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Title	Species and genetic diversity of Bandicota (Murinae, Rodentia) from Myanmar based on mitochondrial and nuclear gene sequences
Author(s)	Mori, Satoko; Thwe, Thidalay; Thu, Wai Min et al.
Citation	Mammal Research, 65(3), 493-502 https://doi.org/10.1007/s13364-020-00491-1
Issue Date	2020-07
Doc URL	https://hdl.handle.net/2115/82108
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Type	journal article
File Information	Mammal Res.v.65(3).pdf

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Species and genetic diversity of *Bandicota* (Murinae, Rodentia) from Myanmar based on mitochondrial and nuclear gene sequences

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Running head: Phylogeny of Myanmar *Bandicota*

Abstract

Bandicoot rats (genus *Bandicota*), widely known as rodent pests, are abundant and widespread throughout the continental part of the Indo-Malayan Realm. However, their evolutionary history is not yet well understood. The molecular phylogenetic relationships of the three bandicoot rat species, *Bandicota bengalensis*, *B. indica*, and *B. savilei*, were assessed based on the gene sequences of specimens collected from Myanmar, where all three species occur, along with database sequences. Early divergence of *B. savilei* (1.5–1.7 million years ago) was inferred from the mitochondrial cytochrome *b* (*Cytb*) gene, and the nuclear interphoto-receptor retinoid binding protein (*Irbp*), and melanocortin 1 receptor (*Mc1r*) gene sequences. The *Cytb* lineage of *B. bengalensis* from Sri Lanka was distinct from the monophyletic lineage of the continental lineages of *B. bengalensis* and *B. indica*. This can be explained by the preservation of ancient mitochondrial DNA (mtDNA) in the insular population owing to female philopatry and male dispersal, given that no substantial intraspecies geographic subdivision was observed in the nuclear markers. The paraphyletic relationship of *B. bengalensis* with *B. indica* may be explained by introgression of the mtDNA from *B. bengalensis* to *B. indica*, but further investigation is required to confirm this. *B. bengalensis Cytb* sequences from a wide area of Myanmar had limited nucleotide diversity ($\pi = 0.00079$), implying that the genetic diversity of *B. bengalensis* in Myanmar was acquired through Holocene human activities.

Keywords: Phylogeny, *Mc1r*, *Cytb*, *Irbp*, bandicoot rats, Myanmar

Introduction

Among the subfamily Murinae, an extremely species-rich group of rodents (over 500 species and 120 genera; Musser and Carleton 2005), the group of rats taxonomically referred to as the Rattini tribe is the most diverse, comprising 35 genera (e.g., *Rattus* and *Bandicota*) and 167 species (Pagès et al. 2010). The Rattini tribe is distributed primarily in the Indo-Malayan Realm and consists of several species with broad geographic distributions (Wilson et al. 2016). Some of the murine rodents are known as commensal rodents and have a substantial impact on human activities, for example by causing agricultural damage and spreading infectious diseases (Kosoy et al. 2015). Several molecular studies have been carried out on murine rodents from the Indo-Malayan realm (Steppan et al. 2005; Pagès et al. 2010; Fabre et al. 2012; Schenk et al. 2013), and in particular on commensal murine rodents such as the roof rat (*Rattus rattus* complex) (Pagès et al. 2010; Aplin et al. 2011) and the Pacific rat (*R. exulans*) (Thompson et al. 2014). However, the evolutionary history of some species remains incompletely understood, partly because several areas, including Myanmar, have been less intensively studied. Investigation of species from less studied areas is urgently required to fill the gaps in our knowledge and provide a comprehensive overview of the evolutionary trends of commensal rodents in the Indo-Malayan Realm.

Bandicoot rats (the genus *Bandicota*) have a large body size (ca. 200 mm head and body length) and live in close proximity with people as domestic pests. The genus *Bandicota* consists of three species: *B. bengalensis* (the Bengal bandicoot rat), *B. indica* (the greater bandicoot rat), and *B. savilei* (Savile's bandicoot rat). *B. bengalensis* and *B. savilei* are distributed in the western and eastern parts of the generic range, respectively, and *B. indica* is sympatric with the other two species. They are closely related to *Rattus rattus* (*Rattus rattus* Complex, RrC), *R. norvegicus*, and *R. exulans* of the Rattini species, as shown by molecular phylogenetic inference (Fabre et al. 2012; Schenket al. 2013). At present, the available molecular data for phylogenetic inference are limited in many geographic areas, especially Myanmar, the central part of the distribution range of the genus wherein all three species occur. The phylogenetic relationship among these three species of *Bandicota* is, therefore, not yet fully understood.

Several phylogenetic inference studies on the *Bandicota* species have been carried out using mitochondrial DNA (mtDNA) markers, cytochrome *b* (*Cytb*) and cytochrome *c* oxidase I (*CoI*) genes, and nuclear markers of interphoto-receptor retinoid-binding protein (*Irbp*) and the melanocortin 1 receptor (*Mc1r*) genes (e.g., Pagès et al. 2010; Kambe et al. 2011; Yasuda et al. 2014). Recently, studies of the *Cytb* sequences of *B. bengalensis* from Sri Lanka revealed that the mtDNA sequences from Pakistan and Sri Lanka were paraphyletic with respect to the lineage of *B. indica* (Yasuda et al. 2014). This can be explained in a number of ways, including by questioning the validity of the taxonomic identification (Yasuda et al. 2014). In general, the paraphyly of mtDNA can be explained either by mtDNA introgression (Shaw 2002; Boratyński et al. 2014; Tosi al. 2019), or by incomplete lineage sorting of ancient polymorphisms (Kearns et al. 2014). To address these issues, it is essential to analyze nuclear gene sequences, and to investigate additional individuals from more localities.

In this study, we focused on the phylogenetic relationships of *Bandicota* from Myanmar, where no molecular phylogenetic study on *Bandicota* has previously been reported. We performed phylogenetic analyses of the three *Bandicota* species collected from Myanmar using the mitochondrial gene *Cytb* and two nuclear genes, *Irbp* and *Mc1r*, to better understand the phylogenetic and phylogeographic status of *Bandicota*. This study addresses the issue of intraspecies genetic variation of *B. bengalensis* in Myanmar.

Materials and Methods

Sample collection

Sample collection in Myanmar was conducted by researchers from the Department of Zoology, University of Yangon in 2013, 2014, 2015, and 2018. Specimens were collected from rural areas near the Ayeyarwady River basin, spanning five Myanmar cities from north to south: Mandalay, Bagan, Taunggyi, Nai Pyi Taw, Pyay, and Yangon, in addition to Kalaymiyo, a town close to the border with India (Fig. 1, Table 1). Our trapping effort yielded specimens of *B. bengalensis* (n = 23), *B. indica* (n = 1), and *B. savilei* (n = 3) from seven sampling localities, together with *Mus cookii* from Kalaymiyo. Liver tissue samples were preserved in 99% ethanol. The taxidermically prepared skin of each specimen was preserved as specimen vouchers in the Department of Zoology, University of Yangon, and labeled with a YUZ series specimen code. We obtained liver tissue samples from a total of 27 *Bandicota* rats. Other DNA samples of *B. bengalensis* from Sri Lanka (Yasuda et al. 2014) were included in this study and subjected to phylogenetic analyses.

DNA extraction, amplification, and sequencing

We targeted the mitochondrial gene marker *Cytb* and two nuclear gene markers, the first exons encoding *Irbp* and *Mc1r*, which are established markers for phylogenetic research on rodents (Serizawa et al. 2000; Jansa and Weksler 2004; Michaux et al. 2002a; Kambe et al. 2011; Yasuda et al. 2014), and *Mc1r*, a coat color-related gene, that has been used to infer phylogeny (Shimada et al. 2009; Kodama et al. 2015). DNA was extracted from tissues using a DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The primer sets used to amplify the *Mc1r* (954 bp), *Irbp* (1,128 bp), and *Cytb* (1,026 or 1,140 bp) genes are listed in Table 2. PCR was performed using AmpliTaq Gold 360 Master Mix (Life Technologies, Carlsbad, CA, USA). PCR products were sequenced with the BigDye Terminator Cycle Sequencing Kit (version 3.1; Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, followed by base detection using an ABI3130 genetic analyzer. Sequence alignment was performed with ProSeqv3.5 (Filatov 2009). Haplotype sequences of the nuclear genes were inferred using PHASE v 2.1 (Stephens et al. 2001). The sequences collected in this study were deposited in the DDBJ/EMBL/GenBank database under accession numbers MK654760–MK654784, MK674609, MK674610, MK695508–MK695525, and MK715435.

Phylogenetic analyses

Phylogenetic analyses were conducted with the maximum likelihood (ML) method (Felsenstein, 1981) using sequence data generated in this study and obtained from DNA databases (DDBJ/EMBL/GenBank). *Cytb* sequences of *B. bengalensis* (AM408336), *B. indica* (HM217378, HM217380, HM217425, HM217426, HM217435, and HM217447), and *B. savilei* (HM217427 and HM217437) were obtained from the databases. ML tree construction and pairwise distance estimation were performed using MEGA version 7.0 (Kumar et al. 2016). For the ML method, the best-fit nucleotide-substitution models and parameters were determined according to the Akaike information criterion (AIC, Posada and Buckley, 2004), as implemented in MEGA 7.0. The GTR+G+I, T92+G, and TN93+G were suggested as the best models for the *Cytb*, *Irbp*, and *Mc1r* data sets, respectively, based on the AIC values. Bootstrap values (Felsentein 1985) were calculated for the ML tree with 1,000 replicates.

The time to the most recent common ancestor (tMRCA) and 95% highest posterior density (HPD) were estimated using BEAST (ver. 1.8.0; Drummond and Rambaut, 2007) with representative haplotype sequences. Bayesian searches were conducted using the Markov chain Monte Carlo (MCMC) method for 10 million generations. The Yule process was used as the tree prior. Among the models compatible with the BEAST software, the GTR+G+I, TN93, and TN93+G models were used for the *Cytb*, *Irbp*, and *Mc1r* data sets, respectively. In the nuclear gene sequences, the divergence between *Rattus* and *Mus*, for which detailed fossil evidence is available, was used as a calibration point, and was set at 11.1–12.3 million years ago (Mya; mean, 11.81 Mya), as suggested by Kimura et al. (2015) based on their fossil examination, in the Bayesian calculations (Rawe et al. 2016; Camacho-Sanchez et al. 2017). In the *Cytb* data set, because deep calibration points are not appropriate for assessing recent divergence events owing to the issue of saturation (e.g., Suzuki et al. 2013), we used the strict-clock model, with an evolutionary rate of 0.031 substitutions/site/million years, in conjunction with a GTR+G+I substitution model. The evolutionary rate was obtained based on the biogeographic calibration point of 0.13 Mya, which represents the onset of the plateau phase of the time-dependent evolutionary rate of mtDNA, and can be applied to the assessment of divergence times on relatively ancient timescales (Hanazaki et al. 2017; Honda et al. 2019). The first 1,000,000 generations were discarded as a burn-in period. Tracer (ver. 1.7; Rambaut et al. 2014) was used to confirm that the effective sample size (ESS) was > 200 for all parameters, which indicated stationarity and was considered an adequate sample size. The trees were summarized using TreeAnnotator v. 1.8.0 (http://beast.community/treeannotator) and displayed in FigTree v. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Molecular phylogenetic analyses

We determined *Cytb*, *Irbp*, and *Mc1r* sequences in the three species of *Bandicota* rats from Myanmar, and constructed trees with the ML method using these sequence data and data from the existing databases (Fig. 2). We observed seven haplotypes (hap 1–7) of *Cytb* in *B. bengalensis*, five of which were collected from the geographic localities of Mandalay/Taunggyi (hap 1), Bagan/Nay Pyi Taw (hap 2), the west- (hap 3) and east- (hap 5) sides of Ayeyarwady River in Pyay, and Yangon (hap 4) (Table 1, Fig. 1d). Haps 6 and 7 were collected from Pakistan (Michaux et al. 2007) and Sri Lanka (Yasuda et al. 2014), respectively.

The *Cytb* tree showed the basal position of *B. savilei*, with a genetic distance of 0.076–0.104 (Fig. 2). The tree displayed the paraphyly of *B. bengalensis* with respect to *B. indica*, exhibiting two markedly divergent clades of *B. bengalensis*; clades I and II contained sequences from Southeast Asia (Myanmar, Pakistan) and Sri Lanka, respectively, with a sequence divergence of 0.058 (*d*, K2P), which was greater than that between the continental *B. bengalensis* and *B. indica* (*d* = 0.042). In *B. savilei*, two distinct clades appeared (clades I and II), with a clear geographic affinity; clade I was from Thailand (Pagès et al. 2010) and clade II was from Myanmar, with a genetic distance of 0.036. Similarly, in *B. indica*, two distinct clades (*d* = 0.032) were seen, between haplotypes (Table 1, Pagès et al. 2010) from southern Thailand and Myanmar (Clade I) and northern Thailand and Laos (Clade II).

In the phylogenetic analyses of the nuclear gene sequences of *Irbp*, the monophyly of *Bandicota* had a high bootstrap value of 99% (Fig. 2). The bootstrap values supporting the basal position of *B. savilei* and species-specific grouping for *B. bengalensis* were low (55–60%). Five haplotypes were observed in *B. bengalensis* (Table 1), divided into two groups; one contained only hap 3 and the other contained haps 1, 2, 4, and 5, all of which have long branch lengths. There was no apparent geographic affinity. Haps 1, 3, and 5 were recovered from both Myanmar and Sri Lanka.

In *Mc1r*, a total of nine haplotypes were recovered in *Bandicota*, and a species-specific grouping was observed with high supporting values for *B. bengalensis* and *B. savilei* (Fig. 2c). Among the three species, the basal position of *B. savilei* was observed with a low bootstrap value (53%). The ML tree showed three distinct lineages in *B. bengalensis*, one of which (lineage for haps 2 and 3) represented only rats from Sri Lanka. The lineage was not as distinct as that of the remaining two rat species, from Myanmar and Sri Lanka, with a genetic distance of 0.003; this was relatively low compared to those between *B. bengalensis* and *B. indica* (*d* = 0.016), and *B. bengalensis* and *B. savilei* (*d* = 0.015).

The time of divergence of *B. savilei* from the other two species was estimated to be 1.7, 1.5, and 1.7 Mya in the BEAST analyses for *Cytb*, *Irbp*, and *Mc1r*, respectively, with large 95% credibility intervals (Fig. 3).

Discussion

Mitochondrial paraphyly and its evolutionary implications

Our current and previous molecular phylogenetic analyses (Yasuda et al. 2014), which used the

mitochondrial (*Cytb*) and nuclear gene (*Irbp*, *Mc1r*) sequences, allowed us to view the phylogenetic relationships of the three species of *Bandicota*, thus aiding assessment of the evolutionary processes of species lineage differentiation and population genetic structuring.

Among the three species of *Bandicota*, we observed the basal position of *B. savilei* in the phylogenetic inference with the three nuclear markers, with a high supporting value (bootstrap value: 96%) but a low value (53–55%) for the nuclear gene markers (Fig. 2). Accordingly, the basal position of *B. savilei* was determined based on previous phylogenetic analyses of *Cytb* (Yasuda et al. 2014), and additional *Irbp* nuclear gene sequences (Fabre et al. 2012). Regarding the results of the nuclear gene analyses, it could be concluded that the genus *Bandicota* began lineage differentiation at 1.5–1.7 Mya, first yielding *B. savilei*, and then undergoing a subsequent divergence at 1.1–1.5 Mya, creating the lineages that led to *B. bengalensis* and *B. indica* (Fig. 3).

In contrast to the nuclear gene sequences, the phylogenetic relationship between *B. bengalensis* and *B. indica* is complicated by the phylogenetic inference with mtDNA. As shown in Fig. 2, *B. bengalensis* is paraphyletic with respect to *B. indica*, as predicted previously (Yasuda et al. 2014). The Sri Lankan lineage of mtDNA (hap 7) is obviously distinct from the other lineages from Myanmar (haps 1–5) and Pakistan (hap 6, Michaux et al. 2007), with an estimated divergence time of ca. 1.1 Mya (Fig. 3a). In contrast to the large amount of diversity observed in the mtDNA, the Sri Lanka population possesses similar nuclear gene sequences to those of the Myanmar population (Fig. 2), providing evidence of gene flow between these geographic regions until recently. Hence, the preservation of the ancient divergent mtDNA haplotype on the remote island of Sri Lanka likely indicates female philopatry and male dispersal (e.g., Hoelzer 1997; Toshi et al. 2010; Nater et al. 2011). The paraphyly of mtDNA can be explained either by mtDNA introgression (Shaw 2002; Boratyński et al. 2014; Tosi al. 2000) or incomplete lineage sorting of ancient polymorphisms (Kearns et al. 2014). Our estimate of the divergence time between *B. indica* and the continental mtDNA lineage of *B. bengalensis* is 0.76 Mya (Fig. 3a), and those for the two nuclear gene sequences are in the range 1.1–1.5 Mya, as discussed above. This may indicate mtDNA introgression between *B. bengalensis* and *B. indica* at around 0.8 Mya, although our phylogenetic results require further validation.

Population dynamics of *Bandicota* species

The current data enabled comparison of the level of mtDNA divergence in each of the three *Bandicota* species, and particularly the continental populations. There were two or three distinct lineages within this group of species, and the tMRCAs were estimated to be 0.25, 0.56, and 0.64 Mya for *B. bengalensis* (Clade I), *B. indica*, and *B. savilei*, respectively, based on the *Cytb* evolutionary rate of 0.031 substitutions/site/million years (Hanazaki et al. 2017; Honda et al. 2019). This implies long residence in the respective geographic areas of the continental *Bandicota* populations.

The two haplotypes of *B. savilei* from Nay Pyi Taw (Locality 5 in Fig. 1d), Myanmar, differed

substantially (d = 0.036) from the two reported haplotypes from Thailand (Pagès et al. 2010). If we assume that these two distinct mtDNA lineages represent the populations of Thailand and Myanmar, the western edge of the current *B. savilei* population in Myanmar may be an ancient residence, dating back to around 0.64 Mya. Similar phylogenetic traits can be seen in other murine rodents in Myanmar; these occur as the endemic species *Mus lepidoides* (Shimada et al. 2010) and *Mus nitidulus* (Shimada et al. 2007; Myat Myat Zaw et al. 2019), and as a distinct population lineage, *Mus caroli* (d = ca. 0.02, Shimada et al. 2007; Myat Myat Zaw et al. 2019).

In *B. bengalensis*, the majority of haplotypes from a broad area of Myanmar belong to subclade Ia (Figs. 1d, 2), in which the level of nucleotide diversity is low (π = 0.00079). A genetic architecture of *B. bengalensis* can be constructed, showing that five *Cytb* haplotypes from Myanmar (haps 1–5) follow a locality-specific pattern (Table 1, Fig. 1d). However, it is important to note the highly polymorphic state of the nuclear markers in the Myanmar population of *B. bengalensis* (Fig. 2). This may suggest that a dramatic change in population size has occurred in the Myanmar *B. bengalensis* population, in prehistoric or more recent times, affecting the genetic diversity of the mtDNA. This may have been caused by human activity (e.g., historical development of agricultural fields in the prehistoric age), or by natural environmental changes. Further research is needed to verify this hypothesis.

ACKNOWLEDGMENTS

We wish to express our gratitude to Pho Kaung, Nyunt Pe, Thet Tun Aung, Nyunt Soe, Maung Thynn, Myinzu Minn, Hla Win, Khin San Wai, and Mie Sein for their encouragement regarding this research collaboration. Our appreciation and thanks also go to Jun J. Sato, Khin Maung Htwe, Khin Maung Than, Mie Kyaw, Myo Myo Aye, Ryo Nakao, and Satoshi Odachi for their assistance with collecting the rat samples. Furthermore, we wish to acknowledge the two anonymous reviewers for their constructive comments. This work was conducted with support from JSPS KAKENHI Grant Number 17H04604. The study was also supported in part by JSPS KAKENHI Grant Numbers JS18H05508, 24405045, 26304035, and 17H04638, and by a grant-in-aid from the Research Program on Emerging and Re-emerging Infectious Diseases, AMED (JP15fk0108005, JP16fk0108117, JP17fk0108217, and JP18fk0108017).

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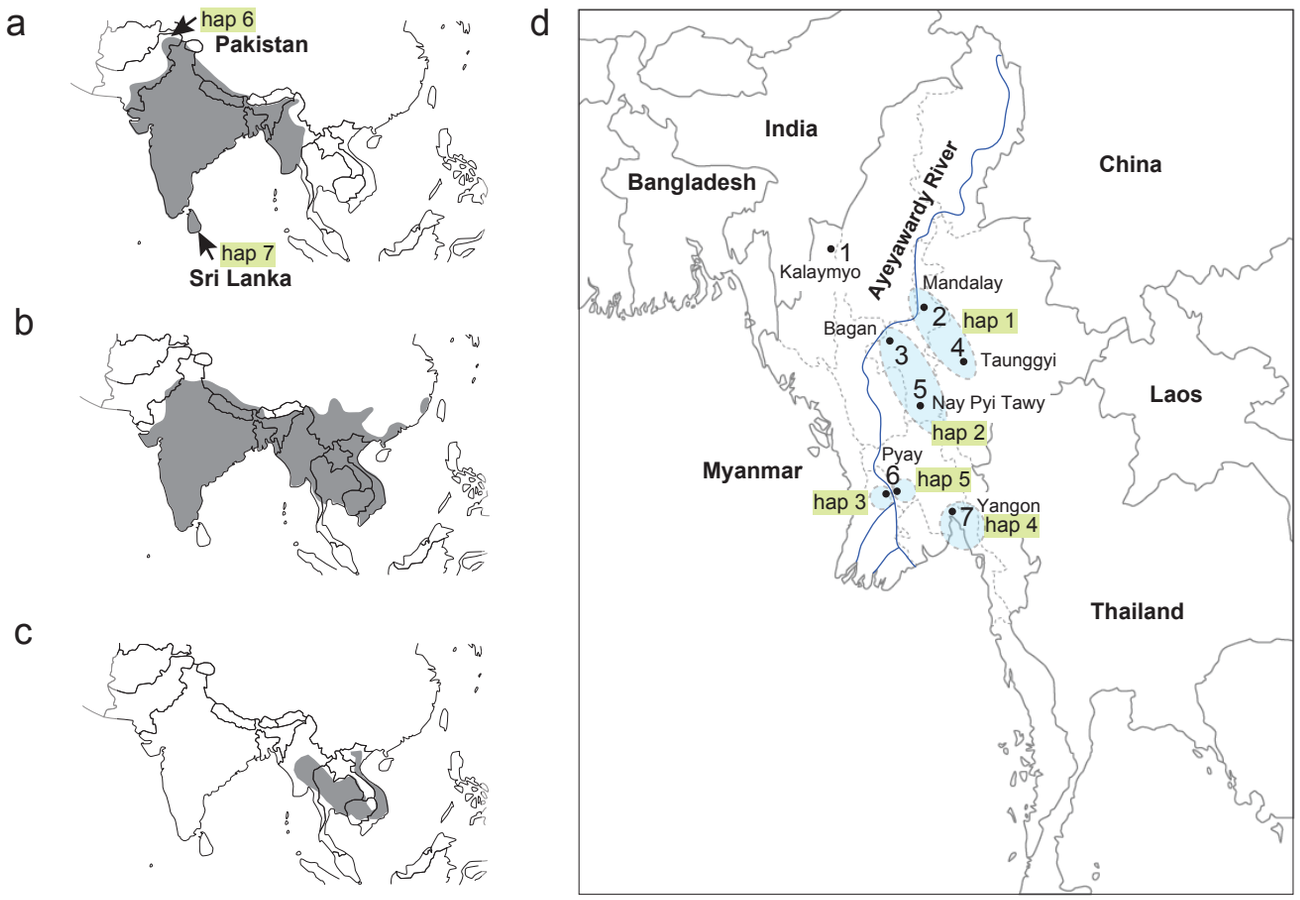
Yasuda SP, Gamage CD, Koizumi N, Nishio S, Isozumi R, Shimizu K, Koma T, Amada T, Suzuki H, Yoshimatsu K, Arikawa J (2014) Distinct genetic characteristics of Sri Lankan *Rattus* and *Bandicota* (Murinae, Rodentia) inferred from mitochondrial and nuclear markers. Genes Genet Syst 89:71–80

Figure legends

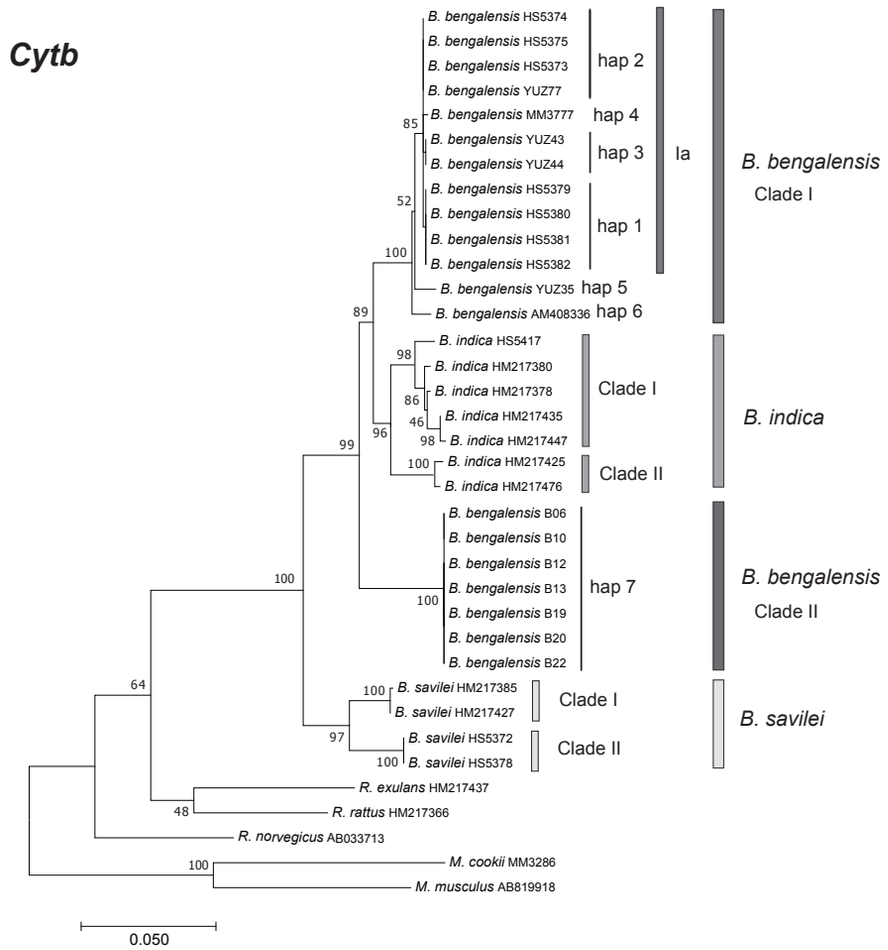
Figure 1. A distribution map of the genus *Bandicota* (Wilson et al. 2016): *B. bengalensis* is distributed in South Asia, including Sri Lanka, and to the east in central Myanmar (**a**); *B. indica* is distributed throughout most of the continental part of the Indo-Malayan Realm (**b**), and *B. savilei* is distributed in mainland South East Asia (**c**). A map of the collection sites (1–7) in Myanmar of the specimens used in this study (**d**). The distribution areas of the *Cytb* haplotypes of *B. bengalensis* from Myanmar (haps 1–5), Pakistan (hap 6), and Sri Lanka (hap 7) are shown.

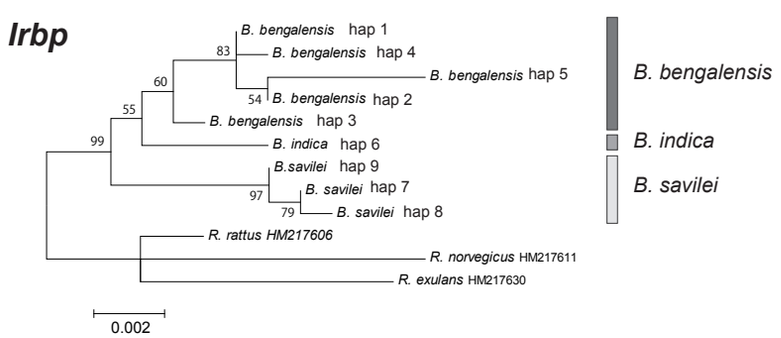
Figure 2. Maximum likelihood (ML) trees of the three species of *Bandicota* based on the mitochondrial *Cytb* (1,026 bp) gene and nuclear *Irbp* (1,128 bp) and *Mc1r* (954 bp) genes. The numbers at each node indicate the bootstrap support values (> 50%).

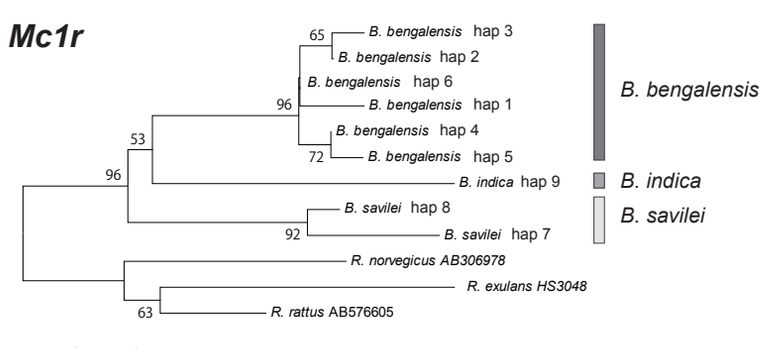
Figure 3. BEAST chronogram of the three species of *Bandicota* based on the mitochondrial *Cytb* (1,026 bp) gene and nuclear *Irbp* (1,128 bp), and *Mc1r* (954 bp) genes. Node bars indicate the 95% credible interval of the posterior density of divergence times. The numbers on the nodes represent the posterior mean divergence times.



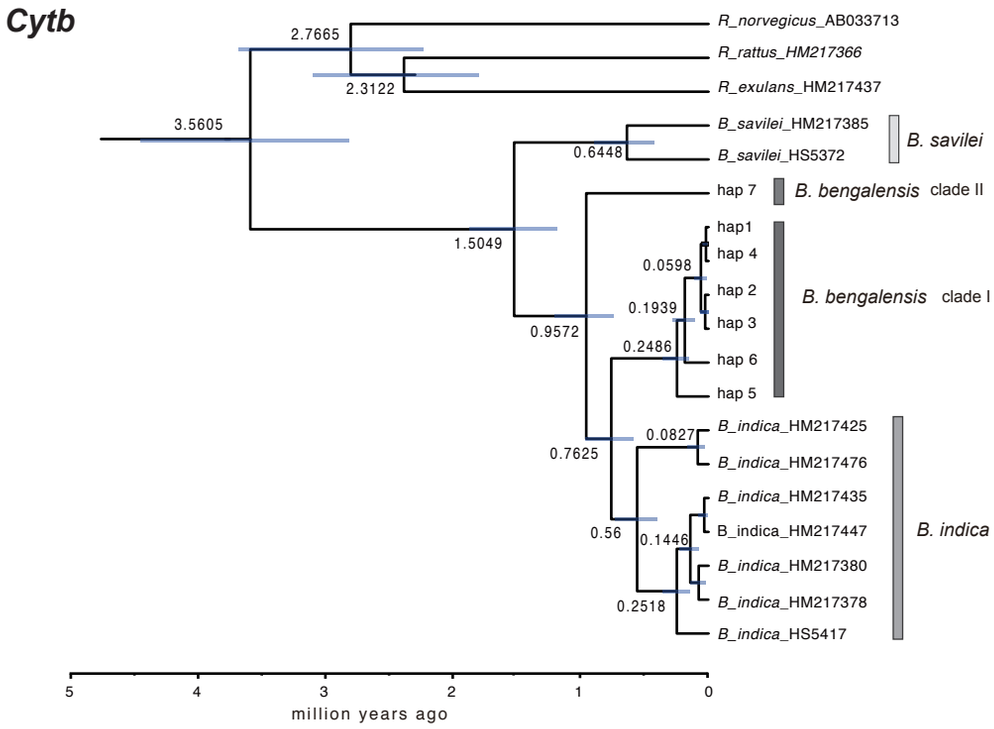
Mori et al. Fig. 1

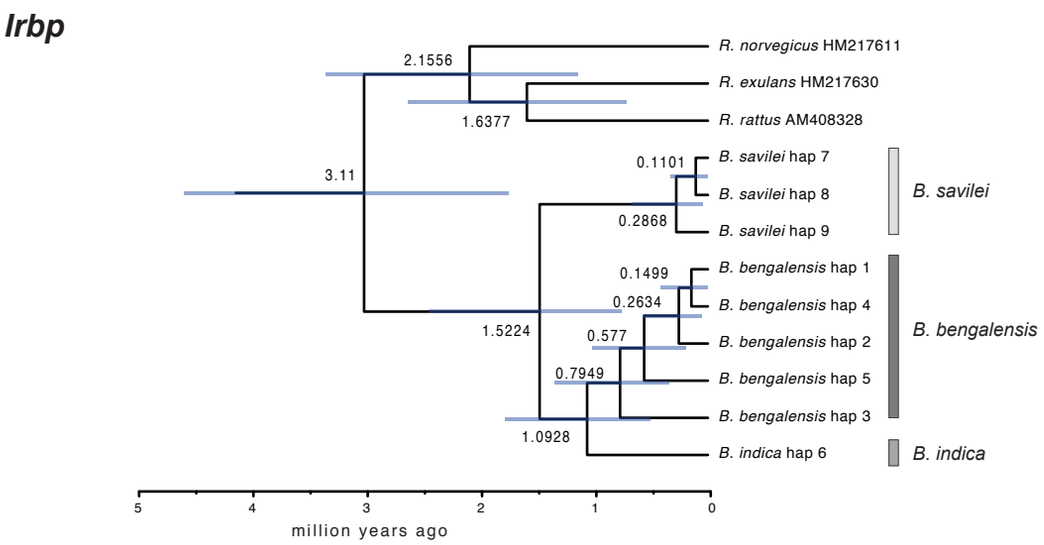


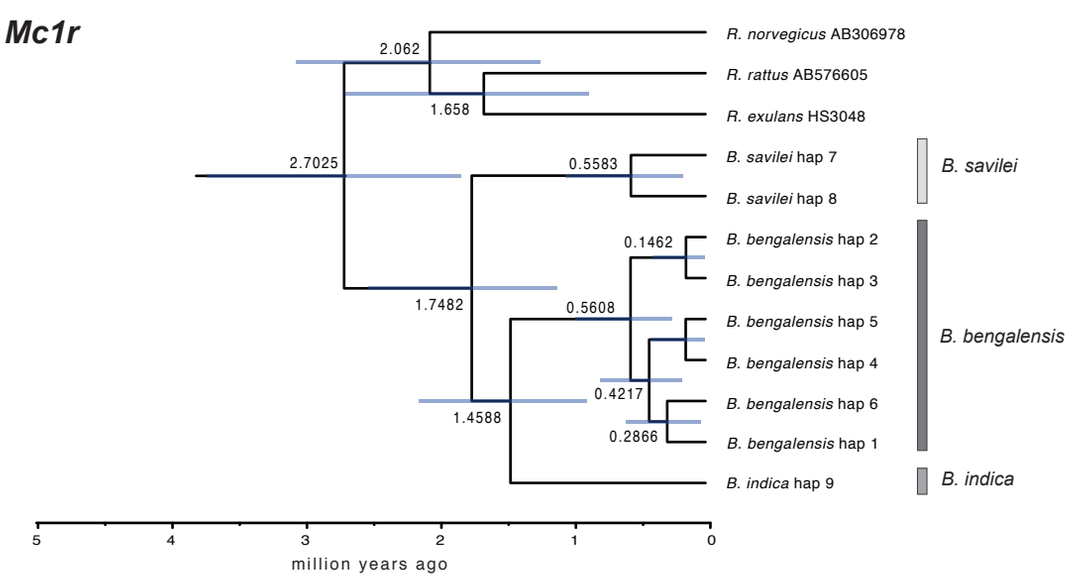




Mori et al., Fig. 2







Mori et al., Fig. 3

Table 1. List of samples used in this study.

Species Country Collection locality	Latitude	Longitude	Altitude (m)	Sample code (DNA code**)	*Cytb* haplotype	*Irbp* genotype	*Mc1r* genotype
Bandicota indica							
Myanmar							
Kalaymyo (#1)	23°11' 21.26"N	94°3'7.63"E	147	1. HS5417/MM3294	hap 1"	6/6	9/9
B. bengalensis							
Myanmar							
Mandaly (#2)	19°48' 51.9"N	96°11'40.0"E	109	2. HS5379/MM3194	hap 1	1/3	4/4
				3. HS5380/MM3195	hap 1	1/3	4/4
	21°59' 34.5"N	96° 11'29.6"E	92	4. HS5381/MM3196	hap 1	1/3	4/5
				5. HS5382/MM3197	hap 1	1/3	5/5
				6. HS5383/MM3198	hap 1	1/1	4/5
				7. HS5384/MM3199	hap 1	1/1	4/5
				8. HS5385/MM3200	hap 1	3/3	4/4
Bagan (#3)	21°3' 58.59"N	94°59'20.38"E	260	9. YUZ 75/MM4064	hap 2		
	21°7' 46.49"N	94°56'51.67"E	160	10. YUZ 77/MM4065	hap 2	4/4	4/4
				11. YUZ 73/MM4062	hap 2		
Taunggyi (#4)	-	-		12. H2772	hap 1		
	-	-		13. H2773	hap 1		
	-	-		14. H2786	hap 1		
	-	-		15. H2774	hap 1		
	-	-		16. H2776	hap 1		
	-	-		17. H2780	hap 1		
Nay Pyi Taw (#5)	19°48' 51.9"N	96°11' 40.0"E	109	18. HS5373/MM3187	hap 2	1/1	1/1
				19. HS5374/MM3189	hap 2	1/1	1/4
				20. HS5375/MM3190	hap 2	1/1	1/1
Pyay (#6)	18°49' 54.40N	95° 12' 2.15"E	20	21. YUZ 35	hap 5	1/4	4/4
	18°47' 49.51N	95° 14' 20.72"E	60	22. YUZ 43	hap 3	1/1	4/6
				23. YUZ 44	hap 3	1/3	4/4
Yangon (#7)	16°47' 58.01N	96° 10' 4.49"E	25	24. MM3777	hap 4		
Sri Lanka							
Kandy District	-	-		25. 73 (B6)	hap 7	2/2	2/3
	-	-		26. 117 (B7)	hap 7	1/3	3/3
	-	-		27. 119 (B12)	hap 7	1/5	2/2
	-	-		28. 120 (B13)	hap 7	2/2	2/3
	-	-		29. 128 (B19)	hap 7	1/3	2/3
	-	-		30. 129 (B20)	hap 7	3/3	3/3
	-	-		31. 131 (B22)	hap 7	5/5	2/3
B. savilei							
Myanmar							
Nay Pyi Taw (#5)	19°48'51.9"N	96° 11'40.0"E	109	32. HS5372/MM3181	hap 1'	7/9	7/7
				33. HS5377/MM3192	hap 1'	7/8	7/8
				34. HS5378/MM3193	hap 1'	9/9	7/8
Mus cookii							
Myanmar							
Kalaymyo (#1)	22°54'47.19"N	93° 38'31.69"E	1200	35. MM3286			

The collection points from Myanmar are indicated in Fig. 1d.

*A bandicoot rat with melanistic coat color.

**HS, personal code of HS; MM, code of NIID (National Institute of Infectious Diseases, Japan); B series, see Yasuda et al. (2014).

Table 2. List of primers used in this study.

Gene name	Primer designation	Nucleotide sequence	Cycling condition	Original publication
Melanocortin-1	**Mc1r1 (Fragment 1)**			
receptor gene	5'*Mc1r* (–52)	GCTCATACCACCTGGAGCTGCAGCC	30 cycle of 95°C/30 s,	Shimada et al. 2009
(*Mc1r*)	3'*Mc1r* (+504)	AAGAGGGTGCTGGAGACGATGCTGACC	55°C/30 s and 72°C/30 s	
	Mc1r2 (Fragment 2)			
	5'*Mc1r* (+131)	ATCCCAGATGGCCTCTTCCT		Shimada et al. 2009
	3'*Mc1r* (+1025)	CCCTTAGACAAATGGAGATCAGG		
Interphotoreceptor	**IRBP1 (Fragment 1)**			
retinoid binding	L1-*Rattus*	ATTGAGCAGGCTATGAAGAG	40 cycle of 94°C/30 s,	Page et al. 2010
proein	J2-*Rattus*	TAGGGCTTGCTCYGCAGG	58°C/30 s and 72°C/47 s	
(*Irbp*)	**IRBP2 (Fragment 2)**			
	L2	ATCCCCTATGTCATCTCCTACYTG	40 cycle of 94°C/30 s,	Poux et al. 2004
	J1	CGCAGGTCCATGATGAGGTGCTCCGTGTCCTG	52°C/30 s and 72°C/53 s	
Cytochrome *b*	**Cytb1 (Fragment 1)**			
(*Cytb*)	L14115	GACATGAAAAATCATCGTTG	35 cycle of 95°C/30 s,	Yasuda et al. 2005
	H655A	TGTGTAGTATGGGTGGAATGG	55°C/30 s and 72°C/60 s	Yasuda et al. 2014
	Cytb2 (Fragment 2)			
	L497A	CCTAGTAGAATGAATCTGAGG		Yasuda et al. 2014
	H15300	GTTTACAAGACCAGAGTAAT		Yasuda et al. 2005

All PCR reactions had an initial denaturation at 95°C for 10 min. The reactions were followed by a final extension at 72°C for 7 min.