Molecular phylogenetic characterization of common murine rodents from Manipur, Northeast India

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The Indian subcontinent and Southeast Asia are hotspots of murine biodiversity, but no species from the Arakan Mountain system that demarcates the border between the two areas has been subjected to molecular phylogenetic analyses. We examined the mitochondrial cytochrome b gene sequences in six murine species (the Rattus rattus species complex, R. norvegicus, R. nitidus, Berylmys manipulus, Niviventer sp. and Mus musculus) from Manipur, which is located at the western foot of the mountain range. The sequences of B. manipulus and Niviventer sp. examined here were distinct from available congeneric sequences in the databases, with sequence divergences of 10–15%. Substantial degrees of intrapopulation divergence were detected in R. nitidus and the R. rattus species complex from Manipur, implying ancient habitation of the species in this region, while the recent introduction by modern and prehistoric human activities was suggested for R. norvegicus and M. musculus, respectively. In the nuclear gene Mc1r, also analyzed here, the R. rattus species complex from Manipur was shown to possess allelic sequences related to those from the Indian subcontinent in addition to those from East Asia. These results not only fill gaps in the phylogenetic knowledge of each taxon examined but also provide valuable insight to better understand the biogeographic importance of the Arakan Mountain system in generating the species and genetic diversity of murine rodents.

Key words: Berylmys, cytochrome b, India, Mc1r, Rattus

INTRODUCTION

Murine rodents, members of the subfamily Murinae, comprise ~500 species and their evolutionary radiation in the major homeland of the Indomalayan Region is an important biogeographic topic. Molecular phylogenetic relationships and population genetic structuring have been assessed in certain groups of murine genera and species, respectively, such as the genus Mus (Shimada et al., 2010), the house mouse M. musculus (Suzuki et al., 2013), and the Rattus rattus species complex (e.g., Pagès et al., 2010; Aplin et al., 2011; Conroy et al., 2013; Yasuda et al., 2014), but a large portion of murine taxa have not yet been examined. Even in extensively examined taxa, some geographic areas, such as Northeast India, remain to be investigated. In addition, two rat species, R. norvegicus and the Himalayan rat R. nitidus, are known as commensal rodents and recognized as invasive alien species, as are the R. rattus SC and M. musculus, but despite their importance the population genetic structure and evolutionary episodes have not yet been assessed.

The zoogeographic Indomalayan Region has a high level of terrestrial animal biodiversity (Srinivasulu and Srinivasulu, 2012; Barley et al., 2014) and its continental part can be divided into two regions, the Indian subcontinent and mainland Southeast Asia, with the Arakan Mountain system on the border of India and Myanmar acting as a geographic barrier. Widespread taxonomic groups are ideal systems for testing biogeographic hypotheses, because they offer the opportunity of examining how biogeographic barriers, as well as dispersal and colonization, affect diversification and determine biogeographic distribution patterns (e.g., Irischick et al., 1997; Barley et al., 2014). Among the taxa occurring in Northeast India, a most attractive taxon is the R. rattus SC, because the geographic area is a possible suture zone of the two major forms, “R. rattus” and “R. tanezumi”, whose native ranges are the Indian subcontinent (mainly in central and southern parts) and the remaining...
Indomalayan Region (e.g., Bangladesh, Cambodia, China, Japan, Korea, Laos, Malaysia, Taiwan, Thailand and Vietnam), respectively (Yosida, 1980; Musser and Carleton, 2005). In addition, generic taxa of purely non-commensal forest-dwelling rodents, such as Berylmys and Niviventer, should be examined to assess the role of the mountain range in speciation processes.

We conducted a molecular phylogenetic study on six rodent species collected from Manipur, in the easternmost part of the union of India. We determined the nucleotide sequences of mitochondrial cytochrome b (Cytb; 1140 bp) in B. manipulus, M. musculus, Niviventer sp., R. nitidus, R. norvegicus and the R. rattus SC collected from Manipur. We also examined the variation in chromosome number in representative individuals of the six species. A nuclear gene, the melanocortin-1-receptor (Mc1r) gene, which is one of the most important genes in the evolution of pigmentation in vertebrates, including rodent species (Nachman et al., 2003; Hoekstra et al., 2005), has been used to assess the phylogenetic groups of the R. rattus SC (Kambe et al., 2011, 2012; Suzuki, 2013; Yasuda et al., 2014), and we examined the variation of Mc1r sequences (954 bp) in this taxon. The present study was performed to fill some of the gaps in our understanding of the inter- and intraspecies phylogenetic relationships of the six species of rats and mice mentioned above. This study also contributed to a better understanding of the biogeographic nature of Northeast India.

MATERIALS AND METHODS

Samples  All of the specimens used in this study were collected from 11 localities in Manipur (Fig. 1). Before initiating any work, each specimen was assigned a reference code and the skull and skin were stored at the Central Agricultural University, Imphal (CAUI), under the code name CAUI (Table 1). Approval was obtained from the Institutional Ethics Committee (IEC) for use of animals, and IEC protocols were followed throughout the study. Morphological and taxonomic studies were carried out according to Corbet and Hill (1992), Agrawal (2000), Aplin et al. (2003) and Alfred (2005). The skulls were prepared according to Herbreteau et al. (2011) to ascertain the true identities of the specimens. All measurements were taken using Fisher’s digital calipers. Tissue samples were preserved in > 95% ethanol for DNA extraction.

In the present study, we collected rat specimens of the genus Rattus from habitats in Manipur (Table 1), using live traps in 2012 and 2013. In Manipur, rats belonging to the R. rattus SC are abundant in the hills and valley regions; in particular, they congregate at Loktak Lake. Rattus nitidus is indigenous to mainland Southeast Asia (Aplin et al., 2003; Musser and Carleton, 2005) and is found exclusively in hilly regions. We collected a single individual, with the tip of its tail cut off, belonging to Niviventer sp. (head and body, 135 mm; tail, > 115 mm; hind foot, 27 mm; ear, 18 mm). Even accounting for the missing tail tip, this individual had a relatively short tail (possible tail ratio < 110%), which differed from other species occurring in the Himalayas (Corbet and Hill, 1992)—N. eha (> 140%), N. fulvescens (> 115%) and N. langbianis (140%)—and was likely to be consistent with that of N. niviventer (100%–115%). Two species of Berylmys are known from Manipur, B. mackenziei and B. manipulus (Corbet and Hill, 1992; Agrawal, 2000). Our young specimens from Singda (Locality 3 in Fig. 1), with incomplete tails, CAUI 2024 (head and body, 115 mm; ear, 23 mm;
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Karyotype analysis

Chromosomes were harvested from the bone marrow cells of the femur using a standard colchicine-hypotonic air-drying technique. The chromosomes were stained with 4% Giemsa for conventional karyotyping. Chromosome number and morphology were recorded from 50 Giemsa-stained metaphase plates from each specimen directly under an Olympus BX-41 microscope at ×100 as well as from 10 photographs of selected cells. The types of chromosome were determined according to Levan et al. (1964) in which the chromosomes were arranged in descending order of chromosome length, the seven small metacentric pairs were separated from the acrocentrics and subtelocentrics were inserted among the acrocentrics according to total chromosome length.

Gene sequence analysis

DNA samples were extracted by the standard phenol/chloroform method (Sambrook et al., 1989) and stored at the Central Agricultural University, Imphal. PCR was performed using Taq DNA polymerase (GeNei Merck, Mumbai, India) and a model 2720 Thermal Cycler (Applied Biosystems). The complete

Table 1. Samples used in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen code*</th>
<th>Collection locality**</th>
<th>Habitat</th>
<th>Date of collection</th>
<th>Diploid number</th>
<th>McIr sequence***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rattus rattus</td>
<td>CAUI 2008</td>
<td>Langol (4)</td>
<td>Valley</td>
<td>2012/7/26</td>
<td>42</td>
<td>AB973118 (I/I)</td>
</tr>
<tr>
<td>Rattus norvegicus</td>
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<td>Keishampat (7)</td>
<td>Valley</td>
<td>2012/2/1</td>
<td>42</td>
<td>AB973121</td>
</tr>
<tr>
<td>Rattus nitidus</td>
<td>CAUI 2012</td>
<td>Ukhrul (2)</td>
<td>Hill</td>
<td>2012/7/25</td>
<td>42</td>
<td>AB973123</td>
</tr>
<tr>
<td>Berylmys manipulus</td>
<td>CAUI 2024</td>
<td>Singda (3)</td>
<td>Hill</td>
<td>2012/9/28</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td>Niviventer sp.</td>
<td>CAUI 2020</td>
<td>Ukhrul (2)</td>
<td>Hill</td>
<td>2012/9/8</td>
<td>46</td>
<td>–</td>
</tr>
<tr>
<td>Mus musculus</td>
<td>CAUI 2007</td>
<td>Imphal (6)</td>
<td>Valley</td>
<td>2012/8/16</td>
<td>40</td>
<td>AB973125</td>
</tr>
<tr>
<td></td>
<td>CAUI 2011</td>
<td>Thoubal (8)</td>
<td>Valley</td>
<td>2012/6/17</td>
<td>40</td>
<td>AB973126</td>
</tr>
</tbody>
</table>

* DNA code of CAUI (Central Agricultural University, Imphal) number.
** Specimens were collected from Manipur, India. The serial numbers are shown in parentheses (see Fig. 1B for details).
*** DDBJ/EMBL/GenBank Accession number. Diploid pairs of haploid groups are shown in parentheses.
mitochondrial Cytb coding sequence (1140 bp) was amplified using the universal primers L14115 and H15300 (Irwin et al., 1991). The amplified fragments were used as templates for sequence determination with the above universal primers and two internal primers. The sequences of the entire coding region of the McIr gene (954 bp) were determined with primers reported previously (Shimada et al., 2009). Both DNA strands of the PCR products were sequenced using an automated method with a BigDye terminator Cycle Sequence Kit (ver. 3.1; Applied Biosystems) and an automated sequencer (model 3130; Applied Biosystems). Sequence fragments obtained with different primers were assembled using MEGA5 software (Tamura et al., 2011), and the sequences were aligned by eye. Novel sequences were deposited in the DDBJ/EMBL/GenBank DNA databases under accession numbers AB973093–AB973126.

Data analysis and tree building We constructed maximum-likelihood (ML) and neighbor-joining (NJ) trees using MEGA5. In ML analyses, the substitution model that better described the substitution patterns of the Cytb (HKY+G; Hasegawa et al., 1985) data was determined by MEGA5. In ML analysis, the tree topology search was evaluated using simultaneous nearest-neighbor interchange (NNI). In NJ analysis, the Kimura two-parameter model (K2) was used. The reliability of nodes was assessed using 1000 bootstrap replicates.

BEAUti and BEAST v1.7.5 (Drummond et al., 2012) was used for Bayesian Markov-chain Monte-Carlo (MCMC) analyses (10000000 chain length) of the Cytb sequence data to estimate the time to the most recent common ancestor (TMRCA) and 95% highest posterior density (HPD). An expected divergence time of 1.7 million years ago (MYA) was used for the ancestral node comprising M. spretus and M. musculus (Suzuki et al., 2004). After a 1000-tree burn-in, 10000 trees were used for the analyses. The Bayesian tree was generated under the relaxed clock model (HKY substitution). The convergence of the MCMC chains and the effective sample size (ESS) values exceeding 200 for all parameters were assessed using Tracer v1.5 (Rambaut and Drummond, 2009).

The population expansion event was assessed by Tajima’s D value using Arlequin version 3.5 (Excoffier and Lischer, 2010). The same software was used for estimation of the expansion parameter τ.

RESULTS

Karyotype We performed karyotype analyses using bone marrow cells and conventional methods (Table 1). The four individuals belonging to the R. rattus SC were shown to have 2n = 42, indicative not of R. rattus, but of R. tanezumi. The R. rattus SC with 2n = 42 showed some simple karyotypic features, such as 13 pairs of acrocentrics, 7 pairs of meta- or submetacentrics, and acrocentric X and Y chromosomes with a fundamental number of 56, with slight deviations. With regard to chromosome variation in the longest chromosome, which is known to be either acrocentric or subtelocentric (Yosida, 1973), our samples from Manipur were of the former type. The “ordinary” chromosome numbers were observed in the other species, B. manipulus (2n = 40), M. musculus (2n = 40), Niviventer sp. (2n = 46), R. nitidus (2n = 42) and R. norvegicus (2n = 42) (Table 1).

Mitochondrial gene sequences We determined Cytb gene sequences (1140 bp) in 24 rats and mice from Manipur (Table 1). We constructed ML and NJ trees using the Cytb sequences, together with database sequences, to assess the phylogenetic relationships within and between species (Fig. 2). The divergence times were assessed by BEAST analysis (Rambaut and Drummond, 2009) and TMRCAs for internal nodes of interest are shown in Fig. 2.

The 13 individuals of the R. rattus SC from Manipur yielded eight distinct haplotypes, with sequence divergence of 0.6%–0.9% (Fig. 2). The eight Manipur haplotypes were integrated into the clade of R. rattus SC Lineage II (Fig. 2) defined in a previous study (Aplin et al., 2011), and they differed from haplotypes of the other Lineage II sequences compared, with sequence divergence of 0.6%–1.5%. The Tajima’s D value for the 18 individual rats of Lineage II was significantly negative (−1.4; P < 0.05), suggesting rapid population growth. The expansion parameter τ was calculated to be 6.9.

In phylogenetic analysis with the Cytb sequence, the sequence of R. norvegicus from Manipur was shown to be virtually identical to those reported for rats from other countries. The Cytb sequences of the three R. nitidus rats differed substantially, yielding two distinct haplotypes with 1.5%–1.7% sequence divergence, one of which was similar to those from Southeast Asia, with 0.2%–0.4% sequence divergence. The single specimen of Niviventer sp. (CAUI 2020) was placed in the clade of the N. confucianus species group, with substantial genetic distances of 10%–12% from any other sequences of this group that have been deposited in the databases with a variety of taxon names (i.e., N. confucianus, N. coxingi, N. bukit, N. tenaster, and N. niviventer) and were apparently distinct from N. fulvescens, with a genetic distance of 17%. Comparison of Berylmys Cytb sequences revealed that B. manipulus, endemic to Northeast India, was equally distinct from B. berdmorei and B. boviersi, with 10%–12% genetic divergence.

The three Cytb sequences of M. musculus from Manipur were integrated into the major subclade of M. m. castaneus, CAS-1. In addition, the Manipur sequences were more closely related to the sequence of mouse from Indonesia
Fig. 2. A NJ tree for six taxa of the murine rodents, including the *R. rattus* species complex (RrC), collected from Northeast India. Numbers at nodes indicate bootstrap values by the NJ and ML methods (1000 replicates, > 50%). The taxon names correspond to those in Table 1. CAUI DNA codes are those of the authors’ personal collections. Shaded boxes indicate taxa collected from Northeast India. Resultant TMRCA values from BEAST analysis are marked on the nodes focused upon in this study. We used a calibration point for dating (the node marked with a star), 1.7 MYA for the basal divergence of *M. musculus* species group, including *M. spretus* (Suzuki et al., 2004). The scale bar indicates the genetic distance.
than to that of the Japanese mouse, representing the two locally restricted phyletic groups CAS-1b and CAS-1a, respectively (Fig. 2) (Suzuki et al., 2013). The Tajima’s D value calculated for the three mice together with 17 mice belonging to CAS-1b (Suzuki et al., 2013) was significantly negative (–2.1; \( P < 0.01 \)), suggesting rapid population growth. The \( \tau \) value was calculated as 1.9.

**Mc1r sequences** We determined the **Mc1r** sequences (954 bp) in four individuals from the *R. rattus* SC, together with *R. nitidus*, *R. norvegicus* and *M. musculus* (Table 1), which will be valuable in future phylogeographic inferences as nuclear markers and for assessing functional effects of each amino acid change on coat color alteration during the course of evolution. In this study, we addressed the phylogeographic relationships of the *R. rattus* SC from Manipur, using the **Mc1r** sequence as a phylogeographic marker. The **Mc1r** sequences of the four rats from Manipur were prone to polymorphic sites and haplotype sequences were created based on minimizing the number of haplotypes. We ignored singletons in the separation of haplotypes and in the construction of a network tree. The neighbor-net tree was generated from the **Mc1r** sequences from Manipur and 12 sequences identified previously (alleles 1–12; Kambe et al., 2011, 2012). The Manipur sequences were located at six different positions in the network, showing their apparent demarcation into two clusters designated “rattus” (Group I) and “tanezumi” (Group II).

**DISCUSSION**

Geographically, Northeast India is potentially a key location to better understand phylogenetic relationships both between and within species of murine rodents in South Asia. Here, we performed phylogenetic analyses on six rodent taxa from Manipur, located at the western foot of the mountain range of the predicted geographic border demarcating the Indian subcontinent and mainland Southeast Asia: two taxa occurring in natural habitats (*Berylmys* and *Niviventer*) and the remaining four showing a trend of human influence in their secondary habitation (*M. musculus*, *R. nitidus*, *R. norvegicus* and the *R. rattus* SC). The present study provided not only data regarding the phylogenetic positions of the rodents from Northeast India but also information valuable for assessing the biogeographic importance of this area.

The genus *Berylmys* comprises four species, *B. berdmorei*, *B. bowersi*, *B. mackenziei* and *B. manipulus* (Corbet and Hill, 1992; Agrawal, 2000). The northeastern part of India is rich in this genus, harboring *B. manipulus* with
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a distribution confined to Northeast India, and B. berdmorei and B. boweri with broad distribution ranges in the Indomalayan Region. The latter two species have been examined in terms of mitochondrial Cytb sequences (Pagès et al., 2010; Aplin et al., 2011); however, no molecular data are available for the two local species. To our knowledge, this is the first reported molecular analysis of the Northeast Indian species B. manipulus (Fig. 2). Comparison of the Cytb sequences revealed that B. manipulus was equally distinct from B. berdmorei (TMRCA: 2.4 MYA, 95%HPD 1.9–3.0) and B. boweri (TMRCA: 2.1 MYA, 95%HPD 1.6–2.6), with 13% and 16% genetic divergence, respectively. These findings suggest that Northeast India has fostered its own lineages of forest dwellers over a long period.

The phylogenetic relationships of the genus Niviventer are difficult to unravel, partly because species misidentifications are abundant in database sequences and also because of the existence of several cryptic species (Balakirev and Rozhnov, 2010; Pagès et al., 2010; He and Jiang, 2013). According to Corbet and Hill (1992), in the Himalayan region and its neighboring region of Northeast India, the two species N. fulvescens and N. niviventer are both present, in addition to N. eha and N. langbianus. In molecular phylogenetic analyses of Niviventer species diversity using more than 200 mitochondrial Cytb sequences, He and Jiang (2013) discussed the presence of four or five major clades, including the two clades of the N. confucianus species group and N. fulvescens species group. To date, no Cytb sequence has been reported for individuals from the Himalayan region and Northeast India. In this study, we have a single sequence determined for Niviventer sp. from Manipur, which provides several valuable insights regarding the zoogeography of this genus. The Cytb sequence is the first determined for a member of this genus in the northeastern peripheral region and suggests the existence of a distinct lineage belonging to the N. confucianus species group with an estimated divergence time of 1.3–1.9 MYA (Fig. 2), indicating that Northeast India was the site of this ancient divergence of the species group of forest-dwelling rats.

Issues related to the evolution of the black rat, the R. rattus SC, for which many molecular studies are available, are of interest. The R. rattus SC is predicted to have homelands in Asia, with six distinct mitochondrial Cytb lineages (I–VI) with different geographic affinity (Chinen et al., 2005; Pagès et al., 2010; Aplin et al., 2011). The area comprising eastern and southern India, representing Lineage I, is the likely geographic focus of the karyotype group of 2n = 38, while the geographic area covering the easternmost part of India to southern China represents the natural range of Cytb Lineage II, representing the chromosome 2n = 42 form, the so-called R. tanezumi (Fig. 1A). We confirmed these conclusions, as our specimens from Manipur had the karyotype 2n = 42, indicative of R. tanezumi, and not R. rattus. The Cytb phylogenetic analysis reported here supported these conclusions (Fig. 2), indicating that all haplotypes recovered fell within the clad of Lineage II with a 100% support value. Notably, the substantial level of nucleotide diversity in the mitochondrial DNA (mtDNA) from Manipur implies ancient habitation of this species in this geographic area, providing robust evidence that the geographic area of the western foot of the Arakan Mountain range functioned as part of the homeland of this species. More interestingly, together with previous results (Chinen et al., 2005; Aplin et al., 2011; Kambe et al., 2012), it is now evident that mtDNA Lineage II dispersed across the Arakan Mountain range, linking the western and eastern sides and covering the far eastern area, the Japanese Archipelago.

From the shallow branching pattern of the phylogenetic tree (Fig. 2) and the result of Tajima’s D test (significantly negative), it is reasonable to assume that the Lineage II phylogroup experienced an ancient range expansion event, as has been noted in our previous study (Aplin et al., 2011), which also stated that such ancient colonization events resulted in the deep and geographically highly structured mitochondrial diversity of the R. rattus species complex in the Indian subcontinent and Southeast Asia.

Contrary to the initial prediction, the eight McIr sequences examined from four individuals from Manipur were split into two clusters representing “R. rattus” (four sequences) and “R. tanezumi” (four sequences), corresponding to the mtDNA Lineages I and II, respectively (Fig. 3). One individual (CAUI 2013) from Loktak was found to have the R. tanezumi type in both the mitochondrial and nuclear sequences, while the other three from Loktak (CAUI 345, 2014) and Langol (CAUI 2008) had the R. tanezumi type of Cytb and the R. rattus type of McIr in their genomes. These results suggest that genetic hybridization is occurring between the two geographic groups of “R. rattus” (2n = 38) and “R. tanezumi” (2n = 42) in the northeastern part of India. This again highlights the specific features of the geographic area that have assisted the dispersal of murine rodents across different geographic domains, perhaps through connectivity via mountain ranges of South Asia, including the Arakan Mountain range.

One of our great concerns related to the murine rodents is the prehistoric human influence on their secondary distributions. Generally, the northeastern tip of India is thought to have been a key corridor for human migrations between these two subcontinental areas (e.g., Nei and Roychoudhury, 1993; Cavalli-Sforza et al., 1994; Reddy et al., 2007). In contrast, Cordaux et al. (2004) suggested that the Northeast Indian passageway acted as a geographic barrier between the Indian subcontinent and East/Southeast Asia, at least since the arrival of Tibeto-Burman speakers in Northeast India, on the basis of
human mtDNA and Y chromosome studies. The two commensal taxa *R. rattus* SC and *M. musculus* have been subjected to intensive phylogeographic analyses of the prehistoric and historical dispersals in association with humans (e.g., Aplin et al., 2011; Suzuki et al., 2013) but few molecular studies had included samples from Northeast India. The house mouse, *M. musculus*, is known to have its homeland in India and the western neighboring countries of Iran, Afghanistan, and Pakistan, and then experienced geographic expansion from three different source areas, including somewhere on the Indian subcontinent, that shaped the current broad distributions of the *M. m. castaneus* (CAS) subspecies (e.g., Suzuki et al., 2013). *Mus musculus castaneus* harbors four distinct mtDNA lineages, one of which (CAS-1) had experienced human-associated geographic expansion from the predicted source area on the Indian subcontinent to its eastern neighboring geographic areas via different routes, resulting in two phyletic groups, CAS-1a from Yunnan and Japan and CAS-1b from a broad area of Southeast Asia and South China (Suzuki et al., 2013). The two *Cytb* sequences of *M. musculus* from Manipur were integrated into the major subclade of CAS-1, further indicating a close relationship with the haplotype from Indonesia (CAS-1b) rather than that from Japan (CAS-1a; Fig. 2). The expansion parameter $\tau$ for CAS-1b with the three Manipur sequences and 17 from the databases (Suzuki et al., 2013) was estimated to be 1.9 (see Results). Notably, the value is substantially lower than the $\tau$ value of 6.9 for the *R. rattus* SC from Manipur and other areas of Lineage II ($n = 18$, Fig. 2), indicating that the two commensal murine rodents—the house mouse and black rat—from Manipur have different population expansion scenarios. Further studies of *M. musculus* will enhance our understanding of the geographic importance of Northeast India with respect to prehistoric human movements.

*Rattus norvegicus* is currently found worldwide, while *R. nitidus* and *R. pyctoris* have broad and enclosed geographic distributions in Southeast Asia and the Himalayan range, respectively, with limited overlapping areas in Northeast India (Agrawal, 2000). Specifically, *R. nitidus* is indigenous to mainland Southeast Asia and occurs in South China (including Hainan Island), Vietnam, Laos, northern Thailand, Burma, Bangladesh, Nepal, Bhutan and northern India; the species is also found on the islands of Central Sulawesi, Luzon Island in the Philippines, Palau, Seram in the Moluccas, the Vogelkop Peninsula of the Province of Papua, and the Palau islands, probably due to human-mediated introductions (Aplin et al., 2003; Musser and Carleton, 2005). Despite its widespread distribution, molecular information about *R. nitidus* is limited (Pagès et al., 2010; Balakirev and Rozhnov, 2012). The exact locations of the homeland and source area(s) for the extensive dispersal events have not yet been addressed by molecular phylogenetic studies. In the present study, the karyotype of *R. nitidus* of the male individuals was shown to have eight small metacentric pairs, two subtelocentric pairs, 10 acrocentrics pairs, and acrocentric X and Y chromosomes, exhibiting similar cytogenetic features to the conspecific Chinese population (Li et al., 2008). Substantial levels of sequence divergence (~1.4%) were found between the two *Cytb* haplotypes recovered from three Manipur *R. nitidus* individuals. This suggests a long-standing presence of this species in this geographic area, which may thus be part of the homeland of this species. On the other hand, one of the lineages (CAUI 2012, 2015; Fig. 2) was closely related to *R. nitidus* from Southeast Asia (Pagès et al., 2011), with which it formed clade A. This may be explained by range expansion through natural population dynamics, associated with prehistoric human movements, or stowaway introduction in the modern age. The numbers of differences are two to four sites among clade A and the degree of sequence divergence (0.2%–0.4%) is comparable to that in the CAS-1 clade of *M. musculus* (0.1%–0.2%), the diversity of which is thought to have been created by prehistoric human movement (Suzuki et al., 2013). In *R. norvegicus*, in contrast, our sequence of *Cytb* from Manipur was identical to that of the laboratory strain SD (Fig. 2). This suggests a modern-age stowaway introduction of this species to Manipur. A worldwide survey of *R. nitidus* and *R. norvegicus* is needed to address these questions more fully.

In summary, the mtDNA lineages of the murine species examined are suggested to have emerged in Northeast India on different time scales, from the ancient (*B. manipulus*, *Niviventer* sp.) to comparatively recent, including the modern age (*M. musculus*, *R. nitidus*, *R. norvegicus*, *R. rattus* SC). Certain groups of commensal murine rodents appear to have been affected by human activities, as seen in the cases of *R. norvegicus* and *M. musculus*, while others, such as *R. nitidus* and the *R. rattus* SC, displayed bimodal patterns, with the presence of both indigenous lineages with long histories and newly introduced lineages, perhaps through human activities. The non-commensal species of *Berylmys* and *Niviventer* showed apparent differences from those of their geographic neighbors in Southeast Asia. The present study provides preliminary information for assessing the biogeographic significance of the region, in terms of whether it acted as a passage corridor or as a barrier for different species. Regardless, it remains true that Northeast India is important geographically as well as for assessment of prehistoric human activities.

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