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Research Article

THE KARYOTYPE EVOLUTION AND SPECIES DIFFERENTIATION IN THE GENUS *RATTUS* OF MANIPUR, INDIA

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ABSTRACT
Rattus is the mostly studied genus all over the world but species of the genus are not thoroughly reported from Manipur. The present paper deals with the morphometric, cytotaxonomic and phylogenetic studies of Manipur, India. The different species of Rattus namely *Rattusrattus, Rattus brunneusculus, Rattus tanezumi, and Rattus nitidus* were studied. *Berylmys manipulus* and *B. mackenziei* were taken from the genus *Berylmys.* The studies of the species reveal that the polymorphic chromosomes in the genus *Rattus* were pair numbers i.e. 1, 9, 10 and 13. The change of acrocentrics which are regarded as primitive/ancestral types of chromosomes into either subtelocentric or small metacentrics leads to speciation or simply new species particularly in Manipur. It is generally accepted cytologically that 2n=40 (*Berylmys manipulus, B. mackenziei, Rattus kandianus* etc) are derived from the 2n=42 through centric fusion/Robertsonian fusion, but the soft palate studies shows affinity of the species i.e. *Berylmys manipulus* and *B. mackenziei* from Manipur towards *Niviventer*. The present study is the first step towards the understanding of the relationship between these two genera-*Rattus* and *Berylmys*.

Keywords: *Rattus, Karyotype, Evolution*

INTRODUCTION
The genus *Rattus* has been defined as a widely distributed and taxonomically mixed group including many species and subspecies throughout the World (Ellerman and Morrison-Scott, 1951; Vinogradov and Argyropulo, 1941; Corbet, 1978; Harrison and Bates, 1991). Out of a total of 4,629 species of mammal known in the world, 372 species occur in the Indian union (Corbet and Hill, 1992). Of these, 69 species are reported in Manipur (Mandal *et al.,* 2005). Mandal *et al.,* (2005) reported five species and seven subspecies: *Rattus rattus brunneusculus* (Hodgson), *Rattus rattus bullocki* Roonwal, *Rattus rattus tistae* Hinton, *Rattus nitidus obsoletus* Hinton, *Rattus mackenziei* (Thomas) and *Rattus manipulus manipulus* (Thomas). The latter two taxa were then included in the genus *Berylmys* as *Berylmys mackenziei* (Thomas) and *Berylmys manipulus* (Thomas) respectively (Agrawal, 2000). As Manipur is one of the passage to the South East Asiafrom Indian continentor *vice-versa*, *Rattus rattus* was originated from South East Asia, whereas the India Continent is the old resident of the species (Suzuki *et al.,* 2012), it is pertinent to study the different species of the genus *Rattus* and some species of the *Berylmys.* The present study is the first step towards the understanding the chromosomal profiles of some species of the *Rattus* and to investigate some of the relationship with *Berylmys* particularly in the context of Manipur.

MATERIALS AND METHODS
A total of 107 (55males, 52 females) specimens of the genus *Rattus* were caught from fourteen localities in five districts of Manipur using live traps during June-October, 2012from fourteen different study sites of the five districts of Manipur (Table 1). Before the work begins, each specimen was given an accession code. The wet specimens, skulls and skins were deposited in the University Museum of Central Agricultural University, Imphal. The morphological studies like fur colours, Head-body Length, Tail length, length of hind foot etc. were carried out according to Agrawal (2000), Aplin *et al.,* (2003) and Alfred (2005). The skulls were set (according to Herbreteau *et al.,* 2011) to study the cranial features according to Agrawal (2000). Chromosomes were collected from the somatic cells from bone marrow cells of femur after treating the rats with colchicine for two hours. The cells were then treated with KCl

for 45 minutes, and fixed in acetic acid and methanol in the ratio 1:3 by volume. Finally the cell suspension was spread on slides pre-chilled in methanol and flame dried. The chromosomes were stained using 4% Giemsa solution. C-banding was done according to Sumner (1972) with some modification. Chromosome number and morphology were recorded from 50 metaphases per specimen directly under a 100X and photographs of selected cells were taken by camera attached in Olympus BX-41. The sorting types of chromosomes and karyotypes were done according to Yosida (1983).
Ethical approval was obtained from the Institutional Ethics Committee (IEC) of the Central Agricultural University whose protocols and guidelines about the using of living animals in Science were strictly followed.

RESULTS AND DISCUSSION

Results

***Rattus rattus* (Linnaeus, 1758)**

1758. *Musrattus* Linnaeus, Syst. nat., 10th ed., 1: 61 (Uppsala, Sweden). (Figure 1. a-c)

General characters: Medium-sized rat, having a completely uncoloured tail, generally longer than head and body length. Fur short and harsh, with many soft flattened spines which were from the plains, and soft without spines in winter from mountain habitats. Dorsum are in various shades of brown or of two colours i.e. (i) greyish, tipped with buff (indoor forms) and (ii) white or creamish, with a medial streak of grey or irregular grey patches (outdoor forms). Mammae generally 5 pairs, if 6th pair present, then postaxial pairs close together (less than 10 mm apart).

Occipitonasal length of skull ranges from 36.9 to 46.5 mm; palate always more than one-half of occipitonasal length ; extending posteriorly behind third upper molars; nasals relatively short, less than 38% of occipitonsal length and not extending behind ascending process of premaxillary tooth. the teeth row ranges from 5.5 to 7.5 mm and forms one of the main characters in the classification of species into subspecies viz. Short-toothed forms (less than 6.6 mm in length) and long-toothed forms (more than 6.6 mm in length).

Table 1: The different rat specimens collected from the different parts of Manipur.

SL. No.	Species	Location	Localities	Number of Specimens	Sex	
					Males	Females
1	*Berylmys mackenziei*	Senapati District	Kharam Waiphei	29	12	17
2	*Berylmys manipulus*	Bishnupur, Senapati District	Keionou, Kharamwaiphei	15	8	7
3	*Rattus rattus*	Imphal West, Senapati District, Thoubal District	Langol, Imphal, Canchipur, Kharam Waiphei, Lilong,	22	16	6
4	*R. r. brunneusculus*	Imphal East District	Singjamei Kshetri Leikai	4	0	4
5	*R.nitidus*	Senapati District,	Mao	4	1	3
6	*Rattus tanezumi*	Loktak Lake Bishnupur District	Karang	18	6	12

7	*Rattus norvegicus*	Imphal West District		Keishampat, Iroisemba, Thangmeiband	25	12	13
Total	7 species	5 district		14 localities	107	55	52

Table 2: The morphometrics and crannial measurement of rat species of Manipur

Sr. no.	Measurements	*B. manipulus*	B. mackenziet	R. Rattus	R. tanezumi	R. nitidus	R. norvegicus
1	Length of head & body length (mm)	165		162	150.3	146	215
2	Length of tail (mm)			190	168.3	157	195
3	Length of hind foot (mm)	31		35	32.8	36	43
4	Length of ear (mm)	24		22	20.7	22	21
5	Length of Occipitonasal (mm)	40.11	46.79	41.77	41.4	41.63	48.83
6	Condylobasal (mm)	39.73	45.61	38.82	39.3	37.93	46.09
7	Height of rostrum (mm)	9.67	10.42	9.7	9.1	9.13	10.43
8	Tympanic bulla (mm)	5.99	6.44	6.91	8.1	6.04	5.67
9	Length of diastema (mm)	13.94	14.11	11.31	11.1	9.82	14.65
10	Length of molar (mm)	6.07	7.4	6.49	6.9	6.93	6.23
11	Length of rostrum (mm)	13.12	15.35	12.41	14.4	13.84	16.42
12	Length of palate (mm)	22.38	24.51	21.92	21.9	20.2	26.56
13	Anterior palatal foramen (mm)	7.26	8.69	7.8	7.5	5.86	8.28
14	Length of nasal (mm)	16.67	17.67	15.31	15.5	17.43	18.42
15	Nasal width (mm)	3.81	4.77	3.84	4.4	4.2	5.66
16	Frontal width (mm)	6.5	6.92	5.93	6.1	6.22	7.26
17	Length of orbit (mm)	12.43	16	14.43	14.4	14.01	17.21
18	Greatest	20.69	23.59	19.25	20.7	20.18	23.41

	zygomatic width (mm)						
19	Cranial width (mm)	15.97	18.06	15.92	15.6	16.77	16.72

Figure 1: *Rattus rattus* (a-e), *Rattus rattus alexandrinus* upper surface-a and lower surface-b, venter-c, *R. rattus elaxandrinus* upper surface-f and lower surface-e, venter-d; venter –g (arrowed T-shaped mark), upper surface-a and lower surface-b -h, i-pes of *R. tanezumi*, j-pes and manus-k *of R. nitidus;* l-pes of *B. manipulus*, soft palates of m- *Rattus rattus alexandrinus*, n- *R. rattus elaxandrinus*, o- *R. tanezumi*, p-*R. nitidus*, q-*B. manipulus*, tail-r, venter-s of *B. manipulus*

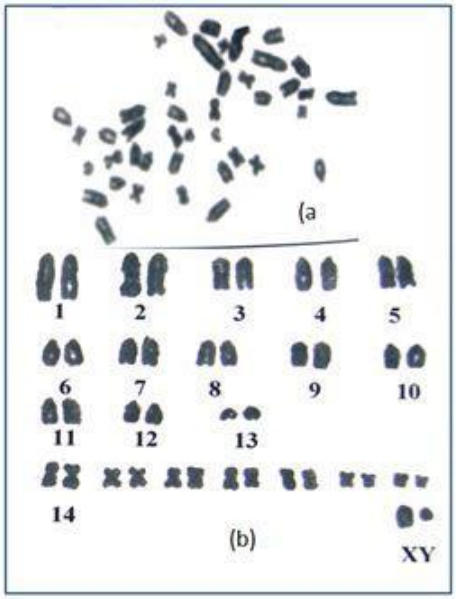

Figure 2: Metaphase a and karyotype b of *Rattus rattus.*

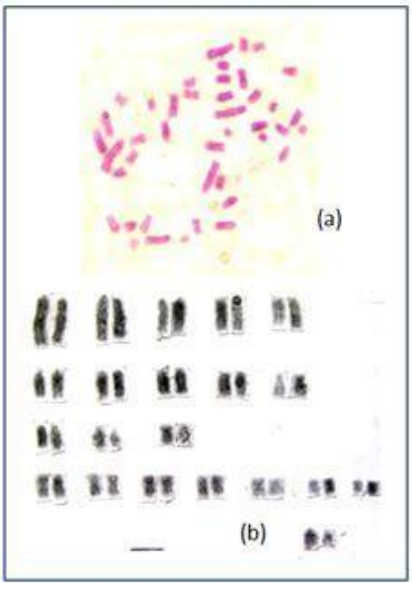

Figure 3: Metaphase a and karyotype b of *Rattus rattus.*

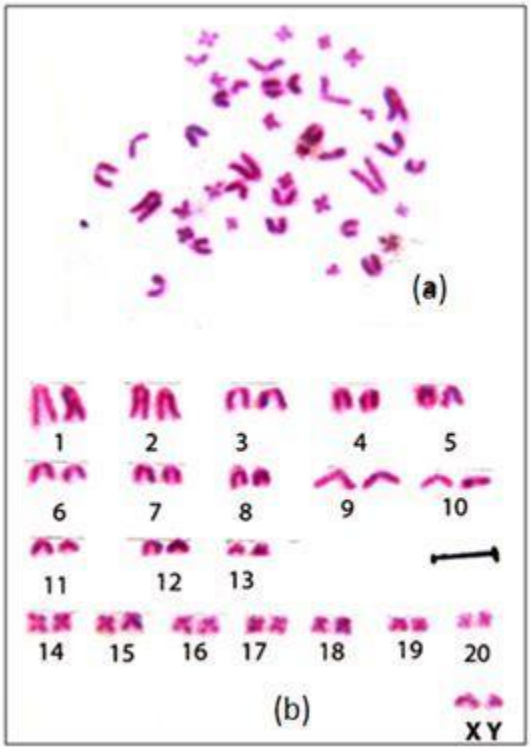

Figure 4: Metaphase a and karyotype b of *Rattus tanezumi*

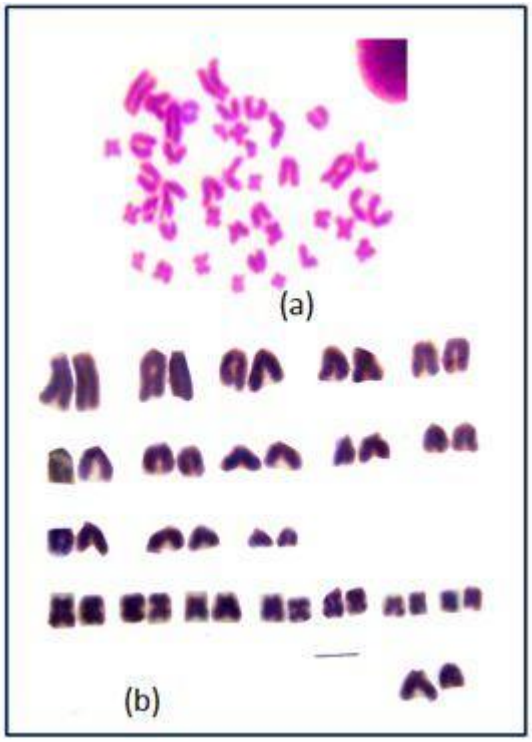

Figure 5: Metaphase a and karyotype b of *Rattus brunneusculus*

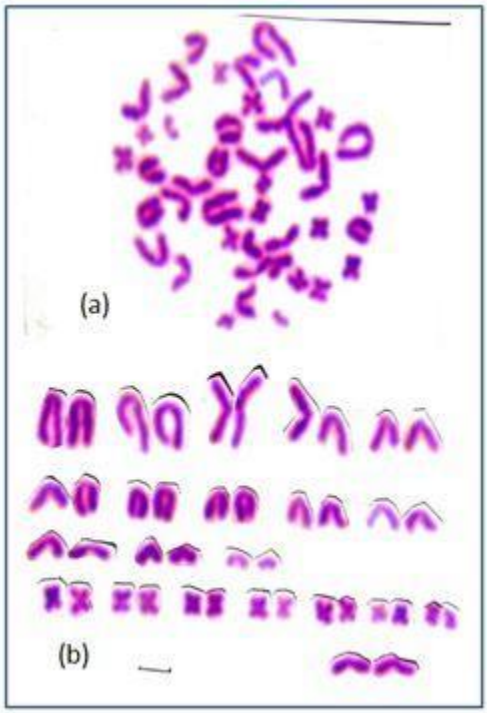

Figure 6: Metaphase a and karyotype b of *Rattus norvegicus*

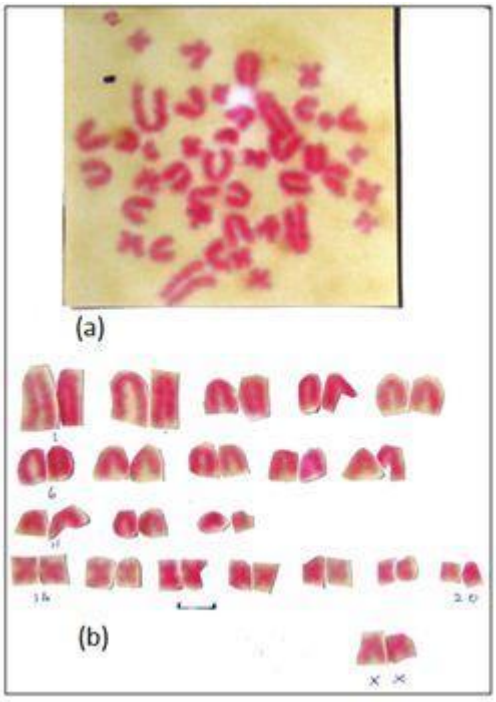

Figure 7: Metaphase a and karyotype b of *Rattus nitidus*

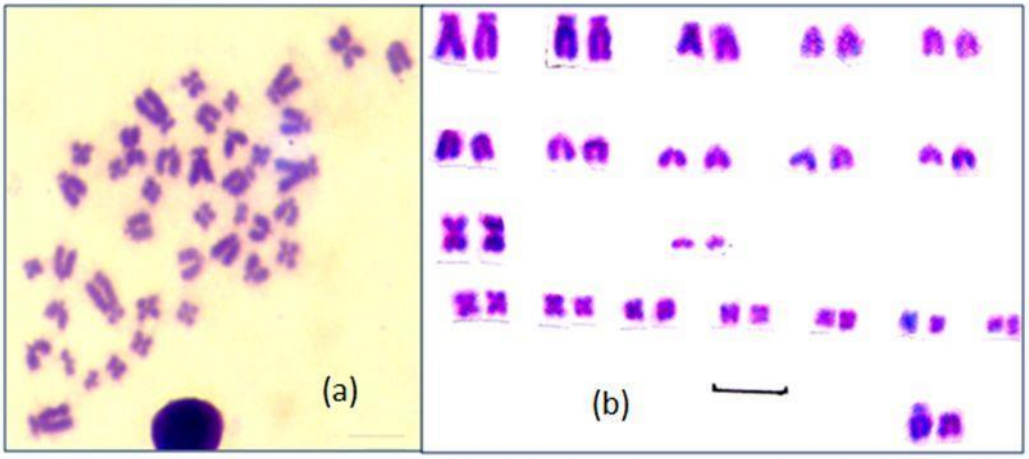

Figure 8: Metaphase a and karyotype b of *Berylmys manipulus*

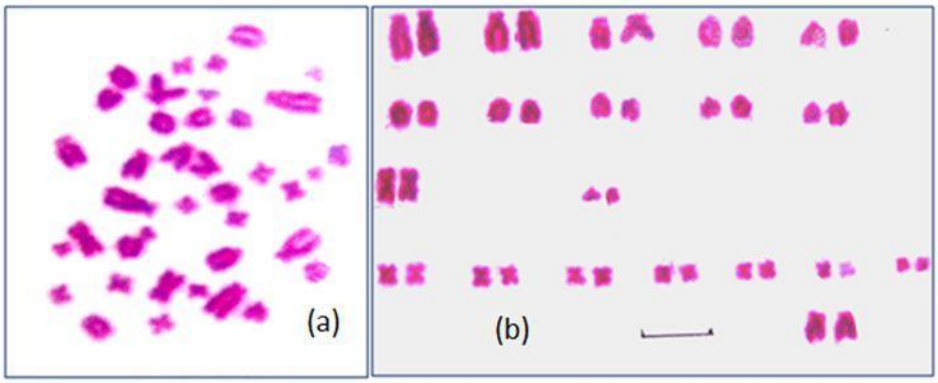

Figure 9: Metaphase a and karyotype b of *Berylmys mackenziei*

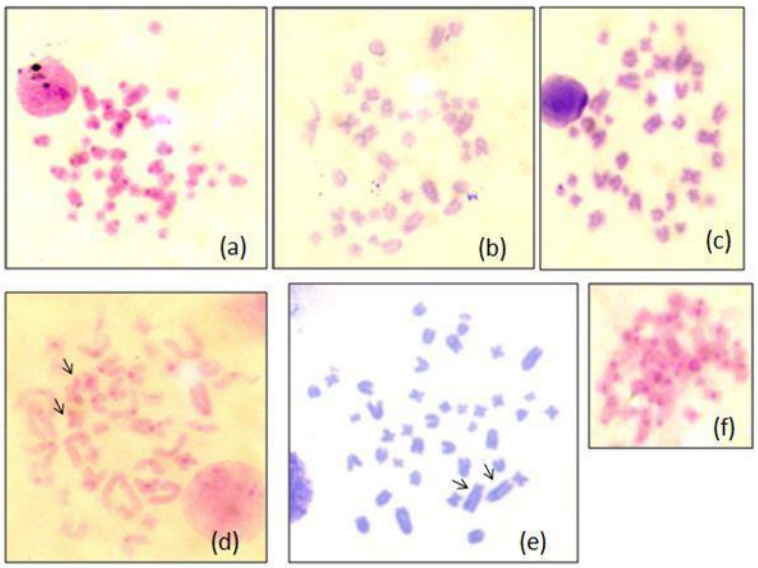

Figure 10: a-C-banded metaphase plate of *B. manipulus*, b-C-banded metaphase plate of *B. mackenziei*, c- G-banded metaphase of *B. manipulus*, d- C-banded metaphase plate of R. nitidus (heteochromatic q- arm of sutelocentric XX, arrowed), e- heteromorphic #1 in R. rattus (arrowed), and f- small centromeric banding in R. rattus

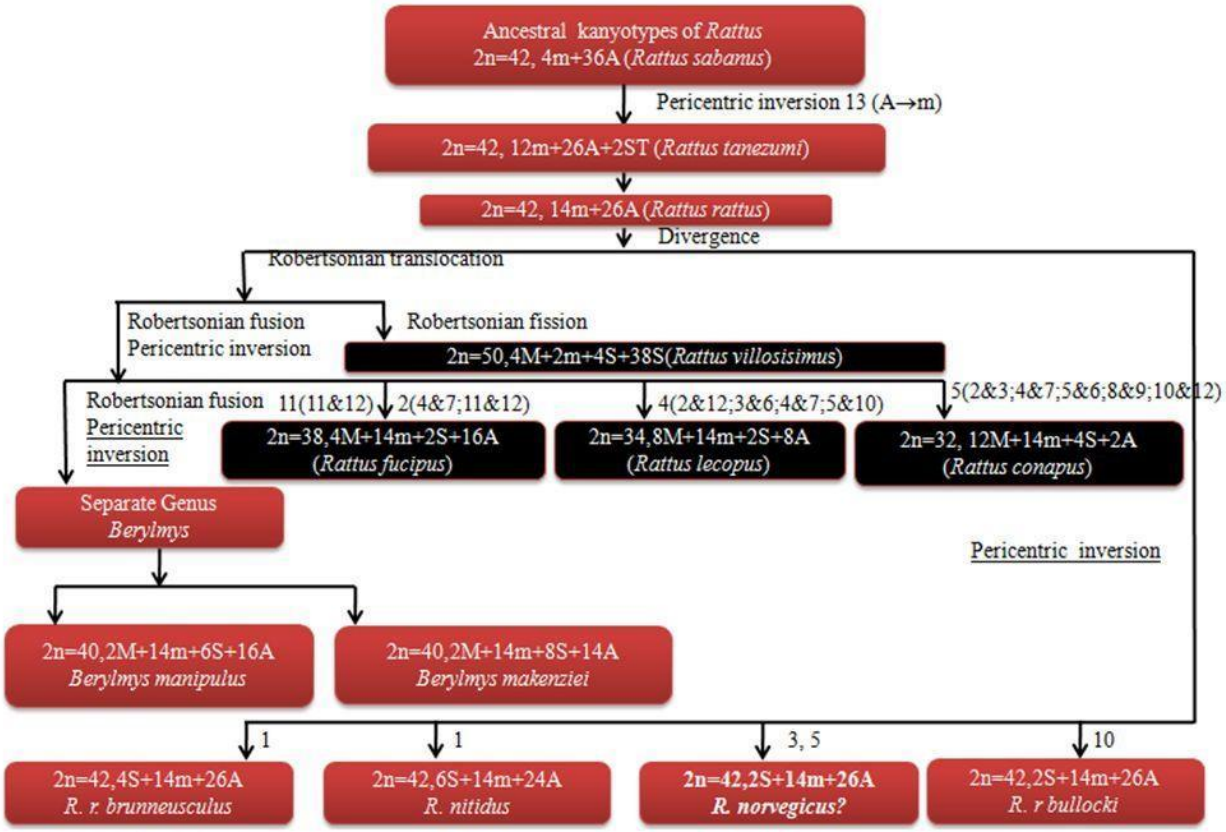

Figure 11: Phylogeny of the genus Rattus (without sex chromosomes), involving pericentric inversion and Robertsonian Fusion. S=Subtelocentric, M=large metacentric, m=small metacentric, A=acrocetric, numeral represents the chromosome numbers, black ones are of other authors

***Rattus rattus* from Kharam Waiphei,**
Senapati Districts and Thoubal District (24^{o} 71.000'', E 93^{o}92.550'')
Morphological features of the rats from the Kharam Waiphei, Senapati Districts and Thoubal were agouti dorsal but the ventral fur is light greyish (Figure 1c).
Cytology: The 2n=42, karyotype was found in ten individuals (8 females and 2 males) collected from Senapati and Thoubal District. It consisted of thirteen acrocentric pairs (#1 to 13), seven pairs of small metacentric pairs (#14 to 20) and acrocentrics X and Y (Figure 2). The X chromosome is comparable in size to the autosomal pair 6. The Y chromosome is the smallest acrocentric chromosome. This type is here considered as ancestral or primitive type.
***Rattus rattus* from Keishampat (**N 24^{0}47.806", E 93^{0}55.926") **and Langol, Imphal West (**N 24^{0}82.798'', E 93^{0}89.965'')**(**Figure 1 d-f).
Morphological features of the rats from the Imphal West were agouti dorsal and venter yellowish white fur (Figure 1 d).
Cytology: The 2n=42 karyotype was found in three individuals (1 females and 2 males) collected from Imphal West district (Langol Locality). Their karyotype consists of eleven acrocentrics pairs (#1 to 9, 11 and 12); pair 10 was homomorphic subtelocentric pair, one heteromorphic pair to subtelocentric/acrocentrics (#13), seven pairs of small metacentric (#14 to 20) elements, and the two acrocentrics X and Y chromosomes. The rats from Keishampat (one male) showed polymorphic in pair #1 due to acrocentric and subtelocentric and rest of the karyotype was quite similar to Langol type. The X chromosome is comparable in size to the 12th autosomal pair while the Y is the shortest element of the male genome (Figure 3).

***Rattus tanezumi* Temminck, 1844 from Karang of Loktak Lake (**N $24^0$54.770'' E $93^0$83.300''**), Bishnupur District**

The rats from the Karang (PBG 345) were identified as *Rattus tanezumi* with the help of mitochondrial DNA (COI). The *Rattus tanezumi* have a unique feature which is the presence of a smoky T-shaped around throat and thoracic region as on upper parts of manus and (Figure 1 g-i)

Cytology: 2n=42, without exception the karyotype consisted of thirteen acrocentrics pairs (#1 to 13), seven pairs of small metacentric pairs (#14 to 20) and acrocentrics XY, and sex chromosomes were acrocentrics. The X chromosome was 10^{th} of autosomal chromosome in length and Y chromosome is the shortest in the genome (Figure 4). Chromosome numbers 15, 19 and 20 were heteromorphic due to metacentric and submetacentrics homologues.

***Rattus rattus brunneusculus* (Hodgson, 1845) from Singjamei, Imphal East (**N $24^0$49.196", E $93^0$56.564"**)**

Diploid count is 42 consisting of chromosome numbers 1 and 9 being subtelocentrics, # 2-5, # 7 to 13 being acrocentrics, The X chromosome is acrocentrics which is comparable to sixth autosomal pair. The Y chromosome is the smallest acrocentric chromosomes (Figure 5).

***Rattus norvegicus* (Berkenhout, 1769) from Keishampat, Imphal West** 1769. *Mus norvegicus* Berkenhout, *Outlines Nat.Hist.Gt. Britain and Ireland*, 1; 5 (Great Britain).

Genaral Characters: A bandicoot –like large rat, having an obscurely bicoloured tail, shorter than head and body length (80-95%) and relatively smaller ears (16-23). Fur course with spines. Dorsum dark brown and venter grey. Skull large, occipitonasal length 45-55 mm; supraorbital ridges powerful, extending backwards fairly straight up to occiput; palate long, more than one half of occipitonasal length (*onl)*, extending posteriorly far behind third upper molars; maxillary too throws less than 15% of *onl* and its width ranges from 2.0-2.3 mm; anterior palatal foramina, on average, 17 % of *onl* and broad (3.0-4.6), ending far ahead of first upper molars; braincase narrow, cranial width less than 28% of *onl*, Mammae 5 or 6 (Agrawal, 2000).

This species is robust and heavily built. The tail is always less than head and body length in adult specimens. The ear is short and when drawn forward, does not reach the eyes. External measurements are given in Table 2. Dorsal fur colour varies slightly from dark brownish to ochre (particularly in old specimens) and the dorsal hair bases are greyish. Tail is slightly is bicoloured and covered with short, sparse, dirty, whitish hairs. The soles of the fore and hind feet are completely naked. The upper surface of both the fore and hind feet are covered with tiny whitish hairs but the base of the hind upper surface has patches of grayish hairs. The hairs on ventral fur are dirty white but bases are greyish. The line of demarcation is quite distinct. The species has six pairs of mammae (2 pairs pectoral, 1 pair abdominal and 3 pairs inguinal).

Cytology: The rats from Imphal West district with the parallel supra-orbital ridge are to be *Rattus norvegicus* (Berkenhout). The species has 2n=42 with exception to karyotype thirteen acrocentrics pairs (#1 to 13), seven small metacentric pairs (14 to 20) and acrocentrics sex chromosomes. The X chromosome was 11^{th} of autosomal chromosome in length and Y chromosome is the shortest in the male genome (Figure 6).

***Rattus nitidus* (Hodgson, 1845) from Mao (**25° 43.520'' E 94° 06.820'')**, Senapati District** 1845. *Musnitidus* Hodgson, *Ann.Mag.nat.Hist.*, **15,** 267.

General character- In this species the fur is soft and quite thick, particularly in the typical race. The tail is nearly naked, 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 a rule (107% average for typical race, Hinton; 99% average for *obsoletus*). Mammae 12, as a rule.

Colour.- 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 dark as a whole (Ellerman, 1961).

The rats from the Mao (PBG 344) are identified as *Rattus nitidus*on the basis of mitochondrial DNA (COI) sequences. The sequence is submitted in the NCBI Gene Bank (NCBI accession number was JQ918374).

Cytology: The 2n of *Rattus nitidus* is 42. The karyotypes comprising two pairs of subtelocentric chromosomes (#1 and 9), 10 pairs of acrocentrics chromosomes (#2-8, 10-12), chromosome pair 13 is metacentric, seven pairs of small metacentric chromosomes (#14 to 20). The X subtelocentrics which are 8th of autosomal chromosome in length (Figure 7) in which the q-arms are always heterochromatic (Figure 10 d).

Berylmys

1947. *Berylmys* Ellerman, *Proc.zool.Soc.Lond*., **117** 261,267.

Type species :*Epimysmanipulus* Thomas.

Genus *Berylmys*is characterized by dense, crisp, iron grey pelage, palate long but not extending posteriorly beyond third upper molars, diastema more than 28% of occipitonasal length, bulla, in Indian species, about 15% of occipitonasal length and lower incisor root forming a prominent knob on outer side of lower jaw.

This genus is represented by four species, *Berylmys manipulus, B.bowersii, B.mackenzie*iand*B.berdmorei*.

Key to Indian species of the Genus Berylmys

1. Size small, occipitonasal length of skull less than 41 mm; diastema long, more than 33% of *onl*; upper surface of hind –foot including toes white……..***B. manipulus***

Skull large, occipitonasal length of skull more than 41 mm; diastema short, less than 33% on *onl*; upper surface of hind-foot brown, only toes white……2

2. Occipitonasal length of skull is more than 52 mm, maxillary toothrows more than 9.6 mm in length; mammae 4 pairs……..***B.bowersii***

Occipitonasal length of skull is less 52 mm; maxillary toothrows less than 9.6 mm in length; mammae 5 pairs……..*B. mackenziei*

***Berylmys manipulus* (Thomas, 1916) from Keinou,(N** 24^{o}64.260'', E 93^{o}78.000'') **Senapati District**

***Berylmys manipulus* (Thomas) 1916.** *Epimys manipulus* Thomas, *J. Bombay nat. Hist. Soc.*, **24**: 413 (Kabow Valley, Kindat, Myanmar). **1948.** *Rattus manipulus kekrimus* Roonwal, *J. Bombay nat. Hist. Soc.*, **14**: 386 (Kekrima, Nagaland, India).

General Characters: It is smallest of the Indian species of the genus *Berylmys*, having the tail equal to or longer than head and body length, body having dense, crisp, iron grey pelage above, and white below; distal one –third of tail white, rest brown; hind foot including the toes white (Figure 1 l, r and s).

Skull small, occipitonasal length less than 41 mm, having lengthened diastema (more than 33% on *onl*), smaller bulla (about 15% of *onl)* and smaller and narrower maxillary tooth rows(length 5.5-6.1 mm and width 1.8-2.0 mm).

The species *Berylmys berdmorei* with which *B. manipulus* was originally confused differs from it by the length of tail which is smaller than head and body and its distal half brown instead of white, and large inflated bulla (more than 7mm or more than 18% of *onl*).

Total length of the tail is 14.5 and white part is 9 cm and % of the white is 62.06%

This rat is initially named as *Rattus manipulus manipulus* (Thomas, 1916) but according Wilson and Reeder (1983), it had changed to *Berylmys manipulus* (Thomas, 1916).

Cytology: The diploid count is 40 consisting of three subtelocentric pairs (# 1, 2, and 3), 8 acrocentric pairs (# 4-10, and 13) and one big metacentric pair (#11) that is formed by the Robertsonian fusion of chromosome numbers 11 and 12 of the ancestral Asian black rat *Rattus rattus*, 7 small metacentric pairs (# 14-20), the X chromosome is acrocentric which is 9th in length of the autosomes and Y chromosome is acrocentrics comparable to 13th of the autosomes (Figure 8).

***Berylmys mackenziei* (Thomas, 1916) from Kharam, Senapati District** (N 24^{o}81.880'', E 93^{o}49.930'')

1916. *Epimysmackenziei* Thomas, *J. Bombay nat. Hist. Soc.*, **24**: 410 (Haingyan ca. 80 km w. Kindat, Chin Hills, Myanmar).

Diagnostic Characters: Smaller than *B. bowersii* in size (Fig. 1 a). This is reflected in length of head and body, hindfoot, occipitonasal and maxillary toothrows; bulla less than 15% of *onl*; diastema less than 31% of *onl* as in *bowersii* but shorter than *manipulus*. Body colour iron grey above, white below; distal one-third to one-half of tail white; hind foot brown, toes white. Mammae 5 pairs.

Cytology: This rat is initially named as *Rattus mackenziei* (Thomas, 1916) but according Wilson and Reeder (1983), it had changed to *Berylmysmanipulus*(Thomas, 1916). The diploid count is 40 consisting of three subtelocentric pairs (# 1, 2, and 3), eight acrocentric pairs (# 4-10, and 13) and one big metacentric pair (#11) that is formed by the Robertsonian fusion of chromosome numbers 11 and 12 of the ancestral Asian black *Rattus rattus*, 7 small metacentric pairs (# 14-20), the X chromosome is subtelocentric which is 6^{th} in length of the autosomes and Y chromosome is acrocentrics comparable to 9^{th} of the autosomes (Figure 9).

The heterochromatic region as evidence from the C-banded pattern points the difference between *B. manipulus* and *B. Mackenziei* (Figure 10 **a** and **b)**.

Soft Palate of the Species in the Study: the soft palate photographs of different species were compared and it can be shown that the *Rattusrattus, R. tanezumi, and R. Nitidus* have three chevron-shaped ridges while the *B. manipulus* has only four tapering ridges arising from the base of the molar teeth (Figure 1 m-q).

Discussion

Ellerman and Morrison-scout (1951) classified *R. rattus* with black coat colour inhabiting the northern part of the world, and *R. rattus alexandrinus* (Geof) with agouti coat colour inhabiting the southern part. Specimens of *Ratus rattus* species collected from hills have agouti as well as black colour while those from valley areas have agouti coat colour agreeing with the above two coat colours of this species. Tolga *et al.,* (1998) reported four main colorations in this species. The first type, has a dark, slate blackish dorsal fur and greyish ventral fur, and is also referred to as *R. rattus rattus*. The second type, has dark brownish dorsal fur with yellowish white ventral fur, and is known as *R. rattus elaxandrinus*. The third type is dorsally the same as in the second type, but the ventral fur is light greyish and the upper sides of the feet are pale brownish, and it is referred to as *R. r. alexandrinus*. In these three types, the hair bases of dorsal and ventral furs are greyish. The fourth type, which is dorsally similar to the second and the third types, has ventral fur that is pure white, and is referred to as *R. rattus frugivorus*. In the first type, the tail and the ear are uniformly covered with short, sparse blackish hairs, the dorsal colour grows paler toward the flanks, but the line of demarcation along the flanks is not very distinct. If available published data are for sure, the present specimen from the Senapati District might be *R. r. alexandrines* while the rats from Thoubal and Imphal West might be *R. rattus elaxandrinus.* The morphological features frequently lead to misidentification in the cryptic species and so the specimen in the present study should be re-examined in future to confirm the true identity through sequencing the mitochondrial COI.

The chromosome compliment of the *Rattus rattus* in the present study shows the polymorphic chromosome numbers of 1 (heteromorphic in Imphal West, homomorphic in the Imphal East), 9 (homomorphic in *Rattus brunneuculus*) and 13 (heteromorphic in *Rattus brunneuculus*) are polymorphic in Manipur. Another polymorphic chromosome pair is number 10 (Imphal West) which is found in *Rattus bullocki*. Hence all the polymorphic chromosomes in the present study are one of the polymorphic chromosomes of the *Rattus rattus* as reported by Yoshida (1983) except pair number #10. Depending upon the length of the acrocentrics and subtelocentrics in the study, it can be referred that the subtelocentrics were derived from the acrocentrics counterparts through the pericentric inversion as reported by Yosida (1983) and Pages *et al.,* (2011).

Gadi and Sharma (1983) reported 2n=42 for *Rattus rattus*, consisting of 7 small metacentrics, 3 subtelocentric, 10 telocentric pairs and telocentric X and Y chromosomes which is agree with the present work except for the number of the subtelocentric. The number of the subtelocentrics may vary due to the pericentric inversion. Wilson and Reeder (2005) synonymised *Rattus brunneusculus* (Hogson, 1945) with *Rattus tanezumi*. In Indian context too *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 it is yet to decide the true taxonomic position of *Rattus tanezumi* particularly in Indian context. A further study in involving the three species through morphology, cytology and COI sequence are much to resolve the taxonomic conflicts of these three species in future.

Here in the present study the *Rattus brunneusculus*is quite different from the *tanezumi* in both morphological and cytological point of view. In *tanezumi* the chromosomes are telocentric in all the autosomal as well as the sex chromosomes bit in the *brunneusculus* the chromosome numbers 1 and 9 were homomorphic subtelocentrics but sex chromosomes are similar. So much work should be done to discriminate these two taxa. Out of the five types of the karyotypes recognised by Yosida (1980a), the Manipuri *Rattus rattus* should be regarded as Japanese types- (2n=42, with low C-banding; *R. tanezumi*). This shows that the heterochromatin diminution is an effective mechanism in the evolution of the karyotypes that led to obvious differentiation among the species and in the speciation process.

Rattus 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; it is also found on the islands of central Sulawesi, Luzon island of the Philippines, PulauSeram 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; Musser and Carleton, 2005).

Li *et al.,* (2008) and Gadi and Sharma (1983) 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 Manipur *Rattus nitidus*is quite similar to the Chinese species in the autosomal compliments but the heterochromatic q-arms in the X-chromosomes are yet to be reported from the other part of the world.

According to Yosida (1983) the Asian black rat with 42 chromosomes is the ancestor of the Ceylonese black rat with 40 chromosomes and the Oceanian black rat with 38 chromosomes. He proposed that 40 chromosomes (*Rattus rattus kandianus* in the Sri Lanka, *Beylmysmanipulus and Berylmys mackenziei* of present studies) and 38 chromosome karyotypes evolved from 42 chromosome karyotype through a first Robertsonian fusion of acrocentric pairs 11 and 12 and a second Robertsonian fusion between acrocentric pairs 4 and 7.

It is considered that the progenitors of the members of the genus *Rattus* originally had acrocentric karyotype and the subtelocentric and metacentric members were derived by pericentric inversion of the acrocentric chromosomes (Yosida, 1983).

Manipur is one of gateways of South Asian to the Indian continent, the ancestral *Rattus rattus* might have passed through, hybridized here and result into diversity of Black rats. The first Robertsonian fusion might had formed here in the Indo-Burma areas and travel through up to Western India and might have occurred there the second Robertsonian fusion and formed the rats with 2n=38. But the comparative studies of the softpalate shows other facts; these structures having three chevron-shaped ridges are present in the different species of the genus *Rattus* but the fingers like soft palate ridges of the *Berylmys* are not at all related to the genus *Rattus*.

The features are quite common in the genus *Niviventer* and morphology too except for the pure white tail tips. The only karyotypic similarity between *Rattus* and *Berylmys* is the 14 metacentric chromosomes and differ in the number of the subtelocentris. Considering the differences in autosomal chromosomes and soft palate, there is not much identical in these two genera. But much works should take up involving the two genera in future.

Phylogeny of the present study begins as *Rattus sabanus* an ancestral karyotype and divergent karyotypes were obtained due to pericentric inversion of acrocentric chromosomes to subtelocentrics and much

deviated karyotypes were seen among the different species of the genus *Rattus* in Manipur (Figure 11). Here the number of the metacentrics is the reference points to decide the primitiveness of the species. In *Rattus tanezumi* the number of metacentric chromosomes is 12 and rest of the *Rattus* species are 14 in number.

The number of subtelocentrics not considered in the study.

The summary of the present study can be as follows:

1. The autosomal chromosome compliments of different species of the genus *Rattus* quite agree with the published data.
2. The chromosomes compliments of different species of the genus *Rattus* are one of the polymorphic forms of the *Rattus rattus*.
3. The studies of the species reveal that the polymorphic chromosomes in the genus *Rattus* were pair numbers 1, 9, 10 and 13.
4. The cytological studies of *Berylmys*species (2n=40) as reported by other authorsneed to be re-evaluated particularly in the Manipur rats.

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