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Research Article

THE MOLECULAR PERSPECTIVE OF MANIPURI RATS

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ABSTRACT

Manipur a tiny state of India is a part of the Indo-Burma hotspots of the World and diversity may be due to the connectivity through the mountain range with the South Eastern Asia particularly the rat species. The diversity of rats of the Manipur remains largely unexplored besides some of the works of the Zoological Survey of India. Here we present the two rat species that were ascertain through the COI (719 bp) sequences, namely *Rattus nitidus* and *Rattus tanezumi*. The diploid of these two species is 42 as reported and grouped as *Rattus tanezumi* group. How much difference from the rest of the world is yet to be ascertained?

Keywords: *Manipur, First Record, Rattus Nitidus, Rattustanezumi, Oriental Rat, Heterochromatic Q-Arms*

Introduction

The rats of Manipur have evolutionary importance as this land is in the junction of Indo-Burma hotspots and South East Asia that overlaps with four hotspots of biodiversity containing exceptional concentrations of endemic species and facing huge habitat loss (Myers *et al.*, 2000). The representatives of the genus *Rattus* have been confirmed to be arise from the South Asian countries and Indian continent is regarded as old habitat of the genus. Some of the taxonomic studies of the rats in India had been initiated through the leadership of Zoological Survey of India (ZSI), Kolkata. The diversity of rats of the Manipur, the easternmost state of India remains largely unexplored besides some of the works of the Zoological Survey of India. Out of a total of 4,629 species of mammals known in the world, 372 species occur in the Indian union. Of these, 69 species are reported from Manipur (Mandal *et al.*, 2005). Five subspecies under the genus *Rattus* viz., *Rattus rattus brunneusculus* (Hodgson), *Rattus rattus bullocki* Roonwal, *Rattus rattus tistae* Hinton, *Rattus nitidus obsoletus* Hinton, and *Rattus manipulus manipulus* (Thomas) besides one species *Rattus mackenziei* (Thomas) had been reported from Manipur. *Rattus mackenziei* (Thomas) and *Rattus manipulus manipulus* (Thomas) were included in the genus *Berylmys* as *Berylmys mackenziei* (Thomas) and *Berylmys manipulus* (Thomas) respectively. In this paper we are reporting the occurrence of two more species of the genus *Rattus* viz. *Rattus tanezumi* (Temminck, 1844) and *Rattus nitidus*, Hinton for the first time from Manipur.

MATERIALS AND METHODS

18 (12 males and 6 females) rat specimens of two different species of the genus *Rattus* were procured death by either killed using slang or poison or alive by trapping and they were taken to the laboratory. Each specimen was given a code of PBG with numbers and the skull and skin were deposited in the University Museum after the experiments. The morphological studies were carried out according to Agrawal (2000), Aplin *et al.*, (2003) and Alfred (2005). The skull preparation was done according to Herbreteau *et al.*, (2011); chromosomes were harvested from the bone marrow cells of femur using standard colchicine-hypotonic-air drying technique. Approval of the Institutional Ethics Committee (IEC), Central Agricultural University was obtained for using live animals and protocols of the IEC were followed throughout the study. The chromosomes were stained with Giemsa for conventional karyotyping. Either liver or kidney tissues were used for DNA extraction by phenol-chloroform technique.

Taxonomic Backgrounds

Morphological Characters of the Rattus Nitidus

In this species the fur is soft and quite thick, particularly in the typical race. The tail is nearly naked, that with signs of growth of hairs throughout its length. Hind foot with five toes, all clawed; the hallux shorter than the fifth, which is shorter than the central three; apparently six plantar pads. Tail little longer than head and body as rule (107% average for typical race, Hinton; 99% average for *obsoletus*). Mammae 12, as rule. The back with is usually dark brown, occasionally with a darker mid-dorsal patch or line. Feet usually yellowish or whitish, rarely dark. Tail normally wholly dark (Ellerman, 1961). *Rattus nitidus* had Harsher- furred; bullae apparently under 15% of the occipitonasal length; tail averages just shorter than head and body. Occipitonasal length not exceeding 45.5 mm. Diastema proportionately shorter than in *Rattus bowersi*.

Morphological Characters of the Rattus Tanezumi

All the measurements were taken from nine fully grown rats collected from Karang, Loktak Lake. Medium-sized rats, dorsal fur slightly reddish grey brown. Both pes and manus had a darker colouration unto the toes. Ventral part of the rat was purely white with clear demarcation between dorsal and ventral that extended onto the cheek with an intervention of smoky T-shaped around throat and thoracic region as on the upper parts of manus and pes in colouration.

Cytological Characters of Rattus Nitidus and Rattus Tanezumi

The 2n of *Rattus nitidus* is 42. The karyotype comprises one pair of subtelocentric (#1), 12 pairs of acrocentrics pairs (#2-13), seven pairs of small metacentric pairs (#14-20) as autosomal compliments. The sex chromosomes are subtelocentrics (Figure 1 B). The q-arms of the X chromosomes were heterochromatic. All the 15 rats available for karyotyping showed without exception 42 chromosomes in which chromosome numbers 14-20 were small metacentric chromosomes and other autosomal and sex chromosomes were acrocentrics. The X chromosome was 10th or 11th of autosomal chromosome in length and Y chromosome was the shortest in the genome (Figure 1A).

Molecular Identity of Rattus Nitidus and Rattus Tanezumi

For confirmation of the molecular identity of the two rats the barcoding gene, cytochrome oxidase subunit I (COI) was sequenced with the primers viz., BatL5310 (5’ ACTTCTGGGTGTCCAAAGAATCA 3’) and R6036R (5’ CCTACTCRGCCATTTTACCTATG 3’). PCR amplification was carried out in 35µl reaction volume with 50 ng of DNA, 0.2 µM of primer pairs BatL5310 and R6036R , 200 µM of dNTP mix, 1.5 mM $MgCl_2$ and 1 Unit of Taq DNA polymerase (Qiagen). The thermal cycling parameters were 4 min at 94°C, 40 cycles of 30 s at 94°C, 30 s at 48°C, 60 s at 72°C, and a final extension of 10 min at 72°C. The PCR products were sequenced bi-directionally and the consensus sequences were compared with homologous sequences available with www.ceropath.org(trees developed may be see-Supplementary I). The two sequences of *Rattus nitidus* and *R. tanezumi* were deposited to the NCBI GenBank with accession numbers JQ906931 and JQ918374. For phylogenetic analysis and cladistic grouping, sequences from NCBI for related species were taken and a phylogenetic tree drawn using MEGA 5.0.Eleven more COI sequences in the Fasta format were downloaded from the NCBI website and the phylogenetic tree to compare the status of the two taxa (Supplimentary II).

RESULTS

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [1]. The tree with the highest log likelihood (-1546.4237) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.3831)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 12 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 50% site coverage were eliminated. That is, fewer than 50% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 675 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [2].

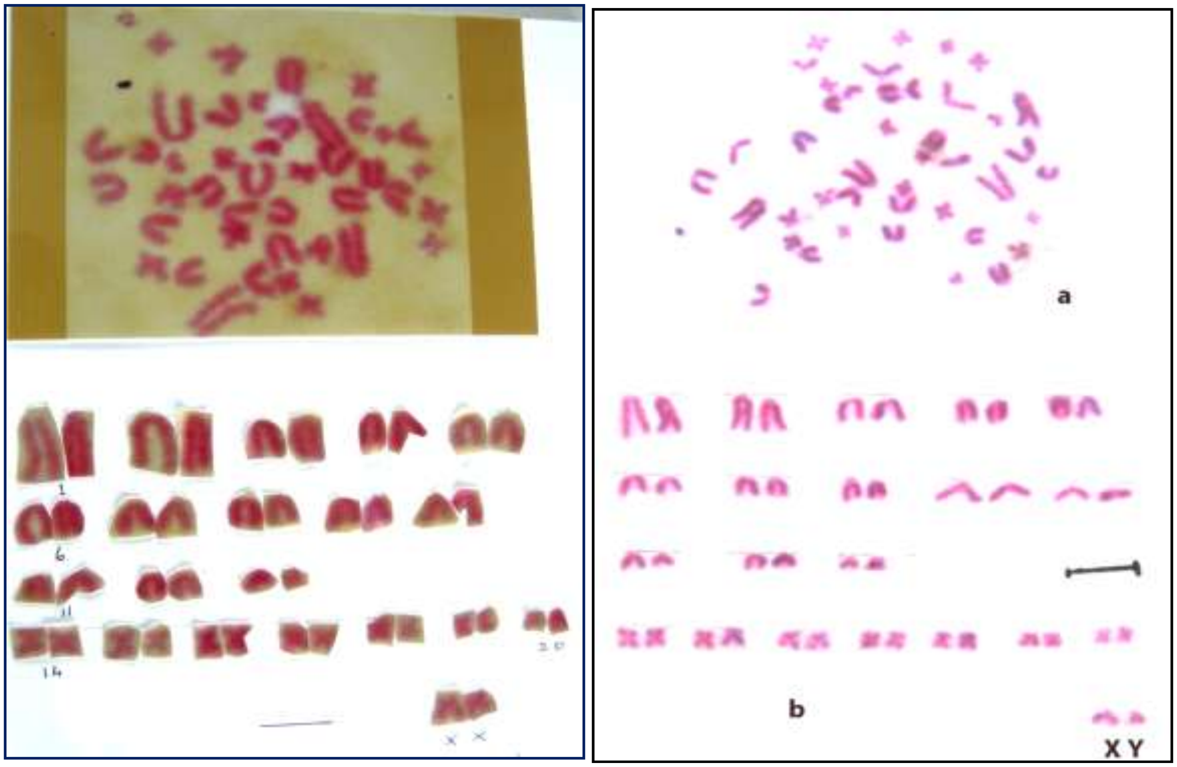

Figure 1: (a) Metaphase and karyotype of *Rattus tanezumi*; (b) *Metaphase and karyotype of Rattus nitidus*

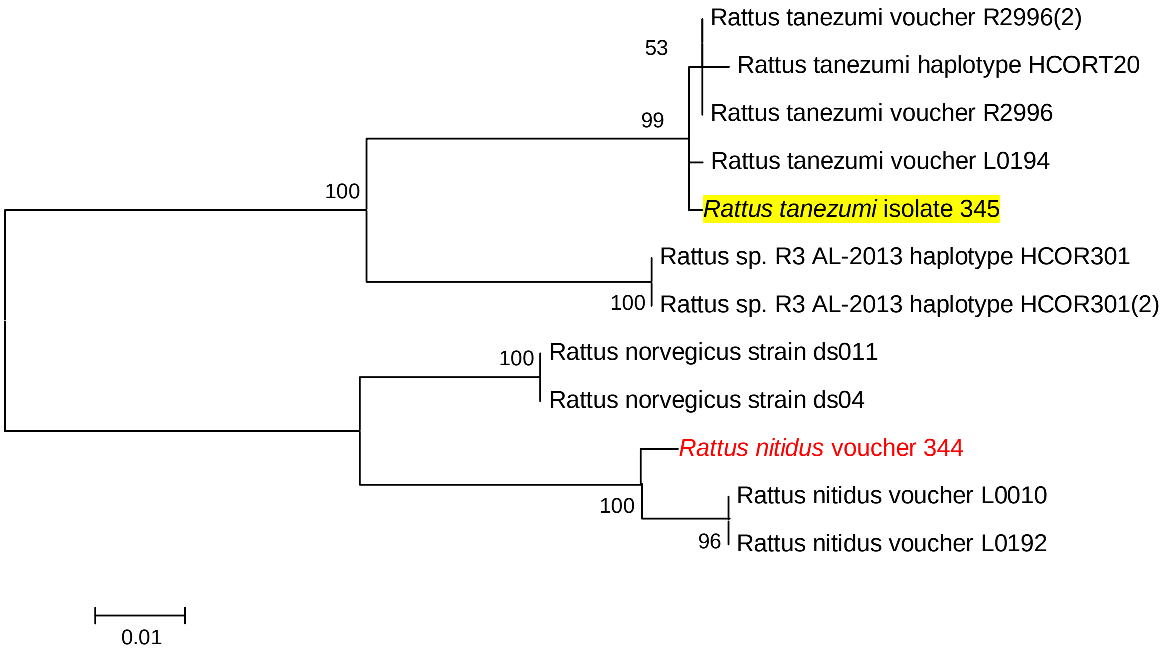

Figure 2: Molecular Phylogenetic analysis by Maximum Likelihood method

The above clearly shows the validity of the species of the Manipur as distincttaxon as compare to different parts of the world but two sequences cannot be taken as the representative of the various species of Manipur. So much works is needed for explaining.

DISCUSSION

Rattus nitidus, also called the Himalayan rat, 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; it is also found on the islands of central Sulawesi, Luzon island in the Philippines, Pulau Seram in the Molluccas, the Vogelkop Peninsula of the Province of Papua, and the Palau Islands, probably due to human-mediated introductions (Aplin *et al.*, 2003). Li *et al.*, (2008) reported the 2n=42 of *Rattus nitidus* comprising of eight metacentric pairs, two subtelocentric pairs, and 10 acrocentric pairs as autosome compliments and acrocentrics XX from China. In the present study also the karyotype is consisting of ten acrocentrics pairs (#2-8, 10-12), two subtelocentric pairs (#1, and 9), eight pairs of small metacentric pairs (# 13, 14-20) and acrocentrics X and Y. Hence the *Rattus nitidus* reported from Manipur is quite similar to the Chinese species even though the chromosome numbers are different. Differences in the karyotype may be due to adoption of different preparation methods or by interpretative differences among studies.

The basic chromosome number of the black rat *Ratus rattus* is 42 (21 pairs) consisting of 13 acrocentric pairs, 7 metacentric autosome pairs and acrocentric X and Y; pairs 1, 9 and 13 are polymorphic due to acrocentric or subtelocentric (metacentric) members. Similarities in length and G-banding pattern between the acrocentric and subtelocentric, and also the frequencies and distribution patterns of the polymorphic chromosomes strongly suggest origin of the subtelocentrics by the pericnetric inversion of the acrocentrics (Yosida, 1983). Subtelocentric pair 1, 9 and 13 of *R. nitidus* could have been derived by pericentric inversion of the original acrocentric pair. The change of subtelocentric X chromosome pairs might involve the duplication of the heterochromatins in the genome as reported in *Niviventer* (Wang *et al.*, 2003).

Hinton (1919) differentiated *R. nitidus obsoletus* from *Rattus nitidus nitidus* on the colour of the under surface of the body, being with grey with a rusty tinge in the former and silvery in the latter. Ellerman (1961) mentioned that this colour-difference seems to be not constant and provisionally differentiated the two subspecies on the length of tail, shorter tail in *obsoletus* (99% of HB) and longer in *nitidus* (107% of HB).

An appraisal of the specimens of *Rattus nitidus* present in ZSI reveals that there is no clear-cut difference between *R. nitidus* and *R. obsoletus* either in the colour of the under surface of the body or in the length of tail. Hence, the subspecies *R. obsoletus* is being treated as a synomym of *Rattus nitidus nitidus* (Agrawal, 2000).

According to the above points it is difficult to come to a conclusion but on the basis of karyotype it will be wise enough to differentiate the two species into as *Rattus nitidus niditus* from Mao and *Rattus nitidus obsoletus* from the Ukhrul. The soft palate ridges strengthened the differentiation of the two species. One thing is certain from the observation that presence of subtelocentric chromosomes there is tendency of having the much darker fur hairs or melanistic forms. Future works should be with more numbers of the specimens and more study sites.

Rattus tanezumi is the oldest name for the 2n = 42 group of Asian house rats that is distinguished from the 2n = 38/40 *Rattus rattus* not only by chromosomal characters but also by morphological and biochemical traits (Wilson and Reeder, 2005). In Manipur the rat is not common in valley but mostly concentrated on the hillock of Karang island in the Loktak Lake. This species is morphologically quite similar to *Rattus rattus* and is very difficult to identify. Hence a barcoding gene (mitochondrial COI region) was sequenced identification purpose. The species is listed as Least Concern due to its wide distribution and tolerance of a wide variety of habitat types (Heaney and Molur, 2008).

Yosida and Sagai (1973) reported subtelocentric chromosome number 1 under the name *Rattus rattus tanezumi* (now named *R. tanezumi*; Wilson and Reeder, 2005) but in our studies (Dhananjoy *et al.*, 2012) the chromosome is acrocentric. They also suggested that subtelocentric chromosomes were derived from acrocentric chromosomes through pericentric inversion. The chromosome numbers 1, 9 and 13 are the hot spots for chromosome polymorphism in genus *Rattus*, but none of these chromosomes are either heteromorphic or homomorphic due to subtelocentric and acrocentric. Instead we found chromosome numbers 15, 19 and 20 to be heteromorphic due to metacentric and submetacentrics. According to Yosida and Sagai (1972), some *Rattus* species having few small metacentrics are included in the third group which might be ancestral type of other species with 7 metacentrics. From the above discussion it can concluded that *Rattus tanezumi* is ancestral to *Rattus rattus* species in order of subtelocentric and small metacentric chromosomes of autosomes and further intensive works should be carried out in order to decide the facts.

Wilson and Reeder (2005) synonymised *Rattus brunneusculus* (Hogson, 1945) with *Rattus tanezumi*. In Indian context also *Rattus brunneusculus* was reported from Manipur instead of the *Rattus tanezumi* (Alfred, 2005) and Ellerman (1961) from The Loktak Lake. Alfred reports that the *brunneusculus* had longer tail, being 123-131% of head and body length but in our study it is 90%. Again Ellerman (1961) synonimised it with *sikkimensis* and argued that the typical *brunneusculus* has the underparts yellowish, not very typical for a wild race, besides this the characteristic feature of both *brunneusculus* and *sikkimensis* is “the bullae are usually less than 17% of the occipitonasal length” but in our study it is 19.51%. So the true taxonomic position of *Rattus tanezumi* is yet to be decided particularly in Indian context. A further study involving the three species through morphology, cytology and molecular approach is suggested to resolve the taxonomic conflicts of these three species in future.

The present trend in the taxonomy of *Rattus* is rather changing. According to Aplin *et al.*, (2011) Rattus rattus (Lineage I) has the diploid number of 38 wheras Rattus tanezumi has2n=42/40. They grouped the entire Black Rats into *Rattus rattus and Rattus tanezumi* based on the DNA sequences for taxonomic rationalization. So the two rats from Manipur belong to the *R. tanezumi* group. According to them India is the long term resident with stable population of Lineage I and the western Indochina and uplands of eastern Indochina is regarded as the likely geographic focus of diversification of Lineage II. The NE region of India is a part of the Indochina hence this region is a precious site both for invention of new species and determine the focus of diversification of rats. The future works on the taxonomic supported by molecular evidences will yield exciting results from this tiny area of the India.

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Supplimentary I

DNA Sequence Identification Results _ CERoPath - Barcoding Rodent South East Asia 5.pdf

Supplimentary II

Sequences of the 13 Specimens used in the study

>1. Rattus nitidus voucher L0010 (COXI) mitochondrial
CCCTCTACCTATTATTTGGAGCCTGAGCAGGAATAGTAGGAACAGCTTTAAGTATTCTAATT
CGAGCTGA
ATTAGGACAACCAGGCGCACTCCTAGGCGATGACCAAATCTATAATGTCATCGTCACAGCCC
ATGCATTC
GTAATAATCTTCTTTATAGTAATACCTATAATAATCGGAGGCTTCGGAAACTGACTTGTACC
ACTAATAA
TTGGAGCCCCTGATATGGCATTCCCACGAATAAATAACATGAGTTTTTGACTGCTCCCTCCAT
CATTTCT
ACTTCTTTTAGCATCCTCCATAGTAGAAGCCGGAGCTGGGACAGGATGGACTGTATATCCTC
CCTTAGCC
GGAAACCTAGCCCATGCTGGAGCATCCGTAGACTTAACTATTTTTTCCCTCCACCTAGCTGGT
GTGTCTT
CTATCTTAGGAGCTATCAACTTTATTACCACTATCATTAACATGAAACCCCCTGCTATAACCC
AGTATCA
GACACCTCTCTTTGTATGATCCGTGCTAATTACAGCTGTCCTACTACTTCTCTCCCTACCCGT
GTTAGCA
GCAGGCATCACTATGCTCCTTACAGACCGAAATTTAAATACTACCTTCTTCGATCCCGCTGG
AGGGGGAG
ACCCAATTCTTTATCAACACCTATT
>2. Rattus tanezumi voucher R4481 (COXI) mitochondrial
CCCTCTATCTATTATTTGGTGCCTGAGCAGGAATAGTAGGAACAGCCTTGAGCATTCTAATTC
GAGCTGA
ACTAGGACAACCAGGAGCACTCCTAGGCGATGACCAAATTTATAATGTCATTGTTACAGCCC
ATGCATTC
GTAATAATTTTCTTTATAGTTATGCCTATGATAATCGGAGGCTTCGGAAACTGGCTTGTACCA
CTAATGA
TTGGAGCCCCTGATATAGCATTCCCACGAATAAACAATATAAGCTTTTGATTGCTTCCCCCAT
CATTTTT
ACTCCTTTTAGCATCATCTATAGTAGAAGCCGGAGCCGGAACAGGATGAACAGTATACCCGC
CCTTAGCC
GGTAACCTAGCCCATGCCGGAGCATCCGTTGACCTAACCATTTTCTCCCTTCACCTAGCTGGT
GTATCCT
CTATCTTAGGAGCTATTAATTTTATCACCACTATCATCAATATAAAACCCCCTGCTATAACCC
AATATCA
GACCCCTCTATTTGTGTGATCCGTATTAATTACAGCTGTACTTCTACTTCTTTCACTACCAGTT
TTAGCA
GCAGGCATTACCATACTCCTCACAGATCGAAACCTAAATACTACTTTTTTTGATCCTGCTGGA
GGCGGAG
ATCCAATTCTCTATCAACATCTATT
>3. Rattus tanezumi voucher R3122 (COXI) mitochondrial
AGGAACAGCCTTGAGCATTCTAATTCGAGCTGAACTAGGACAACCAGGAGCACTCCTAGGC
GATGACCAA

ATTTATAATGTCATTGTTACAGCCCATGCATTCGTAATAATTTTCTTTATAGTTATGCCTATGATAATCG
GAGGCTTCGGAAACTGGCTTGTACCACTAATGATTGGAGCCCCTGATATAGCATTCCCACGAATAAACAA
TATAAGCTTTTGATTGCTTCCCCCATCATTTTTACTCCTTTTAGCATCATCTATAGTAGAAGCCGGAGCC
GGAACAGGATGAACAGTATACCCACCCTTAGCCGGTAACCTAGCCCATGCCGGAGCATCCGTTGACCTGA
CCATTTTCTCCCTTCACCTAGCTGGTGTATCCTCTATCTTAGGAGCTATTAATTTTATCACCACTATCAT
CAACATAAAACCCCCTGCTATAACCCAATATCARACCCCTCTATTTGTGTGATCCGTATTAATTACAGCT
GTACTTCTACTTCTTTCACTACCAGTTTTAGCAGCAGGCATTACCATACTCCTCACAGATCGAAACCTAA
ATACTACTTTTTTTGATCCTGCTGG

>4. Rattus tanezumi voucher R2996 (COXI) mitochondrial

CCCTCTATCTATTATTTGGTGCCTGAGCAGGAATAGTAGGAACAGCCTTGAGCATTCTAATTCGAGCTGA
ACTAGGACAACCAGGAGCACTCCTAGGCGATGACCAAATTTATAATGTCATTGTTACAGCCCATGCATTC
GTAATAATTTTCTTTATAGTTATGCCTATGATAATCGGAGGCTTCGGAAACTGGCTTGTACCACTAATGA
TTGGAGCCCCTGATATAGCATTCCCACGAATAAACAATATAAGCTTTTGATTGCTTCCCCCATCATTTTT
ACTCCTTTTAGCATCATCTATAGTAGAAGCCGGAGCCGGAACAGGATGAACAGTATACCCACCCTTAGCC
GGTAACCTAGCCCATGCCGGAGCATCCGTTGACCTAACCATTTTCTCCCTCCACCTAGCTGGTGTATCCT
CTATCTTAGGAGCTATTAATTTTATCACCACTATCATCAATATAAAACCCCCTGCTATAACCCAATATCA
GACCCCTCTATTTGTGTGATCCGTATTAATTACAGCTGTACTTCTACTTCTTTCACTACCAGTTTTAGCA
GCAGGCATTACCATACTCCTCACAGATCGAAACCTAAATACTACTTTTTTTGATCCTGCTGGAGGCGGAG
ATCCAATTCTCTATCAACATCTATT

>5. Rattus tanezumi voucher R0130 (COXI) mitochondrial

CCCTCTATCTATTATTTGGTGCCTGAGCAGGAATAGTAGGAACAGCCTTGAGCATTCTAATTCGAGCTGA
ACTAGGACAACCAGGAGCACTCCTAGGCGATGACCAAATTTATAATGTCATTGTTACAGCCCATGCATTC
GTAATAATTTTCTTTATAGTTATGCCTATGATAATCGGAGGCTTCGGAAACTGGCTTGTACCACTAATGA
TTGGAGCCCCTGATATAGCATTCCCACGAATAAACAATATAAGCTTTTGATTGCTTCCCCCATCATTTTT
ACTCCTTTTAGCATCATCTATAGTAGAAGCCGGAGCCGGAACAGGATGAACAGTATACCCACCCTTAGCC
GGTAACCTAGCCCATGCCGGAGCATCCGTTGACCTAACCATTTTCTCCCTTCACCTAGCTGGTGTATCCT
CTATCTTAGGAGCTATTAATTTTATCACCACTATCATCAATATAAAACCCCCTGCTATAACCCAATATCA

GACCCCTCTATTTGTGTGATCCGTATTAATTACAGCTGTACTTCTACTTCTTTCACTACCAGTT
TTAGCA
GCAGGCATTACCATACTCCTCACAGATCGAAACCTAAATACTACTTTTTTTGATCCTGCTGGA
GGCGGAG
ATCCAATTCTCTATCAACATCTATT

>6. Rattus rattus voucher MDZ10 (COXI) mitochondrial

CCCTCTATCTATTATTTGGTGCCTGAGCAGGAATAGTAGGAACAGCCTTAAGTATTCTCATTC
GAGCTGA
ACTAGGACAACCAGGAGCACTCCTAGGCGATGACCAAATTTATAATGTCATTGTTACAGCCC
ATGCATTC
GTAATAATTTTCTTTATAGTTATGCCTATGATAATCGGAGGCTTCGGAAACTGGCTTGTGCCG
CTAATGA
TTGGAGCCCCTGATATAGCATTCCCACGAATAAACAACATAAGCTTTTGATTACTTCCCCCAT
CATTTTT
ACTCCTTTTAGCATCATCTATGGTAGAAGCCGGAGCCGGAACAGGATGAACAGTATACCCAC
CCTTAGCC
GGTAATCTAGCCCATGCCGGAGCATCTGTTGATCTAACCATTTTCTCCCTTCACCTAGCCGGC
GTATCCT
CTATCTTAGGAGCTATTAATTTTATCACCACTATTATCAATATAAAACCCCCTGCTATAACCC
AATATCA
GACACCTCTATTTGTGTGATCCGTATTAATTACAGCTGTACTTCTACTTCTTTCACTACCAGT
GTTGGCA
GCAGGCATTACCATACTCCTCACAGATCGAAACCTAAATACTACTTTTTTTGATCCTGCTGGA
GGCGGAG
ATCCAATTCTCTATCAACATCTATT

>7. Rattus nitidus voucher L0192 (COXImitochondrial

CCCTCTACCTATTATTTGGAGCCTGAGCAGGAATAGTAGGAACAGCTTTAAGTATTCTAATT
CGAGCTGA
ATTAGGACAACCAGGCGCACTCCTAGGCGATGACCAAATCTATAATGTCATCGTCACAGCCC
ATGCATTC
GTAATAATCTTCTTTATAGTAATACCTATAATAATCGGAGGCTTCGGAAACTGACTTGTACC
ACTAATAA
TTGGAGCCCCTGATATGGCATTCCCACGAATAAATAACATGAGTTTTTGACTGCTCCCTCCAT
CATTTCT
ACTTCTTTTAGCATCCTCCATAGTAGAAGCCGGAGCTGGGACAGGATGGACTGTATATCCTC
CCTTAGCC
GGAAACCTAGCCCATGCTGGAGCATCCGTAGACTTAACTATTTTTTCCCTCCACCTAGCTGGT
GTGTCTT
CTATCTTAGGAGCTATCAACTTTATTACCACTATCATTAACATGAAACCCCCTGCTATAACCC
AGTATCA
GACACCTCTCTTTGTATGATCCGTGCTAATTACAGCTGTCCTACTACTTCTCTCCCTACCCGT
GTTAGCA
G

>8. Rattus nitidus voucher L0180 (COXI) mitochondrial

CCCTCTACCTATTATTTGGAGCCTGAGCAGGAATAGTAGGAACAGCTTTAAGTATTCTAATT
CGAGCTGA
ATTAGGACAACCAGGCGCACTCCTAGGCGATGACCAAATCTATAATGTCATCGTCACAGCCC
ATGCATTC
GTAATAATCTTCTTTATAGTAATACCTATAATAATCGGAGGCTTCGGAAACTGACTTGTACC
ACTAATAA

TTGGAGCCCCTGATATGGCATTCCCACGAATAAATAACATGAGTTTTTGACTGCTCCCTCCATCATTTCT
ACTTCTTTTAGCATCCTCCATAGTAGAAGCCGGAGCTGGGACAGGATGGACTGTATATCCTCCCTTAGCC
GGAAACCTAGCCCATGCTGGAGCATCCGTAGACTTAACTATTTTTTCCCTCCACCTAGCTGGTGTGTCTT
CTATCTTAGGAGCTATCAACTTTATTACCACTATCATTAACATGAAACCCCCTGCTATAACCCAGTATCA
GACACCTCTCTTTGTATGATCCGTACTAATTACAGCTGTCCTACTACTTCTCTCCCTACCCGTGTTAGCA
GCAGGCATCACTATGCTCCTTACAGACCGAAATTTAAATACTACCTTCTTCGATCCCGCTGGAGGGGGAG
ACCCAATTCTTTATCAACACCTAT

>9. Rattus tanezumi voucher L0100 (COXI) mitochondrial

CCCTCTATCTATTATTTGGTGCCTGAGCAGGAATAGTAGGGACAGCCTTGAGCATTCTAATTCGAGCTGA
ACTAGGACAACCAGGAGCACTCCTAGGCGATGACCAAATTTATAATGTCATTGTTACAGCCCATGCATTC
GTAATAATTTTCTTTATAGTTATGCCTATGATAATCGGAGGCTTCGGAAACTGGCTTGTACCACTAATGA
TTGGAGCCCCTGATATAGCATTCCCACGAATAAACAATATAAGCTTTTGATTGCTTCCCCCATCATTTTT
ACTCCTTTTAGCATCATCTATAGTAGAAGCCGGAGCCGGAACAGGATGAACAGTATACCCGCCCTTAGCC
GGTAACCTAGCCCATGCCGGAGCATCCGTTGACCTAACCATTTTCTCCCTTCACCTAGCTGGTGTATCCT
CTATCTTAGGAGCTATTAATTTTATCACCACTATCATCAATATAAAACCCCCTGCTATAACCCAATATCA
GACCCCTCTATTTGTGTGATCCGTATTAATTACAGCTGTACTTCTACTTCTTTCACTACCAGTTTTAGCA
GCAGGCATTACCATACTCCTCACAGATCGAAACCTAAATACTACTTTTTTTGATCCTGCTGGAGGCGGAG
ATCCAATTCTCTATCAACATCTAT

>***10. 344 Rattus nitidus***

ACCTATGTTCGTAAACCGTTGACTCTTTTCAACTAACCACAAAGAT
ATCGGAACCCTCTACCTATTATTTGGAGCCTGAGCAGGAATAGTAGGAACAGCTTTAAGTATTCTAATTC
GAGCTGAATTAGGTCAACCAGGCGCACTCCTAGGCGATGACCAAATCTATAATGTCATCGTCACAGCCCA
TGCATTCGTAATAATTTTCTTTATAGTAATACCTATAATAATCGGAGGCTTCGGAAACTGACTTGTGCCA
CTAATAATTGGAGCCCCTGATATAGCATTCCCACGAATAAATAACATGAGTTTTTGACTGCTCCCTCCAT
CATTTCTACTTCTTTTAGCATCCTCCATAGTAGAAGCCGGAGCTGGAACAGGATGGACTGTATATCCTCC
CTTAGCCGGAAACCTAGCCCATGCTGGAGCATCCGTAGACTTAACTATTTTTTCCCTCCACCTAGCTGGT
GTGTCTTCTATCTTAGGAGCTATCAACTTTATTACCACTATCATTAACATGAAACCCCCTGCTATAACCC

AGTATCAGACACCTCTCTTTGTATGATCCGTGCTAATTACAGCTGTCCTACTACTTCTCTCCCTACCCGT
GTTAGCAGCAGGTATCACCATACTCCTTACAGACCGAAATTTAAATACTACCTTCTTCGACCCCGCTGGA
GGGGGAGACCCAATTCTTTATCAACACCTATTCTGATTCTTTG

>11. 345 Rattus tanezumi

ACCTATGTTCGTAAACCGTTGACTCTTTTCAACTAACCACAAAGATATCGGAACCCTCTATCTATTATTT
GGTGCCTGAGCAGGAATAGTAGGAACAGCCTTGAGCATTCTAATTCGAGCTGAACTAGGACAACCAGGAG
CACTCCTAGGCGATGACCAAATTTATAATGTCATTGTTACAGCCCATGCATTCGTAATAATTTTCTTTAT
AGTTATGCCTATGATAATCGGAGGCTTCGGAAACTGGCTTGTACCACTAATGATTGGAGCCCCTGATATA
GCATTCCCACGAATAAACAATATAAGCTTTTGATTGCTTCCCCCATCATTTTTACTCCTTTTAGCATCAT
CTATAGTAGAAGCCGGAGCCGGAACAGGATGAACAGTATACCCACCCTTAGCCGGTAACCTAGCCCATGC
CGGAGCATCCGTTGACCTAACCATTTTCTCCCTTCACCTAGCTGGTGTATCCTCTATCTTAGGAGCTATT
AATTTTATCACCACTATCATCAATATAAAACCCCCTGCTATAACCCAATATCAGACCCCTCTATTTGTGT
GATCCGTATTAATTACAGCCGTACTTCTACTTCTTTCACTACCAGTTTTAGCAGCAGGCATTACCATACT
CCTCACAGATCGAAACCTAAATACTACTTTTTTTGATCCTGCTGGAGGCGGAGATCCAATTCTCTATCAA
CATCTATTTTGATTCTTTG

>12. Rattus norvegicus isolate 2 cytochrome oxidase subunit mitochondrial

AACCCTCTACCTATTATTTGGAGCCTGAGCAGGAATAGTAGGGACAGCTTTAAGTATTCTAATTCGAGCT
GAACTAGGACAGCCAGGCGCACTCCTAGGAGATGACCAAATCTATAATGTCATCGTCACAGCCCATGCAT
TCGTAATAATTTTCTTTATAGTAATACCTATAATAATTGGAGGCTTCGGGAACTGACTTGTACCACTAAT
AATTGGAGCCCCTGATATAGCATTCCCACGAATAAATAACATAAGCTTTTGACTGCTTCCTCCATCATTT
CTACTCCTTTTAGCATCCTCCATAGTAGAAGCTGGAGCTGGAACAGGATGAACAGTATATCCCCCCTTAG
CCGGAAACCTAGCCCATGCTGGAGCATCCGTAGATTTAACTATTTTTTCCCTCCACCTAGCCGGGGTGTC
TTCTATCTTAGGAGCTATCAACTTTATCACCACTATCATTAATATAAAACCCCCTGCTATAACCCAATAT
CAGACACCTCTCTTTGTATGATCCGTACTAATTACAGCCGTCCTACTACTTCTCTCACTGCCAGTATTAG
CAGCAGGTATCACTATACTCCTTACAGACCGAAATCTAAATACTACTTTCTTCGACCCCGCTGGAGGTGG
AGACCCAATCCTTTATCAACACCTATTC

>13. Rattus norvegicus mitochondrial

COITTCATAAACCGTTGACTCTTTTCAACT
AACCACAAAGATATCGGAACCCTCTACCTATTATTTGGGGCCT

GAGCAGGAATAGTAGGGACAGCTTTAAGTATTCTAATTCGAGCTGAACTAGGGCAGCCAGGTGCACTCCT
AGGAGATGACCAAATCTATAATGTCATCGTCACAGCCCATGCATTCGTAATAATTTTCTTTATAGTAATA
CCTATAATAATTGGAGGCTTCGGAAACTGACTTGTCCCACTAATAATTGGAGCCCCTGATATAGCATTCC
CACGAATAAATAACATAAGCTTTTGACTGCTTCCTCCATCGTTTCTACTCCTTTTAGCATCCTCCATAGT
AGAAGCTGGAGCTGGAACAGGATGAACAGTATATCCCCCCTTAGCCGGAAACCTAGCCCATGCTGGAGCA
TCCGTAGATTTAACTATTTTTTCCCTCCACCTAGCCGGGGTGTCTTCTATCTTAGGAGCTATCAACTTTA
TCACCACTATCATTAATATAAAACCCCCTGCTATAACCCAATATCAAACACCTCTCTTTGTATGATCCGT
ACTAATTACAGCCGTCCTACTACTTCTCTCACTGCCAGTATTAGCAGCAGGTATCACTATACTCCTTACA
GACCGAAATCTAAATACTACTTTCTTCGACCCCGCTGGAGGTGGAGACCCAATTCTTTATCAACACCTAT
TC