



Greater Bandicoot Rats (*Bandicota indica*) are Not Native to Sundaland Based on Deoxyribonucleic Acid (DNA) Analyses

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Abstract

Bandicoot rats (genus *Bandicota*) are distributed widely across the Indomalay biogeographic realm of tropical East Asia. One widely distributed species, the greater bandicoot rat (*Bandicota indica*), has a disjunct distribution including both north and south of the biogeographic break at the Isthmus of Kra. We compared genetic variation of greater bandicoot rats from north and south of the Isthmus of Kra using mitochondrial cytochrome *b* (cyt *b*, 1140 bp) and nuclear interphotoreceptor retinoid binding protein (IRBP, 801 bp) sequences. We found that the greater bandicoot rat (*B. indica*) is not native to Sundaland, the region south of the Isthmus of Kra. The species was introduced to the region recently as the genetic divergence with other regions is very low and phylogenies of both genes showed Malaysian greater bandicoot rat very closely related to conspecifics from Lao PDR. Haplotype data revealed all individuals from Malaysia are homogenous, which implied that the species was introduced recently. The greater bandicoot rats in Malaysia are so far only reported in the rice producing regions of Kedah and Perlis, but they may be increasing in number and distribution. A more detailed survey on the distribution and population demographics of Malaysian greater bandicoot rats are needed to support a management plan for this invasive species.

Keywords Introduced species · Sundaland · Isthmus of kra · Genetic divergence · Indochina

Introduction

The Indomalay biogeographic realm has been the center of origin for many groups of rats (Murinae), and is particularly rich in species diversity (Musser and Carleton 2005). This biogeographic realm is further divided into biogeographic regions including the Indian subcontinent, Indochina,

Sundaland, and the Philippines (Lekagul and McNeely 1988; Myers et al. 2000). Many genera are endemic to a single biogeographic region (Musser and Carleton 2005; Francis 2008; Woodruff and Turner 2009). One exception is the bandicoot rats (genus *Bandicota*; see Fig. 1).

There are three species in the genus of bandicoot rats, all of which have large distributions in the Indomalay biogeographic realm. One species, Savile's bandicoot rat (*B. savilei*), is endemic to Indochina (Myanmar, Thailand, and Vietnam). The other two species of bandicoot rats, the greater bandicoot rat (*B. indica*) and the lesser bandicoot rat (*B. bengalensis*), are much more widely distributed (Musser and Carleton 2005; Francis 2008). The greater bandicoot rat has the widest distribution, including almost all of the Indian subcontinent and Indochina, a disjunct population on the Malay Peninsula, and a likely introduced population in Java (Fig. 1). The lesser bandicoot rat is primarily distributed across the Indian subcontinent including Sri Lanka, and likely introduced in Sumatra and Java. The populations on the Sunda islands (Sumatra and Java) are widely considered introduced (Aplin et al. 2003), but Musser and Newcomb (1983) suggested that all populations of all species of *Bandicota* in Sundaland may

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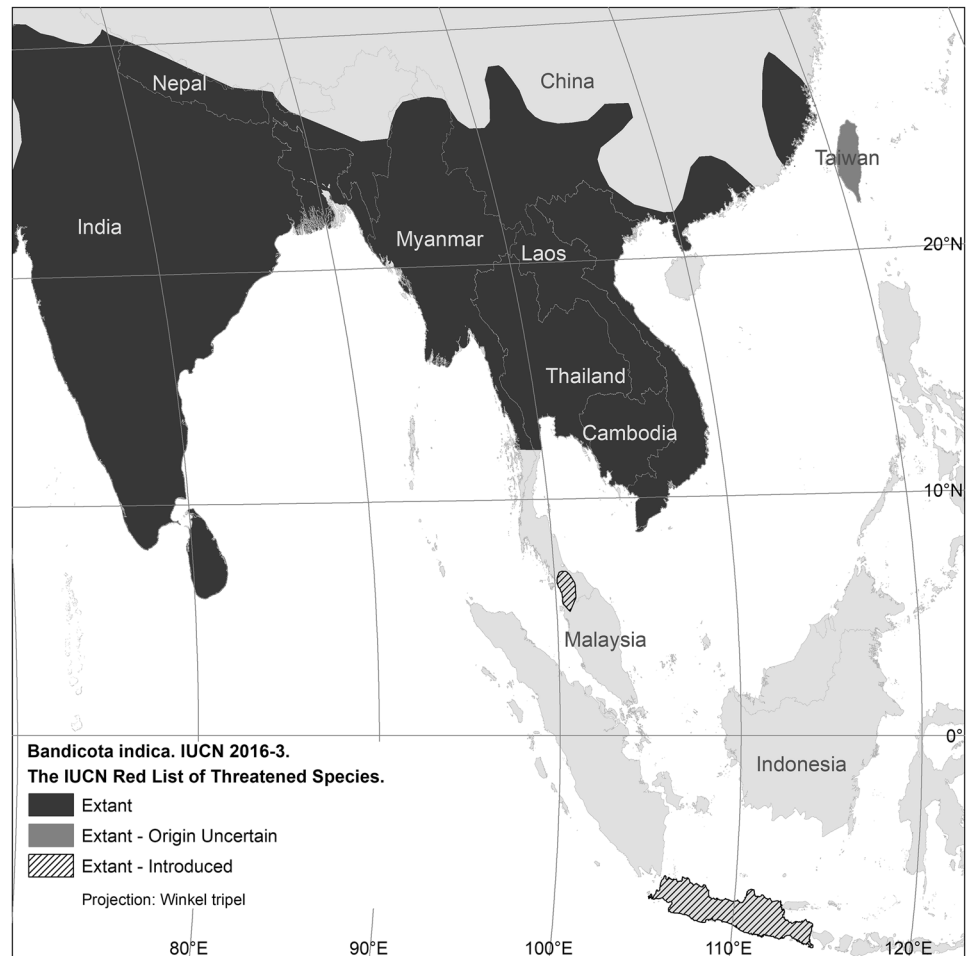
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Fig. 1 Distribution map of the greater bandicoot rat according to the IUCN showing the native distribution in black, and the invasive populations in Sundaland in stippling (modified from Aplin et al. 2016)



have been introduced (Fig. 1), although this remains to be tested. It is important to determine if the greater bandicoot rat is a recently introduced invasive species or a native species in Malaysia, both for the study of biogeography and for practical management, as this species is capable of causing great economic damage.

Sundaland is separated from Indochina by the Isthmus of Kra. This biogeographic barrier is the limit of many species and genera, and it can be hypothesized that there should be deep genetic divergence between populations of species distributed across it, as has been observed in some bird species (Dejtaradol et al. 2015). For this reason we expect there to be deep phylogeographic structure between the greater bandicoot rats north and south of this region, if that is their natural distribution. In this study we aim to see whether there are differences among greater bandicoot rats in Malaysia and other regions. Here, we field sample greater bandicoot rats from the northern region of Malaysia, south of the Isthmus of Kra, and use a phylogeny based on nuclear and mitochondrial DNA sequences to compare them to greater bandicoot rats from Indochina (north of the Isthmus of Kra).

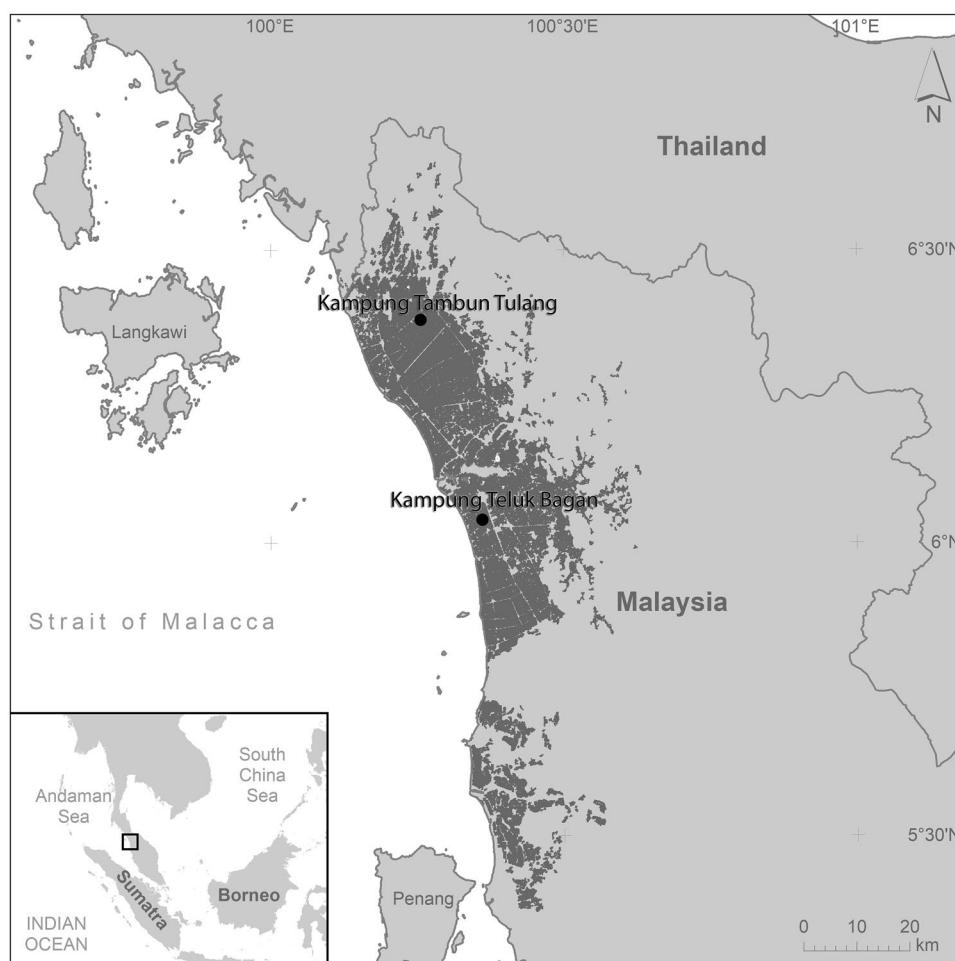
Materials and Methods

Field Sampling

Field work was undertaken under permit #100–24/1.24 Jld 9(8) from the Department of Wildlife and National Parks Peninsular Malaysia and ethical approval S24012019/26,112,018–02/R from the Institutional Animal Care and Use Committee, University of Malaya (UM IACUC). Trapping was undertaken in rice fields in Kampung Teluk Bagan, Alor Setar, Kedah (6°02'22.4"N 100°21'40.0"E) and Kampung Tambun Tulang, Arau, Perlis (6°22'51.1"N 100°15'21.5"E) (Fig. 2). The trapping was conducted using mesh wire box live trap (28 cm x 15 cm x 12 cm) baited with bananas. Thirty to thirty-five traps were set up for five nights at each locality for a total of 150 trap nights in Kedah and 165 trap nights in Perlis during March 2018.

Each trapped rat was visually identified to species and standard morphological measurements were taken. The measurements taken were body weight (g), head-body length (mm), tail (mm), ear (mm), and hind foot length (mm), and sex was noted (Corbet and Hill 1992; Wilson and Reeder 2005). Trapped rats were euthanized first using carbon dioxide before

Fig. 2 Map of rice paddies (in dark grey) in northern Peninsular Malaysia, the habitat in which greater bandicoot rats are tightly associated in this region. The trapping sites used in this study are marked with bold dots and labelled with the district names



the liver were extracted and preserved in 95% ethanol and kept in a -80°C freezer to be used for genetic study. Following Best and Schnell (1974), the greater bandicoot rats were divided into age classes (juvenile, sub-adult, and adult) based on the translucence of the auditory bullae and the convexity of the cranium; see Appendix 1 for additional data.

Genetic Data Collection

Total genomic DNA from the liver was extracted with the Vivantis GF-1 Tissue DNA Extraction Kit (Vivantis Technologies Sdn Bhd) following the manufacturer's protocols. Mitochondrial cytochrome *b* (cyt *b*) and nuclear

interphotoreceptor retinoid binding protein (IRBP) were amplified by polymerase chain reaction (PCR) and sequenced. Each PCR included 1 μl of DNA template (60–110 ng), 1 $\times$ Power Taq PCR MasterMix (Biotek), and 0.4 μM of each primer in 25 μl (Table 1). The cycling conditions were: initial denaturation for 4 min at 94°C , 35 cycles of denaturation for 30 s at 94°C , annealing for 30 s at 52°C and 53.9°C (as in Table 1), and elongation for 1 min at 72°C , with a final extension for 10 min at 72°C in a Mastercycler® (Eppendorf) machine. The PCR products were then checked by electrophoresis using 1% agarose gel in a $1 \times$ TAE buffer and stained with SYBR® Safe DNA Gel Stain. Electrophoresis was run at 80 V, 180 mA in $1 \times$ TAE running buffer for 45 min and checked under ultraviolet

Table 1 Primers used in this study to amplify mitochondrial cyt *b* and nuclear IRBP (genes) with names (primers) and sequences (sequence) as in the original publications (citations), along with fragment length (length), and annealing temperature used in pcrs ($^{\circ}\text{C}$)

Genes	Primers	Sequence (5' – 3')	Length (bp)	$^{\circ}\text{C}$	Citations
Cyt <i>b</i>	L14723	ACCAATGACATGAAAAATCATCGTT	1140	53.9	Irwin et al. (1991)
	H15915	TCTCCATTCTGGTTTACAAGAC			
IRBP	IRBP2F	ATCCCCTATGTCATCTCCTACYTG	892	52	Pagès et al. (2010)
	IRBP2R	CGCAGGTCCATGATGAGGTGCTCCGTGTCCTG			

light (Alpha Imager Gel Documentation System, Siber Hegner, Germany). Lastly, the PCR products were purified and Sanger sequenced at First Base Co. (Selangor, Malaysia). Outgroups from the genus *Rattus* and comparative sequences were obtained from GenBank (Table 2).

Genetic Data Analyses

BioEdit Sequence Alignment Editor (version 7.2.6.1) software was used to check nucleotide bases of the electropherograms. The alignment of sequences was done using ClustalW multiple alignment algorithms (Thompson et al. 1994) in MEGA (version 7.0.26) (Kumar et al. 2016), along with DNA sequences from the Genbank (Table 2). In a study by Pages et al. (2010), the greater bandicoot rat was phylogenetically grouped in the Rattini clade, so multiple species from the genus *Rattus* were selected as outgroups.

Phylogenetic analyses were carried out using neighbor-joining (NJ), maximum-likelihood (ML), and Bayesian Analysis (BA). MEGA software (version 7.0.26) was used to build both NJ and ML trees, while MrBayes software was used for BA (Huelsenbeck and Ronquist 2001). Kimura-2-Parameter model was used to construct NJ tree with 1000 bootstrap permutations to measure confidence of the branches. For ML tree construction, Tamura-Nei model with 1000 bootstrap replicates was performed.

For the Bayesian analysis, the best substitution model (GTR + I + G) for each gene was obtained using Modeltest software (version 3.7) (Posada and Crandall 1998). The trees were constructed using MrBayes software (version 3.0) with one million generations implementing Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC), with trees sampled every 1000 generations. About 535,000 generations (cyt *b*) and 85,000 generations (IRBP) were needed for the tree to reach stationary.

Within population nucleotide and haplotype diversity of the greater bandicoot rat was estimated from the mitochondrial and IRBP sequence datasets using DnaSP (version 5.10.1) (Librado and Rozas 2009). This includes the number of haplotypes (*h*), number of segregating sites (*S*), nucleotide diversity (π), haplotype diversity (θ), and nucleotide divergence (*Da*). The population subdivision (*Fst*) and number of migrants per generation (N_m) were also estimated using DnaSP (version 5.10.1) to determine the gene flow between two populations (Malaysia and Thailand) of greater bandicoot rats. Minimum spanning networks of haplotypes for greater bandicoot rats across three regions (Malaysia, Lao PDR, and Thailand) were generated using NETWORK (version 5.0.0.3) (Brandelt et al. 1999). Median-joining algorithm defined by (Brandelt et al. 1999) was used to construct the haplotype network.

Results

Field Trapping

Eighteen Murinae rats were trapped in 315 trap nights, of which 15 were greater bandicoot rats. The only other species trapped in the same trap lines was the much smaller rice field rat (*Rattus argentiventer*). Four individuals of bandicoot rats were trapped in Alor Setar, Kedah, while the rest were trapped in Kampung Tambun Tulang, Perlis. All the species were identified using identification keys from Aplin et al. (2003) and Francis (2008).

Genetic Analyses

The whole cyt *b* gene was sequenced from 15 greater bandicoot rats, and IRBP from 11 of those (GenBank numbers in Table 2). A total of eight cyt *b* haplotypes were identified in greater bandicoot rats from across their range. All the individuals from Malaysia shared one haplotype, which was closely related to a haplotype from Lao PDR (Fig. 3). The overall haplotype diversity (θ) and nucleotide diversity (π) in Malaysian greater bandicoot rats was 0.000, very much smaller than the diversity observed in a smaller sample from Thailand with seven haplotypes, a haplotype diversity (θ) of 0.9 and nucleotide diversity (π) of 0.02 (Table 3). Two IRBP haplotypes were identified in Malaysian greater bandicoot rats (Fig. 4), with one segregating sites (*S*), 0.5 haplotype diversity (θ), and 0.001 nucleotide diversity (π) (Table 3). The nucleotide divergence (*Da*) (≤ 0.01) and estimation of population subdivision (*Fst*) (< 1.0) between two populations (Malaysia and Thailand) using cyt *b* and IRBP genes were also quite low. Additionally, a small number of migrants per generation ($N_m < 1.0$) were seen. This indicates that populations of greater bandicoot rat in Malaysia were isolated for a period of time after their introduction and very little migration occurred with the populations in Thailand. Thus, the very low genetic diversity suggests a very recent common ancestor for all individuals in this area, followed by isolation.

In the mtDNA cyt *b* dataset of 27 sequences used to construct the phylogenies including all species in the genus, there are 135 variable sites of which 120 are parsimony informative. Phylogenetic trees were estimated by Bayesian analyses (BA), neighbor-joining (NJ), and maximum-likelihood (ML) methods. All sequences grouped according to their species with high support (Fig. 5). The single haplotype from Malaysia was most closely related to a haplotype from Laos ($BI = 1.00$, $BS_{NJ} = 87$, $BS_{ML} = 84$; Fig. 5), from which it differed by 0.3%, suggesting a very recent common origin for these two haplotypes.

For the IRBP gene sequence (801 bp), one tree topology was produced when using BA, NJ, and ML methods. Similar to the tree based on cyt *b*, species of

Table 2 Genbank accession numbers for DNA sequences used in this study, with species of bandicoot rats; greater bandicoot rat (*B. indica*), Savile's bandicoot rat (*B. savilei*), lesser bandicoot rat (*B. bengalensis*), black rat (*R. rattus*), brown rat (*R. norvegicus*), and ricefield rat (*R. argentiventer*). Locations where the species were caught, countries of the locations, and the original published papers were also included

Taxa	Localities	Country	Genbank		Publication
			Cyt <i>b</i>	IRBP	
<i>B. indica</i>	Kalasin	Thailand	HM217435	HM217672	Pages et al. 2010
<i>B. indica</i>	Kanchanaburi	Thailand	HM217408	HM217646	
<i>B. indica</i>	Loei	Thailand	HM217447	HM217684	
<i>B. indica</i>	Nakhon Ratchasima	Thailand	HM217380	HM217618	
<i>B. indica</i>	Phrae	Thailand	HM217425	HM217663	
<i>B. indica</i>	Ratchaburi	Thailand	HM217376	HM217614	
<i>B. indica</i>	Ratchaburi	Thailand	HM217378	HM217616	
<i>B. indica</i>	Nan	Thailand	HM217469	HM217706	
<i>B. indica</i>	Luang Prabang	Lao PDR	HM217476	HM217713	
<i>B. indica</i>	Nakhon Pathom	Thailand	HM217390	HM217628	
<i>B. indica</i>	Nakhon Ratchasima	Thailand	HM217386	HM217624	
<i>B. indica</i>	-	Thailand	JN675475	-	
<i>B. indica</i>	Kedah	Malaysia	MK934298	MK965919	
<i>B. indica</i>	Kedah	Malaysia	MK934299	-	
<i>B. indica</i>	Kedah	Malaysia	MK934300	MK965920	
<i>B. indica</i>	Kedah	Malaysia	MK934301	MK965921	
<i>B. indica</i>	Perlis	Malaysia	MK934302	MK965922	
<i>B. indica</i>	Perlis	Malaysia	MK934303	MK965923	Aplin et al. 2011
<i>B. indica</i>	Perlis	Malaysia	MK934304	-	
<i>B. indica</i>	Perlis	Malaysia	MK934305	-	
<i>B. indica</i>	Perlis	Malaysia	MK934306	-	
<i>B. indica</i>	Perlis	Malaysia	MK934307	MK965924	
<i>B. indica</i>	Perlis	Malaysia	MK934308	MK965925	
<i>B. indica</i>	Perlis	Malaysia	MK934309	MK965926	
<i>B. indica</i>	Perlis	Malaysia	MK934310	MK965927	
<i>B. indica</i>	Perlis	Malaysia	MK934311	MK965928	
<i>B. indica</i>	Perlis	Malaysia	MK934312	MK965929	
<i>B. savilei</i>	Loei	Thailand	HM217455	HM217692	
<i>B. savilei</i>	Phrae	Thailand	HM217427	HM217665	
<i>B. savilei</i>	Nakhon Ratchasima	Thailand	HM217385	HM217623	
<i>B. savilei</i>	Kanchanaburi	Thailand	HM217387	HM217625	
<i>B. savilei</i>	-	-	JN675476	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762733	-	Aplin et al. 2011
<i>B. bengalensis</i>	-	Sri Lanka	AB762732	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762731	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762730	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762729	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762728	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762727	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762726	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762725	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762723	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762722	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762721	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762720	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762718	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762717	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762709	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762707	-	Yasuda et al. 2014
<i>B. bengalensis</i>	-	Sri Lanka	AB762706	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762705	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762704	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762701	-	
<i>B. bengalensis</i>	-	Sri Lanka	AB762700	-	

Table 2 (continued)

Taxa	Localities	Country	Genbank		Publication
			Cyt <i>b</i>	IRBP	
<i>R. rattus</i>	-	India	HM217367	-	Pages et al. 2010
<i>R. norvegicus</i>	Ratchaburi	Thailand	-	HM217611	
<i>R. norvegicus</i>	Ratchaburi	Thailand	HM217370	-	
<i>R. argentiventer</i>	Veal Renh	Cambodia	-	HM217600	

bandicoot rats were grouped within their own species. All Malaysian greater bandicoot rats were grouped together with individuals from Lao PDR and Thailand, with moderate support ($BI = 0.62$, $BS_{NJ} = 63$, and $BS_{ML} = 95$; Fig. 5). Genetic distance between the populations of greater bandicoot rats and among the species of genus *Bandicota* are consistent with the phylogeny (Table 4).

Discussion

Recent Introduction

The expected deep genetic divergence between populations north and south of the biogeographic break of the Isthmus of Kra was not observed. Geographic structure in the genetic diversity north of the Isthmus of Kra was recovered, but the nuclear and mitochondrial haplotypes identified from Malaysian greater bandicoot rats were monomorphic and very closely related to haplotypes found around Lao PDR (cyt *b*); see Fig. 3. The very low amount of genetic diversity observed in the Malaysian greater bandicoot rats suggests that they have a very recent common ancestor. This could be the result of an extreme demographic event (bottleneck) or a recent founder event. The very close relationship between the Malaysian animals and those from Lao PDR suggests a very recent ancestor for the Malaysian animals in or around Lao PDR. Records of *Bandicota* in Global Biodiversity Information Facility (GBIF) from Malaysia are all since 1967, although there is a record in the Zoological Museum of the University of Malaya

(M00769) collected 26th April 1948. It was first recorded as a pest in 1946 (Liat 2015). A local who has owned around 3 hectares (ha) of rice fields since 1986 told us that the greater bandicoot rats have been at the rice fields from the beginning of their ownership. The present number of greater bandicoot rats seems higher than in the 1940s, perhaps due to the presence of snakes at that time such as king cobra (*Ophiophagus Hannah*) and rice paddy snake (*Hypsiscopus plumbae*), which have since declined.

The habitat of the greater bandicoot rat in Malaysia, like India and Thailand, is restricted to strongly human influenced habitats, primarily rice paddies and villages (Herbreteau et al. 2005). All trapping for greater bandicoot rats done in this study was in rice fields, based on discussions with locals in the area.

Together, these ecological and genetic observations suggest that the population of greater bandicoot rats in Malaysia was introduced relatively recently by humans from southwest Indochina. Given the tight association they have with agriculture, especially rice, it is possible that the introduction was accidental and took place in the course of moving rice or other agricultural products across the Isthmus of Kra in historic times.

Biogeographic Implications

The genus *Bandicota* is the sister genus to *Rattus* (Chaimanee and Jaeger 2001). The earliest fossil identified as *Rattus* sensu lato in Thailand is from the late Pliocene or early Pleistocene (Chaimanee 1997). Based on fossil records, *Rattus* including *Bandicota* split

Table 3 Population genetic statistics on two separate populations of greater bandicoot rats between (Malaysia-Thailand). Number of individuals (N), number of haplotypes (h), number of segregating sites (S), nucleotide diversity (π), and haplotype diversity (θ) using cyt *b* and IRBP genes

Populations	N		h		S		π		θ	
Genes	Cyt <i>b</i>	IRBP	Cyt <i>b</i>	IRBP	Cyt <i>b</i>	IRBP	Cyt <i>b</i>	IRBP	Cyt <i>b</i>	IRBP
Malaysia	15	11	1	2	0	1	0.000	0.001	0.000	0.519
Thailand	11	10	7	7	43	11	0.015	0.003	0.909	0.642
Malaysia—Thailand	26	21	8	8	43	12	0.014	0.002	0.662	0.653

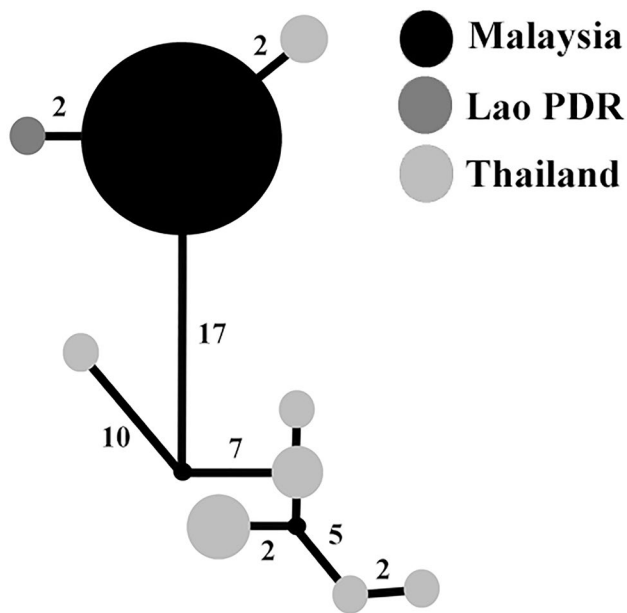


Fig. 3 Minimum spanning network of greater bandicoot rats across the three regions inferred from mtDNA *cyt b* haplotypes. Circle sizes resemble the number of individuals per haplotype. Numbers on branches represent number of base pair changes between haplotypes

from other *Rattini* about 2.5 Mya (Verneau et al. 1997), and while the fossil record in Southeast Asia is very incomplete, the oldest known *Bandicota* fossil was found in Thailand in Snake Cave dated about 170,000 years before present (late middle Pleistocene) (Chaimanee 1997). This suggests an Indochinese, not Sunda, origin for the genus *Bandicota*, which implies that it must have reached Sundaland at a more recent time.

In conclusion, the identification of the Malaysian population of greater bandicoot rats as recently introduced by humans implies that this genus is not native

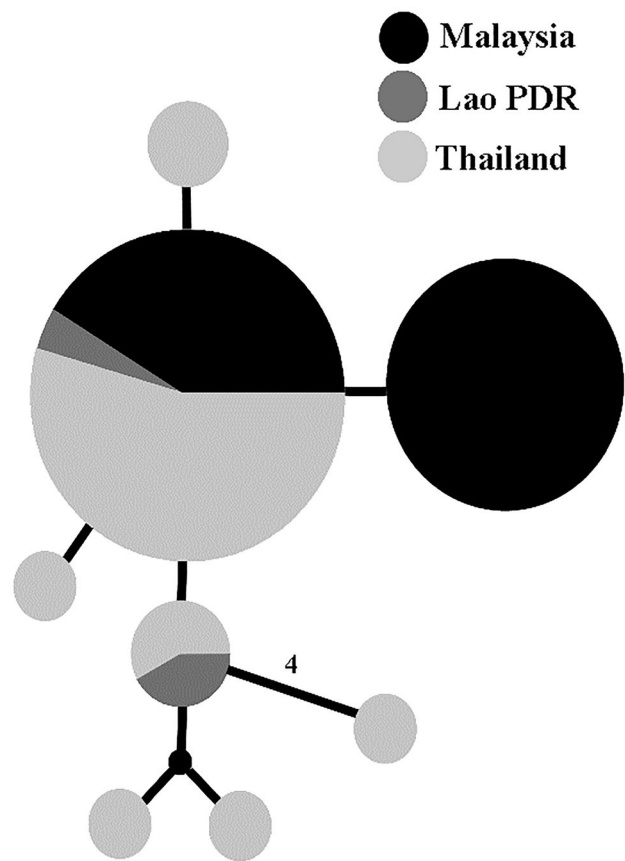


Fig. 4 Minimum spanning network of greater bandicoot rats across the three regions inferred from IRBP haplotypes. Circle sizes resemble the number of individuals per haplotype. Branches represent single base pair changes unless they are labelled with a number, black nodes represent missing alleles

to Sundaland, it is only native to Indochina north of the Isthmus of Kra (Musser and Brothers 1994). This change reduces the number of genera naturally distributed across

Fig. 5 Maximum-likelihood (ML) model phylogeny based on mitochondrial *cyt b* (left) and nuclear IRBP (right) genetic sequences. The same tree topology was produced using Bayesian analyses (BA) model and Neighbor-joining (NJ) methods. Values above the branches correspond to posterior probabilities (PP) and bootstrap support (1000 replicates). The robustness of each node is indicated as follows: BI/BS_{NJ}/BS_{ML}

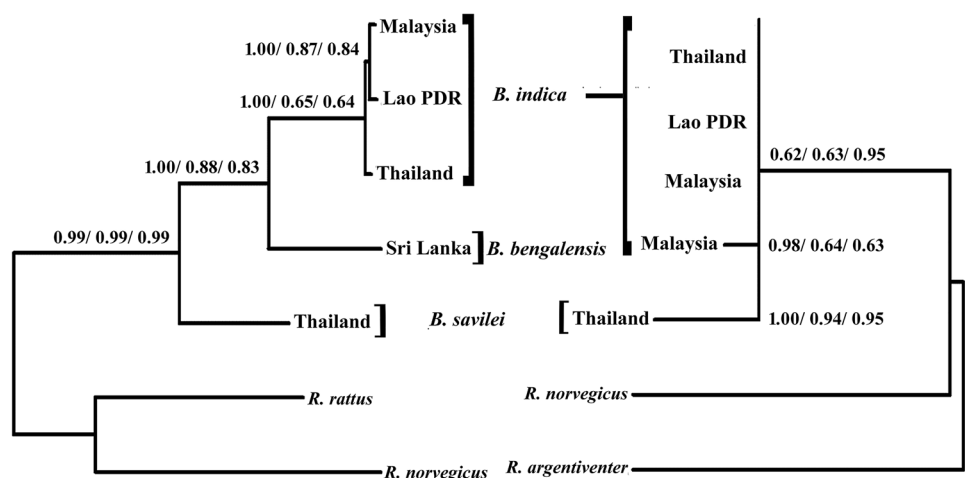


Table 4 Averages of Kimura two-parameter distance values (in percentages) between several populations of greater bandicoot rat (*B. indica*) and among the species in genus *Bandicota*; Savile's bandicoot rat (*B. savilei*) and lesser bandicoot rat (*B. bengalensis*) based on mtDNA cyt *b* (below diagonal) and nuclear DNA IRBP (above diagonal)

	Specimens	1	2	3	4
1	<i>B. indica</i> Malaysia	-	0.1	0.1	0.6
2	<i>B. indica</i> Lao PDR	0.2	-	0.0	0.5
3	<i>B. indica</i> Thailand	2.0	2.2	-	0.5
4	<i>B. savilei</i>	6.0	6.3	6.2	-
5	<i>B. bengalensis</i>	5.4	5.6	6.2	7.8

the Isthmus of Kra, and thus further strengthens its biogeographic importance.

Conservation Implications

By identifying the origin of this population as a recent introduction as opposed to the previously assumed ancient lineage of a native species, the conservation importance of this management unit may decline. As this is a pest species, it fulfils the criteria of being an invasive species. The management goal may therefore be local eradication, if possible. Farmers involved in the rice industry have told us that the number of greater bandicoot rats increases with the growth of the rice and decreases during harvesting season. Therefore, they cooperate with the local agency, Muda Agricultural Development Authority (MADA) in a baiting campaign to reduce the rat's numbers. Although farmers report the distribution of greater bandicoot rats to be stable, recent published reports suggest that counts and distribution have increased since 2015 (Shukor et al. 2018; Burhanuddin and Noor 2019; William-Dee et al. 2019). The species has not yet been reported from neighboring states, such as Perak and Kelantan. However, it is still possible they occur there, but be fewer in numbers and produce less damage to the rice crops than the rice field rats (Singleton and Petch 1994; Sharma 2018).

The current method to control rodents in Malaysia's paddy field includes all species that occurred there, including the rice field rats (*R. argentiventer*), long-tailed rice field mouse (*Mus caroli*), house rat (*R. rattus diardii*), pacific rat (*R. exulans*), and Malaysian wood rat (*R. tiomanicus*) (Amzah et al. 2011; Liat 2015). Even though there are published reports on the methods of control, specific reports on the greater bandicoot rats have yet to be published. The most common practices

are the biological control using barn owl (*Tyto alba*) and conventional methods using anticoagulant rodenticide such as warfarin (Hussain and Prescott 2003), and acute rodenticide such as zinc phosphide (Parshad and Kochar 1995). Together with mechanical (live traps and trap barriers) and cultural (sanitation and synchronized planting) controls, they form an integrated system that helps to minimize the damages to the paddy fields (Amzah et al. 2011).

Rat control towards greater bandicoot rats is still at the preliminary stage due to its limited study in Malaysia as it can only be found in the northern part of the country. A recent study (Burhanuddin and Noor 2019) used a removal sampling technique to estimate the density of the species in Kedah. It was reported by the local farmers that the rice crop loss was up to 50% per planting season (Burhanuddin and Noor 2019). Due to the lack of species ecological information, the effective control strategy is still in the early stage. The economic loss caused solely by the greater bandicoot rats could not be determined, however, the paddy fields lost up to 5–10% of the national produce, equivalent to MYR 43 million per annum. This constitutes a loss of 5% from crop damages (Amzah et al. 2011). A comprehensive study is required to estimate the precise economic loss caused by the greater bandicoot rats. Thus, no accurate comparison based on species can be made, since most of the damages in the rice fields outside of Kedah and Perlis are done by the rice field rats (Wood and Fee 2003; Amzah et al. 2011; Sharma 2018). A detailed survey of the distribution of the invasive population, and an evaluation of the demographic status are urgently needed so that management plans can prevent the spread of this serious economic problem.

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Data Availability Sequences have been submitted to GenBank, accession numbers in Table 2.

Appendix 1

External measurements (in mm) of greater bandicoot rats from Malaysia with field numbers, haplotype distribution based on *cyt b* and IRBP, sexes, captured locations and dates and ranges of age; juvenile (J), sub-adult (SA) and adult(A).

Field numbers	Haplotype distribution		Sex	Localities	Trapping date	Age	Morphological data measurements (mm)					
	Cyt <i>b</i>	IRBP					Hb	T	E	Hf	Ff	Weight (g)
ASK1	Hap 1	Hap 1	M	Alor Setar, Kedah	7/3/2018	A	300	220	28.0	52	26	912
ASK2	Hap 1	-	F	Alor Setar, Kedah	7/3/2018	A	279	224	30.0	53	25	838
ASK3	Hap 1	Hap 1	F	Alor Setar, Kedah	7/3/2018	A	295	193	30.8	49	25	686
ASK4	Hap 1	Hap 1	F	Alor Setar, Kedah	8/3/2018	J	253	203	27.4	49	23	460
TTP01	Hap 1	Hap 1	F	Tambun Tulang, Perlis	8/3/2018	A	348	242	28.5	54	27	759
TTP02	Hap 1	Hap 1	F	Tambun Tulang, Perlis	8/3/2018	A	302	216	23.1	51	25	444
TTP03	Hap 1	-	M	Tambun Tulang, Perlis	8/3/2018	SA	293	200	25.4	48	24	589
TTP04	Hap 1	-	M	Tambun Tulang, Perlis	8/3/2018	J	252	189	22.8	51	27	303
TTP05	Hap 1	-	M	Tambun Tulang, Perlis	8/3/2018	J	196	179	23.2	50	25	249
TTP06	Hap 1	Hap 2	F	Tambun Tulang, Perlis	9/3/2018	J	214	184	23.8	47	22	319
TTP07	Hap 1	Hap 2	M	Tambun Tulang, Perlis	9/3/2018	J	220	172	27.1	48	24	218
TTP08	Hap 1	Hap 2	F	Tambun Tulang, Perlis	9/3/2018	SA	321	184	28.3	49	25	578
TTP09	Hap 1	Hap 1	M	Tambun Tulang, Perlis	10/3/2018	J	235	138	27.4	49	26	337
TTP010	Hap 1	Hap 2	M	Tambun Tulang, Perlis	11/3/2018	J	238	186	26.2	50	24	312
TTP011	Hap 1	Hap 2	F	Tambun Tulang, Perlis	12/3/2018	SA	287	226	29.2	53	25	587

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