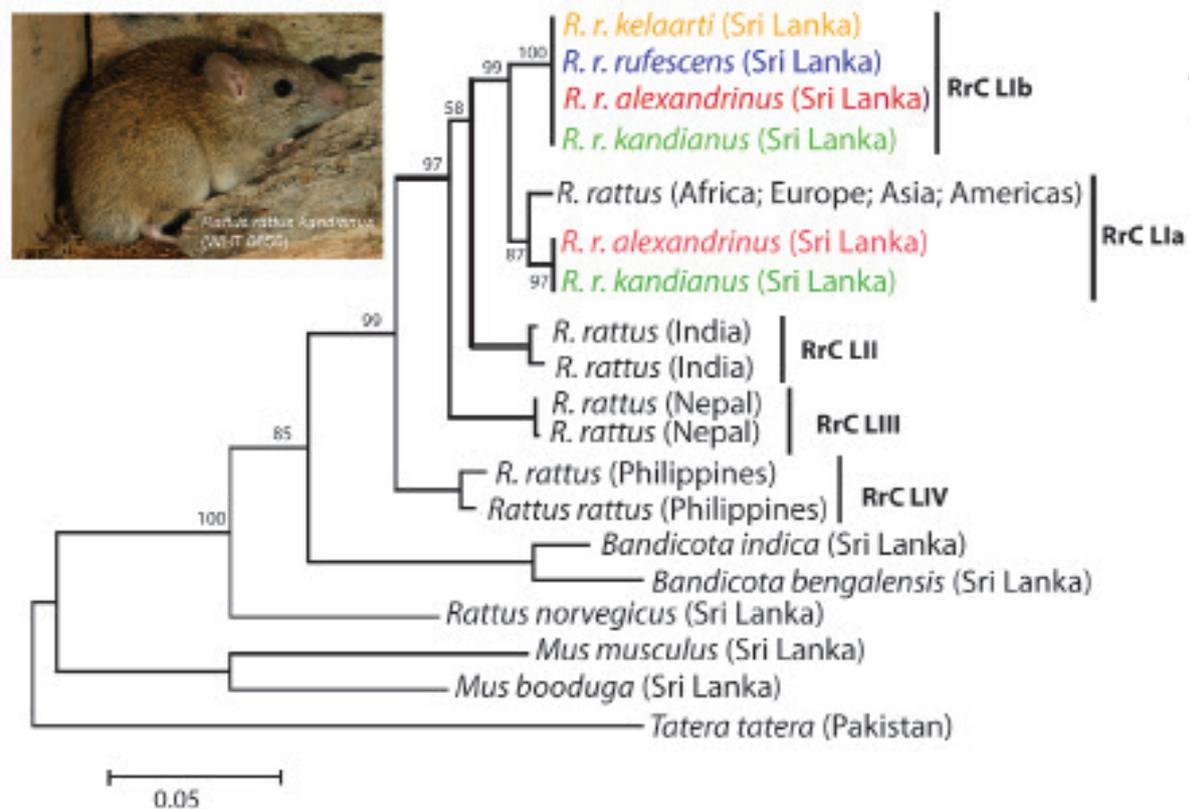


Historic black rat invasions into Sri Lanka lead to hybridization forming two sub-lineages in the *Rattus rattus* species complex

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Highlights

- All Sri Lankan black rats fall into *Rattus rattus* complex lineage I of the phylogeny.
- Four subspecies in Sri Lanka form a unique Sri Lankan sub-lineage within the lineage I.
- Subpopulations of *R. r. alexandrinus* and *R.r. kandianus* are in the main sub-lineage within the lineage I
- There is evidence of introgression of *R. r. kandianus* mitochondrial DNA into other three subspecies.
- There is evidence of introgression of *R. r. alexandrinus* mitochondrial DNA into a subpopulation of *R. r. kandianus*.

RESEARCH ARTICLE

Historic black rat invasions into Sri Lanka lead to hybridization forming two sub-lineages in the *Rattus rattus* species complex

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Abstract: In this work, we used two mitochondrial (16S rRNA and cytochrome-*b*) and one nuclear (Rag 1) gene fragments to analyse phylogeographic history of black rats in Sri Lanka at subspecies level. Of the five subspecies recorded in Sri Lanka three (*Rattus rattus kelaarti*, *R. r. rufescens*, *R. r. kandianus*) are believed to have invaded Sri Lanka in prehistoric times and two (*R. r. alexandrinus*, *R. r. rattus*) in the recent past through trade ships from Europe and India. All subspecies, except *R. r. kandianus*, have restricted distribution with none occurring sympatrically. *Rattus r. kandianus* is widely distributed throughout the country. In the phylogeny, all black rats from Sri Lanka fell into the *Rattus rattus* complex lineage I. But, *R. r. kelaarti*, *R. r. rufescens* most of *R. r. kandianus* and one individual of *R. r. alexandrinus* formed a unique Sri Lankan sub-lineage within the lineage I. The results suggests hybridization with mitochondrial introgression of the endemic *R. r. kandianus* with all other subspecies separately. Introgression of *R. r. alexandrinus* mitochondrial DNA into a subpopulation of *R. r. kandianus* is responsible for placing few individuals of the latter in the widespread sub-lineage in the Lineage I with *R. r. alexandrinus* and *R. rattus* from other parts of the world. The fifth subspecies, *R. r. rattus* which was reported in small numbers from ports in 1930s, has most likely been replaced by *R. r. alexandrinus*.

Keywords: subspecies; mitochondrial introgression; murid rodents; phylogeography.

INTRODUCTION

Genus *Rattus*, comprised of 66 species, is cosmopolitan in distribution and may probably have originated in mainland Asia (Watts & Baverstock 1994; Chaimanee & Jaeger 2001). The black rat, *R. rattus* (Linnaeus 1758), well known as a pest and a disease carrier, is native to the Indian Peninsula and has been introduced world-wide (Musser & Carleton 2005). There are more than 80 subspecies listed under *R. rattus* (Musser & Carleton 2005) however, the subspecies level diversification is largely ignored by taxonomists (Corbet & Hill 1991; Wilson & Reeder 2005).

In general, subspecies are phenotypically distinct, but not distinct enough to prevent hybridization among them. Subspecies has had a long history in taxonomy, dating back to the 19th century. Initially called “varieties”, taxonomists placed any distinct natural population that was not sufficiently different to be called a species in a subspecies

(Mayr 1982). But, with the advent of molecular studies, the failure to identify subspecies as phylogenetically distinct entities started a long-running controversy of recognizing subspecies as a valid taxonomic unit (Mayr 1982; O’Brien & Mayr 1991; Ball & Avise 1992; Burbrink *et al.* 2000; Zink 2004). However, since by definition subspecies interbreed in the zone of contact, it may not always be possible to see phylogenetic distinctness (Patten 2010). Studies which included island subspecies have recovered high level of phylogenetic distinctness, explained by divergence in isolation and smaller population size (Hastings & Gavrillets 1999; Gavrillets 2004; Phillimore & Owens 2006). In addition, some studies have proved that subspecies are useful in estimating historic patterns of divergence among populations (O’Brien & Mayr 1991), populations which may become species with time. Many argue that geographic variation of species can be better understood with use of subspecies (Mayr 1982; Barrowclough 1982). Well-defined subspecies may also serve to track migration, dispersal and geographic origins of introduced populations (Zusi 1982).

With respect to *R. rattus*, numerous studies carried out throughout the world have tried to understand migratory patterns, hybridization or mitochondrial introgression and phylogeography of the group (Chinen *et al.* 2005; Robins *et al.* 2007; Aplin *et al.* 2011; Lack *et al.* 2012; Conroy *et al.* 2013; Yasuda *et al.* 2014). However, only few studies even mention subspecies (Robins *et al.* 2007; Aplin *et al.* 2011; Robins *et al.* 2014).

In Sri Lanka there are three species of *Rattus* (*R. rattus*, *R. norvegicus* and *R. montanus*), of which *R. rattus* is subdivided into 5 subspecies: *R. r. rattus*, *R. r. alexandrinus*, *R. r. rufescens*, *R. r. kandianus* and *R. r. kelaarti*, with the latter two endemic to the country (Phillips 1980). *Rattus r. rattus*, commonly known as the black rat, is a slender, medium-sized rat with a slender tail longer than head and body and large naked ears. Colour is black or blackish grey on dorsal, which pales in to grey black in lower parts. It has been reported in small numbers around dock areas in sea-port towns: Colombo, Galle and Trincomalee. *Rattus r. alexandrinus*, commonly known as Egyptian house-rat or ship rat, can be readily distinguished by its brown or brownish grey dorsal fur and dingy grey lower parts. Size and build is similar to *R. r. rattus*. It is recorded in

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moderate numbers in and around the docks and densely populated areas in Colombo and Trincomalee. Both these subspecies are known to have imported in ships from Europe and Indian ports (Phillips 1980). *Rattus r. rufescens* is a medium-sized long-tailed house rat found commonly in the dry zone. It can be easily distinguished from other subspecies by rusty tinge of grey fur of the belly. It has undoubtedly spread from South India to Sri Lanka (Phillips 1980). *Rattus r. kandianus*, is a subspecies endemic to Sri Lanka but found commonly almost everywhere on the island. Its upperparts are generally dark brown and underparts creamy white to light brown. But the colour is very variable among the individuals that occur in different altitudes. Fur texture, though very variable, is usually short, harsh and spiny in lowland populations and long and soft in highland populations. *Rattus r. kelaarti*, commonly known as Ceylon highland rat, is also endemic to Sri Lanka (Phillips 1980). Its dorsal colour is dark olive brown and underparts are greyish white. It can be distinguished from other subspecies by its longer, softer and darker fur and relatively short tail. It is found commonly but confined to jungles of higher hills of central mountains. Except *R. r. kandianus*, which occur sympatrically with all other subspecies, none of the others cross each other's paths.

Rattus rattus, being a species widely distributed in the world, has diversified into a complex, forming four phylogenetic lineages (Aplin *et al.* 2011). Lineage RrC LI (*Rattus rattus* Complex: Lineage I), mainly centred in western India has a broad distribution throughout Europe, Americas, Africa, Madagascar, Australia and Pacific islands. RrC LIII lineage is distributed in Himalayan foothills of Pakistan and Nepal. Lineages RrC LII and RrC LIV are found in Indo Malayan region. Aplin *et al.* (2011) has included a single sequence of *R. r. kandianus* from highlands of Sri Lanka in their phylogeny, which fell in the lineage RrC LIV. This lineage is typically found in lower Mekong river catchment in southern Laos, Thailand and southern Vietnam. Considering the unique faunal diversity in Sri Lanka, Aplin *et al.* (2011) deduced a prehistoric invasion of Sri Lanka by *R. rattus*. However, a later study based on cytochrome-*b* gene by Yasuda *et al.* (2014) revealed that specimens collected from Kandy district, Sri Lanka, fall in RrC LI not RrC LIV, forming two distinct sub-lineages. One sub-lineage (RrC LIa), which is widely distributed in the world, included few Sri Lankan specimens with *R. rattus* from other parts of the world. The other sub-lineage (RrC LIb), closely related to RrC LIa sub-lineage, included only specimens from Sri Lanka. They inferred two migration events to Sri Lanka, one ancient and the other recent. They also suggested a possible hybridization between the two sub-lineages.

Considering the report of two migration events of *R. rattus* subspecies to Sri Lanka by Phillips (1980), we aimed to determine their phylogenetic relationships and phylogeographic history. Based on distribution of subspecies reported by Phillips (1980) and our own field experience, we hypothesised hybridization or mitochondrial introgression between the widespread *R. r. kandianus* with the other subspecies, including ones introduced through trade ships.

MATERIALS AND METHODS

Rattus rattus subspecies were collected from selected sites throughout the country during field surveys from 2003 to 2005, and 2015 to 2016 (Fig.1; Table 1). One hundred mesh traps and Sherman traps baited with pieces of roasted coconut were used to collect specimens. They were identified using the descriptions given by Phillips (1980). All specimens are deposited in the Department of Zoology, Faculty of Science, University of Peradeniya.

A muscle sample was taken from each specimen and preserved in 90% ethanol for DNA extraction. Two mitochondrial (16S rRNA and cytochrome-*b*) and one nuclear (Rag 1) gene fragments were sequenced for molecular analysis. DNA was extracted from ethanol-preserved tissues using Promega wizard blood and tissue extraction kit following manufacturer's protocols. DNA was amplified by PCR using 25 µl reactions containing 2.0 µl of template, 1.0 µl of each primer (10 µM), and 12.5 µl GoTaq Green master mix and 8.5 µl nano water. Thermal cycling for the cytochrome-*b* fragment was as follows: 35 cycles of denaturation at 94° C for 30 s, annealing at 45° C for 30 s, and extension at 72° C for 1 min, with a final extension of 72° C for 5 min. The same conditions were used to amplify 16S gene, except for the annealing temperature, which was 48° C. Sequence length of cytochrome-*b* and 16S were 1140 and 547 bp, respectively. Thermal cycling for Rag 1 gene (819 bp) was as follows: 35 cycles of denaturation at 95° C for 45 s, annealing at 55° C for 45 s, and extension at 72° C for 1 min, with a final extension of 72° C for 5 min. Cytochrome-*b* and 16S were amplified using the primers MVZ 05/ MVZ 14 and 16S ar/ 16S br, respectively. Primer sequences are as follows: MVZ 05 5' CGA AGC TTG ATA TGA AAA ACC ATC GTTG 3'; MVZ 14 5' GGT CTT CAT CTY HGG YTT ACA AGAC 3'; 16S ar 5' CGC CTG TTT ATC AAA AAC AT 3'; 16S br 5' CCG GTC TGA ACT CAG ATC ACGT 3'. Primers used for Rag 1 PCR were: AmpRAG1 F 5' AGC TGCAGY CAR TAC CAY AAR ATG TA 3'; Amp RAG1R1 5' AAC TCA GCT GCA TTK CCA ATR TCACA 3'. Sequences were cleaned using ChromasPro 1.7.7, aligned using Clustal W in MEGA 6 software and phylogenies were generated using MEGA 6 software. Two species of *Bandicota* Gray, 1873 and two species of *Mus* Linnaeus, 1758 were also used in the analysis as they are closely related to *Rattus* species (Yasuda *et al.* 2014; Pages *et al.* 2010). *Tatera indica* (Hardwicke 1807) was used as the outgroup because of its suggested close relationship to murine rodents (Steppan *et al.* 2004; Jansa and Weksler 2004). Genetic distance values for cytochrome-*b* gene were calculated using MEGA 6 software. The GenBank accession numbers for the sequences generated anew for the study and sequences downloaded from the GenBank are given in the Table 1.

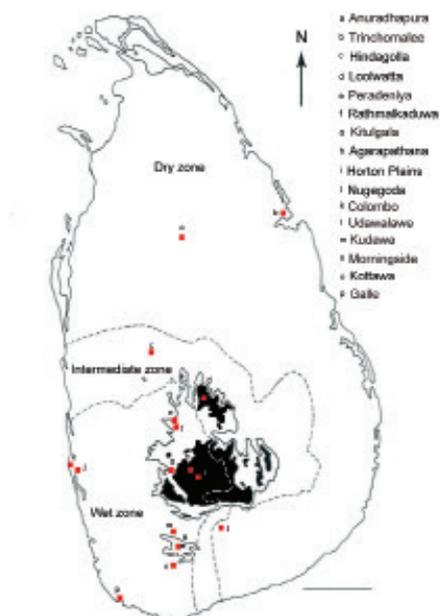


Figure 1: The map of Sri Lanka indicating specimen collection localities.

Table 1: The details of the rodent specimens subjected to phylogenetic analysis.

	Voucher number	GenBank accession number of cytochrome-b/16S sequences/Rag 1	References
<i>Bandicota indica</i>			
Galle; 06°03'N, 80°22'E, 7.2m	PDZ 43	KY697990/KY673247	This study
<i>Bandicota bengalensis gracilis</i>			
Peradeniya; 07°25'N, 80°59'E, 488m	WHT 6831	KY697993/KY673251	This study
<i>Rattus norvegicus</i>			
Galle; 06°03'N, 80°22'E, 7.2m	PDZ 44	KY697996/KY673255	This study
<i>Rattus rattus</i>			
Japan	—	AB211039	Chinen <i>et al.</i> , 2005
Zambia	—	AB752996	Nakamura <i>et al.</i> , 2013
South Africa	—	DQ439852	Bastos <i>et al.</i> , 2011
Tanzania; Oman; India	—	HM217365; HM217366 HM217367	Page's <i>et al.</i> , 2010
France; Europa island	—	JF718277; JF718278	Russell <i>et al.</i> , 2011
California USA	—	JQ814227	Conroy <i>et al.</i> , 2013
Costa Rica; Argentina; Egypt;	—	JQ823417; JQ823422; JQ823431; JQ823524; JQ823534	Lack <i>et al.</i> , 2012
Philippines	—		
Mali	—	JX292875	Schwan <i>et al.</i> , 2012
Madagascar	—	LC147016	Sakuma <i>et al.</i> , 2016
India- Langol; India- Iroisemba	—	AB973103; AB973101	Chingangbam <i>et al.</i> , 2015
Nepal	—	KY002803; KY002804	Unpublished data
<i>Rattus rattus kandianus</i>			
Peradeniya; 07°25'N, 80°59'E, 488m	PDZ 9	MH253704/MH253733	This study
	WHT 6830	MH253718/MH253748	
	WHT 6832	MH253719/MH253749	
Horton Plains: 06°80'N, 80°83'E, 2150m	PDZ 21	MH253705/MH253734;	This study
	PDZ 25	MH253714/MH253735;	
	PDZ 28	MH253706/MH253736;	
	PDZ 34	MH253707/MH253737/	
	WHT M 159	MN160105 MH253716/MH253746	

Loolwatta; 07°37'N, 80°84'E, 980m	WHT H	MH253715/MH253745	This study
Agarapathana; 06°87'N, 80°72'E, 1378.0m	WHT 6805 WHT 6821 WHT 6901	MH253717/MH253747 KY986756/KY986809/ MN160103 MH253724/MH253754	This study
Kitulgala; 06°99'N, 80°42'E, 110.2m	WHT 6856 WHT 6857 WHT 6859 WHT 6861	MH253720/MH253750 KY986758/KY986810 MH253721/MH253751 MH253722/MH253752	This study
Kottawa; 06°10'N, 80°32'E, 38.6m	WHT 6885	MH253723/MH253753	This study
Kudawa; 06°41'N, 80°43'E, 360m	WHT 6902 WHT 6903	MH253725/-/ MN160111 MH253726/MH253755	This study
Anuradhapura: 08°21'N, 80°22'E, 108m	WHT 6916 WHT 6919	KY986757/ MH253756/ MN160104 MH253727/MH253757/ MN160102	This study
Udawalawe; 06°47'N, 80°90'E, 112.2m	WHT 6926 WHT 6927	KY697997/KY673254 MH253728/MH253758	This study
Hindagolla: 07°48'N, 80°41'E, 142m	WHT 6932 WHT 6933 WHT 6935	MH253729/MH253759 MH253730/MH253760 MH253731/MH253761	This study
Morningside: 06°41'N, 80°63'E, 900 m	WHT 6938	MH253732/MH253762	This study
<i>Rattus rattus kelaarti</i>			
Horton Plains: 06°80'N, 80°83'E, 2150m	WHT M 029 WHT M 140 PDZ 32 PDZ 39	K Y 9 8 6 7 6 0 / K Y 9 8 6 8 1 6 K Y 9 8 6 7 5 1 / K Y 9 8 6 8 1 5 KY986750/ MH253738/ MN160109 M H 2 5 3 7 1 2 / M H 2 5 3 7 3 9 / MN160110	This study
<i>Rattus rattus rufescens</i>			
Peradeniya: 07°25'N, 80°59'E, 488m	PDZ 5 PDZ 6	K Y 9 8 6 7 5 3 / K Y 9 8 6 8 1 7 MH253708/MH253740	This study
Nugegoda; 06°86'N, 79°90'E, 7.1m	PDZ 10	K Y 9 8 6 7 5 2 / K Y 9 8 6 8 1 8 / MN160108	This study
Rathmalkaduwa; 07°17'N, 80°56'E, 504m	PDZ 12 PDZ 15 PDZ 16	M H 2 5 3 7 0 9 / M H 2 5 3 7 4 1 M H 2 5 3 7 1 0 / M H 2 5 3 7 4 2 MH253711/MH253743	This study
<i>Rattus rattus alexandrines</i>			
Galle; 06°03'N, 80°22'E, 7.2m	PDZ 3	K Y 9 8 6 7 5 4 / K Y 9 8 6 8 1 9 / MN160107	This study
Galle; 06°03'N, 80°22'E, 8.1m	PDZ 42 PDZ 46	K Y 9 8 6 7 5 5 / K Y 9 8 6 8 2 0 / MN160106 MH253713/MH253744	This study
<i>Mus booduga</i>			
Agarapathana; 06°87'N, 80°72'E, 1378.0m	WHT 6873	KY697998/KY673256	This study
<i>Mus musculus</i>			
Agarapathana; 06°87'N, 80°72'E, 1378.0m	WHT 6886	KY697999/KY673257	This study
<i>Millardia meltada</i>			
Udawalawe; 06°47'N, 80°90'E, 112.2m	WHT 6925	KY986803/ KY986866	This study
<i>Tatera indica</i>			
Pakistan	—	AJ430563/ —	Chevret P.D. (unpublished)
Yala; 06°43'N, 81°31'E, 29.4m	WHT 6893	— /KY673258	This study

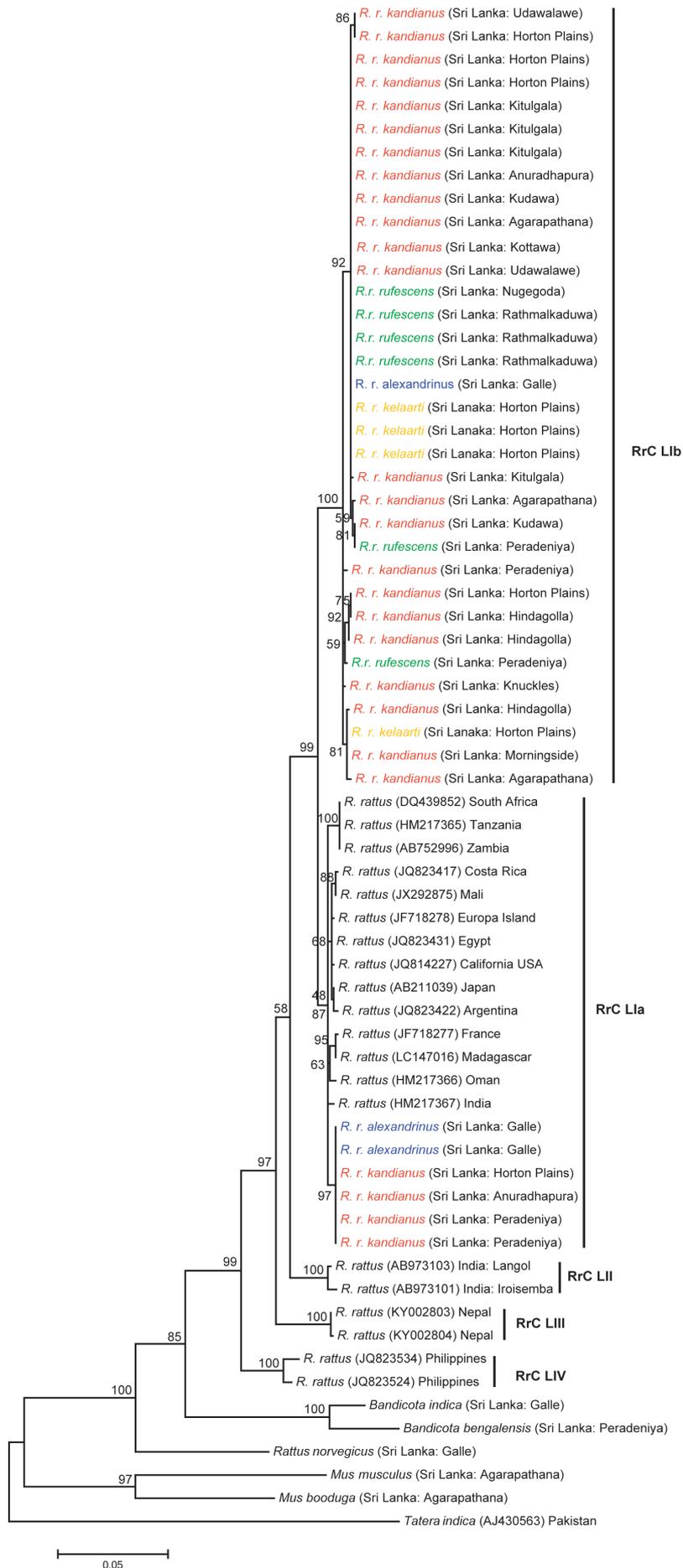


Figure 2: Maximum likelihood phylogram of cytochrome-*b* gene depicting four lineages of *Rattus rattus* in the world and the placement of the *R. rattus* subspecies of Sri Lanka. Bootstrap values are presented on the branches.

DISCUSSION

Only four subspecies of *R. rattus* were captured (*R. r. kandianus*, *R. r. alexandrinus*, *R. r. rufescens* and *R. r. kelaarti*) during this study. We were not able to capture *R. r. rattus* during this study. Early surveys by Phillips (1980) reported the presence of *R. r. rattus* in small numbers around the dock areas in Colombo, Galle and Trincomalee. At present, it is probable that this subspecies is entirely replaced by *R. r. alexandrinus* that occur in docks.

Even though the four subspecies are morphologically distinguishable, genetic differences among them were not substantial (0.00-2.52%). This range does not exactly fall within the average genetic distance value of 0.09-2.34% reported for currently identified subspecies taxa (Bradley & Baker 2001). There were individuals of all four subspecies with zero genetic divergence for cytochrome-*b*. Accordingly, we can deduce that there is hybridization or mitochondrial introgression among all four subspecies. However, this is not the case for these four subspecies.

We have included 40 *R. rattus* individuals collected from around the country representing four subspecies in the phylogeny. They all fell in two sub-lineages of lineage RrC LI as reported earlier (Yasuda *et al.* 2014). The Sri Lankan unique sub-lineage, RrC LIb, contained only the four *R. rattus* subspecies from Sri Lanka, with all individuals of *R. r. kelaarti* and *R. r. rufescens* falling in it. All four subspecies having individuals with zero cytochrome-*b* divergence indicate that each subspecies interbreed with *R. r. kandianus* which is the widespread and common subspecies throughout the island. *Rattus r. kandianus* sharing two cytochrome-*b* haplotypes with *R. r. rufescens* and *R. r. kelaarti* also supports this. *Rattus r. kandianus* is the only subspecies occurring sympatrically with all the other subspecies, facilitating its hybridization with the others. All other subspecies have restricted distributions and none of them occurs sympatrically with each other.

Other sub-lineage, RrC LIa, included four *R. r. kandianus* and two *R. r. alexandrinus* individuals, with *R. rattus* from other parts of the world (Fig. 2). With respect to *R. r. kandianus*, both sub-lineages had individuals from different localities in the country, from lowland dry zone to highland wet zone. All three *R. r. alexandrinus* specimens were from Galle. *Rattus r. rattus* and *R. r. alexandrinus* have been introduced to the island through trade vessels from Europe and India and were reported from in and around the docks in Galle, Colombo and Trincomalee (Phillips, 1980). Other three subspecies had been in the island for a longer period of time and may have invaded Sri Lanka during the glacial sea-level lowstands when there were terrestrial connections between Sri Lanka and India (most recently until ca 10,000 ybp) or with the help of ancient trade boats. Hence, according to our phylogeny, most likely scenario is hybridization or mitochondrial introgression between sub-populations of *R. r. kandianus* and *R. r. alexandrinus*. Introgression appears to have happened in both directions. *Rattus r. kandianus* being a subspecies widespread around the country, occur sympatrically with *R. r. alexandrinus*.

Hence, may have interbreed with *R. r. alexandrinus*. Since we did not find *R. r. rattus* in any of the ports, it is not possible to evaluate its relationship with the other Sri Lankan subspecies. However, it is possible that they also may have hybridized with sub-populations of *R. r. kandianus* and *R. r. alexandrinus*.

Hybridization and mitochondrial introgression between different *Rattus rattus* lineages due to human intervened invasions has been reported in other parts of the world (Chinen *et al.* 2005; Lack *et al.* 2012; Conroy *et al.* 2013). Global picture of invasions and hybridization will be useful in identifying disease transfer paths, pest control methods and even prehistoric human migrations (Matisoo-Smith & Robins 2004).

Rattus rattus rufescens was previously reported only from the dry zone of Sri Lanka (Phillips 1980). During our field collections we found two specimens from Gampola (Rathmalkaduwa) and Peradeniya, indicating the spread of this subspecies towards upland. Though *R. r. rufescens* is reported from Trincomalee, they are not reported to occur sympatrically with *R. r. alexandrinus* and *R. r. rattus* in sea ports. During our field surveys also we did not find them living sympatrically.

Biological invasions result in novel interactions among taxa and can have significant evolutionary implications on both native and invading taxa (Chinen *et al.* 2005; Lack *et al.* 2012). The genetic distance between individuals of the Sri Lankan unique sub-lineage, RrC LIb is 0.00-0.89%. The genetic distance of cytochrome-*b* among the *R. rattus* living in Sri Lanka has increased up to 2.52% due to mitochondrial genes being introgressed from the later introduced *R. r. alexandrinus* into a sub-population of *R. r. kandianus*. A sub-population of *R. r. alexandrinus* has also changed due to mitochondrial introgression from *R. r. kandianus*.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Aplin, K.P., Suzuki, H., Chinen, A.A., Chesser, R.T., Ten Have, J., Donnellan, S.C., Austin, J., Frost, A., Gonzalez, J.P., Herbreteau, V. and Catzefflis, F. (2011). Multiple geographic origins of commensalism and complex dispersal history of black rats. *PloS one* 6(11):26357.
- Ball, Jr, R.M. and Avise, J.C. (1992). Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *The Auk* 109(3):626-636.

- Barrowclough, G.F. (1982). Geographic variation, predictiveness, and subspecies. *The Auk* **99**(3): 601–603.
- Bastos, A.D., Nair, D., Taylor, P.J., Brettschneider, H., Kirsten, F., Mostert, E., Von Maltitz, E., Lamb, J.M., Van Hooft, P., Belmain, S.R. and Contrafatto, G. (2011). Genetic monitoring detects an overlooked cryptic species and reveals the diversity and distribution of three invasive *Rattus* congeners in South Africa. *BMC genetics* **12**(1): 26.
- Bradley, R.D. and Baker, R.J. (2001). A test of the genetic species concept: cytochrome-b sequences and mammals. *Journal of Mammalogy* **82**(4): 960–973.
- Burbrink, F.T., Lawson, R. and Slowinski, J.B. (2000). Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution* **54**(6): 2107–2118.
- Chaimanee, Y. and Jaeger, J.J. (2001). Evolution of *Rattus* (Mammalia, Rodentia) during the plio-pleistocene in Thailand. *Historical Biology* **15**(1–2): 181–191.
- Chinen, A.A., Suzuki, H., Aplin, K.P., Tsuchiya, K. and Suzuki, S. (2005). Preliminary genetic characterization of two lineages of black rats (*Rattus rattus sensu lato*) in Japan, with evidence for introgression at several localities. *Genes & genetic systems* **80**(5): 367–375.
- Chingangbam, D.S., Laishram, J.M. and Suzuki, H. (2015). Molecular phylogenetic characterization of common murine rodents from Manipur, Northeast India. *Genes & genetic systems* **90**(1):21-30.
- Conroy, C.J., Rowe, K.C., Rowe, K.M., Kamath, P.L., Aplin, K.P., Hui L., James, D.K., Moritz, C. and Patton, J.L. (2013). Cryptic genetic diversity in *Rattus* of the San Francisco Bay region, California. *Biological Invasions* **15**(4): 741-758.
- Corbet, G.B. and Hill, J.E. (1991). A World List of Mammalian Species, Third ed. Natural History Museum Publications & Oxford University Press, London and Oxford. V– viii, pp. 1–243.
- Gavrilets, S. (2004). Fitness landscapes and the origin of species (MPB-41) (Vol. 41). Princeton University Press.
- Gray, J.E. (1873). LII.—Notes on the rats; with the description of some new species from Panama and the Aru Islands. *Journal of Natural History* **12**(71): 416-419.
- Hardwicke, T. (1807). IX Description of a species of Jerboa found in the upper provinces of Hindustan, between Benares and Hurdwar. *Transactions of the Linnean Society of London* **08**(1): Pp. 279-281.
- Hastings, A. and Gavrilets, S. (1999). Global dispersal reduces local diversity. *Proceedings of the Royal Society B: Biological Sciences* **266**: 2067–2070.
- Jansa, S.A. and Weksler, M. (2004). Phylogeny of murid rodents: relationships within and among major lineages as determined by IRBP gene sequences. *Molecular Phylogenetics and Evolution* **31**: 256–276.
- Lack, J.B., Greene, D.U., Conroy, C.J., Hamilton, M.J., Braun, J.K., Mares, M.A. and Van Den Bussche, R.A. (2012). Invasion facilitates hybridization with introgression in the *Rattus rattus* species complex. *Molecular Ecology* **21**(14): 3545–3561.
- Linnaeus, C. (1758). *Systema naturae, per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata. Holmiae, ii + 824 pp.*
- Mayr, E. (1982). Of what use are subspecies?. *The Auk* **99**(3): 593–595.
- Matisoo-Smith, E. and Robins, J.H. (2004). Origins and dispersals of Pacific peoples: evidence from mtDNA phylogenies of the Pacific rat. *Proceedings of the National Academy of Sciences* **101**(24): 9167-9172.
- Musser, G.G. and Carleton, M.D. (2005). Super family Muroidea. In: D.E. Wilson, D.M. Reeder (Eds), *Mammal species of the world, a taxonomic and geographic reference*, Johns Hopkins Press, Baltimore Pp. 894–1531.
- Nakamura, I., Hang’ombe, B.M., Sawa, H., Kobayashi, S., Orba, Y., Ishii, A., Thomas, Y., Isozumi, R., Yoshimatsu, K., Mweene, A.S. and Takada, A. (2013). Cross-reactivity of secondary antibodies against African rodents and application for sero-surveillance. *Journal of Veterinary Medical Science* 12-0471.
- O’Brien, S.J. and Mayr, E. (1991). Bureaucratic mischief: recognizing endangered species and subspecies. *Science* **251**(4998): 1187-1189.
- Pagès, M., Chaval, Y., Herbreteau, V., Waengsothorn, S., Cosson, J.F., Hugot, J.P., Morand, S. and Michaux, J. (2010). Revisiting the taxonomy of the Rattini tribe: a phylogeny-based delimitation of species boundaries. *BMC evolutionary Biology* **10**(1): 184.
- Patten, M.A. (2010). Null expectations in subspecies diagnosis. *Ornithological Monographs* **67**(1): 35-41.
- Phillimore, A.B. and Owens, I.P. (2006). Are subspecies useful in evolutionary and conservation biology?. *Proceedings of the Royal Society B: Biological Sciences* **273**(1590): 1049-1053.
- Phillips, W.W.A. (1980). A manual of the mammals of Sri Lanka. Wildlife and Nature Protection Society of Sri Lanka, Colombo, 389+xxxv pp.
- Robins, J.H., Hingston, M., Matisoo-Smith, E. and Ross, H.A. (2007). Identifying *Rattus* species using mitochondrial DNA. *Molecular Ecology Notes* **7**(5): 717–729.
- Robins, J.H., Tintinger, V., Aplin, K.P., Hingston, M., Matisoo-Smith, E., Penny, D. and Lavery, S.D. (2014). Phylogenetic species identification in *Rattus* highlights rapid radiation and morphological similarity of New Guinean species. *PloS one* **9**(5): 98002.
- Russell, J.C., Gleeson, D.M. and Le Corre, M. (2011). The origin of *Rattus rattus* on the Îles Éparses, Western Indian Ocean. *Journal of biogeography*. **38**(9): 1834-1836.
- Sakuma, Y., Ranaroso, M.C., Kinoshita, G., Shimoji, H., Tsuchiya, K., Ohdachi, S.D., Arai, S., Tanaka, C., Ramino, H. and Suzuki, H. (2016). Variation in the coat-color-controlling genes, *Mc1r* and *Asip*, in the house mouse *Mus musculus* from Madagascar. *Mammal study* **41**(3): 131-140.
- Schwan, T.G., Anderson, J.M., Lopez, J.E., Fischer, R.J., Raffel, S.J., McCoy, B.N., Safronetz, D., Sogoba, N., Maïga, O. and Traoré, S.F. (2012). Endemic foci

- of the tick-borne relapsing fever spirochete *Borrelia crocidurae* in Mali, West Africa, and the potential for human infection. *PLoS Neglected Tropical Diseases* **6**(11): 1924.
- Steppan, S.J., Adkins, R. and Anderson, J. (2004). Phylogeny and divergence date estimates of murid rodents based on multiple nuclear genes. *Systematic Biology* **53**(4): 533–553.
- Watts, C.H.S. and Baverstock, P.R. (1994). Evolution in some South-east Asian Murinae (Rodentia), as assessed by microcomplement fixation of albumin, and their relationship to Australian murines. *Australian Journal of Zoology* **42**(6): 711-722.
- Wilson, D.E. and Reeder, D.M. (Eds.) (2005). *Mammal species of the world: a taxonomic and geographic reference* (Vol. 1). JHU Press.
- Yasuda, S.P., Gamage, C.D., Koizumi, N., Nishio, S., Isozumi, R., Shimizu, K., Koma, T., Amada, T., Suzuki, H., Yoshimatsu, K. and Arikawa, J. (2014). Distinct genetic characteristics of Sri Lankan *Rattus* and *Bandicota* (Murinae, Rodentia) inferred from mitochondrial and nuclear markers. *Genes & genetic systems* **89**(2):71-80.
- Zink, R.M. (2004). The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proceedings of the Royal Society of London B: Biological Sciences* **271**(1539): 561-564.
- Zusi, R.L. (1982). Intraspecific geographic variation and the subspecies concept. *The Auk* **99**(3): 606-608.
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