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Crocidura hikmiya, a new shrew (Mammalia: Soricomorpha: Soricidae) from Sri Lanka

SUYAMA MEEGASKUMBURA[1,2,4], MADHAVA MEEGASKUMBURA[1,3], ROHAN PETHIYAGODA[3], KELUM MANAMENDRA-ARACHCHI[3] & CHRISTOPHER J. SCHNEIDER[1]

[1]*Department of Biology, Boston University, 5 Cummington Street, Boston, MA02215, U.S.A.*
[2]*Department of Zoology, Faculty of Science, University of Peradeniya, Sri Lanka.*
[3]*Wildlife Heritage Trust, 95 Cotta Road, Colombo 8, Sri Lanka*
[4]*Corresponding author. E-mail: suyamam@pdn.ac.lk*

Abstract

A new species of crocidurine shrew, *Crocidura hikmiya,* is described from the Sinharaja World Heritage Site, Sri Lanka. The species is diagnosed on the basis of both morphology and mitochondrial DNA sequence data. Morphologically *C. hikmiya* is distinguished from *C. miya*, among other characters, by having a shorter tail, condyles protruding beyond the margin of the braincase, a posterior edge of maxillary bone rounded (dorsal view), an occipital bone triangularly shaped with an obtuse angle (dorsal view) and slightly flattened on the back; a foramen magnum less deep (ventral view); a dorsal posterior brain case not smooth; and an angular process of dentary short and stout. Phylogenetic analysis suggests that *C. hikmiya* is the sister-species of *C. miya*. The uncorrected genetic distance between the two species for the mitochondrial cytochrome-*b* gene fragment is 9.7–10.1%, suggesting species-level divergence. *Crocidura hikmiya* is confined to the mid-montane forests and lowland rainforests in the southwestern Sri Lanka, while *C. miya* is confined to montane forests of the central hills.

Key words: *Crocidura miya*, Sinharaja, phylogenetics, shrew taxonomy

Introduction

Crocidura is the most diverse genus of shrews in the world, comprising 172 species distributed throughout much of Europe, Asia and Africa (Hutterer 2005). Of the nine species of shrews described from Sri Lanka, two are included in this genus: *C. miya* (Phillips, 1929) and *C. horsfieldii* (Tomes, 1856). *Crocidura miya* is a medium-sized shrew with a head-and-body length (HBL) of 75–83 mm and a tail length of 90–100 mm. It is confined to the higher elevations (> 900 m) of the island's central hills (Phillips 1980). *Crocidura horsfieldii* is a smaller shrew with a HBL of 62–68 mm and a 49–55 mm tail. It has been recorded from the lowlands and mid-elevations of Sri Lanka; Mysore (Karnataka State, India); Ladakh (Jammu and Kashmir State, India); and Nepal (Hutterer 2005).

Despite prolonged terrestrial connections to the mainland during successive glacial sea-level lowstands (most recently until *ca* 10,000 ybp), the biota of Sri Lanka's south-western 'wet zone' rainforests (rainfall > 2,000 mm yr^{-1}) shows evidence of significant and prolonged isolation from both the island's dry zone and from peninsular India (Meegaskumbura *et al.* 2002; Bossuyt *et al.* 2004). Most of the island's endemic taxa are restricted to the wet zone, almost all of which was occupied by rain forests until large-scale clearing for coffee, cinchona and tea plantations in the 19th century. The shrews of Sri Lanka also show a high level of endemicity, with five of the nine currently recognized species being restricted to the island: *Solisorex pearsoni, Feroculus feroculus, Crocidura miya, Suncus fellowesgordoni* and *Suncus zeylanicus* (Phillips 1980).

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In 2003–2005 we undertook a survey of small mammals in Sri Lanka, focusing especially on shrews and rodents with a view to establishing the true diversity of these little-explored groups on the island, investigating their phylogeny and clarifying their taxonomy. We located our field sites in all the major habitat types of Sri Lanka: highland and lowland, wet zone and dry zone, and along altitudinal gradients. Extra efforts were made to collect all the hitherto known species from their type localities. During our field studies at the Sinharaja World Heritage Site (WHS), we collected a shrew that we provisionally assigned to *C. miya*, but further morphological and molecular analyses have revealed this to be a distinct species, which we describe here as new.

Materials and methods

Shrews were collected at two sites at the edge of secondary forest (selectively logged *c.*30 ybp) of the Sinharaja WHS (112 km^2, 210–1160 m elevation), using unbaited pitfall traps set between 45 cm high drift fences approximately 30–50 m long. Sherman traps baited with pieces of roasted coconut were also used, but were not effective in capturing shrews. Attempts to trap shrews in the primary forest at the core of the Sinharaja WHS were unsuccessful. A total of seven individuals of the new species described here were captured from the two sites: five from Kudawa (06°26'N, 80°25'E, elevation 460 m) and two from Morningside (06°24. 357'N, 80°86.872'E, elevation 1040 m). Skulls were removed, boiled gently in water, cleaned with a fine forceps and brush, and dried. After samples of muscle and/or liver tissue had been preserved in 90% ethanol for DNA analysis, 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. All specimens collected are deposited in the collection of the Wildlife Heritage Trust (WHT) of Sri Lanka.

DNA was extracted from ethanol-preserved tissues using Qiagen tissue extraction kits following manufacturer's protocols. Two mitochondrial gene fragments, cytochrome-*b* and 16S rRNA, were sequenced for all seven individuals of the new species, four individuals of *C. miya* and six other species (*C. attenuata*, *C. fuliginosa*, *C. horsfieldii*, *Suncus murinus*, *Sorex araneus* and *Euroscaptor longirostris*) included in the phylogenetic analysis. Original sequences of all the individuals used in the phylogenetic analysis were submitted to GenBank under accession numbers EU122195 to EU122226.

DNA was amplified by PCR using 25 µl reactions containing 12.5 µl of c. 5 ng/µl template DNA, 1.25 µl of each primer (10 µM), 2.5 µl of 10 mM dNTPs, 2.5 µl of 25 mM $MgCl_2$, 2.5 µl of 10× PCR buffer, and 0.625 U of Taq DNA Polymerase. 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, except that the annealing temperature was 48° C. Cytochrome-*b* and 16S were amplified using the primers MVZ 05 and MVZ 14 and 16S ar and 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'; and 16S br 5' CCG GTC TGA ACT CAG ATC ACGT 3'. PCR products were gel purified and sequenced on an ABI 3100 automated sequencer following manufacturer's recommendations. A total of 1673 base pairs were sequenced: 1140 bp of cytochrome-*b* and 533 bp of 16S. Sequences of 16S 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. The final dataset comprised 1595 bp including 1140 bp of cytochrome-*b* and 455 bp of 16S.

The mole *Euroscaptor longirostris* was used as outgroup, because moles have been suggested to be proximal to shrews (Stanhope *et al.* 1998; Asher 1999). 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

53 models examined by the hierarchical likelihood ratio test as implemented in Modeltest 3.6 (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 1,000,000 generations. The posterior probabilities were calculated after excluding the first 250,000 generations. 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 for the cytochrome-*b* gene fragment. Since some of the hypervariable regions of the 16S gene fragment were removed from the analysis and because substitution rate for the remaining sites is appreciably lower than for cytochrome-*b*, only the cytochrome-*b* fragment was used to calculate genetic distance.

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), length of forefoot (FFL), length of lower arm (LAL), ear height (EH) and diameter of eyes measured horizontally. The weight (WT) of each specimen was measured using an electronic scale, to the nearest 0.1 g. The following cranial measurements were taken (see Fig. 1): 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 (Table 1). Coefficients of variance (standard deviation/mean expressed as a percentage) were calculated for all measurements taken. Skull and dental characteristics were also observed. Type specimens of *C. miya* and *C. horsfieldii* in the collection of Natural History Museum, London and *C. horsfieldii* collected in the course of the present study were examined (for details see Appendix 1). The measurements of the skull of the holotype of *C. miya* are not included in the statistical analysis because its condition is too poor to facilitate accurate measurement of most of the above cranial measurements.

TABLE 1. Weight (g) and external measurements (mm) for *C. hikmiya* (n=7) and *C. miya* (n=4). Refer to Materials and Methods for abbreviations.

	C. hikmiya		*C. miya*	
	Range	Mean (SD)	Range	Mean (SD)
WT	6.5 – 9.0	7.7 (1.0)	7.7–7.9	7.9 (0.2)
HBL	64.0–76.0	70.1 (3.9)	64.9–73.4	68.6 (3.6)
HL	26.2–28.9 36.0–43.1 % of HBL	27.6 (1.0)	27.0–28.6 37.1–41.6% of HBL	26.6 (0.8)
TL	70.9–77.5 101.4– 114.5 % of HBL	74.9 (2.2)	92.8–100.3 135.0–154.5% of HBL	97.0 (3.4)
HFL	16.1–21.0	17.4 (1.6)	16.1–16.7	16.4 (0.3)
TBL	17.7–19.7	18.5 (0.8)	19.2–19.3	19.3 (0.1)
FFL	9.3 –10.2	9.6 (0.3)	8.7– 9.0	8.8 (0.1)
LAL	11.3–12.4	11.8 (0.3)	11.0–13.3	12.1 (1.0)
EH	7.0–8.9	7.8 (0.7)	5.8– 8.2	7.1 (1.1)

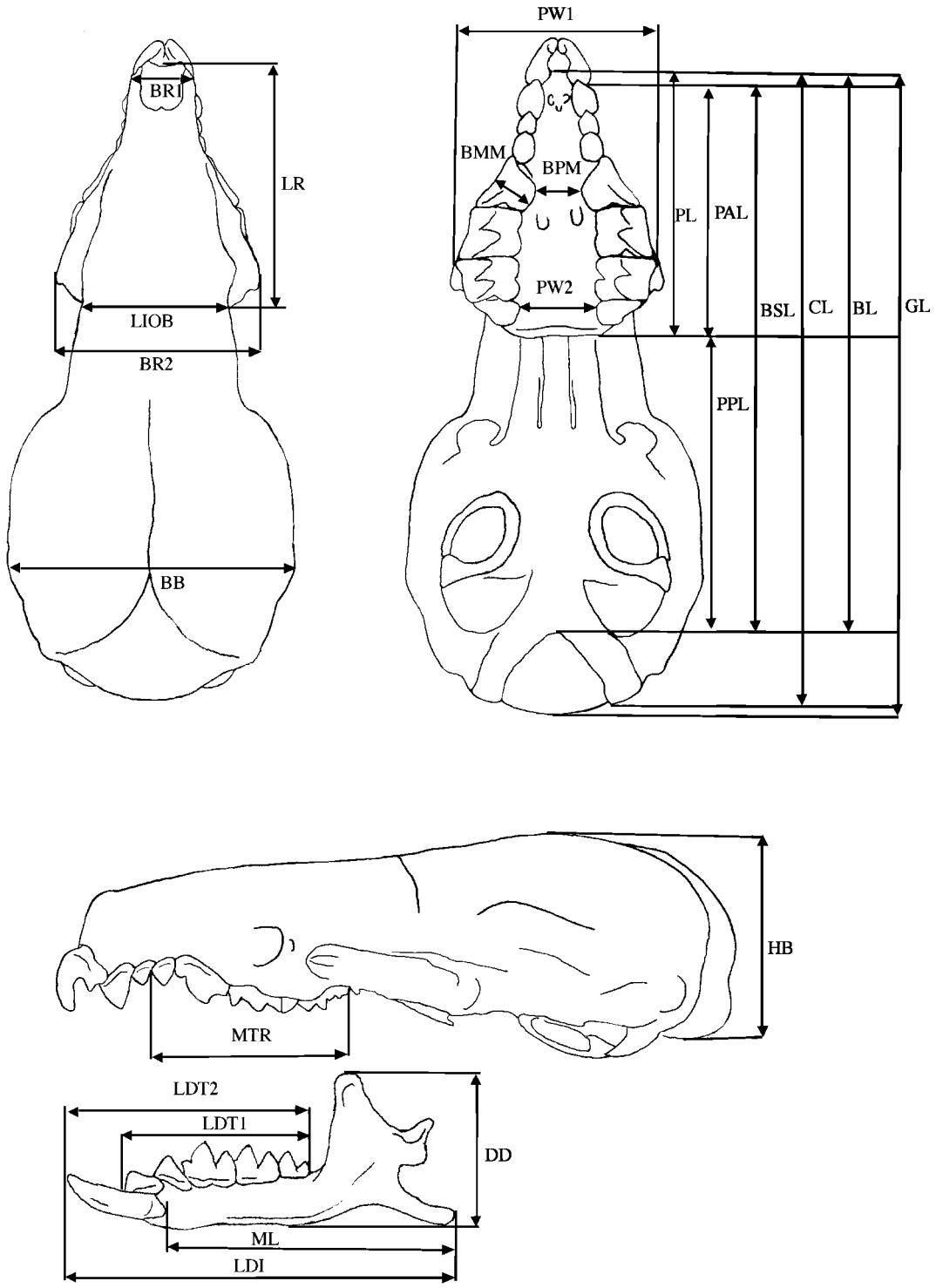

FIGURE 1. Dorsal, ventral and lateral views of cranium (and mandibule in the latter case), illustrating measurements taken in this study (see Materials and Methods for abbreviations).

SYSTAT (Version 11) was used for statistical analysis. Principal Components Analysis (PCA) of external and cranial variables were carried out separately to determine if the new species and *C. miya* occupy separate regions of multivariate morphospace for both external and cranial characters. PCA was performed on correlation matrices of raw morphological measurements with unrotated axes.

We consider it useful to state explicitly the species concept we employ as a testable hypothesis rather than defining a species in a post-hoc, arbitrary manner (Sites and Crandall, 1997). Here we adopt the General Lineage Concept of species (de Queiroz, 1998). This defines species as independent evolutionary lineages that are diagnosed by multiple criteria. We use both molecular and morphological data to define *C. hikmiya* and distinguish it from its congeners.

Results

Crocidura hikmiya sp. nov. (Fig. 2)

Holotype. WHT 6845 (male, 76.0 mm HBL, 7.8 g mass in life), preserved in alcohol; skull removed and preserved separately. Collected by M. Meegaskumbura and M. Bahir on 25 January 2004, at Kudawa, Sinharaja Forest Reserve (06°26'N, 80°25'E, elevation 460 m).

Paratypes. WHT 6836 (male, 64.0 mm HBL), WHT 6837 (female, 73.0 mm HBL), WHT 6843 (female, 69.9 mm HBL), and WHT 6844 (female, 71.0 mm HBL), collected from 20 – 25 January 2004, other collection data same as holotype. WHT 6849 (male, 62.5 mm HBL) and WHT 6853 (male, 67.1 mm HBL), collected by M. Meegaskumbura and K. Manamendra-Arachchi from 31 January- 4 February 2004 at Morningside (06°24.36'N, 80°86.87'E, elevation 1040 m). All specimens preserved in alcohol; skulls removed and preserved separately.

Etymology. The specific epithet 'hikmiya' is Sinhala for 'shrew', applied here as a substantive in apposition.

Diagnosis. *Crocidura hikmiya* is distinguished from *C. miya*, its sister-species (see Fig. 3 and below), by the following external characters (Table 1) and cranial characters (Table 2): shorter tail length 101.4–114.5% of HBL (*vs* 135.0–154.6% of HBL in *C. miya*); longer forefeet, 9.3–10.2 mm (*vs.* 8.7–9.0 mm in *C. miya*); a narrower least inter-orbital breadth 3.9–4.2 mm (*vs.* 4.2–4.4 mm in *C. miya*); broader breadth of rostrum at narrowest point, 1.9–2.2 mm (*vs.* 1.8–1.9 mm in *C. miya*); occipital condyles protrude beyond braincase (*vs.* condyles do not protrude beyond braincase in *C. miya*); posterior edge of maxillary bone rounded (*vs.* triangular in *C. miya*); in dorsal view the occipital bone is triangular shaped with an obtuse angle (*vs.* acute angle in *C. miya*); occipital bone is slightly flattened on the back (*vs.* rounded in *C. miya*); in ventral view foramen magnum less deep (*vs.* deep in *C. miya*); dorsal posterior brain case is not smooth (*vs.* smooth in *C. miya*); angular process of dentary short and stout (*vs.* long and thin in *C. miya*) (Fig. 4). The two species also differ in coloration, *C. hikmiya* is dark grey-brown on the dorsum including the tail and slightly lighter colored on ventral surface of body and tail (*vs. C. miya* which is brown on the dorsum including the tail and slightly lighter colored ventrally). There are no differences in the tooth characteristics between the two species.

Description. *Crocidura hikmiya* is a medium-sized shrew with a 64–76 mm HBL in the type series, which comprises sexually mature individuals. Tail slender, its length slightly exceeding that of head and body (tail length 101.4–114.5% of HBL) (see Table 1), semi-naked, with long, protruding hairs extending along proximal 13–24% of its length. Head length 26.2–28.9 mm (36.0–43.1% of HBL). Ears prominent, naked, height 7.0–8.9 mm. Snout pink, with long bristles interspersed among shorter ones; bristles dark at base, paling to silvery grey at tips. Eyes small (< 1 mm in diameter, measured horizontally). General color of body including tail dark grey-brown. Venter slightly lighter colored than dorsum. Several long, dark, 'guard hairs' interspersed among grey hairs on both dorsal and ventral sides of body; individual hairs grey at base, with brown tips. Dorsal side of hands and feet semi-naked, appearing pinkish brown, with sparse brown hairs on digits.

External and cranial measurements (mm) of the holotype of *C. hikmiya* (WHT 6845): HBL, 76.0; HL, 27.8; TL, 77.5; HFL, 17.0; TBL, 19.7; FFL, 9.5; LAL, 12.4; EH, 8.9; GL, 20.2; BL, 18.2; BAL, 17.1; CL, 20.2; MTR, 6.0; PL, 8.8; PAL, 7.9; PPL, 9.8; LR, 7.1; BB, 9.0; LIOB, 4.2; PW1, 5.9; PW2, 3.1; BR1, 2.2; BR2, 6.1; BPM, 1.6; BMM, 1.0; HB, 5.2; ML, 11.4; LDI, 13.0; LDT1, 6.1; LDT2, 8.3; DD, 5.5.

FIGURE 2. *In-vivo* portrait of *Crocidura hikmiya* paratype, WHT 6849, male, 62.5 mm HBL.

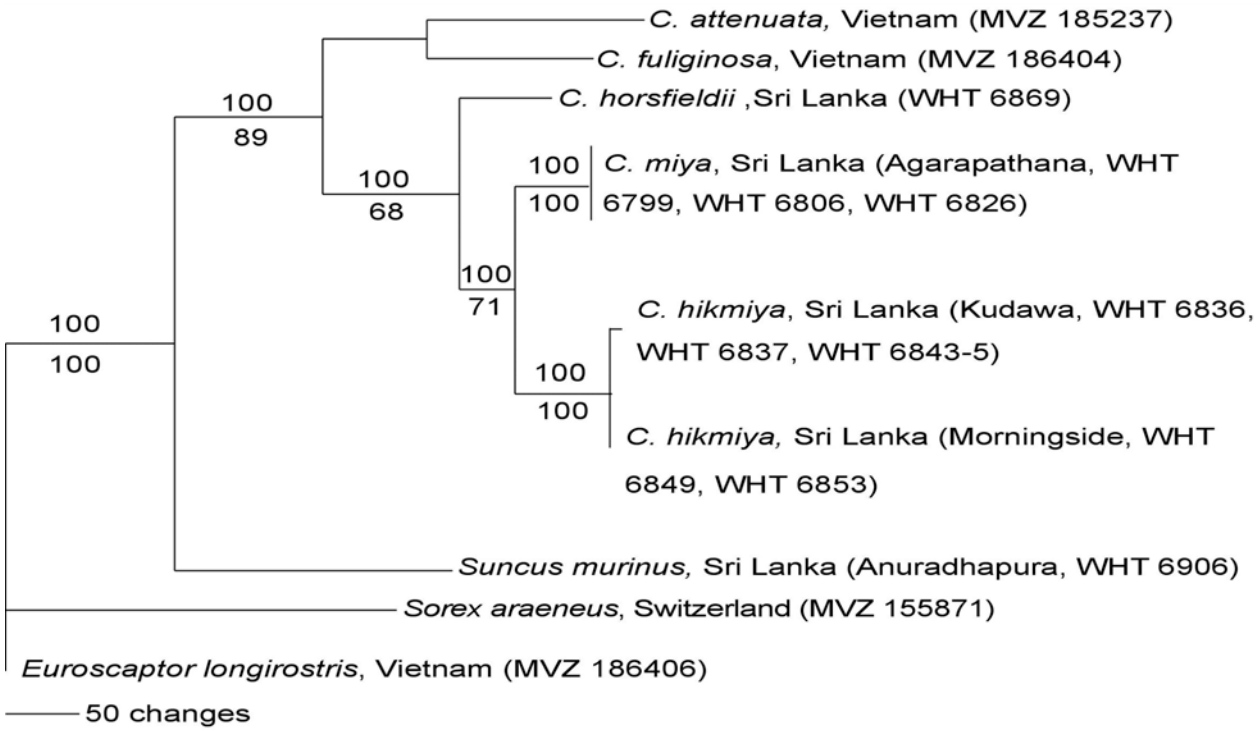

FIGURE 3. Phylogram inferred from Bayesian analysis of combined cytochrome *b* + 16S data sets under model GTR+I+G. Posterior probability and bootstrap values (expressed as percentages) are given above and below branches, respectively.

TABLE 2. Skull measurements of *C. hikmiya* and *C. miya*. Refer to Materials and Methods for abbreviations.

	C. hikmiya		*C. miya*	
	Range	Mean (SD)	Range	Mean (SD)
GL	19.2–20.2	19.7 (0.3)	19.5–20.7	20.1 (0.5)
BL	17.3–18.2	17.6 (0.3)	17.3–18.0	17.6 (0.3)
BSL	16.1–17.3	16.7 (0.4)	16.4–17.2	16.8 (0.3)
CL	19.2–20.2	19.7 (0.3)	19.6–20.3	19.9 (0.3)
MTR	5.6–6.1	5.8 (0.2)	5.6–5.8	5.7 (0.1)
PL	8.3–8.8	8.6 (0.2)	7.9–8.4	8.1 (0.2)
PAL	7.2–7.9	7.5 (0.2)	7.1–7.6	7.4 (0.2)
PPL	9.2–9.8	9.5 (0.2)	9.2–10.1	9.7 (0.4)
LR	6.9–7.1	7.0 (0.1)	6.8–7.3	7.0 (0.2)
BB	8.9–9.0	9.0 (0.1)	8.9–9.2	9.1 (0.1)
LIOB	3.9–4.2	4.1 (0.1)	4.2–4.4	4.3 (0.1)
PW1	5.5–5.9	5.7 (0.2)	5.7–5.9	5.8 (0.1)
PW2	2.6–3.1	2.8 (0.2)	2.7–2.9	2.8 (0.1)
BR1	1.9–2.2	2.0 (0.1)	1.8–1.9	1.9 (0.1)
BR2	5.9–6.2	6.1 (0.1)	6.0–6.2	6.1 (0.1)
BPM	1.4–1.6	1.5 (0.1)	1.4–1.6	1.6 (0.1)
BMM	1.0–1.1	1.0 (0.0)	0.9–1.1	1.0 (0.1)
HB	4.8–5.2	4.9 (0.1)	4.9–5.2	5.1 (0.1)
ML	10.8–11.4	11.1 (0.2)	10.5–11.0	10.8 (0.2)
LDI	12.4–13.1	12.8 (0.2)	12.2–12.9	12.5 (0.3)
LDT1	6.0–6.2	6.1 (0.1)	6.0–6.3	6.1 (0.1)
LDT2	8.0–8.3	8.1 (0.2)	7.9–8.3	8.1 (0.2)
DD	5.2–5.6	5.4 (0.1)	5.0–5.4	5.2 (0.2)

Distribution. *Crocidura hikmiya* is known only from two forest-edge sites in Sinharaja Forest, at Kudawa and Morningside.

Variation. There is no apparent sexual dimorphism among the specimens collected. All specimens from both sites were of approximately the same size (mean mass = 7.7; SD = 1.0); however, both ventral and dorsal colors of the Morningside specimens were comparatively lighter than those from Kudawa. One specimen (WHT 6837) has 5 unicuspid teeth on each side of upper jaw instead of 3, as observed in all other specimens of *C. hikmiya*: the first of these is located immediately posterior to the first incisor, having thrusted the latter forwards and upwards. It has also displaced the tooth immediately posterior to it. The first incisor of this specimen is 0.2 mm smaller than the first incisor in other specimens. This 'extra' tooth is triangular in buccal and occlusal views and the pointed end protrudes towards the palate in occlusal view. The second 'extra' tooth is small, triangular in occlusal view and located at the end of the unicuspid tooth row filling the space between the other unicuspids and the pre-molar. Due to the addition of 'extra' unicuspid teeth, the length of unicuspid tooth-row has increased by 0.2–0.4 mm than other normal specimens. However, due to the smaller size of the first incisor and displaced unicuspid tooth posterior to the first 'extra' tooth, the upper jaw tooth row remains the same length as that of other specimens (Fig. 5).

Phylogenetic analysis. Both Bayesian and maximum parsimony phylogenetic trees based on mitochondrial DNA sequence data strongly supports *C. hikmiya* as the sister species of *C. miya* (Fig. 3). The Bayesian

percentage posterior probability of the clade that includes only *C. miya* and *C. hikmiya* is 100, and the non-parametric parsimony bootstrap value for the same clade is 71% (Fig. 3). The percent pairwise uncorrected distance between these two species for the cytochrome-*b* gene fragment is 9.7–10.1%, while that between the currently-recognized species *C. miya* and *C. horsfieldii* is 11.5–11.6%; the latter, however, are not sister species. The percent pairwise uncorrected distance between *C. hikmiya* and *C. horsfieldii* is 13.3–13.6.

Morphological analysis. Principal Components Analysis of external measurements revealed that *C. hikmiya* and *C. miya* differ in multivariate morphological space along a single axis that represents tail length and forefoot length. PCA of the correlation matrix among variables resulted in four axes that explained a total of 88.7% of the total variance. Factor scores for *C. hikmiya* and *C. miya* overlap substantially on PC2, PC3, and PC4, but differ on PC1 (Fig. 6A). PC1 explained 31.8% of the variance in external morphological variables. Tail length loaded heavily and positively on PC1, whereas forefoot length had a high negative loading. Factor scores from the two species do not overlap on PC1, with *C. hikmiya* having shorter tail and longer forefoot, and *C. miya* having longer tail and shorter forefoot.

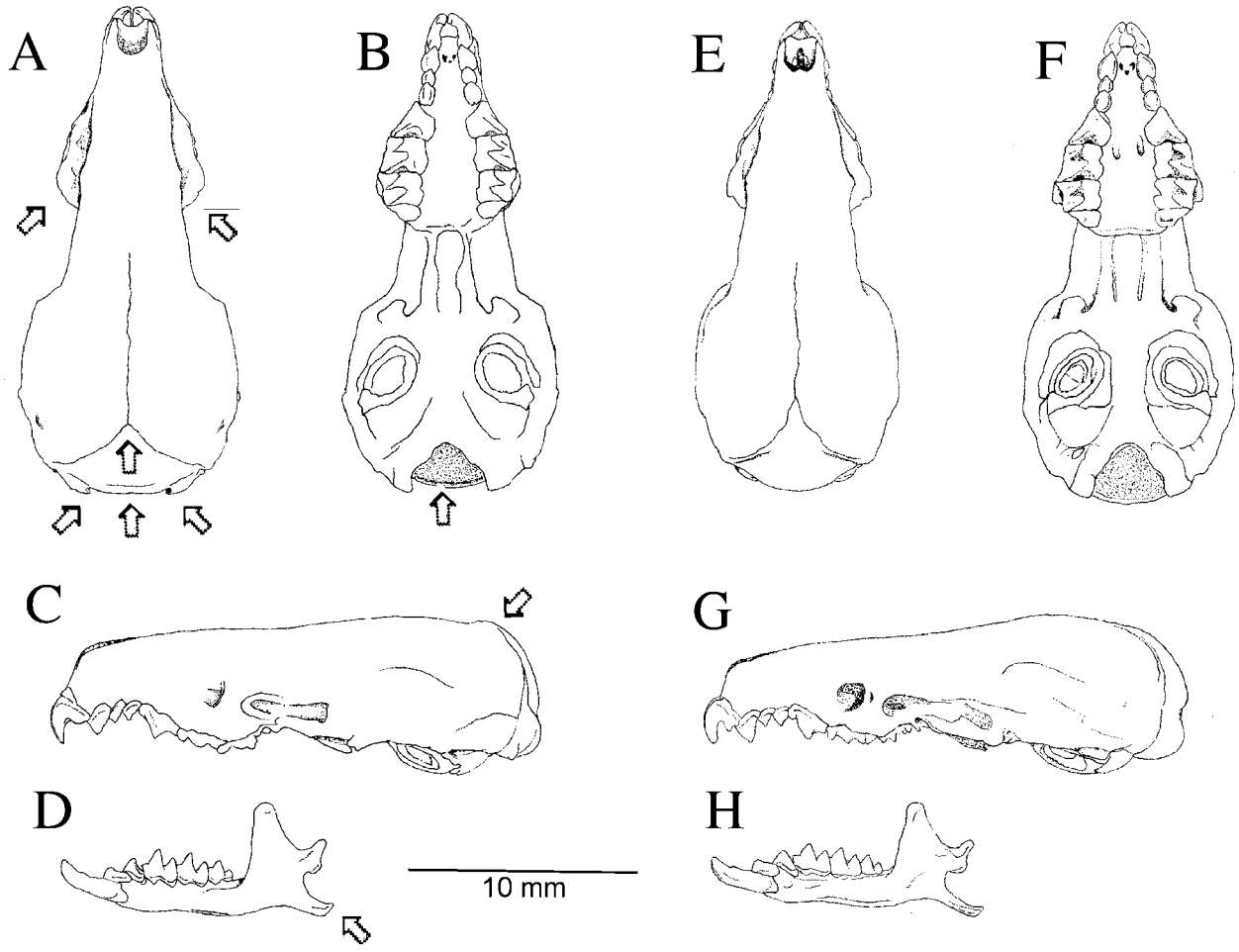

FIGURE 4. Dorsal, ventral and lateral views of crania (and mandibule in the latter case) of *Crocidura hikmiya*: WHT6845 (A–D) and *C. miya* WHT6826 (E–H). Arrows indicate skull characteristics that are different between the two species.

PCA of cranial variables revealed that *C. hikmiya* and *C. miya* differed primarily on PC2, with substantial overlap of factor scores on PC1 (Fig. 6B). PC1, which explained 33.6% of the variation, is largely a size axis, with high positive loadings for linear measurements reflecting skull dimensions (Basal Length, Mandible Length and Dentary Length Excluding Incisors). The two species overlapped substantially on PC1 indicating little variation in overall size. PC2 explained 20.1% of the total variance and had high positive loadings for

Least Interorbital Breadth and Breadth of Braincase and high negative loading for breadth of rostrum at narrowest point and palatal length. Factor scores of the two species do not overlap on PC2, with *C. hikmiya* having a narrower least interorbital breadth, narrower breadth of braincase on average, broader breadth of rostrum at narrowest point and longer palatal length on average than *C. miya* (Fig. 4; Table 2). PC3 and PC4 explained 13.5% and 12.3% of the total variance respectively, but factor scores of the two species overlap completely on these axes (plots not shown). These analyses indicate that *C. hikmiya* and *C. miya* occupy different regions of morphospace for both cranial and external features and are diagnosable morphologically.

In addition, several characteristics of the skull also diagnose the species (see Diagnosis).

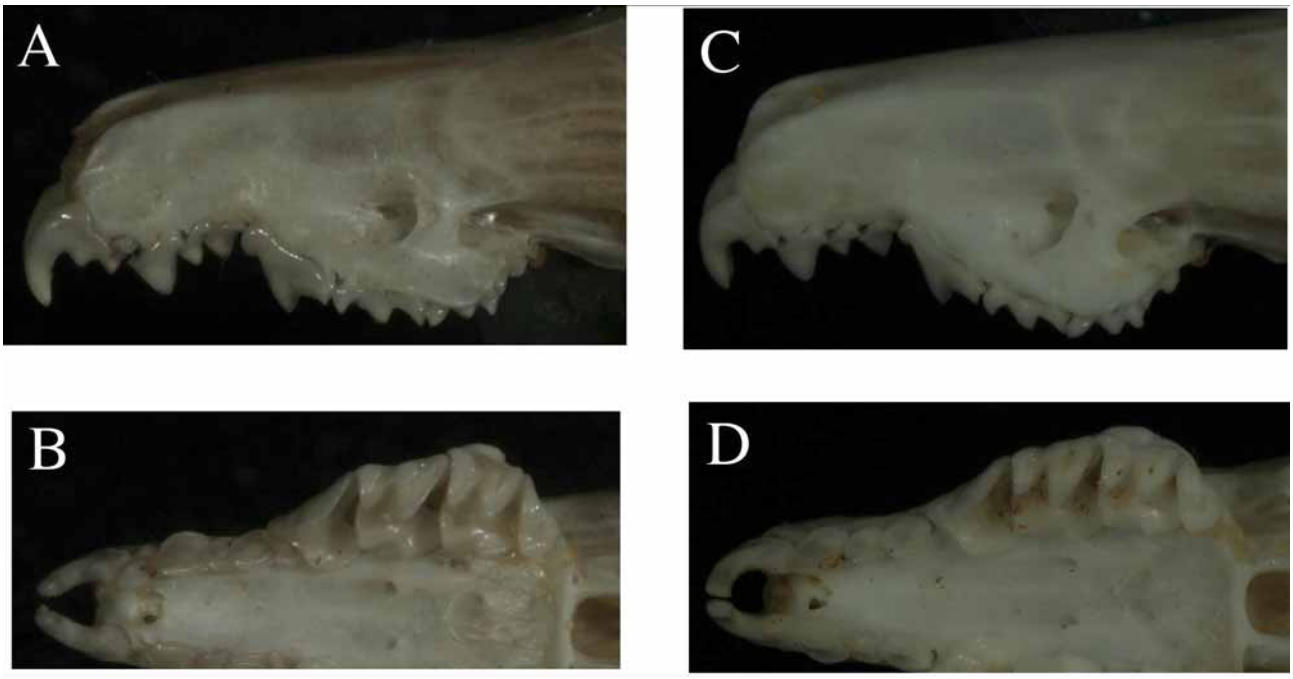

FIGURE 5. Buccal and occlusal views of upper jaw with extra teeth (A and B; WHT 6837) and normal teeth (C and D; WHT 6845) in *C. hikmiya*.

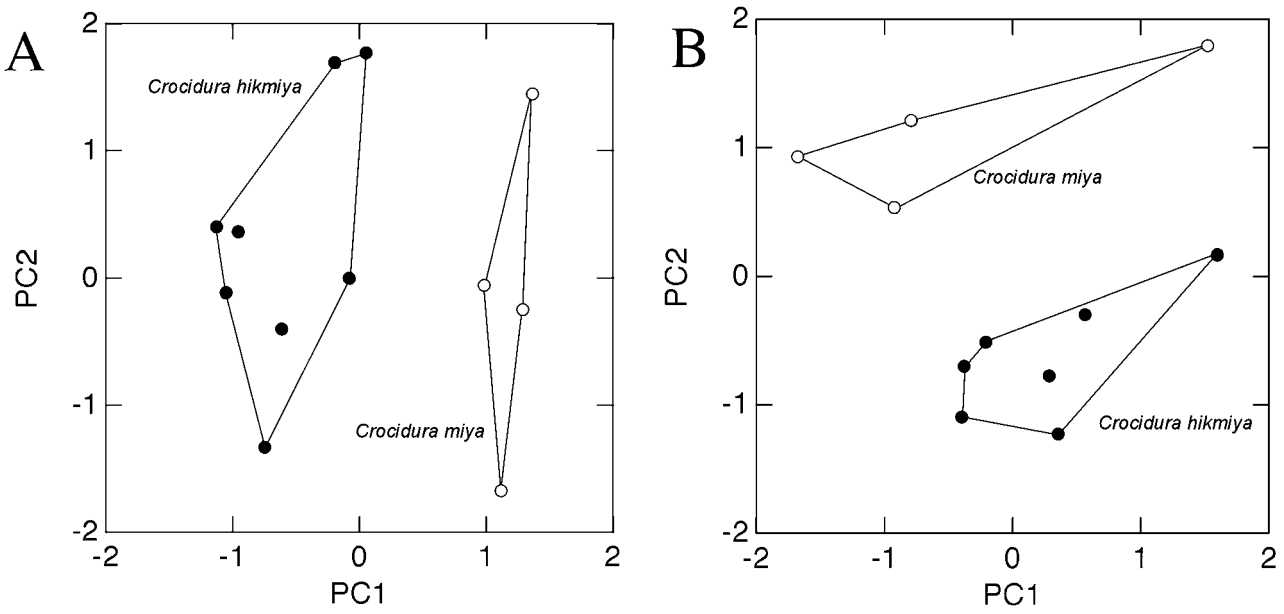

FIGURE 6. Plots of factor scores for PC1 *vs.* PC2 of *C. hikmiya* and *C. miya* for A: external body measurements, and B: cranial measurements.

Discussion

Crocidura hikmiya and its sister-species *C. miya* have overlapping head-and-body lengths. However, the new species is immediately distinguished from *C. miya* by its shorter tail, longer forefeet and, on average shorter tibia (Table 1). Principal Components Analysis of external and cranial measurements revealed that *C. hikmiya* and *C. miya* differ in multivariate morphological space (Fig. 6). *Crocidura hikmiya* is also distinguished from *C. miya* by several cranial characteristics as discussed above. Characteristics of the teeth do not show marked differences between the two species.

Phylogenetically, *C. hikmiya* is the sister-species of *C. miya* (Fig. 3). The genetic divergence between these two species is 9.7–10.1% for the cytochrome-*b* gene. The mitochondrial cytochrome-*b* gene is used extensively in phylogenetic studies, and was also used to validate genetic species identification by Bradley and Baker (2001) and Johns and Avise (1998). It is suggested that a 2–11% divergence of cytochrome-*b* may indicate a species-level divergence among mammal groups (Bradley and Baker 2001). Johns and Avise (1998) noted, however, that 90% of a wide range of commonly accepted vertebrate sister species show more than 2% genetic divergence in the cytochrome-*b* sequence. We did not use these values as strict cut-off points for validating *C. hikmiya*, but its observed genetic divergence from *C. miya* indicates a prolonged independent history of mtDNA lineages in the two species. This, combined with the morphological diagnosis, supports our contention that *C. hikmiya* represents a distinct species of crocidurine shrew.

We have compared the morphology of *C. hikmiya* only with its sister-species *C. miya* because of their phylogenetic and morphological affinities. No other species closely related to *C. miya* have been recorded elsewhere in Sri Lanka (Phillips 1980) or in peninsular India (Corbet and Hill 1992). The only other Sri Lankan congener, *C. horsfieldii*, is much smaller than *C. miya* and *C. hikmiya*, and is more distantly related to them as indicated by mtDNA data. Small-mammal sampling by us at 10 other sites in Sri Lanka failed to record *C. hikmiya*, which appears to be restricted to the vicinity of the Sinharaja WHS.

Crocidura hikmiya is currently known only from the mid- to high-elevation regions of the Sinharaja WHS, where it has a total available range of 110 km^2. Parts of Sinharaja were selectively logged in the 1970s, resulting in recent secondary growth, while a large, unlogged core area persists. We sampled small mammals in both these habitats, but recorded *C. hikmiya* only from secondary forest.

The only other published study of small mammals in Sinharaja is that of Wijesinghe and Brooke (2005), which recorded three species of shrews, and included Kudawa, the type locality of *C. hikmiya*. These authors recorded three species of shrews from this area: *C. miya*, *Feroculus feroculus* and *Suncus zeylanicus*. While they found all three species absent from highly disturbed habitats, they found *C. miya* to be the most abundant shrew in unlogged forest (11 specimens, against two each of *F. feroculus* and *S. zeylanicus*). *Crocidura miya* was, however, present also in logged forest, though only two specimens were recorded.

It seems likely that the individuals of *C. miya* recorded by Wijesinghe and Brooke (2005) were in fact specimens of *C. hikmiya*, as we did not find *C. miya* in the Sinharaja area. We are unable to explain our failure to record *C. hikmiya* from unlogged forest except by noting that our sampling was done in January (the peak of the dry season), whereas Wijesinghe and Brooke (2005) sampled in May–August, which is the wettest time of year in Sinharaja. We also note that Wijesinghe and Brooke (2005) were successful in catching shrews in baited Sherman traps, whereas we were not: all the shrews collected in the present study were trapped in unbaited, drift-fenced pitfall traps. In any event, the data of both Wijesinghe and Brooke (2005) and ourselves suggest that *C. hikmiya* is the most abundant shrew in Sinharaja. As such, we feel it is premature to assign a conservation status to this species until additional information on its Area of Occupancy or Extent of Occurrence becomes available.

Crocidura hikmiya and *C. miya* do not appear to occur in sympatry. The latter is confined to the high elevations (900–1900 m) in the Central Hills (Phillips 1980; pers. obs.). The four specimens used for comparison in this study were collected from Agarapatana, in the island's central hills. *Crocidura hikmiya* was

recorded only from Kudawa and Morningside, located at the western and eastern extremities, respectively, of the Sinharaja WHS. The forest around Kudawa has been classified as a *Mesua–Doona* community, representing, on a regional scale, a mixed dipterocarp forest (Whitmore 1984). The habitat at Morningside, however, is quite different from that at Kudawa. This represents a transition between the lowland wet-evergreen vegetation and low-elevation (~1000 m) tropical montane forest in which recent studies have shown a remarkable degree of microendemism in amphibians, lizards and freshwater crabs (Fernando *et al.* 1994; Pethiyagoda and Manamendra-Arachchi 1998; Bahir and Maduwage 2005; Bahir and Ng 2005; Bahir and Surasinghe 2005; Bahir *et al*. 2005; Batuwita and Bahir 2005; Manamendra-Arachchi and Pethiyagoda 2001, 2005; Meegaskumbura and Manamendra-Arachchi 2005). Given the ecological separation of these two habitats, we predict that *C. hikmiya* occurs throughout most of the ~110 km^2 extent of Sinharaja, and perhaps also in other nearby forested areas.

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Appendix 1. Comparative material examined

Crocidura miya, BMNH 28.9.6.1, holotype, Galaha, Central Province, Ceylon [Sri Lanka], coll. W. W. A. Phillips. WHT 6799, WHT 6806, WHT 6826 Agarapatana, Central Province, Sri Lanka, coll. S. H. Meegaskumbura.
Crocidura horsfieldii, BMNH 52.2.19.12, holotype, Ceylon, ex. Cuming. WHT 6833, Dimbula, Patana, Sri Lanka, coll. M. Meegaskumbura and M. M. Bahir. WHT6869, WHT 6872, WHT 6878, Peradeniya, Sri Lanka, coll. S. H. Meegaskumbura. WHT 6908, Anuradhapura, Sri Lanka, coll. M. Meegaskumbura and K. Mahamendra-Arachchi.
Suncus murinus, WHT 6906, Anuradhapura, Sri Lanka, coll. M. Meegaskumbura.
Crocidura attenuata, MVZ 185237, Vietnam.
Crocidura fuliginosa, MVZ 186404, Vietnam.
Sorex araneus, MVZ 155871, Switzerland.
Euroscaptor longirostris, MVZ 186406, Vietnam.