.294	0.279	0.288	0.294	0.281	0.275	0.278	0.258	0.262	0.267	0.056			
60. NfuNPL195	0.232	0.208	0.208	0.205	0.195	0.208	0.200	0.205	0.203	0.200	0.223	0.279	0.281	0.282	0.284	0.282	0.281	0.283	0.277	0.277	0.285	0.281	0.281	0.278	0.234	0.238	0.235	0.234	0.255		
61. NfuCHN001	0.233	0.209	0.209	0.195	0.186	0.189	0.190	0.195	0.193	0.190	0.197	0.260	0.262	0.263	0.265	0.263	0.262	0.264	0.259	0.256	0.266	0.269	0.269	0.266	0.228	0.229	0.208	0.216	0.242	0.066	

Muridae are usually difficult to identify due to its remarkable biodiversity as well as evolutionary characteristics such as short genetic distance and rapid adaptive radiation (Steppan *et al.*, 2004; Rowe *et al.*, 2011), which revealed the wide range of intraspecific and interspecific genetic distance. The wide range of genetic distance in conspecific individuals could be the existence in different geographical locations. Li *et al.* (2015) suggested that the geographic population differentiation could be the possible reason for relatively large intraspecific distance. murids taxa especially *Rattus* and *Niviventer* have such characteristics (Rowe *et al.*, 2011; Li *et al.*, 2015).

Phylogeny-based identification of species was carried out to further confirmation of the results obtained by BLAST analysis. Forty-four *CytB* haplotypes found in this study and 17 haplotypes determined from the reference sequences taken from NCBI database (Table 9) were used to construct a phylogenetic tree (NJ tree). The haplotypes of *B. bengalensis*, *M. musculus*, *M. booduga*, *N. fulvescens*, *R. nitidus*, *R. pyctoris*, *R. rattus*, and *R. tanezumi*, were clustered together with haplotypes of reference sequences in eight distinct mono-specific clades in the NJ tree (Fig. 9). In the phylogenetic tree, the haplotype of *R. tanezumi* (RtaNPL073) was clustered together with the haplotypes of *R. tanezumi* recorded in Laos (Rta001) and South Korea (Rta002) (Pages *et al.*, 2013; Han *et al.*, 2013). It made confirmation on identification of *R. tanezumi*.

Although, two haplotypes of *Mus* sp. (MspNPL048 and MspNPL049) were clustered together with *R. nitidulus*, they could not confirm at species level because the genetic distance between *Mus* sp. and *R. nitidulus* (0.055) was higher than intraspecific genetic distance usually find in the rodents (Baker and Bradley, 2006). Shimada *et al.* (2007) claimed that *R. nitidulus* is an endemic species of Myanmar which is genetically close with *M. booduga*. In addition, the morphological traits of *M. nitidulus* are not available so it could not compare morphologically. Considering all these facts, it is identified at

genus level only and named as *Mus* sp. in this study. Further specimen collection and integrative study of morphology and molecular are required to confirm its taxonomy.

This study revealed molecular identification based on the *CytB* gene sequence is highly succeeded to species identification in the polytypic and cryptic species occurred in Muridae. Based on the morphology analysis, BLAST results, genetic distance, and phylogeny analysis, eight murids taxa were successfully identified at the species level and one taxon at the genus level. Although, the sample size in some species were low, the DNA sequences generated in those species will be the reference sequences for species identification in future taxonomic studies on murids taxonomy.

The DNA sequences generated in this study have provided baseline information, which will be applicable in further taxonomic studies and understanding the evolutionary phenomenon such species. Furthermore, this study provided invaluable information about the application of molecular identification technique as a potentially valuable tool for taxonomic studies on wildlife of Nepal.

3. Phylogenetic study of *Mus* in Nepal

Altogether, six distinct haplotypes (MmuNPL062-63, MmuNPL077-78, MmuNPL084, MmuNPL093) in the 22 sequences of *M. musculus*, two haplotype (MboNPL057, MboNPL204) in the two sequences of *M. booduga*, and two haplotypes in the two sequences of *Mus* sp. determined in this study. Haplotype distribution of *Mus* species collected in Nepal has shown in Fig. 10. The haplotypes of *M. musculus*, *M. booduga*, and *Mus* sp., have been submitted to NCBI database (Table 10).

Table 9. Genetic distance between different species of murids

Species	Rra	Rta	Rpy	Rni	Rno	Mmu	Mbo	Msp	Mni	Bbe	Nfu
R. rattus											
R. tanezumi	0.048										
R. pyctoris	0.109	0.109									
R. nitidus	0.153	0.164	0.139								
R. norvegicus	0.153	0.159	0.15	0.067							
M. musculus	0.219	0.226	0.263	0.227	0.209						
M. booduga	0.231	0.25	0.256	0.254	0.261	0.188					
Mus sp.	0.219	0.233	0.252	0.214	0.225	0.177	0.126				
M. nitidulus	0.235	0.236	0.253	0.216	0.217	0.179	0.128	0.055			
B. bengalensis	0.17	0.163	0.162	0.193	0.203	0.278	0.281	0.266	0.269		
N. fulvescens	0.218	0.226	0.209	0.197	0.21	0.271	0.273	0.232	0.222	0.236	

Rra, *R. rattus*; Rta, *R. tanezumi*; Rpy, *R. pyctoris*, Rni, *R. nitidus*; Rno, *R. norvegicus*; Mmu, *M. musculus*; Mbo, *M. booduga*; Msp, *Mus* sp.; Mni, *M. nitidulus*; Bbe, *B. bengalensis*; Nfu, *N. fulvescens.*

Table 10. Reference sequences used in molecular identification of murids

Species	Sequence name	Accession no.	Country	Reference
B. bengalensis	Bbe001	AB762700	Sri Lanka	Yasuda *et al*., 2014
M. booduga	Mb001	AB125761	Nepal	Suzuki *et al*., 2004
M. musculus	Mm001	AB649490	India	Suzuki *et al*., 2013
M. musculus	Mm002	KT376789	Iran	Hamid *et al*., 2017
M. musculus	Mm003	AB819914	China	Suzuki *et al*., 2013
M. musculus	Mm004	AB649506	Nepal	Suzuki *et al*., 2013
M. nitidulus	Mni001	AB269819	Myanmar	Shimada *et al*., 2016*
N. fulvescens	Nfu001	KY068720	China	Zhang *et al*., 2016
R. nitidus	Rni001	AB973110	India	Chingangbam *et al*., 2015
R. nitidus	Rni002	HM217479	Laos	Pages *et al*., 2010
R. nitidus	Rni003	FR775884	Vietnam	Balakirev and Rozhnov, 2012
R. norvegicus	Rno001	GU592997	China	Dumont and Payseur, 2011
R. pyctoris	Rpy001	JN675511	Nepal	Aplin *et al*., 2011
R. rattus LIII	RraIII001	JN675599	Nepal	Aplin *et al*., 2011
R. rattus LIII	RraIII002	JN675601	Pakistan	Aplin *et al*., 2011
R. tanezumi	Rta001	JX534065	Laos	Pages *et al*., 2013
R. tanezumi	Rta002	KF011916	South Korea	Han *et al*., 2013*

*, Unpublished reference; N, number of *CytB* sequence.

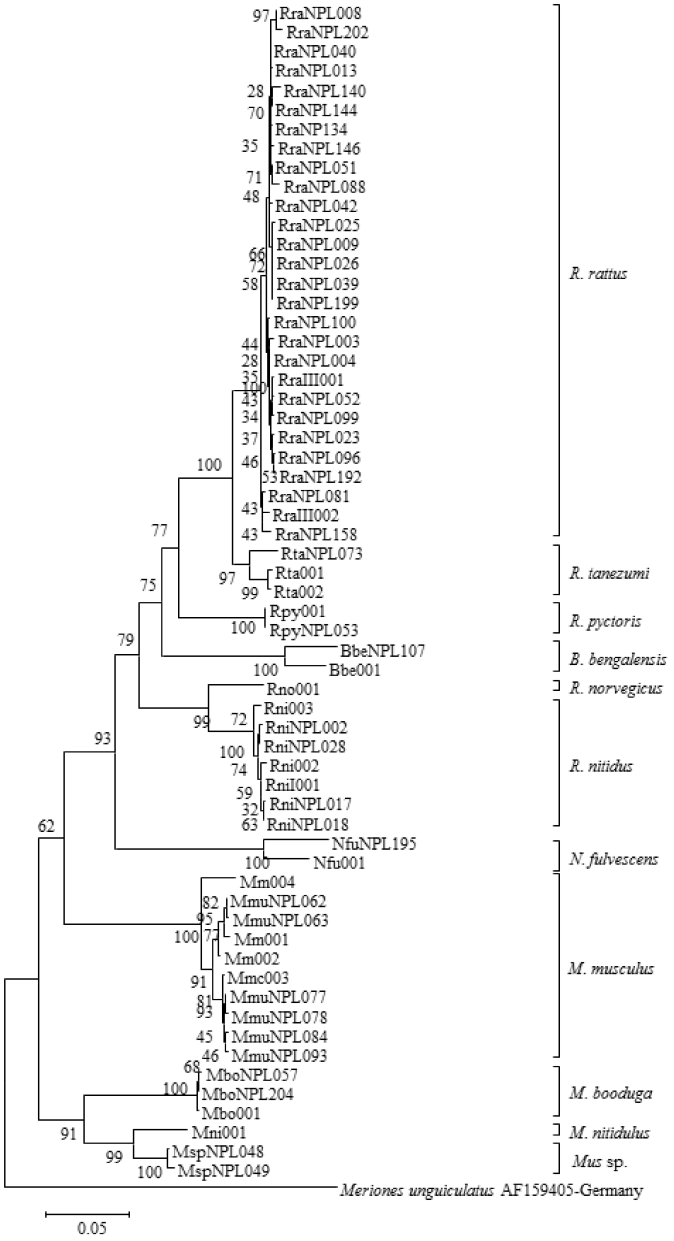

Fig. 9. Phylogenetic tree for the *CytB* haplotypes of murids. The NJ tree was constructed from the genetic distances based on the nucleotide polymorphisms among the *CytB* haplotype sequences. Genetic distances were calculated using Tamura-Nei's model (Tamura and Nei, 1993). Bootstrap values for internal nodes have given at each node. *CytB* sequences of *Meriones unguiculatus* was used as the outgroup. Detail information of haplotypes corresponding to those in the figure has been explained in Tables 6 and 9.

In order to describe the phylogenetic relationship of *Mus* within and between the species, NJ tree was constructed based on the pairwise genetic distance using mitochondrial *CytB* haplotypes of *M. musculus*, *M. booduga*, and *Mus sp.* and the reference sequences of *Mus* species taken from NCBI database (Fig. 11 and Table 10). The NJ tree comprised three distinct groups representing different species groups namely *M. musculus*, *M. cervicolor*, and *M. booduga* of the subgenus *Mus*.

1) *Mus musculus* species group

M. musculus species group composed of the haplotypes of *M. musculus*, *M. spicilegus*, *M. macedonicus*, *M. cypriacus*, and *M. spretus*. The pairwise genetic distance between the haplotypes within the *M. musculus* species group ranged 0.001–0.119, at which haplotypes of *M. musculus* recorded in this study were ranged between 0.001–0.013 (Table 11). This analysis indicates that there was a extensive genetic variation existing among the species present in the *M. musculus* species group.

Out of the four species present in the group, the *M. musculus* is a most abundant species in the world (Prager *et al.*, 1998). Based on the genetic distance it has close genetic relation with *M. cypriacus* (0.080), having tentative divergence times from 3.893–4.353 MYBP calculated using genetic distance and fossils based calibration interval of *Mus* and *Rattus* (Jacobs and Flynn, 2005). Similarly, distant genetic relation with *M. spretus* (0.114) having tentative divergence time approximately from 5.540–6.195 MYBP within the same species group. The genetic distance and tentative divergence time of *M. musculus* with other *Mus* species have been documented in Table 12. The genetic distances between different species of *Mus* determined in this study were comparable with Rudra *et al.* (2016) but the estimations of tentative divergence time were little high.

As like to Suzuki *et al.* (2004) this study also determined a close genetic relation between *M. musculus* species group and *M. booduga* species group (0.176) compared to compared to *M. cervicolor* species group (0.212). Therefore, Suzuki *et al.* (2004) hypothesized these two species group have sibling lineage status. These estimations revealed the speciation of *M. musculus* species group might occur during the late Miocene Period. The estimate of divergence time between two different set of species group was relatively high as compared to Suzuki *et al.* (2004).

The *M. musculus* is a polytypic species displays complex patterns of morphological, genetic, and geographic variation (Suzuki and Aplin, 2012). Schwartz and Schwartz (1943) classified it into more than ten subspecies based on the morphological traits and geographical distribution. However, recent molecular analysis well distinguished into seven subspecies namely *M. m. castaneus*, *M. m. musculus*, *M. m. domesticus*, *M. m. bactrianus*, *M. m. gentilulus*, and *M. m. isaticus* (Prager *et al.*, 1998; Terashima *et al.*, 2006; Searle *et al.*, 2009; Suzuki *et al.*, 2013; Hardouin *et al.*, 2015; Sakuma *et al.*, 2016; Hamid *et al.*, 2017). In this study, altogether six haplotypes were determined in twenty-two mice collected in Nepal. Among them, two haplotypes (MmuNPL062-63) were found in four mice collected in Pokhara, and four haplotypes (MmuNPL077-78, MmuNPL084, MmuNPL093) were found in eighteen mice collected in Lumbini. All the haplotypes were identified at subspecies level and studied their phylogenetic relationship regarding thirty-nine haplotypes determined from the reference sequences of M. musculus collected from the NCBI database (Table 10). The pairwise genetic distance between two haplotypes of different subspecies was ranged 0.001-0.044, at which the haplotypes found in this study was ranged between 0.001 and 0.019 (Table 13). The NJ tree was constructed based on the genetic distances shows the *CytB* haplotypes fall into seven distinct groups (Mm01-07) representing seven subspecies of *M. musculus* (Fig. 12).

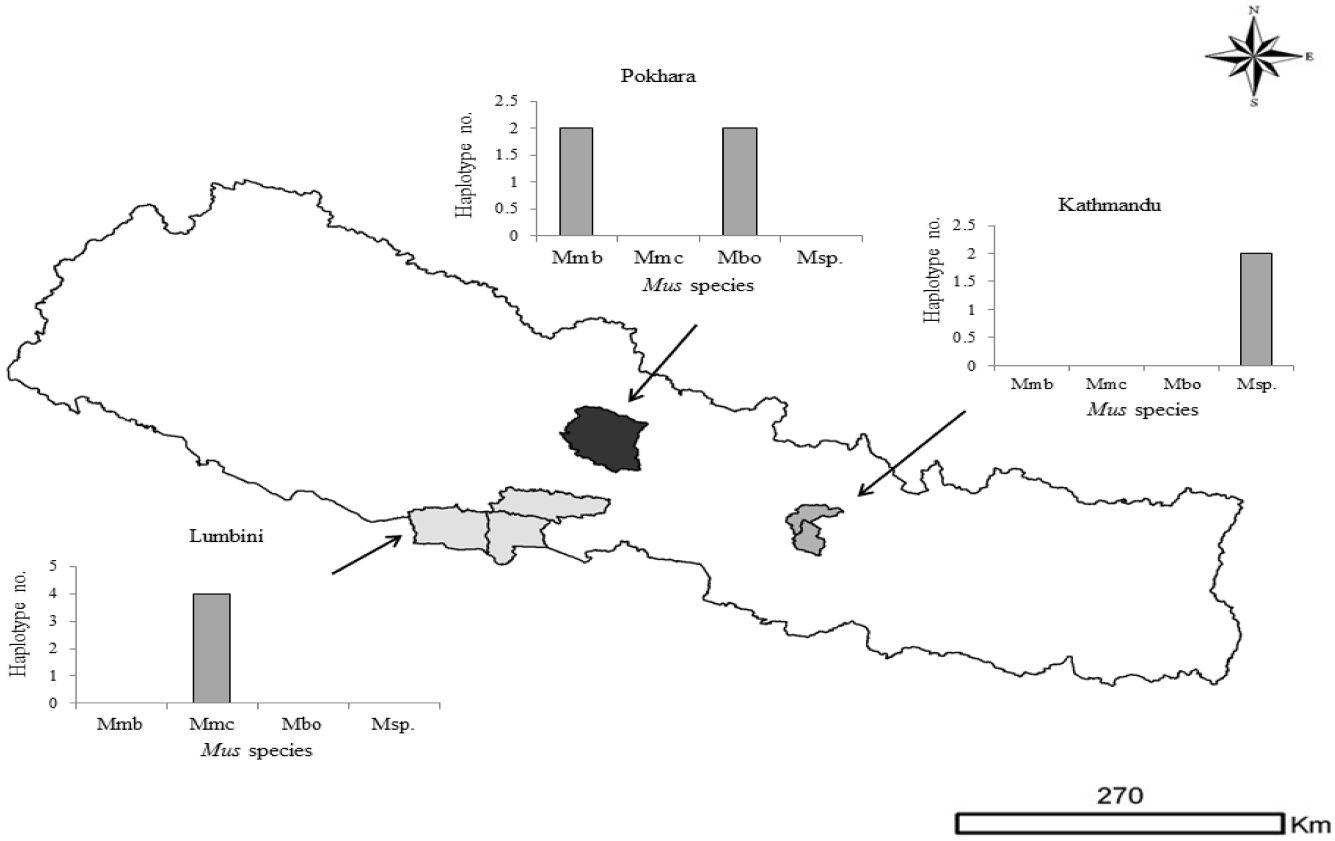

Fig. 10. Distribution of *CytB* haplotypes of *Mus* species collected in Nepal. *CytB* sequences of *M. m. castaneus* (Mmc) in Lumbini was 18, *M. m. bactrianus* (Mmb) and *M. booduga* (Mbo) in Pokhara was four in each taxon, and *Mus* sp. (Msp.) in Kathmandu was two.

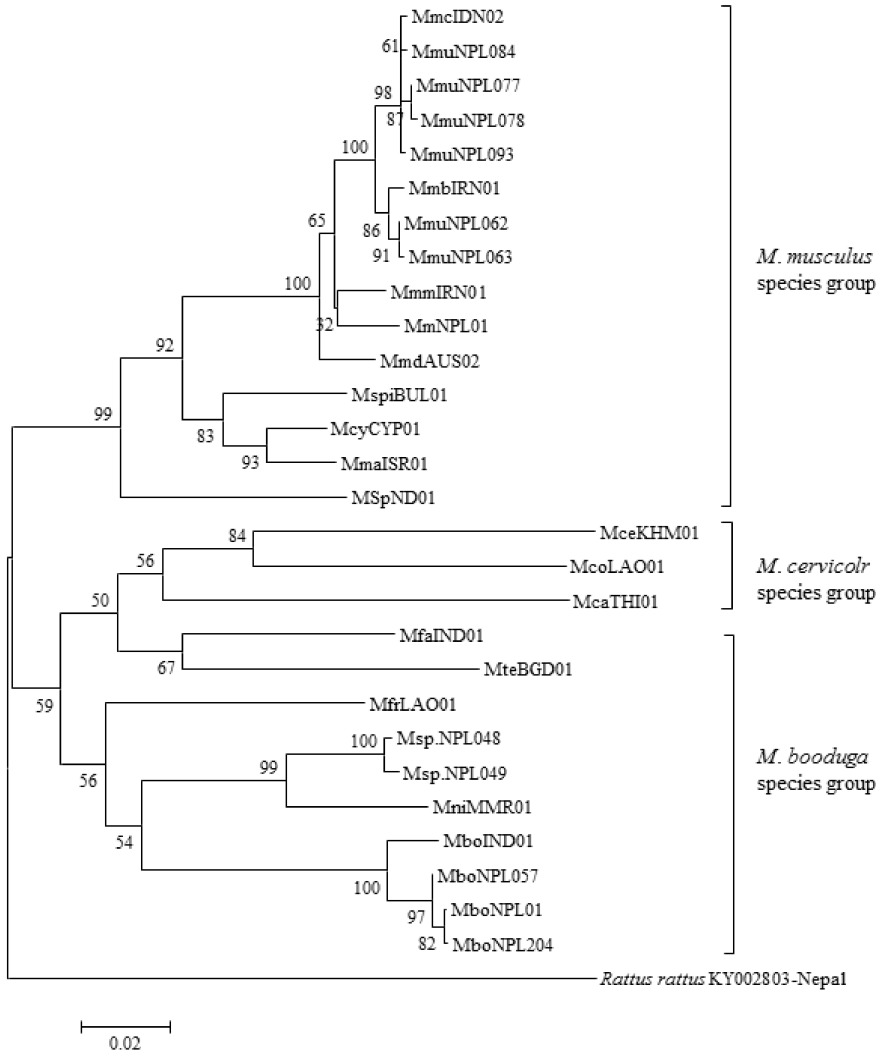

Fig. 11. Phylogenetic tree for the *CytB* haplotypes of *Mus* species. The NJ tree was constructed from the genetic distances based on the nucleotide polymorphisms among the 28 haplotypes sequences of *M. musculus* collected from Nepal and reference sequences taken from NCBI database. Genetic distances were calculated using Tamura-Nei's model (Tamura and Nei, 1993). Bootstrap values for internal nodes have given at each node. CytB sequences of *R. rattus* was used as outgroup. Detail informations of haplotypes corresponding to those in figure have been explainedhaplotypes used in figure have been explained in Tables 10.

Table 11. List of samples used in the phylogenetic study of *Mus* in Nepal

Species (subspecies)	Group	Haplotype	Country	N	Accession no.	Reference
M. m. bactrianus	Mm01	MmuNPL062	Pokhara, Nepal	3	KY418170	This study
		MmuNPL063		1	KY418171	
		MmbIRN01	Iran	1	KT376789	Hamid *et al*., 2017
		MmbIRN02		2	KT376794	
		MmbIRN03		1	KT376752	
		MmbIRN04		2	KT376746	
		MmbIRN06		1	KT376780	
		MmbAFG01	Afghanistan	1	KT376796	Hamid *et al*., 2017
		MmbAFG02		1	KT376771	
		MmbAFG03		1	KT376773	
		MmbAFG04		1	KT376788	
		MmbAFG05		1	KT376760	
		MmbAFG06		1	KT376803	
M. m. castaneous		MmcIND04	India	1	AB649490	Suzuki *et al*., 2013
		MmcTWN01	Taiwan	1	AB125773	
M. m. isatissus	Mm02	MmiIRN02	Iran	1	KT376811	Hamid *et al*., 2017
		MmiIRN03		1	KT376816	
		MmiIRN01		1	KT376818	
M. m. castaneous	Mm03	MmuNPL077	Lumbini, Nepal	11	KY418172	This study
		MmuNPL078		1	KY418173	
		MmuNPL084		5	KY418174	
		MmuNPL093		1	KY418175	
		MmcCHN02	China	1	AB819914	Suzuki *et al*., 2013
		MmcIND021	India	1	AB819910	
		MmcBGD011	Bangladesh	1	AB820905	Suzuki *et al*., 2013
		MmcBGD021		1	AB820904	
		MmcIDN011	Indonesia	1	AB820908	
		MmcIDN02		1	AB820900	
		MmcJPN01	Japan	1	AB820915	
		MmcMMR021	Myanmar	1	AB820906	
		MmcMMR011	Myanmar	1	AB820907	Suzuki *et al*., 2013
M. musculus		MmIND011	India	1	AB973116	Chingangbam *et al*., 2015*
M. musculus	Mm04	MmNPL01	Nepal	1	AB205280	Terashima *et al*., 2006
		MmNPL02		1	AB649506	Suzuki *et al*., 2013
M. m. domesticus	Mm05	MmdAUS032	Australia	1	AB649474	
		MmdDEU022	Germany	1	AB649456	
		MmdGRC02	Greece	1	AB649467	
		MmdGRC01		1	AB649468	
		MmdAUS02		3	AB649475	

[1], identical hapltype with MmuNPL084; [2], identical hapltype with MmdAUS02; *, unpublished reference; N, number of *CytB* sequences; n.d., not determined; *, unpublished reference; N, number of *CytB* sequences.

Table 11. Continued

Species (subspecies)	Group	Haplotype	Country	N	Accession no.	Reference
M. m. domesticus	Mm05	MmdIRN02	Iran	1	KT376844	
		MmdIRN01		1	KT376849	
		MmdITA02	Italy	1	AB649461	
		MmdITA01		1	AB649462	
		MmdZAF01	South Africa	1	HQ157798	
M. musculus		MmmAUS01[2]	Australia	3	EU349766	Rowe *et al*., 2008
		MmDEU01[2]	Germany	1	JF286601	Stewart *et al*., 2008
M. m. musculus	Mm06	MmmCZE01	Czech	1	AB819918	Suzuki *et al*., 2013
		MmmIRN02	Iran	1	KT376872	Hamid *et al*., 2017
		MmmIRN01		1	KT376873	
		MmmKAZ01	Kazakhstan	1	AB649511	Suzuki *et al*., 2013
		MmmRUS01	Russia	1	AB819916	
		MmmKOR01	South Korea	1	AB649560	
		MmmUKR01	Ukraine	1	AB819917	
		MmmUZB01	Uzbekistan	1	AB649514	
M. musculus		MmmCHN01	China	1	AF520626	Li and Zhang, 2002*
M. m. gentilulus	Mm07	MmgMDG01	Madagascar	1	LC147005	Sakuma *et al*., 2016
		MmgMDG02	Madagascar	1	LC147006	
M. booduaga	-	MboNPL057	Nepal	1	KY587423	This study
	-	MboNPL204		1	KY587425	
	-	MboNPL01		1	AB125761	Suzuki *et al*., 2004
	-	MboIND01	India	1	AB125760	
Mus sp.	-	MspNPL048	Nepal	1	-	This study
Mus sp.	-	MspNPL049		1	-	
M. cookii	-	McoLAO01	Laos	1	AB125769	Suzuki *et al*., 2004
M. cervicolor	-	MceKHM01	Cambodia	1	AB125766	Suzuki *et al*., 2004
M. famulus	-	MfaIND01	India	1	AJ698872	Chevret *et al*., 2005
M. fragilicauda	-	MfrLAO01	Laos	1	AB125780	Suzuki *et al*., 2004
M. platythrix	-	MplTHA01	Thailand	1	AJ698880	Chevret *et al*., 2005
M. terricolor	-	MteBGD01	Bangladesh	1	AB125778	Suzuki *et al*., 2004
M. nitidulus	-	MniMMR01	Myanmar	1	AB269819	Shimada *et al*., 2006*
M. caroli	-	McaTHI01	Thailand	1	AB253438	Shimada *et al*., 2007
M. spicilegus	-	MspBUL01	Bulgeria	1	AB125775	Suzuki *et al*., 2004
M. macedonicus	-	MmaISR01	Isral	1	AB125770	Suzuki *et al*., 2004
M. cypriacus	-	McyCYP01	Cyprus	1	FR751074	Cazaux *et al*., 2011
M. spretus	-	MspND01	n.d.	1	AB033700	Suzuki *et al*., 2000

The global distributions of *CytB* haplotypes of subspecies of *M. musculus* have been shown in Fig. 13. The group Mm01 contained the following haplotypes: two new haplotypes (MmuNPL062-63) were found in four mice of Pokhara (Fig. 12), Eleven haplotypes of *M. m. bactrianus* were found in Iran and Afghanistan (Hamid *et al.*, 2017), and two haplotypes of *M. m. castaneus* were found in India and Taiwan (Suzuki *et al.*, 2013). The two haplotypes from India (MmcIND04) and Taiwan (MmcTWN01) present in the Mm01 group were possibly misidentified because of all the haplotypes of *M. m. castaneus* reported by Suzuki *et al.* (2013) were clustered in the Mm03 group. The haplotype sequences showed that the mice found in Pokhara might be *M. m. bactrianus*. Yonekawa *et al.* (1981) identified *M. m. bactrianus* in West Asia for the first time. This subspecies has been found in the Iranian plateau, Afghanistan, Pakistan, India, China, and Malaysia (Ellerman, 1961; Yonekawa *et al.*, 1981; Din *et al.*, 1996; Hamid *et al.*, 2017), but in Nepal, there have not been any authentic reports of the presence of this subspecies before this study. This study could be valuable for understanding the geographical distribution of *M. m. bactrianus*. Therefore, it is predicted that the middle mountainous region, such as in Pokhara, could be an area where this subspecies is most likely found. However, further study is required for generating detailed information regarding the distribution of *M. m. bactrianus* in Nepal and surrounding countries.

Out of the six haplotypes present in the group Mm03, four new haplotypes (MmuNPL077, MmuNPL078, MmuNPL084, and MmuNPL093) were found in Lumbini (Fig. 12), Nepal, and two different haplotypes (MmcCHN02, MmcIDN02) of *M. m. castaneus* determined in three sequences recorded in China (MmcCHN02), Indonesia (MmcIDN02), and Japan (MmcJPN01) by Suzuki *et al.* (2013). The haplotype MmuNPL084 was identical to five sequences found in Lumbini and seven sequences of *M. m. castaneus* recorded in India (MmIND01, MmcIND02), Bangladesh (MmcBGD01-02),

Myanmar (MmcMMR01-02), and Indonesia (MmcIDN01) by Suzuki *et al.* (2013) and Chingangbam *et al.* (2015) (Table 10). This result indicated that all the haplotypes found in Lumbini might be *M. m. castaneus*. Previous studies revealed that the Indian subcontinent, including Nepal, is the homeland for *M. m. castaneus,* and a rapid range expansion of the subspecies occurred in East and South East Asia including Indonesia, China, Taiwan, and Japan approximately 4,650-9,300 years ago (Boursot *et al.*, 1996; Prager *et al.*, 1998; Jing *et al.*, 2014; Suzuki *et al.*, 2015). This result also supports our present finding of *M. m. castaneus* in Lumbini, Nepal. Marshall (1977) has recorded the presence of *M. m. castaneus* in Kathmandu, Nepal, indicating that *M. m. castaneus* could be found in both the low altitude *terai* region and high altitude mountainous regions. The haplotypes distribution of *M. m. bactrianus* and *M. m. castaneus* in Nepal have been shown in Fig. 10. Similar to Yonekawa *et al.* (1981), this study also determined the lowest genetic distance (0.016) between the two subspecies *M. m. castaneus* and *M. m. bactrianus* (Table 14), indicating that these two subspecies have the closest genetic relationship among all the *M. m.* subspecies.

The tentative divergence time between *M. m. castaneus* and *M. m. bactrianus* 0.68 MYBP was higher than the estimation (0.2-0.26 MYBP) of Yonekawa *et al.* (1981). In contrast to Yonekawa *et al.* (1981), this study estimated fossil-based calibration interval of *Mus* and *Rattus* divergence (11-12.3 MYBP), as suggested by Jacobs and Flynn (2005).

Two distinct haplotypes (MmNPL01-02) present in the group Mm04 was reported by Terashima *et al.* (2006) and Suzuki *et al.* (2013). Interestingly, these two haplotypes appeared in different groups in the NJ tree, which seems to be a different subspecies of *M. musculus,* but the authors did not address the morphological status or specimen preservation. Therefore, morphology of these haplotypes could not compare with other subspecies of *M. musculus* recorded in Nepal.

Table 12. Pairwise genetic distance between the haplotypes of *Mus* species

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1. MmuNPL062																												
2. MmuNPL063	0.001																											
3. MmuNPL077	0.013	0.015																										
4. MmuNPL078	0.015	0.013	0.001																									
5. MmuNPL084	0.012	0.013	0.004	0.005																								
6. MmuNPL093	0.012	0.013	0.004	0.005	0.002																							
7. MmbIRN01	0.006	0.007	0.015	0.016	0.013	0.013																						
8. MmcIDN02	0.012	0.013	0.004	0.005	0.002	0.002	0.013																					
9. MmdAUS02	0.031	0.032	0.035	0.036	0.034	0.032	0.032	0.034																				
10. MmNPL01	0.031	0.032	0.032	0.033	0.031	0.031	0.029	0.031	0.028																			
11. MmmIRN01	0.028	0.029	0.029	0.030	0.028	0.028	0.026	0.028	0.025	0.025																		
12. MmaISR01	0.085	0.087	0.086	0.087	0.081	0.082	0.087	0.084	0.079	0.087	0.082																	
13. MSpND01	0.115	0.117	0.117	0.119	0.116	0.115	0.110	0.116	0.100	0.120	0.110	0.100																
14. MspiBUL01	0.087	0.088	0.088	0.089	0.087	0.085	0.090	0.087	0.082	0.090	0.084	0.049	0.092															
15. McyCYP01	0.078	0.080	0.084	0.086	0.083	0.081	0.083	0.083	0.073	0.076	0.076	0.029	0.108	0.055														
16. McoLAO01	0.218	0.219	0.219	0.220	0.219	0.215	0.214	0.219	0.204	0.209	0.212	0.205	0.188	0.197	0.216													
17. McaTHI01	0.209	0.211	0.211	0.213	0.211	0.210	0.203	0.211	0.202	0.200	0.208	0.222	0.202	0.194	0.223	0.172												
18. MceKHM01	0.220	0.222	0.221	0.223	0.219	0.220	0.217	0.225	0.211	0.213	0.225	0.216	0.192	0.196	0.222	0.147	0.196											
19. MboNPL057	0.186	0.187	0.182	0.184	0.182	0.181	0.185	0.182	0.178	0.180	0.173	0.172	0.157	0.166	0.171	0.174	0.197	0.216										
20. MboNPL01	0.191	0.193	0.188	0.189	0.187	0.186	0.191	0.187	0.183	0.186	0.178	0.177	0.162	0.171	0.176	0.174	0.200	0.216	0.004									
21. MboIND01	0.179	0.181	0.181	0.183	0.176	0.180	0.179	0.181	0.177	0.190	0.177	0.171	0.165	0.175	0.180	0.175	0.219	0.200	0.024	0.023								
22. MboNPL204	0.191	0.193	0.188	0.189	0.187	0.186	0.191	0.187	0.183	0.186	0.178	0.177	0.162	0.171	0.176	0.176	0.203	0.216	0.002	0.001	0.021							
23. MfaIND01	0.163	0.164	0.170	0.171	0.170	0.167	0.164	0.170	0.171	0.168	0.173	0.157	0.156	0.157	0.164	0.182	0.169	0.163	0.169	0.172	0.189	0.174						
24. MfrLAO01	0.158	0.159	0.167	0.169	0.167	0.166	0.152	0.167	0.155	0.146	0.167	0.165	0.164	0.158	0.157	0.182	0.188	0.197	0.128	0.126	0.136	0.128	0.169					
25. MteBGD01	0.204	0.205	0.198	0.199	0.198	0.203	0.198	0.204	0.186	0.198	0.193	0.170	0.163	0.168	0.187	0.186	0.165	0.192	0.150	0.153	0.154	0.155	0.114	0.154				
26. Msp.NPL048	0.171	0.173	0.175	0.177	0.174	0.174	0.168	0.174	0.178	0.168	0.179	0.178	0.152	0.155	0.178	0.164	0.183	0.177	0.123	0.125	0.127	0.123	0.129	0.118	0.152			
27. Msp.NPL049	0.174	0.175	0.178	0.179	0.177	0.174	0.170	0.177	0.176	0.170	0.178	0.176	0.156	0.153	0.175	0.165	0.187	0.179	0.127	0.129	0.130	0.127	0.129	0.121	0.156	0.005		
28. MniMMR01	0.181	0.182	0.177	0.178	0.171	0.175	0.175	0.176	0.185	0.164	0.175	0.184	0.152	0.181	0.203	0.186	0.193	0.202	0.128	0.126	0.127	0.128	0.137	0.136	0.147	0.055	0.058	

Table 13. Genetic distance and tentative divergence time between different species of *Mus*

	Divergence time (MYBP)[1]														
Species name	Mbo	Mca	Mce	Mco	Mcy	Mfa	Mfr	Mma	Mmu	Mni	Msp	Mspi	Mspr	Mte	Rra
M. booduga	`-	9.94-11.114	10.31-11.528	8.489-9.492	8.529-9.536	8.535-9.544	6.289-7.033	8.481-9.483	8.942-9.998	6.178-6.908	6.137-6.862	8.292-9.272	7.837-8.763	7.422-8.298	11.00-12.3
M. caroli	0.205	-	9.532-10658	8.372-9.361	10.849-12.131	8.218-9.190	9.11-10.187	10.755-12.026	10.103-11.297	9.38-10.488	8.961-10.020	9.411-10.523	9.825-10.986	8.001-8.947	11.751-13.130
M. cervicolor	0.212	0.196	-	7.112-7.953	10.778-12.052	7.903-8.837	9.558-10.687	10.476-11.714	10.663-11.922	9.828-10.990	8.632-9.652	9.521-10.646	9.323-10.425	9.324-10.425	11.142-12.862
M. cookii	0.175	0.172	0.147	-	10.493-11.732	8.812-9.853	8.855-9.902	9.935-11.109	10.456-11.691	9.009-10.073	7.988-8.931	9.56-10.690	9.141-10.221	9.011-10.076	10.858-12.250
M. cypriacus	0.176	0.223	0.222	0.216	-	7.976-8.918	7.62-8.521	1.406-1.572	3.893-4.353	9.865-11.030	8.556-9.567	2.676-2.993	5.256-5.877	9.076-10.148	9.914-11.086
M. famulus	0.176	0.169	0.163	0.182	0.164	-	8.194-9.162	7.627-8.528	8.17-9.135	6.67-7.458	6.262-7.002	7.64-8.543	7.589-8.486	5.514-6.166	10.882-12.168
M. fragilicauda	0.130	0.188	0.197	0.182	0.157	0.169	-	8.004-8.950	7.822-8.746	6.612-7.393	5.804-6.489	7.69-8.599	7.938-8.876	7.472-8.355	11.155-12.473
M. macedonicus	0.175	0.222	0.216	0.205	0.029	0.157	0.165	-	4.094-4.577	8.937-9.993	8.588-9.603	2.401-2.685	4.841-5.412	8.257-9.233	10.108-11.302
M. musculus	0.184	0.208	0.220	0.215	0.080	0.168	0.161	0.084	-	8.565-9.577	8.47-9.471	4.22-4.719	5.54-6.195	9.65-10.790	10.689-11.952
M. nitidulus	0.127	0.193	0.202	0.186	0.203	0.137	0.136	0.184	0.176	-	2.726-3.049	8.809-9.850	7.398-8.272	7.144-7.989	11.178-12.49
Mus sp.	0.126	0.185	0.178	0.165	0.176	0.129	0.120	0.177	0.174	0.056	-	7.49-8.375	7.456-8.337	7.465-8.347	10.444-11.678
M. spicilegus	0.171	0.194	0.196	0.197	0.055	0.157	0.158	0.049	0.087	0.181	0.154	-	4.459-4.986	8.161-9.125	9.775-10.930
M. spretus	0.161	0.202	0.192	0.188	0.108	0.156	0.164	0.100	0.114	0.152	0.154	0.092	-	7.909-8.843	10.246-11.456
M. terricolor	0.153	0.165	0.192	0.186	0.187	0.114	0.154	0.170	0.199	0.147	0.154	0.168	0.163	-	11.839-13.238
R. rattus	0.227	0.242	0.230	0.226	0.204	0.224	0.230	0.208	0.220	0.230	0.215	0.201	0.211	0.244	-

[1], indicates million years before present and were calculated based on Jacobs and Flynn (2005).

Genetic distances were calculated using Tamura-Nei's model (Tamura and Nei, 1993).

Mbo, *M. booduga*; Mca, *M. caroli*; Mce, *M. cervicolor*; Mco, *M. cookie*; Mcy, *M. cypriacus*; Mfa, *M. famulus*; Mfr, *M. fragilicauda*; Mma, *M. macedonicus*; Mmu, *M. musculus*; Mni, *M. nitidulus*; Msp, *Mus* sp.; Msp, *M. spicilegus*; Mspi, *M. spicilegus*; Mspr, *M. spretus*; Mte, *M. terricolor*; Rra, *R. rattus*.

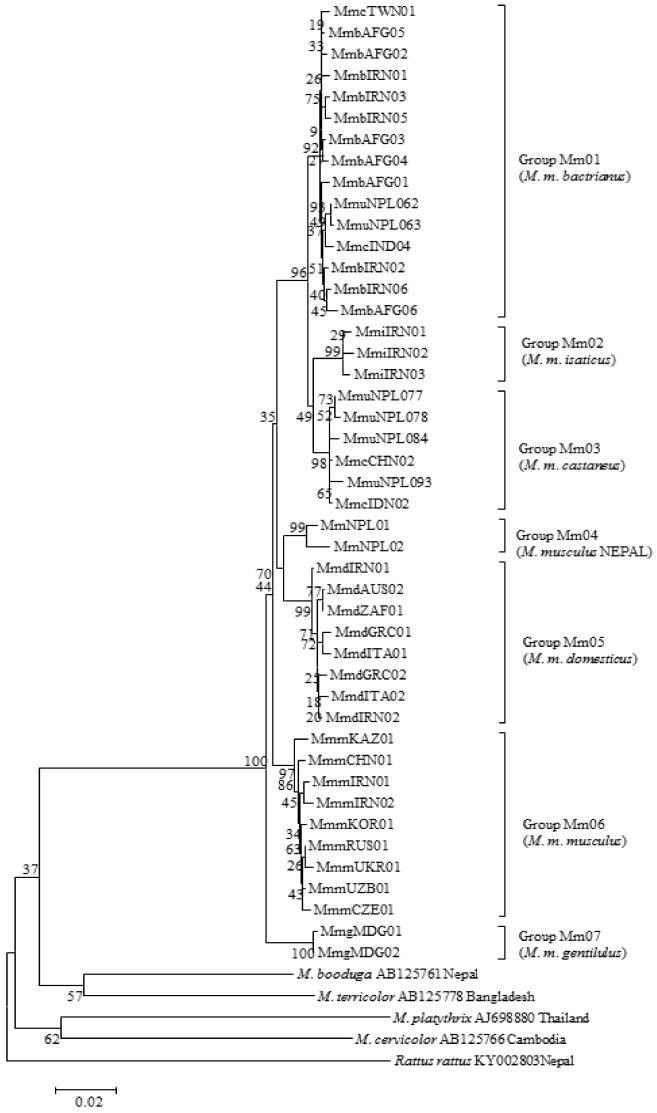

Fig. 12. Phylogenetic tree for the *CytB* haplotypes of *M. musculus*. The NJ tree was constructed from the genetic distances based on the nucleotide polymorphisms among the 45 haplotypes of *M. musculus* collected from Nepal and reference sequences were taken from NCBI database. Genetic distances were calculated using Tamura-Nei's model (Tamura and Nei, 1993). Bootstrap values for internal nodes have given at each node. CytB sequences *M. booduga*, *M. terricolor*, *M. platytrhrix*, and *M. cervicolor*, and *Rattus rattus* were used as outgroups. Detail information of haplotypes corresponding to those in the figure have been explained in Table 10.

Tereshima *et al.* (2006) postulated that there is a possibility that these specimens could be *M. m. bactrianus*. However, the results of phylogenetic analysis in this study showed that these two haplotypes (MnNPL01-02) were not clustered together with *M. m. bactrianus*. In addition, the genetic distance between *M. m. bactrianus* and the group Mm04 (0.033) was greater than genetic distance between *M. m. musculus* and the group Mm04 (0.028), suggesting these two haplotypes they have a closer evolutionary relationship with *M. m. musculus* than with *M. m. bactrianus*. The samples of those sequences were collected from the high-altitude regions (Tukuche and Kathmandu) of Nepal. The Tukuche was not study area for this study and in Kathmandu, the *M. musculus* could not capture. Therefore, this study could not collect the mouse as reported by Tereshima *et al.* (2006) and Suzuki *et al.* (2013). Further studies on morphological and molecular analyses are required to verify their findings and determine the taxonomy of the sample source.

On the other hand, the other four groups (Mm02, Mm05, Mm06, and Mm07) consisted of the reference sequences. The group Mm02 comprises three haplotypes of *M. m. isatissus*, which was reported only in Iran. The group Mm05 comprises eight haplotypes of *M. m. domesticus* reported in Italy, Greece, South Africa, Iran, and Australia; and the group Mm06 comprised nine haplotypes of *M. m. musculus* reported in Russia, Ukraine, Czech Republic, Kazakhstan, Iran, Uzbekistan, China, Russia and South Korea. Similarly, the group Mm07 comprises two haplotypes of *M. m. gentilulus* reported in Madagascar (Fig. 12, Table 10). These results were supported by the subspecies classifications of *M. musculus* by Suzuki *et al.* (2013), Sakuma *et al.*, (2016), and Hamid *et al.* (2017). The global distribution of *CytB* haplotypes of seven subspecies of *M. musculus* have shown in Fig. 13. These molecular data and phylogenetic analysis revealed that at least two subspecies of *M. musculus*, *M. m. bactrianus* and *M. m. castaneus* are present in Nepal.

Table 14. Pairwise genetic distance between the haplotypes of subspecies of *M. musculus*

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1. MmuNPL062																												
2. MmuNPL063	0.001																											
3. MmuNPL077	0.015	0.016																										
4. MmuNPL078	0.017	0.016	0.002																									
5. MmuNPL084	0.017	0.018	0.006	0.006																								
6. MmuNPL093	0.018	0.019	0.006	0.008	0.008																							
7. MmmRUS01	0.030	0.031	0.032	0.034	0.034	0.035																						
8. MmmUZB01	0.030	0.031	0.032	0.034	0.034	0.035	0.002																					
9. MmdGRC01	0.035	0.036	0.040	0.042	0.042	0.042	0.028	0.028																				
10. MmdGRC02	0.034	0.035	0.037	0.039	0.039	0.039	0.027	0.027	0.008																			
11. MmcIDN02	0.014	0.015	0.003	0.005	0.005	0.006	0.031	0.031	0.039	0.036																		
12. MmmKOR01	0.031	0.032	0.033	0.035	0.035	0.036	0.003	0.003	0.029	0.028	0.032																	
13. MmdAUS02	0.033	0.034	0.038	0.040	0.040	0.040	0.024	0.024	0.007	0.005	0.037	0.025																
14. MmmKAZ01	0.031	0.032	0.033	0.035	0.035	0.036	0.006	0.006	0.031	0.030	0.032	0.006	0.029															
15. MmmCZE01	0.032	0.033	0.034	0.036	0.036	0.037	0.004	0.004	0.030	0.029	0.033	0.005	0.026	0.008														
16. MmmUKR01	0.033	0.034	0.035	0.037	0.037	0.038	0.003	0.005	0.029	0.028	0.034	0.006	0.025	0.009	0.006													
17. MmdZAF01	0.032	0.033	0.037	0.039	0.039	0.039	0.025	0.025	0.008	0.006	0.036	0.026	0.001	0.030	0.027	0.026												
18. MmdITA01	0.035	0.036	0.038	0.040	0.042	0.042	0.028	0.028	0.006	0.006	0.039	0.029	0.006	0.031	0.030	0.029	0.006											
19. MmdITA02	0.035	0.036	0.039	0.041	0.041	0.041	0.027	0.027	0.008	0.006	0.038	0.028	0.005	0.030	0.029	0.028	0.006	0.006										
20. MmmCHN01	0.030	0.031	0.032	0.034	0.034	0.035	0.004	0.004	0.028	0.027	0.031	0.005	0.024	0.008	0.006	0.006	0.025	0.028	0.027									
21. MmcCHN02	0.014	0.015	0.003	0.005	0.005	0.006	0.031	0.031	0.039	0.036	0.002	0.032	0.037	0.032	0.033	0.034	0.036	0.039	0.038	0.031								
22. MmcIND04	0.005	0.006	0.016	0.018	0.018	0.019	0.031	0.031	0.036	0.035	0.015	0.032	0.034	0.032	0.033	0.034	0.033	0.036	0.036	0.031	0.015							
23. MmcTWN01	0.006	0.007	0.016	0.018	0.018	0.019	0.029	0.029	0.034	0.034	0.015	0.030	0.033	0.030	0.031	0.032	0.032	0.035	0.034	0.029	0.015	0.007						
24. MmNPL01	0.031	0.032	0.033	0.035	0.036	0.036	0.024	0.024	0.027	0.026	0.032	0.025	0.025	0.025	0.026	0.027	0.026	0.027	0.027	0.024	0.032	0.032	0.030					
25. MmNPL02	0.035	0.036	0.038	0.040	0.040	0.040	0.030	0.030	0.029	0.026	0.037	0.031	0.027	0.033	0.032	0.031	0.028	0.029	0.028	0.030	0.037	0.036	0.034	0.011				
26. MmiIRN01	0.021	0.022	0.021	0.021	0.021	0.024	0.034	0.034	0.042	0.040	0.020	0.033	0.040	0.035	0.036	0.037	0.039	0.042	0.039	0.034	0.020	0.022	0.022	0.038	0.040			
27. MmiIRN02	0.022	0.023	0.022	0.022	0.022	0.025	0.035	0.035	0.043	0.042	0.021	0.034	0.043	0.036	0.037	0.038	0.042	0.043	0.044	0.035	0.021	0.023	0.023	0.041	0.039	0.006		
28. MmiIRN03	0.021	0.022	0.019	0.019	0.019	0.022	0.036	0.036	0.044	0.043	0.018	0.035	0.042	0.037	0.038	0.039	0.041	0.044	0.044	0.036	0.018	0.022	0.022	0.040	0.042	0.006	0.006	
29. MmdIRN01	0.029	0.030	0.033	0.035	0.036	0.035	0.022	0.022	0.007	0.005	0.033	0.023	0.004	0.025	0.024	0.023	0.005	0.006	0.005	0.022	0.032	0.030	0.028	0.021	0.023	0.036	0.039	0.038
30. MmdIRN02	0.032	0.033	0.037	0.039	0.037	0.039	0.025	0.025	0.006	0.004	0.036	0.026	0.003	0.028	0.027	0.026	0.004	0.005	0.004	0.025	0.036	0.033	0.031	0.024	0.026	0.039	0.042	0.041
31. MmmIRN01	0.032	0.033	0.034	0.034	0.034	0.037	0.005	0.005	0.031	0.030	0.033	0.006	0.027	0.009	0.006	0.007	0.027	0.031	0.030	0.006	0.033	0.033	0.031	0.025	0.031	0.034	0.035	0.036
32. MmmIRN02	0.033	0.034	0.035	0.035	0.035	0.038	0.006	0.006	0.032	0.031	0.034	0.006	0.028	0.010	0.007	0.008	0.028	0.032	0.031	0.007	0.034	0.034	0.032	0.028	0.032	0.035	0.034	0.037
33. MmbIRN01	0.007	0.008	0.016	0.018	0.018	0.019	0.029	0.029	0.034	0.034	0.015	0.030	0.033	0.029	0.031	0.032	0.032	0.035	0.034	0.029	0.015	0.007	0.006	0.028	0.033	0.022	0.023	0.022
34. MmbIRN02	0.005	0.006	0.014	0.016	0.016	0.017	0.027	0.027	0.032	0.032	0.013	0.028	0.030	0.028	0.029	0.030	0.030	0.033	0.032	0.027	0.013	0.006	0.006	0.030	0.035	0.018	0.019	0.018
35. MmbIRN03	0.006	0.007	0.016	0.018	0.018	0.019	0.029	0.029	0.032	0.033	0.015	0.030	0.033	0.030	0.031	0.032	0.032	0.035	0.034	0.029	0.015	0.007	0.006	0.030	0.033	0.022	0.022	0.022
36. MmbIRN05	0.008	0.008	0.017	0.019	0.017	0.020	0.030	0.030	0.033	0.033	0.016	0.031	0.033	0.031	0.032	0.033	0.032	0.035	0.035	0.030	0.016	0.008	0.006	0.031	0.034	0.023	0.023	0.023
37. MmbIRN06	0.006	0.006	0.015	0.017	0.017	0.018	0.028	0.028	0.033	0.033	0.014	0.029	0.032	0.029	0.030	0.031	0.031	0.034	0.033	0.028	0.014	0.006	0.006	0.032	0.036	0.019	0.020	0.019
38. MmbAFG01	0.006	0.007	0.015	0.017	0.015	0.018	0.030	0.030	0.035	0.035	0.014	0.031	0.033	0.031	0.032	0.033	0.032	0.035	0.035	0.030	0.014	0.007	0.006	0.031	0.035	0.021	0.022	0.021
39. MmbAFG02	0.006	0.006	0.015	0.017	0.017	0.018	0.028	0.028	0.034	0.033	0.014	0.029	0.032	0.029	0.030	0.031	0.031	0.034	0.031	0.028	0.014	0.006	0.005	0.030	0.034	0.021	0.022	0.021
40. MmbAFG03	0.006	0.007	0.015	0.017	0.015	0.018	0.028	0.028	0.033	0.033	0.014	0.029	0.032	0.029	0.030	0.031	0.031	0.034	0.033	0.028	0.014	0.006	0.005	0.029	0.034	0.021	0.022	0.021
41. MmbAFG04	0.007	0.008	0.014	0.016	0.014	0.017	0.027	0.027	0.034	0.034	0.013	0.028	0.033	0.028	0.029	0.030	0.032	0.035	0.034	0.027	0.013	0.007	0.006	0.028	0.032	0.020	0.021	0.020
42. MmbAFG05	0.005	0.006	0.014	0.016	0.016	0.017	0.027	0.027	0.032	0.032	0.013	0.028	0.030	0.028	0.029	0.030	0.030	0.033	0.032	0.027	0.013	0.006	0.004	0.028	0.032	0.020	0.021	0.020
43. MmbAFG06	0.006	0.007	0.018	0.020	0.020	0.021	0.031	0.031	0.036	0.033	0.017	0.030	0.035	0.030	0.033	0.034	0.034	0.037	0.036	0.031	0.017	0.007	0.009	0.035	0.037	0.022	0.021	0.022
44. MmgMDG01	0.040	0.041	0.040	0.042	0.042	0.043	0.026	0.026	0.037	0.036	0.039	0.025	0.038	0.027	0.028	0.028	0.037	0.037	0.039	0.028	0.039	0.041	0.039	0.033	0.036	0.042	0.041	0.044
45. MmgMDG02	0.039	0.040	0.038	0.041	0.041	0.042	0.025	0.025	0.036	0.035	0.038	0.024	0.036	0.026	0.027	0.027	0.035	0.036	0.038	0.027	0.038	0.039	0.038	0.032	0.035	0.041	0.040	0.043

Table 14. Continued

Haplotype	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
29. MmdIRN01																	
30. MmdIRN02	0.003																
31. MmmIRN01	0.025	0.028															
32. MmmIRN02	0.026	0.029	0.005														
33. MmbIRN01	0.028	0.031	0.029	0.032													
34. MmbIRN02	0.026	0.029	0.029	0.030	0.006												
35. MmbIRN03	0.028	0.031	0.031	0.032	0.006	0.006											
36. MmbIRN05	0.029	0.030	0.032	0.033	0.007	0.006	0.003										
37. MmbIRN06	0.027	0.030	0.030	0.031	0.006	0.003	0.006	0.007									
38. MmbAFG01	0.029	0.030	0.032	0.033	0.007	0.005	0.007	0.006	0.006								
39. MmbAFG02	0.028	0.031	0.030	0.031	0.005	0.005	0.005	0.006	0.006	0.006							
40. MmbAFG03	0.027	0.029	0.030	0.031	0.005	0.005	0.005	0.004	0.006	0.004	0.004						
41. MmbAFG04	0.028	0.030	0.029	0.030	0.006	0.006	0.006	0.005	0.006	0.005	0.005	0.003					
42. MmbAFG05	0.026	0.029	0.029	0.030	0.004	0.004	0.004	0.005	0.005	0.005	0.003	0.003	0.004				
43. MmbAFG06	0.030	0.033	0.033	0.034	0.009	0.006	0.008	0.009	0.005	0.008	0.008	0.008	0.009	0.007			
44. MmgMDG01	0.033	0.036	0.026	0.029	0.037	0.037	0.036	0.040	0.038	0.040	0.038	0.038	0.037	0.037	0.039		
45. MmgMDG02	0.032	0.035	0.025	0.028	0.036	0.035	0.035	0.039	0.037	0.039	0.037	0.037	0.035	0.035	0.038	0.001	

Table 15. Estimates of genetic distance between the different groups of *M. musculus*

Group	Mm01	Mm02	Mm03	Mm04	Mm05	Mm06	Mm07
Mm01							
Mm02	0.021						
Mm03	0.016	0.021					
Mm04	0.033	0.040	0.036				
Mm05	0.032	0.041	0.038	0.026			
Mm06	0.030	0.036	0.034	0.028	0.027		
Mm07	0.038	0.042	0.040	0.034	0.036	0.026	

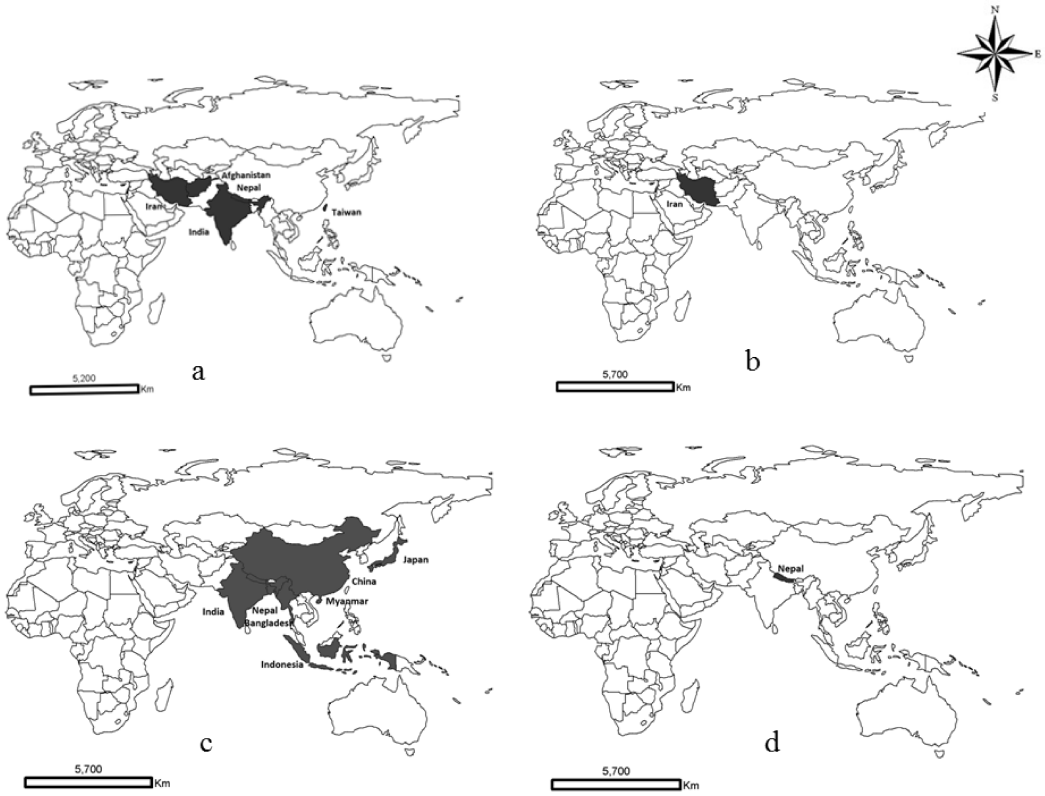

Fig. 13. Global distribution of *CytB* haplotypes of subspecies of *M. musculus*. *M. m. bactrianus*, group MM01 (a), *M. m. isaticus*, group MM02 (b), *M. m. castaneus*, group MM03 (c), *M. musculus* NEPAL, group Mm04 (d), *M. m. domesticus*, group MM05 (e), *M. m. musculus*, group Mm06 (f), and *M. m. gentilulus*, group Mm07 (g).

Fig. 13. Continued.

Before the present study, there have not been any authentic reports of *M. m. bactrianus* in Nepal. However, *M. m. castaneus* was reported in previous studies. The phylogenetic relationship, genetic distance, and a tentative estimation of divergence time suggested that *M. m. bactrianus* and *M. m. castaneus* are the closest taxa and both subspecies could have the sympatric association.

2) *Mus booduga* species group

The *M. booduga* group comprised of the haplotypes of *M. booduga*, *Mus* sp., *M. famulus*, *M. fragilicauda*, *M. terricolor*, and *M. nitidulus.* Initially, Suzuki *et al.* (2004) determined this group including three species of *Mus* namely *M. booduga*, *M. terricolor*, and *M. fragilicauda* using mitochondrial and nuclear gene analysis. However, subsequent studies of Shimada *et al.* (2007, 2009) showed *M. nitidulus* and *M. famulus* embedded phylogenetically within the *M. booduga* species group. The pairwise genetic distance between the haplotypes within the *M. booduga* species group ranged 0.001-0.189 (Table 11) and genetic distance between different species within this species group have tabulated in Table 12. The *M. booduga* and *M. terricolor* are the best-known taxa in the Indian subcontinent (Chatterjee *et al.*, 1994; Sharma, 1996). Thus, Suzuki *et al.* (2004) have introduced this species group under the subgenus *Mus*.

Two unique haplotypes were found in the four mice of *M. booduga* collected in Pokhara, Nepal and two different haplotypes determined from the reference sequenes of *M. booduga* recorded in Nepal and India by Suzuki *et al.* (2004). The pairwise genetic distance between the haplotypes were ranged between 0.001 and 0.023 (Table 11), and all the haplotypes of *M. booduga* were clustered in a group at the phylogenetic tree (Fig. 11). The genetic distance between intra populations of *M. booduga* was similar with Chatterjee

et al. (1994). Among the six taxa accompanied in the *M. booduga* species group, only two were collected in this study. Based on the genetic distance *M. booduga* has found the closest genetic relationship with *Mus* sp. (0.126) with divergence times approximately 6.137-6.862 MYBP and distant genetic relation with *M. cypriacus* (0.176) with divergence time about 8.535-9.544 MYBP. However, Suzuki *et al.* (2004) determined its close genetic relation with *M. fragilicauda* and Shimada *et al.* (2009) determined with *M. terricolor*. The genetic distance and tentative divergence time of *M. booduga* with other *Mus* species have been documented in Table 12. Very few genetic studies were found on these species. Chatterjee *et al.*, (1994) and Sharma (1996) have determined similar genetic distances between *M. booduga* and *M. terricolor* as well as *M. booduga* and *M. musculus*. The earlier study based on the mtDNA restriction fragment length polymorphism showed that *M. booduga* species group evolved simultaneously with other lineages but not before *M. cervicolor* species group (Chatterjee *et al.*, 1994). The inter-population genetic distances in *M. booduga* ranged between 0.021-0.024, which was exactly similar with the estimation of Suzuki *et al.* (2004). These two populations have separated at least 1.06-1.13 MYBP. Hubert and Hanner (2015) suggested that if the intraspecific genetic distance was higher than 0.02, the species might occur different lineages. It indicates that two populations of *M. booduga* found in Nepal and India might be different lineages. Although, the sample size of *M. booduga* was little in this study, the haplotype determined in this study represent the genetic population of *M. booduga* occurring in Nepal and likely to compared with other populations found in South Asia.

Two haplotypes (MspNPL048 and MspNPL049) of *Mus* sp. were determined in two mice collected in Kathmandu of Nepal. The genetic distance was lowest between *Mus* sp. and *M. nitidulus* recorded in Myanmar by Shimada *et al.* (2016). The haplotypes of *Mus* sp. and *Mus nitidulus* were clustered together at the phylogenetic tree (Fig. 11). Two haplotypes (MspNPL048 and

MspNPL049) of *Mus* sp. could not identify yet because they were about 95% identical with *M. nitidulus* but genetic distance was higher than intra-specific genetic distance usually find in the rodents (Baker and Bradley, 2006), which have been discussed in earlier section 'molecular identification of murids'. However, considering the genetic distance and phylogeny of *Mus* sp. it might be accompanied by the *M. booduga* species group.

3) *Mus cervicolor* species group

The *M. cervicolor* group comprised of the haplotypes of *M. cervicolor*, *M. cookii*, and *M. caroli*. It is recognized as the Asian lineage, and the taxa included in this group are distributed in East Asia and countries of the Indian subcontinents. All the haplotypes were determined from the reference sequences taken from the database and compared molecular data with other species group. Two species *M. cervicolor* and *M. cookie* are recorded in Nepal, but their molecular information is still lacking. More sampling required to collect the specimen and determine their genetic information.

The present study provided the phylogenetic relationship of three *Mus* taxa (*M. musculus*, *M. booduga*, and *Mus* sp.) occurred in Nepal. The phylogenetic relationships of these taxa have been discussed regarding three different species groups (*M. musculus*, *M. booduga*, and *M. cervicolor*) of the subgenus *Mus*. In this study, single taxon *M. musculus* was recorded from the *M. musculus* species group. Its phylogenetic analysis revealed two subspecies of *M. musculus*, *M. m. bactrianus* and *M. m. castaneus* are present in Nepal. Although, *M. m. castaneus* was reported in earlier studies, the *M. m. bactrianus* have no authentic record before this study. Based on the genetic distance and phylogenetic relationship this study suggested that *M. m. bactrianus* and *M. m. castaneus* are closest taxa probably diverged about middle Pleistocene Period. Considering the habitat and geographical

distribution, these two species could have the sympatric association. However, further studies required to understand their population status and geographic distribution in Nepal.

Two taxa *M. booduga* and *Mus* sp. were recorded from the *M. booduga* species group. Based on the phylogenetic analysis, it is found that the populations of *M. booduga* occurred in Nepal and India have close genetic relationship. The haplotypes of both *M. booduga* populations were included in the same group at the phylogenetic tree suggesting single lineage of *M. booduga* in two countries. Although the sample size of *M. booduga* was little and have collected from single geographical location of Nepal but based on this study it is predicted that there was no wide diversification on *M. booduga* populations as like to *M. musculus*.

Phylogenetically, the *Mus* sp. found in Nepal has close genetic relation with *M. nitidulus*. This study could not determine sufficient evidence for its identification at the species level but based on the genetic distance, phylogenetic position and estimated divergence time it is concluded that *Mus* sp. belonged to the *M. booduga* species group. It could be a new taxon in *M. booduga* species group but further studies of integrating both morphological and molecular analysis are required to confirm its taxonomy. This study will serve as the baseline for further taxonomic and ecological studies of *M. musculus* and *M. booduga* in Nepal. Detailed and inclusive studies are required concerning morphology and at the molecular level to determine the correct taxonomy and evolutionary relationship of *Mus* species.

4. Phylogenetic study of *Rattus* in Nepal

Altogether, twenty-six distinct haplotypes were found in the 61 sequences

of the *R. rattus*, four haplotypes in the ten sequences of *R. nitidus*, one haplotype in the seven sequences of *R. tanezumi* and one haplotype in the three sequences of *R. pyctoris* (Table 15). The haplotypes of *R. nitidus*, *R. pyctoris*, *R. rattus*, and *R. tanezumi* determined in this study have been submitted to NCBI database, which accession numbers have been tabulated in Table 15. The haplotypes of *R. rattus* comprised of 14 in Pokhara, eight in Lumbini, and four in Kathmandu. Similarly, the haplotypes of *R. nitidus* comprised of two in Kathmandu, one in Pokhara and one in Lumbini. However, the haplotypes of *R. pyctoris* and *R. tanezumi* were recorded only in Kathmandu and Lumbini, respectively. Haplotype distribution of *Rattus* species in Nepal have shown in Fig. 14. The haplotype 'RraNPL040' has represented the highest number of sequences (14 sequences) and found in all three locations.

In order to describe the phylogenetic relationship of *Rattus* within and between the species, phylogenetic tree (NJ tree) was constructed based on the pairwise genetic distance using mitochondrial *CytB* haplotypes of *R. nitidus*, *R. pyctoris*, *R. rattus*, and *R. tanezumi* and the reference sequences of *Rattus* species taken from NCBI database (Fig. 15). In NJ tree, 12 species of *Rattus* are clearly distinguished into 14 groups.

1) Phylogeny of *Rattus rattus*

The haplotypes of *R. rattus* found in this study and determined from reference sequences were clustered into three monophyletic groups at the phylogenetic tree namely *R. rattus* group I, *R. rattus* group II, and *R. rattus* group III (Fig. 15). The *R. rattus* group I is composed of the 26 unique haplotypes found in this study, two haplotypes of *R. rattus*, and three haplotypes of *R. rattus* LIII recorded in Nepal and Pakistan by Aplin *et al.*, (2011), Conroy *et al.*, (2013) and Karmacharya *et al.* (2016) (Fig. 15 and

Table 15). The *R. rattus* group II is composed of the three haplotypes recorded in India (Rra001, Rra006 and Rra007) by Aplin *et al.* (2011) and Page *et al.* (2011) and one haplotype recorded in Japan (Rra002) by Chinen *et al.*, (2005). Similarly, the *R. rattus* group III is composed of the three haplotypes of *R. rattus* LIV (RraIV001-03) recorded in Cambodia, Sri Lanka, and Vietnam by Aplin *et al.* (2011) and one haplotype of *R. rattus* recorded in Malaysia (Rra003) by Tamrin *et al.* (2011). Distributions of *CytB* haplotypes of different groups of *R. rattus* determined in this study have been shown in Fig. 16.

The thirty haplotype present in the *R. rattus* group I, considering one sequence from each haplotype group was used for the calculation of genetic distance by means of pairwise genetic distance, which showed that the genetic distance ranged between 0.001 and 0.019 (Table 16). However, the pairwise genetic distance between the haplotypes of all three groups (I, II and III) of *R. rattus* varied between 0.001 and 0.085.

Table 16. Sample used in this study

Species	Haplotype	N	Accession no.	Locality	Reference
R. rattus	RraNPL003	1	KY985274	Pokhara	This study
R. rattus	RraNPL004	3	KY002796	Pokhara	This study
R. rattus	RraNPL008	6	KY985275	Pokhara	This study
R. rattus	RraNPL009	8	KY985276	Pokhara	This study
R. rattus	RraNPL013	2	KY985277	Pokhara	This study
R. rattus	RraNPL023	1	KY985278	Pokhara	This study
R. rattus	RraNPL025	1	KY985279	Pokhara	This study
R. rattus	RraNPL026	1	KY985280	Pokhara	This study
R. rattus	RraNPL039	1	KY985281	Pokhara	This study
R. rattus	RraNPL040	14	KY002799	Pokhara	This study
R. rattus	RraNPL042	1	KY002801	Pokhara	This study
R. rattus	RraNPL051	3	KY002802	Kathmandu	This study
R. rattus	RraNPL052	1	KY985282	Kathmandu	This study
R. rattus	RraNPL081	1	KY002808	Lumbini	This study
R. rattus	RraNPL088	1	KY985283	Lumbini	This study
R. rattus	RraNPL096	1	KY985284	Lumbini	This study
R. rattus	RraNPL099	1	KY002812	Kathmandu	This study
R. rattus	RraNPL100	2	KY002813	Kathmandu	This study
R. rattus	RraNPL134	3	KY985288	Lumbini	This study
R. rattus	RraNPL140	1	KY985289	Lumbini	This study
R. rattus	RraNPL144	2	KY985290	Lumbini	This study
R. rattus	RraNPL146	1	KY985291	Lumbini	This study
R. rattus	RraNPL158	1	KY985292	Lumbini	This study
R. rattus	RraNPL192	2	KY985285	Pokhara	This study
R. rattus	RraNPL199	1	KY985286	Pokhara	This study
R. rattus	RraNPL202	1	KY985287	Pokhara	This study
R. rattus	Rra001	1	HM217741	India	Pages *et al*., 2011
R. rattus	Rra002	1	AB211039	Japan	Chinen *et al*., 2005
R. rattus	Rra003	1	JF437010	Malaysia	Tamrin and Abdullah, 2011
R. rattus	Rra004	1	KU214581	Nepal	Karmacharya *et al*., 2016*

*, unpublished reference; N, number of *CytB* sequence.

Table 16. Continued

Species	Haplotype	N	Accession no.	Locality	Reference
R. rattus	Rra005	1	JQ814242	Pakistan	Conroy *et al*., 2013
R. rattus	Rra006	1	JN675525	India	Aplin *et al*., 2011
R. rattus	Rra007	1	JN675528	India	Aplin *et al*., 2011
R. rattus LIII	RraIII001	1	JN675599	Nepal	Aplin *et al*., 2011
R. rattus LIII	RraIII002	1	JN675601	Pakistan	Aplin *et al*., 2011
R. rattus LIII	RraIII003	1	JN675602	Pakistan	Aplin *et al*., 2011
R. rattus LIV	RraIV001	1	JN675606	Cambodia	Aplin *et al*., 2011
R. rattus LIV	RraIV002	1	JN675603	Srilanka	Aplin *et al*., 2011
R. rattus LIV	RraIV003	1	JN675611	Vietnam	Aplin *et al*., 2011
R. losea	Rlo001	1	JN675627	Thailand	Aplin *et al*., 2011
R. losea	Rlo002	1	JN675628	Thailand	Aplin *et al*., 2011
R. tanezumi	RtaNPL073	7	KY002823	Lumbini	This study
R. tanezumi	Rta001	1	JX534065	Laos	Pages *et al*., 2013
R. tanezumi	Rta002	1	KF011916	South Korea	Han *et al*., 2013*
R. tanezumi	Rta003	1	JX534118	Thailand	Pages *et al*., 2013
R. tanezumi	Rta004	1	AB355901	Vietnam	Truong *et al*., 2009
R. tanezumi	Rta005	1	JN675554	Bangladesh	Aplin *et al*., 2011
R. nitidus	RniNPL002	2	KY985270	Pokhara	This study
R. nitidus	RniNPL017	1	KY985271	Kathmandu	This study
R. nitidus	RniNPL018	6	KY985272	Kathmandu	This study
R. nitidus	RniNPL028	1	KY985273	Lumbini	This study
R. nitidus	Rni001	1	AB973110	India	Chingangbam *et al*., 2015
R. nitidus	Rni002	1	HM217479	Laos	Pages *et al*., 2010
R. nitidus	Rni003	1	FR775884	Vietnam	Balakirev and Rozhnov, 2012
R. pyctoris	RpyNPL053	3	KY587428	Kathmandu	This study
R. pyctoris	Rpy001	1	JN675511	Nepal	Aplin *et al*., 2011
R. argentiventer	Rar001	1	AB033701	Indonesia	Suzuki *et al*., 2000
R. villosissimus	Rvi001	1	EU349783	Australia	Rowe *et al*., 2008
R. sordidus	Rso001	1	GU570665	Australia	Robins *et al*., 2010
R. exulans	Rex001	1	DQ191486	Philippines	Jansa *et al*., 2006

Table 17. Pairwise genetic distance between the haplotypes of *R. nitidus*, *R. pyctoris*, *R. rattus*, and *R. tanezumi*

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1. Rra004																												
2. RraIII001	0.010																											
3. RraIII002	0.010	0.012																										
4. RraIII003	0.013	0.015	0.012																									
5. Rra006	0.047	0.049	0.049	0.053																								
6. RraIV001	0.061	0.062	0.062	0.066	0.061																							
7. RraIV003	0.061	0.062	0.062	0.066	0.061	0.006																						
8. RrI002	0.044	0.045	0.045	0.049	0.009	0.057	0.057																					
9. RrII001	0.045	0.051	0.047	0.058	0.039	0.068	0.068	0.042																				
10. RrIV001	0.064	0.065	0.062	0.069	0.065	0.012	0.009	0.063	0.073																			
11. RrV001	0.066	0.068	0.067	0.064	0.070	0.035	0.035	0.065	0.080	0.035																		
12. RrV002	0.070	0.072	0.071	0.068	0.070	0.037	0.037	0.066	0.085	0.037	0.004																	
13. Rra001	0.044	0.046	0.045	0.049	0.006	0.057	0.057	0.003	0.039	0.063	0.066	0.066																
14. Rra005	0.010	0.012	0.000	0.012	0.049	0.062	0.062	0.045	0.047	0.062	0.067	0.071	0.045															
15. Rra003	0.060	0.062	0.062	0.066	0.061	0.003	0.006	0.056	0.067	0.012	0.038	0.040	0.057	0.062														
16. Rra002	0.045	0.047	0.047	0.051	0.004	0.062	0.062	0.010	0.040	0.064	0.068	0.068	0.007	0.047	0.062													
17. RraNPL003	0.010	0.007	0.016	0.019	0.047	0.061	0.061	0.047	0.047	0.064	0.066	0.070	0.047	0.016	0.060	0.045												
18. RraNPL004	0.007	0.004	0.013	0.016	0.047	0.061	0.061	0.044	0.049	0.064	0.066	0.070	0.044	0.013	0.060	0.045	0.003											
19. RraNPL008	0.006	0.013	0.013	0.016	0.051	0.060	0.064	0.047	0.052	0.067	0.066	0.070	0.047	0.013	0.064	0.049	0.013	0.010										
20. RraNPL009	0.004	0.012	0.012	0.015	0.045	0.062	0.062	0.042	0.050	0.065	0.067	0.071	0.042	0.012	0.062	0.044	0.012	0.009	0.007									
21. RraNPL013	0.003	0.010	0.010	0.013	0.047	0.060	0.060	0.044	0.049	0.063	0.066	0.070	0.044	0.010	0.060	0.045	0.010	0.007	0.006	0.004								
22. RraNPL023	0.010	0.003	0.015	0.018	0.049	0.062	0.062	0.045	0.049	0.065	0.068	0.072	0.046	0.015	0.062	0.047	0.004	0.004	0.013	0.012	0.010							
23. RraNPL025	0.006	0.010	0.013	0.016	0.044	0.060	0.060	0.040	0.049	0.063	0.065	0.069	0.040	0.013	0.060	0.042	0.010	0.007	0.009	0.001	0.006	0.010						
24. RraNPL026	0.006	0.013	0.013	0.016	0.047	0.064	0.064	0.044	0.052	0.066	0.067	0.071	0.044	0.013	0.063	0.045	0.013	0.010	0.009	0.001	0.006	0.013	0.003					
25. RraNPL039	0.006	0.010	0.013	0.016	0.047	0.064	0.064	0.044	0.052	0.067	0.069	0.073	0.044	0.013	0.064	0.045	0.010	0.007	0.009	0.001	0.006	0.010	0.003	0.003				
26. RraNPL040	0.001	0.009	0.009	0.012	0.045	0.059	0.059	0.042	0.047	0.062	0.064	0.068	0.042	0.009	0.058	0.044	0.009	0.006	0.004	0.003	0.001	0.009	0.004	0.004	0.004			
27. RraNPL042	0.003	0.010	0.007	0.013	0.044	0.057	0.057	0.040	0.045	0.060	0.062	0.066	0.040	0.007	0.056	0.042	0.010	0.007	0.006	0.004	0.003	0.010	0.006	0.006	0.006	0.001		
28. RraNPL051	0.003	0.010	0.010	0.013	0.044	0.057	0.057	0.040	0.046	0.060	0.062	0.066	0.041	0.010	0.057	0.042	0.010	0.007	0.006	0.004	0.003	0.010	0.006	0.006	0.006	0.001	0.003	
29. RraNPL052	0.010	0.003	0.015	0.018	0.049	0.063	0.063	0.046	0.047	0.066	0.068	0.072	0.046	0.015	0.062	0.047	0.007	0.004	0.013	0.012	0.010	0.003	0.010	0.013	0.010	0.009	0.010	0.010
30. RraNPL081	0.004	0.006	0.006	0.009	0.045	0.059	0.059	0.042	0.047	0.062	0.064	0.068	0.042	0.006	0.058	0.044	0.010	0.007	0.007	0.006	0.004	0.009	0.007	0.007	0.007	0.003	0.004	0.004
31. RraNPL088	0.007	0.015	0.015	0.018	0.049	0.063	0.063	0.046	0.047	0.065	0.068	0.072	0.046	0.015	0.062	0.047	0.015	0.012	0.010	0.009	0.007	0.015	0.010	0.010	0.010	0.006	0.007	0.004
32. RraNPL096	0.010	0.006	0.018	0.021	0.049	0.066	0.066	0.045	0.049	0.069	0.071	0.075	0.045	0.018	0.066	0.047	0.006	0.006	0.013	0.012	0.010	0.003	0.010	0.013	0.010	0.009	0.010	0.010
33. RraNPL099	0.010	0.003	0.015	0.018	0.046	0.059	0.059	0.042	0.047	0.062	0.064	0.068	0.042	0.015	0.059	0.044	0.007	0.004	0.013	0.012	0.010	0.003	0.010	0.013	0.010	0.009	0.010	0.007
34. RraNPL100	0.007	0.006	0.012	0.015	0.042	0.059	0.059	0.039	0.047	0.062	0.064	0.068	0.039	0.012	0.059	0.040	0.007	0.004	0.010	0.009	0.007	0.006	0.007	0.010	0.007	0.006	0.007	0.007
35. RraNPL192	0.012	0.004	0.016	0.019	0.047	0.064	0.064	0.044	0.047	0.067	0.070	0.074	0.044	0.016	0.064	0.045	0.006	0.006	0.015	0.013	0.012	0.001	0.012	0.015	0.012	0.010	0.012	0.012
36. RraNPL199	0.004	0.012	0.012	0.015	0.045	0.062	0.062	0.042	0.050	0.065	0.067	0.071	0.042	0.012	0.062	0.044	0.012	0.009	0.007	?	0.004	0.012	0.001	0.001	0.001	0.003	0.004	0.004
37. RraNPL202	0.009	0.016	0.016	0.019	0.054	0.064	0.068	0.051	0.056	0.071	0.069	0.073	0.051	0.016	0.067	0.052	0.016	0.013	0.003	0.010	0.009	0.016	0.012	0.012	0.012	0.007	0.009	0.009
38. RraNPL134	0.003	0.010	0.010	0.013	0.047	0.057	0.057	0.044	0.049	0.060	0.062	0.066	0.044	0.010	0.056	0.045	0.010	0.007	0.006	0.004	0.003	0.010	0.006	0.006	0.006	0.001	0.003	0.003
39. RraNPL140	0.006	0.013	0.013	0.016	0.050	0.064	0.064	0.047	0.052	0.067	0.069	0.073	0.047	0.013	0.064	0.049	0.013	0.010	0.009	0.007	0.006	0.013	0.009	0.009	0.009	0.004	0.006	0.006
40. RraNPL144	0.003	0.010	0.010	0.013	0.047	0.061	0.061	0.044	0.049	0.064	0.066	0.070	0.044	0.010	0.060	0.045	0.010	0.007	0.006	0.004	0.003	0.010	0.006	0.006	0.006	0.001	0.003	0.003
41. RraNP146	0.004	0.012	0.012	0.015	0.049	0.060	0.060	0.045	0.050	0.060	0.062	0.066	0.045	0.012	0.060	0.047	0.012	0.009	0.007	0.006	0.004	0.012	0.007	0.007	0.007	0.003	0.004	0.004
42. RraNPL158	0.010	0.012	0.009	0.012	0.049	0.062	0.062	0.049	0.051	0.065	0.068	0.072	0.049	0.009	0.062	0.047	0.013	0.013	0.013	0.012	0.010	0.015	0.013	0.013	0.013	0.009	0.010	0.010
43. Rta001	0.046	0.047	0.044	0.055	0.036	0.064	0.064	0.039	0.009	0.066	0.073	0.078	0.036	0.044	0.063	0.037	0.046	0.046	0.049	0.047	0.045	0.047	0.045	0.049	0.049	0.044	0.042	0.042
44. Rta004	0.042	0.048	0.044	0.055	0.036	0.065	0.065	0.039	0.009	0.070	0.078	0.082	0.036	0.044	0.064	0.037	0.046	0.046	0.050	0.047	0.046	0.048	0.046	0.049	0.049	0.044	0.042	0.043
45. Rta002	0.041	0.046	0.042	0.053	0.037	0.065	0.065	0.040	0.007	0.067	0.074	0.078	0.037	0.042	0.065	0.035	0.044	0.044	0.047	0.045	0.044	0.046	0.044	0.047	0.047	0.042	0.041	0.041
46. Rta003	0.042	0.047	0.044	0.055	0.039	0.064	0.064	0.042	0.009	0.066	0.073	0.078	0.039	0.044	0.063	0.040	0.046	0.046	0.049	0.047	0.045	0.047	0.045	0.049	0.049	0.044	0.042	0.042
47. RtaNPL073	0.042	0.044	0.044	0.047	0.032	0.063	0.063	0.039	0.034	0.064	0.072	0.076	0.035	0.044	0.062	0.031	0.042	0.042	0.045	0.043	0.042	0.044	0.042	0.045	0.045	0.040	0.039	0.039
48. Rpy001	0.110	0.107	0.111	0.107	0.095	0.118	0.118	0.099	0.112	0.118	0.119	0.120	0.095	0.111	0.113	0.100	0.105	0.105	0.114	0.108	0.109	0.107	0.105	0.107	0.110	0.108	0.105	0.106
49. RpyNPL053	0.110	0.107	0.111	0.107	0.095	0.118	0.118	0.099	0.112	0.118	0.119	0.120	0.095	0.111	0.113	0.100	0.105	0.105	0.114	0.108	0.109	0.107	0.105	0.107	0.110	0.108	0.105	0.106
50. Rni001	0.142	0.137	0.145	0.129	0.156	0.146	0.144	0.151	0.161	0.149	0.143	0.148	0.147	0.145	0.145	0.157	0.145	0.142	0.144	0.139	0.141	0.140	0.136	0.138	0.142	0.139	0.142	0.137
51. Rni003	0.140	0.144	0.141	0.131	0.154	0.145	0.142	0.150	0.159	0.147	0.141	0.147	0.145	0.141	0.144	0.155	0.149	0.146	0.142	0.137	0.139	0.144	0.140	0.137	0.140	0.137	0.135	0.136
52. Rni002	0.143	0.138	0.146	0.130	0.157	0.147	0.145	0.153	0.162	0.150	0.144	0.149	0.148	0.146	0.146	0.158	0.146	0.143	0.145	0.140	0.142	0.141	0.137	0.139	0.143	0.140	0.143	0.138
53. RniNPL002	0.142	0.140	0.148	0.133	0.158	0.143	0.140	0.153	0.162	0.146	0.143	0.148	0.149	0.148	0.142	0.159	0.144	0.142	0.144	0.139	0.141	0.138	0.137	0.139	0.142	0.139	0.142	0.137
54. RniNPL017	0.146	0.140	0.148	0.133	0.160	0.150	0.147	0.155	0.164	0.153	0.147	0.152	0.151	0.148	0.149	0.161	0.147	0.146	0.147	0.143	0.145	0.142	0.140	0.142	0.146	0.143	0.146	0.141
55. RniNPL018	0.144	0.138	0.146	0.131	0.158	0.148	0.146	0.153	0.162	0.151	0.145	0.150	0.149	0.146	0.147	0.159	0.145	0.144	0.145	0.141	0.143	0.140	0.138	0.140	0.144	0.141	0.144	0.139
56. RniNPL028	0.140	0.138	0.146	0.131	0.156	0.145	0.142	0.152	0.160	0.147	0.141	0.147	0.147	0.146	0.144	0.157	0.142	0.140	0.142	0.138	0.139	0.137	0.135	0.137	0.140	0.137	0.140	0.136

Table 17. Continued

Haplotype	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
29. RraNPL052																												
30. RraNPL081	0.009																											
31. RraNPL088	0.015	0.009																										
32. RraNPL096	0.006	0.012	0.015																									
33. RraNPL099	0.003	0.009	0.012	0.006																								
34. RraNPL100	0.006	0.006	0.012	0.009	0.006																							
35. RraNPL192	0.004	0.010	0.016	0.001	0.004	0.007																						
36. RraNPL199	0.012	0.006	0.009	0.012	0.012	0.009	0.013																					
37. RraNPL202	0.016	0.010	0.013	0.016	0.016	0.013	0.018	0.010																				
38. RraNPL134	0.010	0.004	0.007	0.010	0.010	0.007	0.012	0.004	0.009																			
39. RraNPL140	0.013	0.007	0.010	0.013	0.013	0.010	0.015	0.007	0.012	0.006																		
40. RraNPL144	0.010	0.004	0.007	0.010	0.010	0.007	0.012	0.004	0.009	0.003	0.003																	
41. RraNPL146	0.012	0.006	0.009	0.012	0.012	0.009	0.013	0.006	0.010	0.004	0.007	0.004																
42. RraNPL158	0.015	0.006	0.015	0.018	0.015	0.012	0.016	0.012	0.016	0.010	0.013	0.010	0.012															
43. Rta001	0.044	0.044	0.044	0.047	0.044	0.044	0.045	0.047	0.052	0.046	0.049	0.046	0.044	0.047														
44. Rta004	0.044	0.044	0.044	0.047	0.045	0.045	0.046	0.047	0.053	0.046	0.049	0.046	0.047	0.048	0.009													
45. Rta002	0.042	0.042	0.042	0.045	0.043	0.043	0.044	0.045	0.051	0.044	0.047	0.044	0.044	0.046	0.006	0.007												
46. Rta003	0.044	0.044	0.044	0.047	0.044	0.044	0.045	0.047	0.052	0.046	0.049	0.046	0.044	0.047	0.006	0.009	0.006											
47.RtaNPL073	0.044	0.040	0.044	0.043	0.041	0.037	0.042	0.043	0.049	0.039	0.045	0.042	0.043	0.044	0.030	0.031	0.029	0.034										
48. Rpy001	0.108	0.108	0.113	0.107	0.104	0.108	0.105	0.108	0.119	0.110	0.114	0.110	0.110	0.112	0.106	0.106	0.106	0.101	0.103									
49. RpyNPL053	0.108	0.108	0.113	0.107	0.104	0.108	0.105	0.108	0.119	0.110	0.114	0.110	0.110	0.112	0.106	0.106	0.106	0.101	0.103	0.000								
50. Rni001	0.141	0.136	0.141	0.145	0.137	0.140	0.143	0.139	0.143	0.142	0.145	0.142	0.137	0.144	0.147	0.154	0.156	0.147	0.150	0.126	0.126							
51. Rni003	0.145	0.137	0.139	0.149	0.140	0.144	0.147	0.137	0.141	0.140	0.144	0.140	0.135	0.145	0.145	0.153	0.154	0.145	0.148	0.122	0.122	0.006						
52. Rni002	0.142	0.137	0.146	0.146	0.138	0.141	0.144	0.140	0.144	0.143	0.146	0.143	0.138	0.145	0.148	0.156	0.157	0.148	0.151	0.127	0.127	0.003	0.009					
53. RniNPL002	0.141	0.139	0.141	0.143	0.137	0.140	0.141	0.139	0.143	0.142	0.146	0.142	0.137	0.147	0.149	0.156	0.158	0.149	0.152	0.126	0.126	0.006	0.009	0.009				
54. RniNPL017	0.145	0.139	0.145	0.147	0.140	0.144	0.145	0.143	0.147	0.146	0.149	0.146	0.140	0.147	0.151	0.158	0.160	0.151	0.154	0.130	0.130	0.003	0.009	0.006	0.006			
55. RniNPL018	0.143	0.137	0.143	0.145	0.138	0.142	0.143	0.141	0.145	0.144	0.147	0.144	0.138	0.145	0.149	0.156	0.158	0.149	0.152	0.128	0.128	0.001	0.007	0.004	0.004	0.001		
56. RniNPL028	0.139	0.137	0.140	0.141	0.135	0.138	0.139	0.138	0.141	0.140	0.144	0.140	0.135	0.145	0.147	0.155	0.156	0.147	0.150	0.125	0.125	0.004	0.007	0.007	0.001	0.004	0.003	

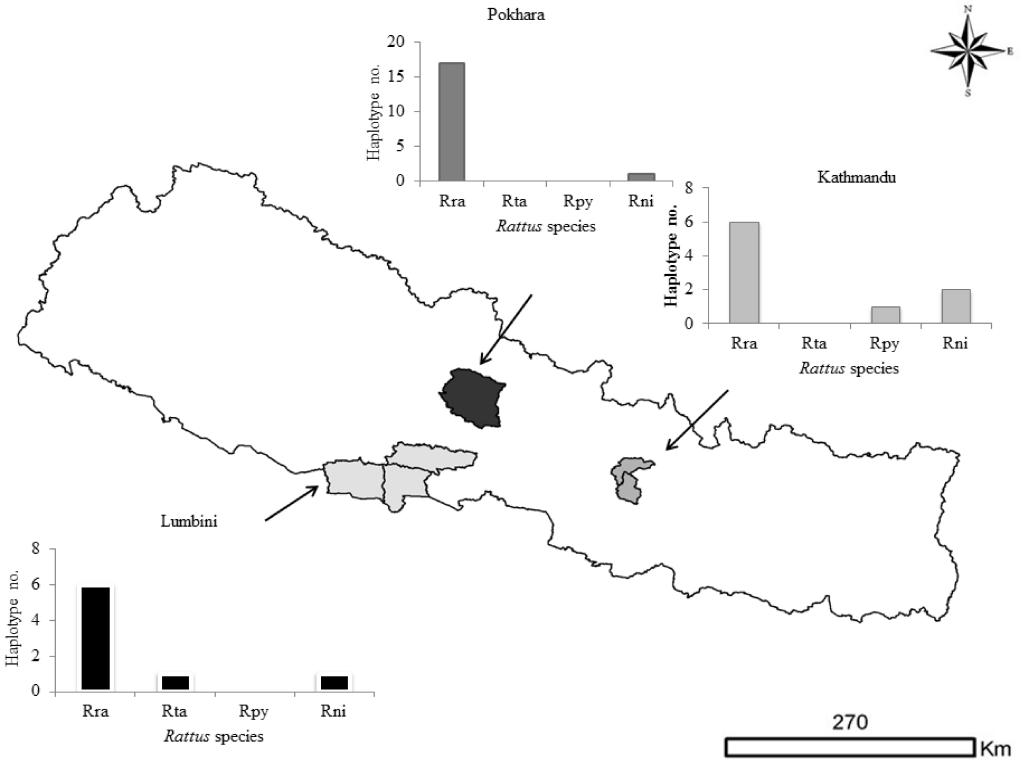

Fig. 14. Distribution of *CytB* haplotypes of *Rattus* species collected in Nepal. In Lumbini, CytB sequences of *R. nitidus* (Rni), *R. rattus* (Rra), and *R. tanezumi* (Rta) were 1, 10, and 7, respectively. In Pokhara, CytB sequences of *R. nitidus* and *R. rattus* were 2 and 42, respectively. In Kathmandu, CytB sequences of *R. nitidus*, *R. pyctoris* (Rpy), and *R. rattus* were 7, 3, and 10, respectively.

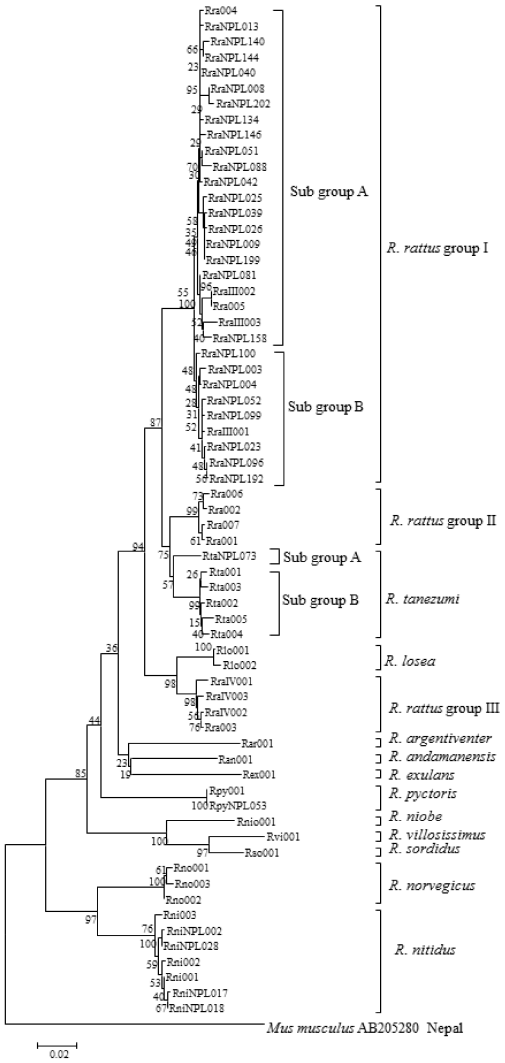

Fig. 15. Phylogenetic tree for the *CytB* haplotypes of *Rattus* species. The NJ tree was constructed from the genetic distances based on the nucleotide polymorphisms among *CytB* haplotype sequences. Genetic distances were calculated using Tamura-Nei's model (Tamura and Nei, 1993). Bootstrap values for internal nodes were given at each node. Detail information of haplotypes corresponding to those in figure have been explained in Table 15.

This analysis showed that there was wide variation in pairwise genetic distance between the haplotypes of *R. rattus* found in the different countries. Similarly, the intergroup genetic distance was calculated for the estimation of the genetic relationship between various groups of the *R. rattus*. The *R. rattus* group I was found genetically closer with *R. rattus* group II (0.045) as compare to *R. rattus* group III (0.062). Yasuda *et al.* (2014) also determined the similar genetic relation between RrC LI (*R. rattus* group I) and RrC LIII (*R. rattus* group II). The mean genetic distance within the group was found 0.009, 0.007, and 0.008 in *R. rattus* group I, *R. rattus* group II, and *R. rattus* group III, respectively, and overall mean distance was 0.085. The tentative divergence time between *R. rattus* group I and *R. rattus* group II was estimated 2.097-2.344 MYBP and *R. rattus* group I and *R. rattus* group III was estimated 2.783-3.112 MYBP.

The *R. rattus* group I have been distinguished into two subgroups consisting of 18 haplotypes (37 sequences) in subgroup IA and eight haplotypes (24 sequences) in subgroup IB (Fig. 15). Both subgroups occupied the haplotypes found in three locations Lumbini, Pokhara, and Kathmandu. The mean genetic distance within the subgroup IA and IB were 0.008 and 0.002 respectively. The inter-group genetic distance between the subgroups IA and IB was 0.012 and the tentative divergence time was estimated 0.529-0.592 MYBP. These results indicate that the *R. rattus* abundant in Nepal could be two different populations, which have a sympatric association and could not distinguish through the morphological analysis. A further molecular study using nuclear markers are required to confirm their taxonomic different.

The genetic distance between 12 species of *Rattus* used in this phylogenetic study revealed that *R. rattus* have the closest genetic relation with *R. tanezumi*

(Table 17) as like to Pages *et al*., (2011), Yasuda *et al*., (2014), Chingangbam *et al*., (2015). The genetic relationship of *R. rattus* was found on the reducing order with other species *R. losea*, *R. andamanensis*, *R. pyctoris*, *R. argentiventer*, *R. exulans*, *R. niobe*, *R. sordidus*, *R. nitidus*, *R. norvegicus*, and *R. villosissimus*, which were ranged between 0.065 and 0.153.

The *R. rattus* is a polytypic species having several mitochondrial DNA lineages have been found in different continents and Islands (Musser and Carleton, 2005; Robins *et al*., 2007; Page *et al*., 2010; Aplin *et al*., 2011). Aplin *et al*. (2003) introduced new term RrC for the indication of some closely related species that presumably arose in different geographical areas but intermixed their ranges. The species under the RrC and their mitochondrial DNA lineages are mostly morphologically indistinguishable and probably resulted from the interbreeding and gene flow, which were defined by various terminologies by different authors (Aplin *et al*., 2003a; Musser and Carleton, 2005, Robins *et al*., 2007, Page *et al*., 2010, and Aplin *et al*., 2011). More recently, Aplin *et al*. (2011) studied the phylogenetic relationship of black rats abundant in Asia and determined the six mitochondrial DNA lineages of *R. rattus*, which were named as *R. rattus* lineage I (LI) to *R. rattus* lineage VI (LVI) at the phylogenetic tree. However, the *R. rattus* LII, *R. rattus* LV and *R. rattus* LVI were already identified and used in many kinds of literature as *R. tanezumi*, *R. losea*, and *R. tiomanicus*, respectively (Pages *et al*., 2010; Chingangbam *et al*., 2014a,b). Therefore, this study has excluded these species from the *R. rattus* group and given particulur species name at the phylogenetic tree. The three groups of *R. rattus* (I–III) used in this study could not describe yet as a different species.

Recent studies on the genetic composition and phylogenetic analysis of black rats (Pages *et al*., 2010; Aplin *et al*., 2011; Yasuda *et al*., 2014) revealed that

these three groups of *R. rattus* have different geographical distribution in the Asia. The *R. rattus* group I, *R. rattus* group II, and *R. rattus* group III have allopatric natural ranges occurred on the Himalayan foothills of Nepal and Pakistan (RrC LIII), southern India (RrC LI), and lowland Indochina including the Islands of the Malay Archipelago (RrC LIV). The three groups of *R. rattus* determined in this study could differentiate by chromosome number. Yosida (1980) has described the karyotypes of Asian population of *R. rattus* (2n=42) but it has not confirmed yet either it included Himalayan population (*R. rattus* group I) or not. Most probably, this group has no karyotype study yet. The *R. rattus* group II and *R. rattus* group III have 2n=38 and 2n=40 karyotypes, respectively (Yosida, 1980). Yosida (1980) demonstrated that the rats occurred in *R. rattus* group III is the transitional state between ancestral 2n=42 (*R. tanezumi*) and 2n=38 karyotypes. Robins *et al.* (2007) suggested it to be the association with *R. rattus diardii* but it could be a different species of *Rattus* because its karyotype is different from native rats found in Indian subcontinents. In addition, the position of *R. rattus* group III located after *R. tanezumi* and *R. losea* from the *R. rattus* groups I and II at the NJ tree determined in this study. Based on the topology of the phylogenetic tree and regional sympatry with *R. tanezumi* abundant in Thailand, Pages *et al.* (2010) also considered it is a new taxon in Muridae. The phylogeny of the *R. rattus* group I have limitedly studied in the past. Aplin *et al.* (2011) first time studied its phylogeny. Yasuda *et al.* (2014) also briefly discussed its phylogeny based on the finding of the Aplin *et al.*, (2011). This study has sampled the *R. rattus* from lowland *terai* region (about 90 m) to the middle mountainous region (up to 2,335 m) in a great scale but could not record other groups of *R. rattus* found in South Asia.

Table 18. Matrix of genetic distance and estimation of divergence time using genetic distance among the different species of *Rattus*

Species	Divergence time (MYBP)[1]												
	Rra	Rta	Rlo	Rpy	Rni	Ran	Rar	Rvi	Rso	Rex	Rni	Rno	Mmu
R. rattus	-	2.370-2.650	3.251-3.635	5.397-6.035	7.105-7.945	4.793-5.360	5.394-6.032	7.575-8.470	7.003-7.831	5.395-6.033	6.677-7.466	7.285-8.146	11.00-12.300
R. tanezumi	0.048	-	3.798-4.247	5.192-5.806	7.547-8.438	4.668-5.22	5.079-5.679	7.585-8.482	7.299-8.162	5.177-5.789	7.324-8.189	7.694-8.603	11.244-12.573
R. losea	0.066	0.077	-	5.880-6.575	7.187-8.036	5.221-5.838	5.127-5.733	7.858-8.786	7.388-8.261	5.406-6.045	6.927-7.746	6.141-6.866	10.902-12.191
R. pyctoris	0.109	0.105	0.119	-	6.216-6.951	5.353-5.986	6.731-7.526	7.475-8.358	6.797-7.601	7.044-7.876	7.263-8.122	6.778-7.579	11.456-12.810
R. nitidus	0.143	0.152	0.145	0.125	-	6.273-7.014	8.282-9.261	8.121-9.080	7.323-8.189	7.647-8.550	6.886-7.700	3.549-3.968	10.089-11.282
R. andamanensis	0.097	0.094	0.105	0.108	0.126	-	5.208-5.824	6.887-7.701	6.491-7.258	4.931-5.514	6.310-7.055	7.148-7.993	11.585-12.954
R. argentiventer	0.109	0.102	0.103	0.136	0.167	0.105	-	7.665-8.571	6.857-7.667	5.539-6.193	8.039-8.989	7.556-8.449	11.600-12.970
R. villosissimus	0.153	0.153	0.158	0.151	0.164	0.139	0.155	-	2.281-2.551	6.724-7.519	3.794-4.242	8.249-9.224	12.813-14.328
R. sordidus	0.141	0.147	0.149	0.137	0.148	0.131	0.138	0.046	-	6.080-6.799	4.059-4.539	7.433-8.311	11.983-13.399
R. exulans	0.109	0.104	0.109	0.142	0.154	0.099	0.112	0.136	0.123	-	7.395-8.269	7.764-8.681	11.112-12.425
R. niobe	0.135	0.148	0.140	0.146	0.139	0.127	0.162	0.076	0.082	0.149	-	6.877-7.690	12.659-14.155
R. norvegicus	0.147	0.155	0.124	0.137	0.072	0.144	0.152	0.166	0.150	0.157	0.139	-	10.504-11.745
M. musculus	0.222	0.227	0.220	0.231	0.203	0.234	0.234	0.258	0.242	0.224	0.255	0.212	-

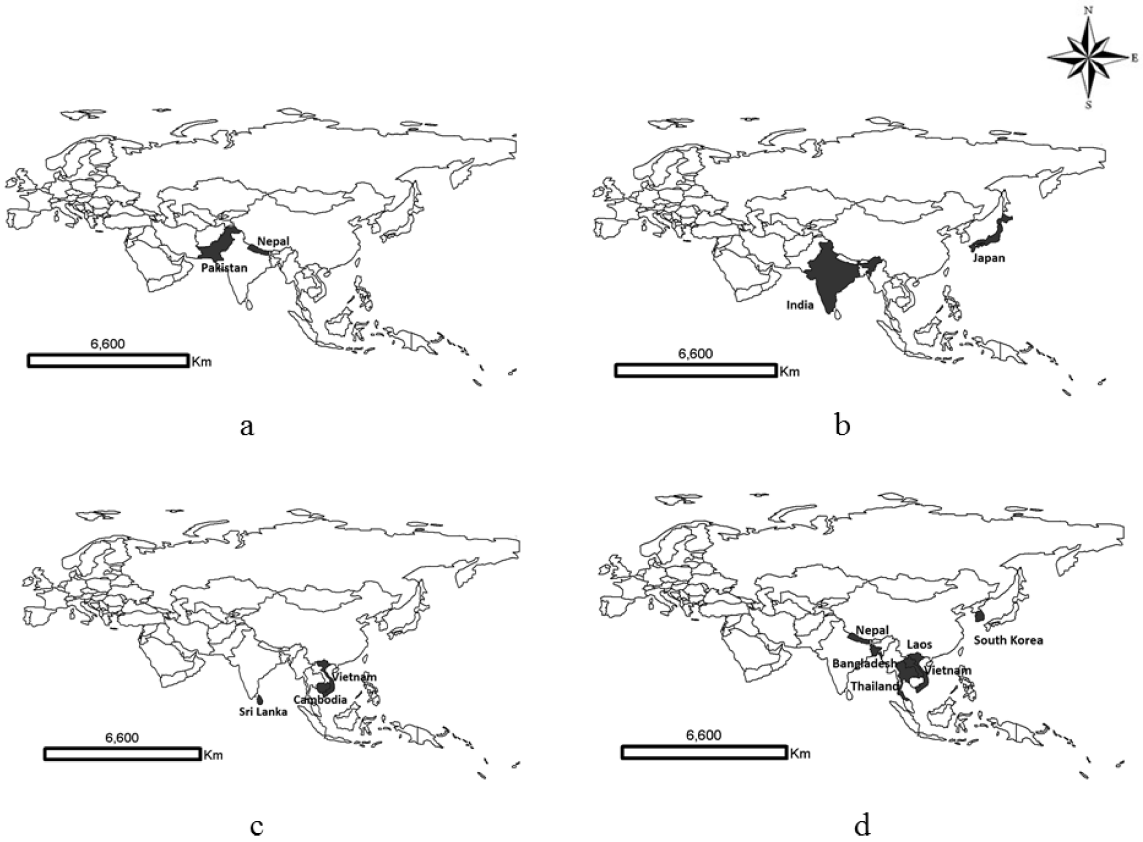

Fig. 16. Distribution of *CytB* haplotypes of *Rattus* species used in this study. Dark color showed the countries having the distribution of *R. rattus* group I (a), *R. rattus* group II (b), *R. rattus* group III (c), *R. tanezumi* (d), *R. losea* (e), *R. pyctoris* (f), and *R. nitidus* (g).

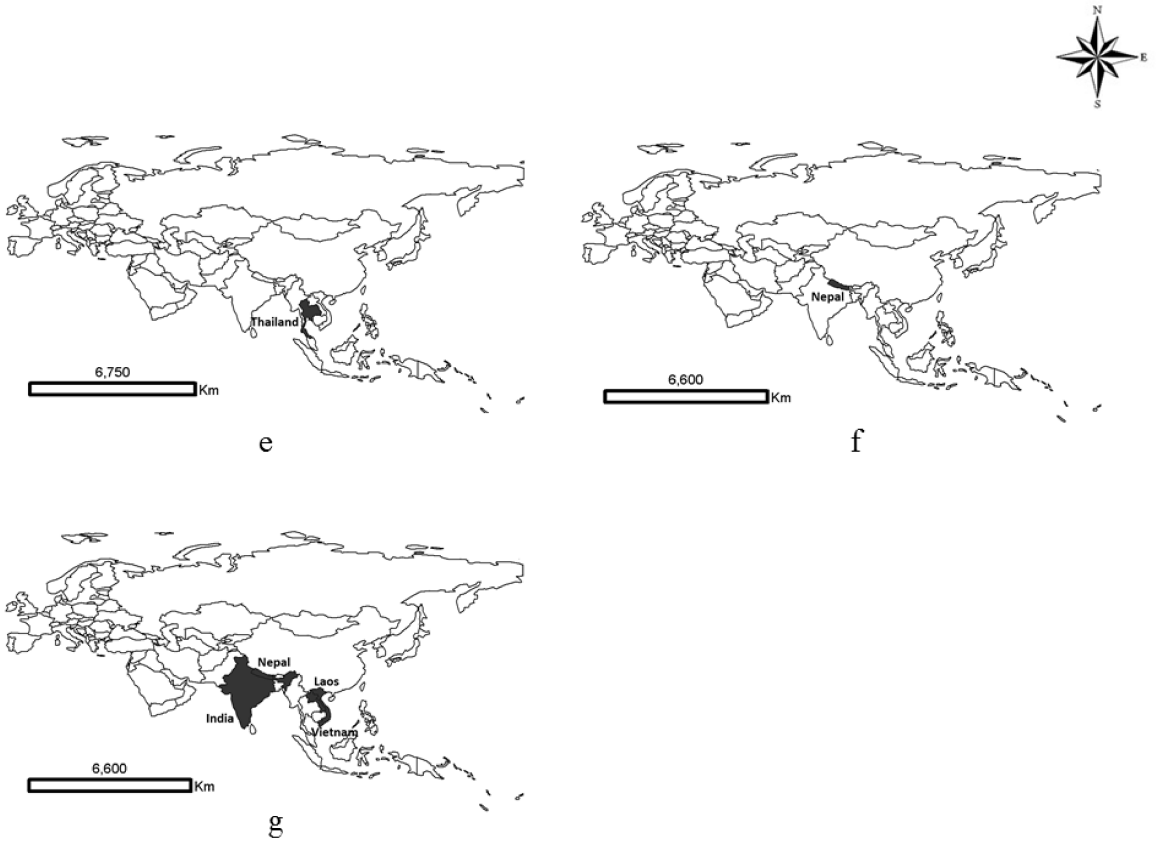

Fig. 16. Continued.

Although, lowland of Nepal is geographically connected to the mainland of India, the *R. rattus* group II found in India could not detect in Nepal.

This study showed that Nepal and Pakistan occupied the genetically distinct population of *R. rattus* (Himalayan population), which are widely distributed in Nepal in various habitat. Based on this study and reporting of Aplin *et al.* (2011), it is assumed that Northern part of South Asia along the Himalayan range including the landmass of Pakistan, India, and Nepal could be the geographical range of this population. Further study required to understand its evolutionary phenomenon and population expansion in Nepal and neighboring countries.

2) Phylogeny of *Rattus tanezumi*

One unique haplotype (RtaNPL073) of *R. tanezumi* was found in seven sequences of *R. tanezumi* recorded in Nepal. In the NJ tree, it has grouped with five haplotypes (Rta001-05) of *R. tanezumi* recorded in South and East Asian countries, Thailand and Laos by Pages *et al.* (2013), Vietnam, Bangladesh, and South Korea by Truong *et al.*, (2009), Aplin *et al.*, (2011), and Han *et al.*, (2013), respectively (Fig. 15 and Table 15). Distribution of *CytB* haplotypes of *R. tanezumi* used in this study have been shown in Fig. 16. The pairwise genetic distance was calculated between the haplotypes of *R. tanezumi*, which was ranged between 0.007 and 0.034 (Table 16). Based on the pairwise genetic distance the *R. tanezumi* recorded in Nepal has closest genetic relation with the *R. tanezumi* recorded in South Korea. Out of the 12 species of *Rattus* used in the phylogenetic analysis, it has closest genetic relationship with *R. rattus* (Table 17). The estimation of the genetic distance between these two species

was found similar to the earlier studies (Tollenaere *et al*., 2010, Page *et al*., 2011, Yasuda *et al*., 2014). The overall mean distance within the *R. tanezumi* group was found 0.009 and tentative divergence time was 2.370-2.650 MYBP with its closest species (*R. rattus*). In contrast to Robins *et al*. (2010), the estimation of tentative divergence time was found higher in this study. The *R. tanezumi* group comprised two subgroups consist of the haplotype RtaNPL073 in subgroup A and the haplotypes Rta001-05 in subgroup B consisting the haplotype determined in Nepal and some East Asian Countries, respectively (Fig. 15). The inter-group genetic distance between these two subgroups was 0.031, overall mean distance was 0.085, and tentative divergence time was estimated 1.420-1.588 MYBP between the subgroups A and B. The intergroup genetic distance was found higher than 0.02, which suggested these two subgroups were different lineages (Hubert and Hanner, 2015).

R. tanezumi is genetically distinct species was distinguished into RrC LII on phylogenetic analysis (Aplin *et al*. 2011; Yasuda *et al*., 2014). It has 2n=42 karyotypes (Yosida *et al*., 1974, 1980; Baverstock *et al*., 1983; Chingangbam *et al*., 2014a). Yosida *et al*. (1974) first time distinguished it from another group of '*Rattus rattus*' (2n=38) and named as Asian type. Musser and Carleton (2005) described *R. tanezumi* complex based on the preliminary work of the Aplin *et al*. (2003a) that have included more than one species. It is believed to be an indigenous species of Southeast Asia (Niethammer and Martens, 1975), that has been introduced to East Asia (Japan, South Korea, Taiwan, Philippines, and Vietnam), New Guinea, Fiji, and Africa through transportation by humans (Musser and Carleton, 2005). Aplin *et al*. (2003b) suggested *R. tanezumi* have two taxa, one taxon is endemic to South East Asia that is recorded in Vietnam, southern Laos, and Cambodia and another is northern and South Asian taxon

recorded in Bangladesh, Hong Kong, northern Laos and northern Vietnam and Japan. They also suggested that these two taxa could have the sympatric association. However, in this study, the haplotypes of *R. tanezumi* recorded in Laos, Vietnam, Bangladesh, and South Korea clustered in the same group at the phylogenetic tree. Although Bangladesh is located in South Asia, it may have South Asian endemic taxon, but the haplotype recorded in Bangladesh took the position with the haplotypes recorded in East Asian countries. Therefore, it's hard to predict the haplotypes present in the subgroup B were either endemic to South East Asia or South Asia. Interestingly, the haplotype of *R. tanezumi* found in Nepal have been placed in a different subgroup A, which could be the sister taxa of the haplotypes present in the subgroup B. Based on this result three possibilities may have on the *R. tanezumi* abundant in Nepal. It could either be the South Asian endemic taxon or be the hybrid taxon between South Asian and East Asian taxa as obtained by Aplin *et al.* (2003b) in Sunda Shelf Island. If both are not it could be the new taxon, which has not studied yet. Single population and recorded only in lowland near to the Indo-Nepal border may suggest the relatively recent introduction of this species. Therefore, the further study required to confirm its taxonomy at the population level.

There was no authenticated record of *R. tanezumi* in Nepal before this study. Musser and Carleton (2005) have mentioned synonyms of *R. tanezumi* based on the findings of Hodgson (1845) (*Mus brunneus* and *Mus brunneusculus*), but a report by Hinton and Fry (1923) argued that those are the subspecies of *R. rattus* rather than of *R. tanezumi.* This molecular identification and the phylogenetic study confirmed its presence in Nepal, which has a sympatric association with *R. rattus* in human settlements and agricultural land.

3) Phylogeny of *Rattus pyctoris*

One unique haplotype (RpyNPL053) of *R. pyctoris* was found in the three sequences of *R. pyctoris* and two reference sequences recorded in Nepal by Aplin *et al.* (2011). The sequences of *R. pyctoris* generated in this study were 100% identical with reference sequences. Until recently, *CytB* sequences of *R. pyctoris* have been reported only from Nepal (Fig. 16). The pairwise genetic distance between the haplotypes of other species ranged between 0.101 and 0.119 (Table 16). Based on the genetic distance the *R. pyctoris* has its closest genetic relation (0.105) with *R. tanezumi* having tentative divergence 5.192-5.806 MYBP. Similarly, it has most distant relationship with *R. villosissimus* (0.151) having tentative divergence 7.475-8.358 MYBP. The *R. pyctoris* (*R. turkestanicus*, *R. rattoides*) have type locality Uzbekistan (Corbet and Hill, 1992) but Musser and Carleton (2005) mentioned its locality is in Kyrgyzstan. It has broad and enclosed geographical distribution in highlands of Middle East to central Asia, Southeast Asia, and Himalayan range including Nepal, North India, and China (Agrawal, 2000; Aplin *et al.*, 2003a). According to Aplin *et al.* (2003a), there is a taxonomic confusion on the Southeastern Chinese populations, which is usually reported as *R. rattoides* but it is unclear whether these populations are typical *R. pyctoris* abundant in central Asia or not. These two populations have variations in chromosome morphology, but they did not mention the type of variations in chromosome between two populations. In Nepal, Hodgson (1845) identified this species as '*Mus*? *pyctoris*' but Nepalese specimens are found to be identified using either *rattoides* or *turkestanicus*. However, the molecular information of Nepalese specimens have been recorded as *pyctoris* in the database. The phylogenetic analysis of *R. pyctoris* has not discussed before this

study. Aplin *et al.* (2011) introduced this species as a *R. rattus* complex and showed in the phylogram but they did not discuss in detail about its phylogeny. In this study, the *R. pyctoris* has a distinct group in the phylogenetic tree, placed between *R. exulans* and *R. niobe* (Fig. 15). This study revealed a single population of *R. pyctoris* exists in Nepal. However, further studies are required to understand its geographical distribution in Nepal, population status, and their phylogenetic relationship.

4) Phylogeny of *Rattus nitidus*

Four unique haplotypes (RniNPL002, RniNPL017-18, RniNPL028) of *R. nitidus* was found in ten sequences of *R. nitidus* recorded in Nepal. In the NJ tree, it has grouped with three haplotypes (Rni001-03) of *R. nitidus* recorded in Laos, Vietnam, and India by Pages *et al.* (2010), Balakirev and Rozhnov, (2012), and Chingangbam *et al.* (2015), respectively (Fig. 15 and Table 15). Distribution of *CytB* haplotypes of *R. nitidus* used in this study has been shown in Fig. 16. The pairwise genetic distance between the four haplotypes found in Nepal ranged between 0.001 and 0.006, but in the overall analysis, it was varied 0.001 to 0.009 (Table 16). It indicates that there was no sharp genetic variation among the haplotypes of *R. nitidus* recorded in four countries (Nepal, India, Laos, and Vietnam). In contrast to this study, Chingangbam *et al.* (2015) found a higher range of genetic distance between the haplotypes of *R. nitidus* found in Manipur, India. The overall mean distance of *R. nitidus* was found 0.005. Before this phylogenetic analysis, there was confusion on the identification of these haplotypes because they have an equal identity with *R. norvegicus* at similarity search. However, the genetic distance and grouping of these haplotypes with *R.*

nitidus identify them at the species level. Similar to Pages *et al.* (2011), this study also determined the lowest genetic distance (0.072) between *R. nitidus* and *R. norvegicus* (Table 17), indicating that these two species have the closest genetic relationship. The tentative divergence time between these two closely related species was estimated approximately 3.549–3.968 MYBP. Similarly, *R. nitidus* has distant genetic relation with *R. argentiventer* having tentative divergence time 8.282–9.261 MYBP. The mitochondrial *CytB* sequences of *R. nitidus* have found virtually identical with *R. norvegicus*. These two species have the same number of karyotypes (2n=42) (Chingangbam *et al.*, 2014a). Thus, there could be confusions on species identification. However, calculation of genetic distance and analysis of phylogeny can distinguish these two species.

R. nitidus is an indigenous species to Southeast Asia currently occurs in hilly regions of Nepal, India, South China, Vietnam, Laos, Thailand, Indonesia (Aplin *et al.*, 2003a). Despite its widespread distribution, molecular information is limitedly available (Pages *et al.*, 2010; Balakirev and Rozhnov, 2012). The origin, homeland, and its dispersal have not been discussed yet by molecular phylogenetic studies (Chingangbam *et al.*, 2015). In Nepal, this study recorded the *R. nitidus* from lowland to the higher mountainous region. Although the sample size of *R. nitidus* was little, it provided the molecular information of Nepalese populations and determined the phylogenetic relationship within and between the species. However, the further study required to understand the population status and geographical distribution.

5) Phylogeny of *Rattus andamanensis and Rattus norvegicus*

R. andamanensis and *R. norvegicus* are recorded in Nepal (Baral and Shah,

2008; Jnawali *et al*., 2011) but mtDNA *CytB* gene sequences of these taxa could not generate in this study. Thus, the phylogenetic position of these species was studied using reference sequences taken from NCBI database. Based on the genetic distance, *R. andamanensis* is genetically close with *R. exulans* having tentative divergence time approximately 4.931–5.514 MYBP (Table 17). As discussed above, the *R. norvegicsus* is genetically close with *R. nitidus*. They were diverged approximately, 3.549–3.968 MYBP.

The present study provided the phylogenetic relation of four species of *Rattus* (*R. rattus*, *R. tanezumi*, *R. nitidus*, and *R. pyctoris*) occurred in Nepal. It is the first molecular phylogenetic study of *R. tanezumi*, *R. nitidus*, and *R. pyctoris* on Nepalese specimens. This study revealed the *R. rattus* found in Nepal and Pakistan have different group compared to the other Asian countries, which comprised two distinct subgroups have the possibility of being two distinct populations. Similarly, *R. tanezumi* also have two subgroups at which the haplotype found in Nepal being placed in a different subgroup, which could be the different population of *R. tanezumi* than the East Asian countries.

In Nepal, several cases of human migration from India and China were found in the history. Together with human migration, murids taxa including *Rattus* probably entered, and hybridization or genetic introgression and adaptive radiation may occur in *R. rattus* and result in the high genetic diversity. Further study required to understand the genetic admixture on this species. The sample size of *R. tanezumi*, *R. nitidus*, and *R. pyctoris* were low, but the molecular information provided on these species will be valuable for understanding their taxonomy and their phylogenetic relationship. This study will be the baseline for the future studies on *Rattus* found in Nepal. However, further studies using nuclear markers and karyotype analysis are recommended for their detail taxonomy.

IV. CONCLUSIONS

Taxonomic studies of murids have been carried out on specimens collected in three locations Lumbini, Pokhara, and Kathmandu of Nepal using morphological and molecular analyses. Five species *B. bengalensis*, *M. booduga*, *N. fulvescens*, *R. pyctoris*, and *T. indica* were identified through morphological analysis. All the morphological identification were further confirmed by molecular analysis except *T. indica*. The *CytB* gene of *T. indica* could not amplify either due to the low quality of DNA or amplification failure. The morphometric comparisons were carried out between male and female of *R. rattus*, *M. musculus* and *T. indica*, which revealed there was no sexual dimorphism except *T. indica*. In *T. indica*, male was significantly bigger dimension than female. Two populations of *M. musculus* found in Lumbini and Pokhara were distinguished by different coat color but there was no significant difference on morphometric comparisons. Two species *R. rattus* and *R. tanezumi* have not consistently discernible coat color difference within and between the species and also have not a significant difference in morphometric measurement.

Molecular technique was used for the identification of murids collected in this study, which successfully identified four genera and eight species (*B. bengalensis*, *M. booduga*, *M. musculus*, *N. fulvescens*, *R. nitidus*, *R. rattus*, *R. pyctoris*, and *R. tanezumi*). Two cryptic species *R. rattus* and *R. tanezumi*, were distinguished clearly, at which *R. tanezumi* was the new record for Nepal. The haplotypes of *B. bengalensis* was over 99.30% identical with the *B. bengalensis* found in Bangladesh. It indicates these two populations are genetically close. The haplotypes of *M. booduga* determined in this study was found 99.88% identical

with *M. booduga* recorded in Gorkha of Nepal. The genetic distances between the subpopulation of *M. booduga* found in Nepal ranged between 0.001-0.004 however, it was varied with Indian population by 0.021-0.024. They were placed in the two different groups at the phylogenetic tree, suggested that these two populations have different lineages. These two populations might be separated long time ago and evolve simultaneously. *M. booduga* is a native species of Indian subcontinent. The phylogenetic study of *M. musculus* revealed two subspecies of *M. musculus*, *M. m. bactrianus* and *M. m. castaneus* are existing in Nepal. The *M. m. castaneus* was reported in earlier studies, but *M. m. bactrianus* is the first record for Nepal. These two subspecies were found in two different locations of Nepal. *M. m. bactrianus* was recorded in Pokhara and *M. m. castaneus* was recorded in Lumbini. The *M. m. bactrianus* have been recorded in West Asia especially in Iran and Afghanistan (Yonekawa *et al.*, 1981; Hamid *et al.*, 2017), but it was the first record for the South Asia. It is predicted that the middle mountainous region, such as in Pokhara, could be an area where this subspecies is most likely found. However, further study is required to understand its distribution in Nepal and surrounding countries. It is also required to study either it is a native taxa of Nepal or introduced along human migration. It is believed that Indian subcontinent including Nepal is a homeland of the *M. m. castaneous* (Prager *et al.*, 1998). Although this study recorded *M. m. castaneous* in low land near to the Indo-Nepal boarder, earlier studies showed it is abundant in higher mountainous region too. Therefore, this study assumed it is a native population of Nepal instead of introduced species. However, additional studies are required for the justification. Two haplotypes of *Mus* species found in Nepal could not identify at species level, but genetically, it was found close with *M. nitidulus* recorded in Myanmar. It has 95% identical

with *M. nitidulus* but genetic distance was higher than intraspecific genetic distance usually find in the rodents (Baker and Bradley, 2006). Therefore, further molecular analysis including nuclear markers and morphometric analysis including cranial analysis required to understand its taxonomic status.

Based on the morphological characteristics described in earlier report *N. fulvescens* has been identified morphologically. However, in molecular analysis it was found 94% identical with *N. fulvescens* recorded in China, with genetic distance 0.066, indicating different lineages. The tentative divergence time between these two populations was estimated 3.3 MYBP. These two populations are geographically isolated due to having higher Himalayan range bordering to the two counties. Therefore, low identity and wide genetic diversity are quite considerable (Li *et al*., 2015). Based on the genetic distance, the haplotypes of *R. nitidus* determined in this study have close genetic relation with Indian population. It is geographically distributed east to west of Nepal.

R. nitidus recorded in Nepal, East India, Laos, and Vietnam has found genetic distance between 0.001 and 0.009 and clustered in a same group at the phylogenetic tree representing close genetic relationship. It is believed to be a native species of Southeast Asia and its rapid range expansion occurred in North India, Bhutan, central and East China. This study showed it is recently dispersed species and have no significant local evolution. In Nepal it has been recorded from central and western region. Additional studies required to understand its subpopulations and distribution in other parts of the country. Phylogeny of *R. pyctoris* has not studied before this study. It has type locality Uzbekistan and distributed in highlands of Middle East to central Asia, Southeast Asia and Himalayan range. Phylogenetic study showed it has close genetic relation with *R. tanezumi* diverged approximately 5.192–5.806 MYBP. In Nepal, it could be

introduced from West towards the East because human migration trend was found in Nepal from West to the East within few thousand years. Further studies with specimens collection from different regions are required to understand its population status and distribution pattern in Nepal.

The *R. rattus* is taxonomically most complex species have wide genetic diversity. In Asia three distinct groups of *R. rattus* found in this study. The *R. rattus* existing in Nepal and Pakistan were clustered together in a group at the phylogenetic tree. The other two other groups composed from the rats of India and Japan in one group and Cambodia, Vietnam, Malaysia and Sri Lanka in another group. Based on the intergroup genetic distance the *R. rattus* abundant in Nepal and Pakistan have close genetic relation rats found in India and Japan, which were estimated to diverge about 2.097–2.344 MYBP. These results indicate that these two groups have simultaneous evolution. Evolution and distribution pattern of the *R. rattus* population found in Nepal and Pakistan are the part of discussion. It is required to study first either *R. rattus* abundant in Nepal is a native species or introduced species. This study assumed it is a native species of Himalayan region, which could have distribution from Eastern part of Nepal up to the Pakistan. Aplin *et al.* (2011) suggested its distribution in lower foothill of Himalaya. Details sampling in different locations of Nepal, North and northern West of India including UP, Bihar and Uttarakhanda and Pakistan required to understand its population expansion and distribution pattern. The haplotypes of *R. rattus* have been clustered into two subgroups representing two genetic subpopulations of *R. rattus*, which have been diverged approximately 0.529–0.592 MYBP. The haplotypes of *R. tanezumi* record in Nepal and South and East Asian countries Bangladesh, Laos, Vietnam, and South Korea grouped into two different subgroups. Considering the genetic distance between two subgroups

these might be different lineages. The *R. tanezumi* is native taxon of East Asia could be introduced in Nepal through transportation system developed by humans. It was recorded near to the Indo-Nepal boarder predicted to be introduced through India. This study was limited only in three locations of Nepal therefore addition studies are required for understanding its taxonomic status and distribution in Nepal.

In this study, species identification was carried based on the integrative approach of morphological and molecular analysis for correct identification. The morphological and molecular dataset generated in this study will be the baseline for the further taxonomic studies. Extensive species collection from different geographical locations and identification using both morphological and molecular techniques are required for determining the taxonomic status of murids found in Nepal. Although several studies have done in murids taxonomy of Nepal but still there was no unification in nomenclature. Thus, correct species identification and unified nomenclature system required in murids taxonomy of Nepal.

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요약

본 연구는 네팔의 Lumbini, Pokhara, Kathmandu 세 지역에서 발견되는 쥐과 동물에 대한 외부형태와 분자유전학적 분석을 통한 분류학적 연구를 수행하였다. 쥐과 동물에 대한 형태학적 동정을 위해서 모색과 발바닥, 꼬리, 귀, 외부생식기와 암컷의 유선 수 등 외부 형태에 대한 관찰과 측정치를 확인하였다. 형태학적 분석결과 전체 5 종(*Bandicota bengalensis*, *Mus booduga*, *Niviventer fulvescens*, *Rattus pyctoris*, *Tatera indica*)이 동정되었으나, 4 종(*M. musculus*, *R. nitidus*, *R. rattus*, *R. tanezumi*)은 동정할 수 없었다. *R. rattus*와 *R. tanezumi* 두 종의 형태학적 형질들을 비교하였으나 지속적으로 식별가능한 모색의 차이는 없었다. 또한 형태 계측 결과의 비교에서도 유의적인 차이는 없었다(Student t-test, n=52, df=50, p>0.05). 반면, 이들 두 종은 꼬리의 길이와 색깔에서 *R. nitidus*, *R. pyctoris*와 구별되었다. 이와 유사하게 *M. musculus*의 형태적 특성을 Lumbini 와 Pokhara에서 수집한 개체군 사이에서 비교하였다. 두 집단은 모색에 의해 구별되었으나, 외부형태 측정치에서는 유의적인 차이가 없었다(Student t-test, n=23, df=21, p>0.05).

분자유전학적 동정은 미토콘드리아 DNA (mtDNA) *Cytrochrome B* (*CytB*) 유전자 서열을 이용하여 수행하였고, 8 가지 분류군(*B. bengalensis*, *M. booduga*, *M. musculus*, *N. fulvescens*, *R. nitidus*, *R. rattus*, *R. pyctoris*, and *R. tanezumi*)은 종(species) 수준에서, 1 가지 분류군 (*Mus* sp.)은 속(genus)에서 성공적으로 동정되었다. 본 연구에서 결정된 분자 정보를 종내, 종간 변이를 구분하기 위하여 이용하였다. *CytB* haplotype을 모든 분류군에서 결정하였고, 쥐과 동물 114 개의 *CytB* 서열에서 총 41 가지의 독특한 haplotype들을 발견하였다. 연구결과에서 *M. booduga* (2), *M. musculus* (6), *Mus* sp. (2), *R. nitidus* (4), *R. rattus* (26) 등은 다수의 haplotype들을 나타내었으나 *B. bengalensis*, *N. fulvescens*, *R. pyctoris*, *R. tanezumi* 등은 하나의 halotype만을 나타내었다. 유전적 거리지수를 산출한 결과 동

일종 내에서 *R. rattus*, *M. musculus*, *R. nitidus*, *M. booduga*의 거리지수는 각각 0.001-0.017, 0.001-0.016, 0.001-0.008, 0.001-004이었다. 동정된 쥐과 동물들에 대하여 산출된 유전적 거리지수 중에서 *B. bengalensis*와 *M. musculus* (0.278)가 최고치, *R. rattus*와 *R. tanezumi* (0.048)가 최소값을 보였다. *CytB* 서열을 이용하여 *Mus*와 *Rattus* 두 속에서 대한 계통유연관계 연구를 수행하였다. 속 내 종들 사이에서 산출된 유전적 거리지수를 바탕으로 계통수(neighbor joining tree, NJ tree)를 작성하였다. Mus 속의 세 종(*M. musculus*, *M. booduga*, *Mus* sp.)은 *Mus* 아속의 두 가지 종-집단로 구분되었고, *M. musculus*는 *M. musculus* species group, 나머지 두 분류군은 *M. booduga* species group에 위치하였다. *M. musculus*에 대한 계통유연관계분석 결과는 mtDNA *CytB* haplotype들이 NJ tree 상에서 *M. m. bactrianus*와 *M. m. castaneus* 두 아종으로 대표되는 두 개의 clade로 구분되었고, 각각 Pokhara와 Lumbini에서 수집된 시료들임을 보여주었다. 이들 두 아종의 분화시간 추정 결과 약 0.68 MYBP(현재부터 약 100만 년 전, MYBP)에 분화된 것으로 추정되었다. 네팔과 인도에 풍부한 M. booduga의 두 집단에 대한 계통 유연관계 분석결과는 이들이 두 개의 다른 계통에서 발생한 것임을 보여주고 있다. 반면 *Mus* sp.는 종 수준까지 동정되지 않았으나, 계통유전학적 분석에서는 미얀마에서 기록된 *M. nitidulus*와 근연임을 나타내었다. 본 연구에서 동정된 *Rattus* 4 종에 대한 계통유전학적 유연관계를 분석하였다. 네팔에서 흔하게 발견되는 *R. rattus*는 계통수 상에서 파키스탄 개체들과 함께 하나의 그룹을 형성함으로써 매우 근연의 관계임을 보였다. 유전학적으로 이들 두 집단들은 인도 남부 집단과 근연관계를 보였으며, 약 2.097-2.344 MYBP에 분화된 것으로 추정되었다. 네팔 내에서도 두 가지 아집단들이 있었고, 약 0.529-0.592 MYBP에 분화된 것으로 추정되었다. *R. tanezumi*에 대한 계통 유연관계 분석에서 두 가지 subgroups(A, B)가 확인되었다; subgroup A는 네팔의 *R. tanezumi* 개체들만 위치하였고, subgroup B는 방글라데시, 라오스, 베트남, 대한민국 등 남아시아와 동아시아 국가들에서 보고된 서열들이 발견되었다. 두 subgroup 사이의 유전적 거리지수는 0.02보다 높은 수준을 보여, 두 subgroup은 *R.*

*tanezumi*의 다른 계통인 것으로 보인다. 네팔, 인도, 라오스, 베트남에서 흔한 *R. nitidus*의 haplotype들 사이의 유전자 거리지수는 0.001-0.009이며 계통수 상에서 하나의 group형성하 여 집단들이 유전적으로 근연임을 나타내었다. *R. pyctoris*는 하나의 haplotype만을 나타내었고, 다른 종들의 haplotype 분석결과와는 달리, 서로 구분되지 않는 형태를 보였다. 유전학적으로는 *R. tanezumi*와 근연이었으며, 5.192-5.806 MYBP의 분화시간을 보였다.

본 연구결과들은 네팔에서 발견된 쥐과 동물의 형태적, 분자적 데이터들을 제공하였다. 연구를 통해 얻어진 분자 데이터 중에는 네팔에서 *M. m. bactrianus* and *R. tanezumi*가 서식한다는 새로운 기록을 제공하였다. 비록 네팔에서 선택된 몇몇 지역에 국한되어 연구되었으나, 연구결과들은 형태학적 연구와 분자적 연구들의 통합적인 연구 수행이 쥐과 동물의 진화적 현상을 이해하고 정확한 종 동정을 하는데 필요하다는 점을 제안하였다. 또한 쥐과 동물의 분류학적 위치와 계통유전학적 유연관계를 결정하기 위해서는 네팔 전체의 다른 지역들에서 시료의 수집과 확장된 조사들이 필요하다.

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