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Systematic relationships and taxonomy of *Suncus montanus* and *S. murinus* from Sri Lanka

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ABSTRACT

Here we use nuclear and mitochondrial DNA sequence data, combined with morphometric analyses, to clarify the systematic relationships and taxonomy of two complex species of shrews, *Suncus montanus* and *S. murinus*, in Sri Lanka. We find that subspecies of *S. murinus*, *Suncus murinus murinus* from Anuaradhapura and *S. m. caerulescens* from Colombo, show little or no genetic difference in the mitochondrial (cytochrome-*b* and 16SrRNA) and nuclear (Rag 1, aldolase C and EF-1 alpha intron) genes, confirming their classification as a single species. However, two populations of *S. murinus* from Peradeniya and Udawalawe are identified as putative hybrids of *S. murinus* and *S. montanus*. Shrews collected from Peradeniya are best described as a population of *S. murinus*, but could be identified as *S. m. kandianus* using morphological features. Nuclear DNA sequence data places this population in a clade with other *S. murinus*, but mtDNA sequences of the population nests within a clade of *S. montanus* haplotypes. This discordant pattern of nuclear and mitochondrial genes suggests either hybridization between *S. murinus* and *S. montanus* or introgression of *S. montanus* mitochondrial DNA into *S. m. kandianus*. *S. m. murinus* from Udawalawe, which shows no distinct morphological difference from *S. m. murinus* from Anuradhapura, falls in the clade of *S. murinus* in both nuclear and mitochondrial trees. In the nuclear gene tree however, S. m. *murinus* from Udawalawe is placed as a sister taxon to the clade including other *S. murinus*. Rag 1 gene sequences in Udawalawe individuals suggest recombination of *S. murinus* and *S. montanus* DNA within the gene. However, additional nuclear genes are necessary to study the extent of the hybridization of *S. murinus* and *S. montanus*.

1. Introduction

Suncus murinus (Linnaeus, 1766) is a medium to large sized shrew known to live in close contact with humans. It is believed to have originated in the forests of central India (Ellerman, 1961; Yosida, 1982; Jenkins et al., 1998) and migrated to other parts of Asia and Africa aided by humans. Early mammalogists classified individual populations as subspecies or races based on morphological variation, mainly in body size and tail length. Recently, however, most of these subspecies have been disregarded (Corbet and Hill, 1991; Hutterer, 2005). In Sri Lanka four other species, which show close morphological affinities to *S. murinus*, were recognized initially as *Sorex* but later synonymized with *Suncus*; *S. zeylanicus* (Phillips, 1928), *S. montanus* (Kelaart, 1850), *S. kandianus* (Kelaart, 1852) and *S. caerulescens* (Shaw, 1800). Of these, the first three were described from Sri Lanka and the latter species from India. All were later reclassified as subspecies of *S. murinus*: *S. m. murinus*, *S. m. montanus*, *S. m. kandianus* and *S. m. caerulescens* (Phillips, 1980). *S. zeylanicus*, an endemic species to Sri Lanka, was described from Kitulgala (275 m elevation) in Sabaragamuwa Province, and its distribution was predicted to extend to the rainforests of hills in the Sabaragamuwa and Central Provinces at altitudes between 150 and 1070 m (Phillips, 1980). A recent study reported it from Sinharaja rainforest in southwest part of the Sabaragamuwa province (Wijesinghe et al., 2005). We did not observe any specimens of *S. zeylanicus* despite substantial trapping effort at the type locality. Therefore we do not include *S. zeylanicus* in our genetic analysis, but we do include morphometric data from the type specimens in our analysis.

All subspecies of *S. murinus* were referred to *S. murinus*, without subspecific designation by Ellerman and Morrison-Scott (1966). Subsequent reviewers classified *S. m. montanus* as a distinct species, *S. montanus* (Corbet and Hill, 1991, 1992), based on its distinctly smaller size, blackish colour and the fact that it is a forest species that does not venture near human habitation. *S. montanus* is also recorded to occur in India. The Indian *S. montanus* was first

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identified as *S. niger* (Horsfield, 1851). Later, it was synonymized with *S. montanus* (Corbet and Hill, 1991) based on morphological similarities (small size and dark colour) shared with the Sri Lankan *S. montanus*. However, a recent phylogenetic study showed that the Sri Lankan and Indian populations constitute two distinct species and suggested the retention of the name *S. montanus* for the Sri Lankan population and *S. niger* for the Indian populations (Meegaskumbura and Schneider, 2008). Here, we refer to *S. montanus* as a distinct species and the others as subspecies of *S. murinus*.

Morphological identification of the subspecies of *S. murinus* and *S. montanus* is mainly based on the head & body length (HBL) and the tail length (TL) while the hind foot length and the ear height are also reported (Phillips, 1980). Among *S. montanus* and the three subspecies of *S. murinus*, *S. m. caerulescens* is the largest, with a HBL of 145–158 mm and TL 88–103 mm. *S. m. kandianus* and *S. m. murinus* are smaller than *caerulescens*, with overlapping HBL and TL (109–124 mm HBL and 63–75 mm TL in *S. m. kandianus* (vs. 109–112 mm HBL and 59–67 mm TL in *S. m. murinus*). However, in *S. m. kandianus* the size range is larger than that of *S. m. murinus*. The distinguishing features of *S. m. kandianus* are: base of the tail not very stout in *S. m. kandianus* (vs. stout in *S. m. murinus*); muzzle not much swollen at the sides in *S. m. kandianus* (vs. muzzle swollen at the sides in *S. m. murinus*); dark ashy-grey to ashy-brown dorsum and lighter grey under parts in *S. m. kandianus* (vs. bluish-grey with a reddish fawn dorsum and paler under parts in *S. m. murinus*) (Phillips, 1980). *S. montanus* is the smallest of all with a HBL of 91–115 mm and TL of 65–72 mm. However, the subspecies of *S. murinus* are known to interbreed readily with *S. montanus* in places they coexist (Phillips, 1980). Phillips (1980) noted that in these areas, individuals might be intermediate in phenotype and not bear typical features of either taxon.

The distribution of *S. montanus* and the subspecies of *S. murinus* in Sri Lanka are correlated with elevation (Fig. 1). *S. montanus*, commonly known as The Ceylon Highland shrew, is recorded from the highland montane forests of Sri Lanka at 914–2524 m elevation, and in the rain forest of Sabaragamuwa in the southwestern and the southern provinces, above 150 m. They rarely enter human dwellings close to forests but, in contrast to *S. murinus*, usually are not associated with human habitation (Phillips, 1980). *S. m. kandianus* (Kandyan shrew) is found at elevations from 366 to 1220 m in the Central and Uva provinces. They do not extend into the lowland rain forest in the southwestern part of Sri Lanka, where it is replaced by *S. montanus*, or other parts of the lowlands, where its place is taken by *S. m. murinus* and *S. m. caerulescens*. This subspecies is sometimes found inside houses and scrub-jungles but appears to prefer grasslands and more open country (Phillips, 1980). *S. m. murinus* (the common Indian musk-shrew or house shrew) is recorded from the dry zone lowlands of Sri Lanka up to mid elevations. This subspecies is very common and is found associated with human habitation. They thrive in towns and villages where they live in crevices, piles of goods, and boxes (Phillips, 1980). *S. m. caerulescens*, commonly referred to as the Indian grey musk-shrew, is recorded from houses in Colombo, Galle, Jaffna, other sea-ports and some major cities such as Kandy at mid elevation. Both of the latter subspecies are reported to have been imported from India through the trade boats many years ago (Phillips, 1980), which may explain their coexistence in the sea-ports.

The genetic and phylogenetic relationships among *S. montanus* and *S. murinus*, and the subspecies of the latter in Sri Lanka, have not been studied. Several recent molecular phylogenetic studies of *S. murinus* outside Sri Lanka report very little genetic variation among populations throughout its range in Asia despite high phenotypic diversity. Ruedi et al., (1996) reported that allozyme variation in *S. murinus* from South India, Japan, Java, Sumatra and the Phillipines was very low, with Nei's genetic distance among populations ranging from 0 to 0.065. Kitchener et al. (1994) reported Nei's genetic distance among populations of *S. murinus* from Bali, Sulawesi, Adonara, Flores and Lembata to be slightly higher, ranging from 0.017 to 0.116, as might be expected of island populations. Despite the generally low genetic divergence among populations, morphological variation can be substantial. Kitchener et al. (1994) recognized three subspecies from Indonesia based on morphological variation in skull, dental and external characters.

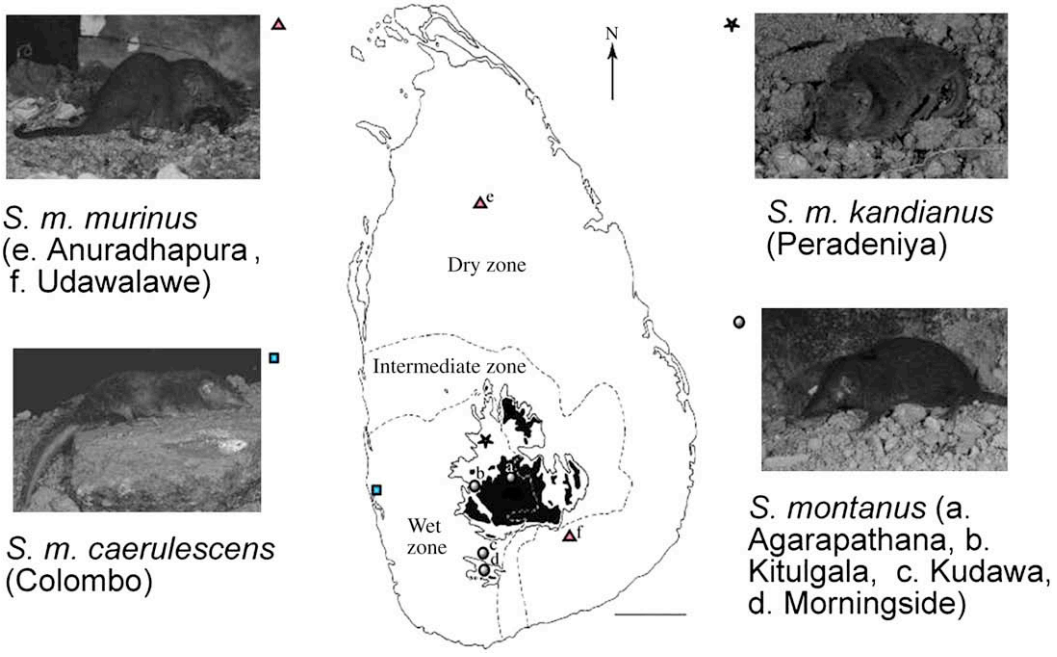

Fig. 1. A map of Sri Lanka showing the collection sites of, *Suncus murinus murinus*, *S. m. caerulescens*, *S. m. kandi'anus* and *Suncus montanus*. On each side of the map are in vivo lateral/ dorsal portraits of each species/subspecies: *S. m. murinus* WHT 6906, male, 111.0 mm HBL; *S. m. caerulescens* WHT 6881, male, 150.0 mm HBL; *S. m. kandianus* WHT 6874, male, 124.2 mm HBL; and *S. montanus* WHT 6841, male, 107 mm HBL. (WHT – Wildlife Heritage Trust, Sri Lanka).

However, two of the subspecies were not distinct genetically from each other.

Previous genetic studies on populations of *S. murinus* in Sri Lanka were restricted to populations from the coastal areas (Yosida, 1982; Yamagata et al., 1987; Ishikawa et al., 1989). Cleavage patterns of mtDNA by restriction endonuclease analysis of eight laboratory lines of *S. murinus* from Sri Lanka, Japan, Indonesia and Bangladesh revealed that the population from a coastal site in Sri Lanka (Koralawella, Western province) is polymorphic for mtDNA (Yamagata et al., 1987). Similarly, Yamagata and colleagues identified three mtDNA haplotypes in a population of *S. murinus* from the same area (Yamagata et al., 1990). In contrast, there was no variation in RFLP patterns of mtDNAs among Japanese and Indonesian populations of *S. murinus* despite their geographical isolation. Yamagata et al. (1990) suggested that the mtDNA polymorphism found in some Sri Lankan populations might have resulted from introduction of *S. murinus* from multiple sources.

Interestingly, several studies report variability in chromosome number among *S. murinus* populations, with values ranging from $2N = 30$ to $2N = 40$. Sri Lankan *S. murinus* from different localities are known to have different number of chromosomes: $2N = 32$ Trincomalee, Northeast Sri Lanka (Yosida, 1982) and $2N = 30$ Koralawella, Moratuwa, South of Colombo (Ishikawa et al., 1989). Yosida (1982) suggested that ancestral *S. murinus* had $2N = 40$ chromosomes, and the number of chromosomes was reduced to $2N = 32$ or $2N = 30$ by Robertsonian fusion and rearrangement. These shrews with the reduced number of chromosomes are believed to have migrated to Sri Lanka. Different populations or subspecies of *S. murinus* with different chromosome numbers are able to interbreed in the laboratory (Ishikawa et al., 1989), but it is not known whether a similar degree of inter-fertility applies in nature. The great variation in chromosome number within a species suggests the presence of a large number of genetic variants (Kurachi et al., 2007) and perhaps cryptic species.

Given the taxonomic uncertainties and both phenotypic and genetic variation in the *S. montanus*/*murinus* complex in Sri Lanka, an analysis of phylogenetic relationships and taxonomy is warranted. Here, we present an analysis of morphology and mitochondrial and nuclear DNA sequence data to determine the phylogenetic relationships of species and subspecies within the *S. murinus*/*montanus* complex in Sri Lanka and to clarify their taxonomy.

2. Materials and methods

2.1. Collections and field methods

S. m. murinus, *S. m. kandianus*, *S. m. caerulescens* and *S. montanus* from several sites in Sri Lanka were collected (Table 1; Fig. 1). An attempt to collect *S. zeylanicus* from its type locality (Kitulgala) was not successful, but we were able to collect *S. montanus* from Kitulgala. The morphological identification of the subspecies followed the descriptions in Phillips (1980).

The shrews were collected using unbaited pitfall traps set between 45 cm high drift fences approximately 30–50 m long. Sherman traps baited with roasted pieces of coconut were also used. Animals were euthanised and tissue samples were taken and stored in 95% ethanol for later genetic analysis. Skulls were removed, boiled gently in water, cleaned with a pair of fine forceps and brush, and dried. The remaining parts were fixed in 5% formalin and preserved in 70% ethanol. Capture and handling of specimens was carried out under Boston University's Institutional Animal Care and Use Protocol number 04-006.

2.2. DNA extraction, amplification, and sequencing

DNA was extracted from ethanol-preserved tissue samples using Qiagen tissue extraction kits following manufacturer's protocols. Two mitochondrial gene fragments (cytochrome-*b* and 16S rRNA) and one nuclear gene fragment (Rag 1) were sequenced for all individuals. Introns from two additional nuclear genes, aldolase C and elongation factor alpha, were also sequenced for a subset of individuals. Original sequences of all the individuals used in this study were submitted to GenBank under accession numbers GQ290320 to GQ290389.

DNA was amplified by PCR in 25 l reactions containing c. Fifty nanograms of DNA template, 1.25 l of each primer (10 M), 2.5 l of 10 mM mixed dNTP, 2.5 l of 25 mM $MgCl_2$, 2.5 l of 10× PCR buffer, and 0.625 U of Taq DNA Polymerase (5U/μl). The 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 60 s, with a final extension of 72 °C for 5 min. The same conditions were used to amplify the mitochondrial 16S rRNA gene fragment, except that the annealing temperature was 48 °C. The following conditions were used to amplify the Rag 1 gene fragment; c. Fifty nanograms of DNA template, 1.2 l of each primer (10 M), 2.5 l of 10 M dNTP, 2.5 l of 25 mM $MgCl_2$, 2.5 l of 10× PCR buffer, and 0.2 l AmpliTaq Gold DNA Polymerase. The thermal cycling 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 60 s, with a final extension of 72 °C for 5 min. Aldolase C and elongation factor alpha were amplified using the same conditions as described for Rag 1 above. Amplification of the aldolase using primers of Lessa and Applebaum (1993) produced two bands of which we sequenced one. PCR products were separated on a 2% agarose gel. The single smaller band, which corresponded to the aldolase C locus, was excised and sequenced. PCR products were gel purified and sequenced on an ABI 3100 automated sequencer following manufacturer's recommendations. Primer sequences and citations are listed in Appendix 1.

Table 1
The species/subspecies of *Suncus* used in the present study, their collection site, and specimen number (WHT – Wildlife Heritage Trust, Sri Lanka).

Genus and Species	Collection site (latitude, longitude, elevation)	Specimen numbers
S. montanus	Agarapathana (06°50′N, 80°41′E, 1814 m)	Male: WHT 6803, WHT 6808, WHT 6809, WHT 6812, WHT 6813, WHT 6814, WHT 6815, WHT 6816, WHT 6822, WHT 6823, WHT 6898. Females: WHT 6791, WHT 6793, WHT 6794, WHT 6795, WHT 6796, WHT 6807
S. montanus	Kudawa, Sinharaja (06°25′N, 80°24′E, 464 m)	Male: WHT 6841; female: WHT 6840
S. montanus	Morningside (06°24′N, 80°86′E, 1040 m)	Male: WHT 6848, WHT6850, WHT6851, WHT 6854; females: WHT 6847, WHT 6852
S. montanus	Kitulgala (06°59′N, 80°24′E, 64 m); (06°59′N, 80°24′E, 105 m)	Male: WHT 6860; female: WHT 6855
S. m. kandianus	Peradeniya (N07°14′, E80°35°, 500 m)	Males: WHT 6874, WHT 6877, WHT 6879, WHT 6828; females: WHT 6875
S. m. murinus	Anuradhapura (N08°21′, E80°22′, 20–60 m)	Male: WHT 6906; female: WHT 6907, WHT 6918
S. m. murinus	Pokunuthanne, Udawalawe (06°34′N, 80°53°E, 60 m)	Male: WHT 6928; female: WHT 6924
S. m. caerulescens	Colombo (06°58′N, 79°52′E, 8 m)	Male: WHT 6881

2.3. Phylogenetic analysis

For phylogenetic analyses of species relationships within the genus *Suncus*, a total of 2492 bp were sequenced, 1140 bp of cytochrome-*b* (complete coding sequence), 533 bp of 16S rRNA and 819 bp of Rag 1 for 26 specimens representing 7 species and 3 subspecies. Mitochondrial 16S rRNA sequences were aligned using Clustal X (Jeanmougin et al., 1998) and adjusted by eye in Se-Al ver.2.0a9 (Rambaut, 1996). Hypervariable regions of ambiguous positional homology were excluded from the analysis. Mitochondrial and nuclear protein coding gene sequences (cytochrome-*b*, Rag 1) were aligned using the translated amino acid sequence. The final data set for phylogenetic analyses comprised 3027 bp including 486 bp of unambiguously aligned 16S rRNA sequence. Four species of *Crocidura* (*Crocidura attenuata*, *C. fuliginosa*, *C. miya* and *C. horsfieldii*) were used as outgroups to root the tree. *S. etruscus* was included as it is closely related to *S. murinus*, *S. montanus* and *Crocidura* (Dubey et al., 2007, 2008).

Bayesian inference as implemented in the program MrBayes v3.0b4 (Huelsenbeck and Ronquist, 2001) was used to estimate evolutionary relationships among taxa. We used the GTR+I+G model of sequence evolution, which was determined as the best-fit model of the 56 models examined by the hierarchical likelihood ratio test as implemented in Modeltest 3.06 (Posada and Crandall, 1998). To search parameter space and determine the posterior probabilities of phylogenetic trees, we ran four Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains for 1000,000 generations. The posterior probabilities were calculated after excluding the first 250,000 generations as burn-in. The data were also analyzed under a maximum parsimony (MP) criterion in PAUP* v.4.0b10 (Swofford, 2000) with all characters unordered and weighted equally. The percent pair-wise uncorrected distances between the species were calculated using PAUP* v.4.0b10. Since some of the hypervariable regions of the 16S gene fragment were removed from the analysis, only the cytochrome-*b* and Rag 1 fragments were used to calculate genetic distance.

2.4. Morphological analysis

External and cranial measurements were made using Vernier calipers to the nearest 0.1 mm. The following external measurements were taken: length of head and body (HBL), length of head (HL), length of tail (TL), length of hindfoot (HFL), length of tibia (TBL) and ear height (EH) (Appendices 2 and 3). The following cranial measurements were taken (see Fig. 2 and Appendices 4 and 5): greatest length of skull (GL), basal length (BL), basilar length (BSL), condylobasal length (CL), length of maxillary tooth row (MTR), palatal length (PL), palatilar length (PAL), post-palatal length (PPL), length of rostrum (LR), breadth of braincase (BB), least interorbital breadth (LIOB), breadth of palate between the buccal margins of second molars (PW1), breadth of palate between the lingual margins of last molars (PW2), breadth of rostrum at narrowest point (BR1), breadth of rostrum at broadest point (BR2), breadth of bony palate at the premolar (BPM), breadth of upper jaw premolar (BMM), height of braincase (HB), mandible length (ML), length of dentary including incisors (LDI), length of dentary teeth excluding incisors (LDT1), length of dentary teeth including incisors (LDT2), and depth of dentary (DD). The external measurements (HL and TL) are also presented as a percentage of HBL. Coefficients of variance (standard deviation/mean expressed as a percentage) were calculated for all measurements. For comparative purposes, a large number of museum specimens, including type specimens of *Suncus* spp. in the collection of Natural History Museum, London, were examined and included in the analysis. Specimens examined, and associated information, are included in Appendix 6.

SYSTAT (Version 11) was used for statistical analysis. Principal components analysis (PCA) of external and cranial variables were carried out separately to determine if *S. murinus* subspecies and *S. montanus* occupy separate regions of multivariate morphospace for both external and cranial characters. PCA was performed on correlation matrices of untransformed morphological measurements with unrotated axes. Since there is marked sexual dimorphism in these species, only data for males were used in PC analysis. Females were not included in the analysis as sample size was too small, but measurements for females are included in Appendices 3 and 5 to make those data available to other researchers.

3. Results

3.1. Phylogenetic relationships of S. montanus and S. murinus subspecies within Sri Lanka

In both Bayesian and maximum parsimony trees based on the nuclear gene Rag1, *S. montanus* formed the sister group to a clade containing all subspecies of *S. murinus* (Fig. 3). The clade comprising the subspecies of *S. murinus* had moderate bootstrap support (63%) but high posterior probability support (100%). A separate analysis of mitochondrial DNA sequences revealed a similar pattern of relationship with one exception. Mitochondrial DNA sequences from *S. m. kandianus* fell within the clade of *S. montanus* mitochondrial sequences with high support, whereas all other *S. murinus* mitochondrial sequences comprised a separate clade (Fig. 4). In the combined analysis (all nuclear and mitochondrial genes) *S. m. kandianus* was placed as sister to the *S. montanus* clade with high support (Fig. 5). Curiously, another two individuals collected from Udawalawe in Southern Province, identified as *S. m. murinus* according to morphology, fell as the sister taxon to the clade including *S. m. kandianus*, *S. m. caerulescens* and *S. m. murinus* (Anuradhapura) with high bootstrap and posterior probability values in the nuclear gene tree. In the mitochondrial and combined data trees the two individuals are placed sister to *S. m. murinus* from Anuradhapura with high posterior probability support but low bootstrap support.

The percent pair-wise uncorrected molecular distance for the Rag 1 gene is 0.5–1.3% between *S. murinus* subspecies and *S. montanus*. But it is lower between *S. m. murinus* (Udawalawe) and *S. montanus* (0.5–0.9) than between the other subspecies of *S. murinus* and *S. montanus* (0.9–1.3%; Table 2). The molecular distance among *S. m. kandianus*, *S. m. caerulescens* and *S. m. murinus* (Anuradhapura) is 0–0.2% (Table 2), but *S. m. murinus* (Udawalawe) has high molecular distance from other subspecies of *S. murinus* (0.4–0.7%).

Consistent with the phylogenetic relationships of mtDNA, the genetic distance for cytochrome-*b* is high between *S. m. kandianus* and the other subspecies of *S. murinus* (6.1–6.5%; Table 3) and low between *S. montanus* and *S. m. kandianus* (0.3–0.7%). Molecular distance between other subspecies of *S. murinus* and *S. montanus* is 6.0–6.5% and among the subspecies of *S. murinus* is 1.8–2.4%.

Aldolase sequences, with four variable nucleotide positions were phylogenetically uninformative and not diagnostic of species or subspecies. In the elongation factor sequences, however, a single, one base indel, separates the two species, *S. murinus* and *S. montanus* (Table 4) and places *S. m. kandianus* with other *S. murinus* subspecies, consistent with the Rag 1 data.

The phylogenetic analysis of sequences from mitochondrial DNA and the nuclear Rag1 gene, and the discovery of a diagnostic deletion in the EFalpha intron of *S. montanus*, suggests that both *S. murinus* and *S. montanus* are genetically distinct and diagnosable taxa, with two exceptions. First, it appears that *S. m. kandianus*

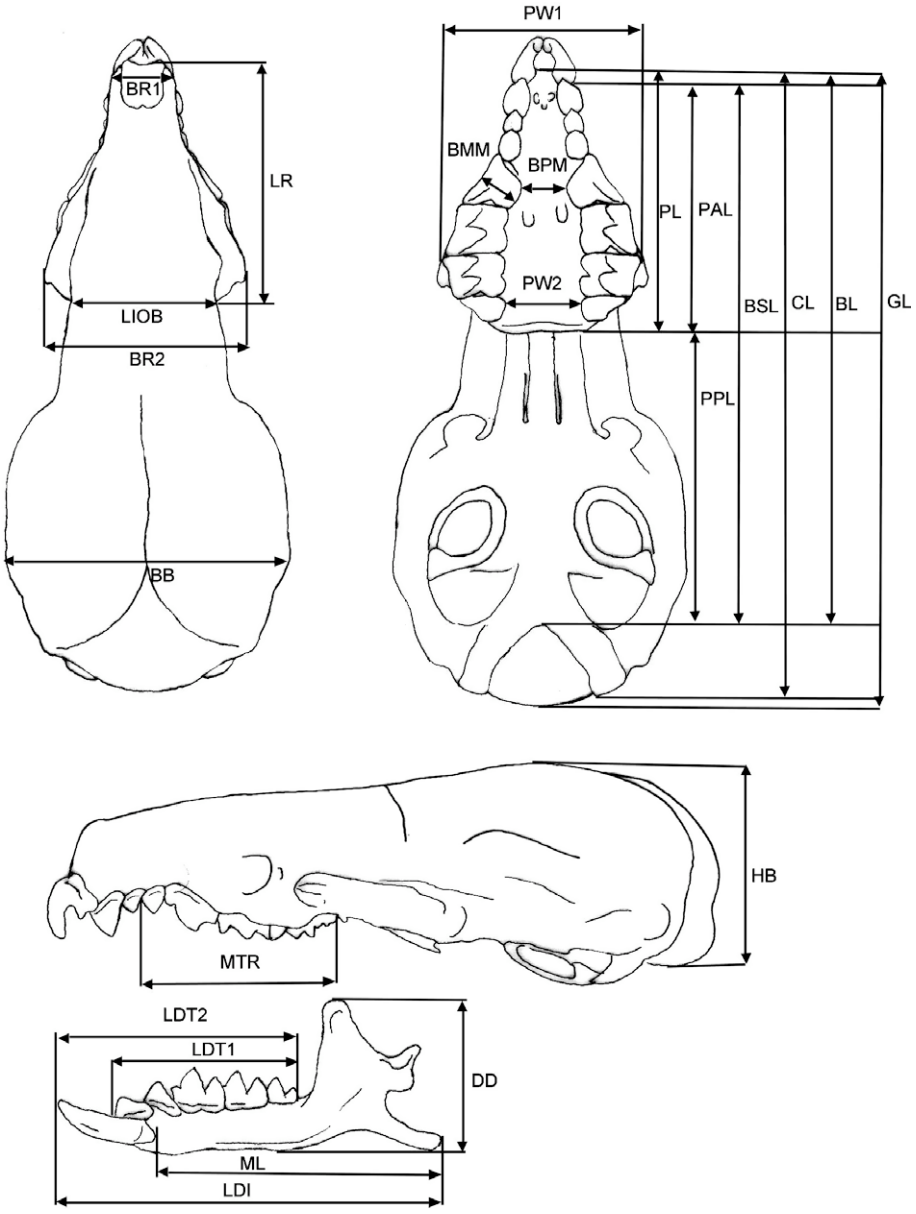

Fig. 2. Dorsal, ventral and lateral views of cranium; and lateral view of dentary, illustrating measurements taken in this study (see Section 2 for explanations of abbreviations).

has mitochondrial haplotypes characteristic of *S. montanus*, though its nuclear genome is like other *S. murinus*, suggesting that mtDNA from *S. montanus* has introgressed into *S. m. kandianus*. Second, *S. m. murinus* from Udawalawe falls with the other *S. murinus* in the mitochondrial gene tree, but is placed as a sister taxon to the other *S. murinus* in the nuclear gene tree. The genetic distances of cytochrome-*b* and Rag 1 also reflect this (Table 2 and 3). It is clear that interbreeding must have occurred between *S. montanus* and *S. murinus* to introduce montanus mtDNA into murinus at Kandy. Additional information, discussed below, suggests that nuclear introgression also may have occurred between *S. montanus* and *S. murinus* at Udawalawe.

3.2. Morphological analysis of *S. murinus* subspecies and *S. montanus*

Principal components analysis of external measurements revealed that *S. montanus* and *S. murinus* subspecies differ in multivariate morphological space along a single axis that represents size. Three axes of the PCA explained a total of 93.7% of the total variance. Factor scores among species and subspecies overlap substantially on PC2 and PC3, but differ on PC1 (Fig. 6A). PC1 explained 80.9% of the variance in external morphological variables. All the external measurements (head and body length, head length, tail length, hindfoot length, tibia length and ear height) loaded heavily and positively on PC1 indicating that PC1 is a size axis (Table 5). Factor scores from *S. montanus* and *S. murinus* subspecies generally do not overlap on PC1, with *S. montanus* being smaller in all dimensions and *S. murinus* subspecies being larger in all dimensions. Among subspecies of *S. murinus*, *S. m. caerulescens*, being the largest of all the subspecies, lies well separated from other *S. murinus* on PC1. *S. m. murinus* (from Anuaradhapara and Udawalawe) and *S. m. kandianus* overlap on PC1 and show no external morphological differences that can distinguish between the two subspecies. *S. montanus* from Kitulgala, a mid elevation site in the Central Hills of Sri Lanka, overlaps with *S. m. murinus* and *S. m. kandianus* on PC1 as it is larger in the length of head and body, head, tail, and

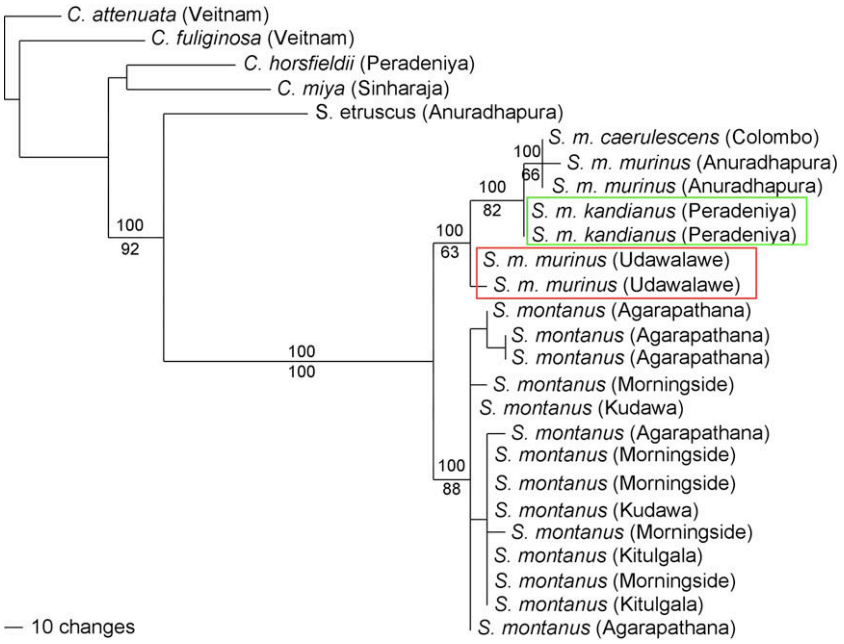

Fig. 3. Phylogram inferred from Bayesian analysis of nuclear data (Rag 1) under model GTR+I+G. Posterior probability and bootstrap values (expressed as percentages) are given above and below branches, respectively.

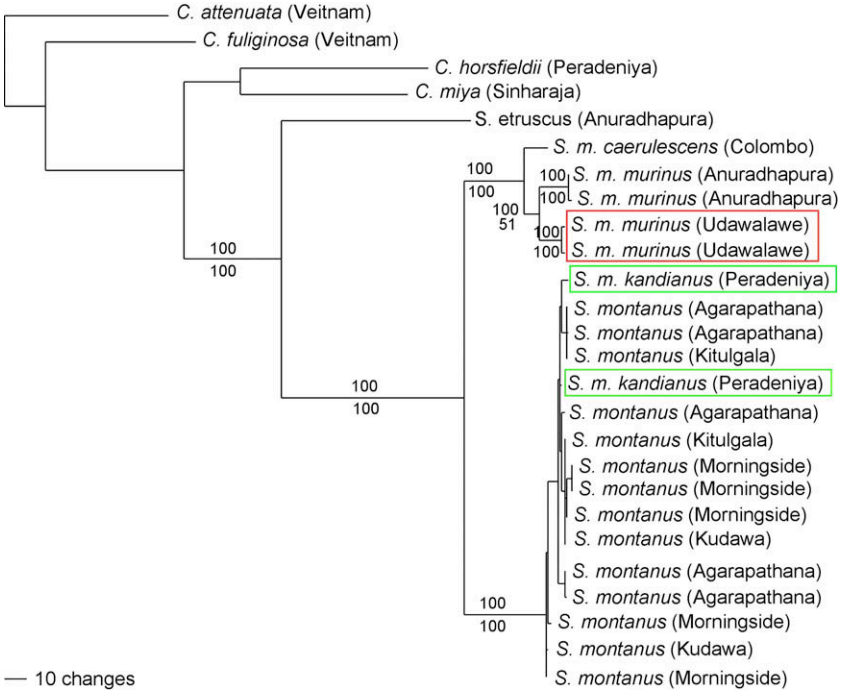

Fig. 4. Phylogram inferred from Bayesian analysis of combined mitochondrial data (cytochrome *b* + 16S) under model GTR+I+G. Posterior probability and bootstrap values (expressed as percentages) are given above and below branches, respectively.

tibia than other *S. montanus* (Appendix 2). *S. montanus* from Kitulgala is separated from *S. murinus* on PC2 by having a smaller ear height, which is the variable weighing most heavily on PC2. However, only one specimen of *S. montanus* from Kitulgala was available, making conclusions regarding this population tentative pending additional collections.

Similar results were obtained from the PCA of cranial measurements. Three axes explained a total of 94.2% of the total variance. Factor scores for the species and subspecies substantially overlap on PC2 and PC3 but differ on PC1 (Fig. 6B). PC1 explained 88.2% of the variance in cranial measurements. All cranial measurements loaded heavily and positively on PC1 indicating that this was primarily a size axis (Table 6). Again *S. montanus* and *S. murinus* subspecies differ in size, just as for external measurements (Fig. 6B). *S. m. caerulescens* lies well separated from the others on PC1 due to its larger size. Based on factor loadings we interpreted PC2 as a

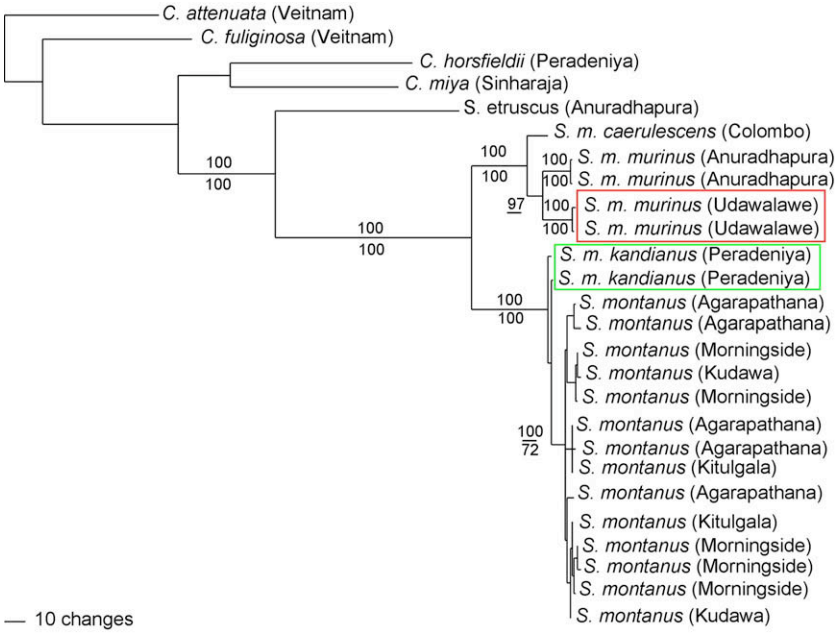

Fig. 5. Phylogram inferred from Bayesian analysis of combined data under model GTR+I+G. Posterior probability and bootstrap values (expressed as percentages) are given above and below branches, respectively.

Table 2
Percent pair-wise uncorrected distance between *S. montanus*, *S. murinus* subspecies of Sri Lanka for the Rag1 gene fragment. (A: Anuradhapura, U: Udawalawe).

	S. m. kandianus	*S. m. murinus* (A)	*S. m. murinus* (U)	*S. montanus*
S. m. caerulescens	0.1	0.0–0.1	0.4–0.5	0.9–1.1
S. m. kandianus	–	0.1–0.2	0.4–0.5	0.9–1.1
S. m. murinus (A)	–	–	0.5–0.7	1.0–1.3
S. m. murinus (U)	–	–	–	0.5–0.9

Table 3
Percent pair-wise uncorrected distance between *S. montanus*, *S. murinus* subspecies of Sri Lanka for cytochrome-*b* gene fragment. (A: Anuradhapura, U: Udawalawe).

	S. m. kandianus	*S. m. murinus* (A)	*S. m. murinus* (U)	*S. montanus*
S. m. caerulescens	6.1	2.3–2.4	2.3–2.4	6.0–6.4
S. m. kandianus	–	6.1	6.4–6.5	0.3–0.7
S. m. murinus (A)	–	–	1.8–2.0	6.1
S. m. murinus (U)	–	-	–	6.4–6.5

Table 4
Part of the elongation factor sequence showing the one base pair indel in *S. montanus*.

S. montanus	(WHT 6816)	3′AAC-ACCCAGGTCAAATCAGTGCT5′
S. montanus	(WHT 6822)	3′AAC-ACCCAGGTCAAATCAGTGCT5′
S. m. kandianus	(WHT 6875)	3′AACCACCCAGGTCAAATCAGTGCT5′
S. m. kandianus	(WHT 6877)	3′AACCACCCAGGTCAAATCAGTGCT5′
S. m. murinus (A)	(WHT 6906)	3′AACCACCCAGGTCAAATCAGTGCT5′
S. m. murinus (A)	(WHT 6907)	3′AACCACCCAGGTCAAATCAGTGCT5′
S. m. murinus (U)	(WHT 6924)	3′AACCACCCAGGTCAAATCAGTGCT5′
S. m. murinus (U)	(WHT 6928)	3′AACCACCCAGGTCAAATCAGTGCT5′

measure of breadth of palate between the lingual margins of last molars (PW2). Based on this, *S. m. murinus* from Anuradhapura differs from that of Udawalawe by having a smaller PW2 (3.7 mm in *S. m. murinus* from Anuradhapura against 4.4 mm in *S. m. murinus* of Udawalawe). But again we have a very small sample size so it is only possible to say that the samples from Anuradhapura and Udawalawe appear morphologically similar to *S. m. kandianus*.

S. montanus and *S. murinus* can be also be distinguished by several skull characteristics (Fig. 7). The braincase of *S. montanus* is rounded (vs. slightly elongated in *S. murinus*); occipital crest less prominent in *S. montanus* (vs. prominent in *S. murinus*); Sagittal crest less prominent in *S. montanus* (vs. prominent in *S. murinus*); Pterygoid region broader in *S. montanus* (narrow in *S. murinus*); Paraoccipital process less prominent in *S. montanus* (prominent in *S. murinus*); angular process in mandible narrow in *S. montanus* (broader in *S. murinus*). Within *S. murinus*, as the skull gets larger from *S. m. murinus* to *S. m. kandianus* to *S. m. caerulescens* the occipital crest gets more prominent.

S. montanus and *S. murinus* are easily diagnosable based on external morphology, cranial dimensions and cranial characteristics. *S. montanus* is distinctly smaller than all the subspecies of *S. murinus* with a slender tail and darker pelage, both dorsal and ventral. *S. montanus* from Kitulgala (the type locality of *S. zeylanicus*) resembles *S. montanus* from other localities in external appearance, but is larger than *S. montanus* from other localities (Fig. 6). However, both nuclear and mitochondrial DNA sequence data place the Kitulgula specimen within *S. montanus*. Within *S. murinus*, *S. m. kandianus* is similar in morphology to other *S. murinus* (Figs. 1 and 6). Hence, the morphology of *S. m. kandianus* is consistent with the nuclear data in placing it within *S. murinus*. *S. m. murinus* from Udawalawe is morphologically indistinguishable from other *S. m. murinus* (Fig. 6). *S.* m. caerulescens is distinctly larger than other *S. murinus* (Fig. 1). However, *S. m. caerulescens* is placed within the *S. murinus* clade by both mitochondrial and nuclear genes (Figs. 3 and 4).

4. Discussion

This study confirms the presence of two species of *Suncus*: *S. murinus* and *S. montanus* in Sri Lanka. *S. m. caerulescens* and *S. m. murinus* from the dry zone (Anuradhapura) constitute a single

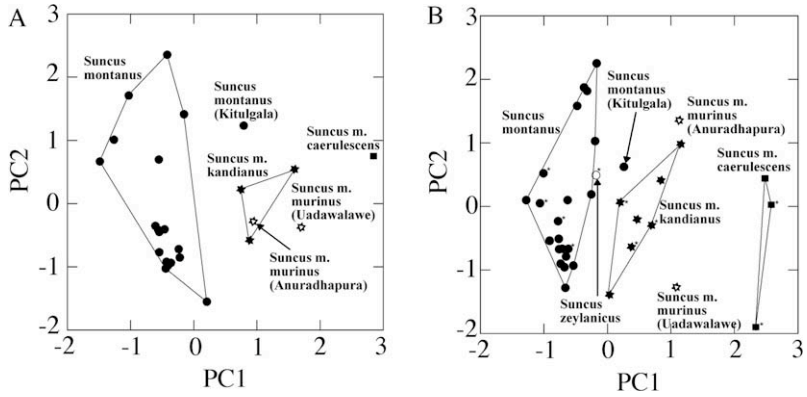

Fig. 6. Plots of factor scores on PC1 vs. PC2 for (A) external body measurements and (B) cranial measurements of *Suncus* species and subspecies of Sri Lanka. *Suncus montanus* (closed circles); *S. zeylanicus* (open circles); *S. m. kandianus* (closed stars); *S. m. murinus* (open stars); *S. m. caerulescens* (closed squares). In plot B type specimens and non-type specimens available at the BMNH are represented by asterisks (∗).

Table 5
Factor-loading for the three PC axis from the PCA of external measurements. Percent of total variance explained by each of the PC axis are also presented. Factor-loading scores above 0.5 are in bold.

	PC1 80.9%	PC2 7.5%	PC3 5.3%
Tail length	**0.935**	−0.034	−0.192
Hind foot length	**0.931**	−0.108	−0.145
Head and body length	**0.915**	0.299	0.124
Head length	**0.897**	0.215	−0.267
Tibia length	**0.889**	0.119	0.408
Ear height	**0.824**	**−0.533**	0.094

Table 6
Factor-loading for the three PC axis from the PCA of cranial measurements. Percent of total variance explained by each of the PC axis are also presented. Factor-loading scores above 0.5 are in bold.

	PC1 88.2%	PC2 3.7%	PC3 2.3%
CL	**0.995**	0.014	0.023
BSL	**0.993**	0.010	0.015
PL	**0.989**	0.079	0.052
DD	**0.987**	−0.001	0.018
GL	**0.984**	0.018	0.027
PPL	**0.980**	−0.080	−0.071
LDI	**0.979**	0.003	0.084
MTR	**0.962**	0.109	0.058
LIOB	**0.955**	−0.080	0.152
PAL	**0.945**	0.118	0.016
BB	**0.942**	−0.118	0.063
HB	**0.921**	-0.088	−0.012
BL	**0.890**	0.074	−0.290
BMM	**0.865**	0.328	0.241
ML	**0.855**	0.145	−0.418
PW2	**0.746**	**−0.622**	−0.004

species, *S. murinus*. Morphologically these two subspecies differ only in body size and colour, *S. m. caerulescens* being larger in body size and lighter in colour than *S. m. murinus* (Phillips, 1980; Figs. 1 and 6). They coexist in sea-ports and other major towns and are the only two subspecies to live inside human dwellings. They are also known to very readily interbreed making it difficult to assign some of the individuals to either of the subspecies convincingly (Phillips, 1980). However, since our sample size is limited to one *S. m. caerulescens*, we are not in a position to comment on this. The genetic distance between them in nuclear and mitochondrial DNA is also low. These facts support their classification as a single species, *S. murinus*, as suggested by many authors. *S. montanus*, the other species, differs from *S. murinus* morphologically, ecologically and genetically. This species is a small dark shrew with a slender tail that is distributed in the montane forests of the highlands, and the rain forest of Sabaragamuwa in the southwestern and southern provinces of Sri Lanka. It is confined to the forested area and very rarely ventures into human habitation. *S. murinus* on the other hand, is larger, with a lighter hue and a broader tail, especially at the base. They live inside houses and rarely venture away from human habitation.

In phylogenetic analyses of genetic data, *S. montanus* and *S. murinus* form two distinct clades. *S. montanus* is also distinct from *S. murinus* with a genetic distance of 0.9–1.3% for the nuclear Rag 1 gene and 6.0–6.5% in the mitochondrial cytochrome-*b* gene. Genetic distances estimated from the mitochondrial cytochrome-*b* gene have been used to define species (Bradley and Baker, 2001; Johns and Avise, 1998). Bradley and Baker (2001) surveyed mammal species and suggested that greater than 2% divergence of cytochrome-*b* sequences may indicate a species-level divergence. Johns and Avise (1998) noted that 90% of vertebrate sister species show more than 2% genetic divergence in cytochrome-*b* sequence and suggested that this level of divergence may indicate species-level divergence. *S. montanus* is a distinct species from *S. murinus* under that criterion.

Phylogenetic analysis of nuclear and mitochondrial gene sequences for Sri Lankan *S. murinus* subspecies and *S. montanus* revealed one instance of incongruence between the nuclear and mitochondrial gene trees (Figs. 3 and 4). Mitochondrial DNA from *S. m. kandianus* falls within a clade of *S. montanus* sequences, whereas nuclear sequences from *S. m. kandianus* fall with those of other *S. murinus*. This incongruence could be explained by homoplasy (McCracken and Sorenson, 2005), chance events of lineage sorting during speciation (Moore, 1995; McCracken and Sorenson, 2005) or introgressive hybridization (Sota and Vogler, 2001; Salzburger et al., 2002; Koblmüller et al., 2007). Homoplasy is unlikely given the marked similarity of mtDNA sequences in *S. m. kandianus* and *S. montanus* despite moderately large (ca. 6%) sequence differences between other haplotypes of *S. murinus* and *S. montanus* in Sri Lanka. Differential sorting of ancestral polymorphism is a possibility. Nuclear genetic differentiation of *S. murinus* and *S. montanus* in Sri Lanka is low (0.5–1.3% among species for the Rag 1 gene), suggesting a relatively recent divergence. Therefore, sorting of ancient polymorphism cannot be excluded. However, the locally restricted

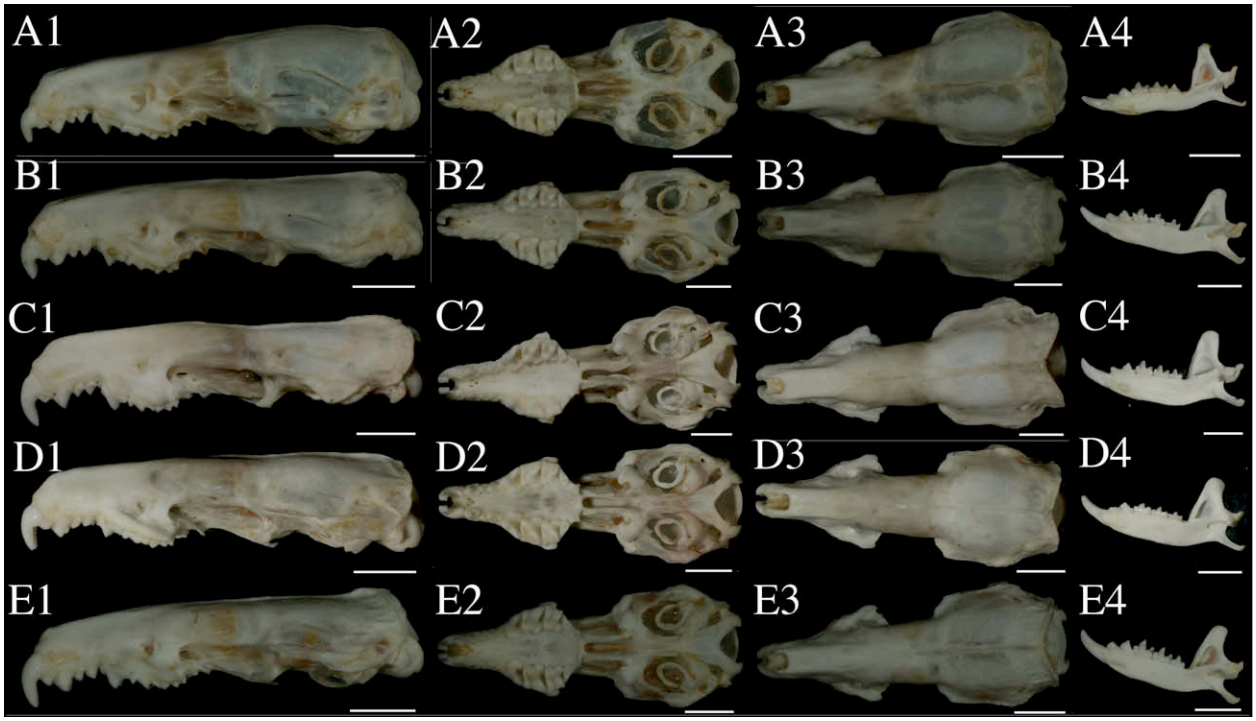

Fig. 7. Lateral, ventral and dorsal views of the crania and lateral view of the mandibles of males of, A1–A4, *S. montanus*; B1–B4; *S. m. murinus* (Anuradhapura); C1–C4, *S. m. caerulescens* (Colombo); D1–D4, *S. m. kandianus*; E1–E4, *S. m. murinus* (Udawalawe). Scale on the figure is 5 mm.

presence of a "*montanus* type" mtDNA in a population of *S. murinus* suggests local introgressive hybridization. Morphological data and nuclear genes are concordant and suggest that *S. m. kandianus* is a form of *S. murinus*. The sharing of mitochondrial genes by *S. montanus* and *S. m. kandianus* is thus likely due to introgressive hybridization. The habitats of *S. m. kandianus* and *S. montanus* overlap and since only the maternally inherited mitochondrial genes are shared by *S. montanus* and *S. m. kandianus*, hybridization must have occurred between one or more females of *S. montanus* and males of *S. m. kandianus* in this population.

When there are discrepancies in mitochondrial and nuclear gene trees there is a possibility that the species or the populations involved are hybrids (Ropiquet and Hassanin, 2006; Meyer et al., 2006). One such classic example is the hybrid origin of swordtail species, *Xiphophorus clemenciae* (Meyer et al., 2006). Here the mitochondrial genes place *X. clemenciae* among swordless platies but the nuclear genes (except the first 749 bp of Rag 1 gene) place it with the other southern swordtails in congruence with the traditional morphological phylogenies. The first 749 bp of Rag 1 gene is congruent with the mitochondrial genes. Sharing parts of the nuclear genes with both parent species confirm the hybrid origin of *X. clemenciae*. A similar situation may pertain to *S. m. murinus* from Udawalawe. The Udawalawe specimens resemble *S. m. murinus* in morphology. Phylogenetic analyses of both nuclear and mitochondrial DNA place it with other *S. murinus* in a single clade. The Udawalawe individuals possess the indel pattern in the EF1-alpha sequence characteristic of *S. murinus*, but the pattern in Rag 1 is less clear. In the Rag 1 gene tree, it is placed as a sister group to rest of the *S. murinus* sequences (Fig. 3) and in the Rag 1 gene sequences, the Udawalawe individuals share four out of eight informative nucleotides with *S. montanus* and four with *S. murinus* (Table 7). The first portion of the Rag 1 sequences shares character states with

Table 7
Informative nucleotide positions among the *S. murinus* subspecies and its populations, and *S. montanus* in the Rag 1 gene.

Species and subspecies (number of individuals)	218	464	564	573	765	768	772	813
S. m. caerulescens (1)	G	T	G	G	A	R	A	A
S. m. murinus – Anuradhapura (2)	G	T	G	G	A	G	A	A
S. m. kandianus – Peradeniya (2)	G	T	G	G	G	G	A	A
S. m. murinus – Udawalawe (2)	G	T	G	G	G	A	C	G
S. montanus – Agarapathana (5)	A	C	A	A	G	A	C	G
S. montanus – Kitulgala (2)	A	C	A	A	G	A	C	G
S. montanus – Kudawa (2)	A	C	A	A	G	A	C	G
S. montanus – Morningside (6)	A	C	A	A	G	A	C	G

S. murinus, whereas the latter portion shares states with *S. montanus*, suggesting that the Rag 1 gene sequence in the Udawalawe individuals may reflect recombination of *S. murinus* and *S. montanus* DNA within the gene. The Udawalawe individuals are found in a place where the distribution of *S. murinus* and *S. montanus* overlap and, therefore, hybridization is possible. Additional specimens and additional nuclear loci are necessary to resolve the genetic relationships of the Udawalawe individuals and to determine the extent of introgression at Udawalawe and Kandy.

S. zeylanicus is an endemic species described from Kitulgala, Sri Lanka and predicted to be distributed in the virgin forests in the Sabaragamuwa and Central Provinces (Phillips, 1980). Only four specimens were used in the description of this species (Phillips, 1928). It was later reported from Sinharaja forest (Wijesinghe et al., 2005). We collected two specimens from Kitulgala, which we initially thought to be *S. zeylanicus* due to the similar body size described for *S. zeylanicus*, even though the tail was not as long as in *S. zeylanicus*. *S. zeylanicus* has a tail 85% the length of the head and body while the two individuals caught in our study had tails 62.1% and 53.5% of the head and body size (Appendices 2 and 3). Phylogenetic analysis of mitochondrial and nuclear DNA sequence revealed that these two individuals were indistinguishable from *S. montanus*. However, the two specimens from Kitulgala are substantially larger than other *S. montanus*. This is reflected in their separation from other *S. montanus* along PC1 in the principal components analysis (Fig. 6). We regard the specimens that we collected at Kitulgala as *S. montanus*. The skull of the type specimen of *S. zeylanicus*, deposited in the British Museum of Natural History in London (BMNH), was used in the PCA of skull measurements, and it fell close to the *S. montanus* specimens (Fig. 6B). *S. montanus* is also recorded from Kitulgala (Phillips, 1928) and it is possible that two species were, or still are, present at that locality. Additional collections at Kitulgala are needed to clarify the identity of *S. zeylanicus* and to determine if this species is still extant in the area. We also failed to collect any specimens of this species from Sinharaja. However, we were able to collect *S. montanus* from this locality as well.

5. Summary

Molecular and morphological data support the recognition of two species of *Suncus* in Sri Lanka, *S. murinus* and *S. montanus*. However, our analyses discovered evidence of possible hybridization between the two species. Additional analyses of multiple nuclear gene markers are necessary to determine the degree to which hybridization and introgression have occurred between the two species, particularly at Kandy and Udawalawe. In addition, we found that *S. m. caerulescens* differs substantially in body size from other *S. murinus*, but the low genetic distance between *S. m. caerulescens* and other *S. murinus* populations suggests that they are either part of a single species, or may represent a very recent divergence event. Again, analyses of multiple nuclear markers are necessary to determine the degree to which *S. m. caerulescens* is exchanging genes with other *S. murinus*. Finally, the status of *S. zeylanicus* remains uncertain. Despite 700 trap nights at the type locality and 1400 trap nights in Sinharaja rainforest, we did not collect any specimens of *S. zeylanicus*. Therefore, this taxon may be rare. Additional field sampling at the type locality, and similar sites, is necessary to determine if *S. zeylanicus* is still present in Sri Lanka.

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Appendix 1

Primers used to amplify and sequence nuclear, mitochondrial and intron DNA loci.

Locus	Primer	5′–3′ primer sequence	Reference
Rag 1	Amp Rag1 F Amp Rag1 R1	AGC TGC AGY CAR TAC CAY AAR ATG TA AAC TCA GCT GCA TTK CCA ATR TCA CA	Murphy et al. (2001)
BDNF	BDNF F BDNF R	CTG ACA CTT TTG AGC ACG TGA TC AGG CTC CAA AGG CAC TTG ACT	Murphy et al. (2001)
Cytochrome-b	MVZ 05 MVZ 14	CGA AGC TTG ATA TGA AAA ACC ATC GTTG GGT CTT CAT CTY HGG YTT ACA AGAC	Smith and Patton (1993)
16S	16S Ar 16S Br	CGC CTG TTT ATC AAA AAC AT CCG GTC TGA ACT CAG ATC ACGT	Palumbi et al. (1991)
Aldolase	ALD 1F ALD 2R	TGTGCCCAGTATAAGAAGGATGG CCCATCAGGGAGAATTTCAGGCTCCACAA	Lessa and Applebaum (1993)
Elongation factor	EF 1a-1 EF 1a-2	GACAACGTTGGCTTCAACGTGAAGAACG ATGTGAGCAGTGTGGCAATCCAA	Palumbi (1996)

Appendix 2

External measurements (in mm) of male *Suncus* spp. *S. montanus* (*n* = 18), *S. montanus* from Kitulgala (*n* = 1), *S. m. kandianus* (*n* = 3), *S. m. murinus* (*n* = 2), *S. m. caerulescens* (*n* = 1) of Sri Lanka and *S. murinus* (*n* = 1) of India. Range, mean (standard deviation), and the range of the values as a percentage of head and body length are presented for each species/subspecies. Refer to the text for the explanations of the abbreviations.

	S. montanus	*S. montanus* (K)	*S. m. kandianus*	*S. m. murinus* (A)	*S. m. murinus* (U)	*S. m. caerulescens*
HB	87.3–107 94.7 (6.5)	121.5	120.5–124.2 122.1 (1.9)	111	127.4	150
H	31.3–37.5 34.0 (1.5) 31.0–38.9%	38.5 31.7%	35.1–42.7 38.4 (3.9) 29.1–34.3%	42.7 38.5%	38.7 30.4%	4.6 29.7%
TL	56.1–70.8 65.3 (3.8) 55.5–77.3%	75.5 62.1%	72–81.1 77.9 (5.1) 59.3–66.8%	76.5 68.9%	76.9 60.4%	84.8 56.5%
HFL	15.6–19.1 17.6 (1.0)	19	20.1–21.1 20.6 (0.5)	21.3	22	23.4
TBL	20.7––23.6 21.9 (0.8)	25.1	23.6–25.4 24.3 (0.9)	21.2	27.8	29.2
EH	8–12.6 9.9 (1.3)	10.6	11.4–12 11.7 (0.3)	11.7	12.9	13.6

Appendix 3

External measurements (in mm) of adult females for *S. montanus* (*n* = 9), *S. montanus* from Kitulgala (n = 1), and *S. m. murinus* (n = 2) of Sri Lanka. Range, mean (standard deviation), and the range of the values as a percentage of head and body length are presented for each species/subspecies. Refer to the text for the explanations of the abbreviations.

	S. montanus	*S. montanus* (K)	*S. m. murinus*(A)
HB	75.7–93.2 84.5 (5.1)	95.6	105.2, 112 108.6 (4.8)
H	32.2–34.75 32.7 (1.0) 35.5–42.7%	31.8 33.3%	33.9, 36.5 35.2 (1.8) 32.4, 32.6%
TL	62.7–67.1 63.5 (2.0) 70.9–79.9%	51.2 53.5%	59.4, 60.4 59.9 (0.7) 53.9, 56.5%
HFL	15.9–17.9 17.0 (0.6)	17.2	16.8, 18.9 17.9 (1.5)
TBL	18–22.3 21.1 (1.4)	20.8	20.4, 22.3 21.3 (1.4)
EH	7.1–10.6 9.2 (1.0)	8.5	11.6, 11.6

Appendix 4

Cranial measurements (in mm) for male *S. montanus* (*n* = 17), *S. montanus* from Kitulgala (*n* = 1), *S. m. kandianus* (*n* = 4), *S. m. murinus* (*n* = 2), *S. m. caerulescens* (*n* = 1) of Sri Lanka and *S. murinus* of India (*n* = 1). Range, Mean (standard deviation), and the range of the values as a percentage of greatest length of skull (GL) are presented for each species/subspecies. Refer to the text for explanation of the abbreviations.

	S. montanus	*S. montanus* (K)	*S. m. kandianus*	*S. m. murinus* (A)	*S. m. murinus* (U)	*S. m. caerulescens*
GL	23.1–27.7 25.2 (1.1)	27.8	27–31.1 29.1 (1.7)	31.4	29.9	34.3
BL	20.5–24.3 22.6 (1.0)	25.3	24.3–27.9 26.4 (1.5)	29	27.8	31.7

(*continued on next page*)

Appendix 4 (*continued*)

	S. montanus	*S. montanus* (K)	*S. m. kandianus*	*S. m. murinus* (A)	*S. m. murinus* (U)	*S. m. caerulescens*
BSL	19.7–23.0 21.4 (0.9)	23.9	22.6–26 24.5 (1.5)	26.9	26.0	29.3
CL	22.9–26.4 24.9 (0.9)	27.8	27–31.1 29.2 (1.7)	31.8	30.2	34.7
MTR	6.8–8.1 7.2 (0.4)	8.1	7.5–8.2 7.9 (0.4)	9.0	8.5	9.4
PL	10.2–12.2 11.1 (0.5)	12.5	11.8–13.8 13.0 (0.9)	14.2	13.6	15.5
PAL	8.9–10.9 9.8 (0.5)	11.3	9.9–11.8 11 (0.9)	12.3	11.7	13.2
PPL	10.6–12.2 11.7 (0.4)	12.6	12.6–13.9 13.5 (0.6)	14	14	16.1
LR	8.4–9.9 9.2 (0.4)	10.3	10.2–11.3 10.7 (0.5)	12	11.3	12.4
BB	9.7–11.4 10.9 (0.4)	12.3	11.3–12.2 11.7 (0.4)	10.3	9.4	15.3
LIOB	4.4–5.1 4.8 (0.2)	5.0	5.2–5.7 5.4 (0.2)	5.3	5.6	6.3
RB	1.9–2.7 2.4 (0.2)	2.9	3.2–3.4 3.3 (0.8)	3.2	3.6	4.3
PW1	6.4–7.7 7.1 (0.3)	7.7	8.2–8.8 8.5 (0.3)	8.6	8.9	9.5
PW2	3.1–3.7 3.5 (0.2)	3.5	3.8–4.0 3.9 (0.1)	3.7	4.3	4
BR1	2.1–2.7 2.4 (0.2)	2.9	3.1–3.3 3.25 (0.1)	3.3	3.5	4.2
BR2	6.8–8.1 7.6 (0.3)	8.3	8.9–9.5 9.1 (0.3)	9.3	9.5	10.7
BPM	1.8–2.1 1.9 (0.1)	2.2	2.1–2.4 2.2 (0.1)	2.2	2.1	2.5
BMM	1.2–1.8 1.5 (0.2)	1.6	1.6–2.0 1.8 (0.2)	1.8	1.9	2.1
HB	5.4–6.2 5.9 (0.2)	6.5	6.3–6.7 6.6 (0.2)	6.1	6.7	7.9
ML	13.2–15.1 14.1 (0.5)	15.3	14.7 17.1 16.0 (1.1)	17.6	15.8	19.1
LDI	15.4–17.5 16.3 (0.6)	18.0	16.9–19.6 18.5 (1.2)	20.3	19.4	22.2
LDT1	7.0–8.4 7.6 (0.4)	8.3	8.1–9 8.6 (0.4)	9.1	9	9.8
LDT2	9.4–11.4 10.3 (0.5)	11.5	11.1–12.1 11.6 (0.5)	12.3	12.4	13.8
DD	5.9–7.6 6.9 (0.5)	8.5	8–9.2 8.8 (0.5)	8.8	8.5	11

Appendix 5

Cranial measurements (in mm) for female *S. montanus* ($n = 8$), *S. montanus* from Kitulgala ($n = 1$), *S. m. kandianus* ($n = 1$), *S. m. murinus* ($n = 1$) of Sri Lanka. Range, mean (standard deviation), and the range of the values as a percentage of greatest length of skull (GL) are presented for each species/subspecies. Refer to the text for the explanations of the abbreviations. K = Kitulgala.

	S. montanus	*S. montanus* (K)	*S. m. kandianus*	*S. m. murinus*
GL	23.2–24.3 23.7(0.4)	23.9	25.7	26.3–27.7 26.9 (0.7)
BL	20.4–21.8 21.1 (0.5)	21.7	23.8	24–25.7 24.8 (0.9)
BSL	19.8–20.9 20.2 (0.4)	20.4	22.5	22.3–24.3 23.2 (1.0)
CL	22.9–24.0 23.5 (0.5)	24	26.1	26.5–27.9 27.1 (0.7)
MTR	6.6–7.3 6.9 (0.2)	7.2	7.4	7.5–7.9 7.6 (0.2)
PL	9.9–10.7 10.4 (0.3)	11.1	11.8	11.8–12.6 12.1 (0.4)
PAL	9.2–9.7 9.4 (0.2)	10.2	10.3	10.3–10.9 10.5 (0.3)
PPL	10.9–11.2 11.1 (0.3)	10.7	12.2	11.7–12.6 12.1 (0.5)
LR	8.3–8.8 8.7 (0.2)	9	9.6	9.9–10.6 10.2 (0.4)
BB	10.1–11.2 10.5 (0.4)	10.5	10.8	10.6–11.3 11 (0.4)
LIOB	4.5–4.9 4.7 (0.1)	4.5	5	4.6–4.9 4.7 (0.2)
RB	2.0–2.5 2.2 (0.2)	2.4	2.6	2.4–2.8 2.6 (0.2)
PW1	6.3–7.5 6.9 (0.4)	7.3	7.7	7.7–8.2 7.9 (0.3)
PW2	3.0–3.7 3.4 (0.2)	3.3	3.7	3.4–3.5 3.46 (0.1)
BR1	7.1–7.9 7.3 (0.3)	7.6	8.2	7.9–8.6 8.3 (0.4)
BR2	2.1–2.3 2.2 (0.1)	2.3	2.5	2.5–2.8 2.7 (0.2)
BPM	1.8–2.0 1.9 (0.1)	1.7	1.9	2.0–2.4 2.2 (0.2)
BMM	1.2–1.7 1.4 (0.2)	1.6	1.7	1.4–1.8 1.6 (0.2)
HB	5.6–6.0 5.9 (0.2)	5.8	6	5.9–6.1 6.0 (0.1)
ML	12.7–13.7 13.3 (0.3)	13.3	14.5	14.2–14.8 14.6 (0.3)
LDI	14.9–15.9 15.4 (0.4)	15.8	17.2	17.1–18 17.5 (0.5)
LDT1	9.5–10.3 9.8 (0.3)	10.5	10.9	10.7–11.6 11.2 (0.5)
LDT2	7.0–7.7 7.4 (0.2)	7.9	7.6	7.7–8.4 8.1 (0.4)
DD	6.1–6.9 6.4 (0.3)	6.5	7.1	7.3–7.8 7.5 (0.3)

Appendix 6

Comparative material examined, their collection site, BMNH registration number and the collector.

Genus	Species (male/female)	Collection site	BMNH Registration number	Collector
Suncus	*zeylanicus*	Kitulgala, Ceylon	28.1.25.2	Phillips
Suncus	*zeylanicus* (type specimen; male)	Kitulgala, Ceylon	28.1.25.1	W.W.A. Phillips
Suncus	(male)	Pachyura, Ceylon	52.2.19.13	Cuming
Suncus	(female)	Ceylon	59.5.31.55	Cuming
Suncus	*montanus*	West Hatupale, Ohiya, Ceylon	31.8.11.1	A.C. Tuthein Nolthenius
Suncus	*murinus montanus* (male)	Craig estate, Bandarawela, Uva hills, Ceylon	1955. 658	P.W. Phillips
Suncus	*montanus* (male)	Pattipola Central province, India	28.8.1.18	Mayor, Bombay nat.his.Soc.
Suncus	*montanus montanus* (male)	New forest estate, Galaha, c. p. Ceylon	28.9.6.5	W.W.A.Phillips
Suncus	*montanus montanus*	Deltotta, new forest estate, c.p. Ceylon	1928.9.6.6	W.W.A.Phillips
Suncus	*murinus montanus* (male)	New forest estate, Galaha, c. p. Ceylon	28.9.6.4	P.W.W.A. Phillips
Suncus	*caeruleus kandianus* (male)	Horton reserve, Kandy, Ceylon	1930.2.11.114	E.W. Mayor
Suncus	*caeruleus kandianus* (male)	Galapitakanda, Namunukula, Uva pro. Ceylon	51. 517	W.W.A. Phillips
Suncus	*c. kandianus* (male)	Tonacombe bungalow Namunukula, Uva Hills, Ceylon	52. 1258	W.W.A.Phillips
Suncus	*murinus kandianus* (male)	Balangoda, Kurunegala, N.W. Province, Ceylon	67. 775	F.C. Fernando
Suncus	*murinus caerulescens* (male)	Maldives	57. 389	
Suncus	*m. caeruleus* (female)	Dehiwala, Colombo, W.P. Ceylon	55. 54	W.W.A. Phillips
Suncus	*murinus*	Ceylon	1862.1.9.4	E. Gerrard
Suncus	*murinus* (male)	Sassawa, Ethiopia	94. 208	C. Hillman, D. Yalden

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